

MINISTRY OF HEALTH OF UKRAINE  
ZAPORIZHZHIA STATE MEDICAL UNIVERSITY  
Pathophysiology Department

# **PATHOGENESIS OF CELL INJURY**

Module № 1. General Pathophysiology  
Submodule 1. General nosology

*Manual for independent work for the students of the 3<sup>rd</sup> course of international  
faculty speciality “General medicine” English medium of instruction*

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## **Introduction**

The cell is an elemental autoregulatory structural and functional unit of tissues and organs. It regulates processes that form the basis of the energy and nutrition supply the functioning tissues and organs. Therefore, the knowledge about cell injury mechanisms is of particular importance in the modern medicine. Any pathological process is accompanied by cell damage and the development of disease onset, disease course and its consequences is to some extent dependent of the mechanisms of cell injury. Study of pathological processes starts with the study of cell injury mechanisms.

The nature and degree of damage depends on the nature and force of the injuring factor, structural and functional features of the organ or tissue, as well as on the reactivity of the organism.

Getting knowledge about the alterations in structure and function of damaged cells is important for a specialist in any medical profile, since there is no disease in which there is no development of alteration. In addition, the alteration is closely related to many other general pathological processes and is the cause of their development, may be one of the components of these processes.

At the heart of any typical pathological processes is the pathology of the cell as a basis for morphogenesis of general pathological manifestations (dystrophy, stasis, thrombosis, heart attack, reparation, metaplasia, neoplasia, etc.). Thus, the pathology of the cell as an integrative concept is a necessary basis for the general pathology, without which it is not possible to understand and to provide an adequate correction of violations at the level of the whole organism.

## **THE AIM AND LEARNING OBJECTIVES OF PRACTICAL CLASS**

- 1. General aim:** to study the pathogenic mechanisms of cell injury:
- 2. Learning objectives** (basic educational and training issues for extracurricular self-study):
  - 1) Students should know:
    - a) the main signs of alterative changes of cells in human organs caused by the action of pathogenic factors;
    - b) the main distinctive features of cell death by necrosis and apoptosis
    - c) mechanisms for protection and adaptation of cells to action damaging agents.
  - 2) Students should be able to:
    - a) to determine the microscopic signs of various types of cell death (necrosis, apoptosis and atrophy);
    - b) determine the reversibility and irreversibility of the revealed structural changes;
    - c) interpret the consequences and significance of alterative changes for the organism;
    - d) interpret the functional significance of the detected changes and the possibility of using them in the diagnosis of diseases of the internal organs;
    - g) To solve situational problems on the basis of pathophysiological analysis of clinical and model situations related to the cell injury.

## **QUESTIONS TO STUDY:**

1. General principles of cell injury
2. Hypoxic (ischemic) cell injury
3. Injury by free radicals
4. Injury by chemicals
5. Pathology of cell signalling
6. Types of cell death: necrosis and apoptosis
7. Mechanisms of cell adaptation to injury

# **THEORETICAL MATERIAL**

## **1. General principles of cell injury**

A cell is an elementary self-regulating structurally functional unit of tissues and organs. Processes underlying energy and plastic maintenance of varied structures and functioning level of tissues and organs proceed in it.

Since most pathological processes originate at the cellular level, an understanding of cell structure and functions, as well as their impairment, is a crucial point for understanding of all the pathological physiology.

The basic concepts of cellular biology are arranged into cell theory including the following issues:

- all free-living organisms are composed of cells and their products;
- all cells are basically similar in their chemical structure;
- new cells are formed from preexisting cells by cell division;
- the activity of an organism is the sum of activities and interactions of its cells.

Basically all the cells consist of two principal parts: the cytoplasm with organelles and cell membrane.

The cell membrane may be either smooth or folded and consists of phospholipids, cholesterol, and other lipids. The hydrophobic portions of the lipid molecules are arranged in a double layer facing one another, the hydrophilic portions jutting out into the watery surroundings. Proteins, many of them mobile, are incorporated into the membrane, some extending through its entire thickness and serving as carriers or pores through which polar (hydrophilic) substances can pass.

Some of the functions of the cell membrane are the protection of the interior of the cell from its surroundings, transport, the recognition of hormones and other

BAS, and the adhesion of cells to one another.

The nucleus contains a fluid known as the karyolymph, as well as the chromatin network and the nucleolus. Chromatin contains the desoxyribonucleic acids (DNA) that are the carriers of the genetic information. Two strands of DNA (double helix) are twisted and folded to form the chromosomes.

The rough endoplasmic reticulum (RER) consists of flat vesicles which are connected to form a network of channels throughout the cell. The ribosomes are attached to the outside of the RER (thus rough ER) or are found free in the protoplasm. They contain transcripts (RNA) of the nuclear DNA. ER without ribosomes is called smooth ER and is chiefly engaged in the synthesis of lipids (lipoproteins).

The mitochondria are the power station of the cell. They contain enzymes of the citric acid (Krebs) cycle and of the respiratory pathway. They are the principal site for oxidative reactions that generate energy. The energy thus produced is stored primarily in chemical form in the adenosine triphosphate (ATP) molecule. Synthesis of ATP provides almost all of the immediately accessible energy stores of the body; breakdown of ATP by various enzymes (phosphatases, ATPases) liberates energy for utilization in cellular reactions.

Lysosomes are enzyme-containing vesicles, arising in most cases from ER and the Golgi apparatus (primary lysosomes). They are also involved in protein transport and the breakdown of substances taken up in the cell by phagocytosis or by pinocytosis (phagolysosomes and secondary lysosomes)

The normal cell is confronted with stresses during all its life. If stresses are not severe the cell tends to maintain normal homeostasis by adjusting its physiological properties, which result in cell's adaptation. When the stress is overwhelming or adaptation is ineffective then cell injury and death occur.

Cellular injury refers to the constellation of metabolic changes that occurs as a result of external stress placed upon a cell. Stressful stimuli include many harmful physical, chemical and biological agents that threaten the maintenance of the stable



internal environment of the cell. The ability of a cell to tolerate a hostile environment depends to a large extent upon internal and external cellular mechanisms that allow the cell to respond to changes in its environment. Such adaptive mechanisms permit a cell to survive and function in the presence of external stress. Adaptation includes hypertrophy, an increase in cell size and function, and atrophy, or loss of cell size and function.

Despite the presence of cellular adaptive mechanisms a state can develop when the capacity of a cell to respond is exceeded, and cellular injury results. In reversible injury the stress is mild and transient, but when stress is severe or persistent, the cell passes the "point of no return" and irreversible injury sets in. The morphologic end-point of such irreversible injury is cell death or necrosis. Thus adaptation, reversible injury, irreversible injury and cell death occur along a continuum in the cellular response to stress.

Every disease process is accompanied by some degree of existing of pre-existing cellular injury. The general manifestations of cell or tissue injury include metabolic, functional and morphologic alterations which are studied both in the course of Pathophysiology and Pathomorphology. However, all injured cells and tissues share certain common characteristics.

Cell damage can occur in many ways.

**Physical agents** responsible for cell and tissue injury include:

- Injury or trauma due to **mechanical force** split and tear tissue, fracture bones, injure blood vessels, and disrupt blood flow. It occurs as the result of body impact with another object. The result of the injury depends on factors such as the amount of force applied and the area which is damaged. In severe trauma, for example crush accidents direct mechanical injury is complicated by systemic effects such as hypovolemia (loss of blood volume), shock (peripheral circulatory failure) and renal failure.

Exposure to the extremes of heat and cold cause damage to the cell, its organelles, and its enzyme systems. Cellular injury or death occurs if tissue

temperature is maintained at over 5 degrees above or over 15 degrees below normal.

- **Exposure to heat** of low-intensity (43° to 46°C), causes cell injury by inducing vascular injury, accelerating cell metabolism, inactivating temperature-sensitive enzymes, and disrupting the cell membrane. It also leads to changes in fluid distribution in the cell nucleus, followed by nuclear swelling, rupture and condensation. The cytoplasm is first granular and then homogeneously coagulated. It manifests as partial-thickness burns and severe heat stroke. With more intense heat protein denaturation and thus coagulative necrosis occurs with typical clinical manifestation of burns. Burns may result from any external heat source (flame, hot liquids, hot solid objects, or, occasionally, steam). They also may be caused by radiation, chemicals, or electrical contact.

- **Exposure to cold** increases blood viscosity and induces vasoconstriction by direct action on blood vessels and through reflex activity of the sympathetic nervous system. The resultant decrease in blood flow may lead to hypoxic tissue injury, depending on the degree and duration of cold exposure. Hypothermia slows the cellular metabolism and ultimately leads to irreversible injury. Exposure to non-freezing temperatures can cause death without injury of single cells individual cells due to circulatory failure and pulmonary edema. Injury from freezing probably results from a combination of ice crystal formation and vasoconstriction. The decreased blood flow leads to capillary stasis and arteriolar and capillary thrombosis. Edema results from increased capillary permeability.

- **Electrical injuries** can also produce thermal injury, because tissue resistance causes electrical energy conversion to heat energy. It can affect the body through extensive tissue injury and disruption of neural and cardiac impulses. The effect of electricity on the body is mainly determined by its characteristics (voltage, the type of current, its amperage), the resistance of the intervening tissue, the pathway of the current, and the duration of exposure.

Lightning and high-voltage wires that carry several thousand volts produce the most severe damage. Alternating current is usually more dangerous than direct

current because it causes violent muscle contractions, preventing the person from releasing the electrical source and sometimes resulting in fractures and dislocations. In electrical injuries, the body acts as a conductor of the electrical current. The current enters the body from an electrical source, such as an exposed wire, and passes through the body and exits to another conductor, such as the moisture on the ground or a piece of metal the person is holding. The pathway that a current takes is critical because the electrical energy disrupts impulses in excitable tissues. Current flow through the brain may interrupt impulses from respiratory centers in the brain stem, and current flow through the chest may cause fatal cardiac arrhythmias.

The resistance to the flow of current in electrical circuits transforms electrical energy into heat. This is why the elements in electrical heating devices are made of highly resistive metals. Much of the tissue damage produced by electrical injuries is caused by heat production in tissues that have the highest electrical resistance. Resistance to electrical current varies from the greatest to the least in bone, fat, tendons, skin, muscles, blood, and nerves. The most severe tissue injury usually occurs at the skin sites where the current enters and leaves the body. After electricity has penetrated the skin, it passes rapidly through the body along the lines of least resistance—through body fluids and nerves. Degeneration of vessel walls may occur, and thrombi may form as current flows along the blood vessels. This can cause extensive muscle and deep tissue injury. Thick, dry skin is more resistant to the flow of electricity than thin, wet skin. It is generally believed that the greater the skin resistance, the greater is the amount of local skin burn, and the less the resistance, the greater are the deep and systemic effects.

- **Ionizing radiation** affects cells by causing ionization of molecules in the cell, directly hitting them, or by producing free radicals. Radiation exerts its effects by transferring its energy to the substance through which it passes. The energy absorbed by living cells ionize intracellular water which then dissociates into free radicals. This sets up a chain reaction with production of more free radicals. Finally the free radicals react with and damage cell membranes, nucleic acids and

enzymes, causing cell injury and death. The injurious effects of ionizing radiation depend on the dose of radiation. Because of the effect on DNA synthesis and interference with mitosis, rapidly dividing cells of the bone marrow and intestine are much more vulnerable to radiation injury than tissues such as bone and skeletal muscle. Over time, occupational and accidental exposure to ionizing radiation can result in increased risk for the development of various types of cancers.

- **Ultraviolet radiation** negative influence depends on the type of UVR, the intensity of exposure, and the amount of melanin in the skin. Ultraviolet rays produce the following effects: thermal effect; photochemical effect (oxidation of molecules, producing free radicals (e.g., through photodynamic effect mediated by photosensitizers); a very mild ionizing (or radicalizing) effect. The local action of ultraviolet rays is limited with the skin and the eyes because of the low penetrating properties of UV radiation in human tissues.

- **Barometric pressure changes**, like mild temperature changes are more injurious to the body as a whole than to individual cells. High atmospheric pressures are better tolerated than decreased atmospheric pressures. Injuries occur during the return from high to normal pressures: too rapid return causes dissolved nitrogen bubbles to resolve from the blood and these may cause death or disability. Decreased atmospheric pressure leads to hypoxia and peripheral vasoconstriction. Blood is shunted into the pulmonary circulatory, causing pulmonary hypertension, damage to alveolar capillary endothelium and pulmonary edema, which is potentially fatal.

- **Chemical agents** The list of injurious chemicals grows at an alarming rate. It include well-known poisons such as arsenic, cyanide, lead; environmental and industrial pollutants such as insecticides, carbon monoxide and chlorinated hydrocarbons; food preservatives and additives such as nitrites; recreational agents such as alcohol and tobacco; drugs, such as chloramphenicol, drugs of abuse such as heroin, cocaine, phencyclidine hydrochloride, Chemicals injure cells directly or indirectly. In direct injury, for example mercuric chloride poisoning, mercury combines with sulfhydryl groups in the plasma membrane and other proteins,

damaging membranes and vital enzyme systems. In indirect injury the toxic agent is not the chemical itself but an intermediate product of its metabolism. The toxic metabolite maybe directly toxic to cell membranes and enzymes as outlined above or indirectly toxic to these components through the generation of free radicals which can injure the cell membrane and other cell structures, block enzymatic pathways, coagulate cell proteins, and disrupt the osmotic and ionic balance of the cell. Corrosive substances such as strong acids and bases destroy cells as the substances come into contact with the body.

**Hypoxic injury** - an extremely important and common cause of cell injury and cell death. It usually accompanies local tissue decrease of oxygen content resulting from ischemia – inadequate organ or tissue blood supply due to decreased bloodflow in arteries. Hypoxia deprives the cell of oxygen and interrupts oxidative metabolism and the generation of ATP. The actual time necessary to produce irreversible cell damage depends on the degree of oxygen deprivation and the metabolic needs of the cell. Well-differentiated cells, such as those in the heart, brain, and kidneys, require large amounts of oxygen to provide energy for their special functions.

Hypoxia can result from an inadequate amount of oxygen in the air, respiratory disease, ischemia (i.e., decreased blood flow due to circulatory disorders), anemia, edema, or inability of the cells to use oxygen. Ischemia is characterized by impaired oxygen delivery and impaired removal of metabolic end products such as lactic acid. In contrast to pure hypoxia, which affects the oxygen content of the blood and affects all of the cells in the body, ischemia commonly affects blood flow through small numbers of blood vessels and produces local tissue injury.

