

# Calcium and Phosphorus Homeostasis II: Target Tissues and Integrated Control **9.8**

## Learning Objectives

- Identify the parts of bone including endosteum, periosteum, diaphysis, epiphysis, and epiphyseal plate
- Identify the three major cell types in bone: osteoblast, osteocyte, and osteoclast
- Describe the organic matrix of bone and its mineralization
- Describe the location and function of osteoblasts
- Describe the recruitment of osteoclasts from hematopoietic stem cells
- Describe the mechanism of osteoclastic resorption of bone
- Describe remodeling of bone
- List the sequential steps in Ca and Pi absorption from the intestine
- Describe how vitamin D increases Ca and Pi absorption from the intestine
- Describe how the intestine adapts to diets containing differing amounts of Ca and Pi
- Describe the actions of PTH, vitamin D, and FGF23 on Ca and Pi reabsorption by the kidney tubule
- Compare the overall effect of PTH on Ca and Pi homeostasis to that of vitamin D and FGF23
- Trace the negative feedback loops involved in the homeostatic response to low plasma Ca

## THE SKELETON GIVES US FORM AND SUPPORT

The skeleton is one of the largest organ system of the body, consisting of 206 bones that vary greatly in size and shape. The larger bones of the limbs are called **long bones**. Smaller bones of the wrist and ankle are called **short bones**. The bones of the skull are classified as **flat bones** and the remainder, such as the vertebrae, are **irregular bones**. All bones consist of a compact **cortex** of bone that surrounds a meshwork of **trabeculae**. Cortical bone is dense or compact bone that comprises about 80% of the mass of the skeleton. The gross appearance of trabecular bone resembles a sponge, and so it is also called **cancellous bone** or **spongy bone**. The interstices between the trabeculae of bone are filled with the red marrow that makes red blood cells. Bones consist mostly of mineralized organic matrix and a small but active cellular component. **Figure 9.8.1** shows the general structure of bone.

The articular surfaces of bones are covered with articular cartilage. Elsewhere the surface is covered by a connective tissue membrane, the **periosteum**. The outer fibrous layers of the periosteum contain periosteal blood vessels. The inner layers of the periosteum contain mesenchymal stem cells that can proliferate and differentiate into **osteoblasts**. Osteoblasts lay down the organic matrix of bone and aid in its mineralization. Therefore, these osteoblasts make bone, either in response to injury or due to remodeling of existing bone. The **endosteum** covers the surfaces of the bone that face the marrow. The endosteum is thinner than the periosteum, but it also contains cells that can make bone (see **Figure 9.8.1**).

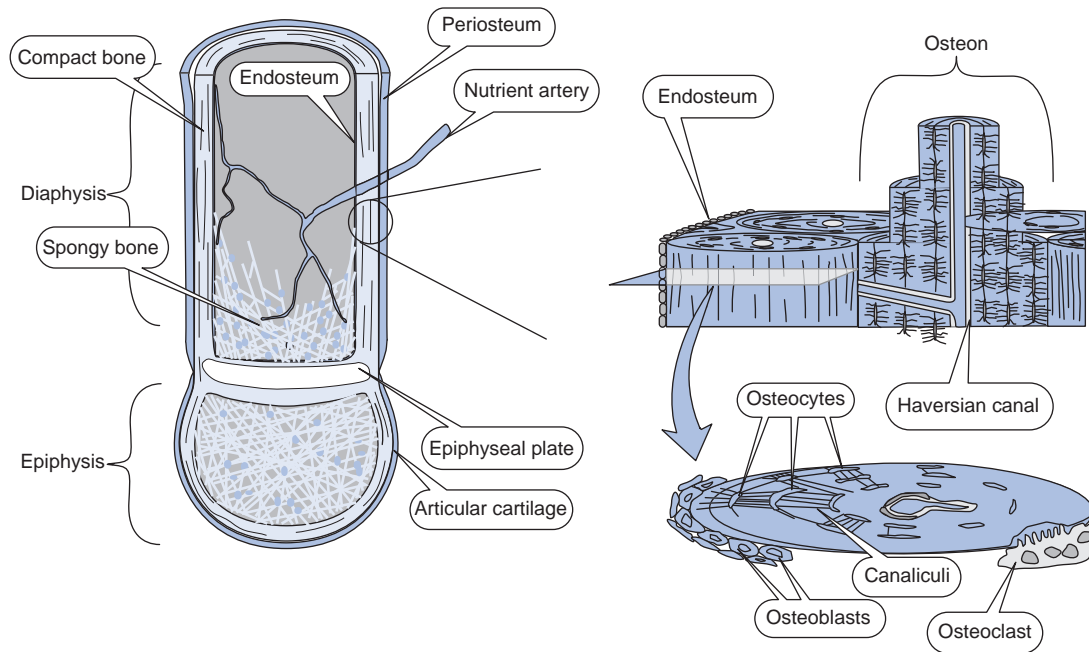
The shaft of the long bones is called the **diaphysis**, whereas the end of the bone is the **epiphysis**. Mineralization of the diaphysis and epiphysis begins independently. The region between contains cartilage, the **epiphyseal plate** responsible for the growth of the long bones. When the epiphyseal plate is mineralized, no more growth occurs. This is referred to as **closure** of the epiphyseal plate.

## OSTEOBLASTS ARE SURFACE CELLS THAT LAY DOWN THE ORGANIC MATRIX OF BONE

**Osteoblasts** form a continuous layer over most of the bone. These cells synthesize and secrete a variety of proteins which together make up the **organic matrix** of bone, called the **osteoid**, which consists primarily of **type I collagen**. Noncollagen proteins make up about 10% of the organic matrix and play important roles in the regulation of mineralization. The collagen fibers line up in regular arrays and form nucleation sites along which  $\text{Ca}^{2+}$  and Pi salts can crystallize. Osteoblasts are recruited from mesenchymal or fibroblast-type cells that form osteoprogenitor cells and then mature osteoblasts. The normal mineralization of the osteoid depends critically on the concentration of  $\text{Ca}^{2+}$  and Pi in the plasma.

## OSTEOCYTES ARE EMBEDDED DEEP WITHIN BONE

Osteoblasts that become completely surrounded by the organic matrix become embedded in the bone and become **osteocytes**. They reside in little pockets called



**FIGURE 9.8.1** Gross and microscopic components of bone. Bone surfaces are completely covered with cells. On the outside, the periosteum is thick and consists of an outer fibrous layer and an inner layer of cells that can proliferate to form osteoblasts. The inner endosteum is thinner. The shaft of mature bones consists of compact bone organized into osteons. An Haversian canal is central to each osteon, which consists of several layers or lamellae of osteocytes, bone cells that are deeply embedded in the bone. These communicate through thin cytoplasmic processes that course through tiny channels in the bone, the canaliculi. Osteoclasts are large, multinucleated cells that resorb bone.

lacunae that communicate through tiny **canaliculi**, which connect with other osteocytes and with surface osteoblasts. These canaliculi provide an enormous surface area over which  $\text{Ca}^{2+}$  and  $\text{Pi}$  can exchange between plasma and bone.

## OSTEOCLASTS DESTROY THE ORGANIC MATRIX OF BONE AND RELEASE BOTH $\text{Ca}^{2+}$ AND $\text{Pi}$

**Osteoclasts** are giant, multinucleated cells that are recruited from a pool of circulating stem cells that also give rise to tissue macrophages. These circulating cells fuse together and attach to the surface of bone. The osteoclast forms a “ruffled border” adjacent to the bone where enzymes and  $\text{H}^+$  ions are secreted onto the bone to dissolve the mineral and digest the organic matrix. Thus osteoclastic activity forms a resorption cavity and completely destroys the bone that is resorbed.

## BONE IS CONSTANTLY BEING REMODELED

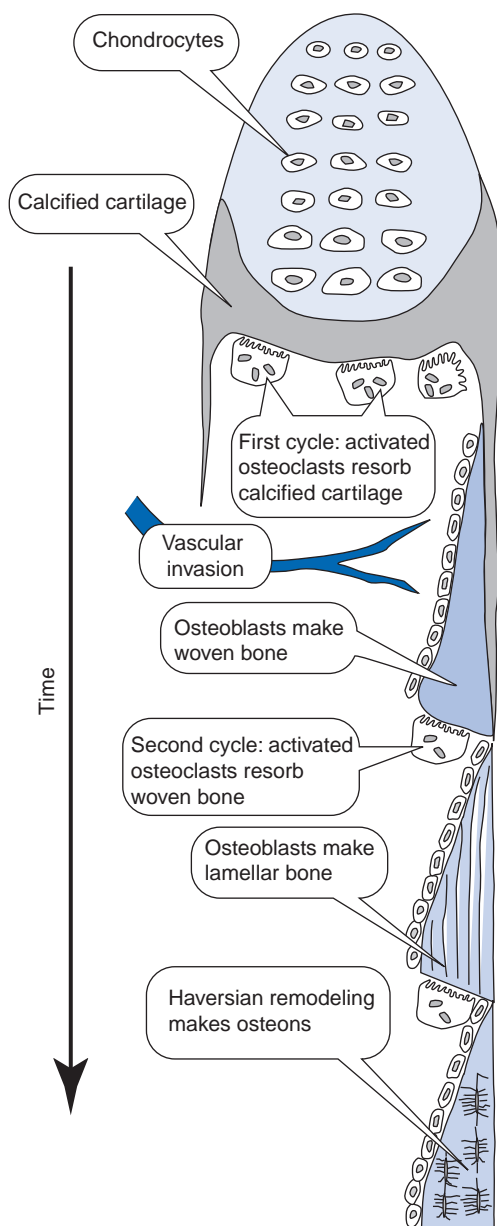
Osteoclasts form a resorption cavity and migrate along, leaving a resorption canal in their wake. Osteoblasts invade the resorbed area and begin laying down new bone, eventually becoming entrapped in the bone as new osteocytes. This process of bone remodeling occurs continuously throughout life. During maturation of bone, the bone forms in a sequence of **woven bone** and **lamellar bone** (see Figure 9.8.2). The balance of bone is

set by the rates of resorption versus rebuilding. If the osteoblasts cannot keep up with the osteoclasts, there will be a net loss of bone and eventually a loss of bone strength.

