Al-Mustaqbal University

جامعة المستقبل
AL MUSTAQBAL UNIVERSITY

College of Medical and Health Techniques

Medical Laboratories Techniques Departments

Biochemistry Lectures for 2nd Year Students

First Semester

(2 Credit Hrs. Theory + 4 Credit Hrs. Practice / Week = 4 Credit Unit

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Lecture No. 7

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Metabolism and Bioenergetics

Significance of Metabolism:

Metabolism is an important theme in medical studies as indicated below:

1. Hereditary enzyme defects
2. Diabetes, obesity, atherosclerosis, gout … …… etc.
3. Antimetabolites in the chemotherapy of cancers and infections
4. Inactivation and elimination of xenobiotics and drugs

Metabolism **is** the sum of all the chemical transformations describe the inter-conversion of thousands chemical compounds taking place in every cell or organism, occurs through a series of enzyme-catalyzed reactions that constitute **metabolic pathways.** The precursor is converted into a product through a series of metabolic intermediates called **metabolites,** Fig. 1.

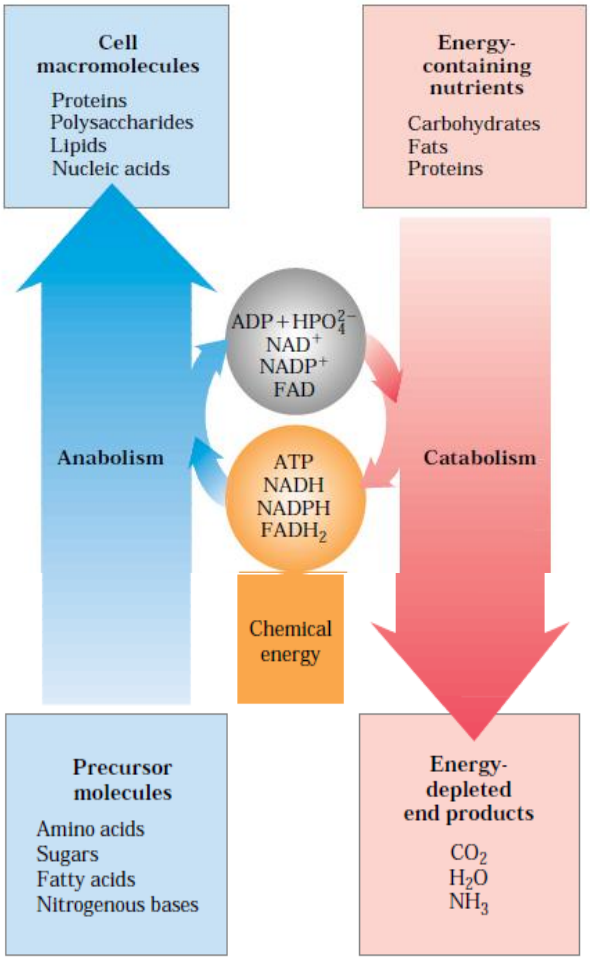


Fig. 1: Energy relationships between catabolic and anabolic pathways. Catabolic pathways deliver chemical energy in the form of ATP, NADH + H^+, NADPH + H^+, and FADH2. These energy carriers are used in anabolic pathways to convert small precursor molecules into cell macromolecules.

The term **intermediary metabolism** is often applied to the combined activities of all the metabolic pathways that interconvert precursors, metabolites, and products of low molecular weight.

Types of Metabolic Pathways:

1. **Catabolism** is the degradative phase of metabolism in which organic nutrient molecules (carbohydrates, fats, proteins and nucleoproteins) are converted into smaller, simpler end products (such as lactic acid, CO_2, NH_3). Catabolic pathways release energy, some of which is conserved in the formation of adenosine triphosphate (ATP) and reduced electron carriers (NADH + H^+, NADPH + H^+, and FADH2); the rest is lost as heat.
2. **Anabolism is** also called biosynthesis, small, simple precursors are built up into larger and more complex molecules, including lipids, polysaccharides, proteins, and nucleic acids. Anabolic reactions require an input of energy, generally in the form of the phosphoryl group transfer potential of ATP and the reducing power of NADH + H^+, NADPH + H^+, and FADH2 **(Fig. 1).**
3. **Amphibolic Pathway** which are seen at cross-roads of metabolism, where both anabolic and catabolic pathways are linked for example, the citric acid cycle.

Some metabolic pathways are linear, and some are branched as shown in **Fig. 2,** yielding multiple useful end products from a single precursor or converting several starting materials into a single product. Generally:

1. Catabolic pathways are converging
2. Anabolic pathways are diverging
3. Cyclic, in which one of the starting materials (ex. oxaloacetate) is regenerated and re-enters the pathway.