**Injury from biological agents.** These agents range from submicroscopic viruses to the larger parasites. Biologic agents injure cells by diverse mechanisms. Viruses enter the cell and become incorporated into its DNA synthesis. Certain bacteria elaborate exotoxins that interfere with cellular production of ATP. Other

bacteria release endotoxins that cause cell injury and increased capillary permeability.

Pathogenic microorganisms include bacteria, viruses, fungi and others. Bacteria cause injury mainly through the release of harmful substance called toxins. Exotoxins are secreted by bacteria while endotoxins are part of their cell wall and are released when the bacteria die. Both *Staphylococcus pyogenus* and *Staphylococcus aureus* secrete exotoxins which damage cell membranes and kill neutrophils and macrophages; the toxin of *Corynebacterium diphtheriae* causes local tissue necrosis and formation of the characteristic pharyngeal membrane in diphtheria.

Viruses cause direct and indirect injuries. Direct cytopathic effects include cell lysis (fragmentation), cell fusion to form multinucleate giant cells and formation of inclusion bodies within infected cells. Viruses can alter cell membrane surface antigens so that immunologic reactions are activated against these cells and they are destroyed.

Compared to bacteria and viruses, fungi have limited pathogenicity because they produce neither exotoxin nor endotoxin and are only weakly antigenic. Hence fungal infections are generally limited to the skin and subcutaneous tissue. In recent years, however, the incidence of fungal infections has increased because of the widespread use of broad spectrum antibiotics and immunosuppressive drugs such as corticosteroids. In patients whose immune systems are compromised by disease or drugs, fungal infections tend to be deep seated and generalized, and often fatal.

Parasites comprise a large category of biologic agents ranging from protozoa such as *Entamoeba histolytica* to the large and complex helminths (worms). Parasites that complete part of their life-cycles in human hosts cause direct injury. The malaria parasite *Plasmodium* replicates in human red blood cells and destroys these cells. Other parasites incite allergic reactions which injure host tissues and impair organ function. An example is the hepatic fibrosis in chronic

schistosomiasis, called 'pipe-stem cirrhosis', which is an allergic reaction against schistosomae deposited in intrahepatic portal veins.

**Injury from nutritional imbalances.** Nutritional excesses and nutritional deficiencies predispose cells to injury. Obesity and diets high in saturated fats are thought to predispose persons to atherosclerosis. The protein and calorie deficiencies that occur with starvation cause widespread tissue damage. In many developing countries protein-calorie deficiency is one of the commonest causes of death, especially among infants. Closely related to calorie deficiency are vitamin, iron and trace metal deficiencies. Vitamin A deficiency, for example, causes corneal softening, scarring and blindness; vitamin B12 deficiency leads to peripheral nerve damage. One effect of vitamin E deficiency or deficiency of trace metal selenium; is the amplification of cellular injury due to toxic free radicals. Toxic free radicals are produced in a number of physiologic conditions such as ageing; and pathologic conditions such as radiation injury. Vitamin E is a natural antioxidant that scavenges free radicals and terminates the free radical-lipid chain reactions that can lead to membrane damage.

**Immunologic reactions.** Immunologic reactions are antigen-antibody reactions that are designed to protect an organism from harmful agents in the environment. Under certain conditions. immunologic reactions may be exaggerated, diminished or so altered that the outcome may be more harmful than beneficial to the organism.

An exaggerated immune response, or anaphylaxis, is a life-threatening situation. It occurs in hypersensitive individuals with administration of, foreign proteins such as penicillin. In immune deficiency states like AIDS on the other hand, the immune response is inadequate, and the patients succumb to infections by agents that are not normally pathogenic to man, such as *Pneumocystis carinii*

Immunologically mediated damage may also be the result of antibodies directed against the body's own tissues that have been altered and so become antigenic. This immune reaction against "self-antigens" is one of the mechanisms

underlying tissue injury in autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus.

### **Cell response to injury**

Cell injury is a disturbance of homeostasis typical for this cell, accompanied by limitation of its adaptation to the environment and shortening of its normal life duration.

There are **acute** (under the effect of strong irritants), and **chronic** cellular injuries, which, in their turn, can be **reversible and irreversible**. The example of **reversible injury** can be the injury of myocardial cells during stenocardia attack, caused by reflex ischemia of myocardium, or the injury of intestinal epithelium under the effect of microbial endotoxins. After cessation of damaging factor effect the homeostasis of the cell is completely restored.

In case of strong and durable effect the cellular injury becomes **irreversible** and results in the death of the cell. Irreversible cellular injuries can be caused by durable ischemia of myocardium, intoxication, etc. When the disturbance of homeostasis in an injured cell reaches the critical level, the death of the cell occurs.

The manifestation of cell injury can be **specific and non-specific**. The example of **specific features of injury**, which is typical for only one certain pathogenic agent, is immune hemolysis of erythrocytes as the result of anti-RBC antibodies synthesis in the organism. The example of **non-specific features** of cell injury is the depression of enzymes' function, cellular pumps, the disturbance of energy metabolism, water and electrolytes metabolism, ABB disorders, changes of intracellular organelles structure and function.

### **Features of cell injury**

In practical medicine the indices of cell injury are used for the diagnosing of different diseases, determining the functional condition of the injured tissue, evaluating the degree of viability of tissues, subjected to conservation and transplantation.



**Morphological signs of injury** manifest as swelling or shrinking of the cell, the disturbance of contact with adjoining cells and supporting structures. The breaking of tubes, vesicles and cisterns of endoplasmic reticulum, decrease of quantity of ribosome located on its membrane, accompany cellular injury. The swelling of mitochondrion may be observed, which is accompanied by the loss of double-structure of external mitochondrial membrane, its damage, and then – by complete destruction of mitochondrion. Nuclear and lysosome membrane damage can be observed too. Due to all these processes in the cell, changes of cell size (enlargement or decrease), shape, color and cell organelles occur.

Cell membrane in the early reversible stage of cell injury is characterized with increased plasma membrane permeability which leads to cell swelling, formation of cytoplasm blebs or protrusions; blunting and distortion of microvillus and degradation of the cytoskeleton. The further development of irreversible injury is associated with damage to the membranes of the subcellular organelles, as described below.

**Mitochondria.** In some forms of injury such as ischemia mitochondrial swelling succeeds plasma membrane damage. This is due to massive influx of calcium ions into the cytosol from the extracellular compartment. Mitochondria take in the calcium, together with fluid and become swollen. Irreversible injury is marked by progressive swelling, and finally rupture, of mitochondrial membranes.

Initial dilatation of the endoplasmic reticulum is followed by detachment of ribosome's and decreased protein synthesis with onset of lethal injury the endoplasmic reticulum fragments and myelin figures are formed.

In early reversible injury little change is observed, in lysosomes. Eventually, however, lysosomal membranes are damaged and enzymes leak into the cytoplasm. Lysosomal enzymes consist of nucleases, proteases phosphates, glycosidase. Activation of these enzymes leads to autodigestion of the cell components.

**Intracellular accumulation of substances** in abnormal amounts which indicate cell injury can occur within the cytoplasm or nucleus of the cell. Such substances can be accumulated: constituents of normal cell metabolism (lipids, proteins etc.), abnormal substances as a result of metabolism disorders (glycogen in glycogenoses), and pigments.

Pigments are colored substances that may accumulate in the cells under different conditions. Pigments may be endogenous or exogenous. Exogenous pigments derived from sources outside the body include: atmospheric pollutants such as carbon or coal dust; metals such as lead, which is deposited as a blue line on the gums; and fungi such as aspergillus and monilia, which produce black and white pigments respectively at the site of infection. A common form of exogenous pigmentation is tattooing, in which coarse pigment particles are forced into the skin for decorative purposes.

Endogenous pigments are produced by the body and include lipofuscin, melanin, hemosiderin and bilirubin. Lipofuscin or lipochrome is a yellow fat-soluble pigment which is normally present in the adrenal cortex, testes, ganglion cells and luteal cells. It is present in cytoplasm as fine granules. Lipofuscin is composed of polymers of lipids and phospholipids in complex with protein, suggesting that it is derived through lipid peroxidation of polyunsaturated lipids of subcellular membranes.

Lipofuscin is also known as the "wear and tear" pigment because of its association with ageing. It is present in excess in patients suffering from malnutrition or cancer cachexia. In these patients the brown discoloration from lipofuscin is usually accompanied by a decrease in size of organs. In the heart this change is known as "brown atrophy."

Melanin is a dark-brown pigment which is normally present in the skin, adrenal cortex, substantia nigra and meningeals. Melanin is derived from oxidation of tyrosine in melanocytes by the enzyme tyrosinase.

Increased melanin pigmentation occurs in many conditions such as: exposure

to sunlight and radiation, pregnancy, Addison's disease. In Addison's disease the adrenocortical insufficiency stimulates increased adrenocorticotrophic hormone (ACTH) from the anterior pituitary. ACTH has melanocytestimulating properties. In hemochromatosis the pigment is a mixture of melanin and hemosiderin. The pigment granules are within macrophages in the lamina propria and consist of melanin and lipofuscin. Benign and malignant tumors of melanocytes, such as nevi and melanomas contain melanin. In poorly differentiated malignant melanomas, however, melanin may only be demonstrable with electron microscopy.

Hemosiderin, a hemoglobin-derived, golden yellow-to-brown, granular or crystalline pigment is one of the major storage forms of iron. Iron is normally carried by specific transport protein called transferrin. In cells, it is stored in association with a protein, apoferritin, to form ferritin micelles. Ferritin is a constituent of most cell types. When there is a local or systemic excess of iron, ferritin forms haemosiderin granules, which are easily seen with the light microscope. Haemosiderin pigment represents aggregates of ferritin micelles. Under normal conditions small amounts of hemosiderin can be seen in the mononuclear phagocytes of the bone marrow, spleen, and liver, which are actively engaged in red cell breakdown.

Local or systemic excesses of iron cause hemosiderin to accumulate within cells. Local caresses result from hemorrhages in tissues. The best example of localized haemosiderosis is the common bruise. Extravasated red cells at the site of injury are phagocytosed over several days by macrophages, which break down the hemoglobin and recover the iron. After removal of iron, the heme mostly is converted first to biliverdin and then to bilirubin. In parallel, the iron released from heme is incorporated into ferritin and eventually hemosiderin.

Hemosiderosis is excessive hemosiderin in organs and tissues. It occurs in: prolonged and excessive iron administration, repeated blood transfusions, hemolytic anemia, and impaired iron utilization. In systemic hemosiderosis the pigment is mainly found in mononuclear phagocytes of the liver, spleen, lymph nodes and bone marrow. Parenchymal cells are only affected in severe cases.

Bilirubin is formed in reticuloendothelial cells from the breakdown of the heme portion of hemoglobin, it is transported in blood to albumin and is carried to the liver. Within hepatocytes bilirubin is conjugated with glucuronic acid. In this water-soluble form it is excreted into the bile canaliculi and ducts and eventually enters the extrahepatic biliary system. Elevations of bilirubin in the blood results in jaundice. Clinically jaundice is evident as yellowing of skin and sclerae..

**Functional signs of cellular injury:**

- Decrease of cell mobility (diagnosing test upon sperms mobility).
- Disturbance or interruption of cellular division (it is manifested as the development of atrophic processes in tissues).
  - Change of cellular membrane permeability to macromolecules (proteins, colloid stains), compounds with low molecular weight (amino-acids, glucose), and ions (is valuated by ability of cells to be stained with vital stains or neutral stains)
  - Appearance of cytoplasmic enzymes in blood as a result of rough injuries of cellular membranes. For example, during the injury of liver cells the release of aspartataminotranspherase (AST) into blood occurs, during injury of heart muscle cells – creatinphosphokynase (KPhK) and lactatdehydrogenase (LDG), which is widely used for diagnosing of myocardial infarction and its severity.
  - Injury is sometimes characterized by appearance of new, qualitatively different functions, which are not typical for non-injured cell. For example, mast cells and macrophages release leukotriens during allergic alteration. Leukotriens cause contraction of smooth muscles of bronchi; leukocytes under the effect of bacterial endotoxins release endogenous pyrogens, which cause the development of fever reaction.
  - Changes of biochemical processes in injured cells with release of suboxidized and toxic products of metabolism into blood.

Cell response depends on kind, severity, and duration of injury. These factors will determine the type of cell injury. Clinical effects of cell injury depend on kind of affected cell, its prior state of health, and what sort of adaptive mechanisms are

available to it. It may manifest at various levels of functional disturbances of organ/tissue or when the cell can not adapt to the consequences of it may be fatal for the whole tissue or organ

The cell components which are most sensitive to injury are: maintenance of cell membrane integrity, aerobic respiration, protein synthesis, genetic integrity. Cellular mechanisms are all interdependent, so no matter what kind of injury first occurs, many cell systems are affected.

Morphologic changes become apparent visually after they occur, with cell swelling being evident within minutes but deeper structural changes – genetic dysfunction, chromatin clumping, taking longer to observe.

**The main processes** of cell injury include the following:

- Decreased ATP production because of decreased cellular respiration is often caused by various stimuli either ischemia or toxins.

- Toxic oxygen radicals may cause cellular injury.

- Influx of calcium into cytosol activates phospholipases, proteases, ATPases and endonucleases which then act to further cellular injury. Calcium gradients are maintained by ATPase pumps.

- Mitochondrial injury: injury to mitochondrial membrane results in formation of nonselective inner membrane channel that disrupts normal proton gradient. Mitochondrial permeability transition may become permanent which indicates impending cell death. Cytochrome C may also be released into the cytosol playing a role in triggering apoptosis.

## **2. Hypoxic (ischemic) cell injury**

Oxygen plays a central role in cell injury. Lack of oxygen underlies the hypoxic cell injury, on the other hand activated oxygen species are important mediators of cell death in many pathologic conditions.

Hypoxic states in the tissues results from:

- loss of blood supply (ischemia) when the arterial flow or the venous drainage is impeded by vascular disease or thrombi; this is the most common cause

of hypoxia;

- inadequate oxygenation of blood (e.g. cardiorespiratory failure);
- loss of the oxygen-carrying capacity of blood, as in anemia or CO poisoning (that blocks oxygen transport).

**Ischemia** is the state of inadequate organ or tissue blood supply due to decreased bloodflow in arteries. It can be caused by:

- external reasons (the compression of artery by a ligature, tumor, foreign body, tourniquet).
- internal reasons (obstruction by thrombus or embolus).
- functional disorder – increase of spastic reactions under the influence of catecholamines, angiotensin, vasopressin.

