Chapter 21: Hormonal Control of Calcium & Phosphate Metabolism & the Physiology of Bone

OBJECTIVES

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After studying this chapter, you should be able to:

- Understand the importance of maintaining homeostasis of body calcium and phosphate concentrations.
- Describe the body 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—vitamin D, parathyroid hormone, and calcitonin—and other factors that regulate calcium and phosphate homeostasis, their sites of synthesis, targets of their action, and consequences of dysfunction.
- Define the basic anatomy of bone and understand how linear bone growth is arrested after puberty.
- Delineate the cell types that regulate bone formation and resorption and their mechanism of action, and discuss diseases that result from abnormalities in bone homeostasis.

INTRODUCTION

Calcium is an essential intracellular signaling molecule and also plays a variety of extracellular functions, thus the control of body calcium concentrations is vitally important. The components of the system that maintains 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 homeostasis. **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 calcium 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 biologic 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 manner, and thus will also be discussed in this chapter.

CALCIUM & PHOSPHORUS BALANCE IN THE BODY

CALCIUM

The body of a young adult human contains about 1100 g (27.5 moles) 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 21–1**). The distribution

of calcium inside the cells and the role of Ca^{2+} as a second messenger molecule are discussed in Chapter 2.

TABLE 21–1

Distribution (mg/dL) of calcium in normal human plasma.

Total diffusible		**5.36**
Ionized (Ca^{2+})	4.72	
Complexed to HCO_3^-, citrate, etc	0.64	
Total nondiffusible (protein-bound)		**4.64**
Bound to albumin	3.68	
Bound to globulin	0.96	
Total plasma calcium		**10.00**

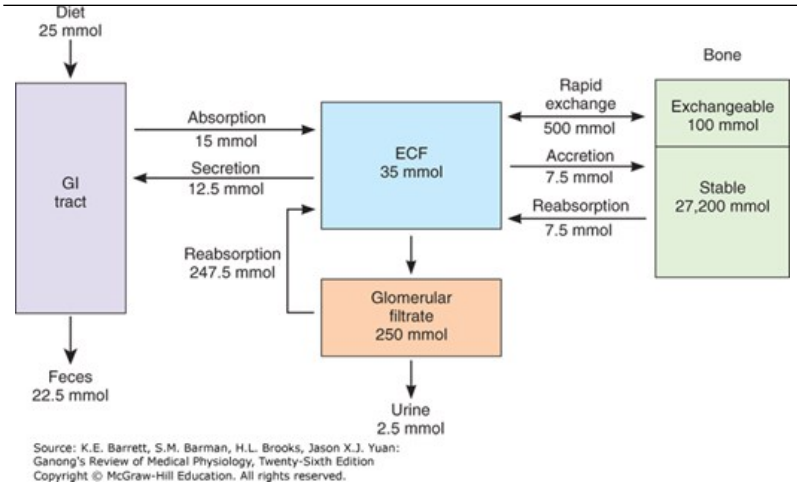
It is the free, ionized calcium (Ca^{2+}) in the body fluids that is a vital second messenger and is necessary for blood coagulation, muscle contraction, and nerve function. A decrease in extracellular Ca^{2+} exerts a net excitatory effect on nerve and muscle cells in vivo (see Chapters 4 and 5). The 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 31), but in vivo, fatal tetany would occur before the clotting reaction would be compromised.

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 can occur 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 anions to bind with and sequester free 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 21–1)**. 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 21–1

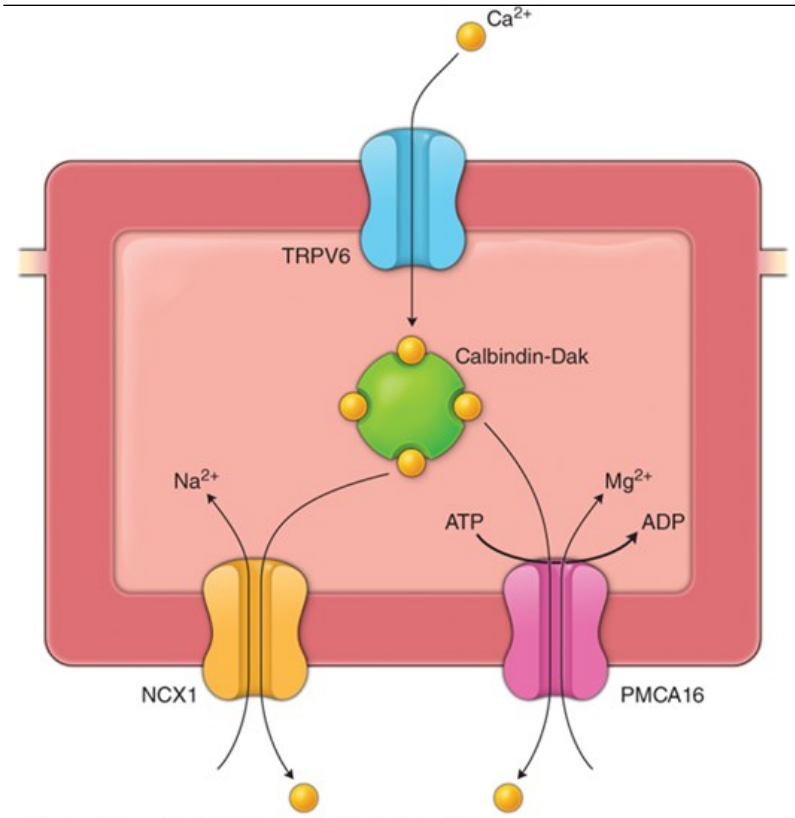
Calcium metabolism in an adult human. A typical daily dietary intake of 25 mmol Ca^{2+} (1000 mg) moves through many body compartments. Note that the majority of body calcium is in bones, in a pool that is only slowly exchangeable with the extracellular fluid (ECF).



