Recent Advances in Calcium and Phosphorus Metabolism

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ABSTRACT

This review considers the most recent developments concerning the metabolism and homeostasis of calcium and phosphorus. The kinetics of the distribution of calcium, theories of calculus formation, hypercalcemia and hypocalcemia are discussed, as well as the role of parathyroid hormone, thyrocalcitonin and 1,25 dihydroxy Vitamin D₃ in maintaining calcium levels and skeletal integrity. In addition, the role of calcium in enzyme activation and inhibition, muscle and nerve function, and intracellular metabolism are considered.

Introduction

Calcium and phosphorus play a major role in skeletal structure, activation and inhibition of enzyme systems, nerve impulse transmission, cellular permeability and, in the case of phosphorus, as a functional entity of muscle and protoplasm and a source of metabolic energy. The purpose of this report is to consider the most recent developments concerning the metabolism of calcium and phosphorus.

Calcification

The skeleton contains 99 percent of the approximate 1,000 g of calcium and 88 percent of the 500 to 600 g of phosphorus in the adult man (table I). Bone itself contains about 57 gram-equivalent calcium and 49 gram-equivalent phosphate. Extraacellular fluid contains about one g of calcium in a supersaturated state and seeding of hydroxyapatite crystals from the extracellular fluid onto bone is associated with mucopolysaccharides. The concentration of calcium in the extracellular fluid is maintained within narrow limits by an equilibrium with calcium at the surface of the bone. The extracellular calcium is ionized, protein bound or complexed in a nonionizable form with citrate or other organic acids. The concentration of the ionized calcium is under the control of parathyroid hormone (PTH), thyrocalcitonin (TCT) and Vitamin D.

PTH increases bone resorption surfaces (osteoclast pool) and decreases the osteoblast pool (bone formation surfaces). TCT increases bone formation and decreases resorption. The active form of Vitamin D, 1,25-dihydroxy-cholecalciferol increases bone resorption surfaces due either to activation of new groups of mesenchymal cells or to an increase in activity of osteoblasts already present. In addition to the old concept that Vitamin D effects the calcium phosphorus ion product, the vitamin acts on bone cells and probably
induces mobilization of calcium and phosphate from underlying calcified bone to osteoid.

In rats, PTH increases both phosphate and cyclic AMP excretion in an inverse relationship to plasma calcium. Infused calcium inhibits the activity of adenyl cyclase but not phosphodiesterase. Calcium inhibits renal action of PTH by action on cyclic AMP generation system. Calcium also may regulate PTH biosynthesis. This regulation may take place at the uptake of amino acids into the cell, synthesis of proPTH, conversion of the prohormone to the active form, secretion of PTH from the cell or the breakdown and destruction of mature secretory granules.

Bone contains both hydroxyapatite and amorphous calcium phosphate. The hydroxyapatite has the formula $\text{Ca}_10(\text{PO}_4)_6(\text{OH})_2$ and consists of right rhombic prisms with a basal plane of 9.432 Å and a height of 6.881 Å with hydroxyls at equidistant intervals one half the height of the cell (3.44 Å). There are three proposed mechanisms for mineralization: (1) a local increase in calcium and phosphate concentration leading to spontaneous precipitation; (2) formation of nucleating sites which lower the energy requirements for bone apatite formation; or (3) formation of a less basic calcium phosphate salt which hydrolyzes into apatite.

Bone cells are involved in calcification. A cellular pumping mechanism possibly raises the calcium and phosphate concentrations to supersaturated levels and allows precipitation of amorphous calcium phosphate. It has been suggested that the osteoblast Golgi apparatus secretes a calcium-phosphoprotein complex which reacts with extracellular phosphatase, freeing phosphate to react with calcium forming amorphous calcium phosphate which becomes the source of apatite.

The role of alkaline phosphatase in calcification has been the subject of conjecture since Robison described its presence in 1923 and suggested that calcification was a simple mass action precipitation resulting from a localized increase in inorganic phosphate resulting from hydrolysis of hexose phosphates. The true role of phosphatase may be to serve as a pyrophosphatase. Inorganic pyrophosphate, in the presence of mucopolysaccharide, appears to inhibit the precipitation of calcium phosphate and the further growth of bone crystals. Pyrophosphate may be the controlling factor in calcification and the physiological action of bone phosphatase may be to lower the concentration of pyrophosphate and thereby to permit crystal formation. It is still a subject of discussion as to whether inorganic pyrophosphatase other than that found in red cells is a different enzyme from other phosphatases. Indirect support for the role of pyrophosphate as a controlling substance in calcification is the fact that patients with hypophosphatasia excrete supranormal amounts of pyrophosphate in their urine; the normal urine concentration is about $10^{-5}$ to $10^{-4}$ mole per liter and in plasma, $10^{-6}$ to $10^{-5}$ mole per liter.

