Chapter 1
CELL INJURY

The concept of cell injury is at the core of the understanding of disease in pathology. Injury refers to damage or pathologic alterations in molecules and structure that can occur in cells and extracellular components of tissue.

Cells composing tissues are normally under homeostatic conditions and are constantly adjusting structure and function to accommodate changing demands and extracellular stresses and maintain a constant intracellular environment.

In response to physiologic stresses and pathologic stimuli or injury cells can undergo:

1. Adaptation:
Changes in function and structure, that maintain a homeostatic state and cell viability.
Cells may revert to previous structure and function when the stress or injury is removed.

2. Reversible Injury:
Pathologic alterations in cell molecules and structure, that are associated with abnormal function and with loss of homeostatic state. Removal of stress or injury results in cell recovery and return to normal function.

3. Irreversible injury and cell death:
Damage to cells reaches magnitude or duration where the cells passes a point of no return. Despite removal of injury or stress, the cells can not recover and dies.
Morphologic and molecular events that occur after the cells is irreversibly injured allow identification of the cells as having undergone:

- Necrosis:
  Pattern of cell death that often follows hypoxic, toxic and some microbial injuries.

- Apoptosis:
  Receptor-mediated pattern of cell death that occurs in programmed cells death during development and following some endocrine-related, toxic and microbially mediated injuries and toxic.

Causes of cell Injury.
A. Hypoxia/hypoxemia:
Examples:
1. Cardi-respiratory failure and neuronal necrosis.
2. Renal vein thrombosis and renal tubular necrosis.
B. Physical Agents:
Examples:
1. Trauma (hit by car and organ rupture).
2. Thermal-induced skin necrosis.

C. Chemicals/Drugs:
Examples:
1. Lead toxicity and neuronal and renal tubular necrosis.
2. Glucocorticoid-induced lymphocyte apoptosis.

D. Infectious Agents:
Examples:
1. Adenovirus-induced epithelial cells necrosis.
2. Endotoxin-induced apoptosis of lymphocytes and hepatocytes in gram-negative bacteriemia.

E. Immunologic reactions:
Examples:
1. Complement-mediated hemolysis in autoimmune hemolytic anemia.
2. T-lymphocyte-induced cells apoptosis in viral infection.

F. Genetic defects:
Examples:
1. Congenital malformations due to the genetic disorders.
2. Lysosomal storage diseases caused by genetically determined enzymatic abnormalities.

G. Nutritional imbalances:
Examples:
1. Obesity in nutritional excesses.
2. Anemia caused by low dietary iron intake.

Pathogenesis of cell injury.
• Intracellular systems are particularly vulnerable to the action of injurious agents:
  1. Intracellular aerobic respiration,
  2. Cell membranes,
  3. Enzymatic and structural protein synthesis, and

• The structural changes of cell injury become apparent only after some critical biochemical derangement has occurred.

The result of cell injury depends on the injury (i.e. the type, duration and severity of the injurious stimulus) and on the cells (i.e. the type, state and adaptability of the injured cell).
FORMS AND MORPHOLOGY OF THE CELL INJURY

A. Reversible cell injury:

- **Degeneration** (cellular dystrophy) refers to morphologic changes in cells caused by sublethal injury.
- The cells can revert to the former state of homeostasis if the noxious agent is removed.
- The changes are associated with the structural alterations in cell organelles or the cytoskeleton and with the intracellular accumulations of the various substances as a result of metabolic derangements in cells. The processes resulting in abnormal intracellular accumulations can be divided into three general types:
  a) storage in cells or/and in the interstitium of some physiologically common structural substances in excessive or decreased amounts;
  b) storage in cells or/and intercellular spaces of some substances that would not normally appear;
  c) appearance and storage in cells or/and in the interstitium of some substances that are not normally present in human body.

These quantitative and qualitative changes of different metabolic products are caused by enzymatic process disorders, and take place through four morphogenic stereotypes: infiltration, decomposition, transformation or pathological synthesis. They are briefly characterized in table 1.