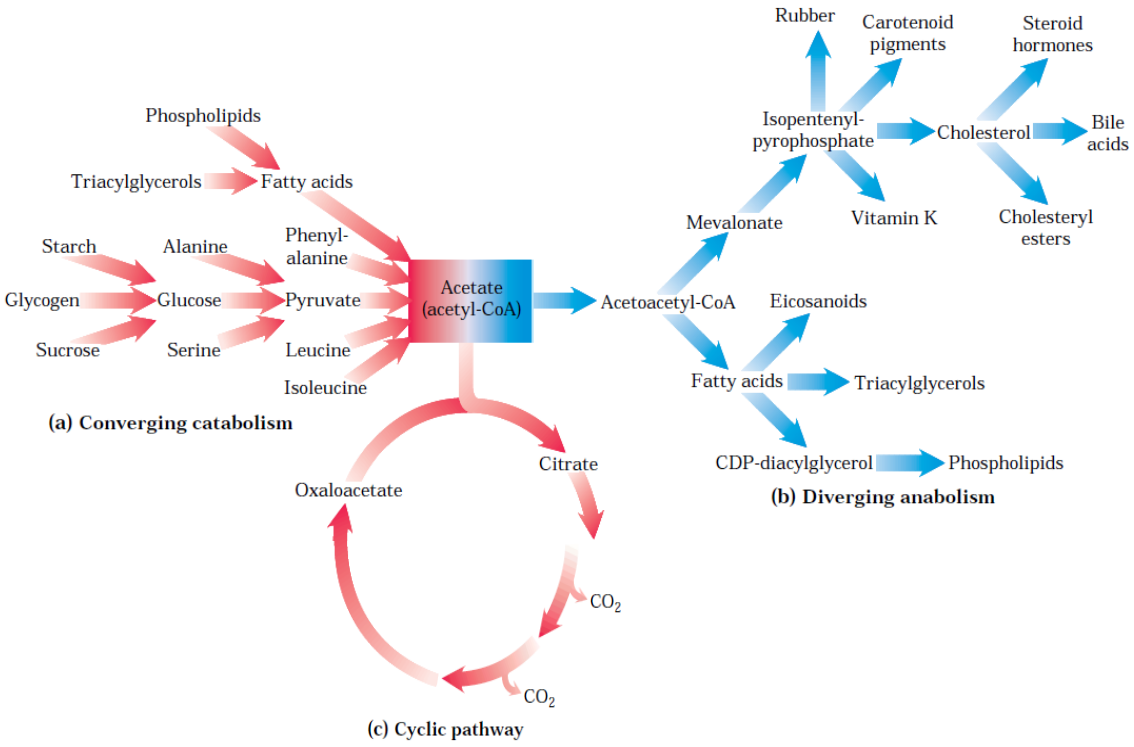


Fig. 2: Three types of non-linear metabolic pathways.

Acetate, a key metabolic intermediate as acetyl-CoA is the breakdown product of a variety of fuels (a), serves as the precursor for an array of products (b), and is consumed in the catabolic pathway known as the citric acid cycle (c).

In ***anaerobic conditions*** i.e., in the absence of oxygen most organisms can depend on ATP that arises in glycolysis. This less efficient type of ATP synthesis is referred to as **fermentation**.

The reducing equivalent NADH + H^+ exclusively supplies two electrons to respiratory chain for ATP generation while, **NADPH + H^+** a very similar coenzyme is the reducing agent for anabolic pathways. **NADPH + H^+** are mainly formed in the pentose phosphate pathway.

Catabolic and anabolic pathways that connect the same two end points (glucose → pyruvate and pyruvate → glucose, for example) may employ many of the same enzymes, but invariably at least one of the steps is catalyzed by different enzymes in the catabolic and anabolic directions, and these enzymes are the sites of separate regulation.

Figure 3 and 4 provides an overview of animal metabolism. The dietary (proteins, carbohydrates, and nucleic acids) cannot be used by the organism directly. Digestive processes first degrade them to monomers **(amino acids, sugars, nucleotides)**. These are then mostly broken down by catabolic pathways into smaller fragments. The products formed (generally referred to as the "metabolite pool") are then either used to obtain energy, or are built up again into more complex molecules by anabolic pathways. Of the numerous metabolites, only **pyruvate, acetyl-CoA, and glycerol** are shown. These molecules represent connecting links between the metabolism of proteins, carbohydrates, and lipids.

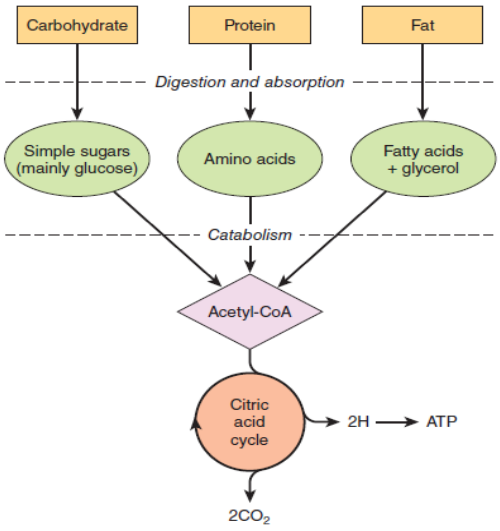


Fig. 3: The metabolite pool also includes the intermediates of the tricarboxylic acid cycle.

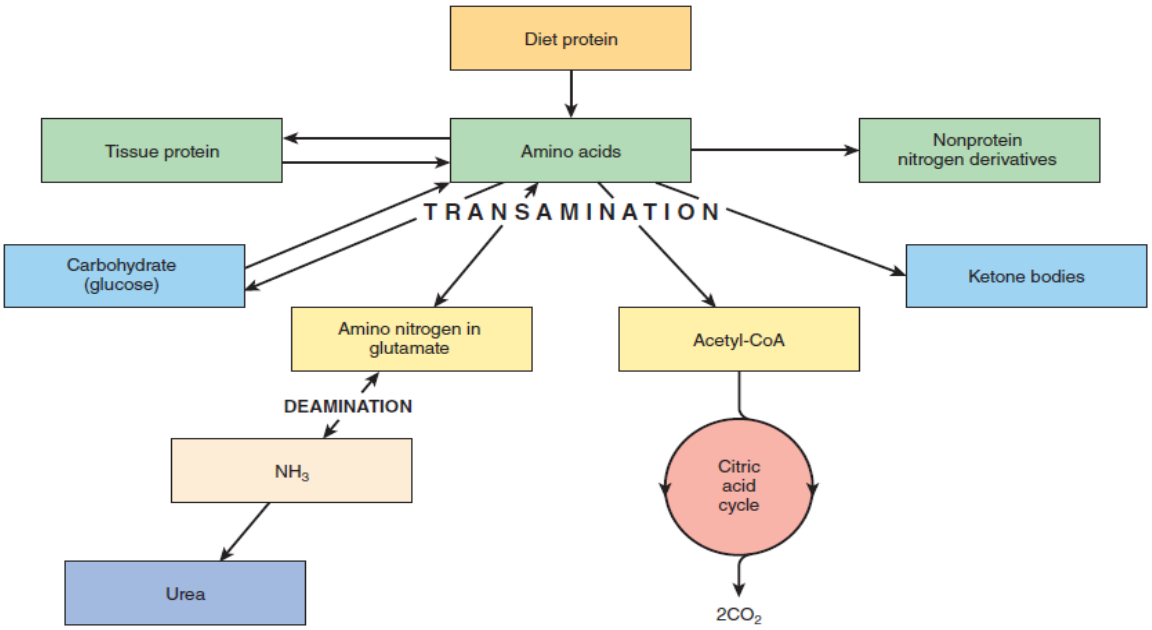


Fig. 4: Overview of amino acid metabolism showing the major pathways and end products.

