LECTURE 12

PATHOLOGICAL CALCIFICATION-MINERALIZATION
And ECTOPIC BONE

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Calcification and mineralization are terms used interchangeably to describe the process by which certain tissues such as bone and teeth are converted to hard substances. Calcium is, however, involved in much more than just providing a rigid structural framework. Calcium in the mammalian body is the most abundant cation and is the 5th most abundant of all the inorganic elements. The great majority of the calcium in the body is stored in bone. The bulk of the remaining calcium is bound to protein or forms small ionic complexes. It is estimated that 1% or less of the calcium in the body is present in ionic form, the active form of the element. Furthermore, the calcium concentration outside of cells is approximately 10,000 fold higher than inside cells. Within cells, there is another gradient; calcium, complexed with proteins, is 10,000 fold higher in the endoplasmic reticulum than in the cytosol.

REGULATION OF INTRACELLULAR CALCIUM

Cells go to great lengths to maintain calcium homeostasis. Homeostasis at the cellular level is maintained by ion channels and transporters in the plasma membrane as well as the membranes of various organelles (Fig 1).

![Figure 1](image)

**Figure 1** Calcium homeostasis in normal cells. Ionized calcium (Ca\(^{2+}\)) in the cytosol is normally maintained at 10–100 nM against steep gradients by transport of the ion to the extracellular space and cisternae of the endoplasmic reticulum (ER), and by protein binding. Physiological increases of cytosolic Ca\(^{2+}\) take place by entry through plasma membrane Ca\(^{2+}\) channels (leak channels, ligand-gated channels, and voltage-gated channels), release of Ca\(^{2+}\) from the ER or sarcoplasmic reticulum (SR) lumina upon binding of inositol trisphosphate (IP\(_3\)) to the inositol trisphosphate receptor (IP\(_3\)R), or release of Ca\(^{2+}\) from the SR (in muscle cells) upon Ca\(^{2+}\) binding to the ryanodine receptor (RyR) by a process called calcium-induced calcium release (CICR). Transport of Ca\(^{2+}\) across the plasma membrane to the extracellular space takes place via the plasma membrane calcium ATPase (PMCA) and the Na\(^+\)/Ca\(^{2+}\) exchanger. The sarco-endoplasmic reticulum calcium ATPase (SERCA) transports Ca\(^{2+}\) into the ER in nonexcitable cells, or the SR in muscle cells. Mitochondrial membranes can also buffer Ca\(^{2+}\) by transport of the ion into the mitochondrial matrix via Na\(^+\)/Ca\(^{2+}\) exchangers. Abnormal increase of mitochondrial matrix Ca\(^{2+}\) concentration is prevented by transport in the reverse direction via mitochondrial Na\(^+\)/Ca\(^{2+}\) exchangers.
Calcium is involved in numerous cellular processes. It is involved in the excitability of cells in the nervous system, contractility of skeletal muscle, and serves many other biochemical functions. Calcium is also considered a ubiquitous second messenger in cells, directing normal cellular functions. The direst consequence from failure to maintain homeostasis is intracellular calcium accumulation, calcium overload, and cell death.

**REGULATION OF EXTRACELLULAR CALCIUM**

Maintenance of calcium homeostasis in the body begins with the diet and involves intake of both calcium and phosphorus. A healthy diet should approximate the normal 2:1 ratio of calcium to phosphorus found in bones. Mammals can tolerate a fairly wide range (1:1 up to 6:1) provided total intake is adequate. Too little calcium or too much phosphorus can lead to disturbances in extracellular calcium levels and be manifest in a variety of disorders. Although this will be a review for most of you, extracellular calcium levels in the body reflect the concerted effort of mainly three hormones.

- **Vitamin D (1,25-dihydroxycholecalciferol):** A little vitamin D is present in the diet and absorbed from the intestine. The bulk is synthesized in sebaceous (oil) glands of the skin via photoactivation of 7-dehydrocholesterol by UV light. Subsequent conversion steps in the liver and then kidney form the fully active vitamin if there is a demand for calcium.