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<th>Morphogenic mechanism</th>
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| **1) Infiltration**   | Excessive penetration in cells (intercellular spaces) of some metabolic products from blood, lymph, urine, and their further storage because of the insufficiency of the enzymatic systems which should normally metabolize them. | a) Infiltration of epithelial cells and renal tubules with glucose in diabetes mellitus.  
  b) Infiltration with lipids of the hepatic lobules in obesity (lipemia). |
| **2) Decomposition**  | The break down (decomposition) of some complex chemical substances and the storage of their components in cells or in the extracellular compartment. | a) Break down of lipoproteic complexes from the membrane structures in hypoxia, intoxications.  
  b) The decomposition of the glicoproteic complexes from the fundamental substance of the connective tissue in rheumatic diseases. |
| **3) Pathologic synthesis** | The synthesis of some substances not normally seen in cells and tissues. | a) The synthesis of abnormal glycogen in some hereditary glycogenosis.  
  b) The synthesis of abnormal protein in cells and of the abnormal proteogluicidal complexes in the intercellular space of the connective tissue in amylloidosis. |

*Table 1*
I. PROTEIC DEGENERATIONS.

1. Granular dystrophy.

It can be found in parenchymatous organs such as kidneys, myocardium, liver. Microscopically it manifests itself by the presence in the cells (nephrocytes, cardiomyocytes, hepatocytes) of a large number of tiny proteic granules.

Macroscopically, the affected organs are somewhat larger in size and weight. They have a weakened capsule, a flaccid consistency, and a whitish color, opaque and pale if sliced like boiled meat, therefore its name of dim intumescence.

*Microspecimen* “Granular dystrophy of the epithelium of contort renal tubules” (fig.1).

The epithelial cells of the contort renal tubules are bigger in size, tumefacted, indistinct, with unclear margins. The cytoplasm has a granular, reticulated aspect containing small proteic granules, uniformly spread, colored with eosin in pink. The lumen of the tubules is thinner than normal and some of them contain proteic masses (proteinuria).

The more frequent morphogenic mechanism of granular dystrophy is infiltration-penetration inside the cell of some chemical liquid substances, which are metabolic products of blood, lymph, urine, etc. The infiltration process can be easily seen using electronic microscopy.

*Electronmicrography* “Granular dystrophy of the epithelium of proximal renal tubules” (fig. 2).

The vesicles of the endoplasmatic reticulum are dilated, forming vacuoles which contain proteic masses; the mitochondria are somewhat tumefied.

A slight dysfunction of kidneys is observed in this type of dystrophy; in urine, proteic masses are present (proteinuria). This process is reversible if the cause is eliminated. In other cases, the changes can evolve to serious lesions, as found in the hyalinecellular dystrophy, hydropic or lipidic dystrophy. The causes of the granular dystrophy are various: disorders of blood and lymph circulation (ischemia, venous stasis), infectious diseases (viral and bacterial), exogen and endogenous intoxication.

It must be mentioned that the appearance of proteic granules in the cytoplasm of cells can be seen also in physiological conditions, reflecting the morpho-functional peculiarities of the cell (e.g., the production of secretion granules in the endocrine cells, physiological resorption of proteins by the epithelium of proximal renal tubules, etc.), the increased function of protein synthesis (for example, in hepatocytes, secretory cells), hyperplasia and hypertrophy of cytoplasmic organelles caused by the overtaxing of the parenchymatous organs.

2. Intracellular hyaline dystrophy (intracellular hyalinosis).

It is manifest by the presence of large drops of proteic origin in the cells. This type of dystrophy is not macroscopically evident in the affected organs. Using electron microscopy, the tumefaction of cytoplasmatic organelles can be seen. The more frequently affected organs are the kidneys (the epithelium of renal tubules) and the liver (hepatocytes).

*Electronmicrography* “Intracellular hyaline dystrophy of the epithelium of proximal renal tubules” (fig. 3).

The destruction of mitochondrial crista as cause for their tumefaction and homogenisation, and their transforming into proteic hyalin structures, can be observed in the nephrocyte cytoplasm.
Using optic microscopy, these changes appear in the cytoplasm as acidophilic, confluent and homogeneous drops of proteic origin (fig. 4).

Intracellular hyaline dystrophy in the kidneys is seen in such diseases as glomerulonephritis, renal amyloidosis, diabetic glomerulopathy, paraproteinemic nephrosis, intoxications, etc., when the permeability of the glomerular filter increases. This is an irreversible process that leads to focal coagulative necrosis of the cell. It is clinically manifested by severe disorders of organ function (e.g., the presence of proteins and cylinders in the urine).

