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Calcium-Regulating Hormones: From Fish to Man and Back Again

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Dedication
This paper is dedicated to the memory of Charles C. Capen, DVM, PhD, DACVP. I am very proud to say that Charles was my mentor and dear friend. Charles had a profound effect on my life and success as a faculty member. It is hard for me to imagine how different my life would be if he had not recruited me to The Ohio State University from mixed animal veterinary practice. His dedication to the profession and excellence in all endeavors, from pathology to photography, were an inspiration to me. He guided my early development and taught me to set the highest goals possible and strive until they were attained. He was a true gentleman with deep integrity in an era where politeness and respect are too often lacking. Charles was committed to education and discovery throughout his life. His knowledge was deep, but he was never satisfied unless he was continually contributing to new biomedical discovery. Charles combined his love for pathologic morphology with the comparative pathophysiological mechanisms of disease. To him, these were inseparable and he instilled this philosophy in his students and peers.

Charles began his research career investigating calcium-regulating hormones, namely the pathogenesis and treatment of post-parturient hypocalcemia (‘milk fever’) in cows. He became an expert on the ultrastructure of the parathyroid gland chief cells and their 100 to 300 nm secretory granules, which demonstrated his early adoption of ‘nanotechnology’. This area of research was apropos for him since he was raised on a dairy farm in Washington state. As with most subjects that he investigated, he changed our perception on the pathogenesis of post-parturient hypocalcemia.

Charles’s challenge to me as a new graduate student was to discover the cause of humoral hypercalcemia of malignancy in dogs with cancer. It was so exciting to be part of the discovery of a new hormone, parathyroid hormone-related protein, which is now known to play many roles in health and disease. It is my goal to honor Charles by retelling the fascinating story of the discovery of calcium-regulating hormones during the past century. I will emphasize the comparative nature of the story, since comparative pathophysiology was a key principle of Charles’s life. He was an advocate of ‘One Medicine’ long before it became a popular catchphrase of the day.

Introduction
The concentration of ionized calcium in the blood and extracellular fluids of the body is under very tight minute-to-minute regulatory control by multiple endocrine and cellular systems. This is imperative for normal homeostasis due the many biologic processes that depend on a stable concentration of extracellular ionized calcium. The multiple hormones, cytokines, receptors, and intracellular messengers involved in calcium homeostasis create challenges in understanding the individual roles and functions of calcium-regulating hormones due to the complicated feedback loops that exist to compensate for alterations in calcium balance. Even mild ionized hypocalcemia or hypercalcemia is an indication that there have been substantial disturbances in the pathophysiology of calcium balance.
Major Hormones Involved in Calcium Balance
- Parathyroid Hormone (PTH)
- Calcitonin (CT)
- Parathyroid Hormone-Related Protein (PTHrP)
- Calcitriol (1,25-dihydroxyvitamin D, active form of vitamin D)
- Stanniocalcin (fishes)
- Prolactin (fishes)
- Somatolactin (fishes)

Major Organs/Tissues Involved in Calcium Balance
- Parathyroid Glands
- C-Cells of the Thyroid Glands or Ultimobranchial Bodies
- Placenta
- Bone
- Gastrointestinal Tract
- Kidney
- Gills in fishes
- Corpuscles of Stannius in fishes
- Skin
- Certain epithelial cancers

Major Receptor Involved in Calcium Balance
- Cell membrane Ca\textsuperscript{2+} receptor (CaR)
  - Chief cells of the parathyroid gland
  - C-cells
  - Renal tubule cells
  - Many other cell types

Consistency of the Serum Concentration of Ionized Calcium in Animals and Humans
Amazingly, most animals have similar concentrations of ionized calcium (Ca\textsuperscript{2+}) in the blood and extracellular fluids. In general, most animals from fishes to amphibians to terrestrial vertebrates to humans have approximately 1.2 – 1.5 mM Ca\textsuperscript{2+} in their blood. This fact emphasizes the evolutionary importance of a constant ionized calcium concentration for proper function of biologic systems even though the environments animals live vary widely in calcium availability and the physiologic systems of calcium balance in animals can be very different.