  - **Stimulus**
    - Low extracellular calcium
    - Parathormone stimulates formation of 1,25-dihydroxycholecalciferol by the kidney
  - **Main Action** – leads to increased extracellular calcium
    - The principal action of vitamin D is to increase the absorption of calcium and phosphorus from the intestine.

- **Parathormone:** Parathormone or parathyroid hormone is synthesized by the endocrine (chief) cells of the parathyroid gland. This is the hormone mainly responsible for minute-to-minute regulation of extracellular calcium

  - **Stimulus**
    - Low extracellular calcium
    - Elevated extracellular phosphorus
  - **Main actions** – lead to increased extracellular calcium
    - Increased mobilization of calcium from bone
    - Stimulates formation of vitamin D by the kidney, ultimately increasing absorption of calcium from the intestine
    - Promotes resorption of calcium from the kidney
    - Promotes excretion of phosphorus in the urine
- **Calcitonin**: This hormone is synthesized by the C-cells (parafollicular cells) of the thyroid gland. This hormone antagonizes the effects of parathormone.
  - **Stimulus**
    - Elevated extracellular calcium
  - **Action** – lowers extracellular calcium
    - Inhibits the parathormone-induced release of calcium from bone
    - Promotes the urinary excretion of phosphorus

The purpose of this lecture is to provide the brief and necessarily superficial overview of calcium homeostasis (above) and some of the disturbances in homeostasis that result in pathological calcification (or mineralization) of soft tissue. A separate portion of this lecture will also cover instances in which actual bone forms in soft tissue, a process unrelated to pathological calcification.

**PATHOLOGICAL CALCIFICATION (slides 6 – 31)**

Pathological calcification is a lesion in which calcium salts, usually in the form of calcium phosphate, are deposited abnormally in soft tissues. There are two forms of pathological calcification.

- **Dystrophic calcification** occurs in dying and dead tissue. Injured and dying cells are not able to maintain normal calcium homeostasis, intracellular calcium levels increase. Figure 2 shows a cell damaged by ischemia with accumulation of calcium seen as electron dense deposits in mitochondria. Elevations in serum or

![Figure 2](image_url)

*Figure 2* High-power electron micrograph of a cell injured by ischemia, showing mitochondrial swelling with electron-dense precipitates. Several mitochondria are shown. Most of the mitochondria are in various stages of swelling. A mitochondrion in condensed configuration is present just adjacent to the left of the asterisk. Two of several markedly swollen, ballooned mitochondria with amorphous electron-dense precipitates are indicated by short red arrows. When such mitochondria are examined by electron-probe microanalysis, the amorphous densities reveal the presence of calcium in pathologically high amounts. Matrix calcium also increases. Structural damage is present in some of the ballooned mitochondria. A mitochondrion with early swelling and aggregates of electron-dense material is indicated by the long red arrow.

Dong Z. et al. 2006.
extracellular calcium are NOT required for dystrophic calcification. Once calcification is initiated deposits of calcium continue. Dystrophic calcification is typically a localized lesion. Dystrophic calcification can occur with any type of tissue necrosis but is not as common in cases where the necrotic tissue undergoes liquefaction. Tissues with often grossly visible calcification include heart and skeletal muscle and caseating granulomas that occur, for instance, in tuberculosis or a disease of small ruminant known as caseous lymphadenitis.

Figure 3. This is a cross section of a lymph node from a goat with caseous lymphadenitis, a disease caused by infection with a bacterium (Corynebacterium pseudotuberculosis). Normal architecture of the node is gradually obliterated by successive waves of necrosis and inflammation creating a laminated appearance. The light zones in this photo are areas of dystrophic calcification.