Similar lesions appear in the cells of the liver in case of alcoholic hepatitis (alcoholic cirrhosis) - so called Mallory corpuscles or the alcoholic hyalin (hyaliniform inclusions situated around the nucleus).

Intracellular hyaline dystrophy is caused by such processes as the destruction of cellular organelles, cytoplasmic protein denaturation, resorption (infiltration) of some macrodispersive abnormal proteins or proteins pathologic synthesis (e.g. Mallory corpuscle).

- Due to entry of excessive isotonic fluid into the cells as a result of functional derangements in the mitochondria and plasma membrane.
- The organ shows pallor, increased turgor and increased weight.
- Individual cells are swollen with accumulation of Na+ and water, and loss of glycogen.
- With progressive influx of water, clear vacuoles appear in the cytoplasm.

Microspecimen “Hydropic degeneration of the renal contort tubule epithelium” (fig.5).
A large number of optically empty vacuoles are present in nephrocytes of the renal contort tubules. These oval or round vacuoles are situated mostly along the basal membrane; the nucleus of these cells is pale and the lumen of the tubules is more narrow than in normal conditions.

Electronmicrography “Vacuolar swelling of the liver”. In the altered hepatocyte (fig.6) a large number of dilated channels of the endoplasmatic reticulum are present; these all form cisterns containing cytoplasmatic liquid.

The basic mechanism of the vacuolar swelling is a disorder of the hydro-electrolyte and proteic metabolism. Modification of the intracellular colloid-osmotic pressure leads to the penetration of water into the cells, or to disorder of water elimination from cells while the redox processes take place. The excessive accumulation of water leads to destruction of cellular ultrastructures and the appearance of vacuoles containing cytoplasmatic liquid. This liquid accumulates in the cisterns of the endoplasmatic reticulum and in the mitochondria. The precise diagnosis of hydropic swelling can be made only after coloring the microspecimen for glycogen and lipids (the negative staining confirms the diagnosis). Vacuolar dystrophy is an irreversible process ending up with the colliquative necrosis of the cells. It can also end up with the swelling of cells as an aspect of focal colliquative necrosis. The affected organs suffer from severe functional disturbances. For example, the hydropic degeneration of the epithelium of contort renal tubules frequently seen in the nephrotic syndrom, is characterized by severe proteinuria (as a result of diminished of tubular resorption function), dysproteinaemia, hypoproteinaemia, hyperlipidaemia and edema. The vacuolar dystrophy of the myocardium manifests itself by a great reduction of the contractile function of the heart.
Hydropic accumulations is also present in some infectious diseases (mostly in variola and viral hepatitis), intoxications (with phosphor, arsen, carbon tetrachloride), inanition, avitaminosis and under the action of penetrating radiation, etc.


This type of dystrophy is mostly found in skin and mucous membranes covered by squamous and transitional epithelium. The excessive keratinization of the pluristratified squamous corneous epithelium of the skin (hyperkeratosis), or the presence of keratin in the mucous epithelium, which in normal condition is not corneous (leukoplakia).

**Macro- and microspecimen “Hyperkeratosis of skin”**

Macroscopically, in the sites of hyperkeratosis, the skin is thick, dry, and has a fish scaled appearance (fig.7). Microscopically (fig.8), the corneous stratum of the epidermis is considerably thickened as a result of the excessive keratin synthesis. Of great importance in the etiology of the lesions are chronic inflammation, viral infections, avitaminosis, especially the lack of vitamin A, chronic irritations, some skin developmental disorders (e.g. inborn hyperkeratosis or ichthyosis).

**Macro- and microspecimen “Leukoplakia of the buccal cavity mucosa”**

Macroscopically, (fig.9) it has a whitish color, with smooth or rough surfaces, and can reach several cm in size. Microscopically, the pluristratified squamous epithelium (fig.10) is thickened, the superficial layer consisting of keratinised anuclear cells and covered with a layer of keratin.

It is more frequently seen on the mucosa of the buccal cavity, tongue, lips, pharynx, larynx, vaginal surface of the uterine cervix, vagina and the bladder. The leukoplakia may appear also on the mucous surfaces covered with unistratified epithelium, following squamous metaplasia of the mucous membrane in bronchi, stomach, intestine, and endometrium. The most frequent causes of leukoplakia are chronic inflammation, chronic irritation, trauma, etc. It is considered to be a precancerous lesion.

