Ministry of Public Health of Ukraine
Zaporizhzhya State Medical University
Biochemistry & Laboratory Diagnostics Department

Introduction into Metabolism and Energy Exchange in Human Organism

Textbook
for independent work at home and in class
for students of international faculty

Speciality: 7.120 10001 «General Medicine»

Zaporizhzhya
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The textbook is created as additional manual for study of biochemistry for students of international faculty.

This textbook is recommended to use for students of international faculty (the second year of study) for independent work at home and in class.

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Chapter 1. The introduction into metabolism and energy exchange. Tissue respiration and oxidative phosphorylation

Metabolism. Catabolic and Anabolic pathways

Metabolism is the sum total of processes that are carried out in human organism. Metabolism consists of metabolic pathways. Metabolic pathway is a sequence of enzymatic reactions, which provides the formation of some important products for human organism. All metabolic pathways provide the constant level of all important substances in a cell (homeostasis).

The most important substances in the cell are: proteins, nucleic acids, lipids, carbohydrates, water, some simple substances: O_2, vitamins, ions and many others. So, first of all, we have to discuss the metabolic pathways for these substances.

All metabolic pathways are divided in three groups: ***Anabolic, Amphibolic and Catabolic processes.***

Anabolism is the sum total of metabolic pathways concerned with combining building block compounds into the complex macromolecules required by the organism. Anabolic processes require energy inputs. Energy can be supplied in two ways: 1) by ATP transferred from the catabolic pathways; 2) in some cases, by high-energy hydrogen in a form of reduced NADPH.

Catabolism is the sum total processes where complex molecules are broken down into simpler ones. There is the free energy formation during these processes. Particularly, this energy (ΔE_1) is used for ATP synthesis, another part of energy is a thermal energy (ΔE_2).

Amphibolic processes are those that have some intermediate metabolites which may be used in anabolic and catabolic processes. The interrelation between all of them is represented in figure 1.1.

Peculiarities of catabolic pathways

All substances that are involved in catabolic processes are divided in two groups: exogenous and endogenous. Exogenous substrates incorporate into human organism during the nutrition, they are in food products. Endogenous substances are produced in human organism. Catabolic pathways are divided in three stages.

The first stage of catabolic processes for exogenous substances is located in the gastrointestinal tract (GIT). There is a conversion of proteins, lipids (triacylglycerols, TG), carbohydrates (polysaccharides and disaccharides), nucleic acids into nucleosides (fig.1.2). Enzymes for this digestion are Hydrolases. There is no energy formation in a form of ATP or NADPH during catalysis by hydrolases. All these products (amino acids, High Fatty Acids (HFA), glycerol,

monosaccharides, nucleosides) are absorbed in the small intestine and there is their intake to the bloodstream. In this way they are transported to all organs and tissues. Complete information about this phase in GIT you will obtain in chapter 2.

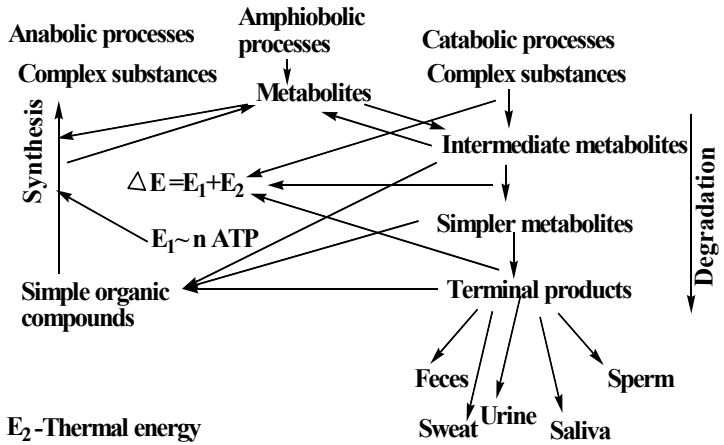


Figure 1.1 Interrelations in different metabolic pathways and energy exchange in human organism

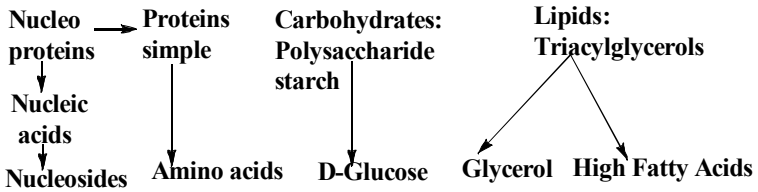


Figure 1.2.The first stage of catabolic processes in GIT

The first stage of catabolic processes for endogenous substances may be in various parts of a cell of any type of tissue: in cytoplasm, endoplasmic reticulum (ER) and lysosomes. Lipids are destroyed in membranes, cytoplasm, lysosomes and mitochondrion; proteins - in cytoplasm and lysosome; carbohydrates – in cytoplasm.

The second stage of catabolic processes is located in cytoplasm, ER and mitochondrion (fig.1.3). The terminal intermediate metabolite for the second catabolic phase is acetyl-CoA. Nucleosides are destroyed with another products formation in this stage: uric acid, ammonium, urea, carbon dioxide. It should be mentioned that the second stage of catabolic processes is carried out by three enzyme classes: Oxidoreductases, Lyases, Transferases. Reduced forms NADPH, NADH and $FADH_2$ are formed in this stage (may be in cytoplasm or in mitochondria).

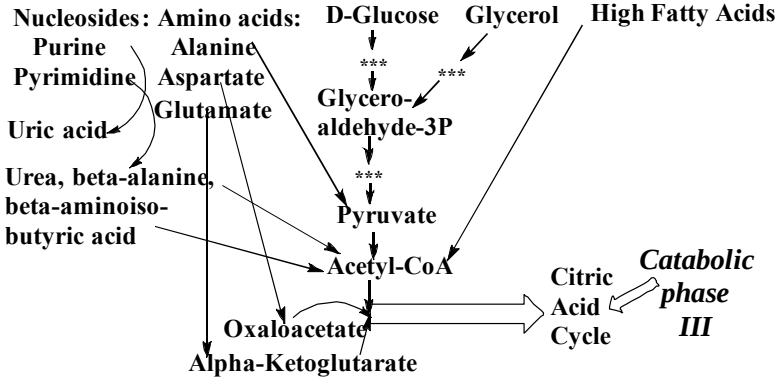


Figure 1.3. The second stage of catabolic processes

The third stage (the last one) of catabolic processes is the Citric Acid Cycle or Krebs Cycle located in the matrix of mitochondria except one reaction (Succinate is converted to Fumarate (fig.1.4.)

Some features of enzymes action in the Krebs cycle

Citrate synthetase catalyzes the synthesis of Citrate from two substrates: Oxaloacetate and Acetyl-CoA. Allosteric Inhibitors: ATP, NADH, Acyl~SCoA of High Fatty Acids in high concentration. It is the first key enzyme for process regulation.

Cis-Aconitase (Fe^{2+}) catalyzes two reactions: dehydration ($-H_2O$; cis-aconitate is formed from citrate) and hydration ($+H_2O$); isocitrate is formed from cis-aconitate). Inhibitor: Fluoride acetate.

Isocitrate dehydrogenase (Mg^{2+}, Mn^{2+}, NAD^{+}) catalyzes the oxidative decarboxylation of isocitrate to form three products: α-ketoglutarate, carbon dioxide and NADH. Allosteric Activator: ADP. Allosteric Inhibitors: ATP, NADH in high concentration. It is the second key enzyme for process regulation.

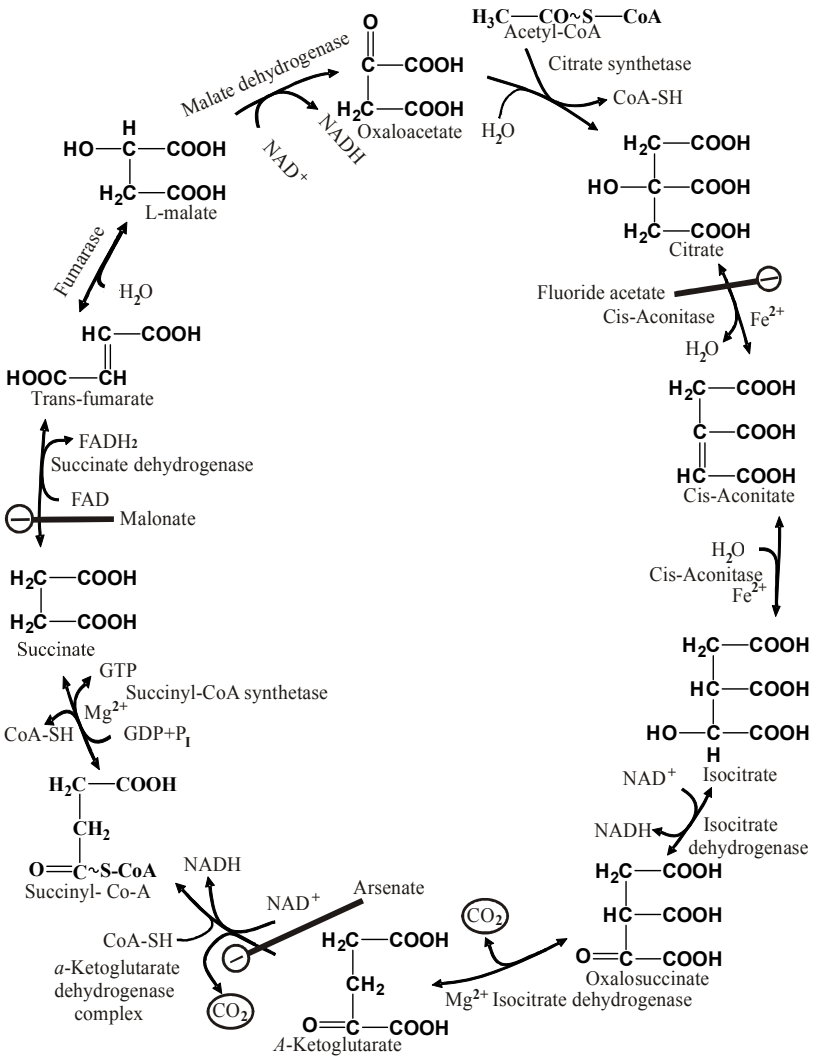


Figure 1.4. Krebs Cycle (chemical reactions)

α-Ketoglutarate dehydrogenase complex contains three enzymes: E_1-TPP (vitamin B_1 –derivative); E_2: CoASH (vitamin B_3-derivative), Amine of Lipoic acid; E_3: FAD (vitamin B_2-derivative, NAD+ (vitamin PP-derivative). It catalyzes the oxidative decarboxylation of α-ketoglutarate to form three products: succinyl~SCoA, carbon dioxide and NADH. Allosteric Inhibitor: Succinyl~SCoA in high concentration.

Succinyl~SCoA thiokinase (Mg^{2+}) catalyzes the formation of two products: succinate and GTP using the energy from Succinyl~SCoA cleavage. The reaction type is substrate phosphorylation.

Succinate dehydrogenase (FAD^+-containing) catalyzes the dehydrogenation of Succinate to form Fumarate and $FADH_2$ as prosthetic group of enzyme. Competitive Inhibitor for it is Malonate. It is single enzyme placed in the inner mmembrane of mitochondria.

Fumarase catalyzes the conversion of trans-fumarate, only, to form Malate due to hydration.

Malate dehydrogenase (NAD^+-containing) catalyzes the dehydrogenation of L-malate, only, to form oxaloacetate and NADH. This reaction explains the cyclicity of the process because of regeneration of oxaloacetate - the initial substrate for first reaction catalyzed by Citrate synthase.

Biological role of Citric Acid Cycle:

- Citric Acid Cycle – the last stage of all catabolic processes in a cell. This process generates per 1 cycle the reduced forms NADH (3 molecule) and $FADH_2$ (1 molecule) which are donors of electrons to respiratory chain.
- One molecule of high-energy bonds containing substance is formed in 1 cycle→ GTP
- There is the utilization of two carbon atoms from acetyl~SCoA in two molecules of carbon dioxide per 1 cycle;
- According last three notions the most important role of this process to provide a cell by energy supply.
- Citric Acid Cycle is amphibolic pathway, because some metabolites of this process are used for anabolic processes: a) synthesis of some essential amino acids from oxaloacetate (Asp, Asn) and α-ketoglutarate (Glu, Gln); b) synthesis of glucose (in gluconeogenesis) from oxaloacetate and its precursors in the cycle; c) at condition of citrate accumulation in the matrix it can move through membranes into cytoplasm. Citrate lyase hydrolyses it in two products: acetyl~SCoA and oxaloacetate in cytoplasm. Acetyl~SCoA is used in cytoplasm for HFA, Cholesterol, Ketone bodies synthetic ways.
- The terminal products for process per 1 mole of acetyl-CoA involved into the process: $2CO_2$, GTP, 3NADH, 1 $FADH_2$·E
- The energy effect for 1 cycle equals 12 ATP:
  1) due to substrate phosphorylation – 1 GTP = 1 ATP;
  2) due to oxidative phosphorylation – 11 ATP

Vitamin provision of Krebs cycle

Nicotinic acid (NAD^+), riboflavin (FAD^+), pantothenic acid (CoA~SH); thiamine (TPP), Lipoic acid are the main important for

normal duration of Krebs cycle reactions. The most severe infringements in human tissues are observed at deficiency of thiamine and nicotinic acid.

The regulation of Krebs cycle duration

The rate of Citric Acid cycle duration depends on the Pyruvate dehydrogenase complex activity that gives Acetyl-CoA as a product. The ratio $\frac{NADH \cdot H^+}{NAD}$>1; $\frac{CH_3CO \sim SCoA}{CoA \sim SH}$>1 are factors for Pyruvate dehydrogenase complex inhibition, so the Citric Acid Cycle is also inhibited using these factors.

It is very important to value the ratio ATP/ADP in a cell. It is called as respiratory control.

If $\frac{ATP}{ADP}$≥1 - the rate of Krebs cycle duration decreases.

$\frac{ATP}{ADP}$<1 - the rate of Krebs cycle duration increases.

$\frac{ATP}{ADP}$≠0, if it equals zero - it means the cell death.

Tissue respiration

It is the sum of oxidation processes producing reduced vitamin derivatives such as NADH, FADH2, which are donors of electrons transported by electron transport chain to molecular oxygen.

The first stage of tissue respiration is the second stage of catabolic processes.

The second stage of tissue respiration is third stage (Citric Acid Cycle) of catabolic processes.

Both two stages are producers of NADH, FADH2 (figure 1.5)

The third (last) stage of tissue respiration is the function of electron transport chain (respiratory chain) in the inner membrane of mitochondria. It is the most important stage for tissue respiration, because the oxygen use may be on the level of respiratory chain function, only.

The electron transport chain is series of highly organized oxidation-reduction enzymes whose reactions can be represented by equation:

Reduced A + Oxidized B→ Oxidized A + Reduced B

Long and short respiratory chains may be found in the inner membrane of mitochondria. Experimental investigation of the components for respiratory chains showed the presence of some complexes which may be allocated and recognized in function.

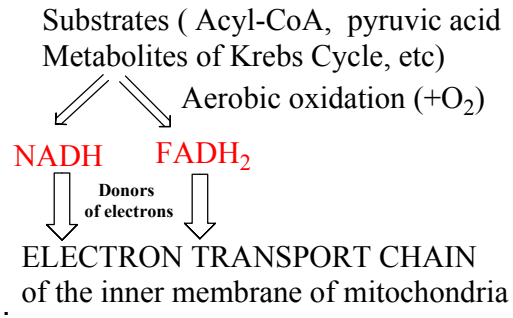


Figure 1.5. Aerobic oxidation in production of NADH and $FADH_2$

Complexes of respiratory chain

All the components of ETC are shown in the figure 1.6.

Complex I: NADH-coenzyme Q reductase (NADH-dehydrogenase). FMN^+ and Iron-Sulfur centers (Fe-S) are represented as the non-protein parts in this complex. It is the initial complex for the long respiratory chain. The function of it is described below:

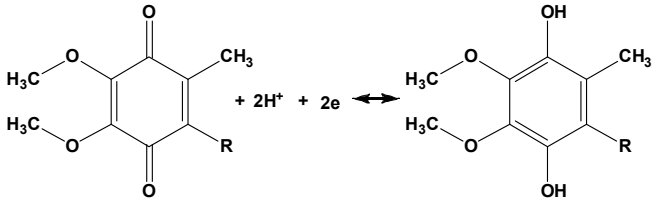
NADH + H^+ + FMN•E_1 → NAD^+ +$FMNH_2$•E_1 (1)

FMN• E_1 – NADH-dehydrogenase (oxidized form)

Then NADH-degydrogenase passes the electrons to Ubiquinone (CoQ):

CoQ + $FMNH_2$•E_1 → FMN•E_1 + $CoQH_2$ (2)

The transformation of CoQ structure to $CoQH_2$ is described below:



Complex II: Succinate dehydrogenase (FAD^+- the non-protein part) linked to Iron-Sulfur proteins (Fe-S). Its function is to oxidize the succinate and to transfer protons ($2H^+$) and 2 electrons to CoQ. It is the initial complex for the short respiratory chain.

Complex III: 2 cytochromes b (Fe^{3+}/ Fe^{2+}), 2 cytochromes c_1 (Fe^{3+}/ Fe^{2+}) linked to Iron-Sulfur proteins (Fe-S). Its function is to oxidize $CoQH_2$ and to transfer 2 electrons from it to 2 cytochromes c (Fe^{3+}/ Fe^{2+}).

$CoQH_2$ + 2 cyt b (Fe^{3+}) → 2 cyt b (Fe^{2+}) + CoQ + $2H^+$ (3)

2 cyt b(Fe^{2+}) + 2 cyt c_1(Fe^{3+}) → 2 cyt b(Fe^{3+}) +2 cyt c_1(Fe^{2+})(4)

2 cyt c(Fe^{3+})+2 cyt c_1(Fe^{2+}) → 2 cyt c(Fe^{2+}) + 2 cyt c_1(Fe^{3+})(5)

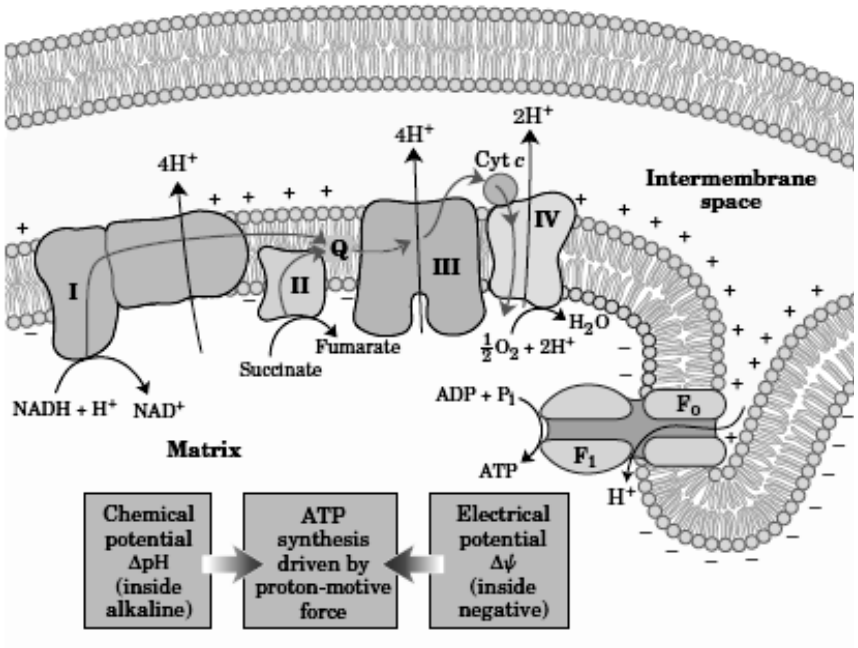


Figure 1.6. Coupling of oxidation with oxidative phosphorylation

All the cytochromes b, c_1, c are hemoproteins containing one heme and one polypeptide chain according to one molecule composition. The exception is for Cytochrome C Oxidase, it is also hemoprotein but more complicated in structure. All the cytochromes can attach electrons,only, and pass the to acceptor.

Complex IV: Cytochrome C Oxidase (cytochrome aa_3), terminal complex of respiratory chain, containing 6 subunits (four *a* with Fe^{3+}/ Fe^{2+}, two a_3 *with* Cu^{+}/Cu^{2+} ions). Its function is to oxidize 2 cytochromes *c* (Fe^{2+}) and to reduce 1 atom of molecular oxygen to form oxide-anion O^{2-} and then H_2O:

$$CChO(Fe^{3+}, Cu^{2+}) + 2\ cyt\ c(Fe^{2+}) \rightarrow 2\ cyt\ c(Fe^{3+}) + CChO\ (Fe^{2+}, Cu^{+}) \quad (6)$$

$$CChO\ (Fe^{2+}, Cu^{+}) + ½\ O_2 \rightarrow CChO\ (Fe^{3+}, Cu^{2+}) + O^{2-} \quad (7)$$

Or:

$$O_2 + 8H^{+} + 4e \rightarrow 2H_2O + 4H^{+} \quad (8)$$

The equations (3, 8) explain the function of complex III, IV as pumps for protons. The same function - to be the pump for protons - was proved for complex I, too

According the description above cytochrome C and ubiquinone are not components of any complex of respiratory chain.

Tasks dissolved by the function of ETC:

1. **The transport of electrons from reduced derivatives of vitamins (NADH, $FADH_2$) to molecular oxygen.**
2. **The release of energy at any step of oxidation in ETC.**
3. **The pumping of protons from the matrix to intermembrane space**
4. **The production of active form of oxygen - oxide-anion O^{2-} and then H_2O**

Some free radicals may be formed from oxygen during the tissue respiration:

Superoxide anion $Ó_2^-$, Superoxide radical $Ó_2$, Hydrogen peroxide radical HOÓ or may be hydrogen peroxide H_2O_2. All these particles are highly reactive that can react with and damage DNA and lipid bilayers of membranes. There are some enzyme systems that can protect cells from free radicals damage:

1) ***Superoxide dismutase*** catalyzes reaction:

$$2\ Ó_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

2) ***Peroxidase and Catalase*** catalyze reaction:

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

3) ***Glutation peroxidase*** catalyzes reaction:

$$H_2O_2 + 2\ GSH \rightarrow 2H_2O + GS\text{-}SG$$

Glutathione reductase reduces the oxidative form of glutathione GS-SG to its reduced form GSH:

$$GS\text{-}SG + NADPH + H^+ \rightarrow 2\ GSH + NADP^+$$

Some vitamins are used as antioxidants to prevent the accumulation of these dangerous particles: Retinol, L-ascorbic acid, vitamin E, vitamin K, $CoQH_2$. Melatonin and Selenium derivatives are able to protect cells under the influence of free radicals and hydrogen peroxide, too.

Oxidative phosphorylation mechanism

The biophysical researches (the researches of red-ox-potentials of each red-ox pair of respiratory chain using polarography method) proved that there is free energy formation due to ETC function. The change of red-ox-potential of each red-ox pair is estimated in volts. This change becomes more and more in the direction of electron transport in the respiratory chain (red-ox potential for pair CChO/ ½ O_2 equals +0,48 volt).

Some part of this energy is thermal energy, and another part may be used for ATP synthesis in mitochondria. This process is named oxidative phosphorylation. The energy released for each electron pair passing through respiratory chain is coupled to the formation of 3 moles of ATP by phosphorylation of ADP (if NADH is a

donor of electrons).The chemiosmotic coupling hypothesis is probably the most widely accepted theory of oxidative phosphorylation. It was proposed by Peter Mitchell in 1961.

Notions of chemiosmotic coupling hypothesis proposed by Peter Mitchell:

1. The inner membrane is not permeable for protons in any site of membrane in the direction from the intermembrane space to the matrix.
2. Some electron carriers act as pumps, which cause directional pumping of protons across the inner membrane from the matrix to the intermembrane space. As the electrons move down the chain protons are expelled, penetrating from the matrix to the intermembrane space. Later it was proved, that the pumps for protons movement from the matrix to the intermembrane space are: complex I, complex III, complex IV (fig. 1.6).
3. Because proton is a charged particle the flow of free energy across the inner membrane is due to the combination of a ***concentration gradient (ΔpH), and a charge gradient (ΔΨ).*** The sum of them is electrochemical potential of the inner membrane:

$$\Delta\mu H^{+} = \Delta pH + \Delta\Psi$$

4. The chemical gradient of protons across the inner membrane of mitochondria serves as the means of coupling the energy flow of electron transport to the formation of ATP . Protons pass back into the matrix at a special site where ATP synthetase resides (proton channel - factor Fo,(fig 1.7).
6. Oxidative phosphorylation is the synthesis of ATP in the inner membrane of mitochondria catalyzed by ATP synthetase due to energy produced by ETC enzymes.
7. ATP formation from ADP and phosphoric acid does not require energy. ATP is obtained in the linked form with F1. Energy is required for ATP removal from F1. This action is due to the moment of protons movement through the inner membrane across the Fo as a channel for protons.
8.The moment of protons movement through the inner membrane across the Fo as a channel is the moment for coupling of phosphorylation (ATP synthesis) with the change of electrochemical potential of the inner membrane (or the use of energy produced by the ETC).

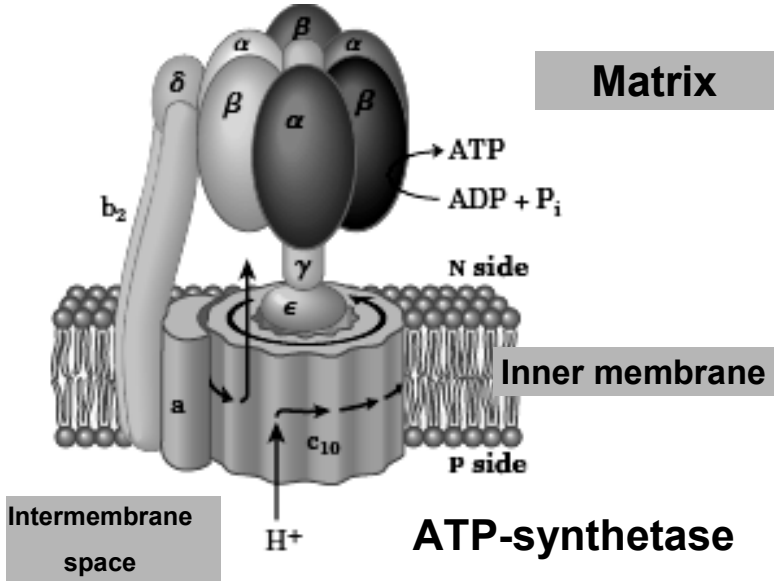


Figure 1.7. Structure of ATP-synthetase

The P/O ratio

The P/O ratio signs how many molecules of ATP is synthesized per 2 electrons transferred across the ETC to one atom of molecular oxygen.

Three sites of coupling in ETC:

1 – site where electrons are transferred from complex I to CoQ;

2 – site where electrons are transferred from cytochrome *b* to cytochrome *c1* ;

3 - site where electrons are transferred from cytochrome *c* oxidase to molecular oxygen.

They are correlated in location with complexes-pumps for protons.

- **Substrates that donate electrons to NAD+ yield P/O ratio of 3**
- **Substrates that donate electrons to FAD or FMN yield P/O ratio of 2**

P/O ratio is changed:

1) **under the influence of substances-uncouplers;**
2) **at the presence of inhibitors of ETC complexes, etc.**

The main factors to control tissue respiration and oxidative phosphorylation

Five states of respiratory control have been defined in which the rate of respiration is limited by different factors:

State 1 is limited by availability of ADP and a substrate (source of electrons).

State 2 is limited by the availability of substrate.

State 3 is limited by the capacity of the electron chain itself, when ADP, oxygen and substrate are saturating.

State 4 is limited by the availability of ADP.

State 5 is limited by availability of oxygen.

ADP/ATP transport is due to ATP/ADP translocase system (figure 1.8). Inhibitors of this system are:

1) Atractyloside; 2) Bongkrekic acid.

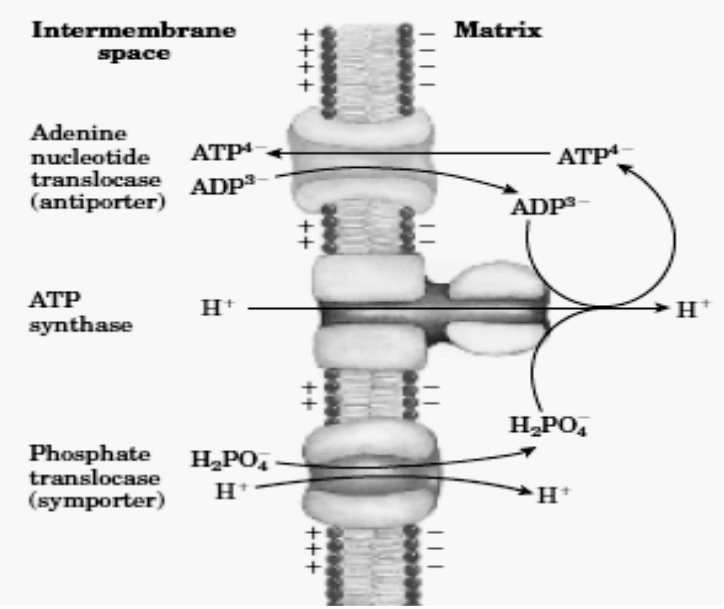


Figure 1.8. Transport systems placed in the inner membrane to promote ATP synthesis

Inhibitors of electron transport chain

Inhibitors for complex I:

- Rotenone, an insecticide;
- Barbiturates (drugs for sleepless treatment);
- Piericidin A, an antibiotic.

Result: NADH is accumulated in the matrix! P/O = 0 for reactions produced NADH.

Inhibitors for complex II:

- Malonic acid (1) – the competitive inhibitor for succinate dehydrogenase (SDH ase);
- Carboxin (2); Thenoyl trifluoride acetone (TTFA, 3).
- Substances (2) and (3) block the electron transport
- from reduced SDHase to CoQ.

Result: P/O = 0 for succinate oxidation.

Inhibitors for complex III:

- Dimercaprol (1);
- Antimycin A, an antibiotic (2)

Result: P/O may be lower then 3 or 2 (respectively oxidized substrate).

Inhibitors for complex IV:

- Carbon monooxide which competes with oxygen for its binding site on Cytochrome *aa*3;
- Hydrogen sulfide;
- Azides (an N–containing organic compounds);
- Cyanides block the heme-centers of Cytochrome *aa*3 binding by covalent bonds

Result: complete block of tissue respiration – the death of a cell.

Blocking of oxidative phosphorylation without ETC inhibition

Uncouplers-protonofores allow the transport of protons across the inner membrane (they are very lipophylic compounds), thus collapsing the proton gradient before it may be used for ATP synthesis.

Uncouplers-iononofores reduce the electrochemical potential because they can accept ions as Na+, K+ to transfer them across inner membrane of mitochondria.

Result under these substances accumulation in a cell:
P/O = 0 in all cases !

The energy produced by ETC is thermal energy, only! It leads to oxerheating of living system.

Uncouplers

- 2,4 - Dinitrophenol (weight-loss drug used in 1970s but was discontinued because of it toxicity);
- Dicumarol that is anticoagulant;
- Chlorcarbonylcyanide phenylhydrazone (CCCP);
- Thyroxin in high abnormal concentration;
- Valinomycin, an antibiotic.