## OSTEOBLASTS MAKE OSTEOID AND SIGNAL BONE RESORPTION

As described earlier and shown in Figure 9.8.2, osteoblasts originate from mesenchymal stem cells which form **osteoprogenitor cells** or **preosteoblasts**. Osteoblasts vary in size and function; they form woven bone in early development and lamellar bone later on. The osteoblasts secrete proteins that form the organic matrix of bone. Table 9.8.1 summarizes some of these proteins and their postulated function. **Osteocalcin** is unusual, in that this 6-kDa protein has three  $\gamma$  carboxy-glutamic acid residues. These amino acids are produced by posttranslational modification involving vitamin K-dependent enzymes (see Chapters 2.3 and 5.1). These residues bind  $\text{Ca}^{2+}$  avidly.  $1,25(\text{OH})_2\text{D}_3$  stimulates the synthesis of this protein, and it is postulated to have some role in mineralization. Osteocalcin also feeds back on osteoblasts, inhibiting further bone formation and osteoblast function.

In addition, cells of the osteoblast lineage are important to the initiation of bone resorption. Most of the hormonal factors that stimulate bone resorption act directly on **osteoblasts** or their precursors. Parathyroid hormone (PTH) and  $1,25(\text{OH})_2\text{D}_3$  stimulate these cells to release **macrophage colony stimulating factor** (M-CSF)



**FIGURE 9.8.2** Maturation of endochondral bone. Chondrocytes lay down the cartilage that eventually forms bone. The calcified cartilage is resorbed by osteoclasts and blood vessels invade the structure. Bone resorption then reverses to form bone by osteoblasts. This first bone is woven bone. A second cycle of bone resorption and formation makes lamellar bone. Cortical bone in the adult undergoes further osteoclastic resorption to form resorption canals, which, when filled in by the osteoblasts, form the osteons.

and express **receptor activator of nuclear factor  $\kappa$ B ligand (RANKL)** on the surface of the osteoblasts.

M-CSF is a factor in the hematopoietic generation of macrophages (see Figure 5.3.3). Osteoblasts make a membrane-bound and soluble form of M-CSF in response to stimulators of bone resorption (1,25(OH) $_2$ D $_3$ , PTH, IL-1, IL-6, TNF, prostaglandins). Hematopoietic cells stimulated by GM-CSF (granulocyte-macrophage colony stimulating factor) and M-CSF differentiate into either monocyte macrophages or

preosteoclasts. The preosteoclasts fuse to form multinucleated osteoclasts.

Osteoblasts also make a membrane-bound RANKL in response to stimulators of bone resorption. Its action is opposed by another osteoblast product, **osteoprotegerin (OPG)**. OPG is a soluble receptor for RANKL that prevents RANKL from interacting with its receptor, RANK, on osteoclast precursors and osteoclasts. OPG thus opposes bone resorption, and the balance between RANKL and OPG sets the activity of the osteoclasts. For many stimulators of bone resorption (PTH, 1,25(OH) $_2$ D $_3$ , PGE $_2$ ), inhibition of OPG secretion accompanies stimulation of RANKL production, so there is reciprocal action on RANKL and OPG that activates osteoclast genesis and bone resorption (see Figure 9.8.3).

## OSTEOCLASTS RESORB BONE

Osteoclasts are giant cells containing between 10 and 20 nuclei. They closely attach to the bone matrix by binding its surface integrins to a bone protein called vitronectin. This close apposition seals off an area of the bone beneath the osteoclast and allows the osteoclast to form a microenvironment that resorbs bone. The area of the osteoclast next to bone forms a “ruffled border” consisting of multiple infoldings of the osteoclast cell membrane. It secretes acid and proteases across the ruffled border, and these dissolve the mineral of bone and destroy the organic matrix (see Figure 9.8.4).

## CALCITONIN SHUTS OFF OSTEOCLAST RESORPTION

According to the previous sections, both PTH and 1,25(OH) $_2$ D $_3$  promote bone resorption, but they do this without directly affecting the osteoclast or its precursors. Indeed, osteoclasts have no receptors for PTH. Instead, PTH and 1,25(OH) $_2$ D $_3$  increase bone resorption by acting on osteoblasts to recruit stem cells to form osteoclasts. Osteoclasts do have receptors for calcitonin (CT). CT binds to a G $_s$  protein on the surface of the osteoclasts. This increases cAMP in these cells and shuts off bone resorption. This effect may be of little importance in adults as there are no diseases associated with CT undersecretion or oversecretion.

## SUMMARY OF HORMONE EFFECTS ON BONE

### PTH INCREASES OSTEOCYTIC OSTEOLYSIS

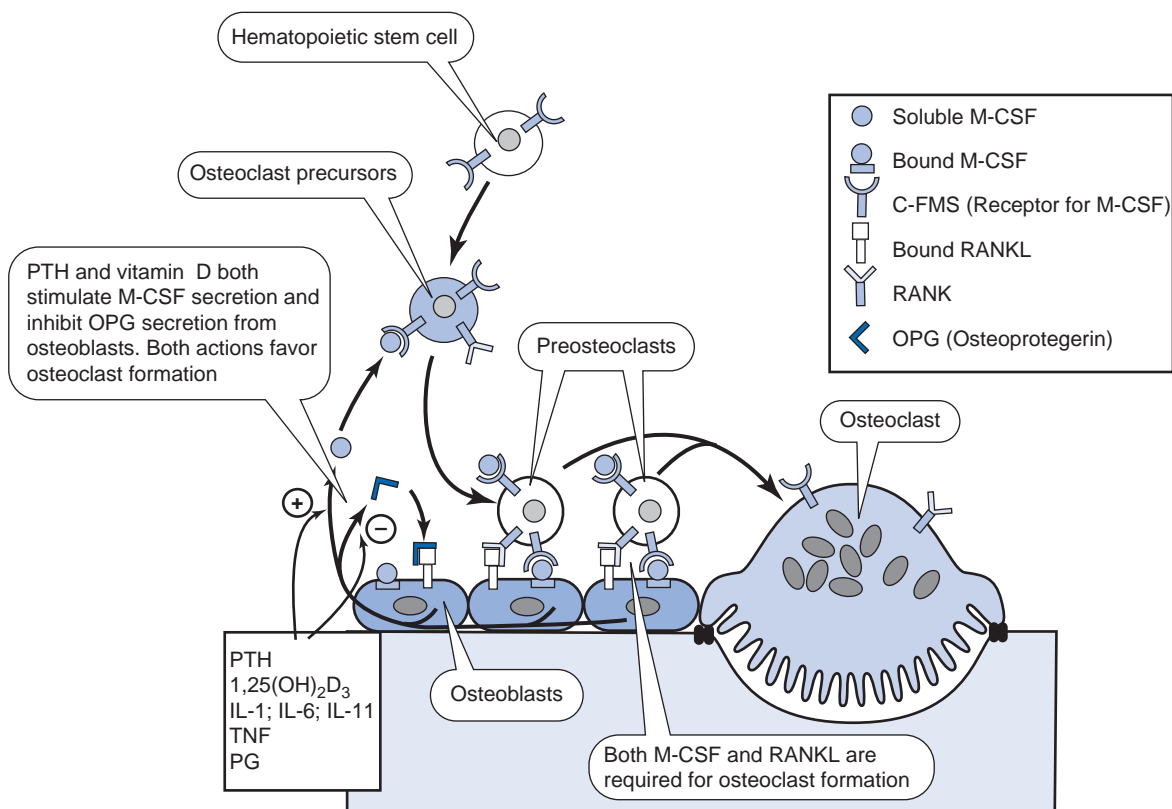
Osteocytes have receptors that respond to PTH by increasing cAMP. This activates them to resorb bone by a rapid **osteocytic osteolysis**. This removes exchangeable Ca $^{2+}$  from bone mineral but does not destroy bone.