Ca^{2+} is taken up into the body 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-D_{9k} 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 Na^+/Ca^{2+} exchanger (NCX1) or the Ca^{2+}-Mg^{2+} ATPase, $PMCA_{1b}$ (**Figure 21-2**). Nevertheless, it should be noted that recent studies indicate that some intestinal Ca^{2+} 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+}.

FIGURE 21–2

Intestinal absorption of calcium. Calcium is taken up across the enterocyte apical membrane via TRPV6 calcium channels. It is sequestered in the cytosol by calbindin-D_{9k} and trafficked to the basolateral membrane, from which it is exported to the bloodstream by either NCX1 (taking advantage of the low intracellular sodium concentration to drive calcium efflux) or $PMCA_{1b}$, with expenditure of cellular energy. The expression levels of TRPV6, calbindin-D_{9k}, and $PMCA_{1b}$ are all upregulated by 1,25-dihydroxycholecalciferol.



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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, whose expression is regulated by PTH.

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 500–800 g (16.1–25.8 moles), 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 remainder as 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 µmol)/kg/d, with an equal amount leaving via resorption.

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 PTH, 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 Na^+ 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 and/or its insertion into the enterocyte apical membrane.

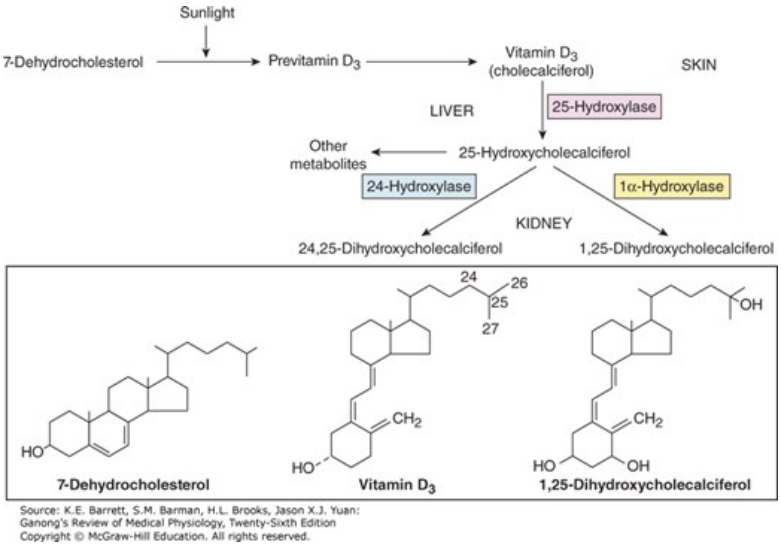
VITAMIN D & THE HYDROXYCHOLECALCIFEROLS

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 21–3**). Vitamin D_3, 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 vitamin D-binding protein (DBP). Vitamin D_3 is also ingested in the diet.

FIGURE 21–3

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



Vitamin D_3 is metabolized by enzymes that are members of the cytochrome P450 (CYP) superfamily (see Chapters 1 and 28). In the liver, vitamin D_3 is converted to **25-hydroxycholecalciferol** (calcidiol, 25-(OH)D_3). The 25-hydroxycholecalciferol is further 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 21–3).

MECHANISM OF ACTION

1,25-Dihydroxycholecalciferol stimulates the expression of a number of gene products involved in Ca^{2+} transport and handling via its receptor (VDR), which acts as a transcriptional regulator in its ligand-bound form. One target 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 9000 and 28,000, respectively. 1,25-Dihydroxycholecalciferol also increases the number of Ca^{2+}–ATPase and TRPV6 molecules in the intestinal cells, and thus, the overall capacity for absorption of dietary calcium.

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 (**Clinical Box 21–1**). The stimulation of osteoblasts brings about a secondary increase in the activity of osteoclasts (see below).

CLINICAL BOX 21–1

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 1α-hydroxylase, or in severe kidney or liver diseases, 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 VDR **(type II vitamin D–resistant rickets),** in which case there is a deficient response to both vitamin D and 1,25-dihydroxycholecalciferol.