- Equivalence of Ca\textsuperscript{2+} concentrations:
  1 mM = 2 mEq = 5 mg/dl

Calcium in the blood exists in a free or ionized form (Ca\textsuperscript{2+}), a protein bound form, and a small amount complexed to anions. Only the ionized form is biologically active. It is best to measure the ionized form, but in most clinical situations total serum calcium is measured. In general, about 50% of the total serum calcium is protein bound. Ca\textsuperscript{2+} binds to serum albumin and some globulins. Striking exceptions to this rule occur. For example, during egg laying in reptiles and birds, total serum calcium can be increased (up to 20 mg/dl or more) due to binding to serum vitellogens produced by the liver. Correction factors exist to estimate Ca\textsuperscript{2+} from concentrations of total serum calcium, albumin, and/or total protein. The correction factors do not have universal acceptance and have not been completely validated under normal and pathological conditions. Ca\textsuperscript{2+} concentrations inside of cells are very low (in the nM range). Therefore, the extracellular to intracellular gradient is large and permits rapid diffusion of Ca\textsuperscript{2+} through cell
membrane Ca channels when they are open or through damaged cell membranes. High intracellular Ca$^{2+}$ concentrations can be toxic, so free intracellular Ca$^{2+}$ is rapidly pumped into organelles or outside the cell by Ca$^{2+}$ ATPases or buffered by protein binding (for example to calmodulin or calbindins).

**Historical Perspectives:**

- **Parathyroid Hormone (84 amino acids)**
  - Parathyroid glands discovered in the late 19th century.
  - 1981 - Gley showed that cats and dogs died of tetany when all four parathyroid glands were removed.
  - 1924 - Biologic activity of parathyroid glands discovered by Hanson in 1924 and refined by Aurbach in 1959.
  - 1948 - Hyper- and hypoparathyroidism described by Albright and Reifenstein.
  - 1965 - Capen begins studies on parathyroid glands of cows and other animals and discovers that post-parturient hypocalcemia in cows is due to suppressed chief cell function from feeding cows too much calcium in the dry period.
  - 1970 - PTH structure discovered by Brewer and Ronan and Niall et al.
  - 1991 - PTH receptor cloned by Jüppner et al.
  - 1990’s - Paradoxical effect of intermittent administration of PTH on stimulation of new bone formation re-discovered and studied extensively.
  - 1995 – Canine PTH cloned and sequenced by Rosol et al.
  - 2002 – Feline PTH cloned and sequenced by Toribio, Rosol, and others.
  - 2002 - Recombinant PTH 1-34 (teriparatide) approved for treatment of human osteoporosis even though it increased the incidence of osteosarcomas in rats in chronic lifetime studies.
  - 2003 - PTH discovered in fish by Danks et al.

- **Calcitonin (32 amino acids)**
  - 1961 - Copp and co-workers discover calcitonin activity based on secretion of a factor that ‘tones’ down blood calcium when the thyroid/parathyroid glands were perfused with high calcium concentrations. Interestingly, it was first thought to be derived from the parathyroid glands.
  - 1963 - Hirsch and others showed that calcitonin originated from the thyroid glands and proposed the name, ‘thyrocalcitonin’.
  - 1967 - Pearse and Carvaheira showed that C-cells of the thyroid were derived from the neural crest.
  - 1968 - Copp collected 100 kg of ultimobranchial glands from 500,000 salmon in Vancouver. Subsequently pure salmon calcitonin was isolated by D’Or et al., sequenced by Guttmann et al., and synthesized by Sandoz Pharmaceutical Company (Basel) in the same year.
  - 1984 – salmon calcitonin approved for use in human osteoporosis. It is also effective to treat Paget’s disease of bone (humans) and acute hypercalcemia.
  - 1995 – nasal salmon calcitonin approved for use in human osteoporosis (however, the effectiveness of calcitonin on reducing fracture risk is still debated).

- **Parathyroid hormone-related protein (PTHrP; 139-141 amino acids)**
  - 1941 - Albright describes clinical syndrome of hypercalcemia (psuedohyperparathyroidism) associated with cancer.
  - 1973 – Osborne describes pseudohyperparathyroidism in the dog.
  - 1977 – Yarrington and others describe pseudohyperparathyroidism in dogs with lymphosarcoma.
  - 1978 – Rijnberk and others describe pseudohyperparathyroidism in dogs with perirectal adenocarcinomas.
1981 - Meuten, Capen and others describe pathogenesis of hypercalcemia in dogs with apocrine gland adenocarcinomas of the anal sac.

