Objectives

After studying this chapter, you should be able to:

- Understand the importance of maintaining homeostasis of bodily calcium and phosphate concentrations, and how this is accomplished.
- Describe the bodily pools of calcium, their rates of turnover, and the organs that play central roles in regulating movement of calcium between stores.
- Delineate the mechanisms of calcium and phosphate absorption and excretion.
- Identify the major hormones and other factors that regulate calcium and phosphate homeostasis and their sites of synthesis as well as targets of their action.
- Define the basic anatomy of bone.
- Delineate cells and their functions in bone formation and resorption.

Hormonal Control of Calcium & Phosphate Metabolism & the Physiology of Bone: Introduction

Calcium is an essential intracellular-signaling molecule and also plays a variety of extracellular functions, thus the control of bodily calcium concentrations is vitally important. The components of the system that maintain calcium homeostasis include cell types that sense changes in extracellular calcium and release calcium-regulating hormones, and the targets of these hormones, including the kidneys, bones, and intestine, that respond with changes in calcium mobilization, excretion, or uptake. Three hormones are primarily concerned with the regulation of calcium metabolism. **1,25-Dihydroxycholecalciferol** is a steroid hormone formed from vitamin D by successive hydroxylations in the liver and kidneys. Its primary action is to increase calcium absorption from the intestine. **Parathyroid hormone (PTH)** is secreted by the parathyroid glands. Its main action is to mobilize calcium from bone and increase urinary phosphate excretion. **Calcitonin,** a calcium-lowering hormone that in mammals is secreted primarily by cells in the thyroid gland, inhibits bone resorption. Although the role of calcitonin seems to be relatively minor, all three hormones probably operate in concert to maintain the constancy of the Ca^{2+} level in the body fluids. Phosphate homeostasis is likewise critical to normal body function, particularly given its inclusion in adenosine triphosphate (ATP), its role as a biological buffer, and its role as a modifier of proteins, thereby altering their functions. Many of the systems that regulate calcium homeostasis also contribute to that of phosphate, albeit sometimes in a reciprocal fashion, and thus will also be discussed in this chapter.

Calcium & Phosphorus Metabolism

Calcium

The body of a young adult human contains about 1100 g (27.5 mol) of calcium. Ninety-nine percent of the calcium is in the skeleton. Plasma calcium, normally at a concentration of around 10 mg/dL (5 mEq/L, 2.5 mmol/L), is partly bound to protein and partly diffusible (Table 23–1). The distribution of calcium inside cells is discussed in Chapter 2.

Table 23–1 Distribution (mmol/L) of Calcium in Normal Human Plasma.

Total diffusible		**1.34**
Ionized (Ca^{2+})	1.18	
Complexed to HCO_3^-, citrate, etc	0.16	
Total nondiffusible (protein-bound)		**1.16**
Bound to albumin	0.92	
Bound to globulin	0.24	
Total plasma calcium		**2.50**

It is the free, ionized calcium in the body fluids that is a vital second messenger (see Chapter 2) and is necessary for blood coagulation, muscle contraction, and nerve function. A decrease in extracellular Ca^{2+} exerts a net excitatory effect on nerve and muscle cells in vivo (see Chapters 4 and 5). The result is **hypocalcemic tetany,** which is characterized by extensive spasms of skeletal muscle, involving especially the muscles of the extremities and the larynx. Laryngospasm can become so severe that the airway is obstructed and fatal asphyxia is produced. Ca^{2+} also plays an important role in blood clotting (see Chapter 32), but in vivo, fatal tetany would occur before compromising the clotting reaction.

Because the extent of Ca^{2+} binding by plasma proteins is proportional to the plasma protein level, it is important to know the plasma protein level when evaluating the total plasma calcium. Other electrolytes and pH also affect the free Ca^{2+} level. Thus, for example, symptoms of tetany appear at higher total calcium levels if the patient hyperventilates, thereby increasing plasma pH. Plasma proteins are more ionized when the pH is high, providing more protein anion to bind with Ca^{2+}.

The calcium in bone is of two types: a readily exchangeable reservoir and a much larger pool of stable calcium that is only slowly exchangeable. Two independent but interacting homeostatic systems affect the calcium in bone. One is the system that regulates plasma Ca^{2+}, providing for the movement of about 500 mmol of Ca^{2+} per day into and out of the readily exchangeable pool in the bone (Figure 23–1). The other system involves bone remodeling by the constant interplay of bone resorption and deposition (see following text). However, the Ca^{2+} interchange between plasma and this stable pool of bone calcium is only about 7.5 mmol/d.

Figure 23–1

Calcium metabolism in an adult human. A typical daily intake of 25 mmol Ca^{2+} (1000 mg) moves through many body compartments.

Ca^{2+} is transported across the brush border of intestinal epithelial cells via channels known as transient receptor potential vanilloid type 6 (TRPV6) and binds to an intracellular protein known as calbindin-D_{9k}. Calbindin sequesters the absorbed calcium so that it does not disturb epithelial signaling processes that involve calcium. The absorbed Ca^{2+} is thereby delivered to the basolateral membrane of the epithelial cell, from where it can be transported into the bloodstream by either a sodium/calcium exchanger (NCX1) or a calcium-dependent ATPase. Nevertheless, it should be noted that recent studies indicate that some intestinal calcium uptake persists even in the absence of TRPV6 and calbindin-D_{9k}, suggesting that additional pathways are likely also involved in this critical process. The overall transport process is regulated by 1,25-

dihydroxycholecalciferol (see below). As Ca^{2+} uptake rises, moreover, 1,25-dihydroxycholecalciferol levels fall in response to increased plasma Ca^{2+}.

Plasma Ca^{2+} is filtered in the kidneys, but 98–99% of the filtered Ca^{2+} is reabsorbed. About 60% of the reabsorption occurs in the proximal tubules and the remainder in the ascending limb of the loop of Henle and the distal tubule. Distal tubular reabsorption depends on the TRPV5 channel, which is related to TRPV6 discussed previously, and whose expression is regulated by parathyroid hormone.

Phosphorus

Phosphate is found in ATP, cyclic adenosine monophosphate (cAMP), 2,3-diphosphoglycerate, many proteins, and other vital compounds in the body. Phosphorylation and dephosphorylation of proteins are involved in the regulation of cell function (see Chapter 2). Therefore, it is not surprising that, like calcium, phosphate metabolism is closely regulated. Total body phosphorus is 500 to 800 g (16.1–25.8 mol), 85–90% of which is in the skeleton. Total plasma phosphorus is about 12 mg/dL, with two-thirds of this total in organic compounds and the remaining inorganic phosphorus (P_i) mostly in PO_4^{3-}, HPO_4^{2-}, and $H_2PO_4^-$. The amount of phosphorus normally entering bone is about 3 mg (97 mol)/kg/d, with an equal amount leaving via reabsorption.