The evolution of keratin dystrophy may lead to the rehabilitation of the affected tissue or to the necrosis of cells. The function of skin and mucous membranes in the affected regions is severely altered.

II. FATTY DEGENERATIONS

- Fatty change refers to any abnormal accumulation of fat within parenchymal cells.
- Liver cells, heart muscle cells and renal tubular cells are most commonly affected.
- The organ is large, pale and greasy.
- Individual cells contain in their cytoplasm membrane-bound fat droplets as clear vacuoles, may fuse, displace the nucleus to the periphery of the cell.

**Macrospecimen “Fatty change of the myocardium (steatosis of myocardium)”**

The heart is larger in all dimensions (fig.11); the chambers are enlarged and dilated. The myocardium has a flaccid consistency, and broadens and stretches at necropsy; if sectioned, it is opaque, and pale yellow. Under the endocardium, especially close to the papillary muscles, alternating fatty yellowish striae and normal tissue can be observed, making the heart look like tiger skin (“tiger heart”). The tiger skin appearance of the myocardium is characteristic for focal fatty dystrophy, because the fat deposition is around the veins and venules.
Electronmicrography “Fatty change of the myocardium” (fig.12)

Drops of fat are found in the myocardial cells sarcoplasm, when electron microscopy is used; they are tightly bound to the membrane of the cytoplasmic organelles, especially of the mitochondria. At the contact zones with the lipidic inclusions, the intracellular membranes become structurally indistinct.

Fatty dystrophy is more frequently found in chronic cardiovascular insufficiency, severe anemia (pernicious anemia), severe infectious diseases (diphtheria), intoxications (with ethanol, phosphor), etc. The contractile function of the heart is decreased. The myocardial steatosis is considered to be morphologic substrate for the functional decompensation of the heart.

The predominant morphological mechanism of myocardial steatosis is decomposition, the degradation of the lipoproteinic compounds of the intracellular membranes.

Macrospecimen “Fatty change of the liver (steatosis of liver)"

The liver is larger in weight and volume (fig.13). The fibrous capsule is weakened and smooth, with rounded margins, and soft and paste-like consistency. If sectioned it has a yellowish (clay) color, which can be either homogeneous or patchy. During necropsy, fat remains on the blade of the necropsy knife. The lobular aspect of the liver is either unchanged, in cases where the dystrophic changes take place only in some parts of the lobule, or is erased, in severe cases with diffuse steatosis of the hepatocytes. In the second case the liver is macroscopically like goose liver.

Microspecimen “Steatosis of liver"

The cytoplasm of hepatocytes (fig.14 and 15), may contain many lipidic drops of different dimensions, without a bordering membrane. These appear to be optically empty if prepared with paraffin (lipids are soluble in alcohol, chloroform, etc.) and of a red-yellow (orange) color if the specimen has undergone cryosectioning (with the congelation microtom) and Sudan III staining (lipophilic colorant). The fat drops are larger at the lobule periphery and smaller in the central region. In some cells from the peripheral regions of the lobule, the fat drops undergo fusion and form one single drop which fills the entire cytoplasm. The nucleus appears to be flattened and pushed towards the cellular membrane.

Electronmicrography “Steatosis of the hepatocyte” (fig.16)

The cytoplasm of the hepatic cells contains numerous small lipidic inclusions, with black-white striations, situated mostly in the perinuclear region.

The most frequent causes of hepatic steatosis are lipidaemia (obesity, excess fats in alimentation, chronic alcoholism, diabetes mellitus, hormonal disturbances), hepatotrophic intoxications (with phosphor, carbon tetrachloride, ethanol, chloroform, etc.), nutrition disorders (lack of proteins or lipotropic factors, avitaminosis, digestive tract affections, etc.), tissular hypoxia (cardiac insufficiency, severe anemia, pulmonary affections), etc.

The predominant morphogenetic mechanism of peripheral zone steatosis of the hepatic lobules (peripheral or periporal steatosis) is infiltration which is observed in case of hyperlipidaemia (the fats reach the liver with the portal blood and infiltrate first in the peripheral zones of the lobules). The morphogenetic mechanism of the central zone steatosis is decomposition, which occurs for example in case of progressive hypoxia of liver.

