**RETROGRESSIVE CHANGES: Part 3**

**Calcification** (***calcificatio)***

* physiological - a desirable process in bones
* pathological - abnormal deposition of calcium salts (mainly calcium phosphate) together with small amounts of iron, magnesium and other minerals.
* it can occur in various organs (outside of the skeleton) 🡪 heterotopic calcification **(*calcificatio heterotopica)***
* calcium salts can be deposited in tissues, they can accumulate in the form of crystals, as deposits, form small grains, lumps or large stones
* calcium deposits show an affinity for alkaline dyes 🡪 dark blue in HE-staining
* histologically, calcification consists in the formation of **intra- and/or extracellular deposits**
* over time, heterotopic bone tissue may form at the calcification site

**Dystrophic calcification (*calcificatio dystrophica*)**

The accumulation of calcium salts in pathologically changed tissues and organs (degenerated, necrotic or atrophic tissues) with normal serum levels of calcium ions. Calcification is often preceded by necrosis or tissue hyalinization. Examples include: caseous lesions, atherosclerotic plaques, thrombi, some cancers, parasites, etc. Often observed in the heart and in skeletal muscles.

**Pathogenesis** of dystrophic calcification includes initiation and growth

Both processes can occur intracellularly and extracellularly, and the final product is a calcium phosphate crystal. Extracellular initiation occurs in membrane-bound vesicles, 200 nm in diameter, originating from degenerating cells. Intracellular calcification is initiated in the mitochondria of dead or dying cells that have lost their ability to regulate intracellular calcium level. After the initiation of the process in any location, the crystal starts to grow.

**Metastatic calcification (*calcificatio metastatica*)**

Can occur in normal tissues in conditions of high serum levels of ionized calcium (hypercalcemia). Causes of hypercalcemia:

* increased parathyroid secretion caused by the primary parathyroid tumour, or hormone production by the tumour in another location (hyperparathyroidism)
* bone resorption at long-term immobilization, bone destruction (primary and metastatic cancers), inflammation of the bone,
* D3 hypervitaminosis, vitamin D intoxication D or sarcoidosis (where macrophages activate vitamin D precursor)
* renal failure, where phosphate retention leads to secondary hyperparathyroidism
* alkalosis
* In herbivores fed mainly on grasses with high phosphorus and low calcium content, parathyroid glands are stimulated and secretion of parathormone increases, which stimulates Ca resorption from bones and hypercalcemia.

The most frequent location of metastatic calcification is the wall of blood vessels, lungs, kidneys (increased pH of these organs). Generally, this does not impair the clinical efficiency of organs. Massive calcifications in the lungs diminish respiratory efficiency, in the kidneys they can cause damage to this organ (excessive accumulation of calcium salts in kidneys due to hyperparathyroidism - ***nephrocalcinosis***).

**Arterial calcification (*calcificatio arteriae)***

Grossly:

* white/greyish white colour of tissues
* sclerosis

Microscopically:

* calcium salts in the form of grains and lumps or amorphous masses, staining dark blue/purple or navy blue (HE)
* fibre necrosis
* decayed areas may be surrounded by an inflammatory process

**Gout *(diathesis urica)***

Gout is a disease caused by excessive accumulation in tissues of salts of uric acid - the final product of purine metabolisms. It is characterized by the presence of inflammation (acute or chronic, degenerative) in tissues, accompanied by the accumulation of large crystalline aggregates formed from sodium and potassium urate - tophi ***(tophi urici)***, which pass from body fluids saturated with them to tissues.

Causes:

* primary - congenital metabolic disorders (enzymatic defects) occurring with excessive uric acid production - mainly in humans, 85-90% of cases
* secondary - increased production and excretion of uric acid (related to excessive nucleic acid metabolism - tissue breakdown, e.g. leukaemia, true polycythaemia, haemolytic anaemia, chemo- and radiotherapy of cancers)
* decreased excretion of uric acid in chronic kidney failure, ketoacidosis or lactic acidosis, some diuretics, high protein diet.

Gout is a significant problem in birds and reptiles because they do not have uricase - the enzyme oxidizing uric acid to allantoin (uricase is found in mammals except for humans, apes and Dalmatian dogs).

Causes:

* excessive supply of protein in feed (animal feed and dog and cat food)
* kidney damage through:
  + insufficient water intake with an oversupply of protein or antibiotics (too low humidity, temperature or lack of access to clean water)
  + excess dietary phosphorus intake
  + bacterial, viral and parasitic diseases
  + toxins and heavy metals

In this case, the concentration of uric acid and its salts in the blood (hyperuricemia) increases, which leads to the precipitation of urate crystals into the internal organs and joints.