Waste products from the degradation of organic substances in metabolism include carbon dioxide (CO_2), water (H_2O), and ammonia (NH_3). In mammals, the toxic substance ammonia is incorporated into urea and excreted in urine.

Generally, metabolism is a sum of the following types of pathways:

1. **Fuel oxidative pathway:** Food materials consumed are converted to energy.
2. **Fuel storage and mobilization pathway:** Stored fuel mobilized when there is no intake of food or during strenuous exercise.
3. **Biosynthetic pathways:** For the synthesis of proteins and other macro-molecules by biosynthetic pathways from certain amino acids, essential fatty acids and vitamins.
4. **Detoxification and waste disposal pathway:** For removal of toxic waste products from the body. Additional pathways, which help these essential pathways, are transport and intercellular signaling pathways.

Normal metabolism includes adaptation to periods of fasting, starvation, and exercise, as well as pregnancy and lactation. Abnormal metabolism may result from nutritional deficiency, enzyme deficiency, abnormal secretion of hormones, or the actions of drugs and toxins.

A 70-kg adult human being requires about 1920-2900 kcal obtained from metabolic fuels each day, depending on physical activity. For human beings, this energy requirement is met from carbohydrates (40%-60%), lipids

(mainly triacylglycerol, 30%-40%), and protein (10%-15%), as well as alcohol. The mix of carbohydrate, lipid, and protein being oxidized varies, depending on whether the subject is in the fed or fasting state, and on the duration and intensity of physical work.

There is a constant requirement for metabolic fuels throughout the day; average physical activity increases metabolic rate only by about 40 - 50% over the basal or resting metabolic rate. However, most people consume their daily intake of metabolic fuels in two or three meals, so there is a need to form reserves of carbohydrate (glycogen in liver and muscle), lipid (triacylglycerol in adipose tissue), and labile protein stores during the period following a meal, for use during the intervening time when there is no intake of food.

If the intake of metabolic fuels is greater than energy expenditure, the excess food is stored, largely as triacylglycerol in adipose tissue, leading to the development of **obesity** and its associated health problems. By contrast, if the intake of metabolic fuels is consistently lower than energy expenditure, there are negligible reserves of fat and carbohydrate, and amino acids arising from protein turnover are used for energy-yielding metabolism rather than replacement protein synthesis, leading to **emaciation,** wasting, and, eventually, death.

The formation and utilization of reserves of triacylglycerol and glycogen, and the extent to which tissues take up and oxidize glucose, are largely controlled by **insulin** and **glucagon hormones.**

Metabolism serves the following purposes:

1. Chemical energy is obtained from the degradation of energy-rich nutrients.
2. Food materials or macromolecules are converted into the building block precursors which later biosynthesize macromolecules, such as proteins, nucleic acids and polysaccharides.
3. Biomolecules required for specialized functions of the cell are synthesized.
4. Metabolic pathways are taking place with the help of sequential enzyme systems. These pathways are regulated at three levels:
   - **a.** Regulation through the action of allosteric enzymes, which increase or decrease the activity under the influence of effector molecules.
   - **b.** Hormonal regulation. Hormones are chemical messengers secreted by different endocrine glands.
   - **c.** Regulation at the DNA level; the concentration of the enzyme is changed by regulation at the level of synthesis of the enzyme.

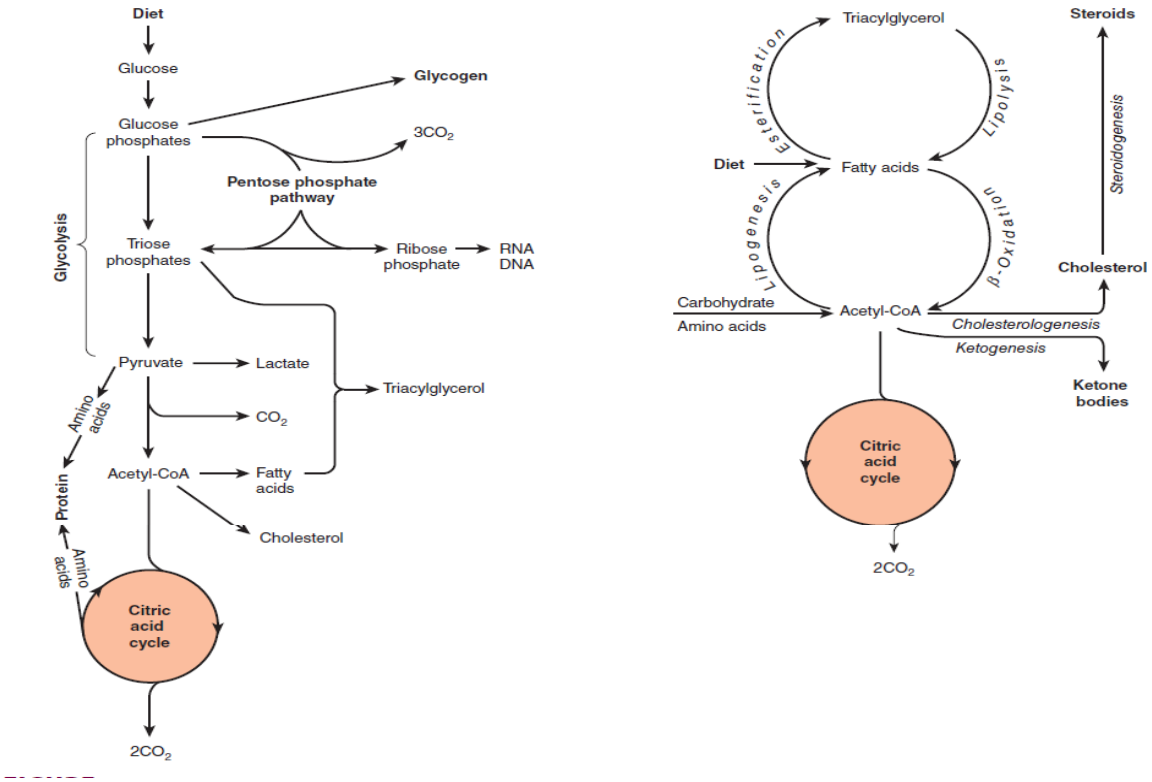


Fig. 5: Overview of carbohydrate and fatty acid metabolism showing the major pathways and end products.

