20. CALCIUM AND PHOSPHOROUS METABOLISM

Many physiological processes are regulated directly or indirectly by calcium. Furthermore, the main physical structure of vertebrates and other species depend on an adequate supply of calcium to maintain the skeleton or exoskeleton. Calcium, therefore, is an important element in the maintenance of life, and understanding its metabolism is crucial to solve many problems which practitioners face on a daily basis.

### STORAGE

The majority of the body calcium is stored in the skeleton. Bones account for 99% of body calcium. This is kept in the form of hydroxyapatite crystals, \( \text{Ca}\text{(PO}_4\text{)}_6\text{(OH)}_2 \), which contain calcium phosphate salts (Fig. 20-1).

The second largest reservoir is intracellular calcium. This can be found in fairly low concentrations in the cytosol when the cell is not engaged in any significant activity. It can also be found stored within the mitochondria bound to different proteins. Finally, it can be found stored within the endoplasmic reticulum (ER) as a source for rapid delivery into the cytosol. In skeletal muscle there is a specialized ER called sarcoplasmic reticulum which is exclusively for calcium regulation. A continuous exchange of calcium takes place between these three pools.

The last compartment in which calcium can be found is the extra-cellular fluid. This pool constitutes the smallest quantity, but it is important in regulating the circulatory concentrations. The concentration in the extra cellular fluid is high enough to cause precipitation of hydroxyapatite, but this is prevented by the presence of an inhibitor which is suspected to be pyrophosphate. The only place where this inhibitor is not active is in the bone tissue.

### ROLE OF CALCIUM

Calcium is needed to successfully conduct a variety of physiological processes. Muscle contraction, nerve impulses, activation of many enzymes and the release of hormones by multiple cells is mediated through diverse roles of calcium. Other events such as the process of blood coagulation and the maintenance of cell membrane stability are also calcium dependent (Fig. 20-2).

#### Calcium movement

The net exchange of calcium between the extra cellular fluids and different organs and systems of the body in a 90 kg animal is approximately 100 mg per day (Fig. 20-3). In total, it is estimated that there are about 1300 mg of calcium in the extra cellular fluid of an animal.

### CALCIUM

- 99% of body calcium is stored in bone as hydroxyapatite
- Second reservoir is intracellular calcium
  - Low concentration in cytosol when cell is inactive
  - Stored bound to proteins in mitochondria
  - Endoplasmic reticulum
- Smallest reservoir in extra-cellular fluid
  - Most important to regulate circulatory concentrations

### ROLE OF CALCIUM

- Required for many intracellular processes
  - Muscle contraction
  - Nerve impulses
  - Enzyme activation
  - Hormonal release
- Also contributes to
  - Blood coagulation
  - Cell membrane permeability
- Structural role in bone and teeth formation
The normal daily intake of calcium in food is about 1 g, of which approximately 35% is absorbed through the small intestine. At the same time about 250 mg of calcium from extra cellular fluids are lost into the digestive tract in the form of secretions such as enzymes and, the normal sloughing of cells from the digestive tract villi. The calcium lost through these modes plus, what was not absorbed, make up about 900 mg of calcium which are excreted in the faeces every day. There is also a relatively constant exchange of calcium between the bones and the extra cellular pool of about 500 mg per day in both directions. This is part of the normal bone remodeling process which goes on continuously in the body. This process is uneven if the animal is growing, because there is a net growth of the bone mass but in an adult, it remains stable. The other point of exchange is in the kidney. A total of about 9980 mg of calcium enters the kidney every day as part of the blood filtration process. Of these 9880 mg are reabsorbed into circulation and only 100 mg are excreted in urine. This loss is compensated by the intake of calcium through the diet.

The metabolism of calcium of a lactating animal or of birds laying eggs is significantly different given the large output of calcium in milk and egg shells. The fundamental difference is that there is a larger calcium intake, which after absorption, is incorporated into the milk or the egg shells. In the case of a cow, the calcium incorporated in each liter of milk is about 1 g, thus a high producer requires a significantly higher intake to make up with this difference without affecting the animal’s reserves. In the case of a hen, the amount of calcium incorporated in the shell of an egg is approximately 3 g.