P_i in the plasma is filtered in the glomeruli, and 85–90% of the filtered P_i is reabsorbed. Active transport in the proximal tubule accounts for most of the reabsorption and involves two related sodium-dependent P_i cotransporters, NaPi-IIa and NaPi-IIc. NaPi-IIa is powerfully inhibited by parathyroid hormone, which causes its internalization and degradation and thus a reduction in renal P_i reabsorption (see below).

P_i is absorbed in the duodenum and small intestine. Uptake occurs by a transporter related to those in the kidney, NaPi-IIb, that takes advantage of the low intracellular sodium concentration established by the Na, K ATPase on the basolateral membrane of intestinal epithelial cells to load P_i against its concentration gradient. However, the pathway by which P_i exits into the bloodstream is not known. Many stimuli that increase Ca^{2+} absorption, including 1,25-dihydroxycholecalciferol, also increase P_i absorption via increased NaPi-IIb expression.

Vitamin D & the Hydroxycholecalciferols

Chemistry

The active transport of Ca^{2+} and PO_4^{3-} from the intestine is increased by a metabolite of **vitamin D.** The term "vitamin D" is used to refer to a group of closely related sterols produced by the action of ultraviolet light on certain provitamins (Figure 23–2). Vitamin D3, which is also called cholecalciferol, is produced in the skin of mammals from 7-dehydrocholesterol by the action of sunlight. The reaction involves the rapid formation of previtamin D_3, which is then converted more slowly to vitamin D_3. Vitamin D_3 and its hydroxylated derivatives are transported in the plasma bound to a globulin vitamin D-binding protein (DBP). Vitamin D_3 is also ingested in the diet.

Figure 23–2

Formation and hydroxylation of vitamin D3. 25-hydroxylation takes place in the liver, and the other hydroxylations occur primarily in the kidneys. The formulas of 7-dehydrocholesterol, vitamin D_3, and 1,25-dihydroxycholecalciferol are also shown below.

Vitamin D3 is metabolized by enzymes that are members of the cytochrome P450 (CYP) superfamily (see Chapters 1 and 29). In the liver, vitamin D_3 is converted to **25-hydroxycholecalciferol** (calcidiol, 25-OHD_3). The 25-hydroxycholecalciferol is converted in the cells of the proximal tubules of the kidneys to the more active metabolite **1,25-dihydroxycholecalciferol,** which is also called calcitriol or 1,25-$(OH)_2D_3$. 1,25-Dihydroxycholecalciferol is also made in the placenta, in keratinocytes in the skin, and in macrophages. The normal plasma level of 25-hydroxycholecalciferol is about 30 ng/mL, and that of 1,25-dihydroxycholecalciferol is about 0.03 ng/mL (approximately 100 pmol/L). The less active metabolite 24,25-dihydroxycholecalciferol is also formed in the kidneys (Figure 23–2).

Mechanism of Action

1,25 dihydroxycholecalciferol stimulates the expression of a number of gene products involved in calcium transport and handling via its receptor, which acts as a transcriptional regulator in its ligand-bound form. One group is the family of **calbindin-D** proteins. These are members of the troponin C superfamily of Ca^{2+}-binding proteins that also includes calmodulin (see Chapter 2). Calbindin-Ds are found in human intestine, brain, and kidneys. In the intestinal epithelium and many other tissues, two calbindins are induced: calbindin-D_{9K} and calbindin-D_{28K}, with molecular weights of 9,000 and 28,000, respectively. 1,25-dihydroxycholecalciferol also increases the number of Ca^{2+}–ATPase and TRPV6 molecules in the intestinal cells, thus, the overall capacity for absorption of dietary calcium is enhanced.

In addition to increasing Ca^{2+} absorption from the intestine, 1,25-dihydroxycholecalciferol facilitates Ca^{2+} reabsorption in the kidneys via increased TRPV5 expression in the proximal tubules, increases the synthetic activity of osteoblasts, and is necessary for normal calcification of matrix (see Clinical Box 23–1). The stimulation of osteoblasts brings about a secondary increase in the activity of osteoclasts (see below).

Clinical Box 23–1

Rickets & Osteomalacia

Vitamin D deficiency causes defective calcification of bone matrix and the disease called **rickets** in children and **osteomalacia** in adults. Even though 1,25-dihydroxycholecalciferol is necessary for normal mineralization of bone matrix, the main defect in this condition is failure to deliver adequate amounts of Ca^{2+} and PO_4^{3-} to the sites of mineralization. The full-blown condition in children is characterized by weakness and bowing of weight-bearing bones, dental defects, and hypocalcemia. In adults, the condition is less obvious. It used to be most commonly due to inadequate exposure to the sun in smoggy cities, but now it is more commonly due to inadequate intake of the provitamins on which the sun acts in the skin. These cases respond to administration of vitamin D. The condition can also be caused by inactivating mutations of the gene for renal 1-hydroxylase, in which case there is no response to vitamin D but a normal response to 1,25-dihydroxycholecalciferol **(type I vitamin D-resistant rickets).** In rare instances, it can be due to inactivating mutations of the gene for the 1,25-dihydroxycholecalciferol receptor **(type II vitamin D-resistant rickets),** in which case there is a deficient response to both vitamin D and 1,25-dihydroxycholecalciferol.

Regulation of Synthesis

The formation of 25-hydroxycholecalciferol does not appear to be stringently regulated. However, the formation of 1,25-dihydroxycholecalciferol in the kidneys, which is catalyzed by the renal 1-hydroxylase, is regulated in a feedback fashion by plasma Ca^{2+} and PO_4^{3+} (Figure 23–3). When the plasma Ca^{2+} level is

high, little 1,25-dihydroxycholecalciferol is produced, and the kidneys produce the relatively inactive metabolite 24,25-dihydroxycholecalciferol instead. This effect of Ca^{2+} on production of 1,25-dihydroxycholecalciferol is the mechanism that brings about adaptation of Ca^{2+} absorption from the intestine (see previous text). Conversely, expression of 1-hydroxylase is stimulated by PTH, and when the plasma Ca^{2+} level is low, PTH secretion is increased. The production of 1,25-dihydroxycholecalciferol is also increased by low and inhibited by high plasma PO_4^{3-} levels, by a direct inhibitory effect of PO_4^{3-} on the 1-hydroxylase. Additional control of 1,25-dihydroxycholecalciferol formation is exerted by a direct negative feedback effect of the metabolite on 1-hydroxylase, a positive feedback action on the formation of 24,25-dihydroxycholecalciferol, and a direct action on the parathyroid gland to inhibit PTH expression.