The clinical manifestations of ischemia are:

1. Ischemic site turns pale.
2. Blood flow speed and temperature decrease.
3. Paresthesia (disorder of sensitivity)
4. Pain.
5. The decrease of blood oxygen level

The disorders of metabolism develop in the site of ischemia are the following:

- the decreasing of energy metabolism
- the decreasing of protein synthesis
- the disturbance of cells function

The most important consequence of ischemia is an infarction. The major determinants of infarction are:

1. the nature of blood supply
2. the rate of occlusion development
3. the vulnerability of tissues to hypoxia
4. the oxygen-carrying capacity of blood

1. The availability of an alternative or newly acquired source of blood supply is the most important factor determining whether occlusion of a vessel will

cause necrosis.

Many organs (lungs, liver, heart and arms) have blood supply from different arteries with numerous anastomoses so that occlusion of one artery can't cause tissue damage.

2. Infarction is usually caused by rapidly developing occlusions. If the occlusion is developing slowly it provides an opportunity for an alternative blood supply with collateral circulation and anastomoses opening.

3. The susceptibility of the tissues to hypoxia is different. Nervous tissue is the most sensitive to hypoxia. 3 –4 minutes of blood supply deprivation causes neurons damage. Myocardial cells are sensitive to hypoxia too. In contrast, the fibroblasts within the myocardium are not affected with hypoxia.

4. The oxygen level of blood is significant in determining ischemic damage too. The patients with cardiac decompensation are more vulnerable to infarction.

**Hypoxia** - (oxygen starvation) is a typical pathological process that may occur when the body is deprived of adequate oxygen supply or due to the lack of oxygen utilization that results in the disturbance of energy metabolism in the organism.

There are several classifications of hypoxic conditions.

Classification of hypoxia based on the causes of occurrence and mechanisms of development.

All hypoxia cases are divided into two big groups: hypoxias caused by exogenous reasons and hypoxias caused by endogenous reasons.

Exogenous hypoxia is another named (hypoxic hypoxia) it is caused by the decrease of the decreased of the partial pressure and percentage of oxygen content in inhaled air. It is subdivided into: hypobaric and normobaric types.

Endogenous hypoxia is divided into 6 types: respiratory, hemic, circulatory, histotoxic, substrate and overload types.

Classification of hypoxia based on the time of appearance and duration of hypoxia features.

1. Fulminant (immediate) – ends up with the death of organism after

several seconds (histotoxic hypoxia during cyanide poisoning).

2. Acute – lasts for several minutes (cardiac arrest, respiratory standstill).
3. Subacute – lasts for several hours or days (during extreme conditions and pathologies that threaten life).
4. Chronic – lasts for months and years.

Classification of hypoxia based on the prevalence of clinical symptoms—local and general.

Classification of hypoxia based on the severity of pathological process: a) light; b) moderate; c) severe; d) critical (lethal) hypoxia.

**Hypoxic hypoxia hypobaric type** develops during the decrease of barometric pressure that is accompanied with decrease of  $P_{O_2}$ . This condition leads to the development of altitude or mountain sickness. It commonly occurs at the altitudes above than 2,500 metres

Mountain sickness develops after getting to high altitudes in mountains. The factors contributing to mountain sickness development are: low partial pressure of oxygen, low barometric pressure, physical loading, cooling, increased exposure to UV rays.

Altitude sickness, also known as acute (AMS) or altitude illness is a pathological condition that is caused by acute exposure to high altitudes. It may develop in opened aircrafts or after rapid depressurization of closed aircrafts. The main pathogenic factors in this case are: low barometric pressure and low partial pressure of oxygen

**Normobaric type of hypoxic hypoxia** occurs as a result of  $O_2$  percentage in air decrease without changes of barometrical pressure. It may develop:

- when persons are situated in small rooms with bad ventilation (elevators, mines, mineshafts);
- in divers, when there are problems with aqualung function;
- incorrect conduction of artificial lungs ventilation during surgical operations.

The leading pathogenic mechanisms of exogenous hypoxia (in spite of its



cause) are: low amount of oxygen and carbon dioxide in the blood, disturbances of acid-base balance (ABB), low ABP.

The starting point of exogenous hypoxia is the decreasing of blood oxygen concentration. It results in low oxygen saturation of the hemoglobin and disturbances of gaseous exchange and metabolic processes in the tissues of the organism.

Carbon dioxide concentration in the blood (hypocapnia) is reduced due to compensatory hyperventilation in the lungs. Hypocapnia leads to ABB disturbances that manifests as gaseous alkalosis. Low carbon dioxide concentration in the blood also results in compensatory constriction of brain and heart blood vessels that only worsens brain and heart blood supply and can result in the disturbances of vital functions, such as fainting of myocardial ischemia.

**Respiratory hypoxia** rises as a result of respiratory insufficiency due to: alveolar hypoventilation, disturbances of lungs blood supply, disturbances of gases diffusion in lungs.

Alveolar hypoventilation is a state in which there is a reduced amount of air entering the pulmonary alveoli. This may occur as a result of impaired lungs function due to obstructive and restrictive violations of lungs ventilation.

The reasons of obstructive violations are: edema, tumors or foreign bodies in the lumen of bronchi and bronchioles.

The reason of restrictive violations is decreased lungs tissue elasticity due to chronic inflammatory or sclerotic processes in the lungs and in the chest.

Alveolar hypoventilation may also be caused with the disturbances of respiratory regulation (toxic substances poisoning, brain trauma and others).

Disturbances of lungs blood supply may occur as a result of heart failure, decreased circulating blood volume (after blood loss).

Disturbances of gases diffusion in lungs is observed in non-specific chronic inflammatory diseases of the lungs, lungs edema.

The development of respiratory hypoxia is accompanied by hypoxemia and decreased hemoglobin saturation with oxygen. Blood level of carbon dioxide is

increased - hypercapnia, pH of the blood is low – acidosis.

**Circulatory hypoxia** (or cardio-vascular) arises during the disturbance of blood circulation due to heart and vessels pathology that lead to insufficient blood supply of organs and tissues. The decrease of blood quantity that flows through tissues per time unit can be caused by:

decrease of heart activity (infarction, cardiosclerosis, myocarditis - these are all causes that decrease the cardiac output);

hypovolaemia - abnormally low intravascular volume with a decreased volume of circulating blood in the body (severe blood loss, dehydration of the organism after burns, cholera, vomiting, etc.);

vascular disorders that manifest as low vascular tone (shock, collapse, aldosterone deficiency).

Circulatory hypoxia may be local due to insufficient blood supply of the organ or tissue (ischemia) or the difficulty of venous outflow (venous hyperemia, stasis) or systemic (caused by the above mentioned reasons)

The characteristics of blood gas content are the following: normal O<sub>2</sub> pressure and its content in arterial blood, the decrease of these indices in venous blood. Usually non-gaseous acidosis develops as a result of circulatory hypoxia.

**Hemic hypoxia** (blood hypoxia) may be connected with Hb quantity or inhibition of its functions. It is observed during anemia of different genesis (anemic type) and also during poisoning with carbon monoxide, nitrates, sulfa drugs and other substances that yield in methemoglobin formation. Methemoglobin is a form incapable of carrying oxygen. In this case we will observe inactivation type of hemic hypoxia.

Expressed symptoms of hypoxia in anemic patients develop only during considerable absolute mass decrease of erythrocytes or acute decrease of Hb content in erythrocytes. Such anemias arise during the exhaustion of bone marrow on the basis of chronic bleedings (tuberculosis, stomach ulcer), erythrocytes hemolysis (during hemolytic toxine poisoning, severe burns, malaria), during erythropoiesis depression by toxic factors (lead, radiation, deficiency of iron and

vitamin B<sub>12</sub>), etc. During carbon monoxide poisoning that has relation to Hb 300 times higher than oxygen, the formation of resistant compound occurs – carboxy-Hb (HbCO). Even the low concentration of CO (less than 0.1%) in inhaled air turns 50% of Hb into HbCO. The gas is especially dangerous because it is not easily detected by human senses. Early symptoms of carbon monoxide poisoning include drowsiness and headache, followed by unconsciousness, respiratory failure, and death.

The indices of gases in the blood are the following: arterial and venous hypoxemia, non-gaseous acidosis.

**Histotoxic hypoxia** is the inability of cells to take up or utilize oxygen from the bloodstream, despite physiologically normal delivery of oxygen to such cells and tissues. Histotoxic hypoxia results from tissue poisoning, such as that caused by alcohol, narcotics, cyanide (which acts by inhibiting cytochrome oxidase), and certain other poisons.

The decreased effectiveness of oxygen utilization by the cells usually is the result of biological oxidation enzymes inhibition, the disturbance of their synthesis or the damage of membrane structures of the cell. Cyanide poisoning can be a typical example of histotoxic hypoxia caused by specific inhibitors of tissue enzymes.

Cyanide ions bind to the iron atom of the enzyme cytochrome c oxidase in the mitochondrial membrane of cells. This deactivates the enzyme and the final transport of electrons from cytochrome c oxidase to oxygen can not be completed. As a result, the electron transport chain is disrupted, meaning that the cell can no longer produce ATP for energy. Tissues that mainly depend on aerobic respiration, such as the central nervous system and the heart, are particularly affected.

One of the causes of histotoxic hypoxia can be the disturbance of respiratory enzymes synthesis as a result of certain vitamins deficiency (thiamin, riboflavin, pantothenic acid).

The disturbance of oxidation processes occurs because of the damage of mitochondrial membranes and other cellular elements, which is observed during

radiation injury, over-heating, intoxication, infection, cachexia, uremia, etc.

During histotoxic hypoxia connected with the tissues inability to utilize  $O_2$ , the pressure, saturation and content of  $O_2$  in blood can stay normal for some time.

A peculiar variant of histotoxic hypoxia rises during acute dissociation of processes of oxidation and phosphorylation in respiratory chain. Consumption of  $O_2$  by tissues can increase, but the significant part of energy is dispersed in the form of heat, which leads to energetic “depreciation” of tissue respiration. Relative insufficiency of biologic oxidation arises, when ATP re-synthesis does not cover tissue needs for energy, despite high intensity of respiratory chain functioning. The agents that dissociate processes of oxidation and phosphorylation are wide range of substances of exogenous and endogenous origin: hormones of thyroid gland, excess of Ca, toxins, etc. In a healthy organism, thyroid hormones - thyroxine and triiodothyronine – carry out the function of physiologic regulator of the association of oxidation and phosphorylation degree, together with other functions.

**Overload hypoxia** arises during excessively strained activity of certain organ or tissue, when functional reserves of transport system or utilization of  $O_2$  or substrates are insufficient to supply acutely increased demands, even without any pathologic changes in these systems. Significant oxygen debt, venous hypoxemia and hypercapnia are typical for overload hypoxia.

This form of hypoxia has its practical meaning as applied to heavy loads of muscular organs: skeleton muscles and myocardium. If the load is excessive, the relative coronary insufficiency, local heart hypoxia and secondary general circulatory hypoxia arise. If muscular work is excessive, skeleton muscles hypoxia is accompanied with the increased bloodflow in the muscles. These events lead to ischemia of other tissues and development of the widely distributed hypoxia.

**Substrate hypoxia.** In absolute majority of cases hypoxia is connected with the insufficient transport or the disturbance of  $O_2$  utilization. In normal conditions the substrate reserve is big enough and very much exceeds the  $O_2$  reserve. But in some cases, when oxygen supply is normal, the state of membrane and enzyme systems is normal; the primary substrate deficiency leads to the disturbance of

biological oxidation. Such hypoxia is usually connected with glucose deficiency in tissues. Thus, the cessation of glucose supply of the brain leads to the death of the most sensitive nervous cells in 5-8 minutes. Carbohydrate starvation and hypoxia of insulin-dependent tissues often occur during diabetes mellitus and other disturbances of carbohydrate exchange.

**Combined hypoxia** is observed most frequently and presents a combination of 2 and more main types of hypoxia. In some cases the hypoxic factor itself affects several links of O<sub>2</sub> transportation system and utilization. For example, CO actively binds to Fe<sup>2+</sup> of hemoglobin, but increased concentrations of it also cause direct toxic effect upon the cell, inhibiting cytochrome oxydase system. Nitrites can also dissociate oxidation-phosphorylation processes along with metHb formation.

In some cases primarily arising hypoxic condition inevitably causes the disturbance of different organs and systems functions that participate in O<sub>2</sub> supply and utilization. During severe hypoxia caused by the insufficiency of external respiration the function of nervous centers regulating vessels tone and conducting system of the heart is disturbed. It results in decreased heart contractions and increased vessels permeability. Thus respiratory hypoxia is exacerbated with additional circulatory hypoxia. Practically every severe hypoxic condition has combined character.

### **Disturbances in the Organs and Physiological Systems**

The first signs of oxygen deprivations are the disturbances in the nervous system. First, euphoria occurs. It is characterized by the emotional and motion excitation, inadequate behavior. The cause of it is the disturbance of the brain internal inhibition.

In prolonged hypoxia the metabolic and functional changes in the nervous system are more severe. The reflex activity of the nervous system is disturbed; the regulation of breathing and blood circulation is impaired too. Loss of consciousness and convulsions are the symptoms of oxygen deprivation.

The disturbances in other organs are interconnected with the disturbed

nervous regulation, energy deprivation and the accumulation of toxic metabolic products.

As to sensitivity to oxygen deprivation, the cardiac muscle takes the second place after the nervous system. The clinical manifestations of the disturbed excitability, conduction and contraction of the myocardium are tachycardia and arrhythmia. Cardiac insufficiency and the reduced vascular tone lead to hypotension and general disturbance of blood circulation.

The disturbance of the external breathing manifests as the disturbance of the lung ventilation. The periodical Chain-Stocks' breathing appears. The development of congestive processes in the lungs leads to decreased diffusion of oxygen from the alveolar air to blood, that impairs hypoxic condition.

The disturbances of kidneys function include qualitative and quantitative changes in the urine.

The disturbance of liver function is usually observed in the cases of chronic hypoxia. It manifests as disturbance of carbohydrate, lipid, protein and vitamin metabolism, inhibition of antitoxic activity of the liver, decreased synthesis of various substances (clotting factors, bile acids).

The disturbances of GIT organs include violation of appetite, inhibition of peristalsis and secretion in the stomach and intestines, development of erosions and ulcers.

Chronic hypoxic states are also accompanied with the decreased activity of the immune system, which manifests as low functional activity of the immune cells, low effectiveness of the innate immunity factors (complement, interferons, natural killers).