The function of the liver in case of fatty degeneration remains normal for a long time. If the action of the pathogenic factor persists, necrotic processes are associated and a portal type cirrhosis develops.
III. GLYCOGENIC DEGENERATION

It is manifested by the excessive accumulation of glycogen in the cytoplasm of cells, mostly found in diabetes mellitus.

Microspecimen “Glycogen infiltration of renal contort tubule epithelium in diabetes”

Glycogen granules of different dimensions staining red with Best carmin, are present in the epithelial cells of the renal tubules (fig. 17). The most severely affected cells are the ones from the narrow segment and from the distal portion of the contort tubules. Glycogen granules can be observed also in the lumen of the tubules. The glomeruli suffer a thickening of the basal membrane of the capillaries and the depositing of polysacharides in the mesangium (intercapillary glomerulosclerosis). The main morphogenic mechanism of renal glyccogenic dystrophy is infiltration.

The renal modifications in diabetes mellitus appear as a result of hyperglycemia and glucosuria. These are caused by the taking over and utilization of glucose by the tissues, associated with the insufficient secretion of insulin by the beta cells of the pancreatic isles (Langerhans). The process can be reversible.

IV. PIGMENTATIONS

A. Exogenous Pigments and Particulates.

Exogenous material often accumulate in the body that are not readily degraded by hydrolytic enzymes in the extracellular space or by macrophages. The most common example of this accumulations is pneumoconiosis.

1. Pneumoconioses:
Definition: Accumulation of particulate matter within the lung.
   a. Small particles (1-5 microns in diameter) settle on the mucosa of the terminal airways (e.g., respiratory bronchioles) and are phagocytosed by alveolar and peribronchiolar macrophages.
   Light microscopy reveals peri-bronchiolar cuffs of particle-laden macrophages (granular, brown-black, intracytoplasmic pigment).
   b. Pathogenicity varies depending on the nature of the particulate material and amount of deposition. Particulates such as silica can cause pulmonary fibrosis, emphysema, and pulmonary inflammatory disease. Particulates such as asbestos can cause fibrosis and neoplasia.
   c. Examples:
      1. Anthracosis: called miners disease, carbon-rich particulates.
      3. Asbestosis: asbestos fibers.

B. Endogenous cell pigments:

Substances produced by the organism/cells accumulate when they are produced in quantities that exceed the cells ability to metabolize or secrete them; or if the cell is lacking enzymatic capacity to metabolize them (example: hereditary storage diseases).
1. Lipofuscin/ceroid (Latin: fuscus = brown, a brown lipid)
   a. Composed of lipid-protein complexes derived from the peroxidation of lipid in cell membranes (free radical damage).
   b. Lipofuscin accumulates in cells over time as a “wear and tear” pigment.
   c. Conditions such as vitamin E deficiency lead to increased tissue deposition.
   d. Most common tissue sites are heart, liver, and brain cells.
   e. The cellular accumulation site are the lysosomes.
   f. Lipofuscin is not toxic to cells except in large quantities.
   g. Morphology:
      1) granular, brown pigment;
      2) autofluorescent and acid-fast;
      3) tissues look brown grossly when high accumulation occurs.

Macrospecimen “Brown atrophy of the heart” (fig. 18).

The heart is smaller in all dimensions, without adipose tissue under the epicardium. The coronary arteries have a meandering pattern; the myocardium is brown on surface and if sectioned.

Microspecimen “Lipofuscinosis of the liver” (fig. 19).

The hepatic trabeculae are thinner than normal because of atrophy of the hepatic cells. The cytoplasm of the hepatocytes in the central zones of the lobules contains granules of lipofuscin, which have a brown color. These granules are situated mainly around the nucleus. The spaces between the hepatic trabeculae are larger than normal.

The accumulation of lipofuscin in organs and tissues (acquired lipofuscinosis) takes place in cachectic conditions, senile atrophy, hypoxia, functional overtaxation (ex. lipofuscinosis of the myocardium in valvular lesions). Therefore lipofuscin is also called the “usage pigment”.