Figure 23–3

Effects of PTH and 1,25-dihydroxycholecalciferol on whole body calcium homeostasis. Note that these hormones are also involved in the regulation of circulating phosphate levels.

(Reproduced with permission from Widmaier EP, Raff H, Strang KT: *Vander's Human Physiology*,10th ed., McGraw-Hill, 2006.)

An "anti-aging" protein called -Klotho (named after Klotho, a daughter of Zeus in Greek mythology who spins the thread of life) has also recently been discovered to play important roles in calcium and phosphate homeostasis, in part by reciprocal effects on 1,25-dihydroxycholecalciferol levels. Mice deficient in -Klotho displayed accelerated aging, decreased bone mineral density, calcifications, and hypercalcemia and hyperphosphatemia. -Klotho plays an important role in stabilizing the membrane localization of proteins important in calcium and phosphate (re)absorption, such as TRPV5 and Na, K ATPase. Likewise, it enhances the activity of another factor, fibroblast growth factor 23 (FGF23), at its receptor. FGF23 thereby decreases renal NaPi-IIa and NaPi-IIc expression and inhibits the production of 1-hydroxylase, reducing levels of 1,25-dihydroxycholecalciferol (Clinical Box 23–1).

The Parathyroid Glands

Anatomy

Humans usually have four parathyroid glands: two embedded in the superior poles of the thyroid and two in its inferior poles (Figure 23–4). Each parathyroid gland is a richly vascularized disk, about 3 x 6 x 2 mm, containing two distinct types of cells (Figure 23–5). The abundant **chief cells,** which contain a prominent Golgi apparatus plus endoplasmic reticulum and secretory granules, synthesize and secrete **parathyroid hormone (PTH).** The less abundant and larger **oxyphil cells** contain oxyphil granules and large numbers of mitochondria in their cytoplasm. In humans, few are seen before puberty, and thereafter they increase in number with age. Their function is unknown. Consequences of loss of parathyroid gland are discussed in Clinical Box 23–2.

Figure 23–4

The human parathyroid glands, viewed from behind.

Figure 23–5

Section of human parathyroid. (Reduced 50% from x 960.) Small cells are chief cells; large stippled cells (especially prominent in the lower left of picture) are oxyphil cells.

(Reproduced with permission from Fawcett DW: *Bloom and Fawcett, A Textbook of Histology,* 11th ed. Saunders, 1986.)

Clinical Box 23–2

Effects of Parathyroidectomy

Occasionally, inadvertent parathyroidectomy occurs in humans during thyroid surgery. This can have serious consequences as PTH is essential for life. After parathyroidectomy, there is a steady decline in the plasma Ca^{2+} level. Signs of neuromuscular hyperexcitability appear, followed by full-blown hypocalcemic tetany (see above). Plasma phosphate levels usually rise as the plasma calcium level falls. Symptoms usually develop 2 to 3 d postoperatively but may not appear for several weeks or more. Injections of PTH can be given to correct the chemical abnormalities, and the symptoms then disappear. Injections of Ca^{2+} salts can also give temporary relief. The signs of tetany in humans include **Chvostek's sign,** a quick contraction of the ipsilateral facial muscles elicited by tapping over the facial nerve at the angle of the jaw, and **Trousseau's sign,** a spasm of the muscles of the upper extremity that causes flexion of the wrist and thumb with extension of the fingers. In individuals with mild tetany in whom spasm is not yet evident, Trousseau sign can sometimes be produced by occluding the circulation for a few minutes with a blood pressure cuff.

Synthesis & Metabolism of PTH

Human PTH is a linear polypeptide with a molecular weight of 9500 that contains 84 amino acid residues (Figure 23–6). It is synthesized as part of a larger molecule containing 115 amino acid residues **(preproPTH).** On entry of preproPTH into the endoplasmic reticulum, a leader sequence is removed from the amino terminal to form the 90-amino-acid polypeptide **proPTH.** Six additional amino acid residues are removed from the amino terminal of proPTH in the Golgi apparatus, and the 84-amino-acid polypeptide PTH is packaged in secretory granules and released as the main secretory product of the chief cells.

Figure 23–6

Parathyroid hormone. The symbols above and below the human structure show where amino acid residues are different in bovine and porcine PTH.

(Reproduced with permission from Keutmann HT, et al: Complete amino acid sequence of human parathyroid hormone. Biochemistry 1978;17:5723. Copyright © 1978 by the American Chemical Society.)

The normal plasma level of intact PTH is 10 to 55 pg/mL. The half-life of PTH is approximately 10 min, and the secreted polypeptide is rapidly cleaved by the Kupffer cells in the liver into fragments that are probably biologically inactive. PTH and these fragments are then cleared by the kidneys. Modern

immunoassays for PTH are designed only to measure mature PTH (1–84) and not these fragments to obtain an accurate measure of "active" PTH.

Actions

PTH acts directly on bone to increase bone resorption and mobilize Ca^{2+}. In addition to increasing the plasma Ca^{2+}, PTH increases phosphate excretion in the urine and thereby depresses plasma phosphate levels. This **phosphaturic action** is due to a decrease in reabsorption of phosphate via effects on NaPi-IIa in the proximal tubules, as discussed previously. PTH also increases reabsorption of Ca^{2+} in the distal tubules, although Ca^{2+} excretion in the urine is often increased in hyperparathyroidism because the increase in the load of filtered calcium overwhelms the effect on reabsorption (Clinical Box 23-3). PTH also increases the formation of 1,25-dihydroxycholecalciferol, and this increases Ca^{2+} absorption from the intestine. On a longer time scale, PTH stimulates both osteoblasts and osteoclasts.

Clinical Box 23–3

Diseases of Parathyroid Excess

Hyperparathyroidism due to injections of parathyroid extract in animals or hypersecretion of a functioning parathyroid tumor in humans is characterized by hypercalcemia and hypophosphatemia. Humans with PTH-secreting adenomas are usually asymptomatic, with the condition detected when plasma Ca^{2+} is measured in conjunction with a routine physical examination. However, there may be minor changes in personality, and calcium-containing kidney stones occasionally form. In conditions such as chronic renal disease and rickets, in which the plasma Ca^{2+} level is chronically low, stimulation of the parathyroid glands causes compensatory parathyroid hypertrophy and secondary hyperparathyroidism. The plasma Ca^{2+} level is low in chronic renal disease primarily because the diseased kidneys lose the ability to form 1,25-dihydroxycholecalciferol. Finally, mutations in the calcium receptor, CaR, gene cause predictable long-term changes in plasma Ca^{2+}. Individuals heterozygous for inactivating mutations have familial benign hypocalciuric hypercalcemia, a condition in which there is a chronic moderate elevation in plasma Ca^{2+} because the feedback inhibition of PTH secretion by Ca^{2+} is reduced. Plasma PTH levels are normal or even elevated. However, children who are homozygous for inactivating mutations develop neonatal severe primary hyperparathyroidism. Conversely, individuals with gain-of-function mutations of the CaR gene develop familial hypercalciuric hypocalcemia due to increased sensitivity of the parathyroid glands to plasma Ca^{2+}.