### Table I

Calcium and Phosphorus Content in Adult

<table>
<thead>
<tr>
<th>Subject</th>
<th>Wt (kg)</th>
<th>Calcium (g)</th>
<th>Corrected to 70 kg (g)</th>
<th>Phosphorus (g)</th>
<th>Corrected to 70 kg (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>70.6</td>
<td>1129</td>
<td>1120</td>
<td>544</td>
<td>540</td>
</tr>
<tr>
<td>B</td>
<td>53.8</td>
<td>1027</td>
<td>1332</td>
<td>495</td>
<td>644</td>
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<tr>
<td>C</td>
<td>73.5</td>
<td>984</td>
<td>947</td>
<td>516</td>
<td>492</td>
</tr>
<tr>
<td>D</td>
<td>62.0</td>
<td>1260</td>
<td>1424</td>
<td>616</td>
<td>696</td>
</tr>
</tbody>
</table>

* Reference 19.

### Calculus and Pathologic Crystal Formation

In nephrolithiasis, brushite (CaHPO$_4$·2H$_2$O) serves as the nidus of calcium phosphate or calcium oxalate stones whether the nucleus forms in an organic matrix or by spontaneous precipitation. Nucleation of solute out of solution results when the extent of supersaturation exceeds the metast-
table supersaturated state. A metastable supersaturated solution has been defined as a biologically supersaturated solution that never reaches the equilibrium state required to achieve precipitation or crystallization. In urine, a nidus of brushite does not form until the urine specimens are at least 2.1 times saturated with respect to brushite. The effectiveness of treatment of such stones by administration of cellulose phosphate is due to a decrease in the activity product ratio of brushite primarily by a reduction in the urinary excretion of calcium. The administration of sodium phosphate increases the excretion of monohydrate phosphate and the formation product ratio (the lowest state of supersaturation supporting crystal formation). However, it appears that the formation product ratio is directly correlated to the concentration of pyrophosphate in the urine and the observed sodium phosphate effect is due to increased excretion of pyrophosphate. In patients with calcium oxalate kidney stones there is about a 20 percent greater urinary excretion of both calcium and oxalate leading to a high degree of supersaturation in the urine.

Inflammation-producing crystals in joints may be either monosodium urate or calcium pyrophosphate dehydrate. Pyrophosphate crystals have been found in the synovial fluid removed from knees of 3.2 percent of examined cadavers. The pyrophosphate crystals are triclinic dimorphs varying in diameter from a few microns to half a centimeter.

The solubility of crystalline pyrophosphate in synovial fluid is about 50 \( \mu \text{mole per l} \) compared to 111 \( \mu \text{mole per l} \) in plasma. The solubility is increased by the presence of albumin or magnesium and decreased by calcium. In synovial fluid from normal persons the pyrophosphate concentration is 3.6 ± 1.4 \( \mu \text{mole per l} \); in osteoarthritis 12.7 ± 2.1 \( \mu \text{mole per l} \); in pseudo-
gout 23.9 ± 3.0 \( \mu \text{mole per l} \); in rheumatoid arthritis 4.6 ± 0.7 \( \mu \text{mole per l} \); and in gout 13.1 ± 3.2 \( \mu \text{mole per l} \). The source of synovial fluid pyrophosphate may be either excessive production or a deficiency of inorganic pyrophosphatase or phosphotransferase.

Despite the fact that pyrophosphate is produced in large amounts during metabolism and more than 40 g are created daily during albumin synthesis with total production in the order of kilograms, only 1 to 4 g are excreted as such in urine. Urinary pyrophosphate is not elevated in gout or pseudogout but is elevated in uremia and certain forms of osteomalacia. In plasma of normal persons the concentration of pyrophosphate is 1.8 ± 0.06 \( \mu \text{mole per l} \) and in plasma from patients with osteoarthritis, 2.62 ± 0.12 \( \mu \text{mole per l} \). In platelets the concentration is 800 times that in plasma and is released on thrombin stimulation.