Tests recommended to answer after study of chapter 1:

1. Name, please, the key metabolite that may be formed in catabolic pathway both from glucose and amino acid alanine in any condition:
   A. Pyruvate
   B. Oxaloacetate
   C. Acetyl-CoA
   D. Lactate
   E. Malate
2. Name, please, the class of organic compounds usually used as energy source for anabolic pathways in humans:
   A. Monosaccharides
   B. Alcohols
   C. Carbonic acids
   D. Nucleosides
   E. Nucleoside triphosphates
3. Name, please, the enzyme class that is the most important to produce coenzymes-donors of electrons for the respiratory chain:
   A. Ligases
   B. Lyases
   C. Hydrolases
   D. Oxidoreductases
   E. Isomerases
4. Name, please, the product of Krebs cycle that is considered as donor of electrons for respiratory chain of mitochondria:
   A. GTP
   B. Oxaloacetate
   C. NADH
   D. CO_2
   E. Malate
5. Name, please, the enzyme of Krebs cycle that is participator both of the second and the third stage of tissue respiration:
   A. Succinate dehydrogenase
   B. Malate dehydrogenase
   C. Isocitrate dehydrogenase
   D. Alpha-ketoglutarate dehydrogenase
   E. Fumarase
6. The complex I of ETC may be inhibited by barbituric acid. Find out, please, the composition of this complex:
   A. NAD and Fe-S-proteins
   B. FAD and Fe-S-proteins
   C. FMN and Fe-S-proteins
   D. NADH-dehydrogenase, FMN, and Fe-S-proteins
   E. Succinate dehydrogenase and Fe-S-proteins

7. Choose, please, the P/O ratio per one mole of isocitrate involved in oxidative decarboxylation at the accumulation of rotenone in the cell:

A. P/O<2
B. P/O<3
C. 0
D. 3
E. 2

8. Name, please, the terminal complex of respiratory chain:

A. Succinate dehydrogenase
B. Cytochrome oxidase
C. Cytochrome *b* and c_1
D. NaDH-dehydrogenase
E. CoQ

9. Choose the reagent used for the investigation of succinate dehydrogenase activity inhibition
in muscles:

A. Amber acid
B. Sodium hydroxide
C. 2,6-dichlor-phenolindophenolate
D. Malonic acid
E. All the reagents named above are in need

10. Catalase activity is determined as catalase number of blood at cachexy state in patients. Name the substrate used for this enzyme in the method:

A. Succinate
B. Hydrogen oxide
C. Hydrogen peroxide
D. NADH
E. Malonate

Chapter 2. Biochemistry of nutrition. Clinical concepts of nutirition

Introduction

A balanced human diet needs to contain a large number of different components. These include **proteins, carbohydrates, fats, minerals and vitamins**. These substances can occur in widely varying amounts and proportions, depending on the type of diet. As several components of the diet are essential for life, they have to be regularly ingested with food. Recommended daily minimums for nutrients have been published by the World Health Organization (WHO) and a number of national expert committees.

Proteins provide the body with amino acids, which are used for endogenous protein biosynthesis. Excess amino acids are broken down to provide energy. Most amino acids are glucogenic, they may be converted into glucose. Proteins are essential components of the diet, as they provide essential amino acids that the human body is not capable of producing on its own.

Nitrogen balance studies show that the average daily requirement is 0.6 g of protein per kilogram of body weight (the factor 0.75 should be used to allow for individual variation), or approximately 50 g/day. Average intakes of protein in developed countries are about 80–100 g/day, ie, 14–15% of energy intake. Because growing children are increasing the protein in the body they have a proportionately greater requirement than adults and should be in positive nitrogen balance. Not only the quantity, but also the quality of protein is important. Proteins that lack several essential amino acids or only contain small quantities of them are considered to be of low value, and larger quantities of them are therefore needed. For example, pulses contain small amounts of methionine only, while wheat and corn proteins are poor in lysine. In contrast to vegetable proteins, most animal proteins are high-value (with exceptions such as collagen and gelatin).

Carbohydrates serve as general and easily available energy source. In the diet, they are present as monosaccharides in honey and fruit, or as disaccharides in milk and in all foods sweetened with sugar (sucrose). Metabolically usable polysaccharides are found in vegetable products (starch) and animal products (glycogen). Carbohydrates represent a substantial proportion of the body's energy supply, but they are not essential.

Fats are primarily important energy suppliers in the diet. They provide more than twice energy as proteins and carbohydrates (per gram of substance). Fats are essential as suppliers of fat-soluble

vitamins absorption in GIT, and as sources of polyunsaturated fatty acids, which are needed to biosynthesize eicosanoids.

Mineral substances and trace elements are very heterogeneous group of essential nutrients. They are usually divided into macroelements and microelements, and are represented as ions mainly in food products and later in the blood of humans after absorption.

Vitamins are also indispensable components of the diet. The animal body requires them in very small quantities (the daily dose is very individual for each vitamin) in order to synthesize coenzymes and some signaling substances. It is imposible to think about them as energy sources or structural components of a cell.

Mechanisms of nutrient substances transformation in the GIT

The digestion and absorption of food sources is a complex process, which depends upon the integrated activity of the organs of the alimentary tract. Food substances are mixed with the various digestive fluids, which contain enzymes and cofactors, and are broken down into small molecules which are absorbed by the intestinal epithelium.

Complex carbohydrates such as starch are converted to mono- and disaccharides, the latter undergoing further hydrolysis by intestinal brush border disaccharidases (e.g. maltase, sucrase) to allow absorption of the constituent monosaccharides. Proteins are broken down by proteases (secreted as inactive precursors) and peptidases to oligopeptides and amino acids.

The absorption of fat is a complex process. Mechanical mixing and the action of bile salts create an emulsion of triacylglycerols and other lipids, which is a substrate for pancreatic lipase. This enzyme converts triacylglycerols to free fatty acids and monoacylglycerols. The products and fat-soluble vitamins are then incorporated with bile salts into mixed micelles, and are absorbed from these into intestinal epithelial cells where products are re-esterified to form lipids needed for humans. The essential enzymes participating in digestion of carbohydrates, lipids and proteins are given in the table 1.

All these processes require the intimate mixing of enzymes, cofactors and substrates, and the maintenance of the optimum pH for enzyme activity.

In the stomach, food mixes with acidic gastric juice, which contains the proenzyme of pepsin, and intrinsic factor, essential for the absorption of vitamin B_{12}. Secretion of gastric juice is under the combined control of the vagus nerve and the hormone gastrin. Gastrin is secreted by C-cells of the stomach and has several physiological functions. It is a polypeptide hormone, present in the bloodstream

mainly in two forms, C-17 and C-34, containing 17 and 34 amino acids respectively. Other gastrin molecules have been identified in the blood, but the physiological significance of this heterogeneity is not known. All the variants have an identical C-terminal amino acid sequence.

Table 2.1. Enzymes of GIT

Place of synthesis	Place of action	pH	Activation of enzymes			Specificity of action
			proenzyme	activator	active enzyme	
Mouth cavity	Mouth cavity	7.0	Absent	NaCl	Salivary amylase	α(1-4)glycoside bonds of starch. glycogen
Stomach	Stomach	1.5-2.5	Pepsinogen	HCl-slowly Pepsin - quickly	Pepsin	-X-Tyr- -X-Phe- -Leu-Glu-
Pancreas	Small intestinal lumen	7.0-8.0	Trypsinogen Chymo-trypsinogen Proelastase Procarboxypepti-dase A. B	Enteropeptidase Trypsin Trypsin Trypsin	Trypsin Chymo-trypsin Elastase Carboxy-peptidase A. B	-Arg-X- -Lys-X- -Trp-X- -Phe-X- -Tyr-X- -Gly-Ala- -X-NH-CH-COOH R
		7.1			Pancreatic α-amylase	α(1-4)glycoside bonds of starch. limit dextrins. maltose
		7.8	Prolipase Prophospho-lipase A_2	Colipase. bile salts. bile phospholipid s. Ca^{2+}	Lipase Phospho-lipase A_2 Sterol esterase	Ester bonds of triacylglycerols and diacylglycerols Ester bond of phospholipids Ester bond of cholesterol ester
Epithelial (Brush border) cells of small intestine	Small intestine lumen, on the border of the intestinal mucosal cells	7.0-8.0	Aminopeptidases. di- & tripeptidases			H_2N-CHR-CO-X Peptide bonds of di- & tripeptides
			Maltase dextrinase (amylo-1.6-glucosidase) Isomaltase (oligo-1.6- glucosidase) sucrase(α-glucosidase)			Glycoside bonds of maltose dextrins Isomaltose sucrose

Disorders of the stomach, pancreas, liver and small intestine can result in the malabsorption of nutrients. In addition to its importance in the absorption of water and nutrients, the mucosal lining of the gastrointestinal tract has an important barrier function, providing protection against the action of hydrogen ions and enzymes, and preventing invasion of its wall by its normal bacterial flora. The small intestine also contributes to this protective function through its immune function. In gastrointestinal disease, this barrier function may be compromised and bacteria may gain access to the circulation and cause a septicaemia. The gut also secretes numerous hormones, most of which act on the gut itself or on related organs.

Disorders and the investigation of gastric function

Biochemical tests are of limited use in the diagnosis of gastric disorders: the stomach can be directly inspected by endoscopy, and contrast radiography can also provide valuable information. **The investigation of gastric juice includes measurement of its volume, basal secretion, concentration of pepsin, total acidity, concentration of hydrochloric acid, presence of lactic acid, blood pigments.**

Gastric juice composition. Daily secretion 2–3 L, pH 1.5-2.5

Components	**Function or substrate**
Water	
Salts	
HCl	Denatures proteins, kills bacteria
Mucus	Protect stomach lining
Pepsins (3.4.23.1-3)	Cleave proteins
Chymosin (3.4.23.4)	Precipitates casein
Triacylglycerol lipase (3.1.1.3)	Cleaves fats
Intrinsic Castle's factor	Formation complex with vitamin B_{12}. It is necessary for vitamin B_{12} absorption in the intestine

Volume of gastric juice on an empty stomach is not exceed 50 ml, rate of **basal secretion** is 50-100 ml/h. Increased acid secretion is important in the pathogenesis of duodenal ulcers. Most peptic ulceration is associated with colonization of the stomach with *Helicobacter pylori*. This organism decreases the resistance of gastric mucosa to acid, and stimulates gastrin, and hence acid secretion. *Helicobacter* can split urea to form ammonia and carbon dioxide, and this is the basis for a breath test, formerly used for diagnosis. The sensitivity of this test is 96% and

specificity virtually 100%. Isotopically labelled (^{13}C- or ^{14}C-) urea is given orally and the isotope is measured in the expired breath. Excretion is increased if infection is present.

Atypical peptic ulceration is a feature of Zollinger-Ellison syndrome, a rare condition in which hypergastrinaemia is caused by a gastrinoma of the pancreas, duodenum or, less frequently, the C-cells of the stomach.

Plasma gastrin concentrations typically exceed 200 ng/L (normal <50). In addition to having recurrent or atypical peptic ulceration, ***patients sometimes have steatorrhea *,*** owing to inhibition of pancreatic lipase by the excessive gastric acid.

Steatorrhea **is the presence of excess fat in feces. Possible biological causes can be lack of bile acids (due to liver damage or hypolipidemic drugs), defects in pancreatic juices (enzymes), and defective mucosal cells. The absence of bile acids will cause the feces to turn gray or pale.**

The first-line biochemical test in such patients is the measurement of fasting plasma gastrin concentration. This is frequently elevated, but some patients with gastrinomas have normal or only slightly elevated plasma gastrin concentrations. If the cause of hypergastrinaemia is in doubt and in patients with atypical peptic ulceration but whose gastrin concentrations are not dearly elevated, it may be helpful to measure plasma gastrin concentration following the administration of secretin. This hormone increases gastrin secretion from gastrinomas, but reduces it or has no effect in hypergastrinaemia from other causes. Measurement of gastric acid secretion may also help to distinguish between the causes of hypergastrinaemia. It is typically >15 mmole/h in patients with gastrinomas but low and resistant to stimulation in patients with achlorhydria. Maximal gastric acid secretion can be measured by the pentagastrin test. Protocols for the test vary but in essence it involves measurement of acid in fluid aspirated through a nasogastric tube in the resting state and after the administration of pentagastrin, a synthetic analogue of gastrin. Basal acid secretion is normally <10 mmole/h in males (<6 mmole/h in females); stimulated secretion is normally <45 mmole/h in males and <35 mmole/h in females.

Another way of stomach function investigation is determination of gastric juice acidity and concentration of hydrochloric acid.

Total acidity of gastric juice for healthy adults (after trial Boas-Ewold breakfast) is 40-60 mmole/L, for newborns – 2.8 mmole/L, for children at the age of 1-12 months – 4-20 mmole/L.

The content of **free hydrochloric acid** changes normally from 20 up to 40 mmole/L (at newborns – 0.5 mmole/L).

The conjugated hydrochloric acid changes from 10 up to 20 mmole/L normally.

The total hydrochloric acid is the sum of all types of hydrochloric acids in the gastric juice. There is increase of free hydrochloric acid content and total acidity during the stomach ulcer or hyperacid gastritis. The decrease of **free hydrochloric acid** and total acidity (hypochlorhydria) is observed during the subacidic gastritis or gastric carcinoma. The condition with complete absence of hydrochloric acid and a considerable lowering of the total acidity is named achlorhydria (observing during gastric carcinoma, chronic gastritis). The condition with complete absence of hydrochloric acid and pepsin in gastric juice is named **achylia** (it's possible during gastric carcinoma).

Concentration of **pepsin** in gastric juice is normal when it contains: on an empty stomach - 0-0.21 mg/ml; after stimulation by cabbage decoction - 0.2-0.4 mg/ml; after stimulation by histamine – 0.5-0.75 mg/ml. Pepsin can completely be absent in gastric juice at achylia state. The quantity of pepsin is increased at stomach ulcer.

Detection of lactic acid in gastric juice specifies an intensification of processes of lactic fermentation in a stomach, the indirect evidence of absence or low concentration hydrochloric acid. There is the hypothesis, that lactic acid in gastric juice may be a metabolite of cancer cells as glycolysis is basic energy source in the tumor cells.

Blood pigments may be found in the gastric juice at ulcer of the stomach.

The pancreas gland functions in digestion of food products. Disorders of pancreas gland associated with infringements in digestion process

The exocrine secretion of the pancreas is an alkaline, bicarbonate-rich juice containing various enzymes essential for normal digestion:

- the proenzyme forms of the proteases: trypsinogen, chymotpypsinogen and procarboxypeptidases A and B;
- the lipolytic enzymes: lipase, co-lipase, phospholipase A_2
- and pancreatic amylase.

The secretion of pancreatic juice is primarily under the control of two hormones secreted by the small intestine: secretin, a 27 amino acid polypeptide, which stimulates the secretion of an alkaline fluid, and cholecystokinin (CCK), which stimulates the secretion of pancreatic enzymes, contraction of the gallbladder, decreases gastric motility. Like gastrin, CCK is a heterogeneous hormone: the predominant form in the gut is a 33 amino acid polypeptide, but an eight amino acid form is present in some parts of the central nervous system and appears to function as a neurotransmitter. Both secretin and CCK are secreted in

response to the presence of acid, amino acids and partly digested proteins in the duodenum.

Pancreatic secretions. Daily secretion 0.7–2.5 L, pH 7.7 (7.5–8.8)

Components	**Function or substrate**
Water	
HCO_3^-	Neutralizes gastric juice
Endopeptidases: Trypsin (3.4.21.4), chymotrypsin (3.4.21.1), elastase (3.4.21.36) Exopeptidase: Carboxypeptidases (3.4.n.n.)	Proteins Peptides
α-amylase (3.2.1.1.)	Starch and glycogen
Triacylglycerol lipase (3.1.1.3)	Neutral fats
Co-lipase	Cofactor for lipase
Phospholipase A_2 (3.1.1.4)	Phospholipids
Sterol esterase (3.1.1.13)	Cholesterol esters
Ribonuclease (3.1.27.5)	RNA
Deoxyribonuclease I (3.1.21.1)	DNA

Pancreatic disorders and their investigation

The major disorders of the exocrine pancreas are acute pancreatitis, chronic pancreatitis, pancreatic cancer and cystic fibrosis. Biochemical investigations are essential in the diagnosis and management of the first of these, of limited use in the second, and of little use in the third. Clinical evidence of impaired exocrine function is usually only seen in advanced pancreatic disease.

Acute pancreatitis. The pancreas becomes acutely inflamed and, in severe cases, haemorrhagic. The initial lesion involves intracellular activation of enzyme precursors, leading to the generation of oxygen free radicals and an acute inflammatory response. This may extend beyond the pancreas and lead to adult respiratory distress syndrome (ARDS), circulatory and renal failure. Sepsis, probably as a result of bacterial translocation from the gut, is a life-threatening complication. Some degree of organ failure occurs in approximately 25% of patients and the mortality is 5-10%.

The clinical diagnosis is supported by finding a high serum amylase activity. This enzyme is secreted by salivary glands and the exocrine pancreas. Its activity in serum is usually (though not invariably) raised in acute pancreatitis, levels >10 **x** ULN (Upper Limit of Normal) being virtually diagnostic. Amylase is a relatively small molecule, and is rapidly excreted by the kidneys (hence the increase in activity in renal

failure); in mild pancreatitis, rapid clearance may be reflected by a normal serum level but increased urinary amylase. Extra-abdominal causes of a raised plasma amylase activity rarely cause increases of more than 5 **x** ULN. **Macroamylasaemia** is an example of a high plasma enzyme activity being due to reduced clearance. In this condition, amylase becomes complexed with another protein (in some cases with immunoglobulin) to form an entity of much greater apparent molecular weight; renal clearance is reduced as a result. This has no direct clinical sequelae but can misleadingly suggest the presence of pancreatic damage.

Measurement of the pancreas-specific isoenzyme of amylase can improve the diagnostic specificity of plasma amylase determinations. Measurement of serum lipase activity has been reported to be a more specific test for acute pancreatitis but the test is little used. A combination of lipase and amylase measurement has been reported to have specificity and sensitivity of approximately 90%.

Chronic pancreatitis. Chronic pancreatitis is an uncommon condition, which usually presents with abdominal pain or **malabsorption** and occasionally with impaired glucose tolerance. The malabsorption is due to impaired digestion of food, but there is considerable functional reserve and pancreatic lipase output must be reduced to only 10% of normal before steatorrhoea is produced. Such a reduction only occurs in extensive disease or if the main pancreatic duct is obstructed. Alcohol is an important aetiological factor and there may be a history of recurrent acute pancreatitis. Measurements of plasma amylase and lipase activities are of no value: they are normal or low in patients with chronic pancreatitis.

Digestion in the small intestine at healthy and diseased persons

The small intestine is a site of absorption of all the nutrients; most of this absorption takes place in the duodenum and jejunum, but vitamin B_{12} and bile salts are absorbed in the terminal ileum. Approximately 8 L of fluid enter the gut every 24 h. This is derived from ingested food and water and from the digestive juices, including those secreted by the small intestine itself. Most of this fluid, and the salts it contains is reabsorbed in the jejunum, ileum and large intestine.

Secretions of the small intestine. Daily secretion 8 L, pH 6.5–7.8

Components	Function or substrate
Aminopeptidases (3.4.11.n)	Peptides
Dipeptidases (3.4.13.n)	Dipeptides
α-Glucosidase (3.2.1.20)	Olygosaccharides
Olygo-1,6-glucosidase (3.2.1.10)	Olygosaccharides
β-Galactosidase (3.2.1.23)	Lactose

Sucrose α-glucosidase (3.2.1.48)	Sucrose
α,α-trehalase (3.2.1.28)	Trehalose
Alkaline phosphatase (3.1.3.1)	Phosphoric acid esters
Polynucleotidases 93.1.3.n)	Nucleic acids, nucleotides
Nucleosidases (3.2.2.n)	Nucleosides
Phospholipases (3.1.n.n)	Phospholipids

Unlike carbohydrates and proteins lipids are insoluble in aqueous solution. The digestive enzymes, however, are present in aqueous medium. Emulsification is essential for effective digestion of lipids. The process of emulsification occurs by three mechanisms:

1) detergent action of **bile salts**;
2) surfactant action of degraded lipids;
3) mechanical mixing due to peristalsis.

Bile consists of watery mixture of organic and inorganic compounds. Lecithin and bile salts are quantitatively the most important organic components of bile. Bile can either pass directly from the liver where it is formed into the duodenum through the common bile duct, or the stored in the gallbladder.

Bile composition. Daily secretion 0.6 L, pH 6.9–7.7

Components	**Function or substrate**
Water	
HCO_3^-	Neutralizes gastric juice
Bile salts	Facilitate lipid digestion
Phospholipids	Facilitate lipid digestion
Bile pigments	Waste product
Cholesterol	Waste product

The small intestine can be affected by many disease processes, but the major effects on function relate to the consequences of impaired absorption of nutrients and fluid, and to disruption of its barrier function.

Lactose intolerance, a common malabsorption syndrome of carbohydrate metabolism, is characterized by nausea, diarrhea, and flatulence after ingesting dairy products or other foods containing lactose. One of the causes of lactose intolerance is a low level of lactase, which decreases after infancy in most of the world population **(**nonpersistant lactase or adult hypolactasia**).** However, lactase activity remains high in some populations (persistent lactase), including Northwestern Europeans and their descendants. Undigested lactose in the intestines increases the retention of water in the colon, resulting in diarrhea.

If more cholesterol enters the bile than can be solubilized by the bile salts and lecithin present, the cholesterol can precipitate in the

gallbladder, initiating the occurrence of cholesterol **gallstone disease**. This disorder may result from:

1) gross malabsorption of bile acids from the intestine;
2) obstruction of the biliary tract;
3) severe hepatic dysfunction, leading to decreased synthesis of bile salts;
4) excess feedback suppression of bile acid synthesis.

Vitamins as nutrition components

Vitamins are essential organic compounds that the animal organism is not capable of forming itself, although it requires them in small amounts for metabolism. It is often noted that vitamins cannot be produced in the body and must, therefore, be supplied in the diet. This statement is valid for many of the vitamins, but is not strictly true for others. For example, vitamin D can be formed in the skin upon adequate exposure to ultraviolet radiation; vitamin K is normally produced in sufficient amounts by intestinal bacteria; and niacin (in a form of NAD^+) can be synthesized in vivo from an amino acid precursor, L-tryptophan. With the possible exception of vitamins D and K, vitamins must be supplied by the diet because they cannot be produced in adequate amounts by the human body. Plants have the ability to synthesize most of the vitamins and serve as primary sources of these dietary essentials.

Most vitamins are precursors of coenzymes; in some cases, they are also precursors of hormones or act as antioxidants.

Since only a few vitamins may be stored (A, D, E, B_{12}), a lack of vitamins quickly leads to **deficiency diseases (hypovitaminosis or avitaminosis).** These often affect the skin, blood cells, and nervous system. The causes of vitamin deficiencies can be treated by improving nutrition and by administration vitamins in tablet form. An overdose of vitamins leads to **hypervitaminosis state** only, with toxic symptoms, in the case of vitamins A and D. Normally, excess vitamins are rapidly excreted with the urine.

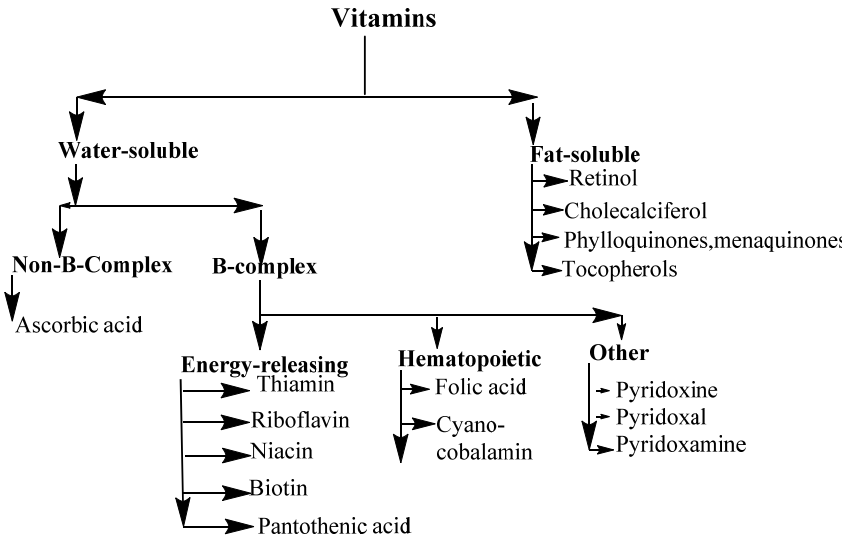
Thirteen vitamins are recognized in human nutrition and these have been classified, according to their solubility, into two groups. The fat-soluble vitamins are represented by vitamins A, D, E and K; also included are carotenoids that possess varying degrees of vitamin A activity. The water-soluble vitamins comprise vitamin C and the members of the vitamin B group, namely thiamine (vitamin B_1), riboflavin (vitamin B_2), niacin (vitamin B_3), pantothenic acid (vitamin B_5), pyridoxine (vitamin B_6), biotin (vitamin B_7), folic acid (vitamin B_9) and cobalamin (vitamin B_{12}).

For several of the vitamins, biological activity is attributed to a number of structurally related compounds known as vitamers. The vitamers pertaining to a particular vitamin display, in most cases, similar

qualitative biological properties to one another, but, because of subtle differences in their chemical structures, exhibit varying degrees of potency.

Most of the B-complex group can be further divided according to general function: energy releasing, hematopoietic (refers to an agent or process that affects or promotes the formation of blood cells) and others (table 2.2). Other vitamins cannot be classified this narrowly because of their wide range of functions.

Table 2.2. Classification of vitamins



The water-soluble vitamins act largely as coenzymes. Water-soluble vitamins, similar to their fat-soluble counterparts, consist of carbon, hydrogen, and oxygen atoms. They also contain nitrogen and metal ions including iron, molybdenum, copper, sulfur, and cobalt. Because of their solubility in water, water-soluble vitamins disperse in the body fluids without being stored to any appreciable extent. If the diet regularly contains less than 50% of the recommended values for water-soluble vitamins, marginal deficiencies may develop within 4 weeks Water-soluble vitamins are absorbed into portal blood; furthermore, with the exception of cyanocobalamin (vitamin B_{12}), they cannot be retained for long periods by the body. Generally, an excess intake of water-soluble vitamins becomes voided in the urine. Water-soluble vitamins exert their influence for 8 to 14 hours after ingestion; thereafter, their potency begins to decrease. For maximum benefit of, for example, vitamin C supplements, they should be consumed at least every 12

hours. Sweating during extreme physical activity can produce negligible losses of the water-soluble vitamins as well.

The fat-soluble vitamins are digested and absorbed with the help of fats presented naturally in the diet. Fat soluble vitamins can be stored in the body for long periods of time. They are stored mostly in the fatty tissues and in the liver. Hence, supplementations are not required as frequently as with water-soluble vitamins. However, in emotionally stressful conditions, supplementation becomes important.

Absorption, transport and formation of vitamin derivatives in human organism

Vitamin B complex

Vitamin B_1. Thiamine can travel by both active (through plasma membrane, requires metabolic energy to "power" the exchange of materials) and passive (through plasma membrane, requires no energy) transport, depending on the amount of the vitamin presented in the intestine for absorption. At low physiological concentrations, thiamine absorption is active and sodium-dependent (type of active transport due to Na^+, K^+-ATPase). Absorption occurs primarily in the upper jejunum but can occur in the duodenum and ileum (portions of the small intestine). When intakes of thiamine are high, absorption is predominantly passive.

Transport of thiamine into red blood cells is thought to occur by facilitated diffusion (diffusion that is assisted by protein transporters). Only free thiamine or thiamine-monophosphate (TMP) is thought to be able to cross cell membranes. In red blood cells, most thiamine exists as thiamine diphosphate (TDP) with smaller amounts of free thiamine and TMP.

The human body contains approximately 30 mg of thiamine, with relatively high but still small concentrators found in the skeletal muscles, heart, liver, kidney, and brain. In fact, skeletal muscles are thought to contain about half of the body's thiamine.

Following absorption, most free thiamine is taken up by the liver and converted to its coenzyme form, thiamine diphosphate (TDP). Conversion of thiamine to TDP requires adenosine triphosphate (ATP) and thiamine pyrophosphokinase, an enzyme found in the liver, brain, as well as other tissues. About 80% of total thiamine in the body exists as TDP, 10% as TTP (thiamine triphospate), and the rest is TMP, which is inactive.

Vitamin B_1 functions in human organism

The active form of vitamin B_1 is **thiamine pyrophosphate** (TPP, trivial name – cocarboxylase), formed by the transfer of pyrophosphate group from ATP to thiamine. TPP serves as coenzyme for the enzymes:

1) pyruvate dehydrogenase (conversion of pyruvate to acetyl CoA);
2) α-ketoglutarate dehydrogenase (Krebs cycle);
3) transketolase (HPM shunt);
4) the branched chain α-ketoacid dehydrogenase (oxidative decarboxylation of amino acids valine, leucine and isoleucine.

So, TPP is intimately connected with the energy releasing reactions.

Thiamine was the first vitamin to be discovered, around 100 years ago. Vitamin B_1 deficiency leads to *beriberi,* a disease with symptoms that include neurological disturbances, cardiac insufciency, and muscular atrophy.

Vitamin B_2. Riboflavin is primarily absorbed in the proximal small intestine by a sodium dependent carrier. Within cells, B_2 is converted to its coenzyme forms, regulated by hormones, such as the thyroid hormone. These coenzymes, than bind to apoenzymes (an enzyme, which needs a co-enzyme to be activated) forming what is called a flavoprotein.

Vitamin B_3. In the human body, niacin is transformed to nicotinamide adenine dinucleotide phosphate ($NADP^+$), and nicotinamide adenine dinucleotide (NAD^+). These are the primary forms which niacin functions within the body.

In the intestine, NAD^+ and $NADP^+$ may be hydrolyzed to release free nicotinamide. This can be absorbed in the stomach, but primarily in the small intestine. In low concentrations, free nicotinamide is absorbed by a sodium dependent carrier (Na^+, K^+-ATPase). In high concentrations, it uses passive diffusion. Most of the niacin in the blood is found as nicotinamide, a small amount of the time as nicotinic acid. From here, they move through the cell via a sodium dependent carrier system.

Vitamin B_5. Pantothenic acid occurs mainly as coenzyme A. It is primarily absorbed in the small intestine via passive diffusion. Transportation in the heart, muscles, and liver cells is done by a sodium active transport. Within the central nervous system, adipose, and renal uptake, facilitative diffusion is used.

Vitamin B_6. For digestion to occur, vitamin B_6 must be broken down, and separated into its 3 main components: pyridoxine (PN), pyridoxal (PL), and pyridoxamine (PM). This occurs in the intestinal brush border, via enzymatic reactions. Absorption occurs primarily in the jejunum (middle portion of small intestine, connects with the duodenum (first portion) and the ileum (last portion) of the small intestine by passive diffusion. In the intestine, PN is converted to pyridoxine phosphate (PNP), PL is converted to pyridoxal phosphate (PLP), and PNP is often converted to PLP, while PM remains the same, and

composes about 15% of the vitamers in the blood. 60% of vitamin B_6 found in the blood is PLP. In order for PLP to cross the cell membrane it must be broken down to PL. The liver stores approximately 10% of vitamin B_6, while muscles store the most at 80%. Other storage houses are the kidneys, brain, and red blood cells.

Vitamin B_7. (H) Biotin binds with high affinity and specificity to *avidin*, a protein found in egg white. Since boiling denatures avidin, biotin deficiency only occurs when egg whites are eaten raw. It is not in need to form derivative form for this vitamin before its use in the structure of enzyme.