Stages or Phases of Metabolism:

The degradation of foodstuffs occurs in three stages as shown in Fig. 6.

1. In the first stage, digestion in the gastrointestinal tract converts the macromolecules into small units. For example, proteins are digested to amino acids. This is called **primary metabolism.**
2. Then these products are absorbed, catabolized to smaller components, and ultimately oxidized to CO_2. The reducing equivalents are mainly generated in the mitochondria by the final common oxidative pathway, citric acid cycle. In this process, NADH or FADH2 are generated. This is called **secondary or intermediary metabolism.**
3. Then these reduced equivalents enter into the **electron transport** chain (ETC, or Respiratory chain), where energy is released. This is the **tertiary metabolism** or cellular respiration.

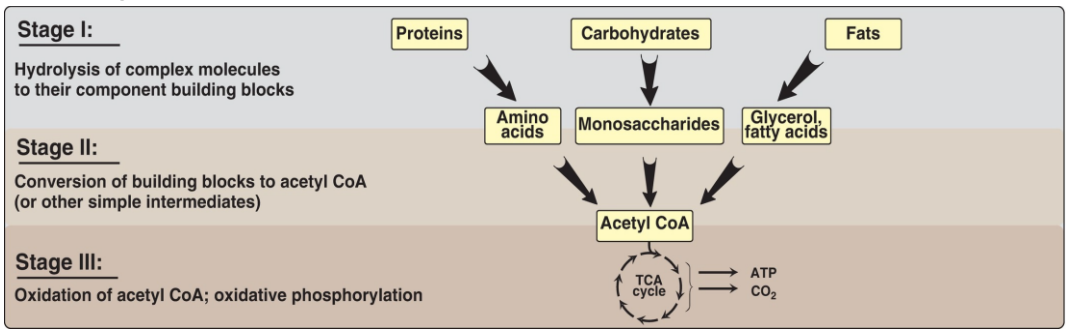


Fig. 6: Stages of Metabolism

Glucose enters glycolysis pathway forming pyruvate and then converted to acetyl-CoA and are oxidized in the citric acid cycle. Carbohydrate metabolism is centered on glucose, and is mainly used for energy production to the body.

Lipid metabolism is centered on fatty acids, which are also oxidized and used for provision of energy.

Amino acids are mainly meant for body building purpose. However, most of the amino acids are eventually transaminated, the carbon skeletons are oxidized providing some energy, but energy production is not the main purpose of amino acid metabolism. Carbohydrate, lipid and amino acid metabolisms are inter-related. Fig. 7- summarized a comparison between catabolic and anabolic pathways.

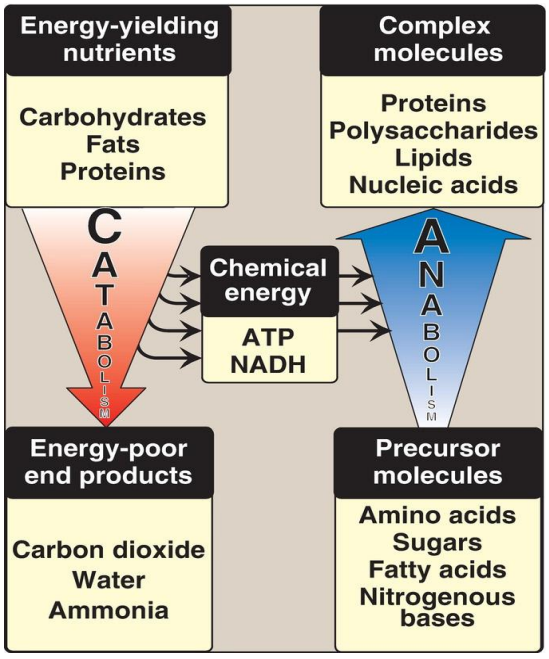


Fig. 7: Comparison between anabolic and catabolic pathways

High Energy Compounds:

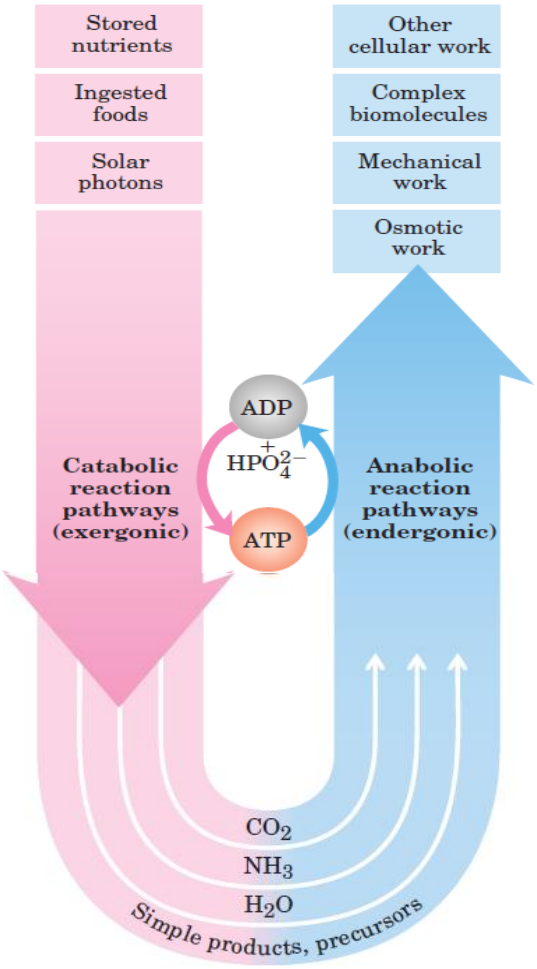
The most important form of storage for chemical energy in all cells is adenosine triphosphate (ATP). ATP synthesis requires energy i.e., the reaction is endergonic. Conversely, cleavage of ATP or hydrolysis into ADP and inorganic phosphate releases energy which is exergonic. The most important pathway for the synthesis of ATP is mitochondrial respiratory chain. In this process, catabolic pathways first form reduced cofactors (NADH + H^+, FADH2 and FMNH2). Electrons are then transferred from these compounds to oxygen catalyzed by cytochrome enzymes of the mitochondrial respiratory chain and used indirectly for the ATP synthesis.