**Hypoxic (ischemic) injury** on the cellular level can be reversible and irreversible.

**Reversible injury.** If oxygen deprivation is of short duration the effects of hypoxia are reversible on rapid restoration of circulation (e.g. ischemic heart disease resulting from coronary artery occlusion). The sequence of event is as follows:

**1. Decreased oxidative phosphorylation by mitochondria and generation of ATP.** ATP is needed for almost all cellular functions such as synthesis of proteins and lipids, membrane transport. The decrease of ATP has widespread effects on many systems within the cell.

**2. Damage to plasma membrane sodium pump.** The lack of ATP results in the decreased activity of the cell membrane ATPase, causing failure of the active membrane "sodium pump". The consequence of this event is intracellular accumulation of sodium, and diffusion of potassium out of the cell. This process is accompanied by an iso-osmotic gain of water, which results in acute cellular swelling.

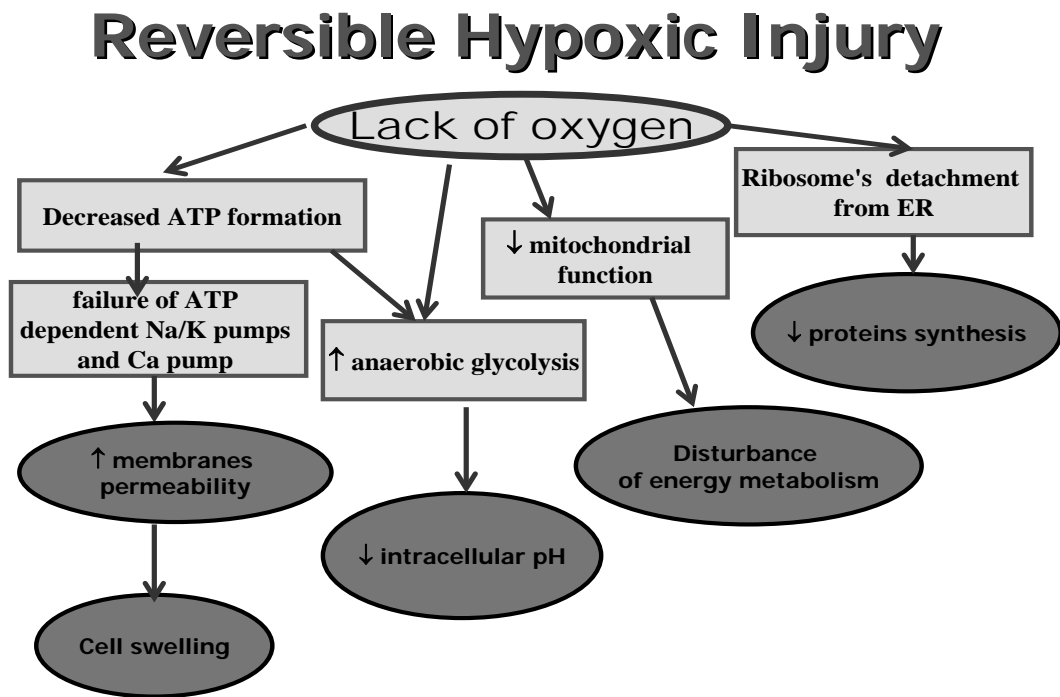


Figure 1. Reversible hypoxic injury

**3. Reduced intracellular pH.** The decrease in cellular ATP and associated increase in AMP result in an increased rate of anaerobic glycolysis. Limited glycogen cell's store is thus rapidly depleted and lactic acid is accumulated in the cell lowering the intracellular pH.

**4. Reduced protein synthesis** occurs due to detachment of ribosomes from

the granular endoplasmic reticulum and dissociation of polysomes into monosomes as a result of continued hypoxia. If hypoxia continues, functional consequences and ultrastructural changes may occur.

All the above disturbances are reversible if oxygenation is restored. However, if ischemia persists, irreversible injury occurs.

**Irreversible injury.** The actual time necessary to produce irreversible cell damage depends on the degree of oxygen deprivation and the metabolic needs of the cell. Well-differentiated cells, such as those in the heart, brain, and kidneys, require large amounts of oxygen to provide energy for their special functions. Brain cells begin to undergo permanent damage after 4 to 6 minutes of oxygen deprivation.

## Irreversible Hypoxic Injury

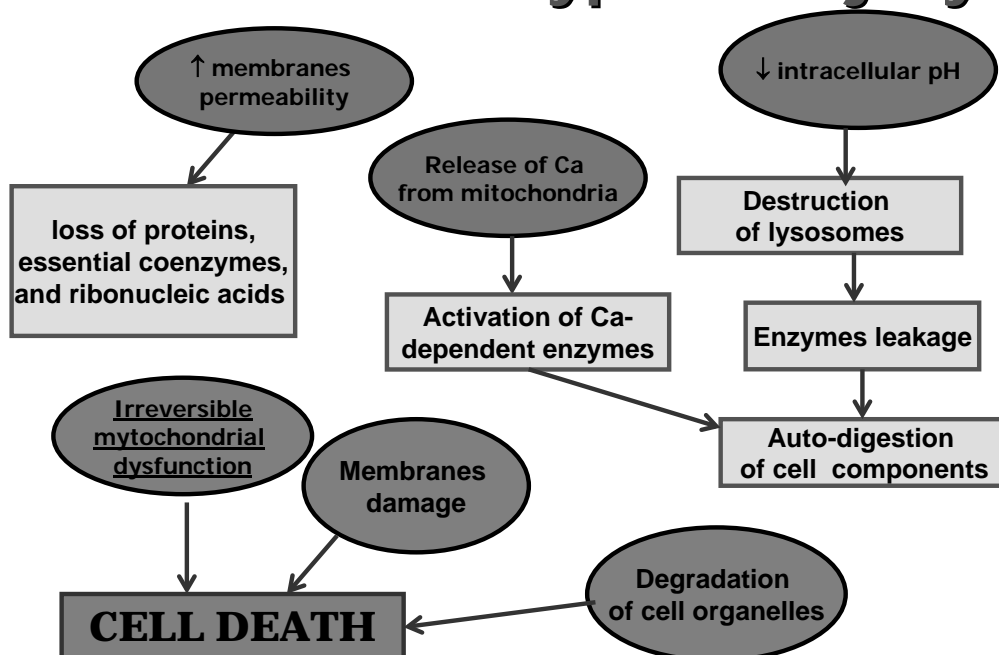


Figure 2. Irreversible hypoxic injury

Two essential phenomena always distinguish irreversible cell injury:

- **inability of the cell to reverse mitochondrial dysfunction** upon reperfusion or reoxygenation;
- **profound disturbance in cell membrane function.**



**Mitochondrial dysfunction.** Irreversible injury is characterized with severe vacuolization of the mitochondria, including their cristae and deposits of amorphous Ca salts in the mitochondrial matrix.

The mechanisms underlying **membrane damage** are following:

- **Accelerated degradation of membrane phospholipids.** Oxygen starvation is known to release calcium sequestered in mitochondria and endoplasmic reticulum, thus raising cytosolic calcium which activate endogenous phospholipases. The latter progressively degrade membrane phospholipids which are the main constituent of the lipid bilayer membrane

- **Cytoskeletal damage.** In the presence of cell swelling, which occurs in ischemia, injury of the cytoskeleton may result in detachment of the cell membrane from the cytoskeleton, rendering the membrane susceptible to stretching and rupture. A potential mechanism in cytoskeletal protein degradation is the activation of intracellular proteases, possibly induced by increased cytosolic calcium or by physical effect of cell swelling.

- **Toxic oxygen radicals.** Partially reduced oxygen species cause injury to cell membranes and other cell constituents. Such oxygen radicals are increased in ischemic tissues upon restoration of blood flow and may be the cause of the so-called reperfusion injury.

- **Lipid breakdown products.** These catabolic products accumulate in ischemic cells as a result of phospholipid degradation and cause further damage to membranes.

There is also continued loss of proteins, essential coenzymes, and ribonucleic acids from the hyperpermeable membranes, conversely, entry of extracellular macromolecules from the interstitial space into the cell occur. The cells may also leak metabolites, which are vital for the reconstitution of ATP, thus cell's energy store will be further depleted. The falling pH leads to injury to the lysosomal membranes, followed by leakage of their enzymes into the cytoplasm and enzymatic digestion of cytoplasmic and nuclear components. Finally, the dead cell may become replaced by large masses, composed of phospholipids. These are then

either phagocytosed by other cells or degraded further into fatty acids.

### **Reperfusion injury**

Depending upon the duration of ischemia (hypoxia) reperfusion may result in the following consequences:

1. When the period of ischemia is of short duration reperfusion restores the structural and functional state of injured cell.

2. When ischemia is for longer duration reperfusion deteriorates the already injured cell. The following mechanisms underlie further cell injury:

- circulation brings neutrophils to re-perfused tissues that release toxic oxygen radicals that do injury to membranes. Damaged cells may express cytokines that attract more neutrophils to them and cause inflammation with additional injury;

- reperfusion brings a massive influx of calcium which leads to activation of phospholipases, endonucleases, proteases, and DNAases. The result of above mentioned enzymes action is progressive destruction of all cell structures.

3. Longer period of ischemia may produce irreversible cell injury during ischemia itself without any role of reperfusion

### **3. Injury by free radicals**

A free radical is a chemical species that has a single unpaired electron in an outer orbital. In this state, the radical is highly unstable and can enter the reactions with key molecules in cell membranes and nucleic acids. Moreover, free radicals can establish chain reactions, as the molecules they react with in their turn form free radicals. Chain reactions may branch, causing even greater damage.

Free radicals may be generated in the following ways:

- By absorbing radiant energy (UVR, x-rays).
- It may be a byproduct of energy generation, breakdown of lipids and proteins, and inflammatory processes (free radical generation is the main mechanism for killing microbes by phagocytic WBC).
- As part of the metabolism of drugs and poisons.

The most important free radicals are the following:

- Superoxide ( $\bar{O}_2$ ) anion may be generated by direct autooxidation of  $O_2$  during mitochondrial electron transport reaction, or it can be produced enzymatically by xanthine oxidase and cytochrome  $P_{450}$

- Hydroxyl radical ( $OH\cdot$ ) is formed by radiolysis of water and by reaction of hydrogen peroxide with ferrous ions.

- Hydrogen peroxide ( $H_2O_2$ ) is reduced to water enzymatically by catalase and glutathione peroxidase.

The effects of these reactive species are wide-ranging, but four reactions are particularly relevant to cell injury.

**1. Lipid peroxidation of membranes.** Free radicals in the presence of oxygen may cause peroxidation of polyunsaturated fatty acids in cell membranes. Such fatty acids possess double bonds between some of the carbon atoms. Such bonds are vulnerable to attack by free radicals. The lipid-radical interactions yield lipid peroxides, which are themselves unstable and reactive too, then chain reaction of oxidation starts, which results in extensive membrane, organellar, and cellular injury.

**2. Nonperoxidative mitochondrial injury.** This effect is not dependent on lipid peroxidation and results in loss of mitochondrial function, mimicking the effects of hypoxia on mitochondria.

**3. Lesions in DNA.** Free radicals cause breaks in the single strands of the DNA. Such DNA injury may be the cause of cells death and their malignant transformation.

**4. Oxidation of proteins.** Oxygen-derived free radicals cause cell injury by oxidation of protein macromolecules, cross linking of such labile amino acids as methionine, histidine, cystine, and lysine. The end result is degradation of cytosolic enzymes and cell destruction.

The effect of free radicals upon the organism depends on the degree of their initiation and their elimination. Stress, aging, polluted air, cigarette smoke and

excessive UV radiation can add to the number of free radicals in the body. Some of the free radicals may spontaneously decay. Superoxide, for instance, is unstable and decays automatically into oxygen and hydrogen peroxide. In other cases free radicals are neutralized by specific substances, named antioxidants. Thus cells are more or less vulnerable to free radical injury, depending on the presence and quantity of antioxidants that serve as protective mechanisms.

**Antioxidants** are the substances that prevent or slow the breakdown of another substance by oxygen. Antioxidative substances are divided into enzymatic and non-enzymatic ones.

Enzymatic antioxidants include:

- The **thioredoxin system**, including thioredoxin and thioredoxin reductase. In its active state, thioredoxin acts as an efficient reducing agent, scavenging reactive oxygen species and maintaining proteins in their reduced state. After being oxidized, the active thioredoxin is regenerated by the action of thioredoxin reductase.

- The **glutathione system**, including glutathione, glutathione reductase, and glutathione peroxidase. Glutathione peroxidase catalyze the breakdown of hydrogen peroxide and protects lipids in cell walls from peroxidation.

- **Superoxide dismutase**, a class of closely related proteins found in almost all living cells and in extracellular fluids. Superoxide dismutase protects cells by catalysing the breakdown of the highly reactive superoxide anion into oxygen and hydrogen peroxide.

- **Catalase**, catalyses the conversion of hydrogen peroxide to water and oxygen at rates of up to 6,000,000 molecules per minute. Catalase can also oxidise toxins including formaldehyde, formic acid and alcohols.

The leading role in non-enzymatic anti-oxidation is played by vitamins.

- **Vitamin A** (Retinol), also synthesized by the body from **beta-carotene**, protects dark green, yellow and orange vegetables and fruits from solar radiation injury, and is thought to play a similar role in the human body. Carrots, broccoli,

sweet potatoes, tomatoes, kale, peaches and apricots are particularly rich sources of beta-carotene.

- **Vitamin C** (Ascorbic acid) is a water-soluble compound that fulfills several roles in living systems. Important sources include citrus fruits (such as oranges, sweet lime, etc.), green peppers, broccoli, green leafy vegetables, strawberries, blueberries, raw cabbage and tomatoes.

- **Vitamin E** is fat soluble and protects lipids from oxidation. Sources include wheat germ, nuts, seeds, whole grains, green leafy vegetables, vegetable oil, and fish-liver oil.

Another group of non-enzymatic antioxidants include **vitamin cofactors and minerals**

- **Coenzyme Q<sub>10</sub>** is an antioxidant which is both water and lipid soluble. It is not classified as a vitamin in humans as it can be manufactured by the body, but quantities decrease with age to levels that may be less than optimal, and levels in the diet are generally low. Supplementation with CoQ<sub>10</sub> has been clinically proven to improve the health of gums.

- **Selenium** must be taken in measured amounts because large doses of the element can be toxic. Good food sources include fish, shellfish, red meat, grains, eggs, sunflower seeds, chicken, turkey, garlic.

- **Zinc** may safeguard red blood cell membranes against oxidative effects of other minerals such as copper or iron.

Antioxidative properties are also peculiar to carotenoids and bioflavonoids.