Vitamin B_{12}. Cobalamin can only be absorbed in the small intestine when the gastric mucosa secretes what is known as *intrinsic factor* – a glycoprotein that binds cobalamin (the *extrinsic factor*) and thereby protects it from degradation. In the blood, the vitamin is bound to a special protein known as *transcobalamin.* The liver is able to store vitamin B_{12} in amounts sufficient to last for several months. The cobalt within the corrin ring of cobalamin can form a bond with a carbon atom. In the body, it reacts with the carbon of a methyl group, forming methylcobalamin, or with the 5'-carbon of 5'-deoxyadenosine, forming 5'-deoxyadenosylcobalamin. The conversion of free vitamin B_{12} to B_{12} coenzymes is effected in the organism with the participation of specific enzymes in the presence of cofactors FAD, NADH, ATP, and glutathione.

Vitamin B_{12} deficiency is usually due to an absence of intrinsic factor and the resulting absorption disturbance. This leads to a disturbance in blood formation known as ***pernicious anemia.***

Vitamin C

When ascorbic acid is consumed in the diet, the ileum and jejunum are major sites of ascorbic acid absorption. The bioavailability of vitamin C is dose dependent. In humans, saturation of transport occurs with dosages of 200–400 mg per day. Approximately 70% of a 500 mg dose is absorbed. However, much of the absorbed dose (50%) is non-metabolized and excreted in the urine. With a dose of 1250 mg, only 50% of the dose absorbed and most (.85%) of the absorbed dose is excreted. Vitamin C is not protein-bound and is eliminated with an elimination half-life of 10–12 hours.

Specific non-overlapping transport proteins mediate the transport of ascorbic acid across biological membranes. Dehydroascorbic acid uptake is via the facilitated-diffusion glucose transporters (GLUT 1, 3, and 4), but under physiological conditions these transporters do not appear to play a major role in the uptake of dehydroascorbic acid due to the high concentrations of glucose that effectively block influx. L-ascorbic acid enters cells via Na^+-dependent systems. Cell accumulation of ascorbic acid occurs, because of cellular dehydroascorbate reduction systems that are capable of rapidly

generating ascorbic acid. Ascorbic acid is not very stable in aqueous media, wherein it can decay within a few hours or even minutes at high pH (10.0). In contrast, ascorbic acid is relatively stable in blood (a day or more), or if stored at acidic pH (3.0).

Vitamin C functions in human organism

Ascorbic acid usually carries out red-ox reactions by mechanisms dependent upon free-radical processes. Ascorbate metabolism is linked to the metabolism of glutathione. Ascorbic acid is also required in animals that lack or have mutations in the gene for L-gulonolactone oxidase. Ascorbic deficiency results in reduced mono- and dioxygenase activities. The consequences of severe deficiency are profound, since growth, extracellular matrix, and hormonal regulation are impaired.

In the body, ascorbic acid serves as a reducing agent in variations reactions (usually hydroxylations). Among the processes involved are:

- collagen synthesis (post-translational modification of protocollagen by lysyl hydroxylase, prolyl hydroxylase);
- tyrosine
  - degradation (p-Hydroxyphenylpyruvate dioxygenase);
  - transformation to catecholamines (Dopamine β-hydroxylase);
- tryptophan transformation to serotonin (tryptophan hydroxylase);
- bile acid formation from cholesterol;
- iron
  - absorption from the intestine;
  - storage form formation (ferritin);
  - in methaemoglobin (Fe^{3+}) reconversion to haemoglobin (Fe^{2+}).

The daily requirement for ascorbic acid is about 60 mg, a comparatively large amount for a vitamin. Vitamin C deficiency only occurs rarely nowadays; it becomes evident after a few months in the form of *scurvy,* with connective-tissue damage, bleeding, and tooth loss.

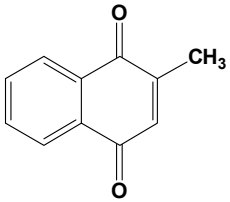
Fat soluble vitamins (A, D, E, K)

Their availability in the diet, absorption and transport are associated with the use of neutral fats, other lipids, and bile acids function. The main components of vitamin A group are retinol, retinal and retinoic acid; in vitamin D group two components D3 and D2 have found which are the precursors for calcitriol formation; in vitamin E group beta-tocopherols are represented. Vitamin K is also the group of substances – naphtoquinones. All of them have special functions usually associated with the function of enzymes, but for calcitriols we can find out the hormone-similar function to regulate calcium and phosphate levels in the blood.

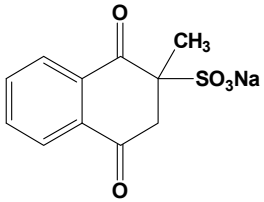
Retinol is the parent substance of the **retinoids**, which include **retinal** and **retinoic acid**. The retinoids also can be synthesized by cleavage from the provitamin β-carotene. Retinoids are found in meat-containing diets, where as β-carotene occurs in fruits and vegetables (particularly carrots). Retinal is involved in visual processes as the pigment of the chromoprotein rhodopsin. Retinoic acid, like the steroid hormones, influences the transcription of genes in the cell nucleus. It acts as a differentiation factor in growth and development processes. Vitamin A deficiency can result in night blindness, visual impairment, and growth disturbances.

Vitamin D3 (calciol, cholecalciferol) is the precursor of the hormone *calcitriol* (1, 25-dihydroxycholecalciferol). Together with two other hormones (parathyrin and calcitonin), calcitriol regulates the calcium metabolism. Calciol is synthesized in the skin from 7-dehydrocholesterol, an endogenous steroid, by a photochemical reaction. Vitamin D3 deficiency occurs when the skin receives insufficient exposure to ultraviolet light or vitamin D3 is lacking in the diet. Deficiency is observed in the form of *rickets* in children and *osteomalacia* in adults. In both cases, bone tissue mineralization is disturbed.

Vitamin K (naphthoquinones: K_1 – phylloquinone, K_2 - menaquinone) are similar substances with modified side chains. A synthetic analog of vitamin K, devoid of the side chain at position 3, is denoted vitamin K_3 (menadoine). It is water insoluble, and has been used as a parent compound for the synthesis of a large number of water-soluble derivatives, one of which has found a wide application in medical practice. This is vicasol, sodium salt of bisulphate derivative of vitamin K_3.



Vitamin K_3



Vicasol

Vitamin K and its derivatives have a high antihemorrhagic activity. They are involved in carboxylation of glutamate residues of some coagulation factors in the liver. The form that acts as a cofactor for carboxylase is derived from this vitamin by enzymatic reduction. Vitamin K antagonists (e. g., coumarin derivatives) inhibit this reduction and consequently carboxylation as well. This fact is used to inhibit blood coagulation in *prophylactic treatment against thrombosis*. Vitamin K

deficiency occurs rarely, as the vitamin is formed by bacteria of the intestinal flora.

Vitamin E (tocopherol) and related compounds are represented in plants (e.g., wheat germ). They contain what is known as a chroman ring. In the lipid phase, vitamin E is located mainly in biological membranes, where as an **antioxidant** it protects unsaturated fatty acid residues against reactive radicals such as superoxide-anion radical, hydrogen peroxide radical, etc.

Tests recommended to answer after study of chapter 2:

1. A steatorrhea is found in the patient. Choose the substance whose deficiency in the bile of patient causes this state mainly:
   A. Glucose
   B. Bile salt
   C. Trypsin
   D. Phosphorylase
   E. Cholesterol

2. Point out the amino acids whose peptide bonds in the composition of protein chain are hydrolyzed by pepsin and chymotrypsin:
   A. Diamino acids
   B. Aromatic amino acids
   C. Dicarboxylic amino acids
   D. Hydroxyaminoacids
   E. Sulfur-containing amino acids

3. The vegetable oils are the obligatory components of man ration because they contain some essential fatty acids. Choose them:
   A. Stearic and palmitic acids
   B. Acetic and butyric acids
   C. Palmitooleic and oleic acids
   D. Linoleic and α-linolenic acids
   E. Citric and fumaric acids

4. Following a fat diet meal of skim milk and yogurt, an adult female patient experiences abdominal distention, nausea, cramping, and pain followed by a watery diarrhea. This set of symptoms is observed each time the meal is consumed. A likely diagnosis is:
   A. Steatorrhea
   B. Lactase deficiency
   C. Maltose deficiency
   D. Sialidase deficiency
   E. Lipoprotein lipase deficiency

5. Point out the group of peptidases which trypsin is belong to:
   A. Aminopeptidase
   B. Exopeptidase
   C. Endopeptidase
   D. Dipeptidase
   E. Carboxypeptidase

6. Find out the right continuation for the notion:”Retinol…:
   A. can be formed from retinoic acid due to enzyme action
   B. is the light-absorbing portion of rhodopsin
   C. influences the transcription of genes

D. is transported from the intestine to the liver in chylomicrones
E. deficiency can result in rickets and osteomalacia

7. Which one of the following statements concerning vitamin D is correct?
A. It is required in the diet of individuals exposed to sunlight
B. Vitamin D opposes the effect of parathyrin and calcitonin
C. 1,25-dihydroxycholecalciferol is the active (hormonal) form of the vitamin
D. A deficiency in vitamin D can result in night blindness
E. Chronic renal failure requires the oral administration of cholecalciferol

8. Find out the right continuation for the notion: “Vitamin K..:
A. Increases the coagulation time of the blood in infants with hemorrhagic disease
B. Plays an essential role in preventing thrombosis
C. Is synthesized by intestinal bacteria
D. Is involved in the deamination of lysine residues of coagulation factors
E. Is a water-soluble vitamin

9. Which one of the following statements concerning vitamin C is correct?
A. It serves as a reducing agent in hydroxylation reactions
B. It decreases bile acids formation
C. It prevents iron absorption from the intestine
D. It provokes to conversion of haemoglobin in methaemoglobin
E. It is coenzyme of α-ketoglutarate dehydrogenase

10. Cocarboxylase serves as coenzyme for the enzymes, excepting one:
A. Pyruvate dehydrogenase
B. α-Ketoglutarate dehydrogenase
C. γ-Glutamyl carboxylase
D. Transketolase
E. The branched chain α-ketoacid dehydrogenase

Chapter 3. Overview of carbohydrate metabolism in healthy humans. Disorders of carbohydrate metabolism (at diabetes mellitus, glycogen storage diseases, etc)

Carbohydrates are aldehydes or ketones of high polyhydric alcohols or components that yield these derivatives in hydrolysis. They occur naturally in plants (where they are produced photosynthetically), animals and microorganisms and fulfil various structural and metabolic roles. Rice, potatoes, bread, corn, candy, and fruits are rich in carbohydrates.

Dietary carbohydrates are cleaved during digestion, forming monosaccharides (mainly glucose) that enter the blood. The major dietary carbohydrate is starch, the storage form of carbohydrate in plants. Starch is cleaved by salivary amylase in the oral cavity and by pancreatic amylase in the intestine to disaccharides and oligosaccharides. Dextrinases, glucosidases, and disaccharidases located on the surface of the brush border of the intestinal epithelial cell complete the conversion of starch to glucose. Ingested disaccharides are cleaved by disaccharidases on the surface of the intestinal epithelial cell. Sucrose (table sugar) is converted to fructose and glucose by sucrase. Lactose (milk sugar) is converted to glucose and galactose by lactase. Some free glucose and fructose are consumed in the diet.

Monosaccharides (mainly glucose, some fructose and galactose) present in the diet or produced by the digestive process, are absorbed by the intestinal epithelial cells and pass into the blood. The products of digestion enter cells from the blood, this transport across cellular membrane insite cytoplasm is stimulated by insulin.

When glucose enters cells, it is converted to glucose 6-phosphate, which is a pivotal compound in several metabolic pathways:

1) glycolysis, which produces pyruvate and generates NADH and ATP;

2) synthesis of glycogen or compounds such as proteoglycans;

3) pentose phosphate pathway or hexose monophosphate shunt (HMP), which produces NADPH for reactions such as biosynthesis of fatty acids and ribose for nucleotide production. (fig.3.1)

The processes described above are stimulated by insulin to promote the decrease of blood glucose levels and the intracellular glucose level, too.

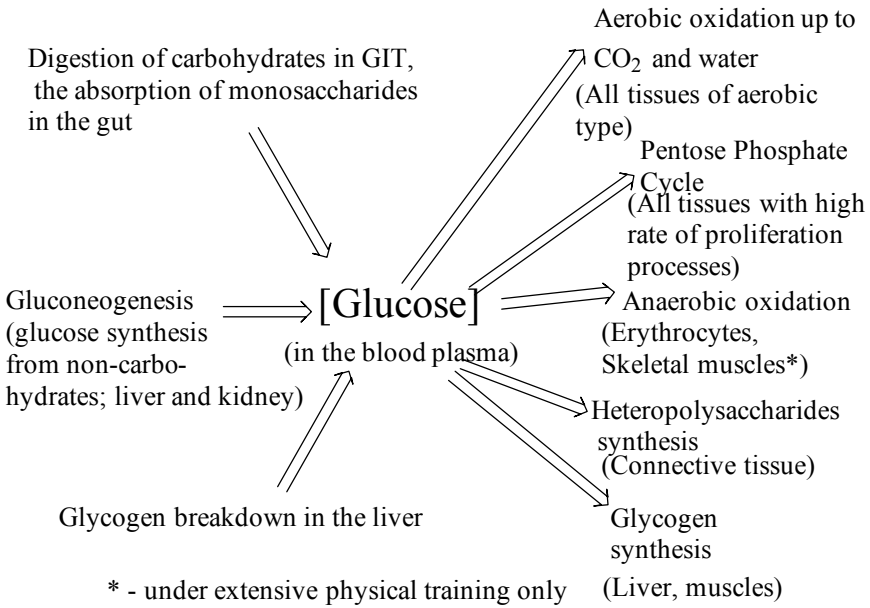


Figure 3.1. Processes which create the glucose pool in the blood plasm of humans.

Monosaccharides leave the intestinal epithelial cells and enter the hepatic portal vein. Therefore, the liver is the first tissue through which these products of digestion pass.

The fate of glucose

in the liver:

a. Glucose is oxidized to CO_2 and H_20 to meet the immediate energy needs of the liver.

b. Glucose is involved in Hexose Monophosphate Shunt to produce NADPH and ribose-5-phosphate (stimulated at S-phase of living cycle of hepatocyte).

c. Glucose is involved in the production of UDP-glucuronic acid to use it in conjugation reactions (stimulated under the accumulation of harmfull substances in the liver).

d. Excess glucose is stored in the liver as glycogen, which is used during periods of fasting to maintain blood glucose.

e. Excess glucose may be converted to fatty acids and a glycerol moiety, which are combined to form triacylglycerols, which are released from the liver into the blood as VLDL, mainly.

in brain - which depends on glucose for its energy needs, glucose is oxidized to CO_2 and H_20, producing 38 ATP per 1 mole of glucose.

in red blood cells - they, lacking mitochondria, oxidize glucose to lactate (anaerobic glycolysis), which is released into the blood.

in muscle cells

a. They take up glucose by a transport process that is stimulated by insulin.

b. Glucose is oxidized to CO_2 and H_20 to generate ATP for muscle contraction (no extensive muscular loading in skeletal muscles or process is considered for myocardium)

c. Glucose is oxidized to lactate under extensive physical loading (anaerobic glycolysis occurs in skeletal muscles, only).

d. Muscles store glucose as glycogen for use of it as glucose source to be involved in glycolysis during contraction.

in cells of adipose tissue – they take up glucose by a transport process that is stimulated by insulin and use it to produce energy and to form the glycerol moiety of its triacylglycerol stores.

in cells of connective tissue – they take up glucose for synthesis of glucose aminoglycans

Production of glucose by the liver

The liver has the major responsibility for maintaining blood glucose levels, initially by the process of glycogenolysis and subsequently by gluconeogenesis.

Glycogenolysis - About 2-3 hours after a meal, the liver begins to break down its glycogen stores by the process of glycogenolysis, and glucose is released into the blood. Glucose then may be taken up by various tissues and oxidized.

Glycogenolysis is stimulated by glucagon and epinephrine.

Gluconeogenesis - After about 4-6 hours of fasting, the liver begins the process of gluconeogenesis. Within 24 hours, liver glycogen stores are depleted, leaving gluconeogenesis as the major process responsible for maintaining blood glucose.

Carbon sources for gluconeogenesis:

a. ***lactate*** produced by tissues such as red blood cells or exercising muscle;

b. ***glycerol*** from breakdown of triacylglycerols in adipose tissue;

c. ***amino acids***, particularly alanine, from muscle protein;

d. ***propionate*** from oxidation of odd-chain fatty acids (minor source).

Glucocorticoids and glucagon stimulate gluconeogenesis.

About 80% of total glucose produced due to gluconeogenesis is synthesized in the liver, other 15% - in kidney tissue.

The fate of fructose and galactose

Although glucose is the most abundant monosaccharide derived from the diet, fructose and galactose are usually obtained in significant quantities. The major dietary source of fructose is the disaccharide sucrose (table sugar), but it is also present as the monosaccharide in fruit and in corn syrup, which is used as a sweetener.

After fructose and galactose enter cells, they are phosphorylated on carbon 1 and converted to intermediates in pathways of glucose metabolism.

Fructose is metabolized mainly in the liver, where it is converted to fructose 1-phosphate and cleaved to produce dihydroxyacetone phosphate and glyceraldehyde, which may be phosphorylated to glyceraldehyde 3- phosphate. These two triose phosphates are intermediates of glycolysis. Fructose may be produced from sorbitol, which is generated from glucose. Normal level of fructose in blood is 5.55-28.75 μmole/L (0.1-0.5 mg%); in urine – 0.17-0.36 mg/day (30-65 mg/day)

Galactose is phosphorylated to galactose 1-phosphate, which reacts with UDP-glucose. The products are glucose 1-phosphate and UDP-galactose, which is epimerized to UDP-glucose. The net result is that galactose is converted to the glucose moieties of UDP-glucose and glucose 1-phosphate, intermediates in pathways of glucose metabolism. UDP-galactose may be used in the synthesis of glycoproteins, glycolipids, and proteoglycans. UDP-galactose may react with glucose in the mammary gland to form the milk sugar lactose. Galactose may be reduced to galactitol.

Common problems associated with carbohydrate metabolism

1. Intestinal lactase deficiency

Intestinal lactase deficiency is a common condition in which lactose cannot be digested and is oxidized by bacteria in the gut, producing gas, bloating, and watery diarrhea.

2. Lactic acidosis

Lactate levels in the blood increase, producing an acidosis. Lactic acidosis can result from decreased utilization of lactate or increased formation of lactate. Anoxia or lack of oxygen may occur in condition that impair blood flow (shock), in respiratory disorders, and in severe anemia. Anoxia is a common cause of high blood lactate levels:

1) Phosphofructokinase-1 is activated in anaerobic condition;
2) Utilization of lactate is reduced without oxygen.

Lactic acidosis can be caused by hypoxia or by alcohol ingestion. Deficiency of oxygen results in increased NADH level, and more pyruvate than normal is converted to lactate. High NADH levels from alcohol metabolism cause pyruvate to be converted to lactate.

Normal blood level of lactate is less then 1,2 mM/lit; with lactic acidosis the blood lactate level may be 5 mM/lit or more. Lactate acidosis results in lowered blood pH and bicarbonate levels.

3. Glucose 6-phosphate dehydrogenase deficiency in RBC

One of the world's most common enzyme deficiencies is glucose-6-phosphate-dehydrogenase deficiency. This deficiency in erythrocytes is particularly prevalent among African and Mediterranean males. A deficiency in glucose-6-phosphate dehydrogenase blocks the pentose phosphate pathway and NADPH production. Without NADPH to maintain glutathione in its reduced form, erythrocytes have no protection from oxidizing agents. Red blood cells lyse and a hemolytic anemia may occur. This X-linked recessive deficiency is often diagnosed when patients develop hemolytic anemia after receiving oxidizing drugs such as pamaquine or after eating oxidizing substances such as fava beans.

4. Diabetes mellitus

High blood glucose levels occur because of either a deficiency of insulin (insulin-dependent diabetes mellitus, IDDM) or the inability of tissues such as adipose and muscle to take up glucose in the presence of normal amounts of insulin (insulin resistance or noninsulin-dependent diabetes mellitus [NIDDM]). If insulin-deficiency diabetes mellitus is untreated, the body responds as if it is starving. Fuel stores are degraded in the face of high blood glucose, and ketoacidosis may occur. Many metabolic pathways are affected.

Rare problems associated with carbohydrate metabolism

1. Essential pentosuria

L-Xylulose reductase (xylitol dehydrogenase) is deficient in essential pentosuria. L-Xylulose (a pentose) appears in the urine and gives a positive reducing-sugar test. The condition is benign.

2. Glycogen storage diseases

Glycogen accumulates primarily in the liver or muscle, or both. Enzyme deficiencies occur mainly in glycogen degradation or conversion to glucose. In the liver, glycogen storage diseases may produce conditions ranging from mild hypoglycemia to liver failure. In muscle, they may cause problems ranging from difficulty in performing strenuous exercise to cardiorespiratory failure.

3. Pyruvate kinase deficiency

Deficiency of pyruvate kinase causes decreased production of ATP from glycolysis. Red blood cells have insufficient ATP for their sodium pumps; their membranes lyse, and a hemolytic anemia results.

4. Essential (familial) fructosuria

While most tissues cannot utilize fructose, the liver, kidneys, intestine, and adipose tissue can. Genetic fructokinase deficiency causes no symptoms. It can be detected by urine measurements of fructose that spills over into the urine. Unless care is taken, this could be

misinterpreted as glucosuria, like that seen in diabetes, since both fructose and glucose are positive for a reducing-sugar test. Liver hexokinase rarely phosphorylates fructose to fructose-6-phosphate because the liver enzyme has a much greater affinity for glucose. However, adipose tissue hexokinase produces fructose-6-phosphate, which then can be acted upon by fructose-1-phosphate aldolase (aldolase B), which splits it into dihydroxyacetone phosphate and glyceraldehyde. Glyceraldehyde and dihydroxyacetone phosphate proceed through glycolysis or gluconeogenesis through the action of triose kinase. Under normal circumstances, liver fructokinase phosphorylates fructose to fructose-1-phosphate, and fructose-1-phosphate aldolase acts upon it.

5. Fructose intolerance

The aldolase that cleaves fructose phosphates is deficient. Fructose 1-phosphate accumulates and inhibits glucose production, causing severe hypoglycemia if fructose is ingested.

6. Galactosemia

Lactose in breast milk and infant formula is converted by intestinal lactase to glucose and galactose that are efficiently absorbed. In galactosemia, deficiency of galactose-1-phosphate uridyl transferase prevents the conversion of galactose into glucose-6-phosphate by the liver or erythrocytes. Most other organs do not metabolize galactose. The severe symptoms of galactosemia are caused by the reduction of galactose to galactitol (dulcitol) in the presence of the enzyme aldose reductase. High levels of galactitol cause cataracts, the accumulation of galactose-1-phosphate contributes to liver disease, and the accumulation of galactose metabolites in urine can be measured as reducing substances. Infants with suspected galactosemia must be withdrawn from breast-feeding or lactose formulas and placed on nonlactose formulas.

The appearance of high concentrations of galactose in the blood after lactose ingestion may be due to a galactokinase deficiency or to a uridyl transferase deficiency. In both conditions, excess galactose may be reduced to galactitol, which can produce cataracts. Uridyl transferase deficiency is more severe, causing elevation of galactose 1-phosphate, which inhibits phosphoglucomutase, interfering with glycogen synthesis and degradation.

7. Mucopolysaccharidoses and gangliosidoses (or sphingolipidoses)

A deficiency of lysosomal enzymes results in the inability to degradate the carbohydrate portions of proteoglycans or sphingolipids. Partially digested products accumulate in lysosomes. Tissues become engorged with these "residual bodies," and their function is impaired. These diseases are often fatal and are associated with impaired nervous system in patients.

Tests recommended to answer after study of chapter 3:

1. Choose the major metabolic product produced under normal circumstances by erythrocytes and by muscular cells during extensive exercises that is recycled through the liver in the Cori cycle:

A. Oxaloacetate
B. Glycerol
C. Alanine
D. Pyruvate
E. Lactate

2. In lung diseases such as emphysema or chronic bronchitis, there is chronic hypoxia that is particularly obvious in vascular tissues such as the lips or nail beds (cyanosis). Poorly perfused areas exposed to chronic hypoxia have decreased metabolic energy for tissue maintenance and repair. An important reason for this is:

A. Increased hexokinase activity owing to increased oxidative phosphorylation
B. Increased ethanol formation from pyruvate on changing from anaerobic to aerobic metabolism
C. Increased glucose utilization via the pentose phosphate pathway on changing from anaerobic to aerobic metabolism
D. Decreased ATP generation and increased glucose utilization on changing from aerobic to anaerobic metabolism
E. Decreased respiratory quotient on changing from carbohydrate to fat as the major metabolic fuel

3 Familial fructokinase deficiency causes no symptoms because:

A. Hexokinase can phosphorylate fructose
B. Most tissues utilize fructose
C. Liver fructose-1-P aldolase is still active
D. Excess fructose does not escape into the urine
E. Excess fructose spills into the bowel and is eliminated in feces

4. A newborn begins vomiting after feeding, becomes severely jaundiced, and has liver disease. Treatment for possible sepsis is initiated, and the urine is found to have reducing substances. A blood screening for galactosemia is positive, and lactose-containing substances are removed from the diet. Lactose is toxic for this kid because:

A. Excess glucose accumulates in the blood
B. Galactose is converted to the toxic substance galactitol (dulcitol)
C. Galactose competes for glucose during hepatic glycogen synthesis
D. Galactose is itself toxic in even small amounts
E. Glucose metabolism is shut down by excess galactose

5. What is the process for glucose utilization that is stimulated first of all by the insulin in the liver under condion of essential hyperglycemia?

A. Glycolysis anaerobic
B. Glycolysis aerobic
C. Glycogenolysis
D. Hexose Monophosphate Shunt
E. Glycogenesis

6. A man goes on a hunger strike and confines himself to a liquid diet with minimal calories. Which of the following would occur after 4 to 5 hours?

A. Decreased cyclic AMP and increased liver glycogen synthesis
B. Increased cyclic AMP and stimulated liver glycogenolysis
C. Decreased epinephrine levels and increased liver glycogenolysis
D. Increased Ca^{2+} in muscle and decreased glycogenolysis
E. Decreased Ca^{2+} in muscle and decreased glycogenolysis

7. A Nigerian medical student studying in the United States develops hemolytic anemia after taking the oxidizing antimalarial drug pamaquine. This severe reaction is most likely due to:

A. Glucose-6-phosphate dehydrogenase deficiency
B. Concomitant scurvy
C. Vitamin C deficiency
D. Diabetes
E. Glycogen phosphorylase deficiency

8. All following compounds can serve as carbon sources for gluconeogenesis except one:

A. Lactate produced by tissues such as red blood cells or exercising muscle;
B. Glycerol from breakdown of triacylglycerols in adipose tissue;
C. Amino acids, particularly alanine, from muscle protein;
D. Propionate from oxidation of odd-chain fatty acids.
E. Acetyl-CoA from oxidation of even-chain fatty acids

9. Two enzymes are used for conversion of glucose into glucose-6-phosphate in human body, but only one from them is inhibited under the accumulation of glucose-6-phosphate in a cell. Name it:

A. Glucokinase
B. Phosphofructokinase-1
C. Hexokinase
D. Phosphofructokinase-2
E. Glucose-6-phosphatase

10. Choose an impossible way (in one step) for the use of pyruvic acid:

A. Oxidative decarboxylation to acetyl-CoA

B. Transformation to phosphoenolpyruvate
C. Transformation to oxaloacetate
D. Alanine formation
E. Lactate formation

Chapter 4. Overview of lipid metabolism and its disorders in humans

Introduction

Lipids are some organic compounds represented in human tissues, non-soluble in the water but soluble in organic solvents. Although the term “lipids” is sometimes used as a synonym for fats, fats are a subgroup of lipids named triacylglyceroles and should not be confused with the term fatty acid. Lipids also encompass molecules such as fatty acids and their derivatives (including tri-, di-, and monoacylglyceroles and phospholipids), as well as other sterol-containing metabolites such as cholesterol (figure 4.1.). The metabolism of lipids is closely associated with fat-soluble vitamins such as vitamins A, D, E and K (their absorption in the gut, the transport across the blood stream to tissues is in the complex with lipids, only).

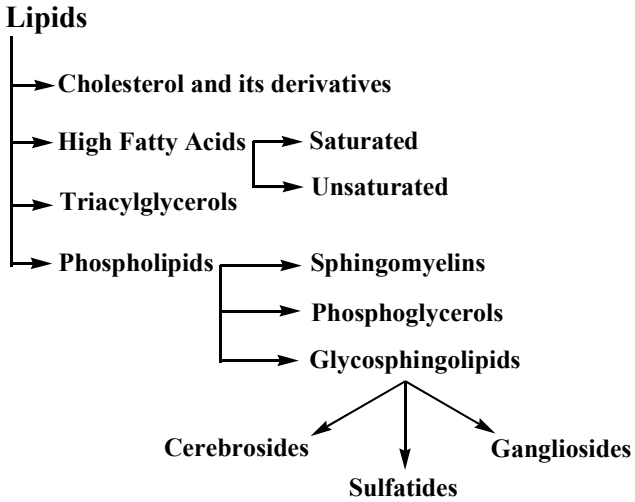


Figure 4.1.. The classification of lipids.

The main biological functions of lipids include:

- to be energy sources energy storage,
- to act as structural components of cell membranes,
- to participate as important signaling molecules.

Common notions about fats digestion and absorption

Most dietary fat is supplied in the form of triacylglycerols which must be hydrolyzed to fatty acids and monoacylglycerols before they can be absorbed. In children and adults, fat digestion is efficient and is nearly completed in the small intestine.

In the newborn, the pancreatic secretion of lipase is low. The digestion of fat in babies is augmented by the lipases secreted from the glands of the tongue (lingual lipase) and a lipase present in human milk.

At adults the beginning of fats digestion is in duodenum because fats entering the duodenum are mixed with bile and are emulsified (micelles from fats are formed). The emulsion is then acted upon by lipases secreted by the pancreas.

Pancreatic lipase catalyzes the hydrolysis of fatty acids from positions 1 and 3 to yield 2-monoacylglycerols (Tso, 1985). Phospholipids are hydrolysed by ***phospholipase A_2*** and the major products are lysophospholipids and free fatty acids (Borgstrom, 1974). Cholesterol esters are hydrolyzed by ***pancreatic cholesterol esterase***.

The free fatty acids and monoacylglyceroles are absorbed by the enterocytes of the intestinal wall. In general, fatty acids which have a chain length of less than 14 carbons enter directly into the portal vein system and are transported to the liver. Fatty acids with 14 or more carbons are re-esterified within the enterocyte and enter the circulation via the lymphatic route as triacylglycerols in the composition of ***chylomicrons*** (lipoproteins class synthesized in the small intestine wall; (figure 4.6). However, the portal route has been described as an absorptive route for dietary long chain fatty acids as well (McDonald et al., 1980). Fat soluble vitamins (vitamins A, D, E and K) and cholesterol are delivered directly to the liver as part of the chylomicron nascent forms.

Diseases that impair the secretion of bile, such as biliary obstruction or liver diseases, lead to severe fat malabsorption, as do diseases that influence the secretion of lipase enzymes from the pancreas, such as cystic fibrosis. The lack of not digested fats with feces is named ***steatorrhea*** state. Medium-chain triacylglyceroles can be better tolerated in individuals with fat malabsorption and these are often used as a source of dietary energy. Complete absorption of lipids from the intestine may be marginally affected by a high amount of fibre in the diet. Fat absorption in the gut is illustrated partially in the figure 4.6. High Fatty Acids (HFA) are transported in the blood as complexes with serum albumin (Alb) (complex ratio 1 Alb : 4 HFA) or as residues in lipids transported by lipoproteins .

