9.7 Calcium and Phosphorus Homeostasis I: The Calcitropic Hormones

Learning Objectives

- Describe the ways calcium is carried in plasma and identify the form that is homeostatically regulated
- Identify the major sources and sinks of plasma calcium
- Explain why calcium and phosphate homeostasis are linked
- Identify the major sources and sinks of plasma phosphate
- List the three hormones involved in calcium and phosphate homeostasis and their sites of origin
- List the three targets of these hormones
- Describe the chemical nature of PTH and its stimulus for release
- List three major actions of PTH
- Describe the chemical nature of CT and its stimulus for release
- List two major actions of CT
- Describe the chemical nature of vitamin D and explain why it isn't a vitamin
- Describe the control of vitamin D activation
- Describe the chemical nature of FGF23 and its stimulus for release
- List the major actions of FGF23

CALCIUM HOMEOSTASIS IS REQUIRED FOR HEALTH

Calcium homeostasis refers to the maintenance of a constant concentration of calcium ions in the extracellular fluid. It includes all of the processes that contribute to maintaining calcium at its "set point." Because plasma $[Ca^{2+}]$ rapidly equilibrates with the extracellular fluid, ECF $[Ca^{2+}]$ is kept constant by keeping the plasma $[Ca^{2+}]$ constant. Maintaining a constant plasma $[Ca^{2+}]$ is important for:

- nerve transmission
- nerve conduction
- muscle contraction
- cardiac contractility
- blood clotting
- bone formation
- excitation–secretion coupling
- cell-to-cell adhesion
- cell-to-cell communication.

Some of these (bone formation, blood clotting, and cell adhesion) depend directly on the extracellular $[Ca^{2+}]$; others depend directly on intracellular $[Ca^{2+}]$. But since ICF $[Ca^{2+}]$ depends indirectly on plasma $[Ca^{2+}]$, all are linked to plasma $[Ca^{2+}]$. Calcium homeostasis can be viewed as having two components: a microcomponent dealing with the intracellular environment and a macrocomponent dealing with the extracellular environment. This chapter concerns regulation of $[Ca^{2+}]$ and phosphate concentrations in the extracellular fluid.

ABOUT HALF OF PLASMA CALCIUM IS FREE: THE OTHER HALF IS COMPLEXED OR BOUND TO PLASMA PROTEINS

Total plasma $[Ca^{2+}]$ is tightly regulated at about 10 mg% (mg per deciliter of blood) or 2.5×10^{-3} M. This total plasma is partitioned among three forms: free Ca^{2+} comprises about half of the total, or 1.2×10^{-3} M; another 40% is bound to plasma proteins, particularly albumin; and the last 10% of the total plasma Ca^{2+} is bound to low-molecular-weight anions such as lactate, citrate, and bicarbonate. The principal plasma proteins are albumin (4 g%), globulin (3 g%), and fibrinogen (0.3 g%). For every g% change in plasma albumin, the total plasma $[Ca^{2+}]$ changes about 0.8 mg%. Thus the total plasma $[Ca^{2+}]$ varies with the plasma protein concentration. The endocrine system regulates the free $[Ca^{2+}]$.

Most of the binding sites for Ca^{2+} on plasma proteins are free carboxyl groups of the side chains of the amino acids, glutamic acid and aspartic acid. When ionized, the negative charge attracts the Ca^{2+} ion and loosely binds it. When two or more of these groups are in close proximity, the binding site has a higher affinity because the negative charges more strongly attract the Ca^{2+} ion. When a hydrogen ion binds to a carboxyl group, it neutralizes its negative charge and so it no longer binds Ca^{2+}. Thus plasma $[H^+]$ competes for Ca^{2+} binding sites. Acidosis (increased plasma $[H^+]$) lowers the protein-bound Ca^{2+} and increases the free $[Ca^{2+}]$. Lowering the $[H^+]$, as in alkalosis, shifts free Ca^{2+} to bound $[Ca^{2+}]$.