**Carotenoids** (Lycopene, Lutein, Alpha-carotene, Beta-carotene) are organic pigments that are naturally occurring in plants. In photosynthetic organisms, carotenoids play a vital role in the photosynthetic reaction centre. They either participate in the energy-transfer process, or protect the reaction center from auto-oxidation. In non-photosynthetic organisms, carotenoids have been linked to oxidation-preventing mechanisms.

**Bioflavonoids**, a subset of polyphenol antioxidants, are present in many dark berries, as well as in certain types of coffee and tea, especially green tea.

#### 4. Injury by chemicals

Chemicals induce cell injury by one of two mechanisms:

**1. Direct cytotoxic effect.** Some chemicals can act directly by combining with components of the cell and produce cytotoxicity without requiring metabolic activation. The cytotoxic damage is usually the greatest to cells which are involved in the metabolism of such chemicals. For example, in mercuric chloride poisoning, mercury produces the greatest damage to the cells of alimentary tract and kidney. Cyanide affect the cell by blocking cytochrome oxidase and breaking oxidative phosphorylation. Many antineoplastic chemotherapeutic agents and antibiotic drugs also induce cell injury by direct cytotoxic effects.

**2. Conversion to reactive toxic metabolites.** Most other toxic chemicals are not biologically active but can be converted to reactive toxic metabolites. Although these metabolites might cause membrane injury and cell injury by direct binding to membrane protein and lipids, the most important mechanism of cell injury involves the formation of reactive free radicals and subsequent lipid peroxidation.

The above mentioned mechanisms of cell injury can produce sublethal and reversible cellular damage or lead to irreversible injury with cell destruction or death.

Classification of chemical agents due to the target organ affected:

- Agents affecting the hematological system affect hematopoiesis and causing leukemia development (leukemogenic agents);
- Immunotoxic agents may affect at any step of immune reaction from antigen presentation to antibodies production;
- Hepatotoxic agents affect liver function and even may cause destruction of hepatic cells(vinyl chloride, carbon tetrachloride);
- Nephrotoxic agents influence may result in ultrastructural damage to any of the principal components of the nephron (mercury);
- Pulmonary toxic agents induce pathological changes in the lungs (tobacco

smoking, air pollution, fumes of chlorine);

- Neurotoxins may affect any aspect of the central, autonomic, and peripheral nervous systems including neurotransmission;
- Agents affecting the cardiovascular system – a variety of therapeutic drugs have adverse effects on cardiovascular system;
- Dermatotoxic agents affect skin function and components;
- Agents affecting the endocrine and reproductive system have the potential for disrupting hormonal pathways, either by site-specific toxicity at the endocrine level, or by interference with the feedback mechanisms.

Another classification of potentially toxic chemicals is according to the use in the human society.

Pesticides are chemicals used to kill harmful animals or plants. Pesticides are used especially in agriculture. Some are harmful to humans, either from direct contact or as residue on food, or are harmful to the environment because of their high toxicity, such as DDT (which is now banned in many countries). Pesticides include fungicides, herbicides, insecticides, and rodenticides.

Direct food or color additives are intentionally incorporated in food and food processing for the purpose of changing, enhancing, or masking color. They are also used for a variety of functionalities ranging from anticaking agents to stabilizers, thickeners, and texturizers. This area falls within the field of food toxicology, and the reader is referred to any of the review articles concerning food ingredients and contaminants (substances which make food impure or unclean).

Toxicological classification of therapeutic agents follows their pharmacological mechanisms of action or their principal target organs of toxicity. It is the subject of clinical pharmacology study.

According to the next classification, chemical may be of botanical (plant poisons) and environmental source. As a result of industrialization, many chemicals are associated and classified according to their continuous presence in

the environment — i.e., water, land, and soil. For example a problem of air pollution includes outdoor and indoor air pollution, presence of atmospheric sulfuric acid, airborne particulate matter, interaction of photochemicals with the environment, and chemicals found in smog (a fog that has become mixed and polluted with smoke and chemical fumes).

#### Exposure to toxins

The route of chemical exposure may be oral, intranasal, parenteral and inhalation. In the proper circumstances, any chemical has the potential for toxicity — i.e., the same dose of a chemical or drug may be harmless if limited to oral exposure but toxic if inhaled or administered parenterally. Thus, the route and site of exposure have a significant influence in determining the toxicity of a substance. More frequently, a therapeutic dose for an adult may be toxic for an infant or child. Similarly, a substance may not exert adverse effects until a critical threshold is achieved. Thus, in order to induce toxicity, it is necessary for the chemical to accumulate in a physiological compartment at a concentration sufficiently high to reach the threshold value. Finally, repeated administration, over a specific period of time, also determines the potential for toxicity.

Acute exposure: In general, any exposure less than 24 h may be regarded as acute. Exposure to most toxic gases requires less than 24 h for toxicity (carbon monoxide, hydrogen cyanide). A single intravenous injection of a chemical is certainly classified as an acute exposure.

Chronic exposure is any relative time period for which continuous or repeated exposure is required for the chemical to induce a toxic response.

Accumulation. A chemical, once absorbed, can distribute and/or bind to one or more of the many physiological sites. The chemicals may be accumulated in whole blood, serum and serum proteins, plasma and plasma proteins, adipose tissue, interstitial and extracellular fluids, alveolar air space, and bone marrow. In addition, any tissue or organ may preferentially accumulate a chemical, thus acting as a discrete compartment. For instance, heavy metals are preferentially



accumulated in adipose tissue. Consequently their toxicity may be experienced for prolonged periods of time as the compounds are slowly released from this compartment, years after exposure has ceased.

Accumulation is determined by the chemical's structure and its interaction within the physiological compartment. In general, at physiological pH, lipid-soluble compounds will be preferentially accumulated in membranes of tissues and organs. Conversely, watersoluble compounds are less prone to tissue binding, the ions are readily available for renal secretion and elimination.

#### Effects of toxins

In general, the effects of most drugs or chemicals are reversible until a critical point is reached — i.e., when vital function is compromised or a teratogenic or carcinogenic effect develops.

Teratogenicity is the ability of toxin to cause defects in a developing fetus. This is distinct from mutagenicity, which causes genetic mutations in sperms, eggs or other cells. Teratogenicity is a potential side effect of many drugs, such as thalidomide. Thalidomide is a sedative drug that when taken between 3rd and 5th week of pregnancy produces a range of malformations of the fetus, in severe cases complete absence of limbs (amelia) or much reduced limb development (phocomelia).

Carcinogenicity is the ability of chemical substances to cause the development of tumor in the organism. Such effects upon the cells of the organism are caused by polycyclic aromatic hydrocarbons, amines, azo compounds, carbamates, etc. In fact, the carcinogenic effect of chemicals, such as those present in tobacco smoke, may be delayed for decades until irreversible cellular transformation occurs.

Reversibility of a chemical effect may be enacted through the administration of antagonists, by enhancement of metabolism or elimination, by delaying absorption, by intervening with another toxicological procedure that decreases toxic blood concentrations, or by terminating the exposure.

The degree of chemical influence is related to the particular dose of it, i.e. the more is the dose the more effect is manifested. For some chemicals LD50 (lethal dose) was established in the experiments on laboratory rodents. The LD50 is a statistically calculated dose of a chemical that causes death in 50% of the animals tested.

Local or systemic effects of a compound depend on site of exposure. The skin or lungs are frequent targets of chemical exposure, since these organs are the first sites of contact with environmental chemicals. Oral exposure requires absorption and distribution of the agent prior to the development of systemic effects.

#### Local effects of chemicals

Chemicals applied on the skin may result in severe local injury in a form of chemical burn. Acid burns produce coagulation necrosis of the skin by denaturing proteins, forming a coagulum (e.g., eschar) that limits the penetration of the acid in deeper tissues. Expressed acidosis, coagulation of proteins occurs, and expressed accumulation of Ca with aggregation of cytoskeleton elements develops in the cell under influence of acids. Necrosis sites are easily calcificated. Nucleus is destroyed, proteolysis is inhibited. The mechanism of this process is connected with the fact that protein molecules have mainly negative electric charge. Therefore interacting with H<sup>+</sup> ions of acids the charge of proteins is neutralized. It leads to coagulation (precipitation and condensation) of proteins. This type of necrosis is also provoked by severe hypoxia in myocardial cells during infarction. It is considered to be typical for tissues rich in proteins and Ca and assumes early and serious damage of mitochondrions.

Acid burns are less severe than alkali burn, which produce liquefactive necrosis. This involves denaturing of proteins as well as saponification (conversion of fats into soap) of fats, which does not limit tissue penetration. Hydrolytic processes of lysosomal autolysis predominate in this type of necrosis. The tissue softens; the process of coagulation and formation of fibrin is less expressed; significant accumulation of active hydroxyl radicals and endogenous

saponification that disrupts cellular membranes are observed. This type of necrosis is accompanied by less significant Calcium excess in cells. It is observed in the brain tissue during stroke and in other tissues rich in lipids.

## **5. Pathology of cell signalling**

When cells communicate with each other then cells communicate with each other, the one that sends the signal is referred to as the signalling cell and the cell receiving the signal is the target cell. Transmission of the information may occur either by the secretion or presentation of signalling molecules, which contact receptors on the target cell membrane (or intracellular).

According to the distance between the signalling cell and the target cell, the humoral pathway of regulation can be graded like this.

1. Endocrine — regulatory substances (in this case called hormones) are produced by the specialized tissue, secreted to the blood, and delivered to the remote target cells throughout the body (e.g. beta-cells of the islets of Langerhans produce insulin that regulates glucose absorption by the majority of the cells in the whole organism).

2. Paracrine — regulatory substances produced by some cell act only on the nearby localized cells (e.g., histamine elaborated by the mast cells causes vasodilation and increases vascular permeability only in the nearest vessels).

3. Autocrine — regulatory substances act on the cell which produces them, cell regulates itself by means of biological active substances (BAS) production (eg interleukin-2 produced by T-cell induces its proliferation).

Normally, the cell accepts the information through receptors of a membrane, the receptors react with biologically active substances (BAS), hormones, enzymes etc (primary messenger). If closely to a cell there are these substances, the cell reacts to them, the receptors are activated and the information is transferred inside of the cell to the secondary messengers. Each secondary messenger activates a specific enzyme (proteinkinase or other), transmits a signal to the executive

systems of cell and regulated different reactions, for example phosphorylation of protein, metabolism of an arachidonic acid, synthesis of hormones or enzymes. The most important secondary (or tertiary) messengers there are: cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), Ca – calmodulin, protein-kinase C, inositol triphosphate, diacylglycerol.

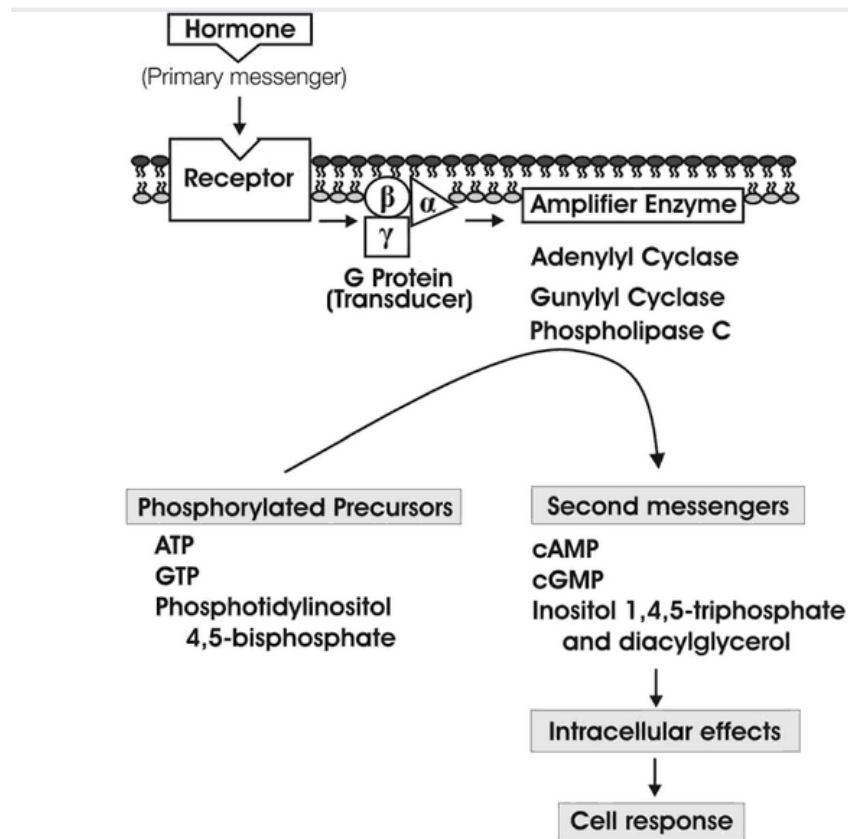


Figure 3. Cell signaling and secondary messengers.

In many cells one hormone initiates the inflow of  $\text{Ca}^{2+}$  while another hormone triggers the formation of cAMP. The two secondary messengers then exert either an antagonistic or a synergistic effect on cellular metabolism. The antagonistic effect might partly be due to the  $\text{Ca}^{2+}$ -calmodulin complex activating the enzyme phosphodiesterase which is responsible for the breakdown of cAMP.

Diacylglycerol remains attached to the inner layer of the plasma membrane and with the assistance of  $\text{Ca}^{2+}$  activates the enzyme protein kinase C (C-kinase).

C-kinase in turn initiates a phosphorylation cascade whose end result is the activation of gene regulatory proteins that initiate transcription of specific genes.

As a rule secondary messengers activated or inhibited reactions which are most typical for this type of cells, For example in the muscle cell this is contraction or relaxation, in the secretory cell - increase or decrease secretion etc. Several stages are present in information sending and receiving and each of them can be damaged resulting into pathology development. These stages are as follows;

1. Signalization — production at signal molecules and their delivery.

2. Reception — recognition of signal molecule by the target cell by means of specific structure called receptor which is localized either on the cell membrane or inside the cell.

3. Intracellular information transfer — activation of the cascade of intracellular regulatory molecules (cAMP,  $Ca^{2+}$  etc) called secondary messengers contrary to first messengers that act on the cell from the outside.

4. Realization of response to stimuli — changes in cellular activity that are specific for every stimulus and based on programs coded by DNA.

From this point of view cell injury can be of two types:

1. Alteration of information content of a cell.
2. Damage of the executive (structural) device of a cell.

Etiology and pathogenesis of the executive (structural) device of a cell damage were discussed previously.

There are following kinds of alteration of information content of a cell:

- Pathology of the signalization
- Alteration of signal reception
- Alteration of the secondary messengers function
- Defects of the cells programs at a stage at realization of a final effect.