1983 - Meuten, Capen and others describe pathogenesis of hypercalcemia in dogs with lymphosarcoma.

1980’s – Laboratories across the world attempt to purify cancer-associated PTH-like factor.

1986 – Rosol, Capen and others develop mouse model of canine anal sac apocrine gland adenocarcinoma.

1987 – PTHrP discovered in human breast and lung cancers by Broadus et al., and Martin et al.

1989 – PTHrP shown to stimulate calcium transport in the placenta by Abbas et al.

1990 – PTHrP demonstrated in canine anal sac apocrine gland adenocarcinomas by Rosol et al.

1992 – PTHrP measured in dogs with cancer-associated hypercalcemia by Rosol et al.

1993 – Danks et al., show that PTHrP is a calcium-regulating hormone from the fish pituitary.

1994 – PTHrP isolated from canine anal sac apocrine gland adenocarcinoma by Gröne, Rosol and others.

1994 – Knockout of PTHrP shown to be lethal at birth by Karaplis et al.

1995 – Canine PTHrP cloned and sequenced by Rosol et al.

1998 – Bovine PTHrP cloned and sequenced by Wojcik, Rosol and others.

1990’s and 2000’s – PTHrP shown to be important endocrine hormone in fetuses, paracrine hormone in most normal tissues, and abnormal endocrine hormone in cancer and metastasis.

2010 – Toribio, Rosol and others show that nuclear localization of PTHrP is necessary for postnatal survival.

Parathyroid Hormone (PTH):

PTH is an 84 amino acid protein secreted from the chief cells of the parathyroid gland. Parathyroid glands are thought to have evolved in terrestrial vertebrates with the move of animals from a high calcium environment (sea water) to a low calcium environment (land). Fishes do not have parathyroid glands. It has recently been discovered that fishes have two PTH genes, but their function is unknown. PTH is responsible for the minute-to-minute regulation of serum Ca^{2+} concentration. Its principal receptor is the PTH1 receptor in bone, kidney, and other organs. Both PTH 1-34 and 1-84 bind and activate the PTH1 receptor. The function of C-terminal PTH 35-84 is unknown and it does not bind to the PTH1 receptor. A nonbiologically active form of PTH 3-84 also circulates in the blood. The most clinically relevant immunoassays for PTH measure intact PTH 1-84 or the N-terminus of PTH 1-34. PTH secretion is regulated principally by serum Ca^{2+} concentration and PTH synthesis and secretion can increase rapidly and dramatically in response to decreased serum Ca^{2+}. PTH stimulates acute Ca^{2+} release from the bone microenvironment and stimulates the formation and function of osteoclasts. Interestingly, the PTH1 receptors are on osteoblasts, which regulate osteoclasts with RANKL (receptor activator of NFkB ligand) and RANK (the receptor for RANKL on osteoclasts). PTH increases calcium reabsorption and phosphate excretion by the kidney and increases the renal synthesis of the active form of vitamin D, calcitriol. Primary and secondary hyperparathyroidism are important clinical diseases in animals. Hypoparathyroidism is uncommon. Daily injections of PTH 1-34 (teriparatide) are used clinically to increase bone mass in postmenopausal women.
Calcitonin:
Calcitonin is a 32 amino acid protein secreted by the C-cells of the thyroid gland or ultimobranchial glands in birds and fishes. Calcitonin has also been identified in unicellular animals, such as bacteria and yeasts. Calcitonin is an important hormone in fishes where it is thought to facilitate calcium excretion from the gills and kidneys. However, there are conflicting investigations on the role of calcitonin in different species of fish. Fish calcitonin is much more biologically active in mammals compared to the native calcitonin and salmon calcitonin is used pharmacologically. Calcitonin secretion is regulated by serum Ca\(^{2+}\) concentration and GI hormones, such as gastrin and cholecystokinin. Calcitonin receptors are present on osteoclasts, renal tubule cells, and the central nervous system. Avian osteoclasts usually do not have detectable calcitonin receptors; however, they have been shown to respond to calcitonin. Calcitonin inhibits osteoclastic bone resorption (although only transiently). It is thought that the major functions of calcitonin are reductions of postprandial hypercalcemia and calcium loss during pregnancy, lactation, and growth. Interestingly, there are no known clinical syndromes due to hypo- or hypercalcitonemia. Calcitonin can serve as a biomarker for C-cell tumors in humans and animals. Salmon calcitonin is used clinically to treat acute hypercalcemia, Paget’s disease of bone (humans), and osteoporosis. Calcitonin can also be used to reduce bone pain and other forms of pain.