### PTH INCREASES OSTEOCLASTIC OSTEOLYSIS

PTH stimulates the formation of new osteoclasts from circulating stem cells and stimulates bone resorption from existing osteoclasts. However, osteoclasts do not have receptors for PTH, so these effects are mediated by

**TABLE 9.8.1** Some Proteins Synthesized and Secreted by Osteoblasts

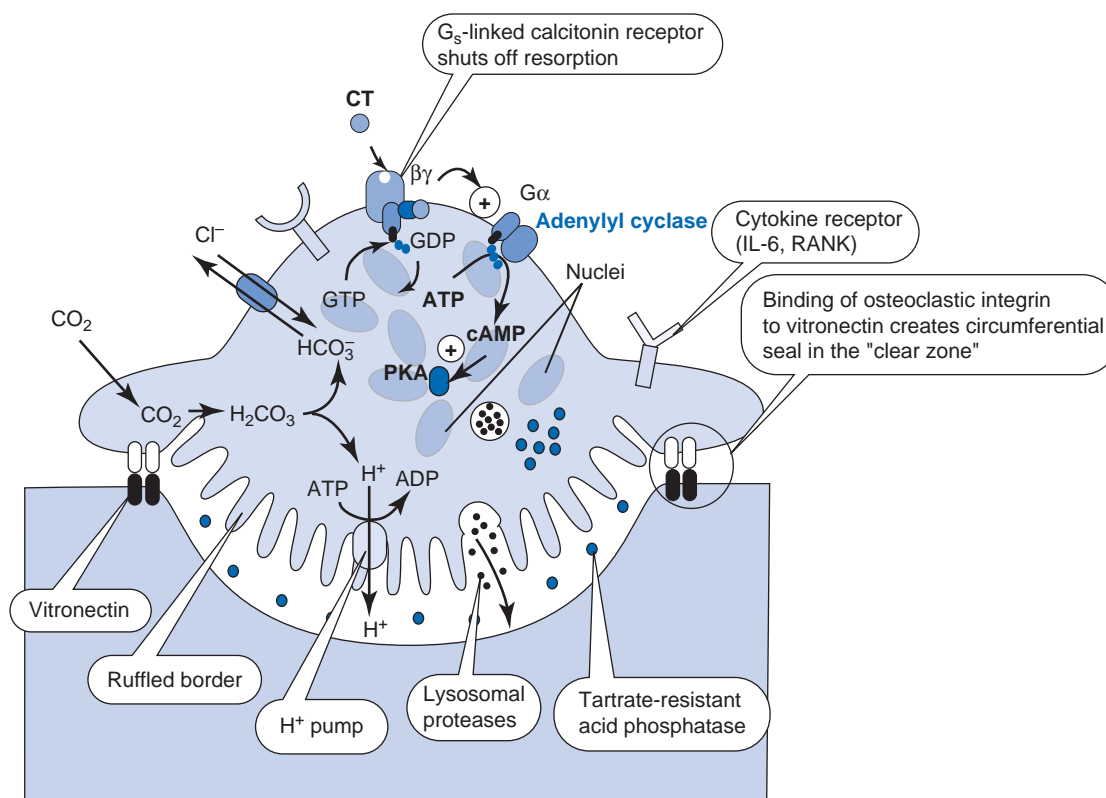
Protein Name	Chemical Nature	Possible Function
Type I collagen	Two $\alpha 1$ chains + one $\alpha 2$ chain; forms triple helix that is stabilized by hydroxylation of lysine and proline	Provides tensile strength to bone; makes nidus for nucleation of Ca and P salts
Osteocalcin	6 kDa; has three $\gamma$ carboxyglutamic acid residues	May regulate mineralization; negative regulator of osteoblasts
Matrix GLA protein	Contains $\gamma$ carboxyglutamic acid residues	Inhibits mineralization
Vitronectin		Attaches cells; binds collagen and heparin
Osteopontin		Binds cells; mediates effects of mechanical stress on osteoblasts and osteoclasts
Alkaline phosphatase		Hydrolyzes inhibitors of mineralization
Osteonectin		May regulate mineralization



**FIGURE 9.8.3** Formation of osteoclasts from hematopoietic precursors. Cells in the granulocyte/macrophage progenitor line have receptors for M-CSF, macrophage colony stimulating factor. When stimulated by factors that favor bone resorption, osteoblasts secrete soluble M-CSF and express it on their surfaces. The hematopoietic stem cells differentiate into monocyte macrophages (not shown) or preosteoclasts. Osteoclasts and their immediate precursors express RANK (receptor activator for nuclear factor  $\kappa$ B, NF- $\kappa$ B). Osteoblasts also express surface RANK ligand (RANKL) that binds RANK on the preosteoclasts. These mononuclear preosteoclasts then fuse to form the giant, multinucleated osteoclasts. Osteoblasts also secrete a soluble protein called osteoprotegerin (OPG) that binds to RANKL, preventing it from activating RANK. Both M-CSF and RANKL are essential for formation of osteoclasts and animals lacking either M-CSF or RANKL develop osteopetrosis (bone completely filling the medullary cavity). PTH and vitamin D stimulate synthesis of both soluble and membrane-bound M-CSF and RANKL. PTH and vitamin D also inhibit synthesis of OPG, which removes inhibition of osteoclast formation.

paracrine signals from osteoblasts. The result is that both  $\text{Ca}^{2+}$  and  $\text{Pi}$  are moved from bone to plasma. As could be expected, recruitment of additional osteoclasts takes some time, so **osteoclastic osteolysis** is a later response of PTH stimulation.

The result of osteocytic osteolysis is to remove the mineral from the osteoid without destroying the osteoid, whereas osteoclastic osteolysis both removes the mineral and destroys the osteoid. In both cases,  $\text{Ca}^{2+}$  and  $\text{Pi}$  are moved from bone to plasma.



**FIGURE 9.8.4** Mechanism of osteoclastic resorption of bone. Osteoclasts form a seal around a resorption area by binding their integrins with a bone protein, vitronectin. The ruffled border incorporates a vacuolar-type H<sup>+</sup> pump that acidifies the extracellular area beneath the osteoclast. Lysosomal proteases and acid phosphatases are released by the osteoclast to break down the organic matrix. Osteoclastic activity is stimulated by cytokines such as IL-6 and RANK and inhibited by calcitonin.

### CT DECREASES BONE RESORPTION

The major effect of CT on bone is to inhibit osteoclastic osteolysis. CT acts through surface membrane receptors that activate adenylyl cyclase and increase cAMP levels in osteoclasts.

### 1,25(OH)<sub>2</sub>D STIMULATES BONE RESORPTION

1,25(OH)<sub>2</sub>D is the active form of vitamin D (cholecalciferol). PTH controls its production, and PTH levels, in turn, respond to plasma [Ca<sup>2+</sup>]. 1,25(OH)<sub>2</sub>D stimulates bone resorption by itself, but it is most potent in stimulating PTH-induced bone resorption. It also induces osteocalcin production and inhibits collagen production by osteoblasts.

### MANY OTHER FACTORS AFFECT THE SKELETON

Factors that alter skeletal metabolism include the following:

- thyroid hormone (hypothyroidism leads to stunted growth);
- glucocorticoids inhibit bone formation (hypercortisolism is associated with osteoporosis);
- gonadal hormones are critical for growth and maintenance;
- insulin is necessary for proper growth of the skeleton;
- paracrine: IL-1, IL-6, TNF-α;
- in youth, growth hormone and FGF-21 regulate the growth of the bones (see Chapter 9.2).

### ONLY 1,25(OH)<sub>2</sub>D DIRECTLY AFFECTS INTESTINAL Ca<sup>2+</sup> AND Pi ABSORPTION

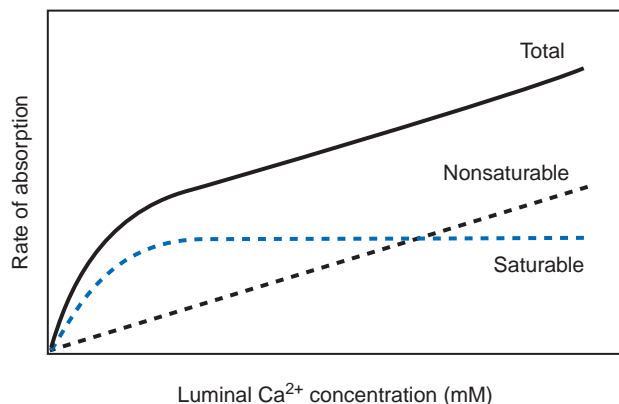
Ca<sup>2+</sup> absorption from the intestine entails the movement of Ca<sup>2+</sup> from the lumen across a sheet of columnar epithelial cells, the **enterocytes**, and into the blood. These epithelial cells are welded together by a ring of connections that goes all the way around the cells near the luminal border. These "welds" have been called the "tight junctions" because in electron micrographs they appeared to hold the cells tightly together. However, some materials can pass between the cells, in what is called the **paracellular** pathway. Alternatively, materials can move from the lumen to the blood by going through the cell, the **transcellular** pathway. The overall Ca<sup>2+</sup> absorption appears to consist of a saturable, active transport pathway and a nonsaturable passive pathway (see Figure 9.8.5). Ca<sup>2+</sup> enters the cells through apical Ca<sup>2+</sup> channels, TRPV5 and TRPV6 (for transient receptor potential vanilloid type 5 and 6). Both of these are expressed in both intestine and kidney, but TRPV6 is the major isoform in the intestine and TRPV5 is the major isoform in the kidney. Both TRPV5 and TRPV6 form heterotetrameric channel complexes with different properties. Both are inactivated by intracellular Ca<sup>2+</sup>. Intestine also possesses an L-type Ca<sup>2+</sup> channel, Ca<sub>v</sub>1.3, that may contribute to apical uptake in the transcellular active Ca<sup>2+</sup> transport.