A pathological condition related to the deposition of urate crystals in:

* internal organs - visceral gout - the crystals are deposited in liver, kidneys, spleen, pericardium, intestines, in the subcutaneous tissue and under the tongue
  + Grossly: organ enlargement, small lumps, grains, chalk-like deposits present on the surface.
* joints - known as articular gout (tophi are formed)

Gout symptoms:

* dejection
* anorexia
* dehydration
* excessive thirst/increased urination
* thermoregulatory disorders
* yellowish urine
* decrease in muscle tone
* joint swelling and lameness (articular gout)
* oedema and dysfunction of internal organs

**Renal gout (*diathesis urica renis)***

Microscopically:

* fan-shaped or radially arranged “needles” of urate crystals forming tophi **(*tophi urici)*** are present in the tubules (first of all the collecting tubules) and glomeruli, contributing to their transformation or damage
* urate deposits demonstrating increased alkalinity - stained **blue** with hematoxylin
* urate clusters are often surrounded by macrophages, lymphocytes, fibroblasts and foreign body giant cells.

**Dystrophy (*dystrophia*)** - dystrophy occurs when different retrograde changes occur in the organ at the same time and may be accompanied by cardiovascular disorders and pigmentation changes.

**Hepatic dystrophy** *(dystrophia hepatis)*

The most common changes in the liver include:

* degenerations: parenchymal, vacuolar and fatty
* hepatocyte necrosis
* pigmentation changes (jaundice)
* cardiovascular disorders;

If the animal survives, repair and recovery processes occur in the organ 🡪 cirrhosis

Causes of hepatocyte damage:

* hypoxia resulting from cardiovascular disorders and anaemia
* dietary errors: insufficient sulphur-containing amino acids (methionine, cysteine), vitamins E and selenium, a mono diet based on fish meat
* alkaloids present in certain plants
* fungal, bacterial toxins, toxic compounds (chlorinated naphthalenes)

Grossly:

* the picture depends on the prevailing change
* organ enlargement
* fragile parenchyma with blurred structure in the cross-section

Microscopically:

* the picture is variable
* various types of degenerations
* cellular necrosis
* vascular congestion
* pigmentation changes

**Fibrous osteodystrophy (*osteodystrophia fibrosa)***

* consists in the resorption of bone tissue and its replacement by fibrous connective tissue and disorderly distributed patches of abnormal bone
* bone tissue is damaged or rebuilt
* the process is slow
* bone resorption is strongest in the subperiosteal areas
* bones become deformed, brittle, slightly flexible
* it occurs in both flat and long bones (bones of the limbs, tail vertebrae, mandible)
* it occurs in different species, both growing and adult animals

Cause: is primary, secondary or pseudohyperparathyroidism

* significant lowering of the serum calcium ion level 🡪 increased parathormone (PTH) secretion
* osteoblasts have receptors for parathormone. At high PTH concentration, osteoblasts stimulate the differentiation of macrophages/osteoclast precursors into osteoclasts, and at the same time, osteoprotegerin (secreted by the stromal cells and osteoblasts - responsible for inhibiting the differentiation of osteoclasts) is inhibited. There is an increase in the number and activity of osteoclasts that resorb the bone, and calcium ions are released into the blood
* additionally, bone marrow stromal cells also have receptors for PTH and with a long-lasting high concentration of this hormone, massive differentiation of these cells into fibroblasts occurs (stimulation of fibroblast growth factor 23; FGF23).
* osteoclasts resorb bone tissue 🡪 fibroblasts and osteoblasts emerge here 🡪 fibrosis and partial bone regeneration
* the marrow also undergoes fibrosis processes - dilated marrow cavities contain an increased amount of loose connective tissue and vessels
* PTH effects on intestines: increased absorption of calcium and phosphate ions (indirectly through activation of vitamin D precursors in kidneys).
* PTH effect on kidneys: decrease in phosphate resorption, increase in calcium ion resorption.