The lipochromes produce the yellow color of the adipose tissue, the corpus luteus of the ovaries, the adrenocortical glands, the testicles, the blood serum, and the transudate.

2. Hemosiderin.
   a. Derived from hemoglobin breakdown.
      - Erythrocyte phagocytosis leads to release of iron from hemoglobin.
      - Iron complexes with apoferritin form micelles that are seen as hemosiderin.
      - Usually locates in the lysosomes.
   b. Normally observed in small amounts in mononuclear phagocytes in the bone marrow, spleen and liver.
   c. Excessive tissue concentrations occur where there is hemorrhage, chronic congestion and diapedesis of erythrocytes into tissue, or excessive hemolysis (autoimmune hemolytic anemia, hemoparasitic diseases)
   d. Morphology:
      1) golden-yellow to brown, granular or crystalline, intracytoplasmic pigment;
      2) prussian blue staining is blue-black.

Note: A tissue artifact that is sometimes mistaken for hemosiderin is acid hematin. Acid hematin has a similar brown appearance in tissue to hemosiderin. Acid hematin is formed when hemoglobin is precipitated in acid formalin fixatives. Use of neutral-buffered formalin avoids this problem.
e. Hemosiderin deposits are not usually toxic.
f. Pathologic accumulation of hemosiderin in parenchymal cells occurs in hemochromatosis, a disease characterized by excessive iron accumulation in cells. Iron builds up in cells such as hepatocytes, and cells injury results from free radical-induced lipid peroxidation and increased lysosomal membrane fragility.

Microspecimen “Hemosiderosis of kidneys” (fig. 20).
The epithelial cells of the renal contort tubules contain hemosiderin granules of brown color, which can be observed in some places in the lumen of the tubes.

Microspecimen “Pulmonary hemosiderosis (pulmonary chronic venous stasis)”. In the alveolar lumen (fig. 21 and 22), in the thickness of the alveolar septum, and in the peribronchovascular connective tissue many macrophages are present. The cytoplasm of the macrophages contains hemosiderin granules of brown-black color, with hematoxylin-eosin and blue color in Perls reaction. This reaction is a specific method for hemosiderin identification: by adding potassium ferrocyanide and hydrochloric acid on the unstained microspecimen, blue granules of iron ferricyanide form (also called Berlin blue or Prussia blue).

Pulmonary hemosiderosis appears in passive (venous), chronic congestion of the lungs, caused by heart diseases (valvulopathy, cardioclerosis, myocarditis, etc.). Hemosiderin is synthesized in alveolocytes and histiocytes. The cells containing hemosiderin are eliminated with sputum and can be analyzed in a laboratory; these cells are called “cardiac cells”. This pathological process is also called “brown induration (hardening) of the lungs” because of the increase of consistency as a result of the connective tissue proliferation under chronic venous hypoxia. Pulmonary hemosiderosis is an illustrative example of localized hemosiderosis resulting from extravascular erythrocyte hemolysis.

Macrospecimen “Cerebral hematoma” (fig. 23).
In the area of subcortical nuclei there can be observed a well-delineated cavity filled with brownish clotted blood. The color is accounted for by hemosiderine.

Microspecimen “Old cerebral hemorrhage” (cerebral hematoma) (fig. 24)
In the central part of the hematoma, hematoidin granules (crystals) can be observed, placed extracellularly in the necrotic masses (detritus). At the peripheral zones of the hematoma, in the surrounding cerebral tissue glial cells, hemosiderin (because of phagocytosis) can be seen.

3. Bilirubin
a. Bilirubin is a breakdown product from heme proteins derived from hemoglobin and from other heme groups such as cytochromes.
b. A major source is from erythrocyte breakdown in mononuclear phagocytes
   - Heme is converted to biliverdin by heme oxygenase.
   - Biliverdin is metabolized to bilirubin by biliverdin reductase.
c. Bilirubin is usually bound to albumin in plasma as the unconjugated form.
d. Unconjugated bilirubin is transported into hepatocytes, conjugated with glucuronic acid in the endoplasmic reticulum and excreted in bile.
e. Jaundice (icterus) is the yellow aspect of the skin, sclera, and mucous membranes due to elevated blood levels of bilirubin (>2-3 mg/dl)
f. Causes of hyperbilirubinemia (icterus) include:
   1) Increased breakdown of heme proteins.
   2) Decreased hepatic uptake of albumin-bound bilirubin.
   3) Impaired conjugation of bilirubin.
   4) Impaired intra-hepatic excretion of bilirubin into bile.
   5) Bile duct obstruction.

   g. Unconjugated bilirubin is highly toxic and is normally bound to proteins.
   h. Conjugated bilirubin is not toxic at low concentrations. It can cause tubular necrosis when concentrated in urine.