In the transcellular route, Ca<sup>2+</sup> is then transported across the cell and is pumped across the basolateral



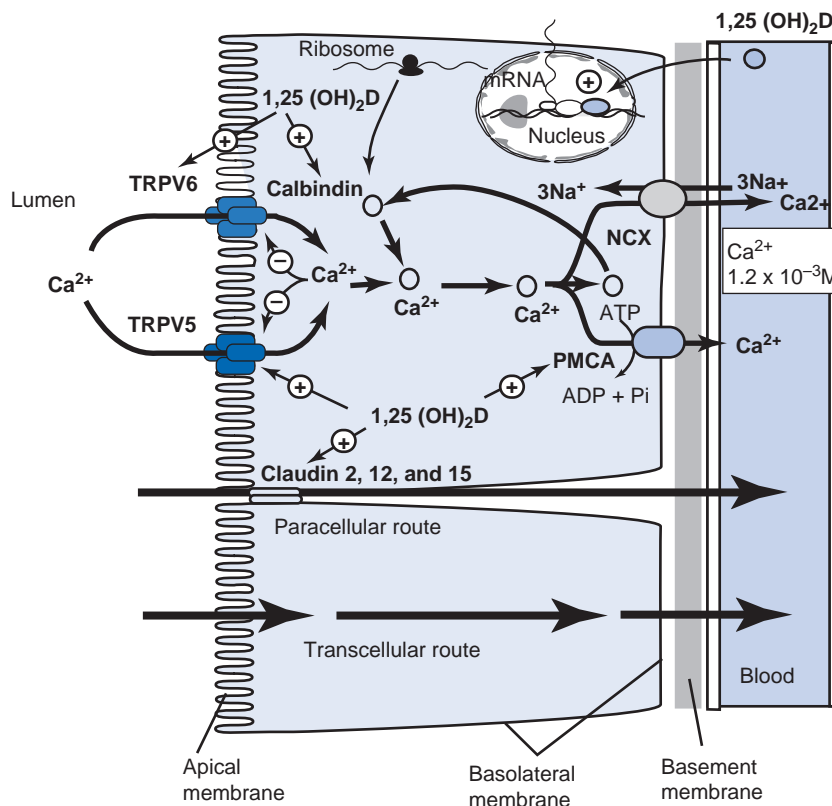
membranes by the **plasma membrane Ca-ATPase (PMCA)** and probably by  $\text{Na}^+ - \text{Ca}^{2+}$  exchange as well.

The active form of vitamin D,  $1,25(\text{OH})_2\text{D}$ , increases  $\text{Ca}^{2+}$  absorption from the intestines by classical steroid mechanisms that are described in Chapter 2.8. The hormone is carried in blood by a 52-kDa globulin, the vitamin D binding protein. The free hormone penetrates the basolateral membranes of the enterocyte and binds to a nuclear receptor that alters its conformation and binds to **vitamin D responsive elements** on the

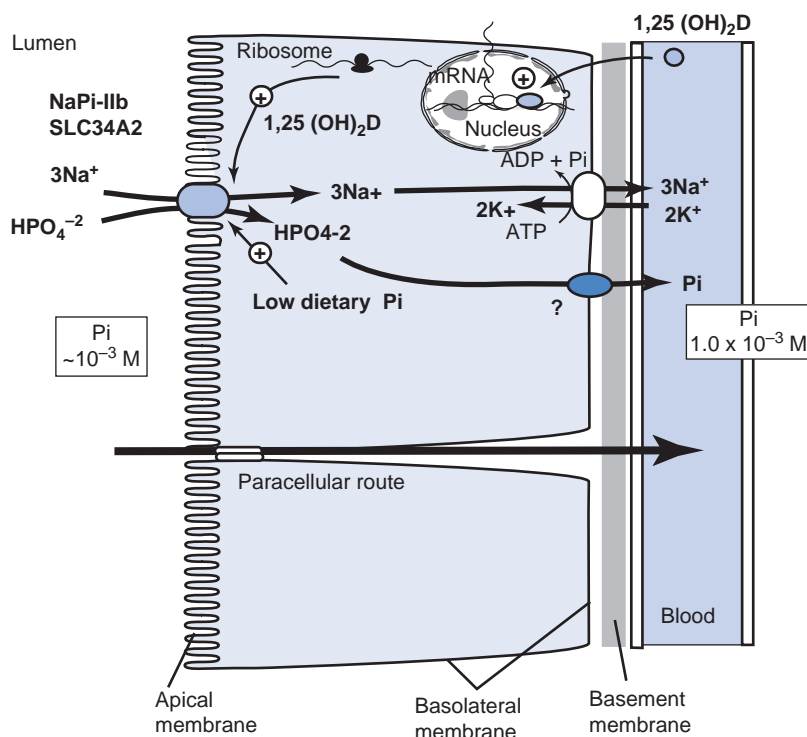


**FIGURE 9.8.5** Kinetics of  $\text{Ca}^{2+}$  absorption from the gastrointestinal tract as a function of luminal  $[\text{Ca}^{2+}]$ . In most parts of the intestine there are two processes, a saturable process that is due to active transport and is transcellular, and a paracellular pathway that obeys passive transport kinetics. The overall transport is a sum of the two processes. The relative importance of each varies with location within the intestine.

DNA, resulting in transcription of mRNA that codes for specific proteins. Vitamin D stimulates the transcription of mRNA that codes for **calbindin**, a soluble, low-molecular-weight protein (9 kDa) that binds two  $\text{Ca}^{2+}$  atoms per molecule with high affinity. Calbindin is found in highest concentration in tissues that actively transport  $\text{Ca}^{2+}$ , such as the intestine and kidney, but it is also found in other cells such as the Purkinje cells of the cerebellum. The function of calbindin is not yet established, but two functions seem likely: it buffers  $\text{Ca}^{2+}$  during its transport across the cell, and it enhances the diffusion of  $\text{Ca}^{2+}$  through the cytosol by carrying it. The total diffusion through the cytosol consists of free  $\text{Ca}^{2+}$  diffusion and diffusion of  $\text{Ca}^{2+}$  bound to calbindin. The diffusive flux is dominated by the slower bound  $\text{Ca}^{2+}$  because its total concentration is much higher than that of the free  $[\text{Ca}^{2+}]$ . Thus the cytosol acts as a permeability barrier because the free  $[\text{Ca}^{2+}]$  is maintained so low, and calbindin acts much like a carrier in facilitated diffusion. The buffering action of calbindin relieves intracellular inhibition of  $\text{Ca}^{2+}$  entry by lowering the  $[\text{Ca}^{2+}]$  immediately adjacent to the apical membrane, and increases transport at the basolateral membrane by increasing  $[\text{Ca}^{2+}]$  to the  $\text{Ca}^{2+}$ -starved PMCA (see Figure 9.8.6).  $1,25(\text{OH})_2\text{D}$  also increases the transcription of genes coding for TRPV6 and possibly TRPV5, and for the exit mechanisms on the basolateral membrane, PMCA and NCX. Evidence is accruing suggesting that  $1,25(\text{OH})_2\text{D}$  also regulates flux through the paracellular pathway by regulating the expression of claudin proteins Cldn-2 and Cldn-12.



**FIGURE 9.8.6**  $\text{Ca}^{2+}$  absorption from the intestinal lumen. The enterocytes line the intestinal lumen, with the microvillar membrane facing the lumen and the basolateral membrane facing the blood.  $1,25(\text{OH})_2\text{D}$  stimulates  $\text{Ca}^{2+}$  absorption by entering the cell and binding to a nuclear receptor that induces the transcription of mRNA coding for a variety of proteins including calbindin, a  $\text{Ca}^{2+}$ -binding protein present in high concentrations.  $\text{Ca}^{2+}$  enters the cells through an epithelial  $\text{Ca}^{2+}$  channel, mainly TRPV6 (previously called ECa2 and CaT1) or TRPV5 (previously called ECa1 and CaT2), binds to calbindin, and is transported across the cytosol bound to calbindin. Binding of  $\text{Ca}^{2+}$  to calbindin relieves inhibition of entry by intracellular  $\text{Ca}^{2+}$ .  $\text{Ca}^{2+}$  dissociates from calbindin near the export mechanism, the plasma membrane Ca-ATPase (PMCA) and the sodium-calcium exchanger (NCX), increasing the local  $[\text{Ca}^{2+}]$  and thereby stimulating these mechanisms. Transport through the paracellular route may also be regulated by  $1,25(\text{OH})_2\text{D}_3$  by regulation of tight junction proteins such as Claudin 2, 12 and 15.



**FIGURE 9.8.7** Absorption of Pi from the intestinal lumen. Pi absorption follows a transcellular route and a paracellular route. In the transcellular route, the NaPi-IIb transporter brings Pi into the cell along with 3 Na<sup>+</sup> ions. Pi exits the cell presumably by facilitated diffusion over a carrier that is not yet identified. 1,25(OH)<sub>2</sub>D stimulates the transcription of the gene coding for the NaPi-IIb transporter. Low dietary phosphate also increases Pi absorption.

Like Ca<sup>2+</sup>, Pi absorption from the intestine appears to have two components: a Na<sup>+</sup>-dependent active component and a Na<sup>+</sup>-independent passive component that probably occurs through the paracellular pathway. The active pathway begins with the cotransport of Pi and Na<sup>+</sup> at the apical or luminal surface of the cell, mediated mainly by NaPi-IIb (SLC34A2). The intestine expresses other NaPi transporters, PiT1 (SLC20A1) and PiT2 (SLC20A2), but their contribution appears to be minor. Pi concentrations in the cell are higher than plasma concentrations, so the exit of Pi at the basolateral cell does not require energy. It is believed that the basolateral membrane contains a carrier for Pi exit into the extracellular fluid adjacent to the cell, from which Pi can then diffuse into the blood (see Figure 9.8.7).