Parathyroid Hormone-Related Protein (PTHrP):
PTHrP is a 141 amino acid protein that was discovered as the principal cause of humoral hypercalcemia of malignancy (cancer-associate hypercalcemia; psuedoerparathyroidism). It was long known that some human and animal cancer patients developed hypercalcemia due to secretion of a peptide that mimicked PTH and bound to the PTH1 receptor. The N-terminal portion of PTHrP has sequence homology to PTH, which enables it to bind and activate the PTH receptor. There is no primary sequence homology between PTHrP and PTH after the first 16 amino acids. PTHrP is an ancestral hormone and it has been speculated that the PTH gene resulted from gene duplication of the PTHrP gene. PTHrP is present in fishes and is secreted by the pituitary gland. It likely functions to regulate calcium balance in fishes in a manner similar to PTH in terrestrial vertebrates. After PTHrP was discovered as a cancer-related factor, it was completely unexpected that PTHrP would play such an important role in normal development and physiology. PTHrP functions as a normal endocrine hormone in the fetus where it regulates calcium absorption from the placenta. The N-terminus and midregion of PTHrP are important in the placenta. The receptor for the midregion is unknown. PTHrP plays an important developmental and regulatory role typically as a paracrine hormone. Most organs have a role for PTHrP. These include bone, brain, endocrine glands, skin, cardiac, skeletal and smooth muscle, epithelial cells, immune cells (including macrophages and lymphocytes), blood vessels, hematopoietic cells, mammary gland, etc. The highest concentrations of PTHrP are found in milk, but its function in milk is unknown. It is thought to play a role in Ca\(^{2+}\) transport in the mammary gland and protect the maternal skeleton from bone loss during lactation. The function and mechanisms of action in most organs is poorly understood. This is because PTHrP knockout mice die in utero or at birth. Normal bone development is dependent on PTHrP because it is necessary for regulation of cartilage proliferation and endochondral ossification. Lack of PTHrP leads to dwarfism and premature ossification of the skeleton. Neonates are unable to respire since their ribs are full ossified and nonexpandable. PTHrP also regulates bone mass in adults. PTHrP is not only a secreted protein, it is also translocated directly to the nucleus when an alternative transcriptional start site is utilized. Nuclear PTHrP likely regulates cell proliferation and apoptosis, but the molecular mechanisms are not known.
Calcitriol (1,25-dihydroxyvitamin D):
Calcitriol is the active form of vitamin D. Calcitriol has long been known to stimulate increased calcium and phosphorus absorption from the GI tract to maintain normal bone growth and prevent rickets due to vitamin D deficiency. Calcitriol also inhibits the synthesis of PTH by chief cells and completes an endocrine feedback loop between PTH and calcitriol. This was an important discovery, because a lack of calcitriol in patients with chronic renal failure often leads to renal secondary hyperparathyroidism. Calcitriol binds to the vitamin D receptor (VDR), which forms a heterodimer with the RXR receptor, translocates to the nucleus, and acts as a transcription factor. There are many tissue-specific co-repressors and co-activators that enable calcitriol to have tissue-specific effects. Many tissues and cells express the VDR even though they do not have a classic role in calcium and phosphorus regulation. Investigations on vitamin D have developed into a well recognized subspecialty with international workshops that occur every 3 years (http://vitamind.ucr.edu/workshop.html). Abundant data is accumulating that calcitriol has an important role in many tissues besides its classic role in bone health and calcium regulation. Calcitriol can regulate cell proliferation and differentiation in normal and neoplastic cells and a relative lack of vitamin D may contribute to the incidence of some cancers. For example, it is known that the incidence of prostate cancer is greater in blacks and those that reside at higher latitudes. It is speculated that lower vitamin D levels in these individuals may play a role in the development of prostate cancer. Calcitriol is also known to be important for the immune system. The concentration of serum 25-hydroxyvitamin D (formed in the liver from dietary or skin-produced vitamin D) is a useful indicator of body vitamin D status. The normal levels of 25-hydroxyvitamin D in humans is currently controversial. It is known that 20 ng/ml is sufficient for normal bone development and mineral homeostasis. However 30 – 40 ng/ml may be required for the nonmineral effects of vitamin D. Some tissues (and cancers) have the ability to synthesize calcitriol locally from 25-hydroxyvitamin D (as accomplished in the kidney) since they express the 1-alpha-hydroxylase enzyme (CYP27B1). There are rare cases where tumors (usually lymphoma) produce excessive calcitriol and induce hypercalcemia. There has been a longstanding interest in developing low hypercalcemic analogues of calcitriol; however, this has met with limited success due to low margins of safety. Toxicity of calcitriol is due to hypercalcemia and soft tissue mineralization, especially of blood vessels. Calcitriol glycoside is an active ingredient produced by some forages and can be a source of toxicity (enzootic calcinosis) for grazing animals (examples include Cestrum diurnum, Solanum sp., Trisetum flavescens, among others). Topical use of calcitriol ointment is effective for treatment of psoriasis.