Mechanism of Action

It now appears that there are at least three different PTH receptors. One also binds parathyroid hormone-related protein (PTHrP; see below) and is known as the hPTH/PTHrP receptor. A second receptor, PTH2 (hPTH2-R), does not bind PTHrP and is found in the brain, placenta, and pancreas. In addition, there is evidence for a third receptor, CPTH, which reacts with the carboxyl terminal rather than the amino terminal of PTH. The first two receptors are coupled to G_s, and via this heterotrimeric G protein they activate adenylyl cyclase, increasing intracellular cAMP. The hPTH/PTHrP receptor also activates PLC via G_q, increasing intracellular Ca^{2+} and activating protein kinase C (Figure 23–7). However, the way these second messengers affect Ca^{2+} in bone is unsettled.

Figure 23–7

Signal transduction pathways activated by PTH or PTHrP binding to the hPTH/hPTHrP receptor. Intracellular cAMP is increased via G_s and adenylyl cyclase (AC). Diacylglycerol and IP_3 (1,4,5-$InsP_3$) are increased via G_q and phospholipase C (PLC).

(Modified and reproduced with permission from McPhee SJ, Lingappa VR, Ganong WF [editors]: *Pathophysiology of Disease*, 4th ed. McGraw-Hill, 2003.)

In the disease called **pseudohypoparathyroidism,** the signs and symptoms of hypoparathyroidism develop but the circulating level of PTH is normal or elevated. Because the tissues fail to respond to the hormone, this is a receptor disease. There are two forms. In the more common form, a congenital 50% reduction of the activity of G_s occurs and PTH fails to produce a normal increase in cAMP concentration. In a different, less common form, the cAMP response is normal but the phosphaturic action of the hormone is defective.

Regulation of Secretion

Circulating ionized calcium acts directly on the parathyroid glands in a negative feedback fashion to regulate the secretion of PTH (Figure 23–8). The key to this regulation is a cell membrane Ca^{2+} receptor, CaR. Activation of this G-protein coupled receptor leads to phosphoinositide turnover in many tissues. In the parathyroid, its activation inhibits PTH secretion. In this way, when the plasma Ca^{2+} level is high, PTH secretion is inhibited and the Ca^{2+} is deposited in the bones. When it is low, secretion is increased and Ca^{2+} is mobilized from the bones.

Figure 23–8

Relation between plasma Ca2+ concentration and PTH response in humans. The set point is the plasma Ca2+ at which half the maximal response occurred (ie, 1.2 mmol/L).

(Modified and reproduced with permission from Brown E: Extracellular Ca2+ sensing, regulation of parathyroid cell functions, and role of Ca2+ and other ions as extracellular (first) messengers. Physiol Rev 1991;71:371.)

1,25-dihydroxycholecalciferol acts directly on the parathyroid glands to decrease preproPTH mRNA. Increased plasma phosphate stimulates PTH secretion by lowering plasma levels of free Ca^{2+} and inhibiting the formation of 1,25-dihydroxycholecalciferol. Magnesium is required to maintain normal parathyroid secretory responses. Impaired PTH release along with diminished target organ responses to PTH account for the hypocalcemia that occasionally occurs in magnesium deficiency (Clinical Box 23–2 and Clinical Box 23–3).

PTHrP

Another protein with PTH activity, **parathyroid hormone-related protein (PTHrP),** is produced by many different tissues in the body. It has 140 amino acid residues, compared with 84 in PTH, and is encoded by a gene on human chromosome 12, whereas PTH is encoded by a gene on chromosome 11. PTHrP and PTH have marked homology at their amino terminal ends and they both bind to the hPTH/ PTHrP receptor, yet their physiologic effects are very different. How is this possible when they bind to the same receptor? For one thing, PTHrP is primarily a paracrine factor, acting close to where it is produced. It may be that circulating PTH cannot reach at least some of these sites. Second, subtle conformational differences may be

produced by binding of PTH versus PTHrP to their receptor, despite their structural similarities. Another possibility is action of one or the other hormone on other, more selective receptors.

PTHrP has a marked effect on the growth and development of cartilage in utero. Mice in which both alleles of the PTHrP gene are knocked out have severe skeletal deformities and die soon after birth. In normal animals, on the other hand, PTHrP-stimulated cartilage cells proliferate and their terminal differentiation is inhibited. PTHrP is also expressed in the brain, where evidence indicates that it inhibits excitotoxic damage to developing neurons. In addition, there is evidence that it is involved in Ca^{2+} transport in the placenta. PTHrP is also found in keratinocytes in the skin, in smooth muscle, and in the teeth, where it is present in the enamel epithelium that caps each tooth. In the absence of PTHrP, teeth cannot erupt.

Hypercalcemia of Malignancy

Hypercalcemia is a common metabolic complication of cancer. About 20% of hypercalcemic patients have bone metastases that produce the hypercalcemia by eroding bone **(local osteolytic hypercalcemia).** Evidence suggests that this erosion is produced by prostaglandins such as prostaglandin E2 from the tumor. The hypercalcemia in the remaining 80% of the patients is due to elevated circulating levels of PTHrP **(humoral hypercalcemia of malignancy).** The tumors responsible for the hypersecretion include cancers of the breast, kidney, ovary, and skin.

Calcitonin

Origin

In dogs, perfusion of the thyroparathyroid region with solutions containing high concentrations of Ca^{2+} leads to a fall in peripheral plasma calcium, and after damage to this region, Ca^{2+} infusions cause a greater increase in plasma Ca^{2+} than they do in control animals. These and other observations led to the discovery that a Ca^{2+}-lowering as well as a Ca^{2+}-elevating hormone was secreted by structures in the neck. The Ca^{2+}-lowering hormone has been named **calcitonin.** In mammals, calcitonin is produced by the **parafollicular cells** of the thyroid gland**,** which are also known as the clear or C cells.

Structure

Human calcitonin has a molecular weight of 3500 and contains 32 amino acid residues (Figure 23–9). Much of the mRNA transcribed from the calcitonin gene is processed to a different mRNA in the nervous system, so that **calcitonin gene-related peptide (CGRP)** is formed rather than calcitonin (see Chapter 4).

Figure 23–9

Human calcitonin. The sequence is shown using the three letter abbreviations for constituent amino acids.