1,25(OH)<sub>2</sub>D stimulates intestinal Pi absorption by increasing the transcription of the gene coding for NaPi-IIb. Low dietary phosphate stimulates Pi absorption by posttranslational modification of the NaPi-IIb. **Alkaline phosphatase**, an enzyme with relatively nonspecific ester phosphatase activity, is located on the intestinal brush-border or microvillar membrane, and 1,25(OH)<sub>2</sub>D increases the activity of this enzyme. Its role in Pi or Ca<sup>2+</sup> absorption is not known.

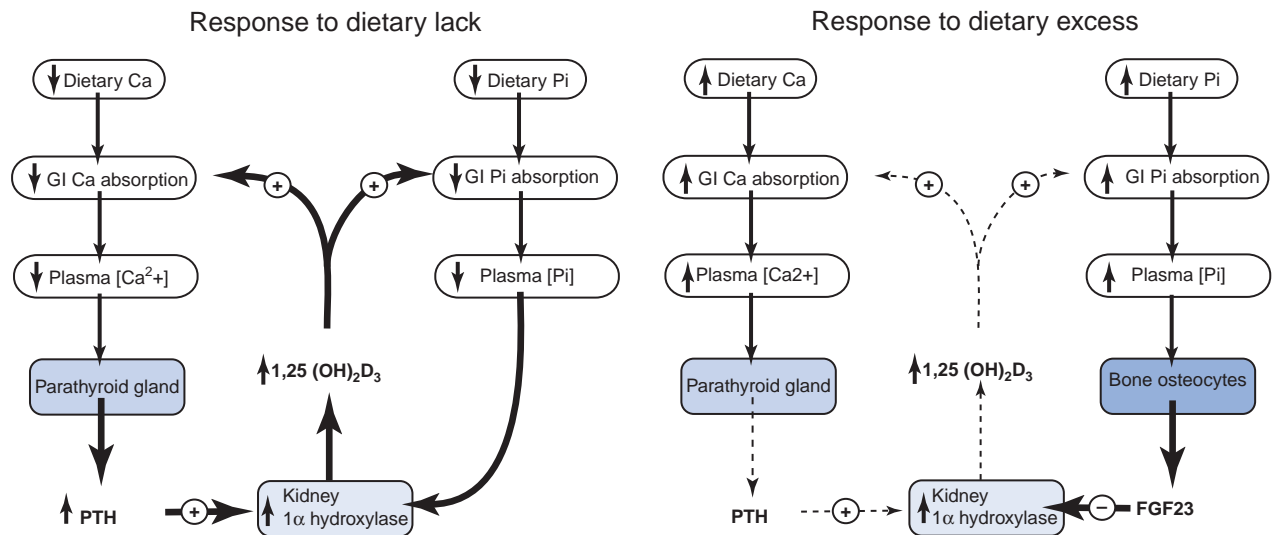
## THE INTESTINE ADAPTS TO DIETS CONTAINING DIFFERING AMOUNTS OF Ca<sup>2+</sup> AND Pi

The body adapts to diets containing differing amounts of Ca<sup>2+</sup> and Pi by adjusting its ability to absorb these minerals from the food. The absorptive capability of the intestine can be expressed as the **fractional absorption**, meaning the fraction of dietary Ca<sup>2+</sup> that is absorbed.

When the diet is low in Ca<sup>2+</sup>, the fractional absorption increases. Thus the fractional absorption measures the affinity by which the intestine absorbs Ca<sup>2+</sup>, but it does not reflect the actual amount of Ca<sup>2+</sup> that is absorbed. All other things being equal, increasing dietary Ca<sup>2+</sup> always results in increased amounts of Ca<sup>2+</sup> that is absorbed. Adaptation blunts the effects of changes in dietary Ca<sup>2+</sup> by increasing the fractional absorption when dietary Ca<sup>2+</sup> is low and by decreasing it when dietary Ca<sup>2+</sup> is high.

The intestinal absorption of Ca<sup>2+</sup> also adapts to the dietary content of Pi. When dietary Pi is low, the absorption of Ca<sup>2+</sup> remains high and decreases little when dietary Ca<sup>2+</sup> changes. When dietary Pi is high, there is a steep response of absorption with dietary Ca<sup>2+</sup>. Thus low dietary Pi stimulates absorption of Ca<sup>2+</sup> by itself, regardless of Ca<sup>2+</sup>, whereas in the presence of adequate Pi, low dietary Ca<sup>2+</sup> can stimulate absorption.

Experiments in animals have established that adaptation to the mineral content of the diet requires functioning parathyroid glands. The intestinal absorption of Ca<sup>2+</sup> is controlled solely by 1,25(OH)<sub>2</sub>D<sub>3</sub> whose synthesis in the kidney is stimulated by low Pi and high PTH and blocked by high FGF23. Low dietary Pi leads to a low plasma Pi regardless of the plasma [Ca<sup>2+</sup>]. This stimulates formation of 1,25(OH)<sub>2</sub>D<sub>3</sub> and intestinal Ca<sup>2+</sup> absorption remains high, even if the dietary content of Ca<sup>2+</sup> is high. If dietary Pi is adequate, then reducing the dietary content of Ca<sup>2+</sup> stimulates PTH secretion, which stimulates formation of 1,25(OH)<sub>2</sub>D and increases intestinal absorption. High plasma phosphate increases FGF23 that inhibits formation of 1,25(OH)<sub>2</sub>D. See Figure 9.8.8 for a schematic representation of these effects.

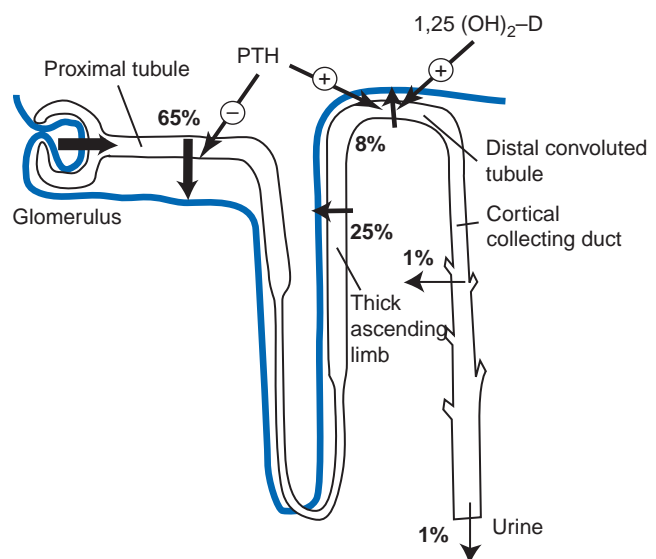


**FIGURE 9.8.8** Adaptation of GI  $\text{Ca}^{2+}$  and Pi absorption to changes in the diet. Low dietary  $\text{Ca}^{2+}$  increases the ability of the gastrointestinal tract to absorb  $\text{Ca}^{2+}$  by PTH-induced stimulation of the kidney vitamin D  $1\alpha$  hydroxylase, to increase the circulating levels of  $1,25(\text{OH})_2\text{D}_3$ . Low dietary Pi also increases the  $1\alpha$  hydroxylase enzyme, but not through PTH. High dietary  $\text{Ca}^{2+}$  reduces the ability to absorb  $\text{Ca}^{2+}$  by removing stimulation of the  $1\alpha$  hydroxylase by lower levels of PTH. High dietary Pi causes the release of fibroblast growth factor 23 (FGF23) from bone, which inhibits the kidney  $1\alpha$  hydroxylase. PTH,  $1,25(\text{OH})_2\text{D}_3$ , and FGF23 also exert effects on kidney handling of  $\text{Ca}^{2+}$  and Pi.

## REGULATION OF URINARY EXCRETION OF $\text{Ca}^{2+}$ AND Pi IS ACHIEVED IN THE DISTAL NEPHRON

The free  $\text{Ca}^{2+}$  and  $\text{Ca}^{2+}$  bound by low-molecular-weight solutes are freely filtered by the glomerulus, whereas  $\text{Ca}^{2+}$  that is bound to plasma proteins such as albumin, globulin, and fibrinogen is retained in the blood. About 60% of the total plasma  $\text{Ca}^{2+}$  is present as free and complexed forms or about 6 mg%. The filtered load of  $\text{Ca}^{2+}$  can be readily calculated as  $\text{GFR} \times [\text{total } \text{Ca}^{2+}]_{\text{ultrafiltrate}}$ . If the GFR is  $125 \text{ mL min}^{-1}$ , the filtered load is about  $7.5 \text{ mg min}^{-1}$ . Of this filtered load, only 1–2% remains in the final urine. The rest is absorbed in various segments of the nephron, the majority being absorbed in the proximal tubule. Significant amounts are absorbed in the thick ascending limb of the loop of Henle and in the distal convoluted tubule. Figure 9.8.9 shows the fraction of filtered  $\text{Ca}^{2+}$  that is reabsorbed in each nephron segment.