**Absorption and Excretion**

Calcium is absorbed through the entire small intestine by diffusion and by active transport in the proximal segment. Vitamin D is essential for active transport and absorption is enhanced by bile salts. Active absorption is inhibited by actinomycin, puromycin or cyclohexamide. These effects support the theory that vitamin D action is more than alteration of the permeability of the intestinal cell membrane and is rather induction of synthesis of a calcium transport protein. Immunofluorescent antibody studies support the presence of a calcium binding protein in the brush border of the cell. The role of a calcium dependent ATPase in these cells is not understood.

The dietary intake of calcium is about 1 g per day and 20 percent of that is absorbed. However, adaptation permits zero balance with a much lower intake. The normal amount of calcium secreted into the intestine is about 130 mg per day, but in the presence of a vitamin D deficiency this
amount is much greater. The urinary excretion of calcium is proportional to the logarithm of the intake and only the plasma diffusable calcium is filtered. A mean of 97.3 ± 1.6 percent of the filtered plasma calcium is reabsorbed with a greater reabsorption of the ionized form. Most of urinary calcium (about 80 percent) is bound to citrate, sulfate, glucuronate and other anions. Urinary calcium excretion parallels sodium excretion, and tubular reabsorption is depressed by inhibitors of aerobic oxidation or phosphorylation. A tubular maximal reabsorbptive capacity (T_M) for calcium has not been precisely established.

The relationship in normal persons between dietary calcium intake (x) and excretion (y) can be expressed by a regression equation. One such equation is: y = 2.5 + 0.71x.45 From equations such as this, it is possible to calculate the theoretical secretion into the intestine at 0 intake. This value has ranged from 8.3 mg per kg to 20.3 mg per kg and 0.40 to 0.95 g per day for the accretion (the difference between the sum of the urinary and fecal excretion and the total turnover). These parameters are increased in hyperparathyroidism and decreased in hypoparathyroidism. In bone metastases there is a two to four fold increase in the total pool and accretion.

Kinetic data with ⁴⁷Ca have indicated that calcium is distributed into a four-compartmental model (table II).21 In normal persons (body surface of 1.73 sq m), the total exchangeable pool was 5.52 g, the accessible (central) compartment was 1.07 g and the three other compartments 0.7 g, 1.50 g and 2.14 g. The total turnover was calculated to be 0.75 g per day and the accretion 420 mg per day. The four-compartment model can also be applied to various pathological conditions including hyperthyroidism and hypoparathyroidism. In other studies, the total pool has reported to range between 4.6 to 6.6 g.

The kinetics of disappearance of intravenously administered ⁴⁷Ca is affected by oral ingestion of calcium. Following the ingestion of calcium there is a temporary rise of the specific activity of serum calcium followed by a more rapid decline or oscillations in the specific activity. These effects have been attributed to the secretion and effect of TCT in inhibiting calcium release from bone and reabsorption in the renal tubule, thereby increasing calcium excretion.21 In experiments in which an oral load of calcium containing a radioactive trace was given to totally thyroidectomized patients and control subjects, it was found that during absorption the thyroidectomized individuals showed significantly higher blood calcium levels than controls. However, the time of appearance of radioactivity in the plasma after ingestion was similar in the two groups of individuals and there did not seem to be any difference in intestinal absorption. It was concluded from these studies (1) that TCT exerted only a minimal effect on calcium homeostasis and (2) that if the action of the hormone continued over several hours during post-absorptive hypercalcemia, the amount of skeletal calcium saved (50 to 100 mg) would be an important long range consideration. In hyperthyroidism, excessive fecal calcium losses are often observed.
TABLE III

Summary of Calcium Homeostasis

1. Low serum calcium stimulates parathyroid hormone
   a. Which increases calcium resorption and
      monohydrogen phosphate excretion in kidney
   b. Which increases calcium resorption in bone

2. High serum calcium stimulates thyrocalcitonin
   a. Which inhibits Ca++ resorption in bone
   b. Which inhibits Ca++ transport in intestine

3. Vitamin D is converted to 1,25(OH)₂ derivative in liver and kidney (25-position) and
   a. Promotes Ca++ resorption in bone
   b. Induces Ca++ transport

It has been reported that there is a significant decrease in calcium absorption in untreated hyperthyroid patients when compared to either normal controls or successfully treated patients. In 42 normals, the mean absorption was 34.5% (range 20 to 75 percent) and in 15 untreated hyperthyroid patients, 18.6% (range 8 to 34 percent).48

Dietary phosphorus is almost entirely absorbed in the intestine. About 10 percent of the amount filtered by the renal tubule is excreted in the urine. Tubular reabsorption of phosphate is affected by Vitamin D and parathyroid hormone. The Tₘ phosphorus is about 2 to 6 mg per minute and the relationship between TₘP (y) and glomerular filtration rate (x) follows the equation

\[ y = 0.043x - 0.76 \]

The fasting plasma phosphate level is related to Tₘ and glomerular filtration rate (GFR).