Phospholipids: structure and function

A 1, 2-diacylglycerol that has a phosphate group esterified at carbon atom 3 of the glycerol backbone is a ***glycerophospholipid*****,** also known as a ***phosphoglyceride*** or a ***glycerol phosphatide*** (Figure 4.2). These lipids form one of the largest classes of natural lipids and one of the most important. They are essential components of cell membranes and are found in small concentrations in other parts of the cell. It should

be noted that all glycerophospholipids are members of the broader class of lipids known as ***phospholipids*.**

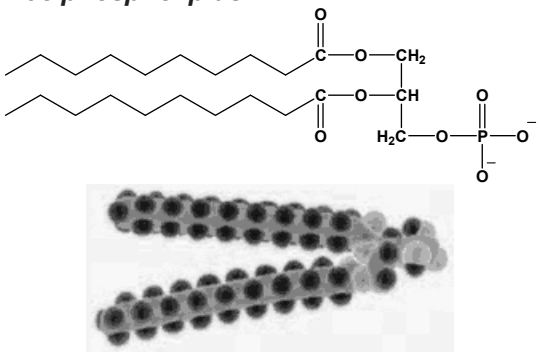
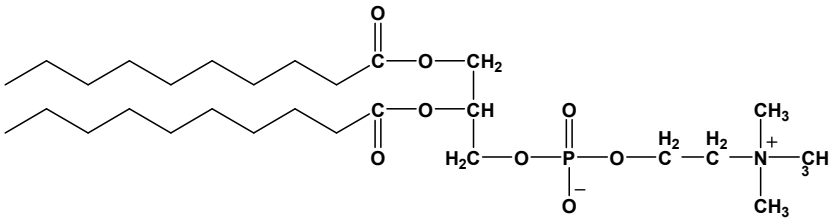


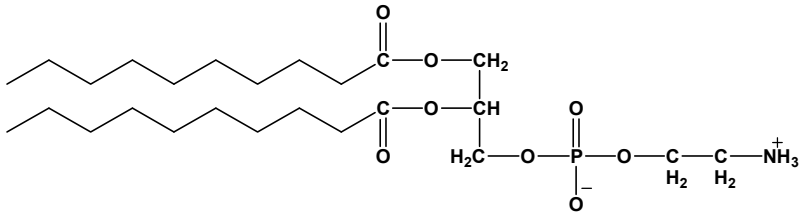
Figure 4.2. Phosphatidic acid, the parent compound for glycerophospholipids

Phosphatidic acid is found in small amounts in most natural systems and is an important intermediate in the biosynthesis of the more common glycerophospholipids (Figures 4.2., 4.3.). In these compounds, a variety of polar groups are esterified to the phosphoric acid moiety of the molecule. The phosphate, together with such esterified entities, is referred to as a “head” group. Phosphatides with choline or ethanolamine are referred to as ***phosphatidyl choline*** (known commonly as ***lecithin***) or ***phosphatidyl ethanol amine*,** respectively. These phosphatides are two of the most common constituents of biological membranes. Other common *head groups* found in phosphatides include glycerol, serine, and inositol. Another kind of glycerol phosphatide found in many tissues is ***diphosphatidylglycerol*.** First observed in heart tissue, it is also called ***cardiolipin*.** In cardiolipin, a phosphatidylglycerol is esterified through the C-1 hydroxyl group of the glycerol moiety of the head group to the phosphoryl group of another phosphatidic acid molecule.

Phosphatides exist in many different varieties, depending on the fatty acids esterified to the glycerol group. As we shall see, the nature of the fatty acids can greatly affect the chemical and physical properties of the phosphatides and the membranes that contain them. In most cases, glycerol phosphatides have a saturated fatty acid at position 1 and an unsaturated fatty acid at position 2 of the glycerol. Thus, ***1-stearoyl-2-oleoyl-phosphatidylcholine*** is a common constituent in natural membranes, but ***1-linoleoyl-2-palmitoylphosphatidylcholine*** is not.



Phosphatidylcholine



Phosphatidylethanolamine

Figure 4.3. Structures of several glycerophospholipids

Cholesterol metabolism in human tissues

All the ways for cholesterol metabolism are represented in the figure 4.4.

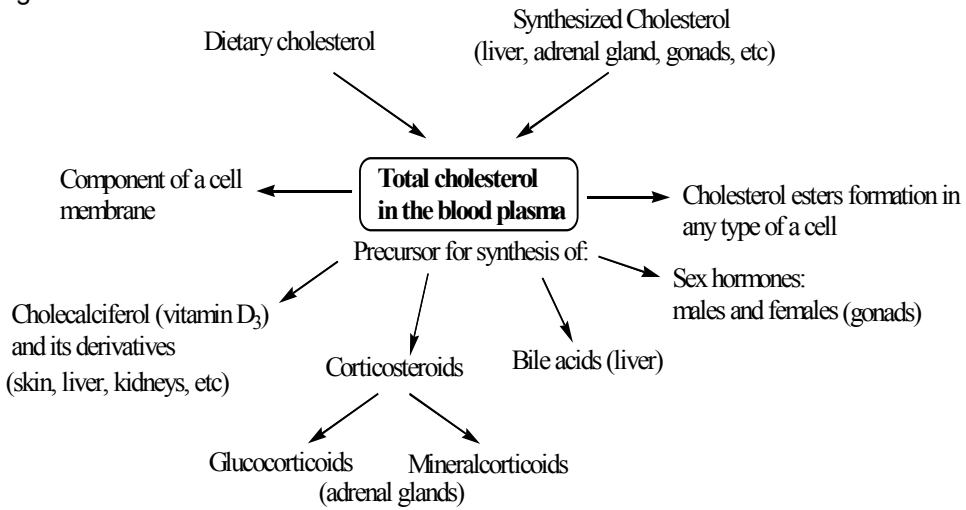


Figure 4.4. All the ways for the formation of cholesterol pool in the blood plasma

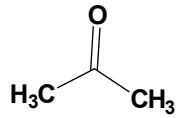
The total cholesterol level of the blood plasma must be in the region 3,9-5,48 mmol/L in healthy adults and it is supplied by all these pathways. All the amount of cholesterol in the blood plasma is in conjugated form as a component of lipoproteins. The dietary cholesterol is the component of a complex with albumins, of VLDL (about 10% are synthesized in the intestine wall), and of chylomicrons. The cholesterol synthesized in the liver and other tissue may be the component of VLDL, LDL, HDL, and may be represented in two forms: free cholesterol and cholesterol esters. So, the metabolism and transport of Cholesterol in humans is associated with metabolism of lipoproteins. The rate of enterohepatic circulation of bile acids is correlated with the level of the total cholesterol in the blood plasm, too.

Tissue lipolysis and β-oxidation of high fatty acids

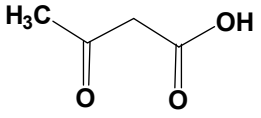
Fatty acids are carried to tissues for the use in the synthesis of triacylglycerols, phospholipids, and other membrane lipids. The main transporters for them in the blood circulation are blood plasm albumins. The mobilization of fatty acids from triacylglycerol stores depends upon hormone-sensitive lipase. This enzyme is activated by cAMP-dependent protein kinase across phosphorylation and moves from the cytoplasm to the surfaces of lipid droplets in a response to catecholamines and other lipolytic hormones (glucagon, STH, ACTH) secretion to the blood. Fatty acids are a major fuel for aerobic cells (except neurons).

Ketone bodies

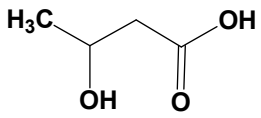
Acetate, Acetone and β-Hydroxybutyrate are named as Ketone bodies. They are preferred as energy source during their conversions in the heart, skeletal muscle and kidney. The end product for their catabolic pathway is acetyl-CoA that is involved in Krebs Cycle to be utilized there. Acetone cannot be utilized in humans, it is very inert molecule.



Acetone



Acetoacetate



β-Hydroxybutyrate

Ketone bodies synthesis is activated in the case of acetyl-CoA accumulation in the cytoplasm of hepatocytes. It may be during extended β-oxidation of HFA and tissue lipolysis.

The ketone bodies level of the blood serum must be in the region 0.034-0.43 mmol/L. In the blood ketone bodies levels increase sufficiently, as they do after about 20 days of starvation, they are a valuable energy substrates for the brain and may account for up to 75%

of brain oxidation. Ketone bodies concentration is elevated in patients with diabetes mellitus, too.

Plasma lipoproteins: classification, composition, metabolism and function

The term lipoprotein can describe any protein that is consisted of lipid molecules (e.g., fatty acids or triacylglycerols, glycerophospholipids, etc.) and protein part; it is most often used for a group of molecular complexes found in the blood plasma of mammals (especially humans). The protein components of lipoproteins are named ***apolipoproteins or apoproteins***.

Plasma lipoproteins transfer lipid molecules (triacylglycerols, phospholipids, and cholesterol) through the bloodstream from one organ to another. Lipoproteins also contain several types of lipid-soluble antioxidant molecules (e.g., fat-soluble vitamins A, D, E, K).

The small particles of plasma lipoprotein, which carry triacylglycerols, can be separated according to their densities by centrifugation. They have been classified into five groups of increasing density: chylomicrons**,** very low density lipoproteins **(VLDL)**, intermediate density lipoproteins (**IDL**), low density **(LDL),** and high density lipoproteins **(HDL)** (Table 4.1). Each lipoprotein particle contains one or more apolipoproteins, whose sizes vary from the enormous 4536-residue apoB-100 to apoC-II and apoC-III, each of which contains just 79 residues, and the 57-residue apoC-I (Table 4.2.).

Lipoprotein particles are spherical micelles (fig. 4.5.) and are consisted of a core of triacylglycerols and fatty acid esters of cholesterol, and a shell of a single layer of phospholipids interspersed with non-esterified cholesterol. Coiled chains of one or more apolipoproteins extend over the surface and, with the amphipatic phospholipids, enable the lipids in the core to be carried in the blood. They also regulate the reaction of the lipid package with specific enzymes or bind the lipoprotein particle to cell surface receptors.

The size of the lipoprotein particles also varies from a 200- to 500-nm diameter for chylomicrons to as little as 5 nm for the smallest HDL particles. The difference in volume is more impressive. If, as has been estimated, a 22-nm diameter LDL particle contains about 2000 cholesterol and cholesteryl ester molecules and 800 phospholipids, a small HDL particle of 7-nm diameter will have room for only about 60 molecules of cholesterol and 90 of phospholipid, while a chylomicron may carry 10 million molecules of triacylglycerol. HDL particles are quite heterogeneous. .

Table 4.1. Classes of Lipoprotein Particles

Class	Diameter (nm)	Density (g/ml)	Composition (weight %)*				
			Surface components			Core lipids	
			Protein	Phospho-lipid	Cholesterol	Cholesterol esters	Triacyl-glycerol
Chylo-microns	75-1200	0.930	2	7	2	3	86
VLDL	30-80	0.930-1.006	8	18	7	12	55
IDL	25-35	1.006-1.019	19	19	9	29	23
LDL	18-25	1.019-1.063	22	22	8	42	6
HDL2	9-12	1.063-1.125	40	33	5	17	5
HDL3	5-9	1.125-1.210	45	35	4	13	3
Lp(a)	25-30	1.040-1.090					

***Data from Havel, R. J., and Kane, J. P. (1995) in *The Metabolic and Molecular Bases of Inherited Disease*, 7th ed., Vol. II (Scriver C. R., Beaudet A.L., Sly W. S., and Valle D., eds), pp. 1841 – 1852, McGraw-Hill, New York.**

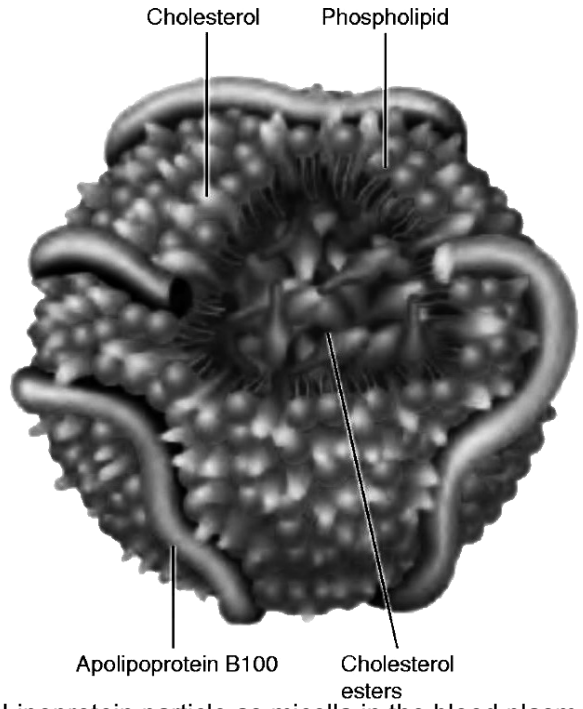


Figure 4.5. Lipoprotein particle as micella in the blood plasm.

They are sometimes divided into HDL2 and HDL3 density groups. In addition, there is a pre-HDL (nascent form) with lower phospholipid and cholesterol contents and discoid forms. Models of a reconstituted lipoprotein disc contain two molecules of apoA-I and ~160 phosphatidylcholines that form a bilayer core.

Table 4.2. Properties of Major Plasma Apolipoproteins

Designation	**№ residues**	**Mass (kDa)**	**Source**	**Function**
A-I	243	29		Major HDL protein, ligand for apo A-1 receptor in the liver
A-II	–	17.4	Liver and intestine	
A-IV	376	44.5		
B-100	4536	513	Liver	VLDL formation; ligand for LDL receptor

B-48	2152	241	Intestine	Chylomicron formation ligand for liver chylomicron receptor
C-I	57	6.6		
C-II	79	8.9	Liver	Cofactor for lipoprotein lipase
C-III	79	8.8		
D	–	31	Many tissues	A lipocalin
E	299	34	Liver, VLDL	Ligand for liver VLDL, chylomicron receptor
(a)	Variable			Ligand for liver chylomicron receptor

Each apolipoprotein has one or more distinct functions. The apoB proteins probably stabilize the lipoprotein micelles. In addition, apoB-100 is essential to recognition of LDL by its receptors. The 79-residue apoC-II has a specific function of activating the lipoprotein lipase that hydrolyses the triacylglycerols of chylomicrons and VLDL. Lack of either C-II or the lipase results in a very high level of triacylglycerols in the blood.

The large apolipoprotein B-100 is synthesized in the liver and is a principal component of VLDL, IDL, and LDL. It is the sole protein in LDL, accounting for nearly 20% of the mass of LDL particles. Partly because of its insolubility in water, its detailed structure is uncertain. If it will be all coiled into α-helix, it would be 680 nm long and could encircle the LDL particle nearly 10 times. The true structure of apoB-100 is unknown.

There is the same apoB gene in intestinal epithelial cells that is used to synthesize apoB-100 in the liver and to make the shorter apoB-48 (48%) protein in the intestine wall. A third form of apoB is found in lipoprotein (a) (LP(a)). This LDL-like particle contains apoB-100 to which is covalently attached by a single disulfide linkage (probably to Cys 3734 of apoB-100) a second protein, apo(a). The presence of high LP(a) is associated with a high risk of atherosclerosis and stroke, many healthy 100-year olds also have high serum LP(a).

Apolipoprotein A-I is the primary protein component of HDL. Most of the 243 residues consist of a nearly continuous amphipathic α-helix with kinks at regularly spaced proline residues. Two disulfide-linked ApoA-I molecules may form a belt that encircles the discoid lipoprotein. ApoA-II is the second major HDL protein, but no clearly specialized function has been identified. ApoA-I, II, and IV, apoC-I, II, and III, and

apoE - all have multiple repeats of 22 amino acids with sequences that suggest amphipathic helices.

The 299-residue apolipoprotein E plays a key role in the metabolism of both triacylglycerols and cholesterol. Like apoB-100 it binds to cell surface receptors. Absence of functional apoE leads to elevated plasma triacylglycerol and cholesterol. The N-terminal domain, from residues 23 to 164, forms a 6.5 nm-long four-helix bundle, which binds to the LDL receptors. There are three common isoforms of apoprotein E (apoE2, apoE3, and apoE4). ApoE3 is most common. The presence of apoE4 in person is associated with an increased risk of atherosclerosis development and Alzheimer disease.

The metabolism of plasma lipoproteins in humans

After the synthesis and release of chylomicrons into the lymphatic circulation, various exchange processes occur by which apolipoproteins, as well as enzymes and other proteins, may be added or removed. These very complex and incompletely understood phenomena are presented in Figure 4.6. Chylomicrons donate apolipoproteins of the A and C families to HDL particles which donate apoE and may also return some apoC protein to chylomicrons.

Both chylomicrons and VLDL particles undergo similar processes in the capillary blood vessels, where their triacylglycerols are hydrolyzed to glycerol and free fatty acids by lipoprotein lipase. This enzyme requires for its activity the apolipoprotein C-II which is present in the chylomicrons and VLDL particles. Lipoprotein lipase is secreted by adipocytes and other cells and becomes attached to heparan sulfate proteglycans on surfaces of capillary endothelial cells, a major site of its action (that is because it may be named as endothelial lipoprotein lipase in some textbooks). Hereditary absence of functional lipoprotein lipase causes chylomicronemia, a massive buildup of chylomicrons in plasma. Hereditary disorder of apoprotein E-receptor in the liver can cause the some state. The condition does not cause atherosclerosis but may lead to pancreatitis if not treated. Restriction of dietary fat to 20 g/day or less usually prevents problems.

Both lipoprotein lipase and the less well understood hepatic lipase (linked with VLDL) are related structurally to pancreatic lipase. In addition to hydrolysis of the triacylglycerols, the uptake of materials from lipoproteins probably involves shedding of intact phospholipids as liposome-like particles. The free fatty acids and glycerol are taken up by tissue cells leaving the cholesterol and some of the phospholipids of the VLDL particles as LDL. In humans intermediate density lipoproteins (IDL) are formed initially, and mainly they are converted to LDL later. Both LDL particles and chylomicron remnants and VLDL remnants are taken up by endocytosis and are degraded by body cells, partially of the

liver. The best known of these receptors is the 839-residue LDL receptor, which has a specific affinity for ApoB-100. The related VLDL receptor (apoE receptor) has a higher affinity for apoE and may function in uptake of both VLDL and chylomicron remnants. The LDL receptor-related protein functions as a third lipoprotein receptor. A series of scavenger receptors, found in abundance in macrophages, take up oxidized lipoproteins and other materials. Scavenger receptor B_1 (SR-B_1), which is also found in liver cells, is involved in uptake of cholesterol from HDL particles by hepatocytes. Liver cells contain lipocalins and fatty acid binding proteins that help to carry these relatively insoluble acids to their destinations within the cells.

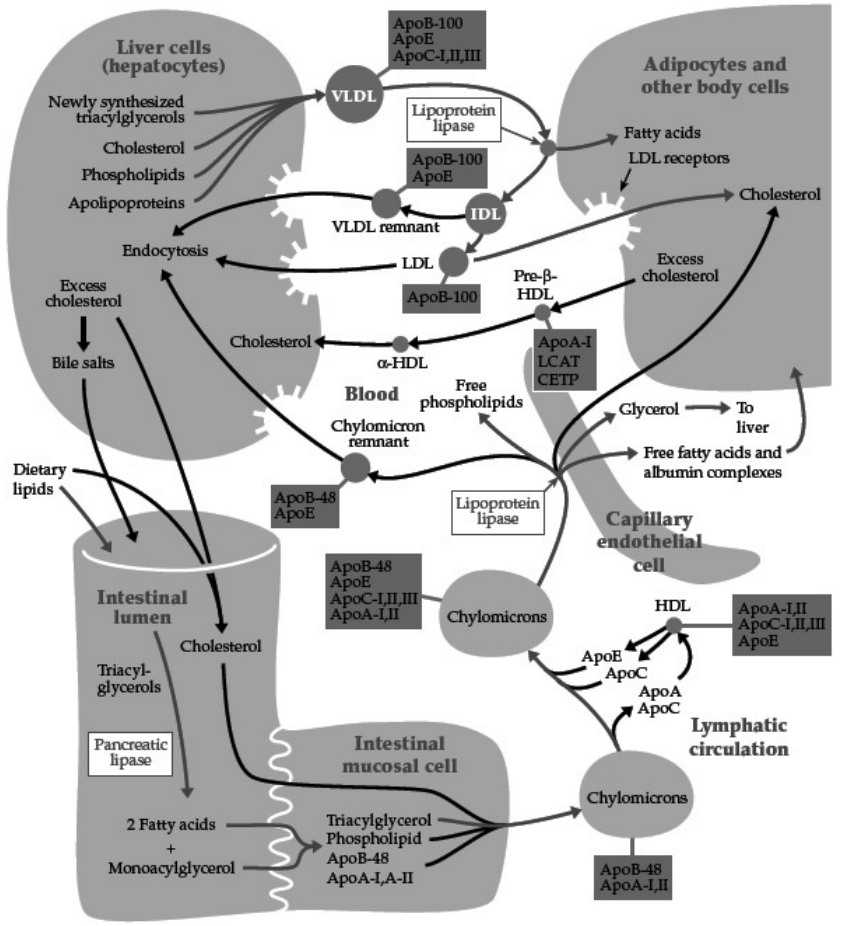


Figure 4.6. The lipids exchange between organs due to lipoproteins of blood plasm

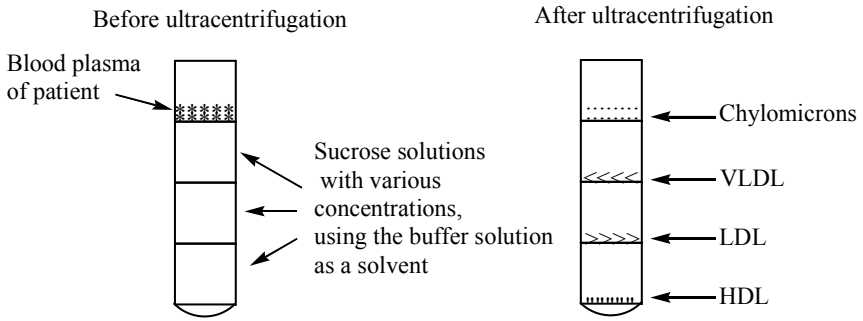
Serum albumin is also a major carrier of free fatty acids. Within the adipocytes the fatty acids are reconverted to triacylglycerols. The low density (LDL) and high density (HDL) lipoproteins are involved primarily in transporting cholesterol to and from cells.

The investigation methods for lipoproteins

There are two main methods for separation of lipoproteins in the blood: ultracentrifugation and electrophoresis.

Ultracentrifugation

Sucrose solutions with various concentrations are plotted step by step into the test centrifugal tube, and the blood plasma (0.2 mL) of patient is plotted on the surface of this mixture. Then there is the centrifugation using special ultracentrifuge that gives 50000g. Centrifugation lasts about 24 hours and lipoproteins of plasma are separated after this time (figure 4.7).



Figur 4.7. The ultrocentrifugation method to separate lipoproteins of blood plasm

Then there is the ability to take any fraction of lipoproteins for further study.

Electrophoresis

Poly Acryl Amide Gel (PAAG) may be used as a carrier for plotting of blood serum (0.2 mL). pH of buffer solution must be about 7,4 and after electrophoresis and the painting of fractions we can see the result (figure 4.8.).

Using the densitometer there is the ability to determine the content of any lipoprotein and to obtain the terminal result for each class of lipoprotein content in the blood plasma.

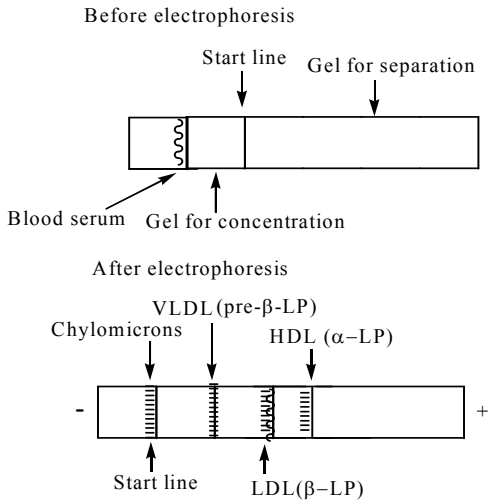


Figure 4.8.Electrophoregram obtained for lipoproteins of blood plasm uder discussed conditions of investigation

Atherosclerosis of blood vessels in humans

Atherosclerosis of blood vessels in humans usually associated with the state of Hypercholesterolemia. It is determined in patients with cholesterol (CHL) level more then 5.72 mmole/ L in the blood plasma. This state is correlated with one of the following abnormalities:

1) elevated concentrations of VLDL with normal concentrations of LDL;
2) elevated LDL with normal VLDL;
3) elevation of both lipoprotein fractions;
4) the inverse relationship between HDL and LDL may be also. The most predictive relationship is the CHL_{LDL}: CHL_{HDL} ratio, and it must be not higher then 3.0 for healthy people.

The accumulation of LDL in the blood may be in patients with genetic defects associated with:

a) defect of synthesis of receptors to LDL – apoB-100 receptors. It results in high levels of cholesterol (about 18 mmol/L) and LDL;
b) decrease of synthesis of lipoprotein lipase linked with VLDL. This defect results the increase of VLDL, then LDL (cholesterol and TG concentrations are increased);
c) the low activity of endothelial lipoprotein lipase (the same results);
d) decrease of synthesis of Lecithin Cholesterol Acyl-CoA Transferase (LCAT) linked with HDL. In this case the surplus of cholesterol can't be transformed into cholesterol ester, and transported from peripheral cell to the liver by HDL.

These changes can cause the atherosclerosis and are considered as primary reasons of this pathological state. The surplus of cholesterol accumulates in the arterial walls. LDL and VLDL (in high levels) can penetrate the vascular wall from the blood plasma to act subsequently as a primary substrate causing the atherosclerotic lesion of arteries.
The secondary reasons of atherosclerosis may be the diseases such as diabetes mellitus, nephritic syndrome, hypothyroidism, and other conditions of hyperlipoproteinemia.

Coronary heart disease is caused by atherosclerosis of arteries and may be finished by myocardium infarction in patient.

The treatment of hypercholesterolemia state depends upon its reason of the development. It may include:

1) Cholesterol free diet with high intake of unsaturated high fatty acids;
2) The use of drugs – inhibitors for β-hydroxy-β-methylglutaryl-CoA reductase: Lovastatin, Mevastatin;
3) The use of drug – Cholestyramine resin to block the reabsorption of bile acids in the small intestine. In this case the cholesterol is utilized in higher quantity up to bile acids;
4) The use of drugs Clofibrate an Gemfibrozil that divert the hepatic inflow of free fatty acids into oxidation, thus decreasing the secretion of VLDL by the liver. This drugs stimulate the hydrolysis of VLDL triacylglycerols by lipoprotein lipase;
5) The use of drug Probucol. It increases the rate of LDL catabolism via receptor-independent pathways, but its antioxidant properties may be more important in preventing accumulation of oxidized LDL in arterial walls;
6) The use of Nicotinic acid to reduce the flux of fatty acids by inhibiting of adipose tissue lipolysis thereby inhibiting VLDL production by the liver.

Hyperlipoproteinemies

Hyperlipoproteinemia is a metabolic disorder characterized by abnormally elevated concentrations of specific lipoprotein particles in the plasma.

According the classification (Fridrickson E., et all) hyperlipoproteinemies are divided in five types:

Type I (Hyperchylomicronemia). Very high level of chylomicrons in the blood serum on empty stomach. Slight higher levels for VLDL and triacylglycerols. Xanthomatosis in patients is associated with this type of hyperlipoproteinemia.

Type II (Hyper-β-lipoproteinemia). Subtype IIa is accompanied with high levels of LDL and total cholesterol in patients. Subtype IIb is associated with high levels of LDL, VLDL, cholesterol and

triacylglycerols. Ischemic heart disease and hypertension is observed at patients.

Type III (Dis-β-lipoproteinemia). High levels of IDL in the blood plasm that are absent at healthy adults, high levels of cholesterol. These changes associated with problems in heart system: atherosclerosis of blood vessels, thrombosis may be at patient too.

Type IV (Hyper-pre-β-lipoproteinemia). High levels of VLDL, but LDL are slight higher or normal. Chylomicrons are absent. Diabetes mellitus with obesity and ischemic heart disease are associated with this type of hyperlipoproteinemia.

Type V (Hyper-pre-β-lipoproteinemia accompanied with Hyperchylomicronemia). High levels of chylomicrons and VLDL are observed in patient's blood plasma. Xanthomatosis is represented, also. This type of state may be in patients with latent form of insulin-independent diabetes mellitus, but ischemic heart disease is not observed at patient.

Tests recommended to answer after study of chapter 4:

1. Chylomicrons, intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and very-low-density lipoproteins (VLDL) are all serum lipoproteins. What is the correct ordering of these particles from the lowest to the highest density?
   A. LDL, IDL, VLDL, chylomicrons
   B. Chylomicrons, VLDL, IDL, LDL
   C. VLDL, IDL, LDL, chylomicrons
   D. Chylomicrons, IDL, VLDL, LDL
   E. LDL, VLDL, IDL, chylomicrons

2. Choose the right continuation of the phrase: “The end product of cytosol fatty acid synthetase in humans is…”:
   A. Oleic acid
   B. Arachidonic acid
   C. Linoleic acid
   D. Palmitic acid
   E. Palmitoleic acid

3. Choose the right continuation of the phrase: “The fatty acid synthase complex of mammals…”
   A. Is a dimmer of two similar subunits
   B. Is composed of seven different proteins
   C. Dissociates into eight different proteins
   D. Catalyzes eight different enzymatic steps
   E. Is composed of covalently linked enzymes with Acyl Carrier Protein to dimmer-multienzyme system

4. Which one of the following apolipoproteins is synthesized in the liver as part of the coat of very-low-density lipoproteins (VLDL)?
   A. A-I
   B. B-48
   C. D
   D. B-100
   E. C-II

5. Which of the following lipoproteins will be absent in blood plasma of healthy person following 12-hours fast?
   A. Very-low-density lipoproteins
   B. High-density lipoproteins
   C. Chylomicrons
   D. Low-density lipoproteins
   E. All the positions above are right

6. Which of the following statements correctly describes the apo B-100-protein?

A. It yields acetyl CoA as a product
B. It has receptors to LDL
C. There are receptors to it in cells where LDL are utilized
D. It forms CoA thioesters as a product
E. It requires lipoprotein lipase to be linked to it

7. Most of the reducing equivalents utilized for synthesis of fatty acids, cholesterol, bile acids formation may be generated from:
A. The pentose phosphate pathway
B. Glycolysis
C. The citric acid cycle
D. Mitochondrial malate dehydrogenase
E. Citrate lyase

8. What proteins of blood plasm are the main transporters of High Fatty Acids?
A. VLDL
B. Albumins
C. HDL
D. Chylomicrons
E. LDL

9. Which one of the following compounds is a key intermediate in the synthesis of both triacylglycerols and phospholipids?
A. CDP-choline
B. Phosphatidic acid
C. Triacylglycerol
D. Phosphatidyl serine
E. CDP-diacylglycerol

10. The synthesis of 3-hydroxy-3-methylglutaryl CoA may be blocked under:
A. Accumulation of cholesterol in a cell
B. ATP accumulation
C. ADP accumulation
D. Increase of cAMP in cytoplasm
E. The use of cholesterol free diet

Chapter 5. Common notions about the metabolic pathways of amino acids and other non-protein nitrogen-containing compounds in humans

Processes supplied the constant level of amino acids in the blood plasma

The evaluation of amino acids blood concentration shows that amino acids Glutamate, Glutamine, Alanine and Serine are in higher concentration then others. That is because their exchange provides very important processes in the brain, muscles, liver, kidneys and gut.