Secretion & Metabolism

Secretion of calcitonin is increased when the thyroid gland is exposed to plasma calcium level of approximately 9.5 mg/dL. Above this level, plasma calcitonin is directly proportionate to plasma calcium. -adrenergic agonists, dopamine, and estrogens also stimulate calcitonin secretion. Gastrin, cholecystokinin (CCK), glucagon, and secretin have all been reported to stimulate calcitonin secretion, with gastrin being the most potent stimulus (see Chapter 26). Thus, the plasma calcitonin level is elevated in Zollinger–Ellison syndrome and in pernicious anemia (see Chapter 26). However, the dose of gastrin needed to stimulate

calcitonin secretion is supraphysiological and not seen after eating in normal individuals, so dietary calcium in the intestine probably does not induce secretion of a calcium-lowering hormone prior to the calcium being absorbed. In any event, the actions of calcitonin are short-lived because it has a half-life of less than 10 min in humans.

Actions

Receptors for calcitonin are found in bones and the kidneys. Calcitonin lowers circulating calcium and phosphate levels. It exerts its calcium-lowering effect by inhibiting bone resorption. This action is direct, and calcitonin inhibits the activity of osteoclasts in vitro. It also increases Ca^{2+} excretion in the urine.

The exact physiologic role of calcitonin is uncertain. The calcitonin content of the human thyroid is low, and after thyroidectomy, bone density and plasma Ca^{2+} level are normal as long as the parathyroid glands are intact. In addition, there are only transient abnormalities of Ca^{2+} metabolism when a Ca^{2+} load is injected after thyroidectomy. This may be explained in part by secretion of calcitonin from tissues other than the thyroid. However, there is general agreement that the hormone has little long-term effect on the plasma Ca^{2+} level in adult animals and humans. Further, unlike PTH and 1,25-dihydroxycholecalciferol, calcitonin does not appear to be involved in phosphate homeostasis. Moreover, patients with medullary carcinoma of the thyroid have a very high circulating calcitonin level but no symptoms directly attributable to the hormone, and their bones are essentially normal. No syndrome due to calcitonin deficiency has been described. More hormone is secreted in young individuals, and it may play a role in skeletal development. In addition, it may protect the bones of the mother from excess calcium loss during pregnancy. Bone formation in the infant and lactation are major drains on Ca^{2+} stores, and plasma concentrations of 1,25-dihydroxycholecalciferol are elevated in pregnancy. They would cause bone loss in the mother if bone resorption were not simultaneously inhibited by an increase in the plasma calcitonin level.

Summary

The actions of the three principal hormones that regulate the plasma concentration of Ca^{2+} can now be summarized. PTH increases plasma Ca^{2+} by mobilizing this ion from bone. It increases Ca^{2+} reabsorption in the kidney, but this may be offset by the increase in filtered Ca^{2+}. It also increases the formation of 1,25-dihydroxycholecalciferol. 1,25-Dihydroxycholecalciferol increases Ca^{2+} absorption from the intestine and increases Ca^{2+} reabsorption in the kidneys. Calcitonin inhibits bone resorption and increases the amount of Ca^{2+} in the urine.

Effects of Other Hormones & Humoral Agents on Calcium Metabolism

Calcium metabolism is affected by various hormones in addition to 1,25-dihydroxycholecalciferol, PTH, and calcitonin. **Glucocorticoids** lower plasma Ca^{2+} levels by inhibiting osteoclast formation and activity, but over long periods they cause osteoporosis by decreasing bone formation and increasing bone resorption. They decrease bone formation by inhibiting protein synthesis in osteoblasts. They also decrease the absorption of Ca^{2+} and PO_4^{3-} from the intestine and increase the renal excretion of these ions. The decrease in plasma Ca^{2+} concentration also increases the secretion of PTH, and bone resorption is facilitated. **Growth hormone** increases calcium excretion in the urine, but it also increases intestinal absorption of Ca^{2+}, and this effect may be greater than the effect on excretion, with a resultant positive calcium balance. Insulin-like growth factor I (IGF-I) generated by the action of growth hormone stimulates protein synthesis in bone. As noted previously, **thyroid hormones** may cause hypercalcemia, hypercalciuria, and, in some instances, osteoporosis. **Estrogens** prevent osteoporosis by inhibiting the stimulatory effects of certain cytokines on osteoclasts. **Insulin** increases bone formation, and there is significant bone loss in untreated diabetes.

Bone Physiology

Bone is a special form of connective tissue with a collagen framework impregnated with Ca^{2+} and PO_4^{3-} salts, particularly **hydroxyapatites,** which have the general formula $Ca_{10}(PO_4)_6(OH)_2$. Bone is also involved in overall Ca^{2+} and PO_4^{3-} homeostasis. It protects vital organs, and the rigidity it provides permits locomotion and the support of loads against gravity. Old bone is constantly being resorbed and new bone formed, permitting remodeling that allows it to respond to the stresses and strains that are put upon it. It is a living tissue that is well vascularized and has a total blood flow of 200 to 400 mL/min in adult humans.

Structure

Bone in children and adults is of two types: **compact** or **cortical bone,** which makes up the outer layer of most bones (Figure 23–10) and accounts for 80% of the bone in the body; and **trabecular** or **spongy bones** inside the cortical bone, which makes up the remaining 20% of bone in the body. In compact bone, the surface-to-volume ratio is low, and bone cells lie in lacunae. They receive nutrients by way of canaliculi that ramify throughout the compact bone (Figure 23–10). Trabecular bone is made up of spicules or plates, with a high surface to volume ratio and many cells sitting on the surface of the plates. Nutrients diffuse from bone extracellular fluid (ECF) into the trabeculae, but in compact bone, nutrients are provided via **haversian canals** (Figure 23–10), which contain blood vessels. Around each Haversian canal, collagen is arranged in concentric layers, forming cylinders called **osteons** or **haversian systems.**

Figure 23–10

Structure of compact and trabecular bone. The compact bone is shown in horizontal section **(top)** and vertical section **(bottom).**

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The protein in bone matrix is over 90% type I collagen, which is also the major structural protein in tendons and skin. This collagen, which weight for weight is as strong as steel, is made up of a triple helix of three polypeptides bound tightly together. Two of these are identical $_1$ polypeptides encoded by one gene, and one is an $_2$ polypeptide encoded by a different gene. Collagens make up a family of structurally related proteins that maintain the integrity of many different organs. Fifteen different types of collagens encoded by more than 20 different genes have so far been identified.

Bone Growth

During fetal development, most bones are modeled in cartilage and then transformed into bone by ossification **(enchondral bone formation).** The exceptions are the clavicles, the mandibles, and certain bones of the skull in which mesenchymal cells form bone directly **(intramembranous bone formation).**

During growth, specialized areas at the ends of each long bone **(epiphyses)** are separated from the shaft of the bone by a plate of actively proliferating cartilage, the **epiphysial plate** (Figure 23–11). The bone increases in length as this plate lays down new bone on the end of the shaft. The width of the epiphysial plate is proportionate to the rate of growth. The width is affected by a number of hormones, but most markedly by the pituitary growth hormone and IGF-I (see Chapter 24).