Phosphoryl Group Transfers and ATP

ATP molecules act as the energy currency that links catabolism and anabolism (see Figure below).

The central role of ATP in metabolism

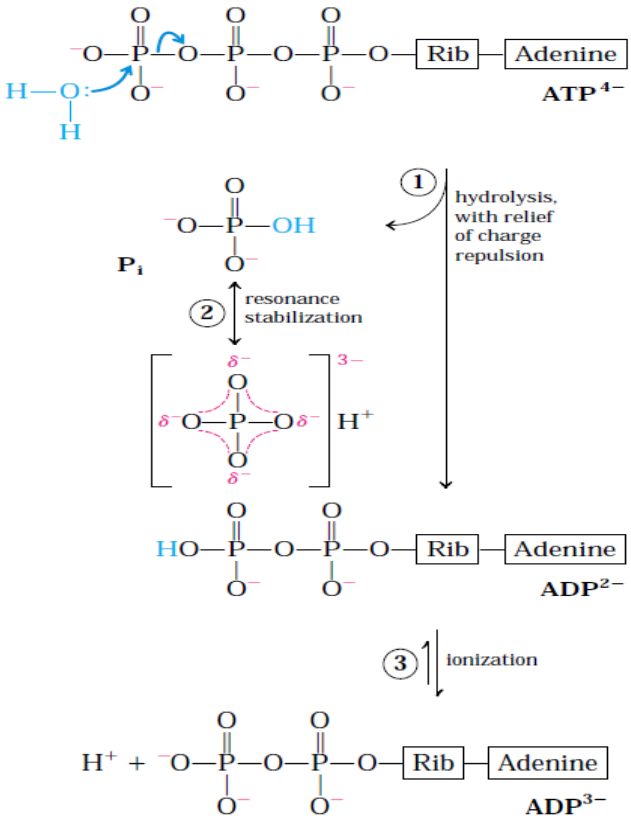
Heterotrophic cells obtain free energy in a chemical form by the catabolism of nutrient molecules, and they use that energy to make ATP from ADP and P_i. ATP then donates some of its chemical energy to endergonic processes such as the synthesis of metabolic intermediates and macromolecules from smaller precursors.



Free-Energy Change for ATP Hydrolysis:

Figure below summarizes the chemical basis for the relatively large, negative, standard free energy of hydrolysis of ATP.

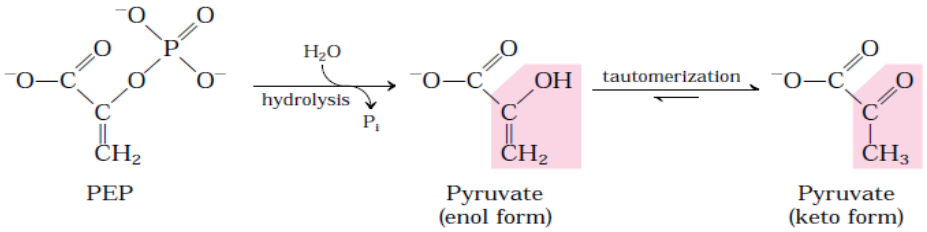
The hydrolytic cleavage of the terminal phosphoric acid anhydride (phosphor anhydride) bond in ATP separates one of the three negatively charged phosphates and thus relieves some of the electrostatic repulsion in ATP; the P_i (HPO_4^{2-}) released is stabilized by the formation of several resonance forms not possible in ATP; and ADP^{2-}, the other direct product of hydrolysis, immediately ionizes, releasing H^+ into a medium of very low [H^+] (~10^{-7} M).



$$ATP^{4-} + H_2O \longrightarrow ADP^{3-} + P_i^{2-} + H^+$$
$$\Delta G'^{\circ} = -30.5 \text{ kJ/mol}$$

Because the concentrations of the direct products of ATP hydrolysis are, in the cell, far below the concentrations at equilibrium, mass action favors the hydrolysis reaction in the cell. Although the hydrolysis of ATP is highly exergonic ($\Delta G'^{o}$ = – 30.5 kJ/mol), the molecule is kinetically stable at pH 7 because the activation energy for ATP hydrolysis is relatively high. Rapid cleavage of the phosphor anhydride bonds occurs only when catalyzed by an enzyme. The free-energy change for ATP hydrolysis is – 30.5 kJ/mol under standard conditions, but the *actual* free energy of hydrolysis (ΔG) of ATP in living cells is very different: the cellular concentrations of ATP, ADP, and Pi are not identical and are much lower than the 1.0 M of standard conditions.

Other Phosphorylated Compounds and Thioesters:



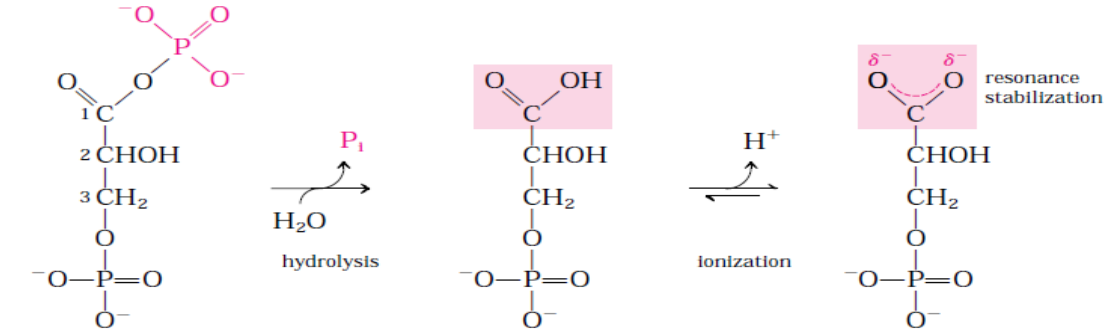
$$PEP^{3-} + H_2O \longrightarrow pyruvate^{-} + P_i^{2-}$$
$$\Delta G'^{\circ} = -61.9 \text{ kJ/mol}$$

Phosphoenolpyruvate, PEP

The PEP contains a phosphate ester bond that undergoes hydrolysis to yield the enol form of pyruvate, and this direct product can immediately tautomerize to the more stable keto form of pyruvate. Because (phosphoenolpyruvate) has only one form (enol) and the product (pyruvate) has two possible forms, the product is stabilized relative to the reactant.