**Phosphates bind calcium ions, therefore hyperphosphatemia also causes relative hypocalcemia!**

Primary hyperparathyroidism:

* hormonally active adenoma and adenocarcinoma
* idiopathic bilateral hyperplasia (rare)

Secondary hyperparathyroidism (more common):

* of nutritional origin:
  + the feeding, especially to growing animals, of feed containing low calcium levels and high phosphorus concentration (e.g. feeding of bran to horses, pigs with feed containing only cereal grains, dogs and cats mainly or exclusively with meat or offal) 🡪 reduction in serum ionized calcium concentration 🡪 increase in PTH secretion
  + of renal origin:
    - chronic kidney failure 🡪 reduced phosphate excretion 🡪 an increase in serum phosphate concentration directly reduces calcium concentration 🡪 stimulation of parathyroid activity 🡪 increase in the parathormone level. Furthermore, in renal failure, the synthesis of the active form of vitamin D decreases, which consequently reduces the absorption of calcium in the small intestine

Pseudohyperparathyroidyzm (paraneoplastic syndrome with hyperparathyroidism)

* some cancers secrete biologically active proteins similar to PTH, e.g. apocrine adenocarcinoma of the circumanal glands or lymphosarcoma in dogs

In fibrous osteodystrophy, the following can be observed microscopically:

* wide bands of fibrous connective tissue
* thin bone trabeculae, randomly arranged in fibrous connective tissue
* the new tissue produced is subject to focal calcification - decalcified bone tissue, which causes bone deformation

**Rickets (*rachitis*)**

Disease of the growing skeleton, in young animals, consisting of abnormal mineralization of the skeleton, abnormal and insufficient calcification of epiphyseal plates, which interferes with the process of maturation of cartilage cells and formation of normal palisade arrangements. It results in impaired bone growth. The main changes occur in the growth zone of long bones, i.e. at the boundary of the shaft and epiphysis (growth plate). The epiphyseal plate becomes thicker (up to 10 times), its edge is uneven and notched. Rickets bones have a lot of decalcified bone tissue, they become light, flexible, deformed (bone shaft bending) and can be cut. The following occur:

* scoliosis of the spine
* ribs bending in an inward direction, causing the breastbone to protrude - the so-called pigeon chest (***pectus gallinaceum***)
* persistent and widened skull sutures
* thinning of the skull bones (***craniotabes rachitica***)
* thickening of the cartilaginous connections of the ribs (rickety/rachitic rosary - ***rosarium rachiticum***) and limb bone epiphyses
* rare: rickety dwarfism

Causes:

* vitamin D3 deficiency - inadequate diet, malabsorption - disorders of bile ducts, pancreatic or intestinal functions - facilitates the absorption of Ca and phosphates from the intestines, stimulates the activity of osteoblasts, stimulates osteocalcin synthesis, facilitates bone mineralization
* Ca deficiency - inadequate diet, malabsorption - mainly in birds
* phosphate deficiency, abnormal Ca:P ratio (norm: 2:1)
* malabsorption - long-term use of antacids that bind phosphates, making them insoluble
* acquired or congenital renal tubular diseases causing excessive excretion
* dietary deficiency - herbivores grazing on pastures with phosphorus deficiency
* alkaline phosphatase deficiency
* excess oxalic acid (beet leaves), lactic, malic and tartaric acid (silage), excess carbohydrates and vegetable fibre

In the case of properly growing bone, the following can be observed:

* a zone of cartilage proliferating cells with regular arrangement of cartilage cavities
* zone of chondrocyte proliferation
* zone of cartilage degeneration cells
* zone of calcification zone and the first bone trabeculae formed by osteoblasts

Microscopically, in rickets, the following are observed:

* abnormal cartilage arrangement - the columnar arrangement of chondrocytes is disturbed, which multiply forming young cartilage lesions
* calcification and ossification lesions in cartilaginous tissue, which impairs the maturation of cartilaginous cells
* irregular ossification line - the newly formed bone trabeculae are faint, less numerous, built of an non-calcified osteoid
* excessive growth of capillaries and fibroblasts in the disarranged zone of growth, resulting from microfractures and pressure on abnormally mineralized, weak and poorly formed bone. The only remaining part left in the place of the trabeculae is the connective tissue stroma - decalcified bone tissue.

**Osteomalacia, bone softening (*osteomalatio*)** - consists in reducing the calcium salt content at the normal behaviour of the matrix - the essence of the process is inhibition of the mineralization processes. Skeleton disease after its completed growth. The number and size of trabeculae is correct, but they are insufficiently calcified, resulting in a wide edge of decalcified bone tissue - osteoid (non-mineralised bone matrix produced by osteoblasts, built of type I collagen, an amorphous substance; ***tela osteoidea***, which is not subject to calcification). Bones become soft and deformed, they bend easily and may crack. The marrow cavity expands, the compact substance becomes spongious.