THERAPEUTIC HIGHLIGHTS

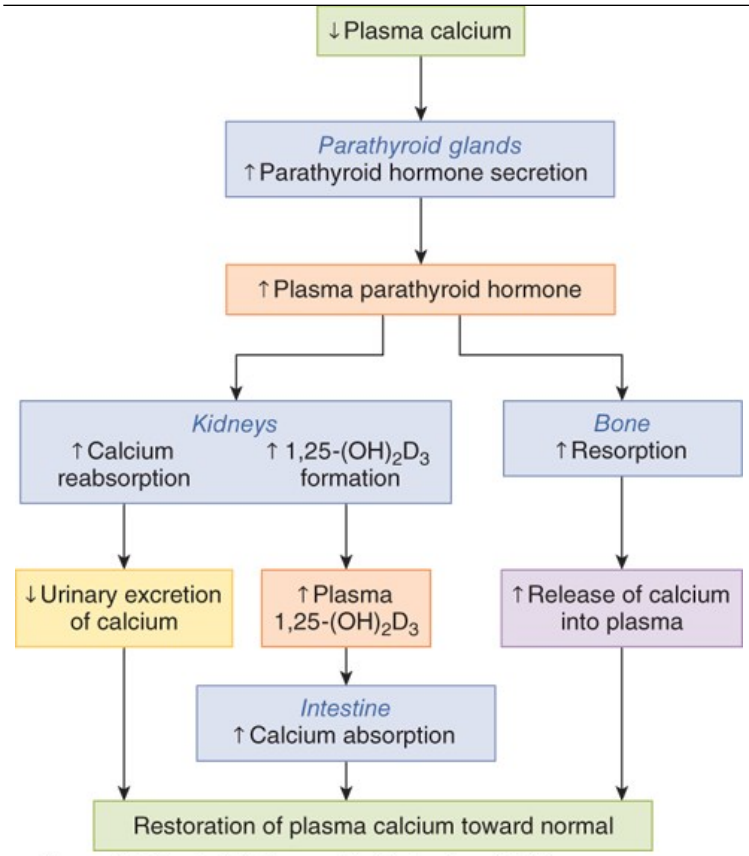
Treatment of these conditions depends on the underlying biochemical basis, as indicated above. Routine supplementation of milk with vitamin D has greatly reduced the occurrence of rickets in Western countries, but the condition remains among the most common childhood diseases in developing countries. Orthopedic surgery may be necessary in severely affected children.

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 1α-hydroxylase, is regulated in a feedback manner by plasma Ca^{2+} and PO_4^{3+} (**Figure 21–4**). 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 thereby reduces the signal for Ca^{2+} absorption from the intestine (see previous text). Conversely, when the plasma Ca^{2+} level is low, PTH secretion is increased, and expression of 1α-hydroxylase is stimulated by PTH. The production of 1,25-dihydroxycholecalciferol is also increased by low plasma PO_4^{3-} levels and inhibited by high plasma PO_4^{3-} levels, by a direct inhibitory effect of PO_4^{3-} on the 1α-hydroxylase. Additional control of 1,25-dihydroxycholecalciferol formation results from its direct negative feedback effect on 1α-hydroxylase activity, a positive feedback action on the formation of 24,25-dihydroxycholecalciferol, and a direct action on the parathyroid gland to inhibit PTH expression.

FIGURE 21–4

Effects of PTH and 1,25-dihydroxycholecalciferol on whole body calcium homeostasis. A reduction in plasma calcium stimulates parathyroid hormone secretion. PTH in turn causes calcium conservation and production of 1,25-dihydroxycholecalciferol in the kidneys, the latter of which increases calcium uptake in the intestine. PTH also releases calcium from the readily exchangeable pool in the bone. All of these actions act to restore normal plasma calcium. (Reproduced with permission from Widmaier EP, Raff H, Strang KT: *Vander's Human Physiology*, 10th ed. New York, NY: McGraw-Hill; 2006.)



An "anti-aging" protein called α-Klotho 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 α-Klotho displayed accelerated aging, decreased bone mineral density, calcifications, and hypercalcemia and hyperphosphatemia. α-Klotho stabilizes 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 1α-hydroxylase, reducing levels of 1,25-dihydroxycholecalciferol.

THE PARATHYROID GLANDS

ANATOMY

Humans usually have four parathyroid glands: two embedded in the superior poles of the posterior thyroid and two in its inferior poles (**Figure 21–5**). Each parathyroid gland is a richly vascularized disk, about 3 × 6 × 2 mm, containing two distinct types of cells (**Figure 21–6**). The abundant **chief cells,** which contain a prominent Golgi apparatus plus endoplasmic reticulum and secretory granules, synthesize and secrete **PTH.** The less abundant and larger **oxyphil cells** contain oxyphil granules and large numbers of mitochondria in their cytoplasm. In humans, few oxyphil cells are seen before puberty, and thereafter they increase in number with age. Their function is unknown. Consequences of loss of the parathyroid glands are discussed in **Clinical Box 21–2**.

FIGURE 21–5

The human parathyroid glands, viewed from behind. The glands are small structures adherent to the posterior surface of the thyroid gland.