**Microspecimen “Biliary stasis in mechanical jaundice” (fig. 25).**

The biliary canaliculae and some interlobular canals are dilated, their lumen contains biliary coagulates of a brown color (biliary “thrombus”). The cytoplasm of some hepatocytes contains granules of biliary pigment. In the center of the hepatic lobules there can be observed necrotic foci of hepatocytes, which are imbued with bile.

Biliary stasis (mechanical jaundice) can be caused by biliary calculus, tumors of the biliary ducts, of the pancreatic head or of the duodenal papilla (Vater’s ampulla), by malformations of the biliary ducts, by cancer metastasis in the lymph node of the hepatic hilus, by scar formations of the biliary ducts, etc. The excess of conjugated bilirubine in blood leads to yellow-greenish coloring of the organs and tissues, including the skin and sclerae. Besides the intense coloring of the skin, the obstructive jaundice has other effects like general intoxication (with biliary acids), hemorrhagic syndrome, dystrophic lesions of the kidneys and hepatorenal insufficiency. The complication of biliary stasis is inflammation of the biliary ducts (cholangitis), but if the evolution of this process is chronic, it can lead to cholestatic biliary cirrhosis.

4. **Melanin** *(Greek melas = black)*

   a. Melanin is formed via oxidation of tyrosine to dihydroxyphenylalanine in melanocytes by tyrosinase.

   b. Melanosis refers to excessive accumulation of melanin in tissue.

**Macrospecimen “Pigmentary nevus” (fig. 26).**

Two nodular structures with a papular appearance, having a diameter of several mm., can be observed on the skin. The structure is pigmented in brown or black-brown color and covered with hair. It has a smooth or verrucous surface and a soft consistency. The color of the nevus is a result of localized excess of melanin.

The pigmentary nevi represent circumscribed, congenital developmental disorders of the skin. They consist of nevus cells, which proceed from lemocytes (Schwann cells) and can synthesize melanin. They usually begin to grow in volume in puberty and during gestation, after which their growth slows down. They are an example of localized melanosis (hyperpigmentation). The pigmentary nevi can evolve into one of the most malignant tumors, the melanoma.

**Macrospecimen “Metastasis of melanoma in bones” (fig. 27).**

On the sectioned bones there can be observed multiple nodules of round or ovoid form, which are well limited from the surrounding tissue. They are black-brown in color due to the great quantity of melanin. The primary tumor can be localized on the skin, in the pigmentary tunica of the eye, and more rarely in leptomeninges and in the medulla of adrenal glands. The metastasis found in bones are spread hematogenous.
5. Calcification

A. Normal deposition of calcium salts occurs in bone and cartilage.

B. Abnormal (pathologic) deposition of calcium salts occurs in other tissues in either intra- or extracellular locations: deposits as calcium phosphate (similar to hydroxyapatite of bone) and deposits as calcium hydroxide in connective tissue: elastin and collagen.

C. Morphology:
   1. Gross: calcified tissue is chalky white to grey, gritty to bone-hard when sectioned.
   2. Microscopic: calcium deposits are usually deeply basophilic, granular and stain positively with Von Kossa stains (black) and alizarin red.

D. Pathogenetic mechanisms:
   1. Dystrophic calcification
   2. Metastatic calcification

E. Dystrophic calcification:
   Abnormal calcium deposition in dead or degenerating tissues.
   1. Intracellular calcification: initially accumulates in mitochondria of dying cells.
   2. Extracellular calcification: calcium has a high affinity for phosphate-rich plasma membrane fragments and basement membrane (acid phospholipids accumulate due to phosphatase activity), calcified fragments usually present extracellularly in membrane-bound vesicles probably derived from degenerating cells.

Sequeiae:
   a. Persists and may result in tissue dysfunction (e.g. loss of tissue elastic properties).
   b. Can serve as a focus for heterotopic bone formation (presence of bone in an abnormal location).