**ROLE OF PHOSPHOROUS**

Phosphorus also contributes to bone formation and without the proper combination with calcium, symptoms of deficiency appear. Phosphorous plays an important role in the maintenance of the integrity of membranes through its involvement in phospholipids. Most enzyme activation or deactivations depend on the process of phosphorylation. Without tightly controlled enzymes, the entire regulation of physiological and metabolic activities would malfunction and life would cease. Other specific common molecules requiring phosphorus are nucleic acids, as well as, ATP, ADP and cAMP (Fig. 20-4).

**REGULATION OF CALCIUM AND PHOSPHOROUS METABOLISM**

Part of the regulation of calcium and phosphorus is mediated through PTH and CT (Fig. 20-5).

As previously indicated, the main reservoir of calcium is the skeleton. Under sub optimal concentrations of circulatory calcium, a hormone produced by the parathyroid called Parathyroid Hormone, activates release of calcium from bones. In opposition to this, when the circulatory concentration of calcium is elevated, a hormone produced by the C cells of the thyroid (Calcitonin) stimulates the absorption of calcium by the bones, thus lowering its circulatory levels (Fig. 20-6).

Acquisition of calcium is done through absorption from the GIT. The process can be passive or active. If the concentration of calcium in the GIT is very high, then, it diffuses passively into the enterocytes and into circulation. If the concentration of
Calcium in the GIT is low, then, an active transport mechanism is used (Fig. 20-7). This mechanism requires support from vitamin D.

Parathyroid hormone

PTH is produced in the Chief or C cells of the parathyroid. It is derived from a 115 amino acid protein called prepro-parathyroid hormone, which is stored in the endoplasmic reticulum. Twenty-five amino acids are then cleaved to produce a 90 amino acid pro-PTH. Then, a section of 6 amino acids is removed to make the biologically active hormone PTH, which is only 84 amino acids long. PTH is packed in the Golgi apparatus and released to circulation by exocytosis when needed. PTH has a half life of 5 to 10 minutes and it is catabolized in the liver by proteases and/or eliminated in the urine (Fig. 20-8).

The general function of PTH is to increase the concentration of calcium and decrease the concentration of phosphate in the extra cellular fluid (Figs. 20-9, 20-10).

One of the effects is to promote or enhance the movement of calcium across the osteoblastic-osteocytic membrane. It also increases osteoclastic activity, and at the same time enhances osteoblastic activity. The net result is an increase in the rate of bone remodeling taking place.
PTH FUNCTION

- Increase calcium and decrease phosphate in extra-cellular fluid
- In bone promotes movement of calcium across osteoblast-osteocyte membrane
- Increase osteoclast activity
- Enhance osteoblast activity

Figure 20-9. Functions of PTH

Calcium absorption

Role of Vitamin D

The second substance contributing to the regulation of calcium metabolism is vitamin D (Figs. 20-11, 20-12)

VITAMIN D

- Promotes intestinal calcium absorption
  - Increase calcium binding protein
  - Increase transport of calcium into enterocytes
    - Prolonged effect
  - Promotes calcium stimulated ATP-ase in brush border
  - Promotes alkaline phosphatase
- Promotes phosphate absorption by intestine
  - Using calcium as transport mediator
- Decreases renal calcium and phosphate excretion
- Lacking Vit D the effect of PTH in bone absorption is negligible
- In small concentrations promotes bone calcification

Figure 20-12. Role of Vit D

CIRCULATORY Ca++ AND PHOSPHATE

Figure 20-10. Changes in circulatory calcium and phosphate in response to an increase in PTH

CHOLECALCIFEROL (VITAMIN D₃)

- Formed in skin by conversion of 7 dehydrocholesterol
- Converted to 25-hydroxycholecalciferol in liver
- Converted to 1,25-dihydroxycholecalciferol in kidney

Figure 20-11. Formation of Vitamin D

It was pointed out previously that when the amount of calcium in the digestive tract is low, the transfer into enterocytes and then into the extra cellular pool for transport, is mediated with the help of Vitamin D or cholecalciferol. Vitamin D does not act as a cofactor but as a hormone. In lay terms we refer to vitamin D as the compound responsible for the metabolic regulation of calcium. In reality Vitamin D has to be first converted in the liver and kidney to the active form 1,25-dihydroxycholecalciferol or 1,25(OH)₂D₃ (Fig. 20-13).