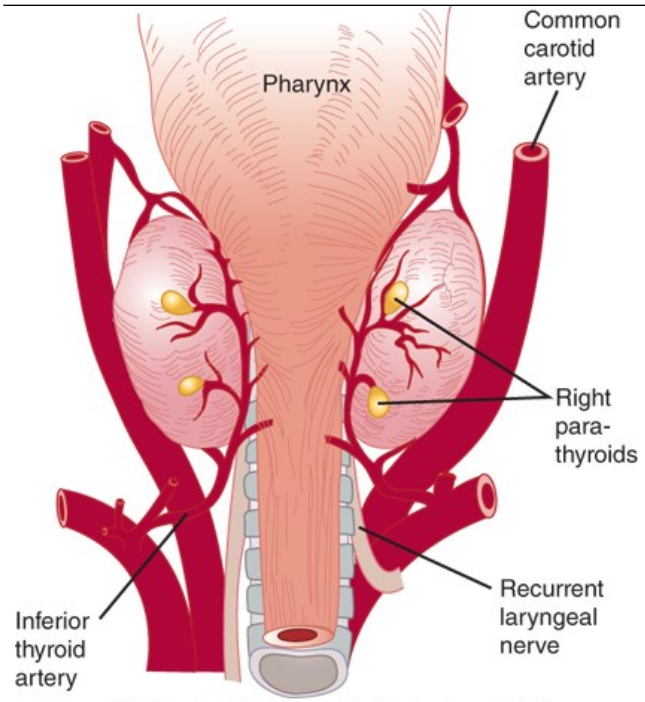
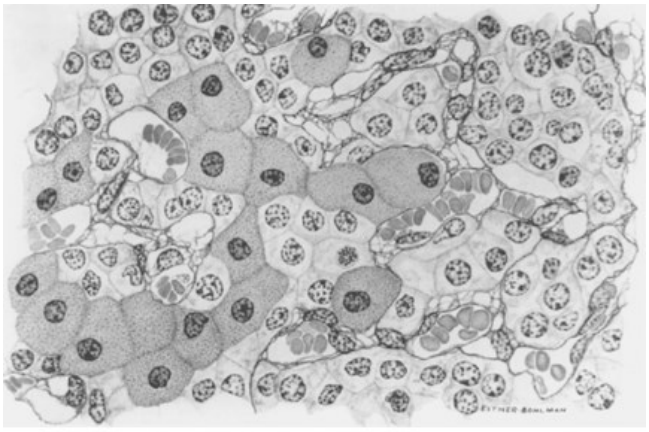


FIGURE 21–6

Section of human parathyroid. (Reduced 50% from ×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 21–2

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 text). Plasma phosphate levels usually rise as the plasma Ca^{2+} level falls. Symptoms usually develop 2–3 days postoperatively but may not appear for several weeks or more. The signs of tetany in humans include **Chvostek sign,** a quick contraction of the ipsilateral facial muscles elicited by tapping over the facial nerve at the angle of the jaw, and **Trousseau 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.

THERAPEUTIC HIGHLIGHTS

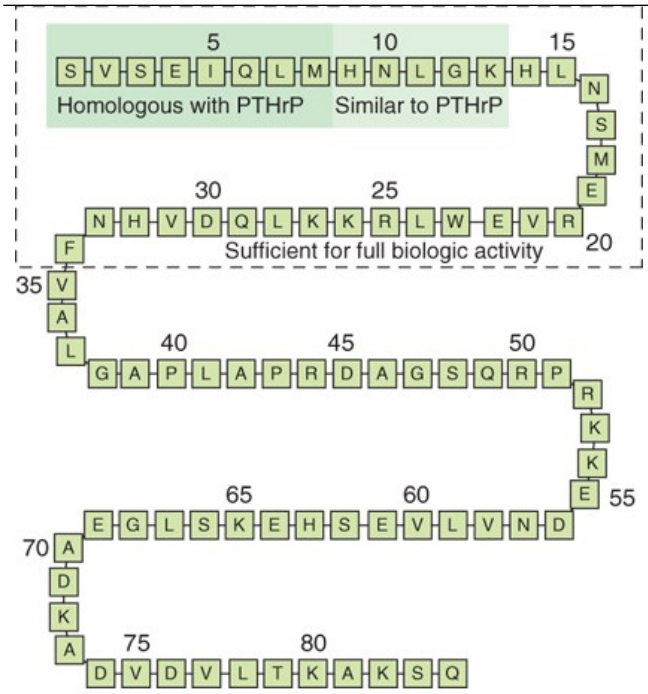
Treatment centers around replacing the PTH that would normally be produced by the missing glands. Injections of PTH can be given to correct the chemical abnormalities, and the symptoms then disappear. Injections of Ca^{2+} salts can also give temporary relief.

SYNTHESIS & METABOLISM OF PTH

Human PTH is a linear polypeptide with a molecular weight of 9500 that contains 84 amino acid residues **(Figure 21–7)**. 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 21–7

Parathyroid hormone. The solid boxes show residues that are identical to, or similar to, those at the N-terminus of parathyroid hormone–related peptide (PTHrP). The dashed box indicates that the first 34 amino acids of PTH are sufficient for full biologic activity. Interestingly, this is also the case for PTHrP, although after the first 13 amino acids, its sequence is divergent from that of PTH.