FAILURE TO REGULATE PLASMA $[Ca^{2+}]$ CAUSES SYSTEM MALFUNCTION

When plasma $[Ca^{2+}]$ falls to 7 mg% or less, the permeability of neuron membranes is increased so that they become more excitable. As a result, **paresthesias** ("pins and needles") may be felt and **tetany** may develop due to increased neuronal excitability both centrally and peripherally. The tetany that develops can be

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fatal. This is called **hypocalcemic tetany**. Plasma [Ca^{2+}] >12 mg% leads to depression of the central nervous system and sluggish reactions. Prolonged increased plasma [Ca^{2+}] can lead to ectopic calcification of the soft tissues with loss of function.

PLASMA Ca^{2+} HOMEOSTASIS RESULTS FROM A BALANCE OF SOURCES AND SINKS

Sources of Ca^{2+} include absorption from the intestinal tract, resorption from bone, and reabsorption from the tubular fluid in the kidney. The major sinks occur in the same organs: secretion into the intestine, accretion onto bone, and ultrafiltration in the kidney. Figure 9.7.1 illustrates these sources and sinks for calcium, along with estimates of the daily magnitude of these sources and sinks. They show a person in **calcium balance**, in which body Ca^{2+} content does not change: ingested Ca^{2+} exactly matches the amount in the feces, sweat, and urine. This is not usually the case. The growing infant or teen excretes less Ca^{2+} than the amount ingested and stores the accumulated Ca^{2+} in bone. These growing youth are in **positive calcium balance**. Ultimately, all of the Ca^{2+} in bone comes either from maternal sources *in utero* or from food. When dietary Ca^{2+} is insufficient to keep up with excretion in feces, urine, and sweat, plasma [Ca^{2+}] falls and bone Ca^{2+} is mobilized to return plasma [Ca^{2+}] to normal. This produces a **negative calcium balance** in which the lost Ca^{2+} derives from bone. Positive and negative calcium balance refer to the long-term averages of intake minus loss. On a day-to-day basis, a given individual can be in either negative or positive calcium balance. What determines the health of the bone is the long-term balance. Figure 9.7.1 shows that **the vast majority, more than 99%, of body Ca^{2+}, resides in bone**. A small, nearly undetectable loss of 40 mg day^{-1} over 30 years loses 440,000 mg, or about 37% of the skeletal Ca^{2+} content. But loss of 40 mg day^{-1} is only 5% of the dietary intake. This small loss can result in **osteoporosis**, a thinning and subsequent weakening of bone.

PLASMA Ca^{2+} HOMEOSTASIS IS LINKED TO P HOMEOSTASIS

Maintenance of cytoplasmic [Ca^{2+}] at very low levels is an axiom of cell biology. Inorganic phosphate, Pi, is an integral part of a variety of important organic constituents of cells including DNA, RNA, ATP, phospholipids, and many intermediates of metabolic pathways. One postulate for the origin of low cytoplasmic [Ca^{2+}] is calcium's insolubility with Pi: Ca^{2+} and Pi solutions produce calcium phosphate crystals. This reaction is governed by a solubility product

$$[Ca^{2+}][Pi] = K_{sp} \quad (9.7.1)$$

According to this postulate, cells evolved Ca^{2+} pumps to prevent Ca–Pi crystallization.

Plasma Ca^{2+} homeostasis is linked to *plasma* Pi homeostasis because the bone mineral is a Ca–Pi salt that is similar to the mineral **hydroxyapatite**, with the empirical formula of $Ca_{10}(PO_4)_6(OH)_2$. When bone is resorbed, both Ca^{2+} and Pi are liberated. When new bone is formed, both Ca^{2+} and Pi are removed from plasma to form the new mineral. Plasma [Ca^{2+}] tends to obey Eqn (9.7.1): rising plasma