## **Pathology of the signalization**

There are plenty of substances that can serve as first messengers: hormones , cytokines, neurotransmitters and other biologically active substances. Both excess and lack of signals may lead to cell injury. Excess of hormones causes such diseases as Cushing disease (excess of adrenocorticotrophic hormone) or Cushing syndrome (primary excess of glucocorticoids). Excessive production of cytokines such as inflammatory mediators can lead to the shock development. Excess of neurotransmitters (acetylcholine) develops at organophosphorous compounds (used as insecticides) poisoning since they are acetylcholine esterase blockers. Deficiency of insulin leads to diabetes mellitus (type I) development. Deficiency of cytokines produced by CD4+-leukocytes leads to immunodeficiency in AIDS.

There is also a specific type of pathology of signalization called mimicry of the signal. In certain cases a molecule can be mistaken for another one. Wrong molecule having physicochemical and biological properties different from normal ligand can either block receptor or cause its overstimulation. Immunoglobulins are the principal molecules that can substitute for another one since they have variable domain in their structure. Blockade of receptor-ligand interaction is involved in the development of myasthenia gravis. In this disease the N-cholinoreceptors of a motor end plate become blocked with antibodies and signal conducting from motoneuron to muscle get impaired which leads to muscular weakness. While antibodies can stimulate receptors as it happens in Grave's disease. This state is characterized by production of antibodies against thyroid stimulating hormone (TSH) receptors of thyrocytes. Antibodies bind to receptor and cause prolonged stimulation of thyrocytes leading to hypertunctioning and hyperplasia of thyroid gland.

## **Alteration of signal reception**

Cell can only recognize signal if it has a specific receptor complementary to the signal molecule structure. There are two primary types of receptors: membrane—bound — localized on the plasma membrane and intracellular —

localized inside the cell. The latter are used by steroid hormones and thyroid hormones, whereas the former are used by the vast variety of signal molecules such as peptides, derivatives of amino acids, and so forth.

Although signals can be present the cell cannot recognize them if specific receptors are absent or their structure is modified. This is quite a frequent pathological situation. For instance, the inability of cells to recognize insulin due to changes or absence of insulin receptors leads to the development of diabetes mellitus type II; atherosclerosis often develops against the background of absence or decreased number of receptors for apoprotein B of low density lipoproteins that leads to hyperlipidemia due to decreased low density lipoproteins absorption and infiltration of vessel walls with lipids.

### **Alteration of the secondary messengers function**

Receptors bound to the plasma membrane need certain mechanisms to conduct received to the intracellular executive structures. There are some mechanisms involved. Sometimes there may develop deficiency of secondary messengers. Another variant is when there is inhibited production of one secondary messenger and activated another. For example: a receptor can be coupled with the G-protein, with the ion channel. and it can have intracellular domain with enzymic activity (commonly tyrosine kinase). Those that coupled with G-proteins produce small molecules called secondary messenger from precursors that reside either in cytoplasm or in the plasma membrane. All G-proteins consist of three subunits: alpha, beta, gamma. Beta and gamma subunits form stable, noncovalently-linked dimer, alpha subunit can bind and hydrolyze GTP (it has GTPase activity). When alpha subunit binds GDP, it associates with beta and gamma subunits to form a trimer that can interact with cytoplasmic domain of receptor. Changes of the receptor conformation occur upon ligand binding and these allow the alpha subunit of G-protein to replace GDP by GTP. This replacement causes alpha subunit to dissociate from beta and gamma subunits and associate with effector molecule such as adenylyl cyclase or phospholipase C and preventing production of CAMP.

Alpha subunit hydrolyses GTP to GDP, detaches from effector molecule and associates with beta-gamma subunits. Then the cycle can repeat.

### **Defects of the cells programs at a stage at realization of a final effect**

This alteration occurs at the normal work of receptors and messengers, when in the cell there are no factors necessary for performance of specific functions (enzymes, vitamins, ions, etc.)

## **6. Types of cell death: necrosis and apoptosis**

Cells death can also occur without the influence of injuring factors either when they have completed a fixed number of division cycles (around 60, **the Hayflick limit**) or some earlier when programmed to do so.

Cell destruction and removal can involve one of two mechanisms: **apoptosis**, which is designed to remove injured or worn-out cells, or **necrosis**, which occurs in irreversibly damaged cells.

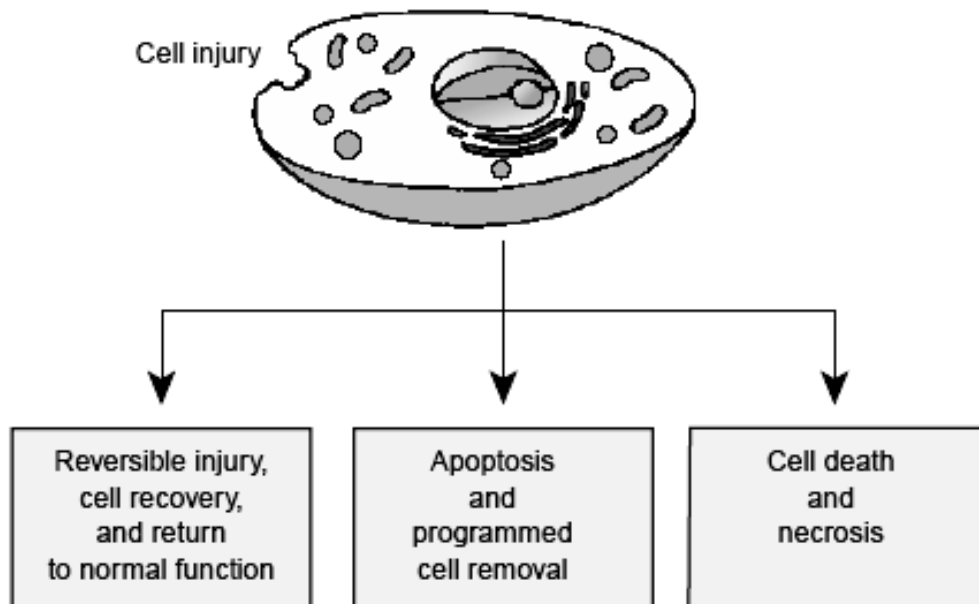


Figure 4. Outcomes of cell injury

The concept of programmed death assumes that certain cells are determined to die at specific stages and specific sites during development.



**Apoptosis** is a programmed cell death as signalled by the nuclei in normally functioning human and animal cells when age or state of cell health and condition dictates. It is an active process requiring metabolic activity by the dying cell.

Apoptosis is thought to be responsible for several normal physiologic processes, including programmed destruction of cells during embryonic development, hormone-dependent involution of tissues, death of immune cells, cell death induced by cytotoxic T cells, and cell death in proliferating cell populations. During embryogenesis, in the development of a number of organs such as the heart, which begins as a single pulsating tube and is gradually modified to become a four-chambered pump, apoptotic cell death allows the next stage of organ development. It also separates the webbed fingers and toes of the developing embryo. Apoptotic cell death occurs in the hormone-dependent involution of endometrial cells during the menstrual cycle and in the regression of breast tissue after weaning from breast-feeding. The control of immune cell numbers and destruction of autoreactive T cells in the thymus have been credited to apoptosis. Cytotoxic T cells and natural killer cells are thought to destroy target cells by inducing apoptotic cell death.

Apoptosis appears to be linked to several pathologic processes. For example, suppression of apoptosis may be a determinant in the growth of cancers. Apoptosis is also thought to be involved in the cell death associated with certain viral infections, such as hepatitis B and C, and in cell death caused by a variety of injurious agents, such as mild thermal injury and radiation injury.

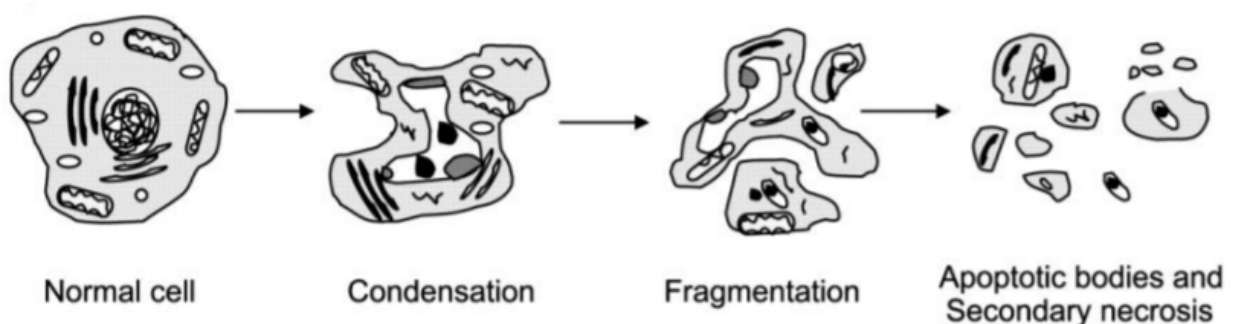


Figure 5. Stages of apoptosis

The characteristic morphologic changes of the cell in apoptosis are:

- Involvement of single cells or small clusters of the cells in the background of viable cells.
- Shrinkage of the cell with dense cytoplasm and almost normal organelles.
- Formation of apoptic bodies (membrane-bound near-spherical bodies containing compacted organelles).
- Condensation of chromatin around the periphery of nucleus.
- Inflammatory response around apoptosis is absent.
- Phagocytosis of apoptic bodies by macrophages.

The early steps in apoptosis are reversible. In some cases, final destruction of the cell is guaranteed only with phagocytosis.

Apoptosis is a genetically determined and biologically meaningful process in which cells that are immunologically reactive against self, infected or genetically damaged are removed to protect the host.

There is phagocytosis though no associated inflammatory response. Morphologically, there is nuclear condensation and fragmentation. The surface membrane becomes irregular and the cell fragments into membrane-bound bodies which may or may not contain nuclear material. These are phagocytized.

Apoptosis can be triggered by a variety of extrinsic and intrinsic signals. It appears to be carried out through the activation of endogenous proteases which disrupt the integrity of the cytoskeleton and endonucleases which degrade nuclear DNA. The key feature of apoptosis is that the plasma membrane remains intact.

Apoptosis is genetically regulated. Early experiments to identify genes that regulated cell death during the development of the nematode *Caenorhabditis elegans* led to the discovery of 3 genes (CED-9, CED-4 and CED-3). It was found that if CED-9 was mutated, apoptosis was prevented. Mammalian homologs for all

three genes have been found. When CED-9 was cloned, it was found to be related to the mammalian oncogene, bcl-2. This gene is present on chromosome 18 and was originally identified because of its involvement in a 14:18 translocation present in 85% of follicular non-Hodgkin's lymphoma. CED-3 is an endogenous protease. When this was cloned, it was also found to have a similar mammalian counterpart called, interleukin 1 $\beta$  converting enzyme (ICE). This was the first member of the mammalian caspase system identified. These caspases are intracellular cysteine proteases that have the novel ability to cleave proteins after an aspartate residue (hence the name). Twelve members of the caspase family have been identified (termed 1-12); caspase 8 (AKA, FLICE...see below) is hypothesized to be essential for apoptosis.

The rate at which apoptotic signaling events initiate or amplify caspase activity is regulated by proteins of the Bcl-2 family. A number of Bcl-2-related proteins have been identified (at least 16 in humans). Paradoxically, some of these proteins have been shown to promote apoptosis (Bax, Bak, Bok/Mtd) whereas others suppress apoptosis (Bcl-2, Bcl-X L).

Apoptosis may also be initiated through extrinsic ligands which bind to cell surface receptors. One of the best characterized is a member of the tumor necrosis factor (TNF) family, Fas. Fas is also known as APO-1 or CD95. Fas is the membrane receptor for Fas ligand (FasL). Fas is ubiquitously expressed in various tissues. FasL on the other hand, is predominantly expressed in activated T lymphocytes, NK cells and macrophages. When FasL binds Fas, an intracytoplasmic protein adaptor (the Fas-associated death domain or FADD) is recruited. This binding eventually leads to activation of one of the caspases referred to as caspase 8 or FLICE for FADD-like ICE. Signaling pathways may induce or prevent apoptosis via transmembrane signals through specific receptors. Growth factors, hormones, and TNF all play a role. TNFR (tumor necrosis factor receptor superfamily) on the surface of the plasma membrane may be activated by TNF or other proteins to initiate programmed cell death.

Control of apoptosis occurs via interrupting the process or allowing it to continue with commitment of the cell to death. Adapter proteins may be utilized to activate mechanisms that cause death. (Example: Fas-Fas ligand mediated apoptosis via Fas receptor and Fas ligand from immune system cells that allows cell killing without immune system activation). Cytochrome c release from mitochondria seems to commit a cell to apoptosis and may be involved in regulation. The Bcl-2 family of genes can act to promote or prevent apoptosis by influencing mitochondrial permeability.

Execution of apoptosis also involves the caspase family (cysteine protease that can cleave aspartic acid residues). Caspases are zymogens which can be activated via hydrolysis through substrate interaction or autocatalytically. Once the cell is committed to cell death, caspases rapidly degrade proteins in the nucleus and cytoplasm. Caspase 9 is involved in the reactions stimulated by cytochrome c release causing cell death, and caspase 8 gets triggered by the Fas-Fas ligand binding. Dead cells are removed by phagocytes because their fragments have signaling molecules targeting them for uptake.

Triggers for apoptosis also include cytotoxic t-lymphocytes recognizing foreign antigens on cell surfaces which causes them to be targeted. Granules with toxic enzymes are then released into the cell. DNA damage also triggers apoptosis unless the damage can be repaired. p53 triggers cell cycle stalling in G1 when there is genetic damage. If it is not repaired, p53 can initiate apoptosis.

Morphological changes: cells are smaller with dense cytoplasm. The chromatin is condensed in fragments near the nuclear periphery. Apoptotic bodies form which are membrane bound with some cytoplasm and organelles and sometimes nuclear fragments. These are taken up by normal cells nearby and degraded in lysosomes.

In addition to ligand/receptor mediated induction of apoptosis, a variety of agents can induce apoptosis through lesser understood pathways. One emerging model has as its critical components caspase 9 and a protein termed Apaf1. In this

model, cytochrome c is released from a mitochondria under stress. Cytochrome c would then associate with a complex of Apaf1 and caspase 9, resulting in the activation of caspase 9. This would lead to the activation of caspase 3 and the initiation of apoptosis. The nematode gene, CED-4 is structurally similar to its mammalian counterpart, Apaf1.