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The normal plasma level of intact PTH is 10–55 pg/mL. The half-life of PTH is approximately 10 min, and the secreted polypeptide is rapidly cleaved by Kupffer cells in the liver into inactive fragments. PTH and these fragments are then cleared by the kidneys. Currently used immunoassays for PTH are designed only to measure mature PTH (ie, 84 amino acids) and not its fragments in order 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 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 21–3**). 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 21–3

Diseases of Parathyroid Excess

Hyperparathyroidism due to hypersecretion from 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 kidney disease and rickets, in which the plasma Ca^{2+} level is chronically low, the associated stimulation of the parathyroid glands causes compensatory parathyroid hypertrophy and secondary hyperparathyroidism. The plasma Ca^{2+} level is low in chronic kidney disease primarily because the diseased kidneys lose the ability to form 1,25-dihydroxycholecalciferol. Finally, mutations in the Ca^{2+}-sensing receptor gene *CASR* 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 in the *CASR* gene develop familial hypercalciuric hypocalcemia due to increased sensitivity of the parathyroid glands to plasma Ca^{2+}.

THERAPEUTIC HIGHLIGHTS

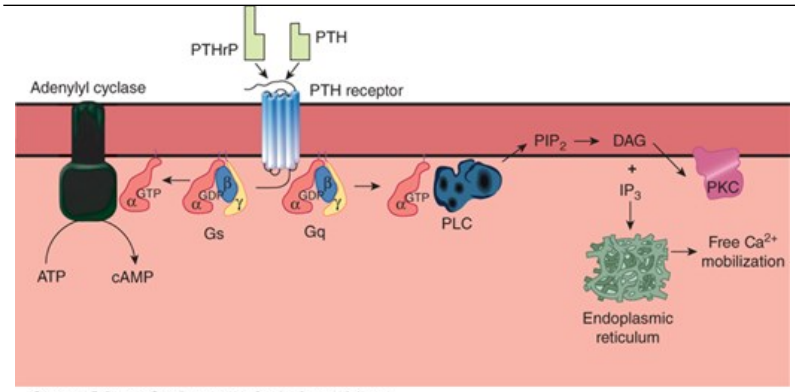
Subtotal parathyroidectomy is sometimes necessary in patients in whom parathyroid adenoma or hyperplasia with associated hypercalcemia and resulting symptoms develops. However, because parathyroid disease is often benign or only slowly progressing, surgery remains controversial in most patients and is typically reserved for those who have experienced life-threatening complications of hypercalcemia.

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+} concentrations and activating protein kinase C **(Figure 21–8)**. However, the way these second messengers affect Ca^{2+} in bone is unsettled.

FIGURE 21–8

Signal transduction pathways activated by PTH or PTHrP binding to the hPTH/PTHrP receptor. Intracellular cAMP is increased via G_s and adenylyl cyclase (AC). Diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3) are increased via G_q and phospholipase C (PLC). DAG and IP_3 activate protein kinase C (PKC) and mobilization of calcium from the endoplasmic reticulum.



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In the disease called **pseudohypoparathyroidism,** the signs and symptoms of hypoparathyroidism develop but the circulating level of PTH is normal or even elevated. Because 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 Ca^{2+} acts directly on the parathyroid glands in a negative feedback manner to regulate the secretion of PTH. The key to this regulation is a cell membrane Ca^{2+} sensing receptor, CaSR. 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 Ca^{2+} is deposited in the bones. When it is low, secretion is increased and Ca^{2+} is mobilized from the bones.

1,25-Dihydroxycholecalciferol acts directly on the parathyroid glands to decrease preproPTH mRNA. Increased plasma P_i 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 Boxes 21–2 and 21–3).

PTHrP

PTHrP, another protein with PTH activity, is produced by many different tissues in the body. 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 additional, 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 hypercalcemia secondary to bone erosion **(local osteolytic hypercalcemia).** Evidence suggests that this erosion is produced by prostaglandins such as prostaglandin E_2 arising from the tumor. Hypercalcemia in the remaining 80% of the patients is due to elevated circulating levels of PTHrP **(humoral hypercalcemia of malignancy).** The tumors responsible for this 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 Ca^{2+}, 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 peptide hormone was secreted by structures in the neck. This 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.

SECRETION & METABOLISM

Calcitonin secretion is increased when the thyroid gland is exposed to a plasma calcium level of approximately 9.5 mg/dL. Above this level, plasma calcitonin is directly proportional to plasma calcium. β-Adrenergic agonists, dopamine, and estrogens also stimulate calcitonin secretion. Gastrin, cholecystokinin (CCK), glucagon, and secretin have also been reported to stimulate calcitonin secretion, with gastrin being the most potent stimulus (see Chapter 25). Thus, the plasma calcitonin level is elevated in Zollinger–Ellison syndrome and in pernicious anemia (see Chapter 25). However, the dose of gastrin needed to stimulate calcitonin secretion is supraphysiologic 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 also 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, after thyroidectomy, there are only transient abnormalities of Ca^{2+} homeostasis when a Ca^{2+} load is injected. 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 OF CALCIUM HOMEOSTATIC MECHANISMS

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 acutely, 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 Ca^{2+} 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