Hypocalcemia

Parathyroid hormone releases calcium and phosphate from bone and produces an increase in plasma calcium and phosphorus. The plasma levels of calcium are the result of an equilibrium between absorption, excretion and movement of calcium into and out of bone.8 A summary of the mechanism of calcium homeostasis is shown in table III. Hypocalcemia is a sustained fall in ionized calcium and is generally due to abnormal parathyroid function or, in the case of severe osteoblastic metastases, thyrocalcitonin over-production by the cancer cells.9 Hypocalcemia can also result from complexing of ionized calcium during therapeutic infusion of phosphate, citrate or EDTA or during acute pancreatitis when calcium complexes with fatty acids. Hypocalcemia, accompanied by hyperphosphatemia and hyperphosphaturia, is seen in about 10 percent of children hospitalized with acute lymphoblastic leukemia. The phosphorus levels in four such children ranged from 5.7 to 9.4 mg per dl and the calcium from 6.6 to 8.3 mg per dl. The hypocalcemia has been explained as the result of a rapid release of a phosphorus load into the circulation during the chemotherapeutic related destruction of lymphoblasts.27,55

Hypercalcemia

Hypercalcemia in cancer patients has been attributed to many factors including (1) destruction of bone and release of calcium into serum during direct invasion of tumor into bone; (2) production of PTH by tumor; (3) production of a vitamin D-like sterol by the tumor; (4) steroid effects caused by adrenal gland metastases; and (5) in myeloma, an increase in total calcium related to an increase in γ-globulin. The role of PTH in cancer related hypercalcemia has been the subject of controversy.4,15,33,38,42,46,47 In normal subjects, there is an inverse relationship between plasma calcium concentration and that of immunoreactive PTH and a similar relationship is observed in patients with hyperplasia or adenoma of the parathyroid. Abnormal concentrations of parathyroid hormone were found in 103 of 108 patients with malignant hypercalcemia.

Tashjian and his associates have described a transplantable mouse tumor that produces a potent bone resorption-stimulating factor. The mice having this tumor have elevated concentrations of calcium and prostaglandin E₂ in their serum. The
administration of indomethacin, a powerful inhibitor of prostaglandin E₂ synthesis, to the animals lowered the serum calcium, as well as the prostaglandin concentration, the size of the tumor and the tumor content of the bone resorption-stimulating factor. The conclusion of the authors was that in this mouse tumor prostaglandin E₂ was the bone resorption factor and the causative agent of the hypercalcemia. The role of prostaglandins in hypercalcemia has been the subject of conjecture. The administration of prostaglandins to rats or dogs does not produce a hypercalcemia, a fact that might be explained by the high rate of clearance of prostaglandins from the plasma.

It is of interest that calcium and inorganic phosphate are elevated in saliva of patients with primary hyperparathyroidism but not in patients with idiopathic hypercalcemia or idiopathic calcium stone formation. Salivary duct cells may respond to PTH in a fashion similar to renal tubular cells. Salivary and renal glands handle sodium and potassium similarly.

Parathyroid hyperplasia with secondary hyperparathyroidism and resulting hypercalcemia and hypophosphatemia is a common finding in patients following renal transplantation. In 22 of 64 renal transplant patients hypercalcemia developed at some time during the post transplant period. The calcium abnormality occurred in most patients within the first 10 days after transplant, but in some not until more than six months after surgery. The hypercalcemia group had higher PTH concentrations (1.63 ± 0.83 ng per ml) than the normocalcemic group (0.46 ± 0.34 ng per ml) and in all patients with hypercalcemia the renal function was good (mean creatinine clearance 62 ml). Hypophosphatemia (<2.5 mg per 100 ml) was seen in 20 of the 64 patients.