Alanine serves as a key glucogenic amino acid. In the liver, the rate of glucose synthesis from alanine is far higher then from all other amino acids. Glutamate, glutamine are extracted by the gut and the kidney, both of which convert a significant portion to alanine.

Glutamine is the main product of ammonium detoxification in brain and also serves as a source of ammonia for excretion by the kidney. In this case it is the donor of ammonia to neutralize acidic products in the blood. Ammonia salts are formed in this case.

Muscles and liver play major roles in maintaining circulating amino acids levels. Alanine and Glutamine are also released by muscles. The kidney provides a major source of Serine for uptake by peripheral tissues, including liver and muscle.

Branched-chain amino acids, particularly Valine, are released by muscle and taken up predominantly by the brain.

The main important pathways for amino acids use and formation are represented in the figure 5.1.:

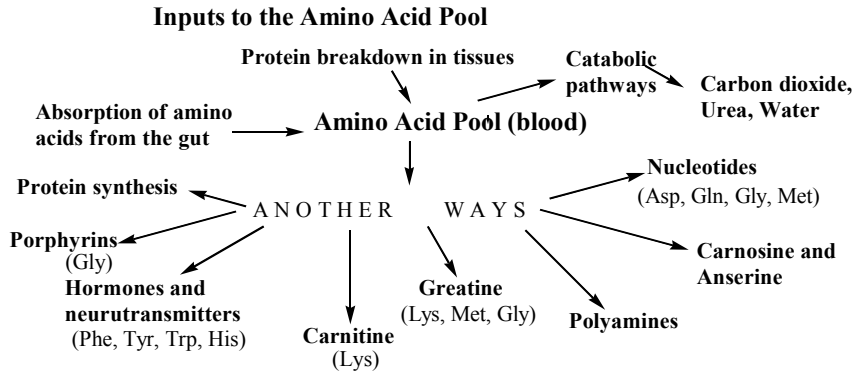


Figure 5.1. The most important pathways for amino acids in human organism

There are four main catabolic pathways for amino acids in tissues (Fig. 5.2):

1. Decarboxylation (carbon skeleton degradation);
2. Transamination (transport of α-amine group to α-keto acid to form new amino acid and new keto acid);
3. Transdeamination (Transamination of amino acid with α-ketoglutarate use and Glutamate Dehydrogenase reaction);
4. Direct Deamination (oxidative, hydrolytic types; for amino acids Glu, Ala, Thre, Ser, Cys only)

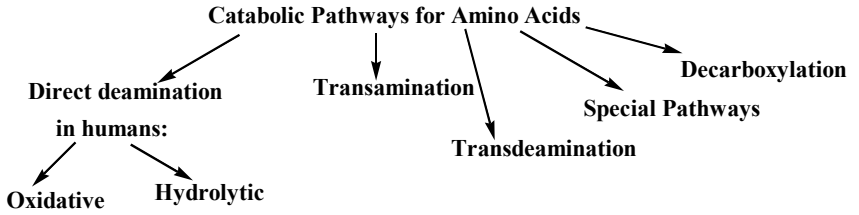


Figure 5.2. Amino acids use in catabolic pathways.

Special metabolic pathways for amino acids are associated with formation of some biologically active organic products that are very individual according the type of amino acid (Fig .5.3)

Catabolic pathways (except transamination reaction type) for most amino acids and organic amines are associated with the formation of ammonia as back-side product. Its formation we can find out during the degradation of any type of nucleotide in human tissues, too. Ammonia is very toxic product that influences the CNS action, changes the pH value of the blood. Concentration of ammonia near 3 mmol/L is lethal (experiments on rabbits). Normally ammonia level in the blood of humans does not exceed to 60 μmole/L. The accumulation of ammonia in the blood is named as hyperammoniemia state. How is this product neutralized in human organism? The metabolic pathways for utilization of ammonia are differ in location in tissues, and the main important one are represented in figure 5.4.

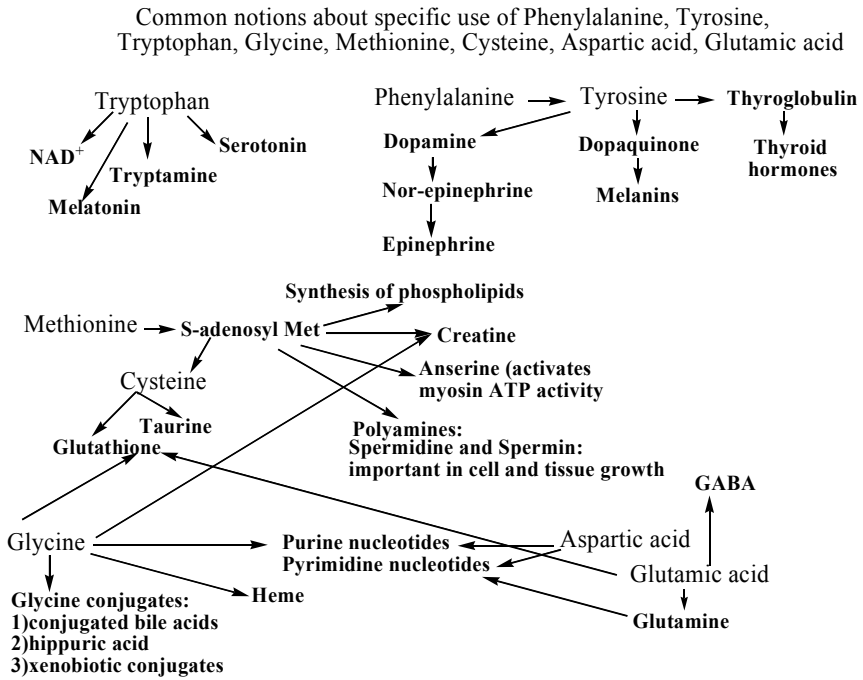
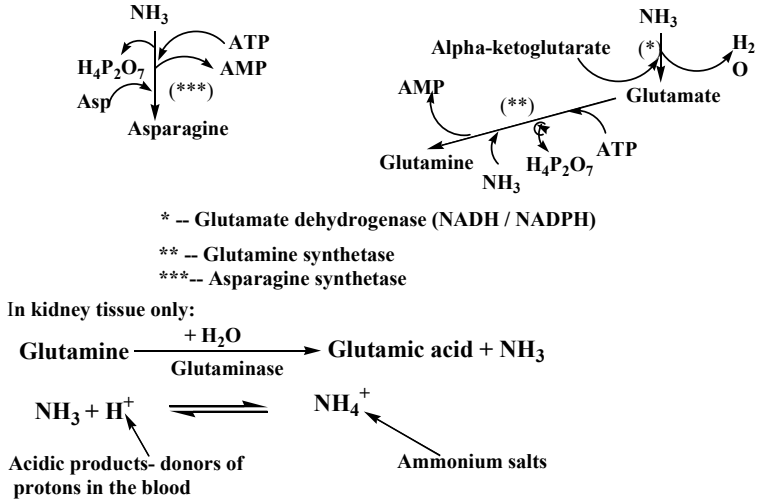


Figure 5.3. Specific ways for some amino acids



There is specific way for ammonia utilization - Urea Cycle- in the liver, only.

Figure 5.4. Ammonia utilization ways in human tissues

Glutamate dehydrogenase reaction

This reaction (Figure 5.5.) is very important for glutamate in all types of tissue. In the liver this enzyme system contains 6 subunits and is considered as multienzyme complex linked with NAD^+ or $NADP^+$. It dissociates if the content of NADH, GTP is increased in a cell, and some steroid hormones can influence inhibiting its activity. The location of this enzyme is in the matrix of mitochondria. NADPH–Glutamate Dehydrogenase takes part in reductive amination of α-ketoglutarate to produce Glutamic acid and to utilize toxic ammonia.

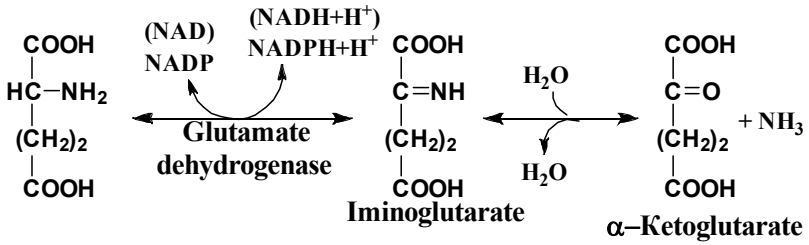


Figure 5.5. Glutamate dehydrogenase reaction in humans

Control of Glutamate dehydrogenase reaction:

1) The reaction above is controlled, therefore, by the relative levels of Glutamate, α-ketoglutarate and ammonia;

2) Allocteric activators: ADP, GDP. Allosteric inhibitors: ATP, GTP and NADH (when they are accumulated in a cell). Steroid hormones can suppress the synthesis of this enzyme.

Biological role of the direct reaction:

a) to produce NADH and α-ketoglutarate as energy sources for a cell;

b) to form NADPH that may be used for synthetic pathways in lipid metabolism and for monooxygenase systems function;

c) it is used as the second step in the transdeamination of a lot of amino acids in a cell.

Biological role of opposite reaction: the utilization of a toxic product – ammonia.

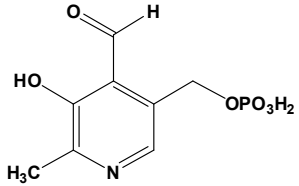
Transamination of amino acids

Transamination involves the transfer of an amino group from an amino acid to an α-keto acid to form a new amino acid and a new α-keto acid (fig.). Enzymes are named: transaminases or aminotransferases.

Mechanism of Transamination

Two steps are considered in this mechanism:

1) the formation of the first product - α-ketoacid and the derivative of coenzyme Pyridoxamine from coenzyme Pyridoxal phosphate (vitamin B_6 derivative);



Pyridoxal phosphate

2) The regeneration of Pyridoxamine to Pyridoxal Phosphate and the formation of the new amino acid as product.

There is the transamination of aspartic acid in the figure 5.6. as the first step for transdeamination

Transdeamination steps for Aspartate:

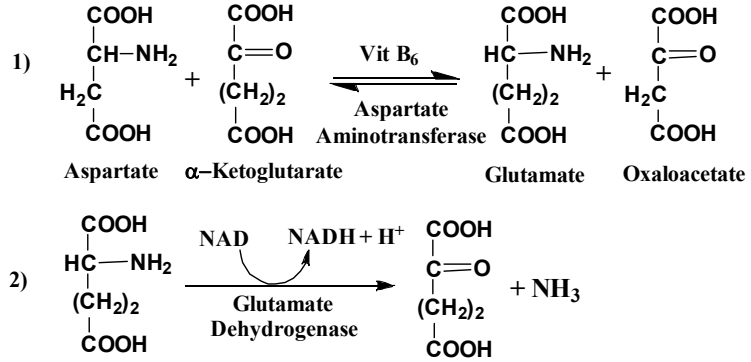


Figure 5.6. The transdeamination of aspartic acid in a cell.

The biological role of amino acid transamination. It is:

- Synthesis of any amino acid if appropriate α-ketoacids are available in the organism;
- The first step for transdeamination of amino acids which can`t be involved in the direct deamination in a cell.
- The way to form α-ketoacids (carbon skeleton) which may be involved in different catabolic pathways to produce energy for a cell or to use them for gluconeogenesis. Amino acids incorporated into gluconeogenesis are named glucogenic one (Ala, Arg, Asn, Asp, Cys, Glu, Cln, Gly, His, Met, Pro, Ser, Thre, Val).

The most active transaminases in the liver, in the myocardium are:

- ***Alanine aminotransferase (AlaAT),***
- ***Aspartate aminotransferase (AspAT).***

It should be noted that level of activity of these two transaminases is not the same in both types of tissue. The most active in the myocardium is AspAT, but in the liver AlaAT is the more active. Normal

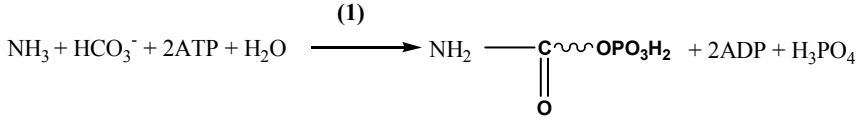
values for AlaAT activity are in the region: 0.1- 0.45 μmole/L•hour. Normal values for AspAT activity: 0.1 – 0.5 μmole/L•hour. At some cases it is useful to determine:

De Ritis coefficient = $\frac{[AspAT]}{[AlaAT]} = 1.33 \pm 0.14$;

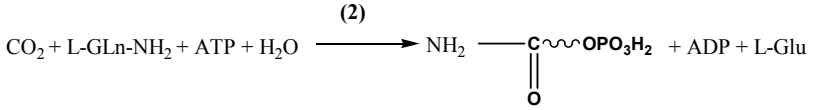
At viral hepatitis (liver parenchyma damage) the blood plasma values for AlaAT are much higher then normal, and De Ritis coefficient is about 0.8. At myocardium infarction (during first 3-4 hours of myocardium tissue damage) the blood plasma values for AspAT may be in 10-100 times higher then normal one. De Ritis coefficient may be 3 or more in this case.

Ammonia neutralization ways in the liver, only

All the ways (fig. 5.7.) are represented in this tissue type, but the main important is Urea Cycle. Primary reasons for development of hyperammoniemia state mainly are associated with genetic disorders of key enzymes in this process. The first reaction is catalyzed by key enzyme Carbomoyl phosphate synthetase (1) (location: matrix of mitochondria). It is activated by N-acetyl-glutamine:



Formation of carbomoyl phosphate may be by the action of another enzyme Glutamine-dependent carbamoyl phosphate transferase (2):



It was discovered in other animal tissues too, and it is used for Pyrimidine nucleotide synthesis usually. Ornithine carbamoyl phosphate transferase (3, figure5.7.) is in need for the formation of Citrulline also in the matrix of mitochondria. The next reaction is catalyzed by Arginine succinate synthetase (4,), it gives the product using the ATP as energy source, and aspartate. Then we have to consider the action of Arginine succcinate lyase (5, figure) that forms two prodicts: Arginine and fumarate. The latter product is involved in Krebs cycle to give in the last reaction of this process oxaloacetate that can be converted in transamination into aspartate. This circulation Fumarate-Oxaloacetate-Aspartate-Arginine succinate is a linkage of two very important processes; Krebs Cycle and Urea synthesis in the liver. The last enzyme in Urea synthesis is called Arginase (6). It gives the end product Urea and again Ornithine that is because we named it as a cycle. Reactions (4), (5), (6) are proceed in the cytoplasma of hepatocytes.

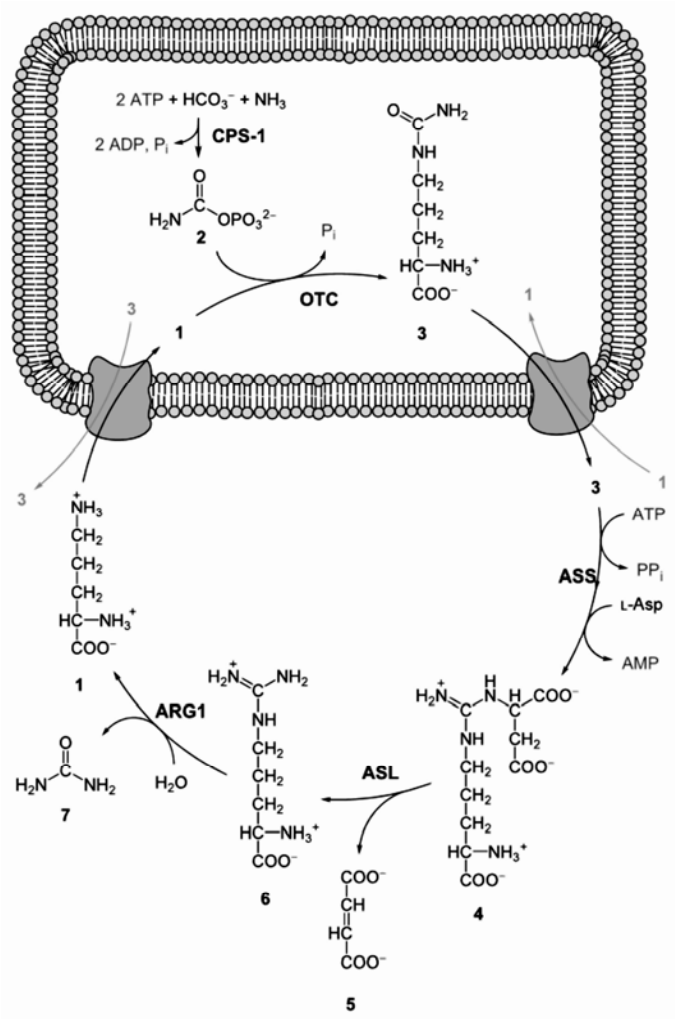


Figure 5.7. The urea cycle duration in the liver tissue
1 L-ornithine
2 carbamoyl phosphate
3 L-citrulline
4 argininosuccinate
5 fumarate
6 L-arginine
7 urea
L-Asp L-aspartate
CPS-1 carbamoyl phosphate synthetase I
OTC Ornithine transcarbamoylase

ASS argininosuccinate synthetase
ASL argininosuccinate lyase
ARG1 arginase 1

Regulation. With the essentially protein free diet, urea excretion account for only 60% of total urinary nitrogen (80% , in a normal diet), and the levels of all Urea cycle enzymes decline. With a high-proteins diet or in starvation the Urea cycle enzymes increase in content.

Genetic defects of enzymes in Urea Cycle causing the hyperammoniemia in patients

1. Hyperammonemia Type I is due to a defect of carbomoyl phosphate synthetase.
2. Hyperammonemia Type II is due to a defect of ornithine carbomoyl phosphate transferase.
3. Citrullinuria is due to a defect of arginine succinate synthetase (Citrulline is absent in the urine of healthy).
4. Argininosuccinic acidemia is due to a defect of arginine succinate lyase.
5. Hyperargininemia is due to a defect of arginase.

The accumulation of ammonia in the blood is the most severe in (1) and (2) diseases. High levels of ammonia are quite toxic and can cause brain damage (the mechanism is not well understood). Episodic encephalopathia, such as convulsions and ataxia may occur and usually cease when protein intake is restricted. Very early diagnosis is critical in preventing mental retardation. The disease is evident by the end of the first week of extrauterine life. The infant is the difficult to feed; may vomit and may be lethargic. Diagnosis prior to 1 week of age is possible only by enzymatic analysis. Extensive brain damage occurs by the end of 1 year.

Normal levels of Urea in the blood plasma are 3,3-6,6 mmol/L. Urea is terminal product for humans , and it is excreted in the urine daily in mass about 25-35 g. The urea content in person correlates with the value of special index of blood - Residual Nitrogen (RN).

Residual nitrogen (non-protein nitrogen)

It is the content of nitrogen in all non-protein nitrogen–containing substances of the whole blood. It is Nitrogen from compounds: urea, free amino acids, creatine, creatinine, uric acid, biogenic amines, peptides, conjugated bilirubin, hippuric acid, indican, nucleosides, etc. Its normal value for adults must be about 25-35 mg% or 15-25 mmol/L (whole blood).

The elevation of this index may be absolute or relative in person. In any case the state is named as azotemia. Absolute azotemia is developed at the accumulation of organic compounds named above due to extensive protein breakdown in tissues (prerenal or productive

subtype of azotemia)) or at renal insufficiency (renal subtype of azotemia). Extensive protein degradation in tissues may be observed at fever, burns, severe form of diabetes mellitus, of cirrhosis of the liver. Renal azotemia is indicated at patients with acute or chronic nephritis. At acute renal insufficiency the urea content may be increased up to 50 – 83 mmol/L, and there is the increase of RN too in this case.

Relative azotemia is associated with extensive dehydration caused by irrepressible vomiting, intense sweating, etc.

The ratio of Urea content to RN must be about 48% in healthy adults. At absolute azotemia state this ratio is increased usually. Concentration of urea is decreased in the blood plasma during the liver diseases (viral hepatitis; cirrhosis), the ratio is decreased in this case and may be about 25%. At renal insufficiency the RN may be about 80% or more.

Creatine, creatine phosphate, creatinine levels in the blood plasma of humans

Arginine, glycine and S-adenosyl methionine are involved in the synthesis of special organic compound creatine (in 85% synthesized in the liver, other content – in kidneys). Its normal levels in the blood serum are 15.25-45.75 micromole/L (for men); 45.75-76.25 micromole/L (for women). Creatine is transported mainly to the muscular tissue (for further transformation (about 10% of its content is utilized in the brain and kidneys). The whole metabolic pathway up to terminal product creatinine is represented in figure 5.8.

It was proved that the way of creatine phosphate utilization in muscles has coupling sites with oxidative phosphorylation in mitochondria, and there are two isozymes of Creatine phosphate kinase (CPK) in myocyte (cytoplasmic and mitochondrial) (figure 5.9..). Creatine phosphate formed due to mitochondrial isozyme of CPK (after coupling with oxidative phosphorylation) may be used in cytoplasm by cytoplasmic isozyme of CPK to produce ATP again for muscular contraction.

The creatine phosphate kinase is represented in three isoforms in humans. BB-isozyme is synthesized in the brain, MB-isozyme – in myocardium, MM-isozyme in muscles, and the highest activity of Creatine phosphate kinase is associated with brain and striated type of muscles, only.

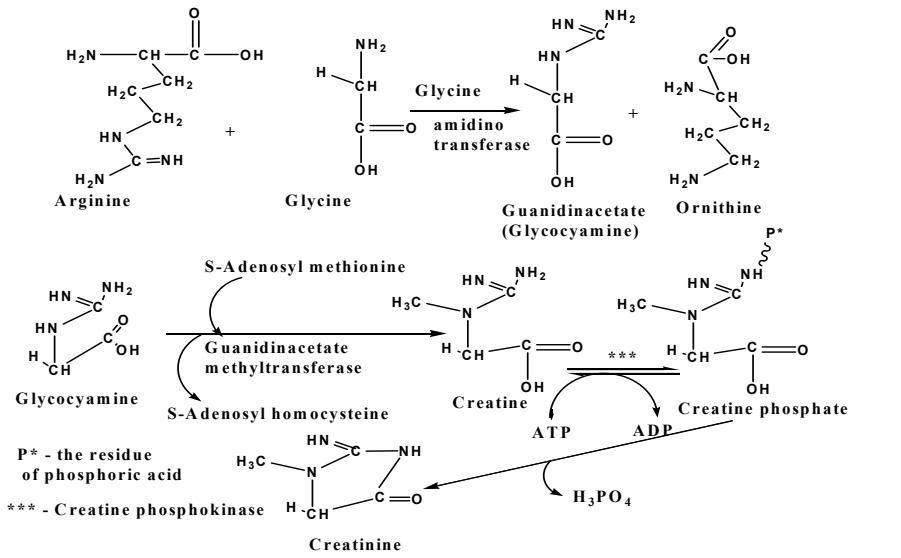


Figure 5.8.The creatine, creatine phosphate and creatinine formation in humans

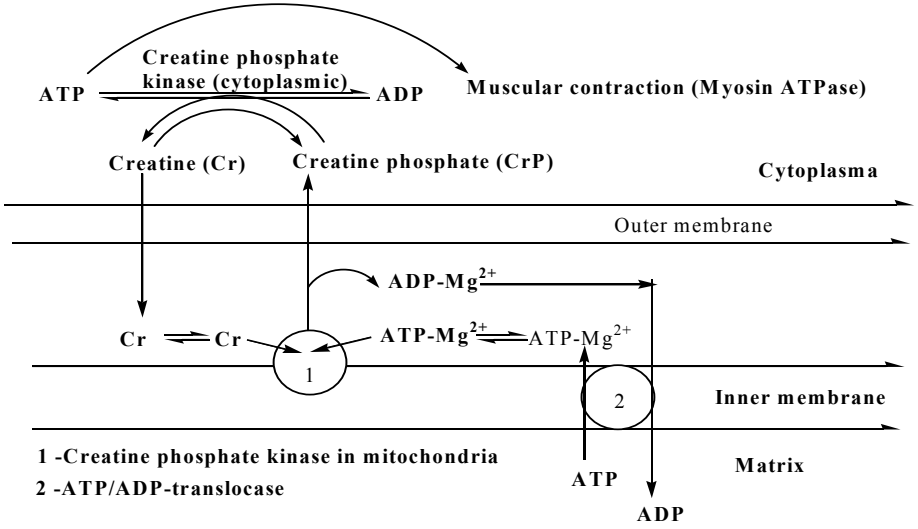


Figure 5.9. The coupling of oxidative phosphorylation with creatine phosphate transformation in cytoplasm of muscular cell

Creatinine is considered as the terminal product that is excreted into the urine. Normally the daily excretion of creatinine in the urine is correlated with muscular mass and equals 8.8-17.7mmole/daily (for

men). This organic compound is not reabsorbed in kidneys, and ratio of its content in the urine to the content in the blood plasma estimates the rate of glomerular filtration in patient. The index is called the clearance of kidney. Normal clearance index in adults equals 120 ml/min. It is usually determined at patients which may be potential donors of kidney; in a case of to choose the initial dose of some toxic drug to treat patients.

The back side product for the first reaction of creatine synthesis is the ornithine, and this is the key metabolite for Urea cycle in duration.

Creatinine and urea contents in the blood plasma are very important indexes for glomeruli function estimation. There is the increase of both indexes in the blood plasma at renal insufficiency in patients. 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) (figure 5.10.).

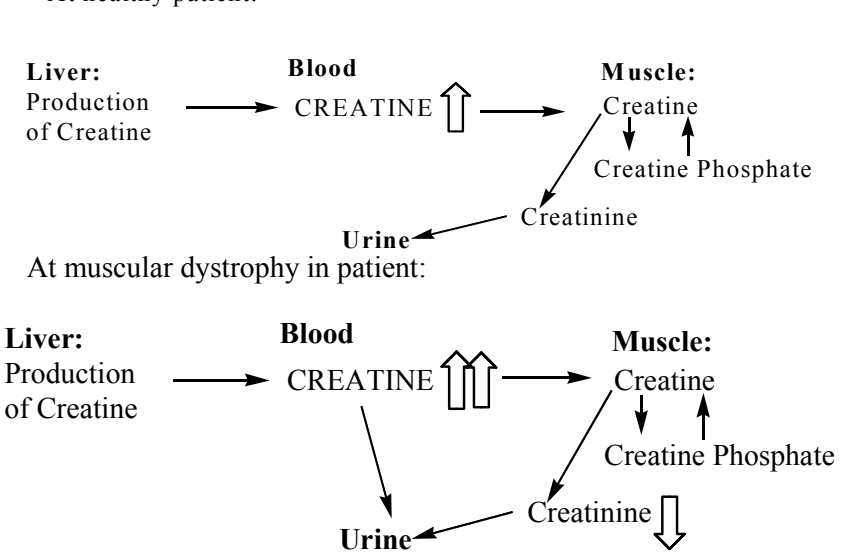


Figure 5.10. Creatine and creatinine levels in the blood and urine of healthy and diseased people (the case of muscular dystrophy, only)

Hippuric acid and indican

These two organic compounds are very important for estimation of liver function to detoxify harmful endogenous metabolites and xenobiotics (foreign compounds for humans), and to recognize the rate of putrefaction in the gastrointestinal tract (GIT).

Tryptophan is the precursor for the formation of indican in the GIT (figure 5.11.).

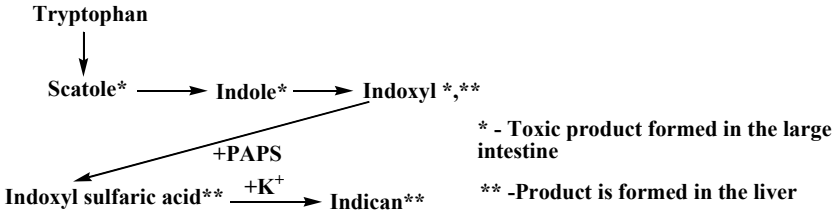
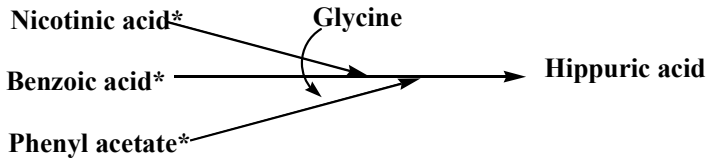


Figure 5.11. Transformation of tryptophan during the putrefaction in the GIT

The normal values for indican in the whole blood are 0.114-0.171 micromole/L, in the urine - 46.99-56.39 micromole/daily. At the accumulation of indican in human organism the urine is colored brown shade. This situation may be at patients with infringements in GIT (twisted bowels, constipation). At prerenal azotemia.

Hippuric acid is formed in the liver mainly at the conjugation of some organic compounds with glycine:



(*) - These substances must be in active form before conjugation step

Quick's test is also used for this purpose (determination of hippuric acid in the urine following the administration of sodium benzoate):

Oral dose of sodium benzoate 3-4 g is administrated for person. After 3 hours the concentration of hippuric acid is determined in the urine of patient. 65%-85% of the initial benzoate is discharged in the composition of hippuric acid (its glycine conjugation product) in the urine of healthy adult patient.

Tests recommended to answer after study of chapter 5:

1. Point out the enzyme, whose activity is determined in the blood plasma during the unicteric period of viral hepatitis:
   A. Phenylalanine hydroxylase
   B. Creatine phosphokinase
   C. Glutamate dehydrogenase
   D. Alanine transaminase
   E. Ornithine carbomoylphoshate transferase

2. Which of the following descriptions related to serotonin pathway **isn't** correct?
   A. Serotonin synthesis lowering in the brain causes depression
   B. The largest amount of serotonin is synthesized in the intestinal cells
   C. The first enzyme of the pathway is tetrahydrobiopterin-dependent hydroxylase
   D. The second enzyme of the pathway is pyridoxal phosphate-dependent aromatic amino acid decarboxylase
   E. Serotonin is synthesized from tyrosine

3. Choose the enzyme of the blood plasma whose activity increases in ten or more times for 3-4 hours after myocardium infarction:
   A. Alanine transaminase
   B. Aspartate transaminase
   C. Alkaline phoshatase
   D. Arginase
   E. Leucine aminopeptidase

4. Which of the following statements related to biological role of transamination is wrong?
   A. Redistribution of amino groups
   B. Production of non-essential amino acids
   C. Formation of biogenic amines
   D. Divergence the excess amino acids towards energy generation
   E. Collection finally nitrogen in glutamate for subsequent deamination and urea synthesis

5. Point out the cofactor, which is used by D-amino acid oxidase in oxidative deamination:
   A. NADPH
   B. NADH
   C. FAD
   D. FMN
   E. TPP

6. Point out the vitamin, whose deficiency causes the violations in the transamination and decarboxylation of amino acids:

A. Vitamin C
B. Vitamin B_1
C. Vitamin B_2
D. Vitamin B_9
E. Vitamin B_6

7. Utilization of amino acids from the body pool is possible in all following ways except one. Choose it:

A. Porphyrins production
B. Purines production
C. Biogenic amine formation
D. Glucose and ketone bodies synthesis
E. Pyridoxal phosphate synthesis

8. Most amino acids undergo transamination to concentrate finally nitrogen in two amino acids only for subsequent urea formation. Choose that amino acids:

A. Lysine, proline
B. Glutamate, aspartate
C. Alanine, phenylalanine
D. Serine, tyrosine
E. Arginine, histidine

9. In course of histidine catabolism a biogenic amine is formed that has powerful vasodilatation effect. Name it:

A. Noradrenalin
B. Dioxyphenylalanine
C. Serotonin
D. Dopamine
E. Histamine

10. According to clinical indications a patient was administered pyridoxal phosphate. What process is this medication intended to correct?