The proximal tubule actively reabsorbs  $\text{Na}^+$ , with water and a variety of substances following passively through the paracellular route. About 80% of the  $\text{Ca}^{2+}$  that is reabsorbed in the proximal tubule is absorbed paracellularly, with another 20% being reabsorbed through transcellular route. PTH binds to a  $\text{G}_s$ -coupled receptor on the surface of proximal tubule cells. The resulting increased cAMP has a number of effects, one of which is to **decrease the reabsorption of  $\text{Na}^+$ , water,  $\text{HCO}_3^-$ ,  $\text{Ca}^{2+}$ , and Pi.** The mechanism of Pi reabsorption in the proximal tubule is shown in Figure 9.8.10. PTH inhibits Pi reabsorption, but this effect is modulated by  $\text{Ca}^{2+}$  in the lumen of the tubule. The proximal tubule contains the extracellular Ca sensor, CaSR, on its apical surface. Increases in  $\text{Ca}^{2+}$  in the lumen inhibit the effects of

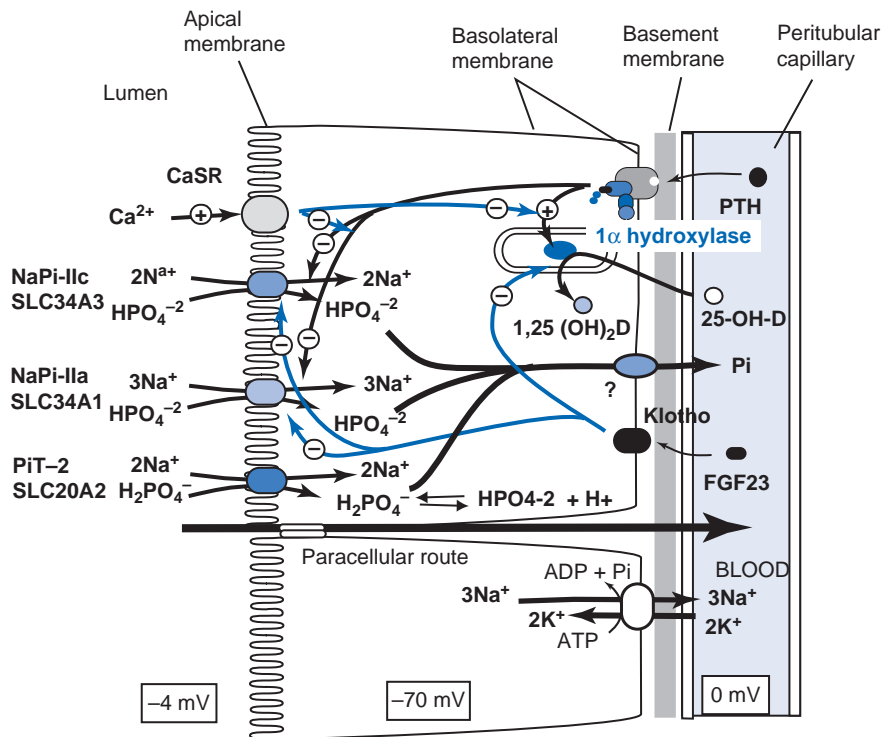


**FIGURE 9.8.9** Fraction of filtered  $\text{Ca}^{2+}$  that is reabsorbed at each nephron segment. Note that PTH **decreases**  $\text{Ca}^{2+}$  reabsorption in the proximal tubule, whereas it **increases**  $\text{Ca}^{2+}$  reabsorption in the distal tubule.  $1,25(\text{OH})_2\text{D}$  increases  $\text{Ca}^{2+}$  absorption in the distal nephron.

PTH on both the Pi transporters and its stimulation of the vitamin D  $1\alpha$  hydroxylase enzyme that activates vitamin D by producing  $1,25(\text{OH})_2\text{D}_3$ . FGF23 inhibits the transcription of the genes that code for NaPi-IIa and NaPi-IIc and for the  $1\alpha$  hydroxylase. Thus FGF23 inhibits both Pi transport in the proximal tubule and synthesis of  $1,25(\text{OH})_2\text{D}_3$ .

The thick ascending limb of the loop of Henle reabsorbs some 25% of the filtered load mainly through





**FIGURE 9.8.10** Regulation of Pi transport in the proximal tubule. NaPi-IIa and NaPi-IIc transport Pi into the proximal tubule cell with some contribution by PiT2. Pi exit across the basolateral membrane is not identified. PTH inhibits these transport mechanisms through a  $G_s$  mechanism. PTH also activates the vitamin D  $1\alpha$  hydroxylase enzyme that converts circulating  $25(\text{OH})\text{D}_3$  to its active form,  $1,25(\text{OH})_2\text{D}_3$ .  $\text{Ca}^{2+}$  in the lumen inhibits these actions of PTH via the extracellular  $\text{Ca}^{2+}$  sensor, CaSR, on the apical membrane of these cells. FSG23 inhibits the transcription of genes for the Pi transporters on the apical membrane, NaPi-IIa and NaPi-IIc, and the gene for the  $1\alpha$  hydroxylase. FSG23 interacts with  $\alpha$ Klotho on the basolateral membrane.

the paracellular route, aided by the lumen positive potential in this segment of the nephron. This reabsorption is passive and is linked to the reabsorption of  $\text{Na}^+$ , but it does not occur by solvent drag. The extracellular  $\text{Ca}^{2+}$  sensor is located on the basolateral membrane of the thick ascending limb cells and inhibits  $\text{Ca}^{2+}$  reabsorption by inhibiting the apical transporters (NKCC2 and ROMK1; see Figure 7.5.5), thereby reducing the transepithelial potential that drives  $\text{Ca}^{2+}$  reabsorption.

The distal tubule consists of a heterogeneous assortment of cells. Some of these cells contain calbindin, produced in these cells like it is in enterocytes. These cells also contain the TRPV5 that is thought to be involved in transcellular  $\text{Ca}^{2+}$  transport. Both PTH and  $1,25(\text{OH})_2\text{D}$  stimulate  $\text{Ca}^{2+}$  absorption in the distal tubule.

In the distal tubule, PTH increases the reabsorption of  $\text{Ca}^{2+}$  but not Pi. Thus PTH causes an increased excretion of Pi or a **hyperphosphaturia**. In the proximal tubule,  $1,25(\text{OH})_2\text{D}$  increases the reabsorption of Pi by increasing expression of NaPi-IIa and NaPi-IIc.

## THE “GOALS” OF PTH, CT, AND VITAMIN D ARE DISTINCT

PTH, CT, and vitamin D, strictly speaking, do not have “goals” because they are inanimate objects without thoughts and anticipatory behavior. The entire physiological system, however, takes on aspects of behavior that are not present in any one part of the system. The systems appear to have goals and if we

speak about them as if they do, their behavior makes sense. This way of speaking is called “**teleological**.” This means to refer to the final causes of behavior or their purpose.

## THE GOAL FOR PTH IS TO RAISE FREE PLASMA $[\text{Ca}^{2+}]$

Hypocalcemia stimulates PTH secretion. PTH then stimulates the bone to release both  $\text{Ca}^{2+}$  and Pi. Pi released from bone complexes  $\text{Ca}^{2+}$  and thus prevents the free plasma  $[\text{Ca}^{2+}]$  from rising as much as it would in the absence of the increased Pi. PTH causes the kidney to retain more of the filtered  $\text{Ca}^{2+}$  and dispose of the Pi. Thus there is a hyperphosphaturia and more of the  $\text{Ca}^{2+}$  that is resorbed from bone is retained in the blood. Because PTH can raise the filtered load of  $\text{Ca}^{2+}$ , the effect of PTH can be either a hypocalciuria or hypercalciuria, depending on the balance of PTH effects on bone resorption and kidney reabsorption of  $\text{Ca}^{2+}$ . Further, PTH stimulates the kidney  $1\alpha$  hydroxylase to increase production of  $1,25(\text{OH})_2\text{D}$ , which stimulates  $\text{Ca}^{2+}$  and Pi absorption from the intestine. The net effect of PTH secreted in response to hypocalcemia is generally not hypophosphatemia because it increases Pi input into the plasma at the same time that it increases Pi output from the plasma.

## THE GOAL OF CT IS TO STOP BONE RESORPTION—PARTICULARLY IN THE YOUNG

The main physiological effect of CT is the rapid cessation of bone resorption. CT exerts no effect on intestinal  $\text{Ca}^{2+}$  and Pi handling and the effect of CT on the

kidney is of minor physiological importance. There are no known abnormalities in  $\text{Ca}^{2+}$  handling associated with known CT undersecretion (in people with thyroidectomies, for example) or hypersecretion (in medullary thyroid carcinomas). On the other hand, it is known that the sensitivity to CT changes with age; young people are much more sensitive than older individuals. Further, the gut hormones are potent secretagogues for CT. This has led to the hypothesis that CT prevents postprandial hypercalcemia in the suckled young. Young mammals get a lot of  $\text{Ca}^{2+}$  in the milk, and this  $\text{Ca}^{2+}$  could cause a transient hypercalcemia when it is absorbed from the intestine. The gut hormones (e.g., gastrin, cholecystokinin, and secretin) increase after eating but before  $\text{Ca}^{2+}$  has a chance to be absorbed. CT assures that young mammals deposit dietary  $\text{Ca}^{2+}$  directly into bone by shutting down bone resorption even before  $\text{Ca}^{2+}$  is absorbed.