Figure 23–11

Structure of a typical long bone before (left) and after (right) epiphysial closure. Note the rearrangement of cells and growth of the bone as the epiphysial plate closes (see text for details).

Linear bone growth can occur as long as the epiphyses are separated from the shaft of the bone, but such growth ceases after the epiphyses unite with the shaft **(epiphysial closure).** The cartilage cells stop proliferating, become hypertrophic, and secrete vascular endothelial growth factor (VEGF), leading to vascularization and ossification. The epiphyses of the various bones close in an orderly temporal sequence, the last epiphyses closing after puberty. The normal age at which each of the epiphyses closes is known, and the "bone age" of a young individual can be determined by x-raying the skeleton and noting which epiphyses are open and which are closed.

Bone Formation & Resorption

The cells responsible for bone formation are **osteoblasts** and the cells responsible for bone resorption are **osteoclasts.**

Osteoblasts are modified fibroblasts. Their early development from the mesenchyme is the same as that of fibroblasts, with extensive growth factor regulation. Later, ossification-specific transcription factors, such as Cbfa1/Runx2, contribute to their differentiation. The importance of this transcription factor in bone development is underscored in knockout mice deficient for the Cbfa1/Runx gene. These mice develop to term with their skeletons made exclusively of cartilage; no ossification occurs. Normal osteoblasts are able to lay down type 1 collagen and form new bone.

Osteoclasts, on the other hand, are members of the monocyte family. Stromal cells in the bone marrow, osteoblasts, and T lymphocytes all express receptor activator for nuclear factor kappa beta ligand (RANKL) on their surface. When these cells come in contact with appropriate monocytes expressing RANK (ie, the RANKL receptor) two distinct signaling pathways are initiated: (1) there is a RANKL/RANK interaction between the cell pairs, (2) mononuclear phagocyte colony stimulating factor (M-CSF) is secreted by the nonmonocytic cells and it binds to its corresponding receptor on the monocytes (c-fins). The combination of these two signaling events leads to differentiation of the monocytes into osteoclasts. The precursor cells also secrete **osteoprotegerin (OPG),** which controls for differentiation of the monocytes by competing with RANK for binding of RANKL.

Osteoclasts erode and absorb previously formed bone. They become attached to bone via integrins in a membrane extension called the **sealing zone.** This creates an isolated area between the bone and a portion of the osteoclast. Proton pumps (ie, H^+-dependent ATPases) then move from endosomes into the cell membrane apposed to the isolated area, and they acidify the area to approximately pH 4.0. Similar proton pumps are found in the endosomes and lysosomes of all eukaryotic cells, but in only a few other instances do they move into the cell membrane. Note in this regard that the sealed-off space formed by the osteoclast resembles a large lysosome. The acidic pH dissolves hydroxyapatite, and acid proteases secreted by the cell break down collagen, forming a shallow depression in the bone (Figure 23–12). The products of digestion are then endocytosed and move across the osteoclast by transcytosis (see Chapter 2), with release into the interstitial fluid. The collagen breakdown products have pyridinoline structures, and pyridinolines can be measured in the urine as an index of the rate of bone resorption.

Figure 23–12

Osteoclast resorbing bone. The edges of the cell are tightly sealed to bone, permitting secretion of acid from the ruffled apical membrane and consequent erosion of the bone underneath the cell. Note the multiple nuclei (n) and mitochondria (mi).

(Courtesy of R Baron.)

Throughout life, bone is being constantly resorbed and new bone is being formed. The calcium in bone turns over at a rate of 100% per year in infants and 18% per year in adults. Bone remodeling is mainly a local process carried out in small areas by populations of cells called bone-remodeling units. First, osteoclasts resorb bone, and then osteoblasts lay down new bone in the same general area. This cycle takes about 100 days. Modeling drifts also occur in which the shapes of bones change as bone is resorbed in one location and added in another. Osteoclasts tunnel into cortical bone followed by osteoblasts, whereas trabecular bone remodeling occurs on the surface of the trabeculae. About 5% of the bone mass is being remodeled by about 2 million bone-remodeling units in the human skeleton at any one time. The renewal rate for bone is about 4% per year for compact bone and 20% per year for trabecular bone. The remodeling is related in part to the stresses and strains imposed on the skeleton by gravity.

At the cell–cell level, there is some regulation of osteoclast formation by osteoblasts via the RANKL–RANK and the M-CSF–OPG mechanism; however, specific feedback mechanisms of osteoclasts on osteoblasts are not well defined. In a broader sense, the bone remodeling process is primarily under endocrine control. Parathyroid hormone accelerates bone resorption, and estrogens slow bone resorption by inhibiting the production of bone-eroding cytokines. An interesting new observation is that intracerebroventricular but not intravenous leptin decreases bone formation. This finding is consistent with the observations that obesity protects against bone loss and that most obese humans are resistant to the effects of leptin on appetite. Thus, there may be neuroendocrine regulation of bone mass via leptin.

Bone Disease

The diseases produced by selective abnormalities of the cells and processes discussed above illustrate the interplay of factors that maintain normal bone function.

In **osteopetrosis,** a rare and often severe disease, the osteoclasts are defective and are unable to resorb bone in their usual fashion so the osteoblasts operate unopposed. The result is a steady increase in bone density, neurologic defects due to narrowing and distortion of foramina through which nerves normally pass, and hematologic abnormalities due to crowding out of the marrow cavities. Mice lacking the protein encoded by the immediate-early gene c-*fos* develop osteopetrosis, and osteopetrosis also occurs in mice lacking the PU.1 transcription factor. This suggests that all these factors are involved in normal osteoclast development and function.

On the other hand, **osteoporosis** is caused by a relative excess of osteoclastic function. Loss of bone matrix in this condition (Figure 23–13) is marked, and the incidence of fractures is increased. Fractures are particularly common in the distal forearm (Colles fracture), vertebral body, and hip. All of these areas have a high content of trabecular bone, and because trabecular bone is more active metabolically, it is lost more rapidly. Fractures of the vertebrae with compression cause kyphosis, with the production of a typical "widow's hump" that is common in elderly women with osteoporosis. Fractures of the hip in elderly individuals are associated with a mortality rate of 12–20%, and half of those who survive require prolonged

expensive care.

Figure 23–13

Normal trabecular bone (left) compared with trabecular bone from a patient with osteoporosis (right). The loss of mass in osteoporosis leaves bones more susceptible to breakage.