A. Deamination of purine nucleotide
B. Synthesis of purine and pyrimidine bases.
C. Transamination and decarboxylation of amino acids
D. Protein synthesis
E. Oxidative decarboxylation of ketoacids

Chapter 6. Common notions about haemoprotein metabolism in human organism. Nucleotide metabolism as the part of nucleoproteins metabolism in humans

Hemoproteins

Haemoprotein contains polypeptide chain and haem as non-protein component. The species character of a haemoglobin is primaly due to its polypeptide moiety, while the haem is the same in haemoproteins of any type. The structure basis of prosthetic group in most haem-containing proteins is the porphyrin ring derived from a tetrapyrrolic compound, porphyrin. The unsubstituted porphyrin is called porphyn. In a haem molecule, porphyn is represented by protoporphyrin IX/\. The chelated comlex of protoporphyrin IX with Fe (II) is called haem.

Examples of Haemoproteins

Haemoglobin is the quantitatively most important haemoprotein**.**

- It is the carrier of oxygen and carbon dioxide in the blood.
- The body contains about 750 grams of hemoglobin; it is replaced every 120 days.
- Normal hemoglobin turnover requires synthesis of new 6-8 grams hemoglobin daily, a process which uses about 14% of the dietary amino acids. 300 milligrams of haem are synthesized daily for conjugation with the newly synthesized globin protein.

Cytochromes are present in the mitochondria and in the endoplasmic reticulum.

- They participate in important electron transfer reactions.
- The most important are *b, c_1, c, aa_3*, b_5, b_{560} ,P450.
- Their half life is about 132 hours.

Catalase and peroxidase utilize toxic hydrogen peroxide.

- These enzymes protect the body against uncontrolled oxidation by hydrogen peroxide.
- Their half life is about 20 hours.

Tryptophan oxygenase is a haemoprotein of intermediary metabolism**.**

- It catalyzes the conversion of tryptophan to N-formylkynurenine, using oxygen as the oxidant.
- Its half life is only about two hours.

These conjugated proteins are only some from many haemoproteins.

Haem Biosynthesis

Mitochondria are not only the powerhouse of the cell, supplying abundant ATP through the coupling of active proton pumping across their inner membrane with the transfer of electrons along the respiratory chain, they are also the alpha and omega of haem biosynthesis. The overall pathway of haem biosynthesis begins in the mitochondria, with the condensation of succinyl CoA coming from the citric-acid cycle with glycine to form δ-aminolaevulinate, which is expedited forthwith into the cytoplasm. There, the synthesis of the tetrapyrroleporphyrin nucleus continues apace, until the oxidative decarboxylation of coproporphyrinogen III to protoporphyrinogen IX is accompanied by its transport back into the mitochondria whence it came, to undergo oxidation of its methylene groups to protoporphyrin IX and insertion of iron to yield the end product, haem. The two major sites of haem biosynthesis are erythroid cells, which synthesize around 85 % of the body's haem groups, and the liver, which synthesizes most of the remainder. A major function of haem in liver is as the prosthetic group of cytochrome P450, the importance of which is detoxification. The liver cell must synthesize cytochrome P450 throughout its lifetime in quantities that vary with conditions. In contrast, the developing erythroid cell only engages in haem synthesis when it differentiates, and then it is a one-time synthesis in vast quantities to accompany globin production and ensure the haemoglobin content that will last for the erythrocyte lifetime. Haem and globin synthesis cease upon red cell maturation. This means that haem synthesis in liver and erythroid cells is regulated in a quite different way. In the liver, the main control site is δ-aminolaevulinate synthase, which is regulated by haemin, the Fe(III) oxidation product of haem, by three mechanisms: *1)* feedback inhibition, *2)* inhibition of transport of the enzyme from its site of synthesis in the cytosol to the mitochondria, and *3)* repression of the enzyme synthesis. In differentiated erythroid cells (reticulocytes), haem stimulates protein synthesis, inducing synthesis of globin to ensure that haem and globin are synthesized in the correct ratio for assembly into haemoglobin, but also induces the synthesis of the haem biosynthetic pathway enzymes. The control of haem synthesis in erythroid cells seems to be at the level of ferrochelatase, and porphobilinogen deaminase, rather than δ-aminolaevulinate synthase. However the translation of δ-aminolaevulinate synthase mRNA is regulated by iron availability, increasing when iron is abundant.

Ferrochelatase (protohaem ferrolyase), is the terminal enzyme in haem biosynthesis, catalysing the incorporation of ferrous iron into protoporphyrin IX. They are monomeric proteins of molecular weight between 36–40 kD. Mutations in the ferrochelatase gene in humans can cause erythropoietic protoporphyria.

Pathological derivatives of haemoglobin

In haemoglobin Fe^{2+} does not change its valency during binding or release of oxygen. But it can be oxidized by oxidation agents to Fe^{3+}, giving rise to haemming. The resulting compound is called **methaemoglobin** and it can't function as oxygen carrier.

Carboxyhaemoglobin (HbCO) is forming during carbon monooxide (CO) poisoning. CO is very toxic gas because it binds with a higher affinity (much tighter) to haeme does oxygen. Since CO has a higher affinity than oxygen, oxygen cannot displace it. In this way, CO acts as much like a potent competitive inhibitor. There can be plenty of oxygen available, but the hemoglobin bond to CO will not carry it; therefore the oxygen is not available for tissues. CO effectively binds irreversibly to the haem in hemoglobin molecule.

The hyperglycemia causes this glucosylation and formation **HbA_{1c}**. This is the formation of Shift (S) base between glucose and the amino group of terminal amino acid of the β-chains. Then the S base becomes the more stable amino ketone. In diabetes mellitus the concentration of HbA_{1c} may reach 12% or more of the total hemoglobin. The amount of HbA_{1c} becomes a good indicator of blood glucose levels over 2-4 month period of disease duration.

Haemoglobinopathies as disorders of haemoglobin synthesis

They are genetic diseases in which haemoglobin subunits are mutated. More then 150 of haemoglobinopathies have been described. There are no symptoms or impairment in some of them, whereas in others there may be severe impairment.

Sickle cell anemia (HbS is instead of HbA_1, homozygous defect) was one of the first hemoglobinopathies to be described. HbS is formed when valine replaces glutamic acid in the sixth position of the β-chains. The α-chains are normal. HbS polymerization and deoxygenation is indicated within red blood cells. The polymerization leads to the characteristic sickle-shaped cell. The symptoms of Sickle cell anemia in homozygous can be severe. The patients are anemic and their red blood cells have an average life span of 10 to 15 days instead of normal 120 days. A sickle cell crisis can be extremely painful and can lead to cumulative organ damage. Death often occurs in early adulthood. Heterozygotes are said to have sickle cell trait. They are usually symptoms free except under condition of low P_{O2}. Persons with sickle cell trait show an increased resistance to malaria.

Thalassemias are hemolytic anemias that arise from insufficient production either the α- or β-chains of haemoglobin.

Thalassemias are classified according to the number of copies of mutated genes that the patient carries. Individuals with β-thalassemias

(insufficient production of β-chain) may have either one (homozygous) or two (heterozygous) mutated genes. Because α-chain genes are duplicated, from one to four α-chain genes may be mutated.

Haem Degradation

Haem is mainly found in the human organism as a prosthetic group in erythrocyte haemoglobin. Most of the haem which is degraded comes from haemoglobin.

Since in the steady state 6-8 grams of haemoglobin are synthesized daily, 6-8 grams must also be degraded, around 100–200 million aged erythrocytes per hour are broken down in the human organism. This gives rise to about 300 milligrams of haem. Haem is not reutilized, so it must be degraded and excreted. The degradation process starts in reticuloendothelial cells in the spleen, liver, and bone marrow.

Although haem is not recycled, **its iron** is conserved.

- Normally, senescent and damaged erythrocytes are sequestered by the spleen, which processes them in a manner that preserves their iron content.
- If haemolysis occurs, haemoglobin (with its iron) is released into the plasma.

Possible causes of **haemolytic anemia** include:

- Genetic defect of some enzymes of haem synthesis.
- Megaloblastic anemia (pernicious anemia) appears when absorption of vitamin B_{12} is prevented by lack of intrinsic factor.
- Folic acid deficiency causes megaloblastic anemia.
- It may by causes by exogenous factors:
  - Prolonged treatment by antibiotics;
  - Poisoning by some products of chemical industry;
  - X-ray radiation.
- Some special disorders of brain marrow: ostheomyelosclerosis, ostheopetrosis.
- It can be caused by deficiency of two enzymes in erythrocytes:
  - Glucose-6-phosphate dehydrogenase,
  - Pyruvate kinase.
- Methaemoglobinemia: intake of excess oxidants (various chemicals and drugs).

In the plasma, oxyhaemoglobin dissociates into alpha-beta dimers, which can escape through the glomerular filtration system of the kidney to appear in the urine. To prevent this, there is a plasma protein, haptoglobin, which binds the dimer and:

- delivers it to the reticuloendothelial system for processing
- activates the haem to prepare it for the degradation.

Any free haem is bound to another plasma protein, haemopexin, which then transports it to the liver for degradation. Turnover of haem proteins, particularly haemoglobin, potentially leads to release of free haem into extracellular fluids, where it can be a source of free radical formation and a major source of iron for invading bacterial pathogens. Protection is afforded by haemopexin, a 60-kD serum protein whose structure has been recently determined, which binds haem with high affinity, and delivers the haem to target cells such as liver via specific receptors. Internalization of the haem–haemopexin complex releases haem for intracellular degradation by haem oxygenase, stimulates intracellular protective mechanisms including induction of haemoxygenase 1 and the anti-apoptotic transcription factor NFkB. In this way, haem binding and transport by haemopexin provides protection against both extracellular and intracellular damage by free haem, limits access by pathogenic organisms to haem, and conserves iron by recycling the haem iron. The importance of haem–haemopexin as a cellular iron source is probably rather limited under normal physiological conditions.

Haem is degraded in two steps to bilirubin, which is conjugated to glucuronic acid and excreted.

The first reaction is cleavage of the haem ring by a microsomal haem oxygenase (HO, fig.6.1.)**.**

The substrates for the reaction are

- haem
- three molecules of oxygen
- NADPH

The reaction is a cleavage of the ring between the I and II pyrrole rings.

- The alpha-methylene group is released.
- The product is symmetric with respect to the propionic acid groups.

The products are

- biliverdin (greencoloured)
- carbon monoxide (this is the only endogenous source of carbon monoxide)
- iron (II)
- $NADP^+$

The enzyme haem oxygenase catalyses the NADPH, O_2 and cytochrome *P*450 reductase-dependent oxygenation of haem to iron, CO and biliverdin. Humans and other mammals have two isoenzymes, HO-1 and HO-2, which are products of separate genes. Human HO-1 is a 288-residue protein which is found at highest levels in the spleen, where recycling of erythrocytes takes place, but is also found in liver, where haem derived from cytochrome *P*450 is degraded, and in other

tissues. It is regulated at the transcriptional level by porphyrins, metals, progesterone, and a variety of other molecules, and is involved in response to oxidative stress, ischaemia, hypoxia and other disease states. Human HO-2, a 316-residue protein, is constitutively expressed at high levels in the testis and some regions of the brain. This has led to the proposal that the principal role of HO-2 is the production of CO as a neural messenger. Both HO-1 and HO-2 have *C*-terminal extensions with membrane anchors that are both localized in the microsomal membrane.

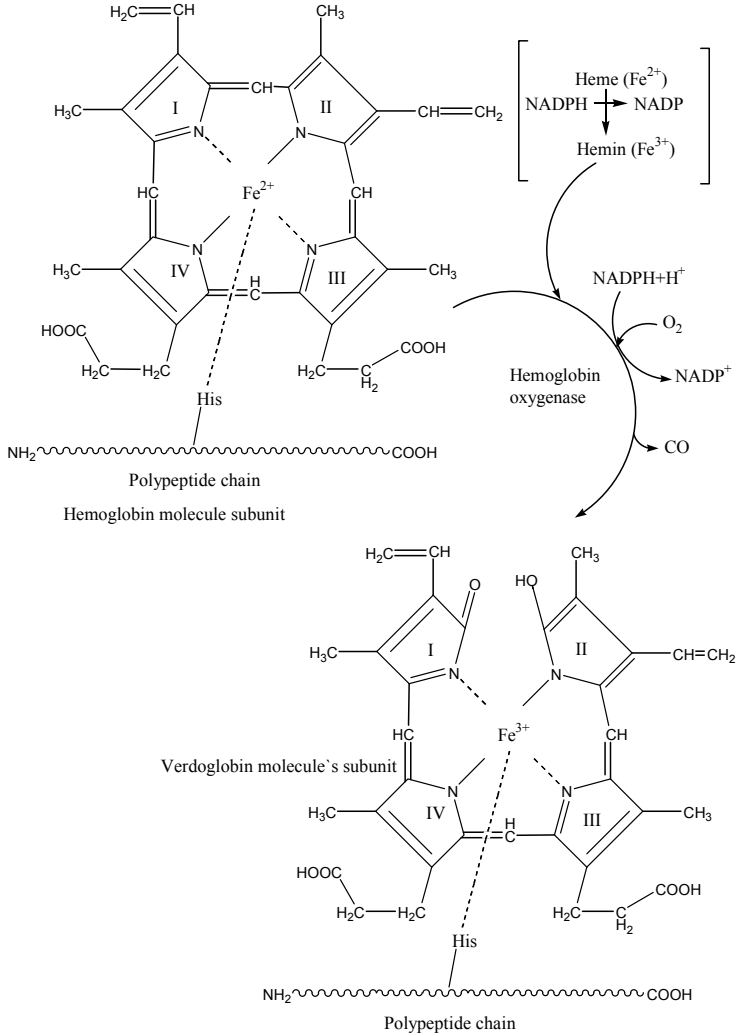


Figure 6.1. Hemoglobin oxygenase action in the breakdown of hemoglobin

In the first step, a ferrous haem–O_2 complex is thought to undergo an internal electron shift to form a ferric peroxyhaem intermediate, which then reacts regiospecifically with the α-bridge carbon to form α-hydroxyhaem. A second O_2 then reacts with α-hydroxyhaem, forming verdohaem and liberating CO. A third O_2 next reacts with verdohaem to form an enzyme-bound Fe(III)–biliverdin complex. On reduction of the iron atom, Fe^{2+} and biliverdin IXa are released from the enzyme. At various steps, reducing equivalents are required. The microsomal haem oxygenases of animals derive these reducing equivalents from NADPH via the microsomal NADPH-cytochrome *P*450 reductase.

In the second reaction biliverdin reductase reduces the central methene bridge of biliverdin, producing orangecolored bilirubin (fig.6.2.). The color change from purple to green to yellow can be easily observed in vivo in a hematoma.

Bilirubin. The high lipid solublity of bilirubin determines its behavior and its further metabolism. **Its lipid solubility dictates:**

- that it is soluble in the lipid bilayers of cell membranes.
- that it must be transported in the blood by a carrier; the physiological carrier is serum albumin. Some drugs that also bind to albumin can lead to an increase in free bilirubin.

Bilirubin must be conjugated to a water-soluble substance. This increased its water solubility, decreases its lipid solubility and makes easier its excretion. Conjugation is accomplished by attaching two molecules of glucuronic acid to it in a two step process.

The substrates are

- bilirubin (or bilirubin monoglucuronide)
- UDP-glucuronic acid (fig.6.2.)

The reaction is a transfer of two glucuronic acid groups sequentially to the propionic acid groups of the bilirubin. The major product is bilirubin diglucuronide.

Glucuronide synthesis is the rate-determining step in hepatic bilirubin metabolism. Drugs such as *phenobarbital,* for example, can induce both conjugate formation and the transport process.

The bilirubin glucuronides are then excreted by active transport into the **bile**, where they form what are known as the **bile pigments**. Some of the bilirubin conjugates are broken down further in the intestine by bacterial *glucuronidases.* The bilirubin released is then reduced further via intermediate steps into colorless **stercobilinogen (fig.6.3.)**, some of which is oxidized again into orange to yellow-colored stercobilin. The end products of bile pigment metabolism in the intestine are mostly excreted in feces, but a small proportion is resorbed. When high levels of haem degradation are taking place, stercobilinogen

appears in the urine, where oxidative processes darken it to form **stercobilin (urobilin, Fig.6.3.)**.

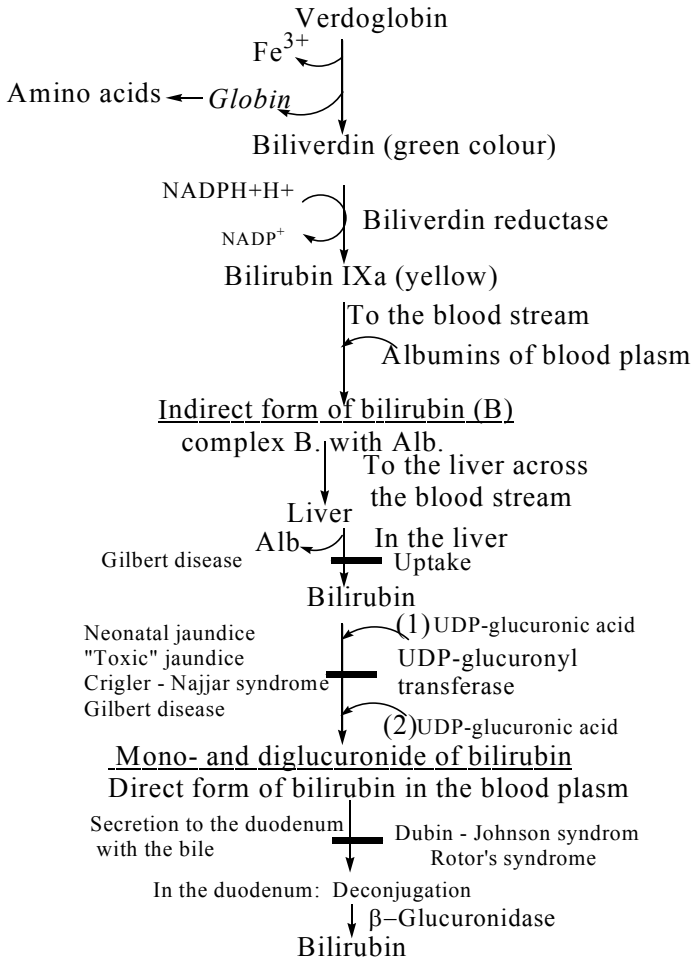


Figure 6.2. The formation of indirect and direct forms of bilirubin

The increased allocation of urobilinogen bodies (urobilin bodies) with urine is called urobilinuria.

Urobilinuria (with determination of urobilinogen) more often occur at hepatocellular diseases of a liver (hepatitis, cirrhosis, poisoning by some toxic compounds, etc.), the cardiovascular pathology accompanying with stagnant damage of the liver. Urobilinogen reabsorbed from intestines and via portal vein does not undergo usual

transformations for it because of functional failure of liver and it is takes out with urine.

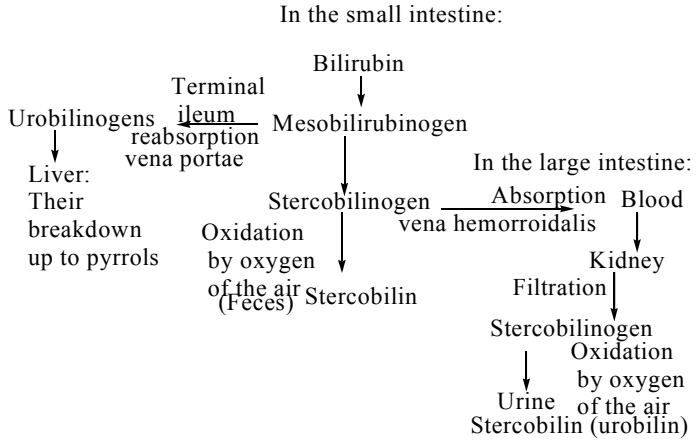


Figure 6.3. The transformation of bilirubin diglucuronides in the intestinal tract

Determination of bilirubins in the blood serum

The determination of total bilirubin and its fractions has a big clinical significance. Blood serum of healthy people contains: total bilirubin 3.5-20.5 μmole/L; non-conjugated bilirubin - < 12 μmole/L; conjugated bilirubin - < 7μmole/L.

It is noticed, that jaundice appears when the total bilirubin level in blood exceeds 27-34 mmole/L (>10 mg/L). An elevated bilirubin level is known as *hyperbilirubinemia.* When this is present, bilirubin diffuses from the blood into peripheral tissue and gives a yellow color (jaundice). The easiest way of observing this is in the white conjunctiva of the eyes. Jaundice can have various causes.

Major causes of juandice

I. Pre-hepatic

Hemolytic jaundice

- is accompanied with increased hemolysis of erythrocytes (incompatible blood transfusion, malaria, sickle-cell anemia and other causes see above);
- results in increased production of bilirubin;
- here more bilirubin is conjugated and excreted than normally, but the conjugation mechanism is overwhelmed, and an abnormally large amount of unconjugated bilirubin is found in the blood.

II. Hepatic

Neonatal jaundice

- results in a temporary condition due to production of insufficient levels of UDP-glucuronyl transferase by the infant;
- currently, the irradiation of jaundiced infants during neonatal life by fluorescent lights is the most common treatment of neonatal hyperbilirubinemia. The products from the irradiation of bilirubin are more soluble than bilirubin and can be excreted by the liver into the bile without conjugation with glucuronic acid.

Gilbert's disease

- may be caused by an inability of the hepatocytes to take up bilirubin from the blood or reduced activity of UDP-glucuronyltransferase;
- as a result, unconjugated bilirubin accumulates.

Neonatal "Physiological jaundice"

- are conditions in which conjugation is impaired due to a deficiency in UDP-glucuronyl transferase or there probably is reduced synthesis of the substrate for that enzyme, UDP-glucuronic acid. UDP-glucoronate is formed under NAD-dependent UDP-glucosyl dehydrogenase;
- *Neonatal jaundice* (physiologic jaundice) usually resolves after a few days by itself;
- in severe cases, however, unconjugated bilirubin can cross the blood–brain barrier and lead to brain damage (*kernicterus*).

Crigler-Najjar syndrome

- Crigler-Najjar syndrome, type I: deficiency in UDP-glucuronyl transferase; the desease is fatal within the first 2 years of life; children have been treated with phototherapy; phenobarbital has no effect;
- Crigler-Najjar syndrome, type II: some activity of the UDP-glucuronyl transferase is retained; addition of second glucuronyl group is defective; treatment with Phenobarbital;
- unconjugated bilirubin is retained by the body.

Dubin-Johnson syndrome

- is associated with inability of the hepatocytes to secrete conjugated bilirubin after it has been formed;
- conjugated bilirubin returns to the blood.

III. Post hepatic

Biliary obstruction

- obstruction in the bile duct (gall stones, tumors);
- blood levels of conjugated bilirubin increase.

Common notions about nucleoprotein metabolism in the human organism

DNA is the chemical basis of heredity. Genes are the fundamental units of genetic information. Knowledge of the structure and function of nucleic acids is essential in understanding genetics and many aspects of pathophysiology as well as the genetic basis of disease.

A typical human cell contains 46 chromosomes, whose total DNA is aporoximately one meter long. The double-stranded DNA helix in each chromosome has a length that is thousands of times the diameter of the cell nucleus. Such a large amount of genetic material can be effectively packaged into a volume the size of a cell nucleus due to interaction of DNA with a large number of proteins termed histones as well as a smaller amount of nonhistone proteins (most of which are acidic and larger than histones) and a small quantity of RNA**.** The nonhistone proteins include enzymes involved in DNA replication, such as DNA topoisomerases. Also included are proteins involved in transcription, such as the RNA polymerase complex. One purpose of the molecules that comprise chromatin, particularly the histones, is to condense the DNA. Electron microscopic studies of chromatin have demonstrated dense spherical particles called nucleosomes**,** which are approximately 10 nm in diameter and connected by DNA filaments.

Histones are the most abundant chromatin proteins. Nucleosomes contain four classes of histones: H2A, H2B, H3, and H4. These proteins are positively charged at physiologic pH as a result of their high content of lysine and arginine. Because of their positive charge, they form ionic bonds with negatively charged DNA. H1 histones are the ones least tightly bound to chromatin and are, therefore, easily removed with a salt solution, after which chromatin becomes soluble.

The features of nucleotide digestion in GIT and nucleotide functions in humans

Nucleotides serve numerous functions in different reaction pathways. For example, nucleotides are the activated precursors of DNA and RNA. Nucleotides form the structural moieties of many coenzymes (examples include reduced nicotinanide adenine dinucleotide [NADH], flavin adenine dinucleotide [FAD], and coenzyme A [CoA]). Nucleotides are critical elements in energy metabolism (adenosine triphosphate [ATP], guanosine triphosphate [GTP]), and nucleotide derivatives are frequently activated intermediates in many biosynthetic pathways. In addition, nucleotides act as secondary messengers in intracellular signaling cascades (e.g., cyclic adenosine monophosphate [cAMP], cyclic guanosine monophosphate [cGMP]).

Finally, nucleotides act as metabolic allosteric regulators. Think about all of the enzymes that are regulated by levels of ATP, adenosine diphosphate (ADP), and AMP.

Dietary uptake of purine and pyrimidine bases is minimal. The diet contains polymeric nucleic acids and the exocrine pancreas secretes deoxyribonuclease and ribonuclease, along with the proteolytic and lipolytic enzymes. This enables digested nucleic acids to be converted to nucleotides. The intestinal epithelial cells contain alkaline phosphatase activity, which will convert nucleotides to nucleosides. Other enzymes within the epithelial cells tend to metabolize the nucleosides to uric acid or to salvage them for their own needs. Approximately 5% of ingested nucleotides will make it into the circulation, either as the free base or as a nucleoside. Because of the minimal dietary uptake of these important molecules, de novo synthesis of purines and pyrimidines is required.

Purine metabolism - key points

Synthesis:

- Purine nucleotides usually are synthesized de novo or salvaged from existing bases.
- De novo purine synthesis is complex, requiring 11 steps and 6 molecules of adenosine triphosphate for every purine synthesized. Purines are initially synthesized in the ribonucleotide form.
- The precursors for de novo purine synthesis are glycine, ribose 5-phosphate, glutamine, aspartate, carbon dioxide, and N^{10}-formyltetrahydrofolate, N^5, N^{10}-methenyltetrahydrofolate.
- The initial purine ribonucleotide synthesized is inosine monophosphate. Adenosine monophosphate and guanosine monophosphate are each derived from inosine monophosphate.
- Since de novo purine synthesis requires a large amount of energy, purine nucleotide salvage pathways exist such that free purine bases can be converted to nucleotides.
- Mutations in purine salvage enzymes are associated with severe diseases, such as Lesch-Nyhan syndrome and severe combined immunodeficiency disease.
- Deoxyribonucleotides are derived by reduction of ribonucleotides, as catalyzed by ribonucleotide reductase. The regulation of ribonucleotide reductase is complex.
- Degradation of purine containing nucleotides results in uric acid production, which is eliminated in the urine. Elevated uric acid levels in the blood lead to gout. The elevation of uric acid in the blood is caused mainly due to overproduction of purine nucleotides.

The salvage pathways

Most of the de novo synthesis of the bases of nucleotides occurs in the liver, and to some extent it also takes place in the brain, neutrophils, and other cells of the immune system. Within the liver, nucleotides can be converted to nucleosides or free bases, which may be transported to other tissues via the red blood cell in the circulation. In addition, the small amounts of dietary bases or nucleosides that are absorbed also enter cells in this form. Because the de novo pathway requires six high-energy bonds per purine produced, a salvage pathway, which is used by many cell types, can convert free bases and nucleosides to nucleotides (fig.6.4.).Thus, most cells can salvage these bases to generate nucleotides for RNA and DNA synthesis. For certain cell types, such as the lymphocytes, the salvage of bases is the major form of nucleotide generation.

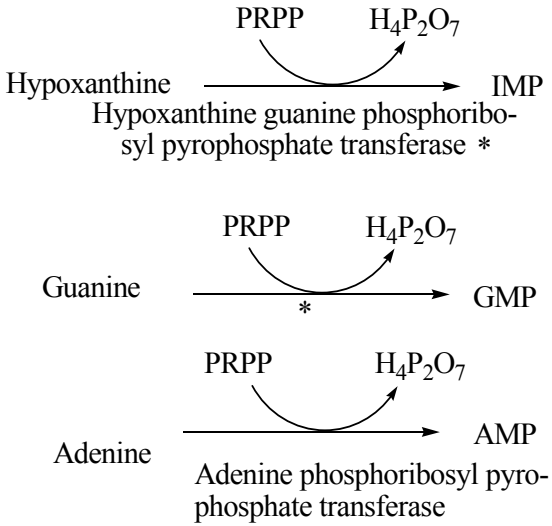


Figure 6.4. Salvage reactions

The salvage pathways allow free bases, nucleosides, and nucleotides to be easily interconverted. The major enzymes required are purine **phosphoribosyl transferases, nucleoside phosphorylase**, and **deaminases**.

The phosphoribosyl transferase enzymes catalyze the addition of a ribose 5-phosphate group from PRPP to a free base, generating a nucleotide and pyrophosphate. Two enzymes do this: adenine phosphoribosyl transferase and hypoxanthine-guanine phosphoribosyl transferase (HGPRT). The reactions they catalyze are the same, differing only in their substrate specificity.

Nucleotides are converted to nucleosides by nucleotidase. Free bases are generated from nucleosides by purine nucleoside phosphorylase. Purine nucleoside phosphorylase catalyzes a phosphorolysis reaction of the N-glycosidic bond that attaches the base to the sugar moiety in the nucleosides guanosine and inosine. Thus, guanosine and inosine are converted to guanine and hypoxanthine, respectively, along with ribose 1-phosphate. The ribose 1-phosphate can be isomerized to ribose 5-phosphate and the free bases then salvaged or degraded, depending on cellular needs.

Adenosine and AMP can be deaminated by adenosine deaminase and AMP deaminase, respectively, to form inosine and IMP. Of the purines, only adenosine can be directly phosphorylated back to a nucleotide, by adenosine kinase. Guanosine and inosine must be converted to free bases by purine nucleoside phosphorylase before they can be converted to nucleotides by HGPRT.

A portion of the salvage pathway that is important in muscle tissue is the purine nucleotide cycle. The net effect of these reactions is the deamination of aspartate to fumarate (as AMP is synthesized from IMP and then deaminated back to IMP by AMP deaminase). When the muscle must generate energy, the fumarate derived from the purine nucleotide cycle is used anaplerotically to replenish tricarboxylic acid (TCA) cycle intermediates and to allow the cycle to operate at a high speed. Deficiencies in enzymes of this cycle lead to muscle fatigue during exercise.

Pyrimidine metabolism - key points

Synthesis:

- Pyrimidine nucleotides just as purine ones can be synthesized de novo or salvaged from existing bases.
- The pyrimidine ring, unlike the purine ring, is not built on a molecule of PRPP. Instead, the pyrimidine ring is formed, and then it reacts with PRPP to form the nucleotide.
- Aspartate and cytoplasmic carbamoyl phosphate are the precursors for pyrimidine ring synthesis. Carbamoyl phosphate formation is catalyzed by carbamoyl phosphate synthase II (CPS II). In contrast to mitochondrial CPC I cytoplasmic CPS II uses as substrate glutamine (it is ammonia for CPS I) and is activated by ATP (N-acetyl-glutamate is activator for CPS I). CPS II is inhibited by UTP.
- The initial pyrimidine nucleotide synthesized is orotate monophosphate, which is converted to uridine monophosphate. The other pyrimidine nucleotides will be derived from a uracil-containing intermediate.

Regulation of nucleotide synthesis

AMP, ADP GMP, GDP, TDP are considered as allosteric inhibitors for PRPP-synthetase when they are accumulated in cytoplasm (figure 6.5.).