### THE GOAL OF VITAMIN D IS TO RAISE BOTH $[\text{Ca}^{2+}]$ AND $[\text{Pi}]$ TO MINERALIZE THE BONES

The “goal” of vitamin D differs from that of PTH because it acts on bone, kidney, and intestine to increase both plasma  $[\text{Ca}^{2+}]$  and  $[\text{Pi}]$ . Its goal is to assure adequate mineralization of the skeleton, and this mineralization requires both  $\text{Ca}^{2+}$  and Pi. Thus vitamin D activation to  $1,25(\text{OH})_2\text{D}$  is stimulated not only by low plasma  $[\text{Ca}^{2+}]$  but also by low plasma  $[\text{Pi}]$ .

### THE GOAL OF FGF23 IS TO PREVENT HYPERPHOSPHATEMIA

FGF23 secretion is stimulated by high plasma Pi and by  $1,25(\text{OH})_2\text{D}_3$ . Its main actions are to inhibit Pi reabsorption from the kidney and to decrease formation of  $1,25(\text{OH})_2\text{D}_3$ . Thus FGF23 and  $1,25(\text{OH})_2\text{D}_3$  exhibit a negative feedback loop within the other homeostatic feedback loops for plasma  $[\text{Ca}^{2+}]$  and  $[\text{Pi}]$  regulation. The main effect here appears to be avoidance of hyperphosphatemia when bone is resorbed.

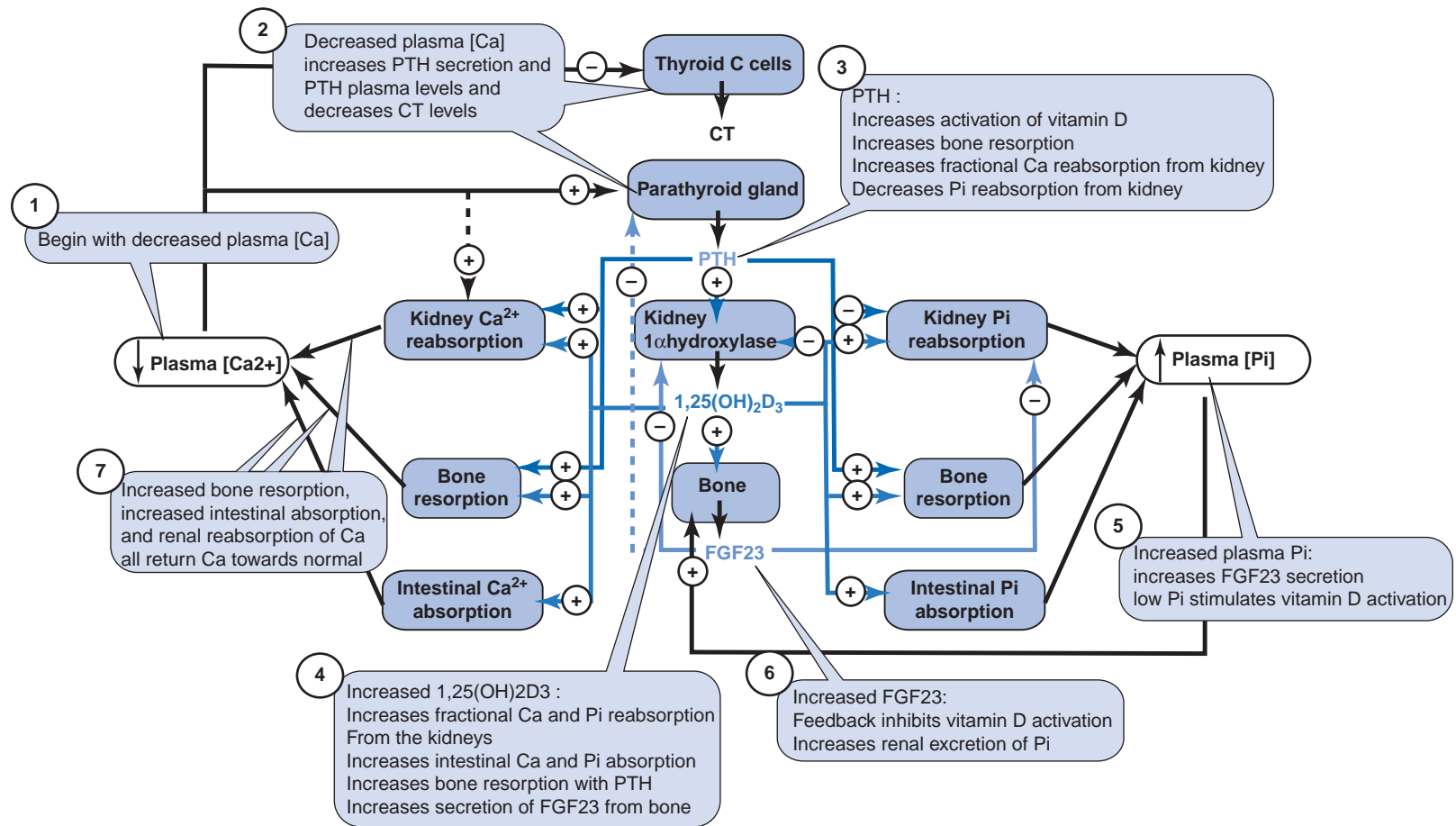
### INTEGRATED CONTROL OF PLASMA $[\text{Ca}^{2+}]$ AND Pi INVOLVES MULTIPLE NEGATIVE FEEDBACK LOOPS

The integrated control of plasma  $[\text{Ca}^{2+}]$  and  $[\text{Pi}]$  is shown in Figure 9.8.11. Here the negative feedback loops are indicated in bold arrows. As an example, consider the physiological response to a lowering of the free plasma  $[\text{Ca}^{2+}]$ . Several events occur. We trace the negative feedback loops, beginning with:

1. Plasma  $[\text{Ca}^{2+}]$  falls.
2. The low plasma  $[\text{Ca}^{2+}]$  stimulates PTH secretion.
3. PTH has several effects:
  - PTH induces a rapid osteocytic osteolysis that resorbs both  $\text{Ca}^{2+}$  and Pi into plasma.
  - The plasma  $\text{Ca}^{2+}$  originating from bone is retained to a greater degree by the kidneys because PTH stimulates  $\text{Ca}^{2+}$  reabsorption. This tends to raise the plasma  $[\text{Ca}^{2+}]$  back toward normal.
  - PTH stimulates the kidney  $1\alpha$  hydroxylase enzyme to increase  $1,25(\text{OH})_2\text{D}$ .
4.  $1,25(\text{OH})_2\text{D}_3$  levels increased by PTH stimulation of the kidney  $1$  hydroxylase increase more slowly and have several effects:
  - $1,25(\text{OH})_2\text{D}_3$  stimulates intestinal absorption of both  $\text{Ca}^{2+}$  and Pi. The increased  $\text{Ca}^{2+}$  absorption tends to restore plasma  $[\text{Ca}^{2+}]$  toward normal.
  - $1,25(\text{OH})_2\text{D}$  is synergistic with PTH in resorbing bone. This helps raise plasma  $[\text{Ca}^{2+}]$  toward normal.
  - $1,25(\text{OH})_2$  helps retain  $\text{Ca}^{2+}$  and Pi in the kidney, which tends to raise plasma  $[\text{Ca}^{2+}]$  toward normal.
  - $1,25(\text{OH})_2\text{D}_3$  increases the secretion of FGF23 from bone cells.
5. The increased bone resorption, increased intestinal absorption, and mixed effects on the kidney can lead to increased plasma  $[\text{Pi}]$ . This increases the secretion of FGF23.
6. Increased FGF23 has several effects:
  - FGF23 inhibits Pi reabsorption from the kidney
  - FGF23 inhibits  $1,25(\text{OH})_2\text{D}_3$  production by the proximal tubule of the kidney.
7. All of these effects work to increase the plasma  $[\text{Ca}^{2+}]$  from its low value: the negative feedback loop is complete.

The effects of PTH on the kidney and on osteocytic osteolysis are rapid, whereas the effects acting through  $1,25(\text{OH})_2\text{D}$  or osteoclastic osteolysis take much longer to develop and last longer. It takes considerable time to change the circulating levels of  $1,25(\text{OH})_2\text{D}$  and for this hormone to regulate the genetic expression of intestinal absorptive cells.

“Normal” plasma  $[\text{Ca}^{2+}]$  can be maintained at identical levels but at different physiological states. For example, the plasma  $[\text{Ca}^{2+}]$  can be within normal ranges when a person consumes a diet that is high in readily available  $\text{Ca}^{2+}$ . Under these conditions, we would expect the PTH levels to be relatively low and  $1,25(\text{OH})_2\text{D}$  levels would also be low. The plasma  $[\text{Ca}^{2+}]$  is being maintained primarily through intestinal absorption of  $\text{Ca}^{2+}$  and the person is likely to be in positive  $\text{Ca}^{2+}$  balance. A person who consumes a low  $\text{Ca}^{2+}$  diet might also have a normal plasma  $[\text{Ca}^{2+}]$ , but the PTH levels and  $1,25(\text{OH})_2\text{D}$  levels would be high because it takes these high values to achieve a normal plasma  $[\text{Ca}^{2+}]$ . In this case, plasma  $[\text{Ca}^{2+}]$  would be maintained by resorption of bone  $\text{Ca}^{2+}$ , and the person would be likely to be in negative  $\text{Ca}^{2+}$  balance.