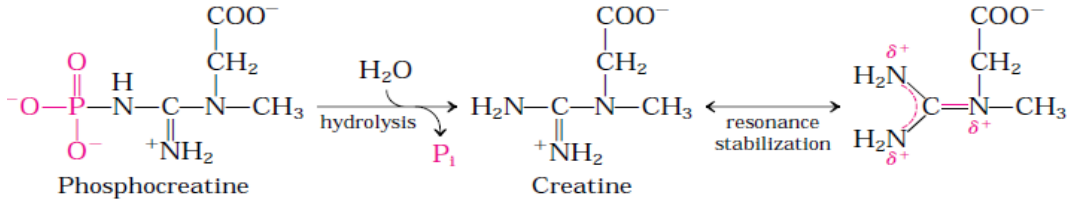
This is the greatest contributing factor to the high standard free energy of hydrolysis of phosphoenolpyruvate: $\Delta G'^{o} = -61.9$ kJ/mol.

Another three-carbon compound, **1,3-bisphosphoglycerate** (Figure below), contains an anhydride bond between the carboxyl group at C-1 and phosphoric acid. Hydrolysis of this acyl phosphate is accompanied by a large, negative, standard free-energy change ($\Delta G'^{o} = -49.3$ kJ/mol).



$$1,3\text{-Bisphosphoglycerate}^{4-} + H_2O \longrightarrow 3\text{-phosphoglycerate}^{3-} + P_i^{2-} + H^+$$
$$\Delta G'^{\circ} = -49.3 \text{ kJ/mol}$$

Hydrolysis of -1,3- bisphosphoglycerate.



$$\text{Phosphocreatine}^{2-} + H_2O \longrightarrow \text{creatine} + P_i^{2-}$$
$$\Delta G'^{\circ} = -43.0 \text{ kJ/mol}$$

Phosphocreatine

In phosphocreatine (Figure above), the standard free-energy change of phosphocreatine hydrolysis is again large, – 43.0 kJ/mol.

Thioesters, in which a sulfur atom replaces the usual oxygen in the ester bond, also have large, negative, standard free energies of hydrolysis. Acetyl-coenzyme A, or acetyl-CoA (Figure below), is one of many thioesters important in metabolism. The acyl group in these compounds is activated for transacylation, condensation, or oxidation-reduction reactions. In both cases, hydrolysis of the ester generates a carboxylic acid, which can ionize and assume several resonance forms. Together, these factors result in the large, negative $\Delta G'^{o}$ (– 31 kJ/mol) for acetyl-CoA hydrolysis.

Generally, the hydrolysis of these compounds is accompanied by release of large amount of free energy. Since ATP is a high energy compound, the energy released during transfer of electrons from reduced coenzymes to O_2 is conserved in the form of ATP. The energy released when a high energy compound is hydrolyzed is not due to bond that is hydrolyzed. It is due to large difference in the free energy content of reactant and product. $\Delta G'^{o'}$ for the hydrolysis of ATP is given below

$$ATP \rightarrow ADP + Pi \qquad \Delta G^{o'} = -7.3 \text{ Kcal/mol } (-30 \text{ KJ/mole})$$

ADP is also energy rich compound because $\Delta G^{o'}$ for ADP hydrolysis is –7.3 Kcal/mol. By convention, high energy bond is shown with (~) symbol. So, the ATP is written as:

Adenine-Ribose- P ~ P ~ P

Significance of ATP:

1. It is involved in the transfer of energy in the cells. It is often called as *energy currency* of the cell.
2. In the cells, the energy released in an exergonic reaction is used to form ATP and energy required for endergonic reactions is supplied by hydrolyzing ATP. Therefore, in biological systems ATP serve as link between the exergonic and endergonic reactions.
3. Energy of ATP hydrolysis is also used for muscle contraction, transport of ions and molecules across cell membrane, motility of sperm cells etc.

Other nucleoside triphosphates like GTP, UTP, CTP, TTP, dATP, dGTP and dTTP are also high energy compounds. The electronic structure of these compounds is responsible for the release of large free energy on hydrolysis.

Table lists below indicate Standard Free Energies of Hydrolysis of Some Phosphorylated Compounds and Acetyl-CoA (a Thioester).

	$\Delta G'^{\circ}$	
	(kJ/mol)	*(kcal/mol)*
Phosphoenolpyruvate	−61.9	−14.8
1,3-bisphosphoglycerate ($\rightarrow$ 3-phosphoglycerate + P_i)	−49.3	−11.8
Phosphocreatine	−43.0	−10.3
ADP ($\rightarrow$ AMP + P_i)	−32.8	−7.8
ATP ($\rightarrow$ ADP + P_i)	−30.5	−7.3
ATP ($\rightarrow$ AMP + PP_i)	−45.6	−10.9
AMP ($\rightarrow$ adenosine + P_i)	−14.2	−3.4
PP_i ($\rightarrow$ 2P_i)	−19.2	−4.0
Glucose 1-phosphate	−20.9	−5.0
Fructose 6-phosphate	−15.9	−3.8
Glucose 6-phosphate	−13.8	−3.3
Glycerol 1-phosphate	−9.2	−2.2
Acetyl-CoA	−31.4	−7.5

Regulatory Mechanisms:-

Fundamental mechanisms of metabolic regulation:

An overview of the regulatory mechanisms of metabolic pathways (**synthesis and degradation**) is presented here. Metabolite flow along a metabolic pathway is mainly determined by the activities of the **enzymes** involved. To regulate the pathway, it is sufficient to change the activity of the enzyme that catalyzes the ***slowest* step** in the reaction chain which is a **key enzymes** that regulate the mechanisms of pathway. The activity of key enzymes is regulated at **three** independent levels:

1. **Transcriptional control**. The biosynthesis of the enzyme protein is influenced at the genetic level. Interventions in enzyme synthesis mainly affect synthesis of the corresponding mRNA i.e., ***transcription*** of the gene coding for the enzyme.