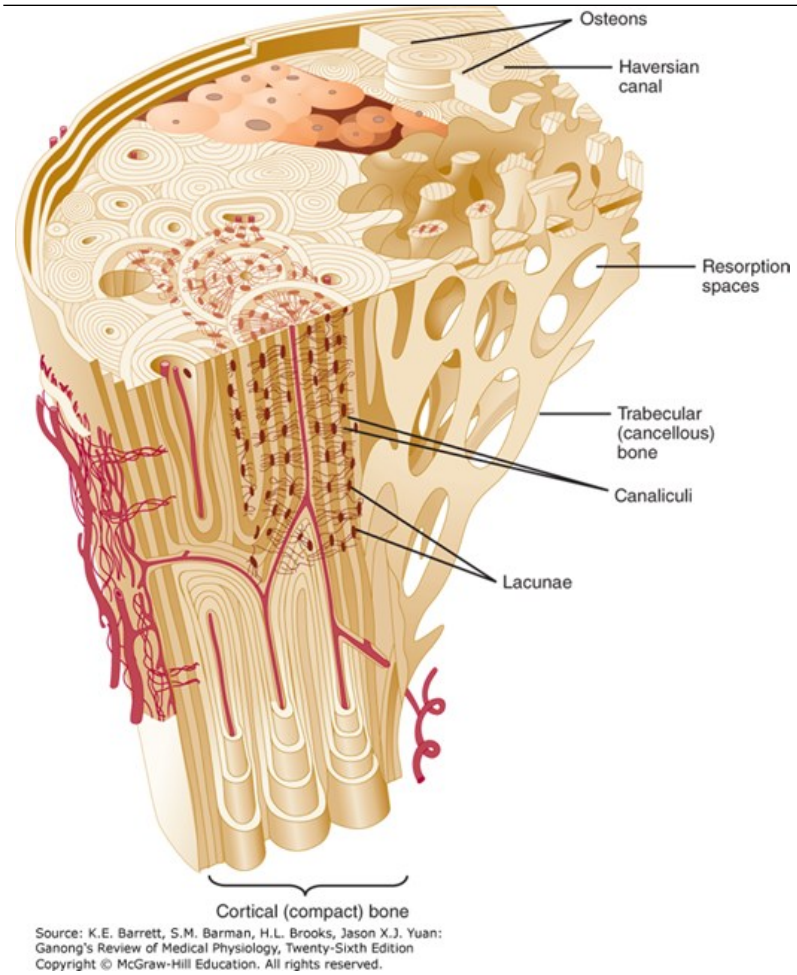
Bone is a special form of connective tissue with a collagen framework impregnated with Ca^{2+} and PO_4^{3-} salts, particularly **hydroxyapatites,** which have the general formula $Ca_{10}(PO_4)_6(OH)_2$. Bone is also involved in overall Ca^{2+} and PO_4^{3-} homeostasis. It protects vital organs, and 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 200–400 mL/min in adult humans.

STRUCTURE

There are two types of bone: **compact** or **cortical bone,** which makes up the outer layer of most bones **(Figure 21–9)** and accounts for 80% of the bone in the body; and **trabecular** or **spongy bone** 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 21–9). 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 21–9), which contain blood vessels. Around each haversian canal, collagen is arranged in concentric layers, forming cylinders called **osteons** or **haversian systems.**

FIGURE 21–9

Structure of compact and trabecular bone. The compact bone is shown in horizontal section (top) and vertical section (bottom). (Reproduced with permission from Williams PL et al (editors): *Gray's Anatomy,* 37th ed. Churchill Livingstone; 1989.)



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 α_1 polypeptides encoded by one gene, and one is an α_2 polypeptide encoded by a different gene. Collagens make up a family of structurally related proteins that maintain the integrity of many different organs.

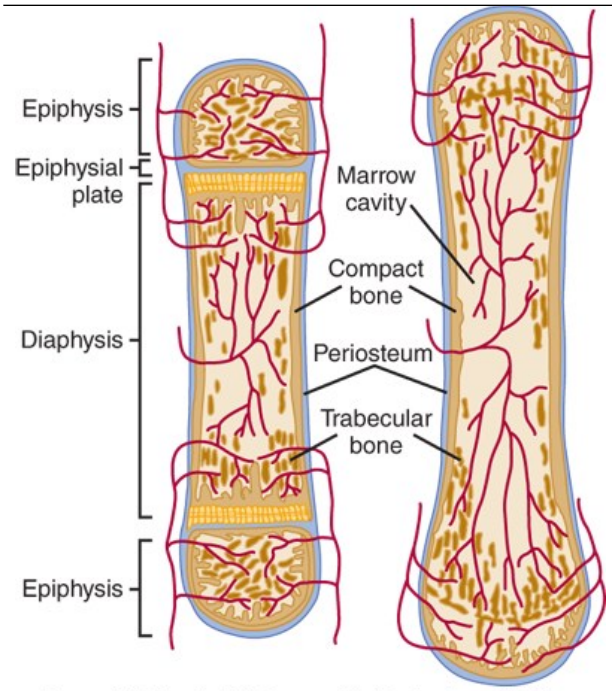
BONE GROWTH

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 21–10)**. 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 proportional to the rate of growth. The width is affected by a number of hormones, but most markedly by growth hormone and IGF-I (see Chapter 18).