The kidney plays a major role in calcium homeostasis. In addition to the role of PTH on renal tubules, the kidney stimulated by PTH is the only organ capable of converting 25-hydroxycalciferol to 1,25-dihydroxycholecalciferol (1,25-(OH)₂-D₃), the most active form of vitamin D. In normal persons, administration of 0.14 to 2.7 µg per day of the 1,25 (OH)₂-D₃ produced an increase in calcium absorption and urinary excretion, as well as a decrease in fecal calcium. In uremic patients 0.68 to 2.71 µg per day was required. The overall calcium balance remains essentially the same suggesting the presence of bone resorption.

Non-Skeletal Calcium Function

In addition to its role in the skeleton, calcium has specialized functions in muscle, nerve and secretory tissues. During muscle contraction, calcium is released into muscle cell cytoplasm and presumably activates the reaction between actinomysin and ATP. Activation of actinomysin in skeletal and cardiac muscle requires a 10⁻⁷ mole per l concentration of calcium. In nerve, excitation may be the result of removal of calcium from binding sites on the plasma membrane of the nerve cells into the axoplasm. The decrease in the membrane calcium concentration results in an increase in permeability to sodium and potassium. It has also been observed that the release of acetylcholine and epinephrine requires the uptake of calcium into the functional nerve tissue. A similar phenomena occurs in the adrenal during secretion of epinephrine, antidiuretic hormone in the pituitary and amylase from the pancreas. The role of calcium in changing membrane permeability is well known and the presence of a calcium pump for the active transport of calcium across the mitochondrial membrane has been described. Calcium transport via the mitochondria is an energy-requiring step and involves electron transport or ATP breakdown. During calcium transport, 1.3 moles of inorganic phosphate is produced from ATP per mole of ATP transported. PTH stimulates adenyl...
cyclase, increases cyclic AMP and enhances the rate of entry of calcium into the cell. Intracellular calcium is also increased by AMP which stimulates efflux of calcium from mitochondria. Intracellular calcium is relatively low; in erythrocytes it is $6 \times 10^{-5}$ moles per l compared to $2.5 \times 10^{-3}$ moles per l in plasma. It has been suggested that calcium of nerve metabolism, after entering the nerve terminal, combines with fixed negative charges at the internal surface of the axonal membrane and thus allows the approach of negatively charged vesicles to the membrane releasing sites. The release of norepinephrine from sympathetic nerve terminals is critically calcium dependent. It has been theorized to be related to either an inward movement of membrane calcium or a charged calcium complex upon arrival of a nerve action potential. There is an inverse relationship between the action of prostaglandin and calcium. In the cat spleen, it has been shown that increasing calcium in the perfusion medium countered the inhibitory action of prostaglandin E₂ on norepinephrine release in response to nerve stimulation.

It has been suggested that prostaglandin reacts directly on membrane calcium reducing its influx. Prostaglandins decrease the potential of the terminal membrane, possibly by increasing sodium permeability and, thereby, upon arrival of a nerve action potential, reduce the influx of calcium or the charged calcium complex necessary for the ultimate release of the impulse transmitter. The positive myocardial inotropic activity of prostaglandin E₁ or E₂ is related to myocardial calcium metabolism and can be most probably explained by an increase in the membrane permeability to calcium and an increase in the ionic calcium available to the contractile apparatus. Prostaglandin increases intracellular myocardial cyclic AMP, adenylyl cyclase and phosphoprotein phosphatase. It has been known for many years that calcium plays an important role in mitosis. In 1967, Perris and Whitfield demonstrated that injection of calcium chloride stimulates mitotic activity in the thymus gland of the rat, and Rixon observed that mitotic activity in rat bone marrow was directly related to plasma calcium levels and parathyroid extract stimulated mitotic activity. The proposed mechanism involves calcium acting on the cell membrane and on the systems which organize the formation and destruction of cyclic AMP. Cyclic AMP then stimulates phosphorylation of histone which disrupt a nucleohistone complex and permits replication of DNA.

Calcium is an activator or inhibitor of numerous enzymes. Brain adenylyl cyclase and a protein kinase require a very small amount of bound calcium for activity, but are inhibited by low concentrations of calcium (less than $10^{-4}$ moles per l). Amylase contains one g atom of intramolecular calcium per mole of enzyme. Cyclic AMP phosphodiesterase and phosphoprotein phosphatase are inhibited by calcium as are phosphoglucomutase and red cell or prostatic acid phosphatase. Creatine phosphokinase and glucose 6-phosphate dehydrogenase are activated by calcium and both inhibitory and activating effects have been reported for lipase. Calcium accelerates amidase and esterase activity of pancreatic trypsin and increases its stability at alkaline pH.

References

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