- Other examples of dystrophic calcification include vitamin E/selenium deficiency in animals, a disease known as nutritional myopathy (slides 9, 10), cardiac necrosis due to drug toxicity (slide 11), and a disorder known as periventricular leukomalacia (slides 12, 13). In humans, periventricular leukomalacia is a serious disorder that occurs in late term infants and newborns and is one of the more common causes of cerebral palsy. Intrauterine infection and hypotension are proposed as causative factors. Dystrophic calcification also occurs in two unrelated skin conditions. In calcinosis circumscripta (slide 15), large calcium deposits occur commonly at sites of persistent trauma such as over boney prominences and have the gross appearance of a tumor, hence the alternative name of tumoral calcinosis. The other form of dystrophic calcification in the skin is termed calcinosis cutis that occurs in the context of hyperadrenocorticism (Cushing’s disease). Here, the calcium deposits occur in collagen bundles (slide 17) and can be sufficiently severe as to be grossly visible (slide 18).

- Metastatic calcification IS associated with elevated extracellular levels of calcium (hypercalcemia), exceeding the homeostatic capacity of cells and tissues. Metastatic calcification is typically a generalized phenomenon but there are
specific tissues that are prone to become mineralized. Within these tissues, mineralization can involve cells as well as extracellular matrix components such as collagen in basement membranes and elastic fibers in arterial walls. Tissues prone to metastatic calcification include:

- Gastric mucosa – the inner epithelial lining of the stomach
- Kidneys and lungs
- Cornea
- Systemic arteries
- Pulmonary veins

You might find it difficult to understand why the calcium deposits have a predilection for these tissues. The deposition of calcium favors organs and tissues where there is an acid to alkaline interconversion or those organs that ‘lose’ acid and have an underlying alkaline compartment. Hence, the lungs and kidneys are two organs involved in control of acid-base balance (see lecture 26), the gastric mucosa secretes hydrochloric acid, venous blood (except pulmonary veins) is more acid than arterial, etc. Hypercalcemia sufficient to cause metastatic calcification mainly occurs in three situations: 1) hyperparathyroidism, 2) hyper-vitaminosis D, and 3) diseases with extensive destruction of bone.

**Hyperparathyroidism** is due to excessive production and secretion of parathormone or a parathormone-like substance. Hyperparathyroidism can be primary, secondary, or associated with certain types of paraneoplastic syndromes.

- **Primary hyperparathyroidism** is due to excessive production by the parathyroid gland(s). This is certainly not common in either humans or animals. Excessive parathormone due to hyperplasia and hypersecretion is observed in German shepherd puppies and is a genetic disease with simple autosomal recessive inheritance. Another cause of primary hyperparathyroidism is production of excessive parathormone by parathyroid gland neoplasms (slide 20). Parathyroid neoplasms do not respond normally to negative regulatory feedback. Despite persistent hypercalcemia, the cells in these tumors continue to produce parathormone.

- **Secondary hyperparathyroidism** can be classified as either nutritional or renal.
  - **Nutritional secondary hyperparathyroidism** is classically a disease of herbivores and is due to too much phosphorus in the diet which, as you will remember, is a stimulus for parathormone production. This disorder is due to prolonged feeding of grain-based rations that are high in phosphorus and low in calcium; hence the name *bran disease* in horses. Hyperparathyroidism results in removal of calcium from bone so in addition to metastatic calcification, bone disease also occurs.
  - **Renal secondary hyperparathyroidism** is due to chronic kidney disease and renal failure (slides 23 – 27). The effects of renal failure are potentially two-fold. First, the failing kidney is unable to excrete phosphorus and hyperphosphatemia develops. Secondly, the active form
of vitamin D (1,25-dihydroxycholecalciferol) may not be synthesized by the failing kidney. The resultant hyperphosphatemia and, to a limited extent, hypocalcemia stimulate parathormone production. Hyperparathyroidism results in removal of calcium from bone so in addition to metastatic calcification, bone disease also occurs (slide 28).

- **Paraneoplastic syndromes** can also lead to significant hypercalcemia and metastatic mineralization. A variety of these syndromes are recognized. In these syndromes, the neoplastic cells may produce substances not normal for the cell type. Pulmonary epithelial cells do not normally produce parathormone. One type of lung cancer, a carcinoma in humans, produces a metabolically active parathyroid hormone or parathormone-like substance. In animals, a parathyroid-like hormone is produced by neoplastic lymphocytes (lymphoma) in multiple species and by the neoplastic epithelial cells of apocrine adenocarcinoma of the anal sacs in dogs (slides 30, 31).