Stanniocalcin:
The corpuscles of Stannius (CS) were discovered as an organ on the ventral surface of the fish kidney in 1839 by Stannius. Stanniocalcin-1 (STC-1) is typically a glycosylated dimer of a 233 amino acid monomer. STC-1 inhibits calcium transport in the gills and is likely the major hypocalcemic hormone in fishes. The CS cells are sensitive to serum Ca\(^{2+}\) concentration and have a calcium-sensing receptor (CaR). Serum concentrations of STC-1 in marine fishes are higher than freshwater fishes, possibly due to the higher concentration of Ca\(^{2+}\) in sea water. STC-1 is also produced in other tissues, such as the kidney, gonad and uterus but there are likely unique functions of STC-1 in these organs. STC-1 was long considered a hormone of fishes; however, in 1995 mammalian STC-1 was discovered. Mammalian genetic analysis also revealed a second gene encoding STC-2, which was subsequently shown to occur in fishes. STC homologues also occur in invertebrates. Only STC-1 is found in the serum and is widely expressed during embryogenesis and in adults. STC-2 is not expressed in embryos and has a limited expression in adults. Transgenic mice with high levels of STC-1 have hyperphosphatemia, normocalcemia, dwarfism, and reproductive impairment in females. STC-1 and -2 likely have overlapping functions. STC-1 is often sequestered in intracellular organelles.
(e.g., mitochondria in hepatocytes, lipid storage droplet in adipocytes, and nuclei of lactating mammary epithelial cells).

**Prolactin:**
Removal of the pituitary gland in fishes results in hypocalcemia, which is restored to normal by treatment with prolactin.

**Somatolactin:**
Somatolactin is produced in the pars intermedia of fishes. It functions during gonad growth and spawning and it may have a role in calcium and phosphate mobilization during vitellogenesis.

### Important Gaps in Current Knowledge:
- Function and receptor of C-terminal PTH
- Direct effect of PTH on the gastrointestinal tract
- Function and receptors of midregion and C-terminal PTHrP
- Function and mechanism of action of nuclear localization of PTHrP
- Metabolism of PTHrP
- Regulation of PTHrP gene expression and secretion in cancer
- Function of PTHrP in normal tissues and development, especially epithelial-mesenchymal interactions
- Function of PTH, PTHrP, and calcitonin in fishes
- Isolation and identification of the putative cell membrane receptor for calcitriol
- Comparative function and tumorigenicity of calcitonin secretagogues in rodents compared to higher mammals and humans (particularly important for glucagon-like peptide-1 receptor agonists)
- Clinical utility of calcitonin for osteoporosis
- Role of PTHrP in bone and soft tissue metastases of epithelial cancers
- Development of potential tissue-selective VDR agonists or antagonists
- Role and function of STC-1 and -2 in fishes and mammals

### Conclusion:
The calcium-regulating hormones continue to be an active and important area of investigation with relevance to comparative medicine and new endocrine therapy for human and animal diseases.

### References and Additional Reading:


Capen CC, Young DM. The ultrastructure of the parathyroid glands and thyroid parafollicular cells of cows with parturient paresis and hypocalcemia. Lab Invest 17:717-37, 1967.


Capen CC, Rosol TJ. Recent advances in the structure and function of the parathyroid gland in animals and the effects of xenobiotics. Toxicol Pathol 17:333-45, 1989.