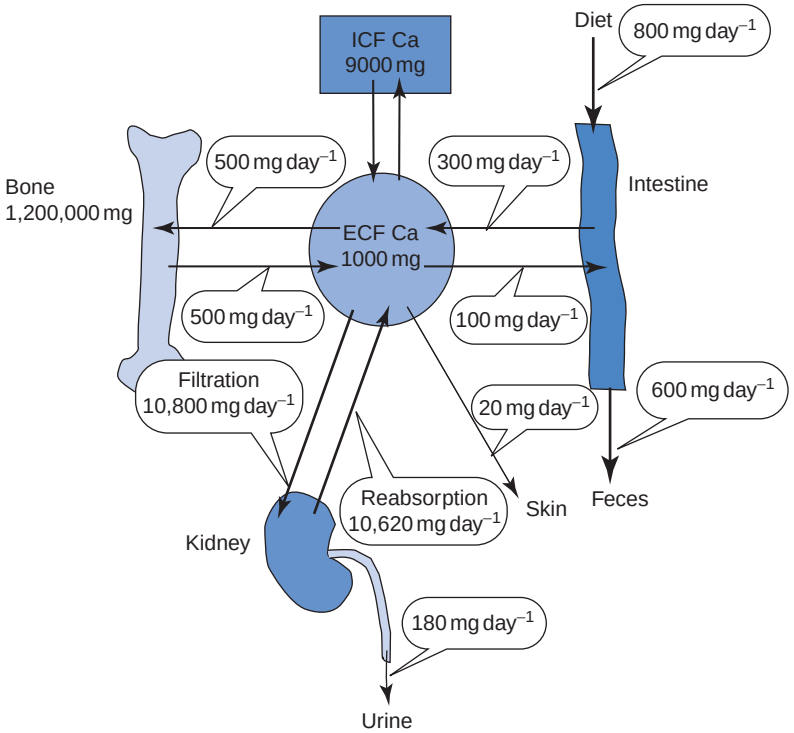

FIGURE 9.7.1 Sources and sinks of plasma Ca^{2+}. Flows given are in mg elemental Ca^{2+} per day, whereas pool sizes are in mg. The numbers in this figure are estimated averages that vary daily and individually with age, gender, and diet.

[Ca^{2+}] tends to lower plasma [Pi], and rising plasma [Pi] tends to lower plasma [Ca^{2+}]. However, plasma [Ca^{2+}] and [Pi] do not obey a solubility product rule, as this would imply that it is in equilibrium with a Ca^{2+}-Pi precipitate, and there is no such equilibrium being obeyed.

PLASMA PI IS PRESENT IN MULTIPLE IONIZED FORMS

Plasma inorganic phosphate participates in a number of reactions with H^+:

$$\begin{aligned} &H_3PO_4 \rightleftarrows H_2PO_4^- + H^+ K_D = 7.5 \times 10^{-3}\ M \\ &H_2PO_4^- \rightleftarrows H_2PO_4^{-2} + H^+ K_D = 6.2 \times 10^{-8}\ M \\ &HPO_4^{-2} \rightleftarrows PO_4^{-3} + H^+ K_D = 4.8 \times 10^{-13}\ M \end{aligned} \quad (9.7.2)$$

Each of these dissociation reactions is characterized by the given equilibrium constant. These dissociation constants correspond to the [H^+] at which the reaction is half complete, when [A^-] = [HA]. Thus the first dissociation is half complete at pH 2.1, the second is half complete at pH 7.2, and the third at pH 12.3. Because of these reactions, the form of Pi in the plasma depends on the pH. Because of this ambiguity, plasma concentration of phosphate is expressed in terms of the mg% *phosphorus*. In adults, the normal value is 3.5–4 mg%; in the growing child it is 4–5 mg%.

PLASMA [Pi] IS SET BY A BALANCE BETWEEN SOURCES AND SINKS

Like Ca^{2+} balance, Pi balance results from the flows between the intestine, kidney, bone, and intracellular compartment. Estimates of the flows in a typical adult are shown in Figure 9.7.2. Note that the magnitude of the numbers is near those for Ca^{2+} balance. The difference is that bone contains more Ca^{2+} than P on a weight basis, and P is higher in the intracellular compartment compared to Ca^{2+}.

OVERALL Ca^{2+} AND Pi HOMEOSTASIS IS CONTROLLED BY FOUR HORMONES ACTING ON THREE TARGET TISSUES

Plasma [Ca^{2+}] is controlled by four hormones:

1. parathyroid hormone (PTH)
2. calcitonin (CT)
3. vitamin D (cholecalciferol)
4. FGF23.