FIGURE 21–10

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).



Source: K.E. Barrett, S.M. Barman, H.L. Brooks, Jason X.J. Yuan: Ganong's Review of Medical Physiology, Twenty-Sixth Edition

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 radiographing the skeleton and noting which epiphyses are open and which are closed.

The **periosteum** is a dense fibrous, vascular, and innervated membrane that covers the surface of bones. This layer consists of an outer layer of collagenous tissue and an inner layer of fine elastic fibers containing cells that contribute to bone growth. The periosteum covers all surfaces of the bone except for those capped with cartilage (eg, at the joints) and serves as a site of attachment of ligaments and tendons. As one ages, the periosteum becomes thinner and loses some of its vasculature. This renders bones more susceptible to injury and disease.

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 initial development from the mesenchyme is the same as that of fibroblasts. Later, ossification-specific transcription factors, such as runt-related transcription factor 2 (Runx2; also known as core binding factor subunit alpha-1), contribute to their differentiation. The importance of this transcription factor in bone development is underscored in knockout mice deficient for the *RUNX2* gene. These mice develop to term with their skeletons made exclusively of cartilage; no ossification occurs. Normal osteoblasts, on the other hand, are able to lay down type 1 collagen and form new bone.

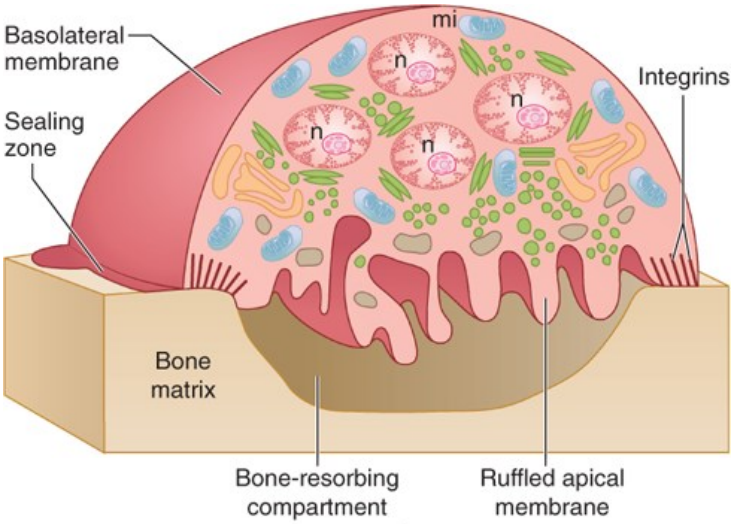
Osteoclasts are members of the monocyte family. Stromal cells in the bone marrow, osteoblasts, and T lymphocytes all express receptor activator for nuclear factor kappa beta ligand (RANKL) on their surface. When these cells come in contact with appropriate monocytes expressing RANK (ie, the RANKL receptor) two distinct signaling pathways are initiated: (1) There is a RANKL/RANK interaction between the cell pairs and (2) mononuclear phagocyte colony stimulating factor (M-CSF) is secreted by the nonmonocytic cells and it binds to its corresponding receptor on the monocytes (colony stimulating factor 1 receptor, CSF1R). The combination of these two signaling events leads to differentiation of the monocytes into osteoclasts. The precursor cells also secrete **osteoprotegerin (OPG),** which limits 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 (bone-resorbing compartment) 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. Thus, 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 21–11)**. The products of bone 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 21–11

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). (Used with permission 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. The shape of bones may also change as bone is resorbed in one location but 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 cellular level, there is some regulation of osteoclast formation by osteoblasts via the RANKL–RANK and the M-CSF–CSF1R 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. Not surprisingly, hormonal control of bone metabolism can be quite complex, and this can be illustrated by examining effects of the weight-associated hormone leptin on bone metabolism. When administered intracerebroventricularly, leptin decreases bone formation. This is thought to occur via release of various substances from the hypothalamus that can reduce osteoblast function. However, circulating leptin can increase bone mass through osteoblast and pre-osteoblast cell signaling pathways. More generally, PTH accelerates bone resorption, and estrogens slow bone resorption by inhibiting the production of bone-eroding cytokines.

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.

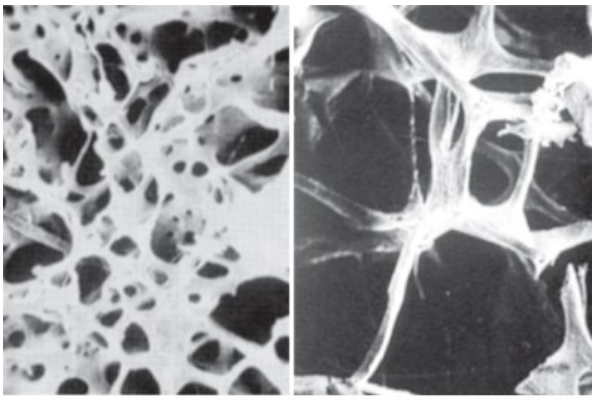
In **osteopetrosis,** a rare and often severe disease, the osteoclasts are defective and are unable to resorb bone in their usual manner so the osteoblasts operate unopposed. The result is a steady increase in bone density, neurologic defects due to narrowing and distortion of foramina