1,25(OH)₂D₃ also promotes phosphate absorption in the intestine and decreases renal calcium and phosphate excretion into urine, but the mechanisms are not clear yet.

The process of transporting calcium into circulation from the GIT is mediated in the following manner. Vitamin D diffuses from circulation into the enterocytes. As a steroid derivative, Vitamin D freely crosses cell membranes. In the cytoplasm of the enterocyte, it binds a receptor forming a hormone.
Figure 20-13. Overall regulation of calcium metabolism

receptor complex which moves into the nucleus (Fig. 20-14).

The nucleus activates translation of DNA and forms mRNA, which will encode a protein called calcium-binding protein that is capable of moving towards the apical membrane of the enterocyte and, transport calcium from the lumen of the GI tract into the enterocyte. This calcium will cross the enterocyte where will be discarded through the basolateral membrane by facilitated diffusion. Then, it reaches the intracellular space and eventually gets into capillaries where finally enters the main circulation. The activity of the calcium-binding protein remains functional for several weeks after 1,25(OH)2D3 has been catabolized.

BONE FORMATION

Intimately related with the overall metabolism of calcium and phosphorous, is the process of bone formation and the ongoing bone remodeling that takes place thereafter. It is estimated that approximately 4% of all bone surfaces are being remodeled at any given time in an adult animal. Several specialized cells contribute and interact to carry out this process (Fig. 20-15). The osteoclasts are multi nucleated cells which have the capability of eroding bone to incorporate the calcium into the extracellular fluid and circulation. The osteoblasts are responsible for depositing a gelationous bone matrix to which calcium attaches and crystalizes.

OSTEOCYTIC MEMBRANE SYSTEM

- Continuous layer of osteocytes and osteoblasts
- Cover bone surface
- Interrupted by osteoclasts
- Bone fluid between osteocytic membrane and bone

Figure 20-16. Role of osteocytic membrane

BONE FORMATION

- Ongoing process
- Osteoclast
- Erode bone
- Incorporate calcium into ECF
- Osteoblast initially form bone matrix
- Then become osteocyte

Figure 20-15. Changes taking place during bone formation

Osteocytic membrane

All bone surfaces are covered with a cellular layer composed of both, osteocytes and osteoblasts. This is called the osteocytic membrane (Figs. 20-16, 20-17).

The functional importance of this continuous layer is that it provides a physical barrier between the bone area and the
extracellular fluid of the body. This barrier is only interrupted in areas where an invasion of osteoclasts has taken place. Between the osteocytic membrane and the solid bone, there is bone material which is not fully crystallized and it is called the bone fluid. The osteocytic membrane also serves other purposes, such as having calcium pumps which permit to actively transport calcium from the bone to the extracellular fluid.

**Rapid phase of osteolysis**

When some calcium has been pumped out of the bone fluid, the consequent decrease is sensed by the osteoclasts and they start actively digesting or extracting more calcium from the bone. This process is known as osteolysis and it is divided in two phases. The rapid phase of osteolysis is mediated through PTH and causes an increase in pumping activity which removes calcium from the osteocytic membrane (Fig. 20-18).

This in turn triggers permeability to calcium in the bone fluid side of the membrane, thus reducing the concentration of calcium in the bone fluid pool.

**Slow phase of osteolysis**

The event that follows is known as the slow phase of osteolysis and consists of the activation of osteoclasts. The mechanism which triggers this activation is not known, but is assumed that both, osteocytes and osteoblast, play a role in the process which involves two sequential stages. First, starts with the activation of the present population of osteoclasts, which work on eroding the bone. This is followed by the formation of new osteoclasts, which further erodes calcium out of bone. Once stimulated the second phase may last several days. If there is an overestimation, then the bones can be weakened. Under normal circumstances, a concentrated area of bone is eroded by osteoclastic activity leaving small tunnels measuring less than a millimeter in diameter and several millimeters long.