Osteoporosis has multiple causes, but by far the most common form is **involutional osteoporosis.** All normal humans gain bone early in life, during growth. After a plateau, they begin to lose bone as they grow older (Figure 23–14). When this loss is accelerated or exaggerated, it leads to osteoporosis (see Clinical Box 23–4). Increased intake of calcium, particularly from natural sources such as milk, and moderate exercise may help prevent or slow the progress of osteoporosis, although their effects are not great. Bisphosphonates such as etidronate, which inhibit osteoclastic activity, increase the mineral content of bone when administered in a cyclic fashion and decrease the rate of new vertebral fractures. Fluoride stimulates osteoblasts, making bone more dense, but it has proved to be of little value in the treatment of the disease.

Figure 23–14

Total body calcium, an index of bone mass, at various ages in men and women. Note the rapid increase to young adult levels (phase I) followed by the steady loss of bone with advancing age in both sexes (phase III) and the superimposed rapid loss in women after menopause (phase II).

(Reproduced by permission of Oxford University Press from Riggs BL, Melton LJ III: Involutional osteoporosis. In Evans TG, Williams TF (editors): *Oxford Textbook of Geriatric Medicine.* Oxford University Press, 1992.)

Clinical Box 23–4

Osteoporosis

Adult women have less bone mass than adult men, and after menopause they initially lose it more rapidly than men of comparable age do. Consequently, they are more prone to development of serious osteoporosis. The cause of the bone loss after menopause is primarily estrogen deficiency, and estrogen treatment arrests the progress of the disease. Estrogens inhibit secretion of cytokines such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor (TNF-), and these cytokines foster the development of osteoclasts. Estrogen also stimulates production of transforming growth factor (TGF-), and this cytokine increases apoptosis of osteoclasts. However, it now appears that even small doses of estrogens may increase the incidence of uterine and breast cancer, and in carefully controlled studies, estrogens do not protect against cardiovascular disease. Therefore, the decision to treat a postmenopausal woman with estrogens depends on a careful weighing of the risk–benefit ratio. Bone loss can also occur in both men and women as a result of inactivity. In patients who are immobilized for any reason, and during space flight, bone resorption exceeds bone formation and disuse osteoporosis develops. The plasma calcium level is not markedly elevated, but plasma concentrations of parathyroid hormone and 1,25-dihydroxycholecalciferol fall and large amounts of calcium are lost in the urine.

Chapter Summary

- Circulating levels of calcium and phosphate ions are controlled by cells that sense the levels of these electrolytes in the blood and release hormones, and effects of these hormones are evident in mobilization of the minerals from the bones, intestinal absorption, and/or renal wasting.
- The majority of the calcium in the body is stored in the bones but it is the free, ionized calcium in the cells and extracellular fluids that fulfills physiological roles in cell signaling, nerve function, muscle contraction, and blood coagulation, among others.
- Phosphate is likewise predominantly stored in the bones and regulated by many of the same factors that influence calcium levels.
- The two major hormones regulating calcium and phosphate homeostasis are 1,25-dihydroxycholecalciferol (a derivative of vitamin D) and parathyroid hormone; calcitonin is also capable of regulating levels of these ions, but its full physiologic contribution is unclear.
- 1,25-dihydroxycholecalciferol acts to elevate plasma calcium and phosphate by predominantly transcriptional mechanisms, whereas parathyroid hormone elevates calcium but decreases phosphate by increasing the latter's renal excretion. Calcitonin lowers both calcium and phosphate levels.
- Deficiencies of 1,25-dihydroxycholecalciferol or mutations in its receptor, lead to decreases in circulating calcium, defective calcification of the bones, and bone weakness. Disease states also result from either deficiencies or overproduction of parathyroid hormone, with reciprocal effects on calcium and phosphate.
- Bone is a highly structured mass with outer cortical and inner trabecular layers. The larger cortical layer has a high surface to volume layer with haversian canals that provide nutrients and gaps (lacunae) inhabited by bone cells that are connected by a canaliculi network. The smaller trabecular layer has a much higher surface to volume layer that relies on diffusion for nutrients supply.
- Regulated bone growth through puberty occurs through epiphysial plates. These plates are located near the end of the bone shaft and fuse with the shaft of the bone to cease linear bone growth.
- Bone is constantly remodeled by osteoclasts, which erode and absorb bone, and osteoblasts, which lay down new bone.