Therapeutic opportunities for the treatment of certain types of cancer are arising with further study of apoptosis regulatory genes. Follicular lymphoma is an example in which the neoplastic cell expansion is primarily caused by failed apoptosis rather than by rapid cell division. In addition to its involvement in a wide variety of hematologic malignancies, the bcl-2 oncogene has also been associated with prostate, lung, melanoma, breast and other solid organ malignancies. The Bcl-2 family proteins have also been implicated in resistance to cancer therapies, which have the induction of the apoptotic pathway as their common basis.

In fact, there is now abundant evidence that Bcl-2 may act as a multi-drug resistance protein that prevents or markedly delays apoptosis induction by radiation therapy and a variety of anti-cancer chemotherapies. The p53 tumor suppressor gene may play an important role in this regard. Wild-type p53 induces apoptosis following DNA damage. Bax is transcriptionally activated by wild type p53 binding. The overexpression of Bax (and other pro-apoptotic members of the Bcl-2 family) renders tumor cells more sensitive to many chemotherapeutic agents. Conversely, ablating Bax expression reduces drug-induced apoptosis. In addition, Bcl-2 appears to have an inhibitory effect on p53, impairing nuclear import of p53 following genetic damage, thus, inhibiting apoptosis.

Though apoptosis is an important mechanism used by the body to eliminate damaged cells, dysregulated apoptosis has been implicated in many human pathologies. When it is inhibited, cells may survive longer as in cancer, autoimmune disorders. Apoptosis also plays role in pathogenesis of ischemic heart disease. When it is accelerated, cells may die sooner as in AIDS depletion of lymphocytes and degenerative neurological disorders.

Autophagy is an evolutionally conserved, genetically controlled cell survival pathway eukaryotic mechanism, which is classically defined as the degradation of cytoplasmic constituents in the lysosomes in mammals. The general targets of autophagy vary from long-lived proteins to protein complexes and even entire organelles. Morphologically, autophagy begins with the formation of cup-shaped double membranes, which expand to form autophagosomes engulfing malfunctioning or unneeded macromolecules and organelles and transport them for degradation inside the vacuole. Upon arrival of the autophagosomes to the vacuoles, their outer membranes fuse with the tonoplast, creating single-membrane vesicles inside the vacuole, termed “autophagic bodies.” The autophagic bodies and their contents are then degraded inside the vacuole, providing recycled materials to build new macromolecules. Nutrient starvation has been one of the hallmark inducers of autophagy in humans. The autophagy mechanism is presumed to play a role in recycling during starvation, thus supplying the cell with nutrients during the stress period. It has been shown to be involved in protein and lipid degradation, as well as to be associated with several metabolic disorders.

Autophagy plays a critical role in cellular homeostasis by eliminating excessive, damaged or long-lived proteins and organelles, thus preserving the quality of essential cellular components. Autophagy is intricately implicated in both health and disease. The process when autophagy lead to the destruction of the main cell structures is named as autophagic programmed cell death (also known as cytoplasmic cell death). Lysosomes and lysosomal enzymes play an important role in this process. Many researches indicate that autophagy, once considered a simple maintenance pathway, plays an important role in preventing metabolic dysfunction and illness. Its function can vary slightly in different systems throughout the body, and thus autophagy has great potential to be foundational in future treatments in the medical field. Defects of autophagy plays a role in pathogenesis of several diseases, including myopathy neuronal degeneration, microbial infection, inflammatory bowel disease, ageing and cancer.

Table 1. Differential features of apoptosis, necrosis and autophagy

Feature	Apoptosis	Autophagy	Necrosis
Cellular membrane	membrane blebbing, no loss of integrity	cell membrane stays intact, weak membrane blebbing	loss of membrane integrity
Nuclear chromatin	aggregation of chromatin at the nuclear membrane	marginal chromatin condensation	no chromatin condensation
Initial event	begins with shrinking of cytoplasm and condensation of nucleus	begins with sequestration of cytoplasmic material in autophagosomes and autolysosomes	begins with swelling of cytoplasm and mitochondria
Mitochondria	mitochondria preserve normal ultrastructure, outer membrane permeabilisation	damaged mitochondria degraded within autophagosomes	disintegration (swelling) of organelles
DNA	Non-random internucleosomal DNA fragmentation	No DNA fragmentation	Random digestion of DNA
End result	ends with fragmentation of cell into apoptic bodies	no cell lysis, ends with cell-digestion	ends with total cell lysis

**Table 2. Physiological importance of apoptosis, necrosis and autophagy**

	Apoptosis	Autophagy	Necrosis
Physiological role	Serves to eliminate damaged, transformed or infected cells; functions in organ development and regulation of immune responses	Serves primarily to maintain cellular energy and to recycle damaged organelles	Physiological role unclear, potential backup mechanism during apoptosis failure.
Regulation	Tightly regulated process involving mediators and enzymes	Tightly regulated process involving various regulatory steps	Loss of ion homeostasis
Initiation	Induced by physiological stimuli (death ligands), growth factor depletion and different cellular stresses	Triggered by nutrient deprivation and other cellular stresses.	Evoked by severe injuries (complement attack, lytic pathogens, hypoxia, ischemia, toxins)
Phagocytosis	Phagocytosis by adjacent cells or macrophages	Self-digestion, late heterophagy by other cells	Phagocytosis by macrophages
Inflammation	No inflammatory response	No inflammatory response	Severe inflammatory response
Energy dependence	Energy (ATP)-dependent (active process)	Generates ATP in the absence of exogenous energy supply	No energy requirement (passive process)



**Necrosis** is defined as a focal death along with degradation of tissue by hydrolytic enzymes released by the cells. Necrosis differs from apoptosis in that it involves unregulated enzymatic digestion of cell components, loss of cell membrane integrity with uncontrolled release of the products of cell death into the intracellular space, and initiation of the inflammatory response. In contrast to apoptosis, which functions in removing cells so that new cells can replace them, necrosis often interferes with cell replacement and tissue regeneration.

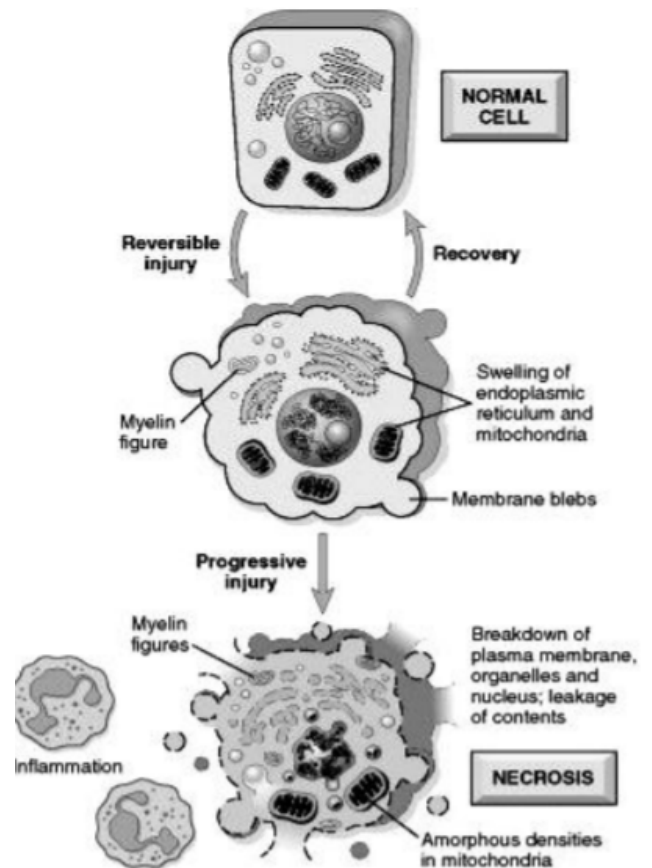


Figure 6. Stages of necrosis

Necrosis can be caused by variety of agents – hypoxia, physical and chemical agents, microbial agents etc. With necrotic cell death, there are marked changes in the appearance of the cell. The nuclear changes include condensation of nuclear chromatin (pyknosis) which may either undergo dissolution (karyolysis) or fragmentation into many granular clumps (karyorrhexis). Cells and their organelles swell (because the ability of the plasma membrane to control the passage of ions and water is disrupted); the cell contents leak out, leading to inflammation of surrounding tissues. There are five major types of necrosis: coagulative, liquefactive (colliquative), caseous, fat and fibrinoid necrosis.

During **coagulation necrosis** (the most common type of necrosis) acidosis develops and denatures the enzymatic and structural proteins of the cell. This type of necrosis is characteristic of hypoxic injury (in infarcted areas) and less often

from chemical agents (acids).

**Liquefaction or colliquative necrosis** occurs due to degradation of tissue by the action of powerful hydrolytic enzymes. An example of liquefaction necrosis is the softening of the center of an abscess with discharge of its contents.

**Caseous necrosis** combines features of both coagulative and liquefactive necrosis. It is most commonly found in the center of tuberculosis granulomas, and results from immune mechanisms

**Fat necrosis** is a term for necrosis in fat, caused either by release of pancreatic enzymes from the pancreas (acute pancreas necrosis) or by trauma to fat, either by a physical blow or by surgery.

**Fibrinoid necrosis** is characterized by deposition of fibrin-like material usually as a result of immunologic tissue injury. The same process occurs in arterioles in hypertension, in peptic ulcer development etc.

## **6. Mechanisms of cell adaptation to injury**

### **Intracellular mechanisms of cell adaptation**

As it was concerned earlier, cells must constantly adapt, even under normal conditions, to changes in their environment. These **physiologic adaptations** usually represent responses of cells to normal stimulation by hormones or endogenous chemical substances. For example, as in the enlargement of the breast and induction of lactation, by pregnancy. **Pathologic adaptations** may share the same underlying mechanisms, but they provide the cells with the ability to modulate their environment and perhaps escape injury. Cellular adaptation, then, is a state that lies intermediate between the normal, unstressed cell and the injured, overstressed cell.

Cellular adaptation is provided both with intracellular and intercellular mechanisms of cellular adaptations. The most important of them provide:

#### **Compensation of energy metabolism disturbance**

Decrease in ATP production due to mitochondrial dysfunction starts the

following compensatory processes:

- increased ATP production by the means of glycolysis;
- increase of enzymes activity which are taking part in reduction-oxidation reactions;
- activation of ATP transport and increase effectiveness ATP use in the cell;
- decrease of cell's functional activity and decrease of anabolic processes in the cell.

**Protection of cells membranes** is realized with activation of:

- antioxidants action – enzymatic and non-enzymatic antioxidants inactivate free radical preventing their pathogenic effects;
- cells buffer system activation – leads to neutralization of intracellular acidosis, which occurs due to high activity of enzymes;
- endoplasmic reticulum enzymes activation – it increases inactivation of toxic substances which were formed in the cell;
- activation of cellular structures reparation which help to repair membranes of the cell and cellular organelles.

**Compensation of water-ion imbalance** is provided with

- activation of ion “pumps” energy supply;
- increase of ion-transporting enzymes activity;
- activation of cell's buffer system.

**Repair of cell genome** is provided with the following mechanisms:

- revealing and elimination of damaged DNA fragment;
- replacement of damaged DNA fragments;
- elimination of DNA ruptures;
- normalization of DNA transcription and translation.

### **Intracellular mechanisms of cell adaptation**

Cells also may adapt by undergoing changes in their size, number, and type. These changes, occurring singly or in combination, may lead to atrophy,

hypertrophy, hyperplasia, metaplasia, and dysplasia.

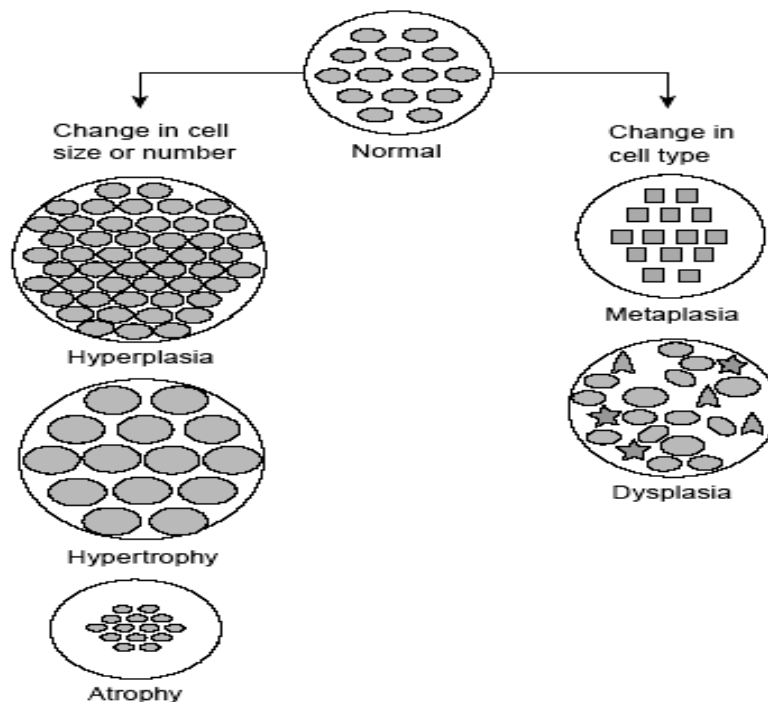


Figure 7. Intracellular mechanisms of cell adaptation

**Atrophy.** When confronted with a decrease in work demands or adverse environmental conditions, most cells are able to revert to a smaller size and a lower and more efficient level of functioning that is compatible with survival. This decrease in cell size is called atrophy. Cells that are atrophied reduce their oxygen consumption and other cellular functions by decreasing the number and size of their organelles and other structures. When a sufficient number of cells are involved, the entire tissue or muscle atrophies. The general causes of atrophy are: decreased workload, loss of innervation, diminished blood supply (ischemia), inadequate nutrition, loss of endocrine stimulation and aging.

Atrophy is a normal physiologic process of **aging** in some tissues which could be due to loss of endocrine stimulation or arteriosclerosis. For example, atrophy of lymphoid tissue in thymus and lymphatic nodes.

Others stimuli are clearly pathologic. The **decreased workload** upon the skeletal muscles results in disuse atrophy. An extreme example of it may be seen in the muscles of extremities that have been encased in plaster casts. Because

atrophy is adaptive and reversible, muscle size is restored after the cast is removed. **Denervation atrophy** occurs in the muscles of paralyzed limbs. **Ischemic atrophy** occurs when there is gradual diminishment of blood supply due to atherosclerosis; it may result in shrinkage of affected organ (e.g. atrophy of brain in cerebral atherosclerosis). In the case of **inadequate nutrition** or even starvation cells decrease their size and energy requirements as a means of survival. **Loss of endocrine regulatory mechanisms** results in reduced metabolic activity of the tissue and hence its atrophy. For example, in women, the loss of estrogen stimulation during menopause results in atrophic changes in the reproductive organs.