   When synthesis of a protein is increased by transcriptional control, the process is referred to as **induction**; when it is reduced or suppressed, it is referred to as **repression**. Induction and repression processes take some time and are therefore not immediately effective.
2. **Interconversion** of key enzymes takes effect considerably faster than transcriptional control. In this case, the enzyme is already present at its site of effect, but it is initially still inactive. It is only when needed that it is converted into the catalytically active form, after signaling and mediation from second messengers through an ***activating enzyme*** (E1). If the metabolic pathway is no longer required, an ***inactivating enzyme*** (E2) returns the key enzyme to its inactive resting state. Interconversion processes in most cases involve **ATP-dependent phosphorylation** of the enzyme protein by a ***protein* kinase** or **dephosphorylation** of it by a ***protein phosphatase***. The phosphorylated form of the key enzyme is usually the more active one, but the reverse may also occur.
3. **Modulation by ligands.** An important variable that regulates flow through a metabolic pathway is **precursor availability** (metabolite A). The availability of precursor A increases along with the activity of the metabolic pathways that form A and it decreases with increasing activity of other pathways that also consume A. Transport from one cell compartment to another can also restrict the availability of A.

   Coenzyme availability can also often have a limiting effect. If the coenzyme is regenerated by a second, independent metabolic pathway, the speed of the second pathway can limit that of the first

one. For example, glycolysis and the tricarboxylic acid cycle are mainly regulated by the availability of NAD^+. Since NAD^+ is regenerated by the respiratory chain, the latter indirectly controls the breakdown of glucose and fatty acids (Fig. 8).

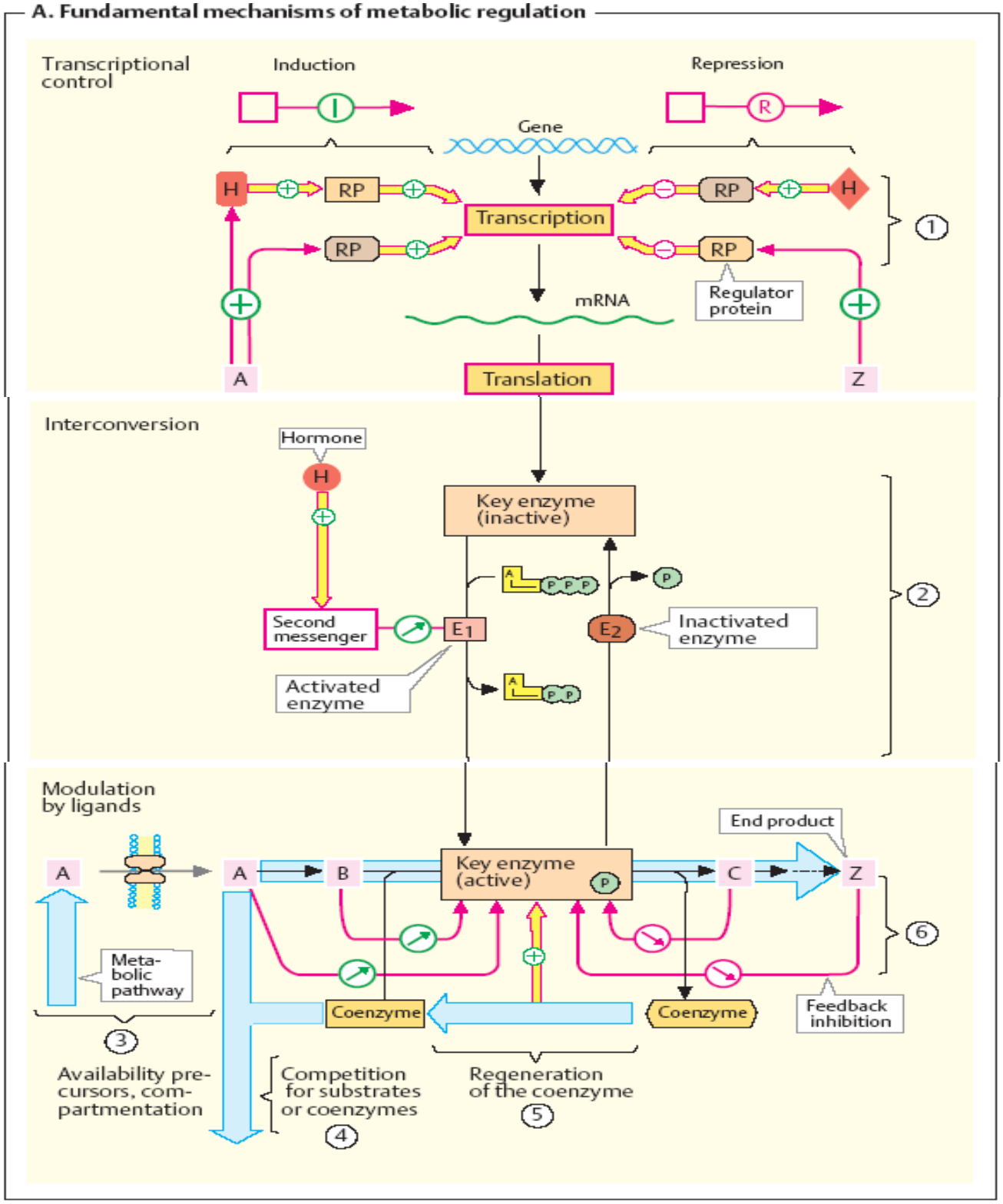


Figure 8. Mechanisms of metabolic regulations

Finally, the activity of key enzymes can be regulated by ***ligands*** (**substrates, products, coenzymes, or other effectors**), which as *allosteric effectors* do not bind at the active center itself, but at another site in the enzyme, thereby modulating enzyme activity.