High concentration of GMP is allosteric inhibitor for Phosphoribosyl aminotransferase in the purine nucleotide synthesis.

GTP accumulation causes the stimulation of AMP synthesis, ATP accumulation causes the stimulation of GMP synthesis (the regulation of second stage of synthesis, only).

The accumulation of PRPP is a positive factor in stimulation of Carbamoyl phosphate synthesis and then the synthesis of UMP. But the accumulation of UDP in a cell is considered as a factor for inhibition of discussed reaction. The accumulation of CTP is the factor for inhibition of Carbamoyl aspartate formation during the synthesis of UMP.

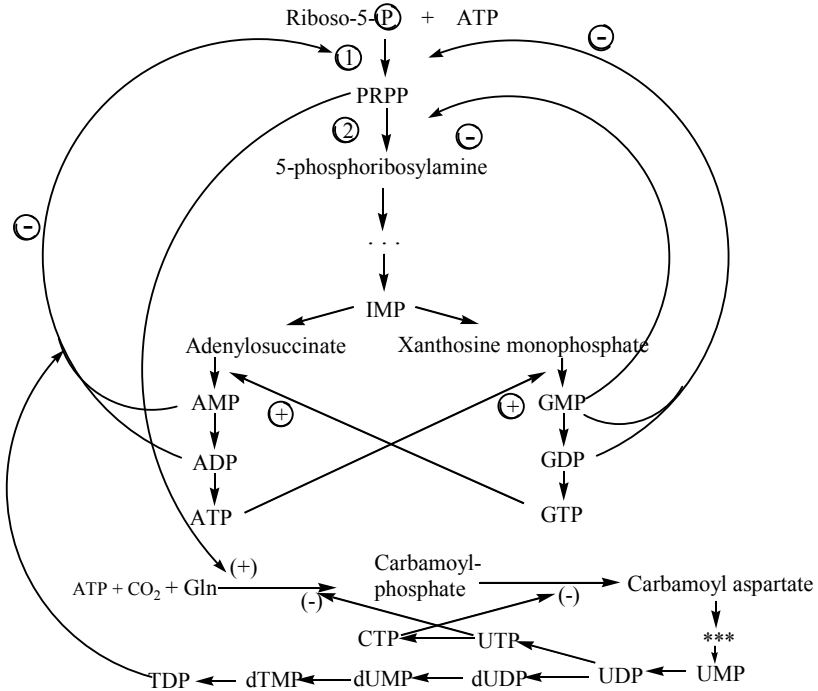


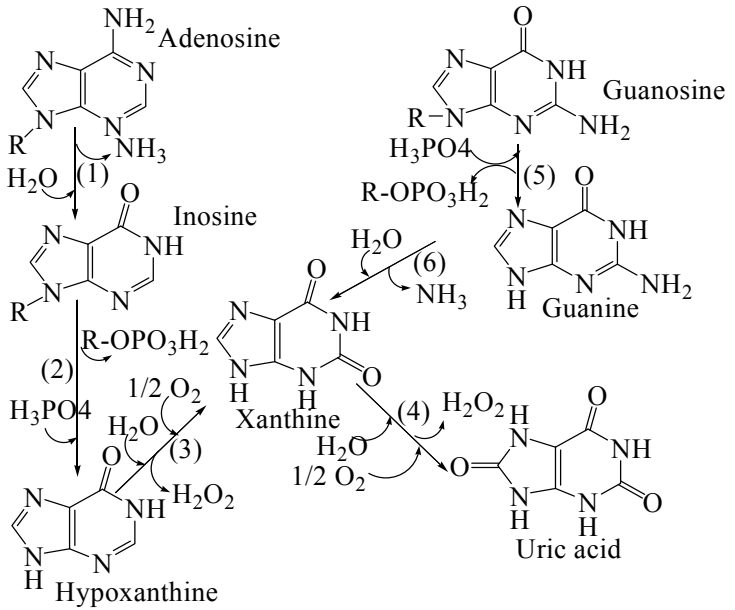
Figure 6.5. The regulation of nucleotide synthesis

Degradation of purine nucleotides

The degradation of the purine nucleotides (AMP and GMP) occurs mainly in the liver. Salvage enzymes are used for most of these reactions.

AMP is first deaminated to produce IMP (AMP deaminase). Then IMP and GMP are dephosphorylated and the ribose is cleaved from the base by purine nucleoside phosphorylase. Hypoxanthine, the base produced by cleavage of IMP, is converted by xanthine oxidase to xanthine, and guanine is deaminated by the enzyme guanase to produce xanthine. The pathways for the degradation of adenine and guanine merge at this point. Xanthine is converted by xanthine oxidase to uric acid, which is excreted in the urine. Xanthine oxidase is a molybdenum-requiring enzyme that uses molecular oxygen and produces hydrogen peroxide.

Uric acid has a pK of 5.4. It is ionized in the body to form urate. Urate is not very soluble in an aqueous environment. The quantity of urate in normal human blood is very close to the solubility constant.



R - the fragment of ribose

Deseases associated with purine metabolism

Gout. It is a disorder characterized by high levels of uric acid in the blood, as a result of either **over-production** or **underexcretion** of uric acid. Hyperuricemia results in the deposition of crystals of sodium urate in tissues, especially the kidney and joints, causing first acute and progressing to chronic gouty arthritis. Hyperuricemia does not always lead to gout, but gout is usually preceded by hyperuricemia. The deposition of needle-shaped monosodium urate crystals initiates an

inflammatory process involving the infiltration of granulocytes that phagocytize the urate crystals. This process generates oxygen metabolites that damage tissue, resulting in the release of lysosomal enzymes that evoke an inflammatory response. In addition, lactate production in the synovial tissues increases, resulting in decrease in pH that fosters further deposition of urate crystals.

Hyperuricemia may be secondary to other disease such as cancer, psoriasis, chronic renal deficiency.

Factors, which can cause the gout in patients with hyperuricemia:

1. Overcooling of human organism. The solubility of sodium urate is lower under low temperature. The rate of urate accumulation in joints is higher in this case.
2. The sharp change of diet in patient with hyperuricemia. If you are patient with hyperuricemia and have the diet with animal food products you cannot become strong vegetarian before consultation with doctor.

Inherited disorders of purine metabolism.

The following are the important metabolic defects (enzymes) associated with primary gout:

1. PRPP synthetase can have abnormal features:

1. Superactive (increased V max) → purine overproduction → gout
2. Resistance to feedback inhibition → purine overproduction → gout
3. Low Km for ribose-5-P→ purine overproduction → gout

2. Hypoxanthine guanine phosphoribosylpyrophosphate transferase (HGPRT):

1. Partial deficiency → purine overproduction → gout
2. Complete deficiency. Lesch-Nyhan syndrome: several forms of HGPRT deficiency have been identified:
   a) in one form patients have normal levels of this enzyme, but the enzyme is inactive;
   b) the patients have en enzyme that is apparently unstable; its activity is higher in young red cells than in old.

The symptoms: hyperuricemia, gout, urinary tract stones, and neurological symptoms of mental retardation, self-mutilation, and then death in young age. The basis of neurological symptoms is unknown. However, brain cells normally have much higher levels of purine salvage enzymes than other cells and may normally use salvage pathways to a greater extent.

Treatment by allopurinol reduces the uric acid formation but does not alleviate the neurological symptoms.

3. Adenosine deaminase (ADA) is expressed in the cytosol of all cells, but, in humans, lymphocytes have the highest activity of this anzyme. A deficiency of ADA results in an accumulation of adenosine, which is converted to its ribonucleotide or deoxyribonucleotide forms by

cellular kinases. As dATP levels rise, ribonucleotide reductase is inhibited, thus preventing the production of all deoxyribose nucleotides. Consequently, cells cannot make DNA and divide. In its most severe form this disorder causes severe combined immunodeficiency disease (SCID)**.** In the severe form of combined immunodeficiency, both T cells (which provide cell-based immunity) and B cells (which produce antibodies) are deficient, leaving the individual without a functional immune system. Children born with this disorder lack a thymus gland and are subject to many opportunistic infections because of the lack of a functional immune system. Death results if the child is not placed in a sterile environment. Administration of polyethylene glycol modified adenosine deaminase has been successful in treating the disorder, and the ADA gene was the first to be used in gene therapy in treating the disorder.

4. P**urine nucleoside phosphorylase.** The deficiency of this enzyme is associated with impairment of T-cell function but has no effect on B-cell function. Uric acid synthesis is decreased and the tissue levels of purine nucleosides and nucleotides are higher. It is believed that dGTP inhibits the development of normal T-cell. Children lacking this activity have recurrent infections, and more than half display neurological complications. Symptoms first appear at 6 months to 4 years of age.

5. Xanthine oxidase: Complete deficiency → hypouricemia is observed at genetic defect of xanthine oxidase that is accompanied by accumulation of xanthines in blood of the patients and their excretion with urine in big concentration (xanthinuria).

6. Glucose-6-phosphatase deficiency - von Gierke's disease. Purine overproduction and hyperuricemia in von Gierke's disease occurs secondarily to enhanced generation of the PRPP precursor ribose-5-phosphate. In addition, associated lactic acidosis (von Gierke's disease is associated with increased activity of glycolysis) elevates the renal threshold for urate, elevating total body urates.

7. **Glutathione reductase**: Increased glutathione reductase generates more NADP+ which is utilized by HMP shunt. This causes increased ribose 5-phosphate and PRPP synthesis, causing hyperuricemia.

8. Aldolase B - Hereditary fructose intolerance (HFI). A severe disturbance of liver and kidney metabolism is a result of **aldolase B** deficiency, which causes fructose 1-phosphate accumulation in cells. Because of it ATP and inorganic phosphate levels fall significantly, with adenine being converted to uric acid, causing hyperuricemia.

Treatment of gout

Normally, as cells die, their purine nucleotides are degraded to hypoxanthine and xanthine, which are converted to uric acid by xanthine oxidase. Allopurinol (a drug, structural analogue of hypoxanthine) is a competitive substrate for xanthine oxidase. It is converted to oxypurinol (also called alloxanthine), which remains tightly bound to the enzyme at the active site, preventing further catalytic activity. Thus, allopurinol is a suicide inhibitor. It reduces the production of uric acid and hence its concentration in the blood and tissues. Xanthine and hypoxanthine accumulate, and urate levels decrease. Overall, the amount of purine being degraded is spread over three products rather than appearing in only one. Therefore, none of the compounds exceeds its solubility constant, precipitation does not occur, and the symptoms of gout gradually subside.

Colchicine is an anti-inflammatory drug that is used only to treat gout. It inhibits leukocyte movement by affecting microtubules. Other anti-inflammatory drug such as aspirin to provide pain relief.

Degradation of pyrimidine nucleotides

The pyrimidine nucleotides are dephosphorylated and the nucleosides are cleaved to produce ribose 1-phosphate and the free pyrimidine bases cytosine, uracil, and thymine. Cytosine is deaminated, forming uracil, which is converted to CO_2, ammonium ion (NH_4^+), and β-alanine. Thymine is converted to CO_2, NH_4^+, and β-aminoisobutyrate. These products of pyrimidine degradation are excreted in the urine or converted to CO_2, H_2O, and NH_4^+ (which forms urea). They do not cause any problems for the body, in contrast to urate, which is produced from the purines and can precipitate, causing gout.

Inherited disorders of pyrimidine metabolism

In hereditary orotic aciduria, orotic acid is excreted in the urine because the enzyme that converts it to uridine monophosphate, **orotate phosphoribosyltransferase** and **orotidine-phosphate decarboxylase** (both enzyme activities are present on a single protein as domains – bifunctional enzyme), is defective. Pyrimidines cannot be synthesized, and therefore, normal growth does not occur. Oral administration of uridine is used to treat this condition. Uridine, which is converted to UMP, bypasses the metabolic block and provides the body with a source of pyrimidines, as both CTP and dTMP can be produced from UMP.

When **ornithine transcarbamoylase** is deficient (urea cycle disorder), excess carbamoyl phosphate from the mitochondria leaks into the cytoplasm. The elevated levels of cytoplasmic carbamoyl phosphate lead to pyrimidine production, as the regulated step of the pathway, the

reaction catalyzed by carbamoyl synthetase II, is being bypassed. Thus, orotic aciduria results.

Once nucleotide biosynthesis and salvage were understood at the pathway level, it was quickly realized that one way to inhibit cell proliferation would be to block purine or pyrimidine synthesis. Thus, drugs were developed that would interfere with a cell ability to generate precursors for DNA synthesis, thereby inhibiting cell growth. This is particularly important for cancer cells, which have lost their normal growth regulatory properties. Development of these drugs would not have been possible without an understanding of the biochemistry of purine and pyrimidine salvage and synthesis. Such drugs also affect rapidly dividing normal cells (such as the intestinal epithelia), which brings about a number of the side effects of chemotherapeutic regimens.

Tests recommended to answer after study of chapter 6:

1. Protoporphyrin IX is:
   A. Intermediate in heme synthesis
   B. Intermediate in heme degradation
   C. Intermediate in purines synthesis
   D. Intermediate in pyrimidine degradation
   E. Intermediate in purines degradation

2. A patient is with blockage of hepatic biliary network. What diagnostics results wil be for him?
   A. Increased "Indirect" bilirubin in patient's blood
   B. Increased "Direct" bilirubin in patient's blood
   C. Increased stercobilinogen in patient's feces
   D. Increased stercobilinogen in patient's blood
   E. Increased mesobilirubinogen in patient's blood

3. A rise in serum "direct" bilirubin would be expected in:
   A. Hemolytic jaundice
   B. Insufficient levels of glucuronyl transferase as in the newborn
   C. Decreased hepatic uptake of bilirubin (Gilbert's disease).
   D. Biliary obstruction
   E. Insufficient levels of NAD-dependent UDP-glucosyl dehydrogenase

4. A newborn develops jaundice (yellow skin and yellow scleras) that requires laboratory evaluation. Which of the following porphyrin derivatives is conjugation product, reacts directly with diazoreagent, and is a major component of bile?
   A. Bilirubin diglucuronide
   B. Stercobilin
   C. Bilirubin
   D. Urobilinogen
   E. Heme

5. A 4-year-old girl presents in the clinic with megaloblastic anemia and failure to thrive. Blood investigations reveal orotic aciduria. Enzyme measurements of white blood cells reveal a deficiency of the pyrimidine biosynthesis enzyme orotate phosphoribosyltransferase and abnormally high activity of the enzyme aspartate transcarbamoylase. Which one of the following treatments will reverse all symptoms if carried out chronically?
   A. Blood transfusion
   B. White blood cell transfusion
   C. Dietary supplements of phosphoribosylpyrophosphate (PRPP)
   D. Oral thymidine
   E. Oral uridine

6. Which of the following would rule out hyperuricemia in a patient?
   A. Lesch-Nyhan syndrome
   B. Gout
   C. Xanthine oxidase hyperactivity
   D. Carbamoyl phosphate synthase deficiency
   E. Purine overproduction secondary to Von Gierke's disease

7. Which one of the following contributes nitrogen atoms to both purine and pyrimidine rings during their synthesis?
   A. Aspartate
   B. Carbamoyl phosphate
   C. Carbon dioxide
   D. Glutamate
   E. Tetrahydrofolate

8. Which statement is the best to describe compound xanthine?
   A. It is a direct precursor of guanine
   B. It covalently binds to allopurinol
   C. It is a substrate rather than a product of the enzyme xanthine oxidase
   D. It is oxidized to form uric acid
   E. It is oxidized to form hypoxanthine

9. Which of the following compounds is an analogue of hypoxanthine?
   A. Methyl thymidine
   B. Allopurinol
   C. Ribose phosphate
   D. 5-phosphoribosylpyrophosphate (PRPP)
   E. 5-fluorouracil

10. Which of the following compounds is not a haemoprotein?
   A. Cytochrome p450
   B. Tryptophan oxygenase
   C. Catalase
   D. Peroxidase
   E. PRPP synthetase

Chapter 7. Hormonal regulation of carbohydrate, lipid and protein metabolism

Key points for consideration of carbohydrate and lipid metabolism regulation

- Three key controlling elements determine whether a fuel is metabolized or stored: hormones, concentration of available fuels, and energy needs of the body.
- Key intracellular enzymes are generally regulated by allosteric activation and inhibition, by covalent modification, by transcriptional control, and by degradation.
- Regulation is complex in order to allow sensitivity and feed-back to multiple stimuli so that an exact balance can be maintained between synthesis of a product and need for the product.
- The insulin/glucagon ratio is responsible for the hormonal regulation **of carbohydrate and lipid metabolism.**

The balance and integration of the metabolism of carbohydrates and lipids are mediated by the hormones **insulin, glucagon, epinephrine, norepinephrine, and glucocorticoids** mainly. The primary control hormones of metabolism are insulin and glucagon.

Glucagon stimulates gluconeogenesis and blocks glycolysis. When blood sugar levels get low, the α-cells of the pancreas release glucagon. The main targets of glucagon are the liver and adipose tissue. In the liver, glucagon stimulates the cyclic AMP–mediated cascade that causes phosphorylation of glycogen phosphorylase and glycogen degradation. This effectively turns off glycogen synthase and turns on glycogen phosphorylase, thereby causing a breakdown of glycogen and a production of glucose in liver, which ultimately raises blood glucose levels.

Insulin and glucagon are two antagonistic hormones that maintain the balance of sugar and fatty acids in blood. Insulin is produced by the β-cells of the pancreas and its release is stimulated by high levels of glucose in the blood. It has a number of effects, but its major effect is to allow the entry of glucose into cells. Insulin also allows the dephosphorylation of key regulatory enzymes. The consequence of these actions is to allow glycogen synthesis and storage both in muscle and liver, suppression of gluconeogenesis, acceleration of glycolysis, promotion of the synthesis of fatty acids, and promotion of the uptake and synthesis of amino acids into protein. All in all, insulin acts to promote anabolism. Blood levels of glucose, amino acids, fatty acids, and ketone bodies are maintained by variations in the **[insulin]/[glucagon] ratio**. When blood sugar is high, the ratio increases and insulin signals the fed state, promoting anabolic activities.

The ratio decreases as glucagon is released to direct catabolic activities when blood glucose falls between meals, during fasting, and during starvation. Under normal conditions, the very precise interplay between insulin and glucagon maintains homeostatic blood fuel levels at about: glucose - 4.5 mM/L; fatty acids - 0.5 mM/L; amino acids - 4.5 mM/L; ketone bodies - 0.02 mM/L. Blood levels of ketone bodies and fatty acids rise during fasting or during starvation with blood glucose levels being maintained. However, during uncontrolled juvenile diabetes, blood glucose levels rise greatly. The lack of insulin in this disease otherwise mimics starvation.

Epinephrine has effects similar to those of glucagon, except that glucagon has a greater effect on the liver while epinephrine has a greater effect on muscle. Epinephrine or norepinephrine is released during exercise to promote catabolism of glucose and fat that supports muscular activity.

The rest hormones regulating carbohydrate and lipid metabolism are glucocorticoids, thyroid hormones, somatotropin, ACTH, β-lipotropin.

I. Regulation of carbohydrate and lipid metabolism in the fed state

A. Mechanisms that affect glycogen and triacylglycerol synthesis in the liver

After ingestion of energy sources, the liver synthesizes glycogen and triacylglycerols. The level of glycogen stored in the liver can increase from approximately 80 g after an overnight fast to a limit of approximately 200 to 300 g. Although the liver synthesizes triacylglycerols, it does not store this fuel but rather packages it in very low density lipoprotein (VLDL) and secretes it into the blood. The fatty acids of the VLDL triacylglycerols secreted from the liver are stored as adipose triacylglycerols. Adipose tissue has an almost infinite capacity to store fat, limited mainly by the ability of the heart to pump blood through the capillaries of the expanding adipose mass. Although we store fat throughout our bodies, it tends to accumulate in places where it does not interfere too much with our mobility: in the abdomen, hips, thighs, and buttocks.

Both the synthesis of liver glycogen and the conversion by the liver of dietary glucose to triacylglycerol (lipogenesis) are regulated by mechanisms involving key enzymes in these pathways.

Glucokinase

After a meal, glucose can be converted to glycogen or to triacylglycerol in the liver. For both processes, glucose is first converted to glucose 6-phosphate by glucokinase. **Synthesis of glucokinase is**

induced by insulin, which is elevated after a meal, **and repressed by glucagon** which is elevated during fasting. In keeping with the liver function in maintaining blood glucose levels, this system has been established such that the liver can metabolize glucose only when sugar levels are high, not when sugar levels are low.

Phosphofructokinase-1 and pyruvate kinase

For lipogenesis, glucose 6-phosphate is converted through glycolysis to glycerol-3-phosphate, and later to pyruvic acid. Key enzymes that regulate this pathway in the liver are phosphofructokinase 1 and 2 (PFK-1 and PFK-2), and pyruvate kinase. PFK-1 is allosterically activated in the fed state by fructose 2,6-bisphosphate (F-2,6-BP) and adenosine monophosphate (AMP). PFK-2, the enzyme that produces the activator fructose 2,6-bisphosphate, is dephosphorylated and active after the meal. The levels of fructose 2,6-bisphosphate are critical in regulating glycolysis versus gluconeogenesis in the liver. Under conditions of high blood glucose and insulin release, fructose 2,6-bisphosphate levels are high because PFK-2 is in its activated state. The fructose 2,6-bisphosphate activates PFK-1 and inhibits fructose 2,6-bisphosphatase, thereby allowing glycolysis to proceed. When blood glucose levels are low and glucagon is released, PFK-2 is phosphorylated by the cAMP-dependent protein kinase and is inhibited, which lowers fructose 2,6-bisphosphate levels and inhibits glycolysis while favoring gluconeogenesis.

Pyruvate kinase is also activated by dephosphorylation, which **is stimulated by the increase of the insulin/glucagon ratio** in the fed state.

Pyruvate dehydrogenase and pyruvate carboxylase

The synthesis of fatty acids requires a source of acetyl coenzyme A (CoA) in the cytosol. Pyruvate enters mitochondria and can be converted to acetyl CoA through the pyruvate dehydrogenase reaction. This multienzyme complex is dephosphorylated and most active when its supply of substrates and adenosine diphosphate (ADP) is high, its products are used, and **insulin** is present.

Pyruvate is also converted to oxaloacetate. The enzyme that catalyzes this reaction, **pyruvate carboxylase**, is activated at the accumulation of acetyl CoA in the matrix of mitochondria, and its synthesis is stimulated by **glucocorticoids**. Because acetyl CoA cannot directly cross the mitochondrial membrane to form fatty acids in the cytosol, it condenses with oxaloacetate, producing citrate. The citrate that is not required for tricarboxylic acid (TCA) cycle activity crosses the membrane and enters the cytosol due to special transport system.

Glycogen synthase

In the way of glucose 6-phosphate conversion to glycogen, the key regulatory enzyme is glycogen synthase. This enzyme is activated

by dephosphorylation, which occurs when insulin is elevated and glucagon is decreased and by the increased level of glucose

Citrate lyase, malic enzyme, and glucose 6-phosphate dehydrogenase

In the cytosol, citrate is cleaved by citrate lyase, an inducible enzyme, to form oxaloacetate and acetyl CoA. The acetyl CoA is used for fatty acid biosynthesis and for cholesterol synthesis, pathways that are activated by insulin. Oxaloacetate is recycled to pyruvate via cytosolic malate dehydrogenase and malic enzyme, which is inducible. Malic enzyme generates reduced nicotinamide adenine dinucleotide phosphate (NADPH) for the reactions of the fatty acid synthase complex. NADPH is also produced by the two enzymes of the pentose phosphate pathway, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. Glucose 6-phosphate dehydrogenase is also induced by insulin.

Acetyl CoA carboxylase

Acetyl CoA is converted to malonyl CoA, which provides the two-carbon units for elongation of the growing fatty acyl chain on the palmitate synthase complex.

Acetyl CoA carboxylase, the enzyme that catalyzes the conversion of acetyl CoA to malonyl CoA, is controlled by three of the major mechanisms that regulate enzyme activity:

1) It is activated by citrate, which causes the enzyme to polymerize, and inhibited by long-chain fatty acyl CoA;
2) A special phosphatase stimulated by insulin activates the enzyme by dephosphorylation;
3) The AMP-activated kinase phosphorylates and inactivates acetyl-CoA carboxylase (that phosphorylates and inactivates HMG-CoA reductase also). It has been suggested that, when cellular ATP levels are depleted causing AMP levels to increase, the resultant activation of the kinase would inhibit cholesterol and fatty acid biosynthetic pathways, thus conserving energy;
4) It is regulated through the induction: the quantity of the enzyme is increased in the fed state.

Malonyl CoA, the product of the acetyl CoA carboxylase reaction, provides the carbons for the synthesis of palmitate on the fatty acid synthase complex. Malonyl CoA also inhibits carnitine palmitoyltransferase I (also known as carnitine acyltransferase I), the enzyme that prepares long-chain fatty acyl CoA for transport into mitochondria. In the fed state, when acetyl CoA carboxylase is active and malonyl CoA levels are elevated, newly synthesized fatty acids are converted to triacylglycerols for storage rather than being transported into mitochondria for oxidation and ketone body formation.

Fatty acid synthase complex

In a well-fed individual, the quantity of the fatty acid synthase complex is increased. The genes that produce this enzyme complex are induced by **the increase in the insulin/glucagon ratio**. The amount of the complex increases slowly after a few days of a high-carbohydrate diet.

Glucose 6-phosphate dehydrogenase, which generates NADPH in the pentose phosphate pathway, **and malic enzyme**, which produces NADPH, **are also induced by the increase of insulin.**

The palmitate produced by the synthase complex is converted to palmityl CoA and elongated and desaturated to form other fatty acyl-CoA molecules, which are converted to triacylglycerols. These triacylglycerols are packaged and secreted into the blood as VLDL.

B. Mechanisms that affect triacylglycerol storage in adipose tissue

Insulin stimulates adipose cells to synthesize and secrete lipoprotein lipase (LPL), which will be linked later to endothelium of blood vessels, and will hydrolyze the chylomicrons and VLDL triacylglycerols. Fatty acids released from chylomicrons and VLDL by LPL are stored as triacylglycerols in adipose cells. The glycerol released by LPL is not used by adipose cells because they lack glycerol kinase. Glycerol can be used by liver cells.

Insulin causes the number of glucose transporters in adipose cell membranes to increase. Glucose enters these cells and is oxidized, giving energy and providing the glycerol-3-phosphate moiety for triacylglycerol synthesis via the dihydroxyacetone phosphate intermediate of glycolysis.

II. Regulation of carbohydrate and lipid metabolism during fasting

A. Mechanisms in the liver that serve to maintain blood glucose levels

During fasting, the insulin/glucagon ratio decreases. Liver glycogen is degraded to produce blood glucose because enzymes of glycogen degradation are activated by cyclic AMP (cAMP) directed phosphorylation. **Glucagon stimulates adenylate cyclase to produce cAMP**, which activates protein kinase A. Protein kinase A phosphorylates phosphorylase kinase, which then phosphorylates and activates glycogen phosphorylase. Protein kinase A also phosphorylates but in this case inactivates glycogen synthase.

Gluconeogenesis is stimulated because the synthesis of phosphoenolpyruvate carboxykinase, fructose 1,6-bisphosphatase,

and glucose 6-phosphatase is induced by glucagon and glucocorticoids. Induction is due to phosphorylation and activation of a particular set of transcription factors. These hormones also increase the availability of substrates for gluconeogenesis. Lactate is derived from glycolysis in muscle; alanine is the primary substrate derived from proteolysis; glycerol is derived from lipolysis.

Fructose 1,6-bisphosphatase is also activated because the levels of its inhibitor, fructose 2,6-bisphosphate, are low. The corresponding enzymes of glycolysis are not very active during fasting. The rate of glucokinase is low because it has a high K_m for glucose, and the glucose concentration is low. PFK-1 is not very active because the concentration of its activator fructose 2,6-bisphosphate is low. Pyruvate kinase is inactivated by cAMP-mediated phosphorylation.

Table 7.1. Flowchart of changes in liver metabolism

When blood sugar increases	**When blood sugar decreases**
Insulin is released, which leads to the dephosphorylation of:	**Glucagon is released, which leads to the phosphorylation of:**
PFK-2 (now active (kinase activity)	PFK-2 (now inactive (kinase activity)
Pyruvate kinase (now active)	Pyruvate kinase (now inactive)
Glycogen synthase (now active)	Glycogen synthase (now inactive)
Phosphorylase kinase (now inactive)	Phosphorylase kinase (now active)
Glycogen phosphorylase (now inactive)	Glycogen phosphorylase (now active)
Pyruvate dehydrogenase (now active)	Pyruvate dehydrogenase (now inactive)
Acetyl CoA carboxylase (now active)	Acetyl CoA Carboxylase (now inactive)
Which leads to the activation of:	**Which leads to the activation of:**
Glycolysis	Glycogenolysis
Fatty acid synthesis	Fatty acid oxidation
Glycogen synthesis	Gluconeogenesis

B. Mechanisms that affect lipolysis in adipose tissue

During fasting the level of cAMP rises in adipose cells because blood insulin levels fall and glucagon levels rise. Consequently, protein kinase A is activated and causes phosphorylation of hormone-sensitive lipase (HSL). The phosphorylated form of this enzyme is active and cleaves fatty acids from triacylglycerols. Other

hormones (e.g., **epinephrine, adrenocorticotropic hormone, growth hormone) also activate this enzyme.**

C. Mechanisms that affect ketone body production by the liver

As fatty acids are released from adipose tissue during fasting, they travel in the blood complexed with albumins. These fatty acids are oxidized by various tissues, particularly muscle. In the liver, fatty acids are transported into mitochondria because acetyl CoA carboxylase is inactive, malonyl CoA levels are low, and carnitine palmitoyltransferase I is active. Acetyl-CoA, produced by β-oxidation, is converted to ketone bodies. Ketone bodies are used as an energy source by some tissues (muscular, nervous tissues). The high levels of acetyl-CoA in the liver (derived from fat oxidation) inhibit pyruvate dehydrogenase (which prevents pyruvate from being converted to acetyl CoA) and activate pyruvate carboxylase, which produces oxaloacetate for gluconeogenesis. The oxaloacetate does not condense with acetyl CoA to form citrate for two reasons. The first is that under these condition (a high rate of fat oxidation in the liver mitochondria), energy levels in the mitochondrial matrix are high; that is, there are high levels of NADH and adenosine triphosphate (ATP) present. The high NADH level inhibits isocitrate dehydrogenase. As a result, citrate accumulates and inhibits citrate synthase from producing more citrate. The second reason to depress citrate synthesis is that the high $NADH/NAD^+$ ratio also stimulates the conversion of oxaloacetate into malate, such that the malate can exit the mitochondria (via the malate-aspartate shuttle system) for use in gluconeogenesis.

D. Regulation of the use of glucose and fatty acids by muscle

During physical exercises, the fuel that is used initially by muscle cells is muscular glycogen. As exercise continues and the blood supply to the tissue increases, glucose is taken up from the blood and oxidized. Liver glycogenolysis and gluconeogenesis replenish the blood glucose supply. However, as insulin levels drop, the concentration of specific glucose transporters (GLUT4) in the membrane is reduced, which reduces glucose entry from the blood circulation into the muscle. However, muscle GLUT4 transporters are also induced by high AMP levels through the actions of the AMP-activated protein kinase. It is logical for AMP to be a critical regulator. As a cell uses ATP in energy-requiring pathways, the levels of AMP accumulate more rapidly than that of ADP because of the adenylate kinase reaction (2 ADP = ATP + AMP). The rise in AMP levels signals that more energy is required (usually through allosteric binding sites on enzymes and the activation of the AMP-activated protein kinase), and the cell will switch to the activation of catabolic pathways. As AMP levels drop and ATP levels rise, the anabolic pathways are activated to store the excess energy.