Causes (similar to the one causing rickets):

* phosphorus, calcium and vitamin D3 deficiency (pregnancy, lactation, malabsorption syndrome, hepatic failure, renal failure, nutritional deficits)
* impaired D3 metabolism (liver diseases, chronic renal disease)
* abnormal Ca:P ratio
* hormonal disorders of the ovaries
* chronic fluoride intoxication

Microscopically:

* normal bone trabeculae insufficiently calcified or devoid of calcium and deformed - a wide edge of decalcified bone tissue is formed

**Necrosis** - local, sudden death of cells, tissues, organs, in the living organism.

Morphological changes in the necrotic lesion are the result of two opposing processes:

1. dissolution of the tissue by enzymes of its own lysosomes (autolysis) released as a result of damage to their membranes or proteolytic enzymes derived from neutrophils (heterolysis)
2. protein denaturation as a result of increased tissue acidity

The prevalence of dissolution is demonstrated in **liquefactive necrosis *(necrosis colliquativa)*** - the tissues affected by this process are soft, smear-like, e.g. brain, gastric mucosa), with the prevalence of denaturation - in **coagulative necrosis *(necrosis coagulativa)*** - the tissues affected by this process are light gray or light yellow, with firm consistency.

Causes:

* ischaemia with infarction
* the effects of toxic compounds (exo- and endotoxins)
* the effects of infectious agents (viruses, bacteria, fungi)
* nutritional deficits

**Morphological changes in a dead cell**

|  |  |  |
| --- | --- | --- |
| **Liquefactive necrosis** | **Cellular structure** | **Caseous necrosis** |
| Plasmolysis, vacuolisation | Cytoplasm | Concentration, increase in acidity |
| Karyolysis, vacuolation, obliteration of the chormatin structure **(*chromatolysis*)** | Cell nucleus | Pyknosis, disintegration, chromatin concentration |
| Vacuolation | Mitochondria, endoplasmic reticulum, Golgi apparatus | The formation of myelin-like structures, condensation |
| Disintegration (intercellular boundaries invisible) | Cell membrane | Disintegration or shrinkage with the cytoplasm (outline of the cell is preserved) |
| Swelling, cell dissolution **(*cytolysis*)** | Cell | Cellular shrinkage |

The tissues surrounding the dead lesion react to its presence with an inflammatory reaction to remove altered tissue fragments. Thus, there is congestion and infiltration of inflammatory cells (neutrophils, macrophages) at the periphery of the lesion, with the aim to clean, and if it is impossible - to replace the dead tissue with connective tissue ***(organizatio)*** and to produce a scar ***(cicatrix)*** or an encystment ***(sequestratio)***.

***Encephalomalacia* (*encephalomalatio*)**

Causes:

* generalised or local ischaemia 🡪 pale infarct, hypovolemic shock
* E-avitaminosis in hen (cerebellum), B1 avitaminosis in carnivores and horses (beriberi disease in humans)
* copper deficiency
* excess of unsaturated fatty acids in the feed (chickens), heavy metals (Pb, Hg, As)
* exogenous toxins (mycotoxins, bacterial toxins: Clostridium perfringens type D, E. coli) and endogenous toxins

Grossly:

* liquefactivenecrotic lesions are usually located in the white matter of the cerebellum and in the brainstem
* microscopic image in the first few hours is not very characteristic
* next, the tissue in the cross-section becomes pale, dull, and may also take the form of a soft-hemorrhagic lesion (red infarct when blood enters the necrotic lesion). After 7-14 days, the necrotic lesion turns yellow-red (yellow softening) as a result of the transformation of extravasated blood
* blurred boundary between the grey and the white matter
* reduced consistency of the cerebral tissue

Microscopically (ischaemic form):

* nerve cells are very sensitive to damage. Necrosis starts with vacuolar degeneration, swelling of mitochondria and fragmentation of nerve cell nucleus. Lysis of the entire cell may also occur due to damage to plasma membranes
* the cribriform state **(*status cribrosus)*** is found as a result of the disintegration of myelin sheaths of nerve fibres and the formation of cavities (empty spaces) filled with serous fluid
* glial proliferation, glial scars emerge over time
* in the cerebellum, oedema of the molecular and granular layers with subsequent formation of free space under the Purkinje cell layer
* in areas of necrosis, numerous active macrophages packed with lipid bodies are present
* in numerous Purkinje cells and granule cells, cytoplasm homogenisation occurs, the nucleus and processes disappear, resulting in complete cell disintegration