The key enzymes are often inhibited by immediate reaction products, by end products of the reaction chain concerned (***"feedback" inhibition***), or by metabolites from completely different metabolic pathways. The precursors for a reaction chain can stimulate their own utilization through enzyme activation.

The pathways of metabolism must be coordinated so that the production of energy or the synthesis of end products meets the needs of the cell. Furthermore, individual cells function as part of a community of interacting tissues, not in isolation. Regulatory signals that inform an individual cell of the metabolic state of the body as a whole include hormones, neurotransmitters, and the availability of nutrients. These, in turn, influence signals generated within the cell (Fig. 9).

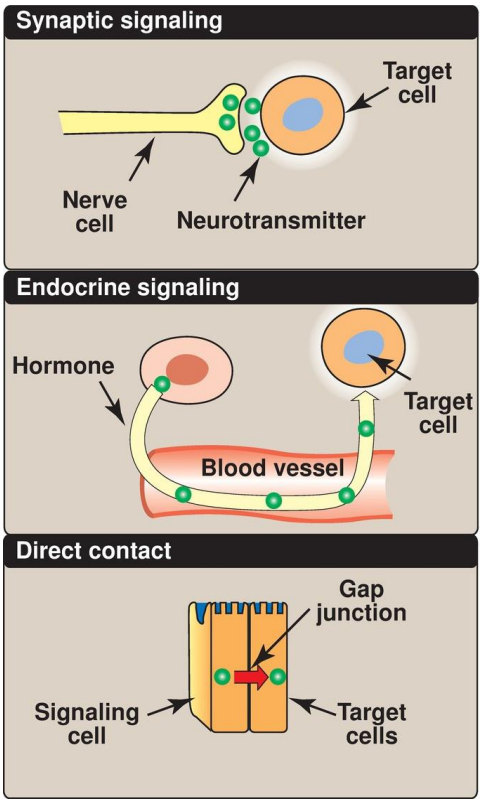


Fig. 9. Hormonal signaling

Hormonal Control:

In higher organisms, metabolic and other processes (growth, differentiation, control of the internal environment) are controlled by **hormones, Fig. 10.**

A. Principles of hormone action:

Depending on the type of hormone, hormone signals are transmitted to the target cells in different ways. Apolar (**lipophilic**) hormones penetrate the cell and act in the cell nucleus, while polar (**hydrophilic**) hormones act on the external cell membrane.

Lipophilic hormones, which include the steroid hormones, thyroxine, and retinoic acid, bind to a specific *receptor protein* inside their target cells. The complex formed by the hormone and the receptor then influences *transcription* of specific genes in the cell nucleus.

The group of **hydrophilic hormones** consists of hormones derived from amino acids, as well as peptide hormones and proteohormones. Their *receptors* are located in the plasma membrane. Binding of the hormone to this type of receptor triggers a signal that is transmitted to the interior of the cell, where it controls the processes that allow the hormone signal to take effect (**signal transduction**).

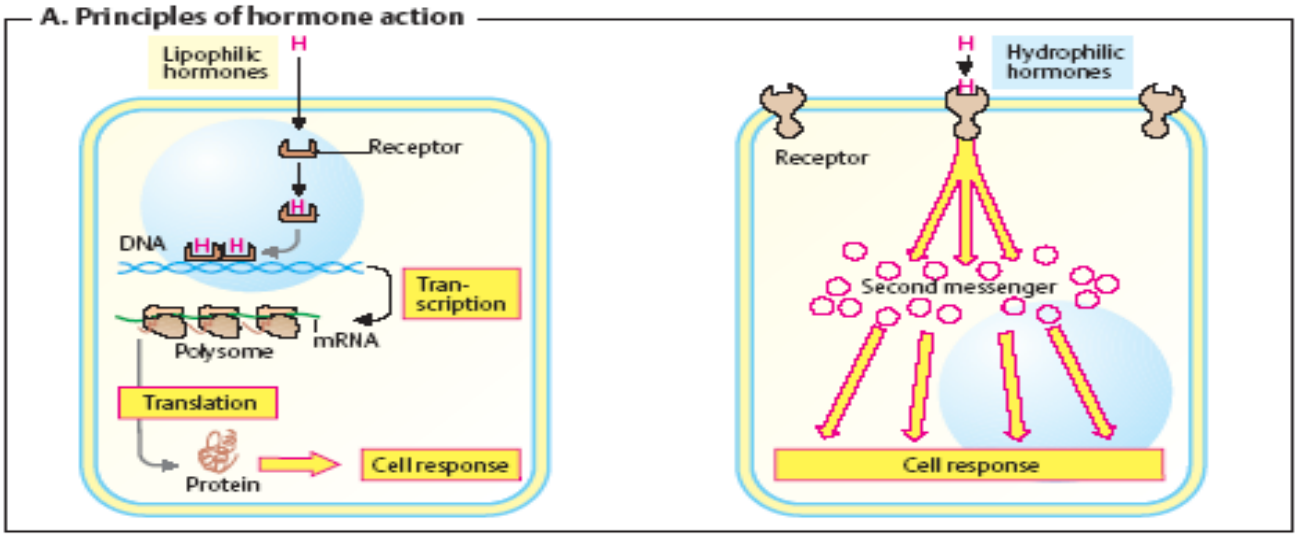


Fig. 10. Hormonal regulation.

B. Hormonal regulation of glucose metabolism in the liver:

The liver plays a major role in glucose homeostasis in the organism. If glucose deficiency arises, the liver releases glucose into the blood, and when blood sugar levels are high, it takes glucose up from the blood and converts it into different metabolites. Several hormones from both groups are involved in controlling these processes. **Glycogen** is the form in which glucose is stored in the liver and muscles. The rate of glycogen synthesis is determined by ***glycogen synthase*** (bottom right), while its breakdown is catalyzed by ***glycogen phosphorylase*** (bottom left).