**Hypervitaminosis D** causes hypercalcemia by increasing absorption of calcium from the intestine. Cases can occur from overzealous supplementation (slide 33). Some rodenticides also contain very potent vitamin D analogs. Lastly, some plants including Cestrum diurnum and Solanum malacoxylon, and many others around the world are associated with hypercalcemia and metastatic mineralization in herbivores.

**Destruction of bone**, if widespread, is a significant cause of hypercalcemia and metastatic calcification in humans but occurs less commonly in animals. Primary (multiple myeloma) and metastatic cancers of bone are one cause. Hypercalcemia can also occur in severe forms of Paget’s disease (osteitis deformans), a disorder of humans; the cause is currently undetermined. As one author puts it, “At the outset, Paget’s disease is characterized by regions of furious osteoclastic bone resorption that are followed by a period of hectic bone formation.” (AE Rosenberg: In Robbins Pathological Basis of Disease, 5th Edition).

**THE APPEARANCE OF SOFT TISSUE CALCIFICATION**

Pathological calcification can only be detected grossly if extensive (slides 9, 10, 12, 13, 15, 18, 23, 24, 33). If grossly evident, calcification appears as pale chalky areas in the tissues. Even if not visible, calcification can sometimes be detected by the coarse gritty feel of the tissues when scraped or incised with a knife or scalpel blade. Microscopically, calcification appears as primarily basophilic (blue) deposits in tissues with H&E staining (see slides 11, 13, 15, 25, 26, 33). In larger deposits, only the rim stains basophilic while the bulk of the internal core is eosinophilic (pink). The von Kossa stain can be used which stains calcifications a brownish black color (slides 13, 27).

**ECTOPIC BONE** (Slides 34 - 41)

Ectopic bone is not related in any way to pathological calcification. It is briefly covered here for convenience and because, in some instances, ectopic bone can be mistaken for pathological calcification. Additionally, ectopic ossification can occur at the same site as
pathological calcification. This lecture will not cover specific neoplasms of primary bone forming tissues (osteoma, osteosarcoma, and others). Ectopia means displacement or malposition. Ectopic bone then, is bone in a tissue where it is not normally found. Ectopic bone can develop in two ways. Heterotopic bone is essentially normal osseous tissue that is believed to originate from embryonic cells that persist in tissues during development and maturation. Common sites include the lungs of mainly dogs and cattle (slide 36), and the dura mater of dogs (slide 37). Heterotopic bone has really no clinical significance. In dogs with dural ossification (also erroneously called ossifying pachymeningitis), this lesion is sometimes blamed for back pain and paresis but it is generally considered an incidental finding. The other mechanism for bone formation at abnormal site is osseous metaplasia. Metaplasia was covered in lecture 8. Osseous metaplasia most commonly develops from other types of connective tissue, i.e. fibrous connective tissue, at sites of chronic inflammation and in florid reparative processes. Ossification can also develop in certain types of connective tissue neoplasms such as fibrosarcoma and ossifying fibroma. Ossification can also develop in certain types of epithelial neoplasms. In this case, the osseous component can develop from true metaplasia or from pluripotential cells in the neoplasm such as myoepithelial cells. Epithelial tumors such as those from mammary gland (slide 40), salivary gland, thyroid gland (slide 41) and apocrine (sweat) gland can have an osseous component. When this occurs, the lesions are called mixed tumors, i.e. mixed mammary tumor, mixed thyroid tumor, etc. Mixed tumors can contain bone as well as cartilage. In most instances, the osseous component is of little clinical significance. In some cases, however, the osseous component can also become neoplastic (see slide 41, actually taken from a pulmonary metastasis of a mixed thyroid carcinoma). Another epithelial tumor that often contains bone is the pilomatrixoma, a neoplasm that forms from the hair bulb of hair follicles.