**FIGURE 9.8.11** Integrated control of plasma  $\text{Ca}^{2+}$ . Some components of this regulatory system are omitted, but the main ones are shown by arrows with plus or minus signs. The plus sign means that when the event at the beginning of the arrow occurs, such as decrease in plasma  $[\text{Ca}^{2+}]$ , the effect is an increase in the event at the end of the arrow. The minus sign on an arrow means that when the event at the beginning of the arrow occurs, there is a decrease in the event at the end of the arrow.

### Clinical Applications: Dual Energy X-ray Absorbimetry and Bone Density

Diagnosis of some bone diseases, such as osteoporosis, requires reliable and accurate estimations of bone density. Dual energy X-ray absorbimetry provides one such method. Known as DEXA and now as DXA this method uses high- and low-energy X-rays to provide estimates of both the soft tissue and bone mineral composition.

The equation describing absorption of X-rays by materials follows an analogue of the absorption of visible light, the Beer–Lambert Law. The equation is

$$(9.7.1) \quad I = I_0 e^{-\mu x}$$

where  $I$  is the transmitted intensity of the X-ray beam,  $I_0$  is the incident intensity (prior to absorption),  $\mu$  is a constant called the **linear attenuation coefficient**, and  $x$  is the distance through which the beam passes. Absorption of X- and gamma-rays by materials occurs by photoelectric mechanisms and Compton scattering, in which orbital electrons interact with the radiation and are ejected from the atom, while the radiation loses some energy and changes its direction. These effects are much larger as the Z-number of the absorber increases. Thus,  $\mu$  is much greater for calcium than it is for hydrogen, oxygen, and carbon. The linear attenuation coefficient also depends on the density of the atoms within the radiation beam: more atoms per unit cross-sectional area mean more opportunities to absorb the radiation. The linear attenuation coefficients also vary with the energy of the radiation, becoming smaller with increasing energy. When layers of soft tissue and bone are both present, the absorption of radiation is given by

$$(9.7.2) \quad I = I_0 e^{-(\mu_t x_t + \mu_b x_b)}$$

where the subscript t denotes tissue and b denotes bone. These attenuation coefficients for standard tissue and bone can be determined and tabulated. For a single radiation beam, there is one equation and two unknowns:  $x_t$  and  $x_b$ . If we use two energies, however, we have two equations:

$$(9.7.3) \quad \begin{aligned} I_1 &= I_{0,1} e^{-(\mu_{t1} x_t + \mu_{b1} x_b)} \\ I_2 &= I_{0,2} e^{-(\mu_{t2} x_t + \mu_{b2} x_b)} \end{aligned}$$

where the subscripts 1 and 2 refer to the low- and high-energy beams. If the sets of coefficients are known, measurement of  $I_1/I_{0,1}$  and  $I_2/I_{0,2}$  allows calculation of  $x_t$  and  $x_b$ . However, the linear attenuation coefficients can be expressed as **mass attenuation coefficients** by dividing by the density of the materials. In this case, the equations become

$$(9.7.4) \quad \begin{aligned} I_1 &= I_{0,1} e^{-\left(\frac{\mu_{t1}}{\rho_{t1}} m_t + \frac{\mu_{b1}}{\rho_{b1}} m_b\right)} \\ I_2 &= I_{0,2} e^{-\left(\frac{\mu_{t2}}{\rho_{t2}} m_t + \frac{\mu_{b2}}{\rho_{b2}} m_b\right)} \end{aligned}$$

where  $m_t$  is the mass of tissue and  $m_b$  is the mass of bone. Solution of these equations gives the mass of either bone or tissue within the radiation beam. This allows calculation of the **areal density**: g of bone mineral per cm<sup>2</sup>. These areal densities are reported in T-scores, the number of standard deviations from the normal bone density of a 30-year-old person of your gender and race. A T-score of  $-2.5$  (2.5 standard deviations below normal) is considered to be diagnostic of osteoporosis.

## SUMMARY

The calciotropic hormones affect Ca and Pi handling in three main target organs: the bone, the intestine, and the kidneys.

Osteoblasts line the surfaces of the bone and secrete the organic matrix that gives bone its form and serves as nucleation sites for mineralization. When these cells become embedded in the matrix they become osteocytes. Osteocytes remain in communication through cytoplasmic processes that travel through tiny canaliculi that link osteocytes to osteoblasts or other osteocytes. Osteoclasts are giant, multinucleated cells that resorb both mineral and organic matrix of bone. Regulation of the number and activity of these bone cells determines the fate of mineral deposition or resorption from bone. Osteoblasts have receptors for PTH and  $1,25(\text{OH})_2\text{D}_3$  and respond by increasing secretion of M-CSF, a factor that induces differentiation of hematopoietic stem cells to form mononuclear preosteoclasts. PTH and  $1,25(\text{OH})_2\text{D}_3$  also stimulate osteoblasts to make RANKL—a ligand that binds to *receptor activator of nuclear factor  $\kappa\text{B}$*  (RANK) expressed on the surface of preosteoclasts and osteoclasts. The two factors M-CSF and RANKL are essential for

formation of osteoclasts.  $1,25(\text{OH})_2\text{D}_3$  also increases osteocalcin formation, inhibits collagen synthesis, and inhibits secretion of OPG.

Osteoclasts resorb bone by sealing off an area of the surface and pumping acid and enzymes into the restricted space. The acid dissolves the mineral away from its nucleation sites on collagen, and the enzymes destroy the organic matrix. Osteoclasts make a resorption cavity and release both Ca and Pi into the blood.

Osteoclasts have no receptors for  $1,25(\text{OH})_2\text{D}_3$  or PTH, but they do possess receptors for CT. CT binds to a  $\text{G}_s$ -linked receptor that increases cAMP in these cells. The result is cessation of bone resorption. This does not appear to be important in the adult but may aid the young growing mammal in making strong bones during youth.

The intestine responds to  $1,25(\text{OH})_2\text{D}_3$  but not to either PTH or CT.  $1,25(\text{OH})_2\text{D}_3$  binds to a nuclear receptor in enterocytes and increases the transcription of genes coding for calbindin, TRPV6 and NaPi-IIb. Calbindin is a small molecular weight protein that binds  $\text{Ca}^{2+}$  ions with high affinity and likely either buffers  $\text{Ca}^{2+}$  during its transcellular transport or increases its diffusion through the cytoplasm of the enterocytes.

$1,25(\text{OH})_2\text{D}_3$  increases both Ca and Pi transport across the intestine.

Osteocytes and osteoblasts release FGF23 in response to high Pi or  $1,25(\text{OH})_2\text{D}_3$ . FGF23 signals through  $\alpha\text{klotho}$  to inhibit Pi reabsorption from the kidney tubule by inhibiting Pi entry.

The kidney responds to PTH,  $1,25(\text{OH})_2\text{D}_3$ , and CT, but the effect of CT appears to be unimportant. PTH acts through a  $G_s$  mechanism in the kidney. PTH decreases absorption of both Ca and Pi in the proximal tubule but increases Ca absorption in the distal tubule. The result is an increased fraction of reabsorption of Ca and increased Pi excretion.  $1,25(\text{OH})_2\text{D}_3$  increases reabsorption of both Ca and Pi in the distal nephron. FGF23 inhibits Pi reabsorption by decreasing NaPi-IIa and NaPi-IIc.

These actions of PTH,  $1,25(\text{OH})_2\text{D}_3$ , and CT show different "goals." PTH's goal is to maintain plasma  $[\text{Ca}^{2+}]$ , and it achieves this by increasing bone resorption to place both Ca and Pi into the blood. It then discards Pi at the kidney and retains Ca.  $1,25(\text{OH})_2\text{D}_3$ , on the other hand, increases intestinal absorption of both Ca and Pi, resorbs both Ca and Pi from bone, and attempts to retain both at the kidney. Thus  $1,25(\text{OH})_2\text{D}_3$  keeps both Ca and Pi levels high to promote bone mineralization. Although CT has definite effects, there are no disease states from known underproduction or overproduction of CT, suggesting that the body can adjust well regardless of CT levels.

## REVIEW QUESTIONS

1. What are the endosteum, periosteum, diaphysis, epiphysis? What is the difference between compact bone and cancellous bone?
2. What are osteocytes, osteoblasts, and osteoclasts?
3. Both PTH and CT increase cAMP in bone, but PTH causes bone resorption and CT causes cessation of bone resorption. Explain how this can be.
4. Describe the origin of osteoclasts and the role PTH and  $1,25(\text{OH})_2\text{D}_3$  play in their formation.
5. What is bone made of? What forms bone? What degrades bone? How is bone resorbed? The continual resorption and reformation of bone is called what?
6. How does Ca get absorbed from the ingested food? What regulates intestinal Ca and Pi absorption?
7. What happens to intestinal Ca absorption in persons who consume a low Ca diet? What is the mechanism that brings this about?
8. What does PTH do to Ca and Pi reabsorption in the proximal tubule? In the distal tubule? What are the overall effects of PTH? Would urinary Ca excretion go up or down? Would urinary Pi excretion go up or down?
9. What does  $1,25(\text{OH})_2\text{D}_3$  do to Ca and Pi handling in the kidney?
10. What effect does CT have on gut and kidney Ca and Pi handling?