Chapter Resources

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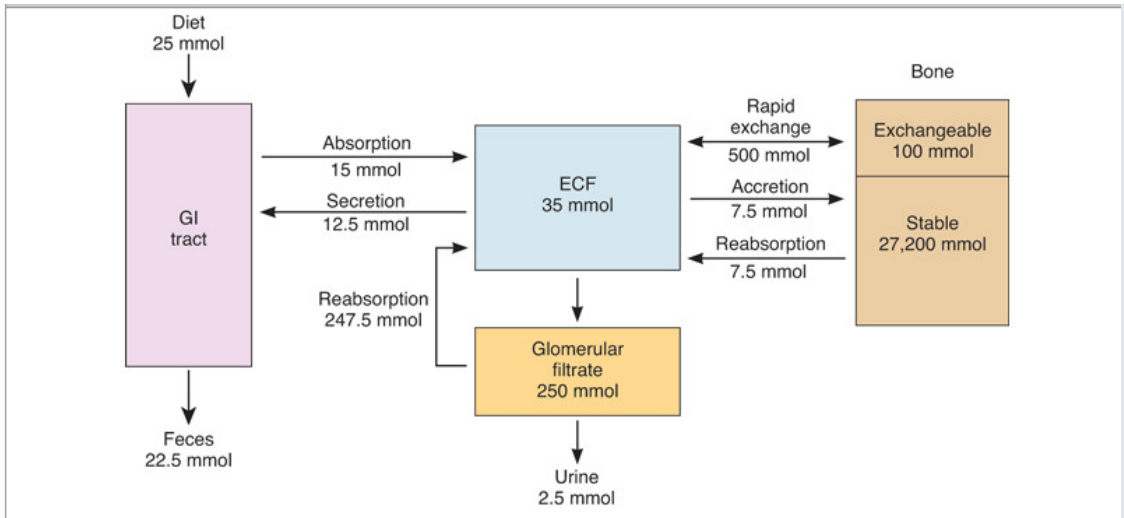
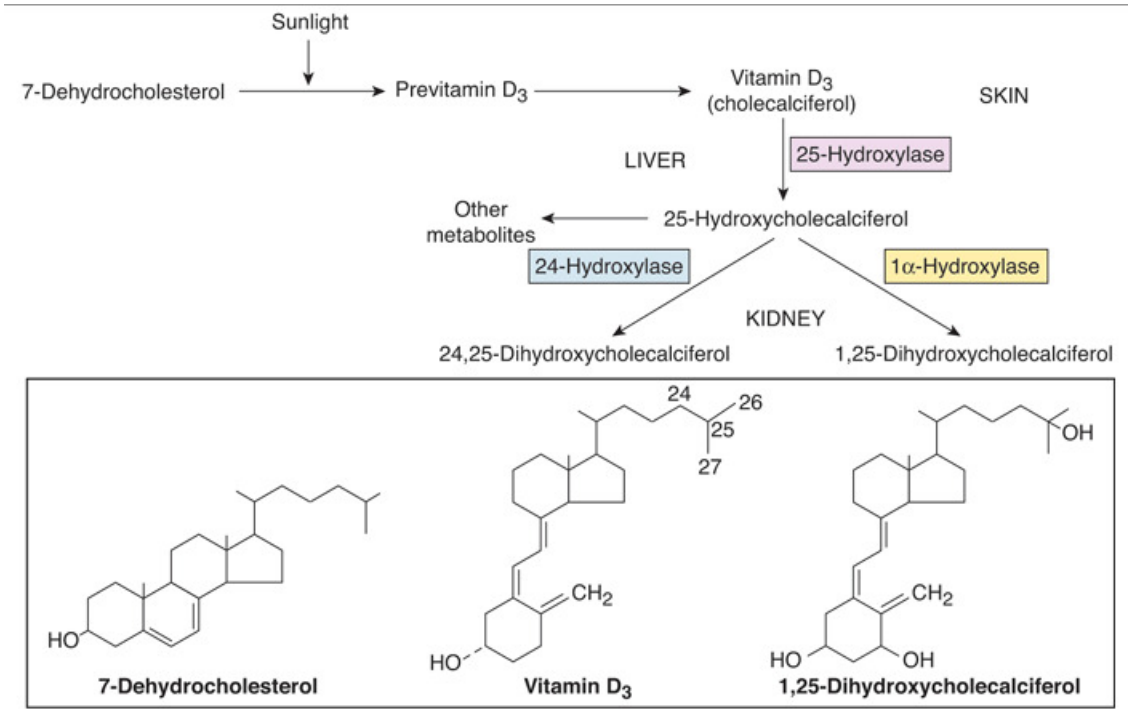
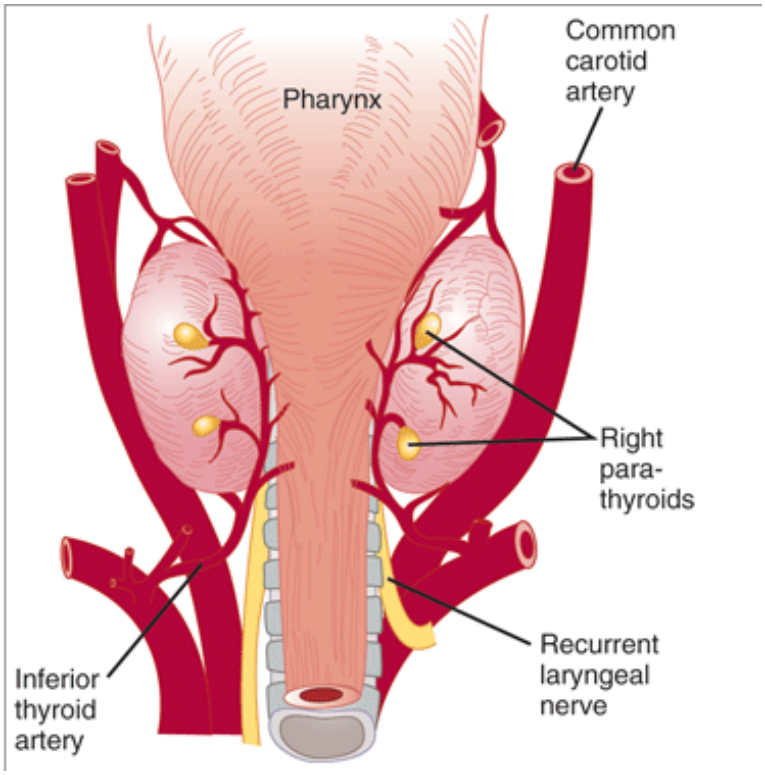
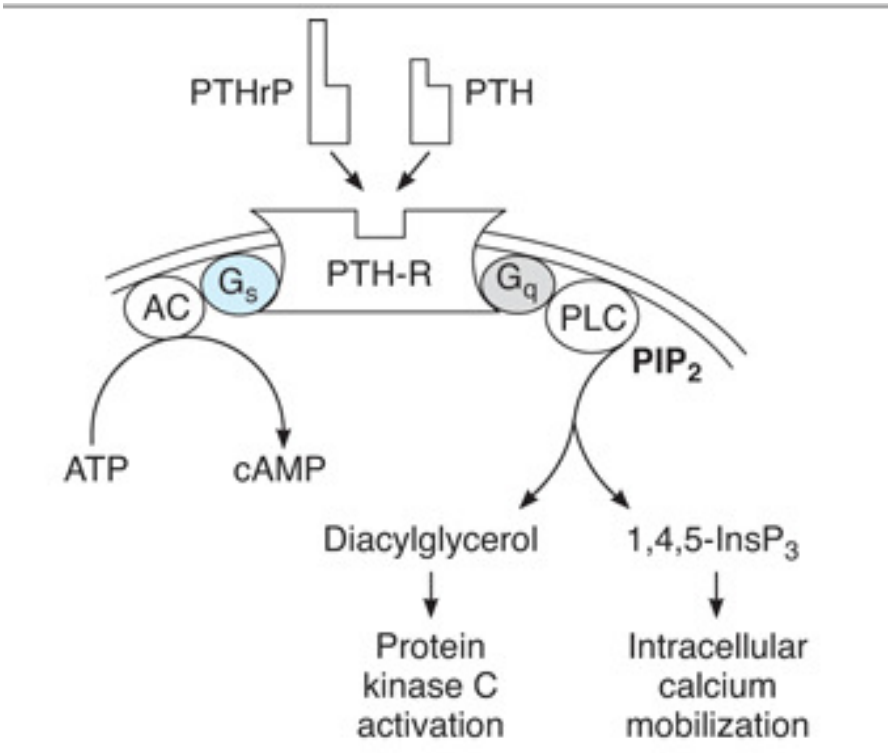





Common carotid artery
Pharynx
Right para-thyroids
Recurrent laryngeal nerve
Inferior thyroid artery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PTH
PTH-R
G_s
G_q
AC
PLC
PIP_2
ATP
cAMP
Diacylglycerol
$1,4,5\text{-}InsP_3$
Protein kinase C activation
Intracellular calcium mobilization