Table 7.2. Regulation of liver enzymes involved in glycogen, glucose, and triacylglycerol metabolism

Liver Enzymes Regulated by Activation/Inhibition

Enzyme	Activated by	State in which it is active
PFK-1	Fructose-2,6-bisP, AMP	Fed
Pyruvate carboxylase	Acetyl CoA	Fed and fasting
Acetyl CoA carboxylase	Citrate	Fed
Carnitine palmitoyl-transferase I	Loss of inhibitor (malonyl CoA)	Fasting

Liver Enzymes Regulated by Phosphorylation/Dephosphorylation

Enzyme	Active Form	State in which it is active
Glycogen synthase	Dephosphorylated	Fed
Glycogen phosphorylase kinase	Phosphorylated	Fasting
Glycogen phosphorylase	Phosphorylated	Fasting
PFK-2[1]	Dephosphorylated	Fed
PFK-2[2]	Phosphorylated	Fasting
Pyruvate kinase	Dephosphorylated	Fed
Pyruvate dehydrogenase	Dephosphorylated	Fed
Acetyl CoA carboxylase	Dephosphorylated	Fed

Liver Enzymes Regulated by Induction/Repression

Enzyme	State in which it is induced	Process affected
Glucokinase	Fed	Glucose → TG
Citrate lyase	Fed	Glucose → TG
Acetyl CoA carboxylase	Fed	Glucose → TG
Fatty acid synthase	Fed	Glucose → TG
Malic enzyme	Fed	Production of NADPH
Glucose-6-P dehydrogenase	Fed	Production of NADPH
Glucose 6-phosphatase	Fasted	Production of blood glucose
Fructose 1, 6-bisphosphatase	Fasted	Production of blood glucose
Phosphoenolpyruvate carboxykinase	Fasted	Production of blood glucose

Phosphofrucrokinase 2 (PFK2) is bifunctional enzyme:
[1] – Phosphofructokinase / Fructose 2,6-bisphosphatase (PFK/F2,6-BP-ase) acts as a kinase, increasing fructose 2,6-bisphosphate levels.
[2] Fructose 2, 6-bisphosphatase / Phosphofructokinase (F2,6-BP-ase/PFK) acts as a phosphatase, decreasing fructose 2,6-bisphosphate levels.

Thus, if energy levels are low and the concentration of AMP increases, glucose can still be transported from the circulation into the muscle to provide energy. This will most frequently be the case during periods of exercise.

As fatty acids become available because of increased lipolysis of adipose triacylglycerols, the exercising muscle begins to oxidize fatty acids. β-Oxidation produces NADH and acetyl CoA, which slow the flow

of carbon from glucose through the reaction catalyzed by pyruvate dehydrogenase. Thus, the oxidation of fatty acids provides a major portion of the increased demand for ATP generation and spares blood glucose.

III. Regulation of carbohydrate metabolism by epinephrine (under the stress, physical exercises)

Epinephrine stimulates both muscle and liver adenylate cyclase to produce cyclic AMP. In the liver, the increased cyclic AMP levels activate a phosphatase activity of PFK-2 that dephosphorylates fructose-2,6-bisphosphate (F-2,6-BP) while deactivating a kinase activity of PFK-2 that produces F-2,6-BP. Thus, F-2,6-BP levels are decreased and PFK-1 activity is decreased. In liver and muscle, F-2,6-BP is the major allosteric activator of PFK-1. In skeletal muscle, however, the PFK-2 responsible for the synthesis of F-2,6-BP is activated, not inhibited, by cyclic AMP. Thus, muscle sees an increase in glycolysis following epinephrine stimulation, while the liver experiences a decrease in glycolytic activity. In both tissues, glycogen phosphorylase is activated and glycogenolysis occurs. Under these conditions, glucose is utilized in muscle for ATP production relative to contractile activity, while the liver produces glucose for export to the blood.

IV. Regulation of carbohydrate and lipid metabolism by glucocorticoids, sex steroids and thyroid hormones

Glucocorticoids are steroid hormones secreted by the adrenal cortex in response to stress or starvation. Glucocorticoids stimulate gluconeogenesis at the expense of phosphoenolpyruvate kinase induction in the liver. They also induce glucogenic amino acids transforming enzymes: tyrosine aminotransferase and tryptophane pyrrolase.

Glucocorticoids display a permissive effect on lipolysis stimulated by catecholamines. Glucocorticoid response elements have been observed in the upstream regions of β_1 and β_2 adrenoreceptor genes and, in fact, an increase in numbers of expressed β-receptors in response to glucocorticoids has been reported. Additionally, the activity of the stimutatory G-protein is enhanced by glucocorticoids. Glucocorticoids probably ensure maintenance of catecholamine-induced lipolysis by enhancing transcription of the genes involved in that signal cascade.

Sex steroids (primarily estrogen in females, which is synthesized by the ovaries, and testosterone in males, synthesized by the testes) like glucocorticoids, also affect gene transcription by binding to nuclear

Zn-finger transcription factors that recognize steroid response elements. In female rats, ovariectomy was shown to diminish lipolysis by decreasing the effectiveness of the adenylyl cyclase catalytic activity. Lipolysis was restored to normal levels in these animals by administration of estrogen but not progesterone. Castrated male rats exhibited decreased lipolysis that appeared to be caused both by defective adenylyl cyclase catalysis and a decreased number of β-adrenergic receptors, again implying desensitization to catecholamines. Normal lipolytic levels could be restored by administration of testosterone.

Thyroid hormones have the unique effect of increasing both synthesis and the oxidation of fatty acids. The effect of elevated thyroid hormone is increased lipolysis, which appears to be mediated by an increase in β_1/β_2-adrenergic receptor expression and a decrease in inhibitory G-protein expression. These alterations effectively sensitize the adipocyte to catecholamine stimulation.

Hormonal regulation of protein metabolism

Introduction

Protein digestion in GIT, protein synthesis and protein degradation in tissues are controlled by hormones. The protein intake from food products promotes the use of essential amino acids formed after digestion of proteins for the purpose to be involved in the cellular synthesis of proteins needed for human body. Protein degradation is used for the utilization of protein molecules whose function is not in need for the cell or, may be, the accumulation of those protein molecule can cause the disturbances in cellular homeostasis. Various functions of proteins may be represented in any type of a cell such as:

- a structural component of cellular compartment;
- a transport system across the membrane for a substance;
- a receptor for hormone or other type of biologically active compound;
- an enzyme for any type of chemical reaction;
- autocrine hormone in a cell.

These functions may be done usually if:

- a protein molecule will be synthesized, and this is stimulated by special hormone. It should be noted that the protein synthesis may be controlled by the hormone on the level of:

1) gene expression regulation (the synthesis of mRNA keeping the genetic information about primary structure of synthesized protein);
2) translation across the regulation of some enzymes involved in translation process;

3) a covalent modification of polypeptide chains or synthesis of the non-protein part if the protein is conjugated one.

- the synthesized protein is keeping up to the end of its use by the cell. All the proteins that are not in need for the cell must be degradated.

Pituitary gland hormones in the regulation of protein metabolism

Hypophyseal hormones are the remarkable example of hormones stimulating protein synthesis in their target tissues. Protein whose synthesis is stimulating by pituitary gland hormone as a rule may be:

- the hormone-protein of peripheral endocrine gland;
- the enzyme involved in the synthesis of the hormone of peripheral endocrine gland.

But any rule has some exceptions. Certainly, we can find out some hypophyseal hormones whose function is associated with the control of mineral metabolism in tissues, or these hormones regulate some metabolic pathways across the stimulation or suppression of the enzyme activity due to secondary messengers function in the target cell. Let us consider some examples for hormones in the control of protein metabolism.

Thyroid gland stimulating hormone (TSH) and thyroid hormones in the protein metabolism regulation

TSH regulates thyroid gland hormones Triiodothyronine (T_3) and Thyroxine (T_4) secretion. The increased concentration of T_3, T_4 inhibits secretion of TSH, because hypophyseal cells are sensitive to thyroid hormones.

T_3, T_4 are iodinated derivatives of tyrosine (Tyr), and may be considered as products of thyroglobulin proteolysis in lysosomes of thyroid gland follicle cells. The transformation of iodide ion to iodine, the modification of Tyr-residues of protein thyroglobulin (iodination by iodine ions) is due to the TSH influence in the follicle cells of the thyroid gland. T_3 and T_4 are hydrolyzed off the thyroglobulin and secreted into the blood stream if TSH is attached with receptors in thyroid gland cells. All the effects of TSH in the thyroid gland are shown in the figure 7.1. All these effects of TSH promote the ripening of thyroglobulin whose molecule contains linked T_3 and T_4, their release from thyroid gland:

- modification of thyroglobulin molecule (iodination step);
- the stimulation of Na^+, K^+-ATP-ase involved in the transport of iodide ion across the cellular membrane;
- the activation of thyroperoxidase producing the iodine ion.
- the release of thyroid hormones T_3, T_4 after proteolysis of thyroglobulin.

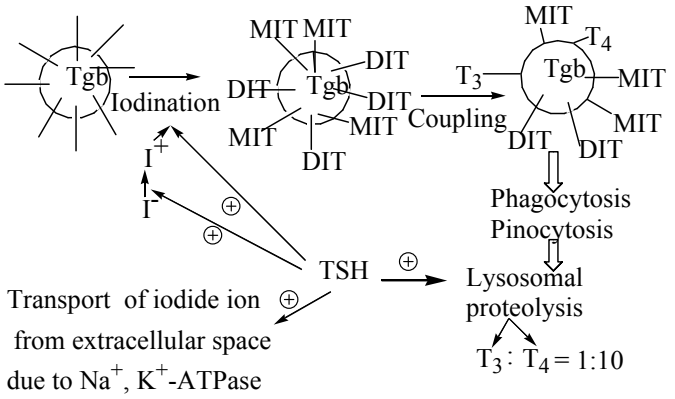


Figure 7.1. TSH effects on thyroid gland

Effects of thyroid hormones in their target tissues

It should be noted that T_3 and T_4 are lipophilic hormones, and their receptors are found in cytoplasm, matrix of mitochondria, and nuclear membrane in target cells. T_3 has 10 time greater biological activity then T_4, and about 80% of circulating in the blood T_4 is converted to T_3. The circulation of thyroid hormones in the blood stream is due to two specific binding proteins, ***thyroxine-binding globulin (TBG)*** and ***thyroxine-binding prealbumin (TBPA)***. TBG is synthesized in the liver, and it is also the subject to regulation, an important consideration in diagnostic testing of thyroid function, since most assays of T_3 or T_4 measure the total amount in the blood plasma rather than the free hormone. It is mentioned that only 0.3% of total T_3 and 0.03% of total T_4 are in a free (active) form in the blood, and the content of TBG and TBPA in the blood is very important factor to regulate the rate of T_3 linkage with receptors.

The major effect of T_3 and T_4 is to enhance general protein synthesis and to cause positive nitrogen balance. Thyroid hormones, like steroids, induce or repress protein synthesis across the increase or decrease of gene transcription rate.

Genes positively regulated by T3:

Sarcoplasmic reticulum calcium ATPase, alpha-myosin heavy chain, Malic enzyme, Myelin basic protein, rat growth hormone.

Negatively regulated genes:

TSH beta-subunit, beta-myosin heavy chain

It is proved that T_3 and glucocorticoids enhance transcription of growth hormone (GH), so that more GH is produced; and pituitaries of T_3-deficient animals were found to lack GH. But very high concentration

of T_3 inhibits the protein synthesis and causes negative nitrogen balance.

The main metabolic function of T_3 and T_4 in target tissues is to increase the saturation of oxygen by the cell. This effect is observed in all targets except brain tissue, reticular endothelial system and gonads. It is discussed their induce effect on mitochondrial alpha-glycerol phosphate dehydrogenase that may be explained due to thyroid hormones effect on oxygen saturation.

Growth hormone and protein metabolism

The concentration of GH in the blood varies widely through the day and it may be undetectable (<1 mU/L) with present assays for long periods. Physiological secretion occurs in sporadic bursts, lasting for one or two hours, mainly during sleep. Peak concentrations may be as high as 40 mU/L. Secretion can be stimulated by stress, exercise, a fall in blood glucose concentration, fasting, and ingestion of certain amino acids. Such stimuli can be used in provocative tests for diagnosing GH deficiency. GH secretion is inhibited by high level of glucose in the blood and this effect provides the rationale for the use of the oral glucose tolerance test in the diagnosis of excessive GH secretion.

GH stimulates the transport of amino acids across the cellular membrane from the blood stream to the intracellular space (experiments with muscular tissue). The rate of DNA and RNA synthesis is also increased, and as the result there is the increase of protein synthesis. GH causes positive nitrogen balance, the decrease in plasma and urinary levels of amino acids and urea. All the growth-related effects of GH are primarily mediated by ***insulin growth factor I (IGF-I, somatomedin C),*** the synthesis of this polypeptide in the liver is controlled by GH. The type of protein whose synthesis will be regulated by GH across the gene transcription stimulation depends on:

- type of target tissue;
- type of the receptor in the target cell;
- type of secondary messenger involved in the cellular mechanism of GH action (diacylglycerol, inositol 1,4,5-triphosphate, cAMP, Ca^{2+}-ions complex with a specific protein Calmodulin, etc).

Adrenocorticotropic hormone (ACTH) effects on protein metabolism

ACTH secretion is pulsatile and also shows diurnal variation, the plasma concentration being highest at approximately 0800 h and lowest at midnight, secretion is greatly increased by stress and is inhibited by cortisol. Thus cortisol secretion by the adrenal cortex is controlled by negative feed-back, but this and circadian variation can be overcome by

the effects of stress. The normal range for plasma ACTH at concentration at 0900 h is < 50 ng/L.

The effects of ACTH in the adrenal cortex are associated mainly with the stimulation of glucocorticoids synthesis across: 1) the change of intracellular levels of cAMP to activate special protein kinases, and to phosporylate inactive forms of enzymes involved in the formation of glucocorticoids from cholesterol esters; 2) the activation of gene expression for enzymes involved in the synthetic way for glucocorticoids. Secondary effect for ACTH is the stimulation of melanins formation from tyrosine in melanocytes, and this effect occurs very extensive at excessive ACTH levels in the blood (Addison`s desease).

Gonadotropins, sex hormones and protein metabolism

Follicle-stimulating hormone and (FSH) and Lutenizing hormone (LH) are regulated in secretion by hypothalamic gonadotropin-releasing hormone (GnRH), thes effects being modulated by circulating gonadal steroids (sex hormones). GnRH is secreted episodically, resulting in pulsatile secretion of gonadotropins with peaks in plasma concentration occurring at approximately 90-minute intervals.

The effects for FSH and LH are different according the sex of person. In males LH stimulates testosterone secretion by Leydig cells: both testosterone and oestradiol, derived from the Leydig cells themselves and from the metabolism of testosterone, feed-back to block the action of GnRH on LH secretion. Cellular effects of LH are across the stimulation of gene transcription of mRNAs for enzymes involved in steroid synthesis. FSH, in concert with high intratesticular testosterone concentrations, stimulates spermatogenesis. The formation and ripening of spermatozoids requires high rate of duration for protein synthesis, and all the other compounds needed for the formation of a cell.

In the female, the relationships are more complex. Estradiol secretion by the ovaries is stimulated primarily by FSH in the first part of the menstrual cycle; both hormones are necessary for the development of Graafian follicles, and certainly there is the stimulation of protein synthesis, too. As estrogen levels in the blood rise, FSH secretion declines until estrogens trigger a positive feed-back mechanism, causing the release of LH and, to a lesser extent, FSH. The increase of LH levels in the blood stimulates ovulation and development of corpus luteum, but rising concentrations of estrogens and progesterone then inhibit FSH and LH secretion. Inhibin (the hormone whose synthesis is stimulated in ovaries by FSH) inhibits also FSH secretion. If conception does not occur, declining concentrations of estrogens and progesterone from regressing corpus luteum trigger menstruation and LH and FSH release, initiating the maturation of further follicles in a new cycle.

Conclusion: **Gonadotropins and sex hormones have anabolic effects in their target cells to stimulate synthesis of proteins, too.**

Pancreatic hormones in the regulation of protein metabolism

Insulin

All the effects of insulin in its target tissues are promoted across the formation of insulin-receptor complex in the cellular membrane. Insulin receptor usually consists of two α- and two β-subunits, α-subunits are used for the contact with hormone and β-subunits have tyrosine kinase activity at the moment of the hormone–receptor complex formation (figure 7.2).

The half-time life ($t\frac{1}{2}$) for insulin receptor is 7-12 hours. Hormone–receptors complexes can bind each to another and move into cytoplasma. After autophosphorylation insulin-receptor complex can phosphorylate some proteins to activate them (figure 7.2).

Phosphatases whose activity may be increased by phosphorylation are in need to dephosphorylate some conjugated enzymes, and dephosphorylation may be considered for inhibition of some enzymes using the same mechanism: glycogenphosphorylase *a* and hormone-sensitive triacylglycerol lipase.

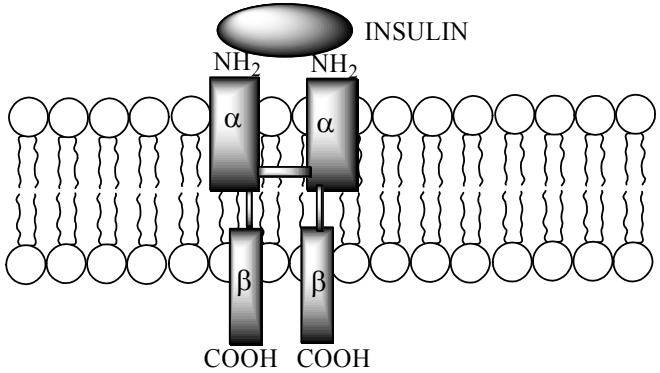
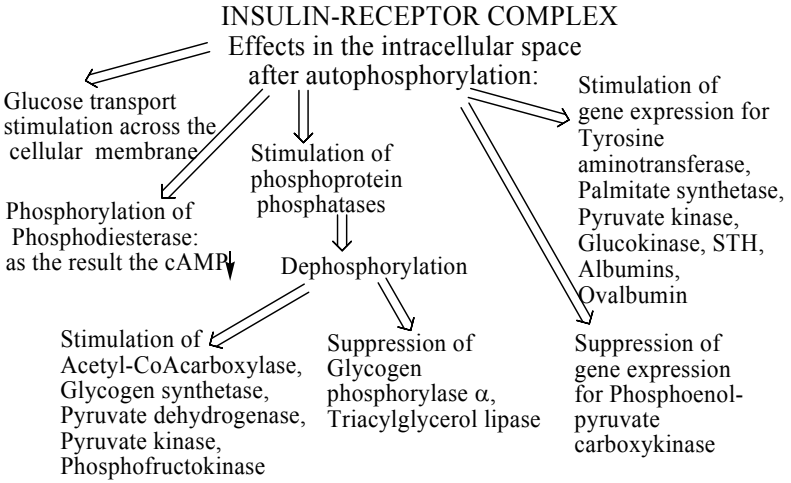


Figure 7.2. The structure of insulin receptor and its function in the intracellular space

It should be noted that some small “adopter” proteins have been found in target tissues for insulin. They can be phosphorylated by insulin-receptor complex and are named as insulin-receptor substrates (IRSs). IRS-1 which is present in most cells is phosphorylated on several tyrosines usually within special sequences. Phosphotyrosine within such sequences is known to be a ligand for the recognition domain SH2, which is present in many proteins. Binding of SH2

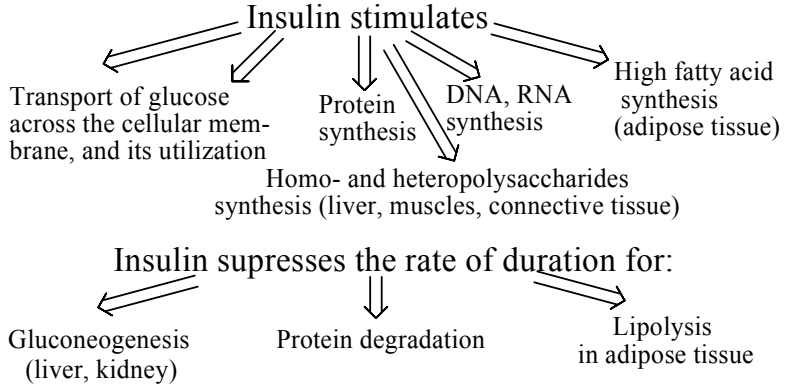
domains of other proteins to IRS-1 allows the insulin signal to be passed to several different proteins in branched signaling pathways. IRS-2 function is thought to mediate the mitogenic (growth promoting) effects of insulin and also to promote phosphorylation of serine and threonine side chains of many proteins in the cytoplasm, the cytoskeleton, ribosomes, membranes, and the nucleus.



Insulin effects gene expression using these "adopter" molecules. Insulin-receptor complex can inhibit or stimulate gene expression:

1) stimulates gene expression for : Tyrosine aminotransferase, Fatty acid synthetase, Pyruvate kinase, Glucokinase, albumins, growth hormone, ovalbumin;

2) suppresses gene expression for Phosphoenolpyruvate carboxykinase (PEPCK).



Glucagon

Glucagon is polypeptide (29 amino acid residues) and is synthesized from precursor proglucagon. It is noted that only 30-40 % of the immunoreactive "glucagon" in plasma is pancreatic glucagon; the rest consists of biologically inactive large molecules. Glucagon is in a free form in the blood plasma, $t\frac{1}{2}$ =5 min. It is utilized by the liver across limited proteolysis.

Secretion of glucagon is stimulated at hypoglycemia state and inhibited under hyperglycemia state. The increase of insulin and IGF1 levels in the blood is the factor to inhibit the secretion of glucagon. Some amino acids, ketones, gastrointestinal tract hormones and neurotransmitters affect glucagon secretion. The main targets for glucagons are liver and adipose tissue.

Glucagon is an insulin antagonist. It means all the processes that are stimulated by insulin are inhibited by glucagon. But the mechanism of its action is membrane-intracellular, cAMP is the major secondary messenger for its action on a target cell. cAMP levels in the cell can stimulate gene expression of some enzymes in the gluconeogenic pathway (e.g.: PEPCK). Lipolysis and glycogen breakdown are stimulated by glucagon across the activation of cAMP-dependent protein kinases in target tissues. For each metabolic process in any target tissue we have to take into account the influence of the **insulin/glucagon ratio** in the blood stream because both two hormones are involved in the regulation of the same processes.

Adrenal hormones in the regulation of protein metabolism

Glucocorticoids

The ability to regulate the protein metabolism is found for these hormones in kidney and in the liver, mainly. Their influence on the regulation of gene expression was studied for some enzymes involved in the gluconeogenesis duration such as Phosphoenolpyruvate carboxykinase (PEPCK)

The effects of hormones to regulate protein metabolism depend on the type of target tissue. Try to look at some factors regulating the protein metabolism in skeletal muscles (figure 7.3.)

Factor name	*Synthesis*	*Degradation*
Insulin	↑	↓
Growth hormone	↑	-
Thyroid hormones	↑	↓
Glucocorticoids	↓	↑
Prostaglandin	↑	-

$PGF_{2\alpha}$		
Prostaglandin PGE_2	-	↑
β-Adrenergic transmitters (epinephrine, etc.)	-	↓
Serotonin	-	↓
Glucose	-	↓
Amino acids	↑	↓
Ketone bodies	-	↓
Free fatty acids	↑	↓
Exercise	↓	↑, ↓
Starvation	↓	-
Fever	-	↑
Trauma	-	↑

Figure 7.3. Factors affecting the protein synthesis and protein degradation in skeletal muscular tissue (adapted from Kenneth L. Becker et al, Principles and practice of endocrinology and metabolism, 2000)

Hormones of GIT in the regulation of protein digestion

These hormones are involved in the control of gastric or pancreatic juice secretion thus to regulate the activity or secretion of enzymes needed for the degradation of proteins in GIT. Function of those hormones is described below in the table 7.3.

Table 7.3. Hormones of GIT involved in protein digestion

The name of a hormone	**Location**	**The major function**
Gastrin	Gastric antrum, duodenum	Stimulation of HCl and pepsinogen secretion
Secretin	Duodenum, jejunum	The neutralization of HCl across the stimulation of bicarbonate secretion, and stimulation of pancreatic juice secretion
Cholecystokinin	Duodenum, jejunum	The stimulation of pancreatic enzymes and bicarbonate secretion, the increase of the bile flow into duodenum
Gastric inhibitory polypeptide	Small bowel	The inhibition of gastric acid secretion
Somatostatin	Stomach, duodenum	It prolongs gastric emptying, decreases gastrin secretion

		and therefore gastric acid production, decreases pancreatic digestive enzyme secretion
Bombesin-like immunoreactivity factor	Stomach, duodenum	Stimulates secretion of gastrin and cholecystokinin

The rate of protein digestion promotes the rate of amino acid levels increase in the blood after absorption process. Certainly, the protein synthesis duration depends upon the availability to use these amino acids in the intracellular space of tissues. In this case it is in need to estimate:

1) the function of transport systems for amino acids across the intestinal wall during absorption, across the membrane;
2) the activity of amino acyl-tRNA synthetases.

All these processes are regulated by hormones whose function may be described by the term “anabolic action”.

Tests recommended to answer after study of chapter 7:

1. Which of the following hormones stimulates gluconeogenesis?
   A. Progesterone
   B. Glucagon
   C. Insulin
   D. Calcitriol
   E. Thyroxine

2. Which one of the following processes is simultaneously stimulated by epinephrine in muscle and inhibited by epinephrine in the liver?
   A. Fatty acid oxidation
   B. Glycogenolysis
   C. Cyclic AMP synthesis
   D. Glycolysis
   E. Pentose phosphate cycle

3. Choose from listed enzymes a bifunctional enzyme possessing both kinase and phosphatase activities:
   A. Glucose 6-phosphatase
   B. Glucokinase
   C. Hexokinase
   D. Phosphofrucrokinase 2
   E. Phosphofrucrokinase 1

4. Choose the hormone whose secretion to the blood stream will cause the increase of glucose levels across the stimulation of gluconeogenesis in the liver:
   A. Cortisol
   B. Estradiol
   C. Insulin
   D. Prostaglandin E2
   E. Somatotropin

5. Chose the metabolic pathway which will be stimulated in skeletal muscular tissue by insulin:
   A. Protein synthesis
   B. Glycogen synthesis
   C. Glycolysis
   D. Glycogen breakdown
   E. All the metabolic pathways placed in position A, B,C

6. Name hormones with anabolic affects on tissues
   A. Insulin
   B. Growth hormone
   C. Testosterone
   D. Gonadotropins

E. All the proposed

7. What ratio of hormones is the main important for the control of glucose levels in the blood of patient at first 24 hours of starvation?

A. Insulin/Epinephrine ratio
B. Insulin/Glucocorticoids ratio
C. Insulin/Glucagon ratio
D. TSH/STH ratio
E. Epinephrine/Glucocorticoids ratio

8. Name the process located in parenchyma of kidney whose duration is promoter for the increase of blood glucose levels:

A. Glycogenolysis
B. Gluconeogenesis
C. Aerobic glycolysis
D. Glycogenesis
E. Hexose Monophosphate Shunt

9. Name the hormone involved in the regulation proteins digestion in GIT:

A. Parathyroid hormone
B. Vasopressin
C. Aldosterone
D. Atrial natriuretic peptide
E. Gastrin

10. Name the most important messenger for Growth hormone influence on different tissues except liver:

A. Somatomedin C
B. Somatostatin
C. Insulin
D. Releasing factor
E. Corticotropin

Right answers for tests for all chapters

Chapter 1. The introduction into metabolism and energy exchange. Tissue respiration and oxidative phosphorylation

1	2	3	4	5	6	7	8	9	10
A	E	D	C	A	D	C	B	E	C

Chapter 2. Biochemistry of nutrition. Clinical concepts of nutirition

1	2	3	4	5	6	7	8	9	10
B	B	D	B	C	D	C	C	A	C

Chapter 3. Overview of carbohydrate metabolism in healthy humans. Disorders of carbohydrate metabolism (at diabetes mellitus, glycogen storage diseases, etc)

1	2	3	4	5	6	7	8	9	10
E	D	A	B	E	B	A	E	C	B

Chapter 4. Overview of lipid metabolism and its disorders in humans

1	2	3	4	5	6	7	8	9	10
B	D	E	D	C	C	A	B	B	A

Chapter 5. Common notions about the metabolic pathways of amino acids and other non-protein nitrogen-containing compounds in humans

1	2	3	4	5	6	7	8	9	10
D	E	B	C	C	E	E	B	E	C

Chapter 6. Common notions about haemoprotein metabolism in human organism. Nucleotide metabolism as the part of nucleoproteins metabolism in humans

1	2	3	4	5	6	7	8	9	10
A	A	D	A	E	D	A	D	B	E

Chapter 7. Hormonal regulation of carbohydrate, lipid and protein metabolism

1	2	3	4	5	6	7	8	9	10
B	D	D	A	E	E	C	B	E	A

Literature

1. Berezov T.T. ,Korovkin. B.F. Biochemistry.-M.: Medicine, Russia, 1992.- 542 p
2. Becker MA. Hyperuricemia and gout. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The Metabolic and Molecular Bases of Inherited Disease, vol II. 8th ed. New York: McGraw-Hill, 2001: 2535 p.
3. Bettelheim F.A.. Laboratory Experiments for General, Organic and Biochemistry. – 4th edition.- 1997. – 568p.
4. David J. Holme, Hazel Peck. Analytical Biochemistry. 3rd ed. London:Prentice Hall.- 1998- - 488 p.
5. Davidson V.L., Sittman D.B. Biochemistry. USA:Harwal Publishing. -1994. – 584 p.
6. Dawn B. Marks, Ph.D. Biochemistry. - 2nd ed. - Philadelphia, Pennsylvania:"Harwal Publishing".- 1994. – 337 p.
7. Koolman Jan, Roehm Klaus-Heinrich. Color Atlas of Biochemistry 2d ed. - Stuttgart · New York:Thieme. -2005. – 467 p.
8. Lieberman Michael; Marks Allan; Smith Colleen. Marks' Essential Medical Biochemistry, 2nd Edition. - Lippincott Williams & Wilkins. – 2007. – 540 p.
9. Marshall W.J., Bangert S. K. Clinical chemistry. Fifth edition.- Mosby, 2004.-422 p.
10. Reitman S., Frenkel S.- Amer. J. Clin. Pathol., 1957, 28,56 (AlAT)
11. Murray R. K., Granner D.K., Mayes P.A., Rodwell V.W.. Harper`s Illustrated Biochemistry., LANGE medical books, 26-edition, India, 2006.-868 p.
12. Richard A. McPherson, Matthew R. Pincus. Henry's Clinical Diagnosis and Management by Laboratory Methods. - 20th edition.- Philadelphia:Saunders.- 2001.- 1472 p.
13. Ronald A. Sacher, Richard A. McPherson. Widmann's Clinical Interpretation of Laboratory Tests. - 11th edition, F.A. Davis Company, 2000.- 1092 p.
14. Satyanarayana U., Chakrapani U. Biochemistry. – 3rd ed. - Kolkata:Books and Allied. – 2006. – 792 p.
15. Smith Colleen, Marks Allan, Lieberman Michael. Marks' Basic Medical Biochemistry: A Clinical Approach, 2nd Ed. - Lippincott Williams & Wilkins . 920 p.
16. William J Marshall, Stephen K Bangert. Clinical Chemistry. Fifth edition. – China:"Mosby". -2004. – 422 p.

FOR NOTES