These hormones are collectively called the **calciotropic** hormones. They accomplish Ca^{2+} and Pi homeostasis by acting on three separate targets:

1. intestine
2. bone
3. kidney.

These hormones also exert effects on other tissues that are not directly involved with Ca^{2+} and Pi homeostasis.

HYPOCALCEMIA STIMULATES PTH SECRETION

One pair of the four parathyroid glands is embedded in each half of the thyroid gland. The glands consist of chief cells and oxyphile cells. The chief cells synthesize a 115 amino acid precursor called preproPTH. The N-terminal signal sequence of 25 amino acids is clipped within the ER to produce a 90 amino acid polypeptide called proPTH. The N-terminal 6 amino acids are clipped again to form an 84 amino acid polypeptide,

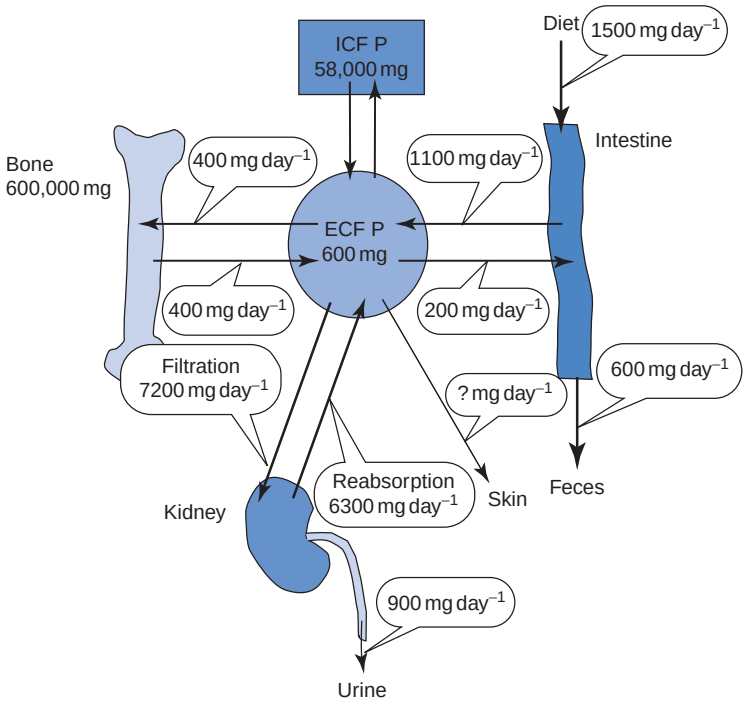

FIGURE 9.7.2 Pi balance in a typical adult. Flows are in units of mg *phosphorus* per day; pool sizes are in units of mg. These numbers differ individually and daily.

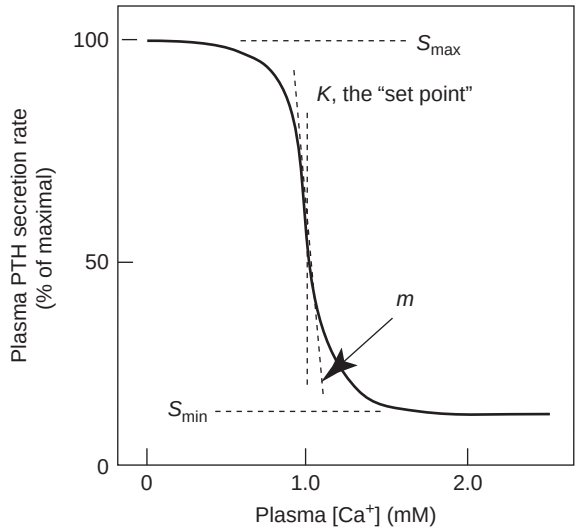

FIGURE 9.7.3 Sigmoidal control of PTH secretion by plasma $[Ca^{2+}]$. Decreases in plasma $[Ca^{2+}]$ below the normal value cause a steep rise in PTH secretion. Conversely, rises in plasma $[Ca^{2+}]$ decrease in PTH secretion. The curve can be approximately described by the empirical equation:

$$S = [S_{max} - S_{min}]/[1 + ([Ca]/K)^m] + S_{min}$$

where S is the rate of secretion; S_{max} is its maximum rate of secretion and S_{min} is the minimum rate; K is the "set point" at which S is half way between S_{max} and S_{min}; m is the slope of the response at K.