F. Metastatic calcification:
   Abnormal calcium deposition in abnormal tissues secondary to hypercalcemia. This pathogenetic mechanism is complicated because high levels of calcium are capable of causing cells injury. In hypercalcemia, cells injury may underlie the cellular mineralization. Elevated serum calcium is present and abnormal calcium metabolism occurs.
   Causes or examples include:
   a. Hypervitaminosis D.
   b. Ingestion of toxic plants, some plants such as Solanum malacoxylon and Cestrum diurum, contain agents identical to or similar to 1,25-dihydroxycholecalciferol (vitamin D$_3$).
   c. Primary Hyperparathyroidism: increased levels of parathyroid hormone due to parathyroid neoplasia causes bone resorption and release of calcium into the circulation.
   d. Secondary (nutritional) hyperparathyroidism: increased production of parathyroid hormone due to areal or relative decrease in serum calcium, which is the result of inadequate levels of calcium in the diet. Bone resorption occurs to maintain adequate calcium levels.
   e. Renal secondary hyperparathyroidism occurs in chronic uremia, which results in phosphate retention by dysfunctional kidney and impaired uptake of calcium.
   f. Hypercalcemia of malignancy (multiple myeloma, metastatic cancer, osteosarcoma, lymphosarcoma).

Common sites of metastatic calcification: Blood vessels (intima and media) and connective tissue of the kidney, lungs, and stomach.
Macrospecimen “Petrification in the lung” (fig. 28).
When the lung is sectioned, a focus of calcium salts in round form, whitish color and hard consistency can be seen. It has a chalklike appearance. This petrification has appeared as a result of dystrophic calcification of a caseous necrotic site in a case of pulmonary tuberculosis.

Microspecimen “Calcification (calcinosis) of the coronary artery in atherosclerosis” (fig. 29).
In the wall of the coronary artery there can be observed multiple focuses of calcinosis of a violet color, if the specimen is stained using hematoxylin-eosin. The accumulation of calcium salts takes place in the atherosclerotic plaque, a circumscribed, prominent thickening of the arterial intima. This leads to reduction in size of arterial lumen.

6. Nucleoproteic degenerations
The disorders of the nucleoproteic metabolism are manifest by the excessive formation of uric acid and of its salts, which can accumulate in tissues. It is seen in gout, urinary lithiasis and in the uratic infarct in newborn.

Microspecimen “Gout toph” (fig. 30).
Foci of crystalline or amorphous accumulations of sodium urates, surrounded by necrotic tissues, inflammatory infiltrates containing gigantic multinucleated cells of foreign bodies (which are responsible for the resorption of the uric salts). The proliferation of the connective tissue can be observed in the subcutaneous tissue. Macroscopically, these sites have the appearance of painful nodules in the region of the finger, knee and tibiotarsal articulations, etc.

7. Calculogenesis (lithiasis)
The classification, morphogenic mechanisms and the consequences of lithiasis are represented in table 2.

Macrospecimen “Biliary calculi” (fig. 31).
In the cavity of the gallbladder there can be observed multiple calculi of various forms and dimensions. They have a smooth, rough or granular surface, of a yellowish-white color and a hard consistency.
Considering their chemical structure the calculi can be cholesterolic, pigmentary, calcific or mixed. The biliary calculosis can evolve into obstruction of the cystic canal, retention of bile and the developing of hydropsy, mucocelle or even of the vesicular empyema, the apparition of the acute or chronic inflammation of the vesicle, the perforation of the wall and the overflowing of bile into the peritoneal cavity, leading to consecutive biliary peritonitis.

Macrospecimen “Renal calculi” (fig. 32).
In the cavity of the renal pelvis there can be observed multiple calculi of irregular form, branchlike (corallike), with a rough surface and a whitish color. The cavities of the pelvis and of the calyces are dilated, and the renal parenchyma is atrophied.
Considering their chemical composition, the most frequent are the uratic calculi (consisting of uric acid and its salts), calcium oxalate and phosphate calculi. The pelvic
Calculi cause retention of the urine, distention of the pelvis and calyces, atrophy of the renal parenchyma, and repeated installation of hydronephrosis. It is associated with chronic pyelonephritis.

Table 2