**Coagulative liver necrosis (*necrosis coagulativa hepatis*)**

The macroscopic image of necrosis depends on its cause, the length of the process and the number of cells involved. The most commonly occurring include:

* **focal liver necrosis (necrosis hepatis focalis)** - infectious diseases (salmonellosis, tularemia, listeriosis, pasterellosis, necrobacillosis, Aujeszky’s disease), parasites, biliary obstruction. Numerous white-grey lesions of various sizes are present in the liver, clearly visible against the brown parenchyma
* **massive necrosis**-affects large areas of the liver, sometimes whole lobes are covered or the whole liver degenerates. This is caused by toxins damaging hepatic cells, dietary errors, volvuli and dislocation of organs

Morphological patterns of necrosis/degeneration of hepatocytes

1. Random - typical for many infection factors - viruses (e.g. equine herpesvirus type 1 infection in foals; EHV-1), bacteria, protozoa

* single-cell necrosis
* multifocal necrosis - numerous foci of necrotic hepatocytes
* piecemeal necrosis - with the participation of lymphocytes - viral infections, autoimmunization

1. Zone necrosis - involving hepatocytes in different parts of the lobules

* ***centrilobular***- very frequent; the central part of the lobule is most sensitive to hypoxia (it is the last one to receive oxygen-rich blood) and has the highest enzymatic activity. Causes: anaemia, right ventricular failure, passive congestion.
* **paracentral (*periacinar*)**-the form of a wedge around the central vein, usually reflects direct toxic damage by factors requiring bioactivation; severe hypoxia - acute anaemias or right ventricular failure, in such cases it precedes centrilobular necrosis
* ***midzonal***– rare in domestic animals; reported in pigs and horses with aflatoxicosis, in cats exposed to hexachlorophene, cortisone (anti-inflammatory, anti-allergic drug)
* ***periportal***– rare. Causes – the effect of toxic substances, e.g. phosphorus, which do not require metabolism (oxidase activity) to cause damage
* ***bridging*** - the result of the merging necrotic areas of adjacent lobules, e.g. centrilobular with centrilobular **(*central bridging*)***,* or centrilobular with periportal, portal with portal

1. Massive

The microscopic image of coagulative liver necrosis depends on the time elapsed since the beginning of the necrosis:

* in fresh lesions, a typical organ structure can be recognized
* coagulative necrosis affects hepatocytes, endothelial cells, Kupffer-Browicz cells
* hepatocytes affected by necrosis stain lighter with eosin, cell nuclei are damaged, the outline of cells is preserved
* over time, damaged cells become homogeneous, cell nuclei become invisible
* on the perimeter of the necrosis lesion the process of cleaning (macrophage and neutrophil infiltration) and repair begins

**Waxy necrosis of muscles (*necrosis cerea musculorum*)** - is a type of coagulative necroses It most often occurs in striated muscles as Zenker’s degeneration **(*necrosis cerea, s. Zenkeri*)**

Causes:

* vitamin E deficiency, selenium deficiency
* stress (stress-related myopathies)
* equine exertional rhabdomyolysis

Grossly:

* the muscles are dry, friable, watery, matt (waxy) or with hyaline appearance (resembling fish meat) - in poultry
* white, pale pink or greyish yellow

Microscopically:

* loss of cross striations of muscle fibres
* sarcoplasm is disintegrated into lumps/grains or is hyalinised in segments
* the nuclei of muscle fibres are subject to pyknosis or karyolysis
* sarkolemma remains unaffected
* phagocytic cell infiltrations - macrophages and neutrophils (removal of dead sections)
* regeneration processes or calcification are present
* blood vascularisation and plethora may occur

**Enzymatic necrosis of fat tissue, fat necrosis (*necrosis adiposa s. Balseri*)** - this type of coagulative necrosis occurs as a result of damage to pancreatic parenchyma or after hemorrhagic necrosis when pancreatic juice (lipase) enters the fatty tissue through lymphatic or blood vessels or through direct contact. On-site lipase decomposes fats into glycerol and fatty acids, which form soaps when combined with cations, e.g. Ca++, K+, Na+. Glycerol, sodium and potassium soaps are water-soluble and are absorbed. Calcium soaps, which do not dissolve, remain and are deposited in the fatty tissue. This results in hard, dry, chalk-like lesions of various sizes.

Grossly: clearly outlined, pale yellowish, matt, hard lesions, resembling wax drops are visible in the fat tissue

Microscopically:

* pale pink/blue calcium soap deposits of varying colour saturation, with the shape corresponding to dead fat cells
* phagocytic cell infiltrations
* calcification foci