PTH, that is stored in secretory granules within the gland, and secreted in response to hypocalcemia.

The parathyroid cells express an extracellular Ca^{2+} sensor (CaSR), a homodimeric G-protein-coupled receptor, that interacts with a variety of G proteins in various tissues. In the parathyroid gland, CaSR activates phospholipase C and inhibits adenylate cyclase. When plasma $[Ca^{2+}]$ rises, phospholipase C activity in parathyroid cells increases, thereby increasing the hydrolysis of phosphatidylinositol bisphosphate, in turn increasing the cytoplasmic concentration of IP_3 that releases Ca^{2+} from stores in the endoplasmic reticulum. Thus cytoplasmic $[Ca^{2+}]$ rises at the same time that cAMP levels decrease, and PTH secretion also decreases. When plasma $[Ca^{2+}]$ falls, the reverse occurs: cell $[Ca^{2+}]$ falls, cAMP rises, and PTH secretion is increased. Thus parathyroid cells are an exception to the rule that rises in cytoplasmic $[Ca^{2+}]$ cause secretion. The overall result (see Figure 9.7.3) shows an impressive, sigmoidal response to plasma $[Ca^{2+}]$ that ranges from maximum to minimum over a range from 0.8 to 1.5 mM. The plasma $[Ca^{2+}]$ that results in 50% of the maximum plasma [PTH] is called the "set point." It is near the normal plasma $[Ca^{2+}]$ levels.

PTH SECRETION IS AN EXAMPLE OF DERIVATIVE CONTROL

At least three different functional relationships between plasma $[Ca^{2+}]$ and the rate of PTH secretion have been considered. In **direct control**, the rate is directly proportional to the plasma $[Ca^{2+}]$. In **derivative control**, the rate of PTH secretion is proportional to $d[Ca^{2+}]/dt$. In this case, the rate of change of plasma $[Ca^{2+}]$ influences the rate of PTH secretion. In **integrative control**, the rate of PTH secretion is proportional to $\int[Ca^{2+}]dt$. In this case, the gland responds to some average plasma $[Ca^{2+}]$ and therefore it would be sensitive to slow but prolonged changes in $[Ca^{2+}]$. Experiments suggest that PTH is under derivative control. When plasma $[Ca^{2+}]$ falls, there is a higher rate of PTH secretion when the rate of fall is faster and the rate of secretion is measured at the same plasma $[Ca^{2+}]$. At the same plasma $[Ca^{2+}]$, PTH secretion is higher when $d[Ca^{2+}]/dt$ is negative compared to when it is positive.

PTH IS DESTROYED RAPIDLY AFTER SECRETION

The normal circulating levels of PTH, about 10–65 ng/L, are a heterogeneous mixture of intact PTH and PTH fragments. Only the N-terminal 34 amino acids are biologically active. A variety of PTH fragments are produced by proteolysis, particularly in the kidney. The half-life of circulating PTH is extremely short, about 2 minutes. Thus there must be a continuous secretion of PTH to maintain plasma $[Ca^{2+}]$, and the circulating levels reflect the rate of secretion by the parathyroid glands. Because of this rapid turnover, only a tiny fraction of circulating PTH ever binds to its receptors on target tissues.