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; 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 21–12)** 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 21–12

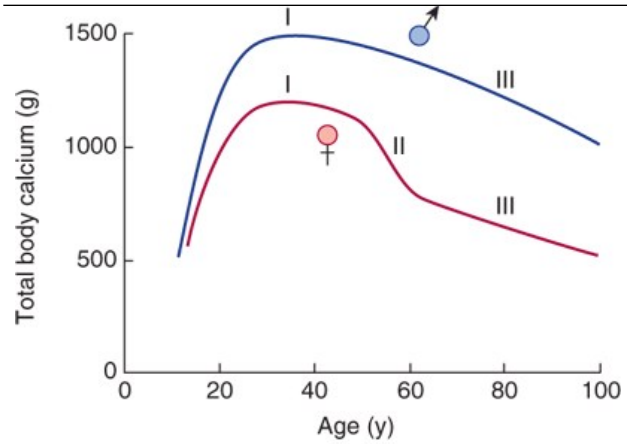
Normal trabecular bone (left) compared with trabecular bone from a patient with osteoporosis (right). The loss of mass in osteoporosis leaves bones more susceptible to breakage.



Osteoporosis has multiple causes, but by far the most common form is **involutional osteoporosis.** Humans normally gain bone during growth early in life. After a plateau, they begin to lose bone as they grow older **(Figure 21–13)**. When this loss is accelerated or exaggerated, it leads to osteoporosis **(Clinical Box 21–4)**. 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 very large. Bisphosphonates such as etidronate, which inhibit osteoclastic activity, increase the mineral content of bone and decrease the rate of new vertebral fractures when administered in a cyclical manner. Fluoride stimulates osteoblasts, making bone more dense, but it has proven to be of little value in the treatment of the disease.

FIGURE 21–13

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 with permission from Evans TG, Williams TF (editors): *Oxford Textbook of Geriatric Medicine.* Oxford University Press; 1992.)



Source: K.E. Barrett, S.M. Barman, H.L. Brooks, Jason X.J. Yuan: Ganong's Review of Medical Physiology, Twenty-Sixth Edition

CLINICAL BOX 21–4

Osteoporosis

Adult women have less bone mass than adult men, and after menopause they initially lose it more rapidly than men of comparable age. Consequently, they are more prone to development of serious osteoporosis. The cause of 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-α), cytokines that otherwise foster the development of osteoclasts. Estrogen also stimulates production of transforming growth factor (TGF-β), and this cytokine increases apoptosis of osteoclasts.

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 PTH and 1,25-dihydroxycholecalciferol fall and large amounts of calcium are lost in the urine.

THERAPEUTIC HIGHLIGHTS

Hormone therapy has traditionally been used to offset osteoporosis. **Estrogen replacement therapy** begun shortly after menopause can help maintain bone density. However, it 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, treatment of a postmenopausal woman with estrogens is no longer used as a primary option. **Raloxifene** is a selective estrogen receptor modulator that can mimic the beneficial effects of estrogen on bone density in postmenopausal women without some of the risks associated with estrogen. However, this too carries risk of side effects (eg, blood clots). Other hormone treatments include the use of **calcitonin** and the PTH analogue **teriparatide.** An alternative to hormone treatments is the **bisphosphonates.** These drugs can inhibit bone breakdown, preserve bone mass, and even increase bone density in the spine and hip to reduce the risk of fractures. Unfortunately, these drugs also can cause mild to serious side effects and require monitoring for patient suitability. In addition to hormones and medications listed above, **physical therapy** to increase appropriate mechanical load and improve balance and muscle strength can significantly improve quality of life.

CHAPTER SUMMARY

- Calcium and inorganic phosphate ions fulfill physiologic roles in cell signaling, nerve function, muscle contraction, and blood coagulation, among others. 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 is physiologically relevant. Phosphate is likewise predominantly stored in the bones.

- Calcium and phosphate are absorbed from the diet in a regulated fashion, enter the extracellular fluid, and move into and out of the bones. Circulating calcium and phosphate are filtered by the kidneys, and typically largely reabsorbed unless renal wasting is called for when circulating levels of the minerals are elevated.
- Circulating levels of calcium and phosphate ions are controlled by cells that sense the levels of these electrolytes in the blood and are thereby stimulated to release hormones, and these hormones mobilize the minerals from the bones, increase intestinal absorption, and/or modulate renal wasting.
- The major hormones regulating calcium and phosphate homeostasis are 1,25-dihydroxycholecalciferol (a derivative of vitamin D that is produced cooperatively by the skin, liver and kidneys) and parathyroid hormone, secreted by the parathyroid glands. 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, also 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. 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. An imbalance between the activities of these two cell types can lead to a steady increase in bone density when osteoblasts function unopposed (osteopetrosis) or a loss of bone mass when there is a relative excess of osteoclast activity (osteoporosis).