**Hypertrophy** refers to an increase in the size of cells and, with such change, an increase in the size of the organ. It is usually seen in muscles because this type of tissue is not capable of mitotic activity. Hypertrophy can be caused by increased functional demand or by specific hormonal stimulation and may occur under both physiologic and pathologic conditions. The increase in muscle mass associated with exercise is an example of **physiologic hypertrophy**. Another example of physiologic hypertrophy affected by hormonal stimulation is enlarged size of the uterus in pregnancy. The cellular hypertrophy is stimulated by estrogen through smooth muscle estrogen receptors.

**Pathologic hypertrophy** occurs as the result of disease conditions and may be adaptive or compensatory. Example of **adaptive hypertrophy** is the myocardial hypertrophy that results from valvular heart disease or hypertension. **Compensatory hypertrophy** is the enlargement of a remaining organ or tissue after a portion has been surgically removed or rendered inactive. For instance, if one kidney is removed, the remaining kidney enlarges to compensate for the loss.

Hypertrophy eventually reaches a limit beyond which enlargement of muscle mass is no longer able to compensate increased workload, and cardiac failure, for example, ensues. At this stage a number of "degenerative" changes occurs in the myocardial fibers. The limiting factors for continued hypertrophy are due to limitation of the vascular supply to the enlarged fibers, to diminished oxidative

capabilities of mitochondria, or to alterations in protein synthesis and degradation.

**Hyperplasia** is an increase in the number of cells of a tissue or organ. It occurs in tissues where cells are capable of mitotic division such as the epidermis, intestinal epithelium, and glandular tissue. Hyperplasia is a controlled response to an appropriate stimulus and ceases once the stimulus has been removed.

The stimuli that induce hyperplasia may be physiologic or pathologic. There are two common types of physiologic hyperplasia: hormonal and compensatory. Breast and uterine enlargement during pregnancy are examples of a **physiologic hyperplasia** that results from estrogen stimulation. The regeneration of the liver that occurs after partial hepatectomy (i.e., partial removal of the liver) is an example of **compensatory hyperplasia**. Hyperplasia is also an important response of connective tissue in wound healing, during which proliferating fibroblasts and blood vessels contribute to wound repair.

Most forms of **pathologic hyperplasia** are due to excessive hormonal stimulation or the effects of growth factors on target tissues. Excessive estrogen production can cause endometrial hyperplasia and abnormal menstrual bleeding. Skin warts are an example of hyperplasia caused by growth factors produced by papillomaviruses.

**Metaplasia** is a reversible change in which one adult cell type (epithelial or mesenchymal) is replaced by another adult cell type. Metaplasia is thought to involve the reprogramming of undifferentiated stem cells that are present in the tissue undergoing the metaplastic changes in response to chronic irritation and inflammation. Metaplasia is seen in the respiratory tract in the habitual cigarette smoker. The normal columnar ciliated epithelial cells of the trachea and bronchi are replaced by stratified squamous epithelial cells. Stones in the excretory ducts of the salivary glands, pancreas, or bile ducts may cause replacement of the normal secretory columnar epithelium by nonfunctioning stratified squamous epithelium. Squamous epithelium is more able to survive under circumstances in which the more fragile specialized epithelium most likely would have succumbed.

**Dysplasia** is characterized by deranged cell growth of a specific tissue that results in cells that vary in size, shape, and organization. Minor degrees of dysplasia are associated with chronic irritation or inflammation. Although dysplasia is abnormal, it is adaptive in that it is potentially reversible after the irritating cause has been removed. Dysplasia is strongly implicated as a precursor of cancer. In cancers of the respiratory tract and the uterine cervix, dysplastic changes have been found adjacent to the foci of cancerous transformation. However, dysplasia does not necessarily lead to cancer.

## **Examples of situational tasks and tests**

### **Task 1**

Patient was made blood biochemical test in order to confirm hepatitis. Increased level of alanine transaminase (ALT) and aspartate transaminase (AST) was found in blood serum.

1. Which cellular changes could lead to this situation? Prove your answer.
2. Explain possible mechanism of enzymes appearance in the blood.
3. Which clinical importance does this test have?

### **Task 2**

Patient with gastritis and increased acidity had fibrogastroscopy, during which the tissue was taken from the place of mucous coat erosion. Tissue histology: increased cells size with their form and coloring changing without features of cell organoids and membranes damage.

1. What features of cell injury (morphological or functional) are described here?
2. Point out cell adaptation feature to damage.

### **Task 3**

Patient M., 50 years old complains of pain and burning sensation just below the xiphoid process. Sensations suddenly appear and also suddenly disappear. After a big meal pain usually intensifies, sometimes occurs even at night. Patient takes milk or soda solution to relieve this pain. The patient is overweight, smokes 15-20 cigarettes a day, drinks alcohol several times a week, prefers spicy and fatty foods. Upper GIT endoscopy: hyperemia of the lower third of the esophageal mucosa, the gastric mucosa is not changed. Biopsy data: a fragment of the mucosa of the esophagus is partially covered with stratified squamous epithelium epithelium, partially with prismatic epithelium of the gastric type.

1. Describe the possible mechanism of cell injury in this patient.



2. Name the features of cells adaptation to injuring factor

#### **Task 4**

A 30-year-old man sustained a fracture of his leg 2 months ago. The leg had been encased in a cast, which was just removed. The patient is amazed at the degree to which the muscles in his leg have shrunk.

1. Would you consider the changes in the patient's muscles to be a normal adaptive response? Explain.
2. What type of measures can be taken to restore full function to the leg?

#### **Task 5**

During ECG examination of the patient there were found the features of intoxication (sinus bradycardia, negative asymmetric T wave, ventricle extrasystoles, atrioventricular transmission delay). The patient was treated with heart glycosides (Strophanthinus).

1. What features of heart muscle violation does the patient have? Point out.
2. What mechanism of heart muscle violation does the patient have?

#### **Examples of tests**

1. Microscopy of biopsy material shows the signs of cellular damage. Which of the signs from listed below can be related to morphologic signs of cellular damage?
  - a. disturbance of cellular division
  - b. \*change of cell's color
  - c. increase of cellular membrane permeability for proteins
  - d. release of intracellular enzymes into blood
  - e. increase of suboxidized substances in blood
2. The increase of organ volume due to increased number of the cells in response to different stimuli can be estimated as ...
  - a. \*hypertrophy

- b. hyperplasia
  - c. hyperactivity
  - d. hypereactivity
  - e. overnutrition
- 3.** The increase of organ volume due to increased volume of the cells in response to different stimuli can be estimated as ...
- a. hypertrophy
  - b. \*hyperplasia
  - c. hyperactivity
  - d. hypereactivity
  - e. overnutrition
- 4.** Microscopy of biopsy material shows the signs of cellular damage. Which of the signs from listed below can be related to functional signs of cellular damage?
- a. change of cellular organoids quantity and structure
  - b. swelling of cell
  - c. changed cell's color
  - d. \*release of intracellular enzymes into blood
  - e. accumulation of calcium in the cell
- 5.** Which signs of cellular damage can be related to functional?
- a. damage of nuclear membrane
  - b. destruction of structure of mitochondria
  - c. swelling of cell
  - d. change of color
  - e. \*disturbance of cellular division
- 6.** Which is the most typical morphological sign of cell death by apoptosis?
- a. \*condensation of nucleus and cytoplasm
  - b. presence of inflammatory reaction
  - c. compensatory increase of DNA-synthesis
  - d. swelling of mitochondria
  - e. increase of cell's size

- 7.** Which is the most typical morphological sign of cell death by necrosis?
- a. condensation of nucleus and cytoplasm
  - b. \*presence of inflammatory reaction
  - c. compensatory increase of DNA-synthesis
  - d. shrinking of the cell
  - e. increase of cell's size
- 8.** Which is the most typical morphological sign of cell death by necrosis?
- a. condensation of nucleus and cytoplasm
  - b. \*swelling of the cell
  - c. compensatory increase of DNA-synthesis
  - d. shrinking of the cell
  - e. increase of cell's size
- 9.** Each cell of the organism has limited abilities to adapt to pathogenic factors influence. What will happen to the cell if the pathogenic factor will be of extreme strength?
- a. apoptosis
  - b. adaptation
  - c. \*necrosis
  - d. reversible injury
  - e. necrobiosis
- 10.** Osmotic fragility test is carried out by adding hypotonic solution to the sample of patient's blood. We observe hemolysis of erythrocytes as a result of test. Which process underlies hemolysis in this case?
- a. \*swelling and rupture of RBC
  - b. shrinking and apoptosis of RBC
  - c. activation of lipid peroxidation and membranes destruction
  - d. decrease of ATP synthesis
  - e. destruction of lysosomal membranes
- 11.** Chose the example of specific cell injury from listed below:
- a. myocardial ischemia

- b. intestinal epithelial injury due to bacterial toxins
- c. \*immune hemolysis of RBC
- d. liver cell injury due to chemicals
- e. skin damage due to mechanical trauma

**12.** Ischemic heart disease develops in the patients due to hypoxic injury of myocardial cells. But even when the bloodflow is restored to the site of ischemia, the degree of myocardial fiber injury may increase. Which factor is playing the leading role in reperfusion injury?

- a. cytoskeletal filament loss
- b. activation of anaerobic glycolysis
- c. \*increase in toxic oxygen radicals
- d. mitochondrial swelling
- e. nuclear chromatin clumping and decreased protein synthesis

**13.** Disturbance of which process is primary observed in hypoxic injury:

- a. detachment of ribosomes from EPR
- b. reduction of intracellular pH
- c. \*oxidative phosphorylation by mitochondria
- d. sodium pump activity
- e. activation of glycolysis

**14.** Which factor directly causes the decrease of intracellular pH in the case of hypoxic injury?

- a. detachment of ribosomes from EPR
- b. decreased oxidative phosphorylation by mitochondria
- c. failure of sodium pump
- d. \*activation of anaerobic glycolysis
- e. increased membranes permeability

**15.** Which process is initiated by calcium ions in hypoxic cell injury?

- a. detachment of ribosomes from EPR
- b. disturbance of cells aerobic respiration
- c. disturbance of sodium pump

- d. activation of glycolysis
  - e. \*activation of intracellular enzymes
- 16.** Reperfusion injury is developed mostly due to massive inflow to the cell of:
- a. \*calcium
  - b. sodium
  - c. potassium
  - d. aminoacids
  - e. enzymes
- 17.** Free radicals cause the cell's injury by the mechanisms listed below EXCEPT OF:
- a. lipid peroxidation of membranes
  - b. nonperoxidative mitochondrial injury
  - c. \*disturbance of cells aerobic respiration
  - d. DNA lesions
  - e. cross-linking of proteins
- 18.** Which mechanism of cellular adaptation is provided with anti-oxidants action?
- a. compensation of energy metabolism disturbance
  - b. \*protection of cell's membranes
  - c. compensation of water-ion misbalance
  - d. repair of cell genome
  - e. decrease of cell's functional activity
- 19.** Which factors determine the type of cell's response to injuring stimuli?
- a. kind of injuring factor
  - b. injuring factor severity and time of duration
  - c. prior state of the cell
  - d. type of the affected cell
  - e. \*all is correct
- 20.** Which process distinguishes irreversible hypoxic injury from reversible one?
- a. \*inability to reverse mitochondrial dysfunction

- b. damage to plasma membrane sodium pump
- c. inability to re-start protein synthesis
- d. extremely low pH
- e. depletion of ATP store in the cell

**21.** Which tissue cells are most sensitive to hypoxic injury?

- a. skeletal muscles
- b. smooth muscles
- c. myocardial cells
- d. \*brain cells
- e. liver cells

**22.** Give the correct definition of apoptosis. Apoptosis is...

- a. a process of virus infected cell killing
- b. \*a programmed cell death
- c. a death of the cell after injuring factor influence
- d. a cell's death as a result of enzymes action
- e. an irreversible cell injury

**23.** People who have had a heart attack may experience additional damage once blood flow has been restored, a phenomenon referred to reperfusion injury. Which blood cells from listed below take active part in reperfusion injury development?

- a. lymphocytes
- b. eosinophils
- c. \*neutrophils
- d. erythrocytes
- e. thrombocytes

**24.** Patient was made blood biochemical test in order to confirm hepatitis.

Increased levels of alanine transaminase (ALT) and aspartate transaminase (AST) were found. It has been defined as a functional sign of hepatic cells injury. Which from the listed may be the reason of it?

- a. \*cell's membrane damage
- b. damage to plasma membrane sodium pump

- c. depletion of ATP store in the cell
- d. nonperoxidative mitochondrial injury
- e. disturbance of cells aerobic respiration

**25.** Cells may adapt to external and internal stimuli by undergoing changes in their size, number and type. What happens to other kidney when one is damaged?

It undergoes....:

- a. \*hypertrophy
- b. atrophy
- c. hyperplasia
- d. metaplasia
- e. dysplasia

**26.** Cells may adapt to external and internal stimuli by undergoing changes in their size, number and type. What happens to muscles of extremities that have been encased in plaster casts? The muscles undergo....:

- a. hypertrophy
- b. \*atrophy
- c. hyperplasia
- d. metaplasia
- e. dysplasia

**27.** A 30-year-old man sustained a fracture of his leg 2 months ago. The leg had been encased in a cast, which was just removed. The patient is amazed at the degree to which the muscles in his leg have shrunk. Which is the reason of it?

- a. inadequate nutrition
- b. loss of innervation
- c. loss of endocrine stimulation
- d. \*decreased workload
- e. diminished blood supply

**28.** Sperm analysis in a 40-year-old childless man shows low sperm motility. Which sign of cell damage is observed?

- a. morphological

- b. \*functional
- c. chemical
- d. biochemical
- e. pathological

**29.** Activation of lipid peroxidation in the patient with acute hepatitis is observed. Which mechanism is leading in cell injury in this case?

- a. decrease in ATP synthesis
- b. disturbance of RNA synthesis
- c. \*damage of membranes
- d. release of inflammatory mediators
- e. damage of MHC molecules

**30.** Every day, blood cells in our body become senescent and die without producing signs of inflammation, and yet, massive injury or destruction of tissue, such as occurs with a heart attack, produces significant signs of inflammation.

Why it happens?

- a. \*due to necrosis of heart muscle
- b. due to apoptosis of heart muscle
- c. due to atrophy of heart muscle
- d. due to swelling of heart muscle
- e. due to disturbances in calcium metabolism

## **RECOMMENDED LITERATURE**

### ***Basical:***

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