PTH DEFENDS AGAINST HYPOCALCEMIA BY ACTIONS ON BONE AND KIDNEY

The mechanism by which PTH alters Ca^{2+} and Pi handling by bone, intestine, and kidney will be discussed in Chapter 9.8. Here, we summarize its actions:

- PTH causes resorption of bone mineral. This releases both Ca^{2+} and Pi from bone into plasma. By itself, this action would tend to raise both plasma $[Ca^{2+}]$ and [Pi].
- PTH increases the reabsorption of Ca^{2+} from the renal tubular fluid, whereas it decreases the reabsorption of Pi. This helps retain the Ca^{2+} that is removed from bone and discards the Pi. This would tend to raise the plasma $[Ca^{2+}]$ alone while decreasing plasma [Pi].
- PTH activates vitamin D. Vitamin D exerts its major hormonal influence on intestinal Ca^{2+} and Pi absorption, but it must be activated to exert these effects. By contributing to the control of vitamin D activation, PTH *indirectly* stimulates intestinal Ca^{2+} absorption.
- PTH increases release of FGF23 from osteoblasts and osteocytes.

CT IS SECRETED IN RESPONSE TO HYPERCALCEMIA AND GASTROINTESTINAL HORMONES

C-cells of the thyroid gland, also called parafollicular cells because they reside in between the thyroid follicles, synthesize a glycosylated precursor with an M_r of about 17 kDa. These cells secrete **CT**, an polypeptide hormone 32 amino acids long with an M_r of 3.4 kD, in response to hypercalcemia. Because of its origin in the thyroid gland, CT is often called **thyrocalcitonin**. Figure 9.7.4 shows the relationship between CT secretion and plasma [Ca^{2+}]. The sensor for extracellular Ca^{2+} in the C-cells is the same CaSR that is present in chief cells of the parathyroid gland, but in this case increases in plasma [Ca^{2+}] result in an increase in secretion rather than an inhibition. Thus CaSR in different tissues are connected to the cellular mechanisms for secretion in different ways, producing inhibition in one case (PTH) and stimulation in another (CT). In addition to hypercalcemia, injections of the gastrointestinal hormones **gastrin** or **cholecystokinin** powerfully stimulate CT release. C-cells have CCK2R (cholecystokinin 2 receptor) on their surfaces. The normal circulating levels of CT are 5–40 ng/L. Circulating CT is degraded by proteolysis mainly in the kidney and liver, with a half-life less than 10 minutes.

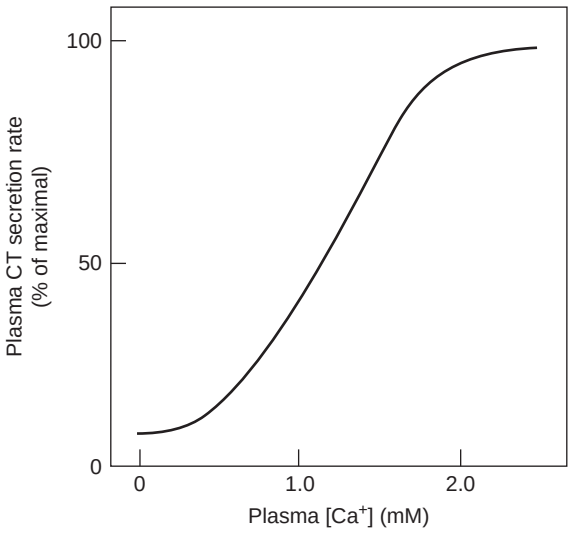

FIGURE 9.7.4 Relationship between CT secretion and plasma [Ca^{2+}].

CT TENDS TO LOWER PLASMA [Ca^{2+}]

CT was discovered relatively recently, in 1961, partly because there are no clinical symptoms resulting from CT overproduction or underproduction. Thyroidectomy does not produce hypercalcemia, and massive overproduction of CT in medullary thyroid tumors does not cause hypocalcemia. Nevertheless, injection of CT promptly lowers plasma [Ca^{2+}] in experimental animals. Lack of pathology associated with abnormal CT levels does not mean that CT is unimportant in human physiology. The body might adjust for these abnormal levels. For example, lack of symptoms in medullary thyroid carcinoma, when CT levels are high, could be due to "CT escape." Downregulation of the CT receptors could result in tissues that no longer respond to CT, whereas they would remain responsive if CT levels were normal. The main effects of CT are to:

- inhibit bone resorption;
- increase Ca^{2+} and Pi excretion in the urine.