The counterpart of osteolysis is the deposition of calcium in the bones or bone calcification. Once the erosion of bone ceases, the vacated space is invaded by osteoblasts, which initially deposit collagen molecules or collagen monomers, as well as, proteoglycans, also known as ground substance. The collagen polymerizes forming collagen fibers which eventually establishes a cartilage-like net to which calcium precipitates. This material is called osteoid which will end up surrounding the osteoblasts. The result is that the osteoblast activity ceases and the cell becomes an osteocyte. It is not clear why calcium precipitates in osteoid tissue. It is suspected that a substance that neutralizes the inhibitor in charge of preventing precipitation in the rest of the body is released by the osteoblasts, thus permitting precipitation. The formation of new bone is done in concentric circles called lamellae. These circles start in the perimeter of the tunnel and continue layering centrally until the cavity is almost completely refilled with bone. The process slows down once the growing bone starts obstructing the irrigation of the area. At the end, all that is left, is a small channel called the Haversian canal, which is surrounded by concentric deposits of bone. This newly deposited section of bone is called an ostion (Figs. 20-19, 20-20).
The remodelling process contributes to the regulation of the bone strength, needed to carry the load or strain to which the bone is exposed. In this manner, if the animal gets heavier, the bones have a mechanism to grow thicker to support the new weight. Remodelling also serves to replace old bone that becomes brittle. In the cases of bone fracture, when a limb is in a cast and does not support weight, the bone can lose up to 30% of its mass in comparison to the functioning limb.

Role of calcitonin

The third hormone involved in calcium metabolism is Calcitonin (Fig. 20-21). As previously indicated, this hormone is secreted by the parafollicular or C cells of the thyroid gland. Its function is to reduce plasma calcium concentration, which produces the opposite effect to that of PTH.

The stimulus for CT secretion is elevated circulatory calcium concentration. The effect is rapid and mediated through two known mechanisms. The first is to decrease the activity of osteoclasts and to slow down the osteolytic activity of the osteocytic membrane. This automatically enhances the process of deposition of calcium in bone. The second mechanism is a decrease in the generation of osteoclasts, that eventually will reduce the need for osteoblastic activity. The net result is a transient effect in decreasing calcium concentration.
ABNORMALITIES

The consequences of hypocalcemia are rapidly observed in the CNS. Under low calcium concentration the CNS becomes easily excitable, as a result of an increase in membrane permeability to Na ions, which leads to initiation of action potentials. As concentration drops below 50% the peripheral nerve fibers start discharging spontaneously normally causing tetany (Fig. 20-22).

Contrarily, an increase in calcium concentration leads to a depression in the responsiveness of the CNS resulting in a sluggish animal (Fig. 20-23).

A great elevation over the normal may lead to calcium precipitation in tissue leading to deposits in lung alveoli, kidney tubules, and artery walls.

PATHOPHYSIOLOGY

- Hyperparathyroidism resulting from exaggerated PTH production
  - Increased osteoclastic activity
  - Increased circulatory Ca++
  - Bone weakness due to decalcification (osteitis fibrosa cystica)
  - Increased compensatory osteoblastic activity
    - Osteoblasts produce alkaline phosphatase

- Hypercalcemia
  - Depression of CNS, muscle weakness, constipation

Figure 20-23. Pathophysiology of hyperparathyroidism

PATHOPHYSIOLOGY

- Hypoparathyroidism
  - Reduced osteoclastic activity
  - Removal of thyroid can cause Ca++ drop in 2 to 3 days
    - Signs of tetany appear (laringeal muscles specially sensitive)

- Treatment
  - Supplementary PTH or massive dosages of Vit D or 1, 25-DHC and Calcium

Figure 20-22. Pathophysiology of hypoparathyroidism