"VITAMIN D" IS A HORMONE, NOT A VITAMIN, SYNTHESIZED IN THE SKIN

The naturally occurring vitamin D is called **cholecalciferol** and is derived from ultraviolet radiation of **7-dehydrocholesterol** in the skin. The 7-dehydrocholesterol is an intermediate in the synthesis of cholesterol from lanosterol. The conjugated diene group of 7-dehydrocholesterol absorbs UV radiation in the 250–310 nm range, which causes the B ring of the steroid nucleus to open up to form previtamin D. This then isomerizes in a nonenzymatic reaction to form cholecalciferol, also known as **vitamin D3** (see Figure 9.7.5).

A deficiency of cholecalciferol causes **rickets** in the young and **osteomalacia** in the adult. These diseases are characterized by the **failure to mineralize bone**. This results in a weakened bone that is pliable, and hence children afflicted with rickets often have painful, bowed legs. The noun is "rickets" and the adjective for the disease is "rachitic."

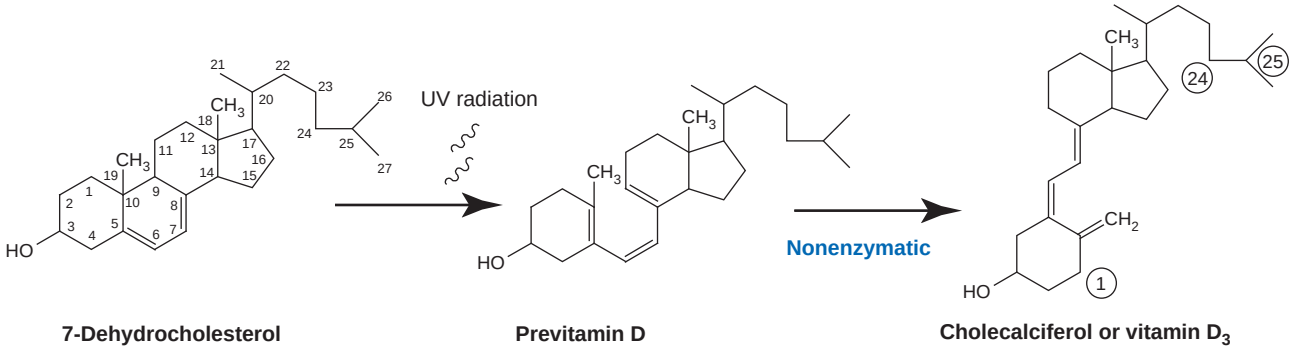

FIGURE 9.7.5 Conversion of 7-dehydrocholesterol in the skin to cholecalciferol. The numbering system at the far right of the figure indicates three important positions for activation and deactivation of vitamin D at the 1, 24, and 25 positions.

The distinction between vitamin D as a hormone or as a vitamin goes back to research performed at the beginning of the 20th century. In 1919, Huldschinsky in Vienna exposed one arm of a rachitic child to the UV light from a mercury vapor lamp and demonstrated by X-rays that mineralization began in both irradiated and nonirradiated arms. This was clear proof that something traveled (presumably via the blood) from the irradiated area to the nonirradiated area. In that same year, Sir Edward Mellanby produced rickets in puppies and showed that cod liver oil cured them. At that time it was known that cod liver oil contained vitamin A, so Mellanby attributed the cure to vitamin A. In 1922, McCollum, the discoverer of vitamin A, showed that vitamin A in cod liver oil was destroyed by bubbling oxygen through the oil, but the antirachitic factor was still present. McCollum named the new antirachitic factor "vitamin D." At that time there was enormous interest in the micronutrients and the "vitamine" concept. The name "vitamin D" stuck. Obviously, if vitamin D cured rickets, it must be a vitamin deficiency disease! It is only now in hindsight that we can set the historical record straight.

VITAMIN D DOES NOT FIT STANDARD DEFINITIONS OF EITHER VITAMIN OR HORMONE

A vitamin is defined as an "organic substance, present in minute amounts in natural foodstuffs, which is essential to normal function and lack of which in the diet causes deficiency diseases." "Vitamin D" fails to meet many of these requirements. First, it is not naturally present in most foodstuffs. Milk contains 400 USP units of vitamin D per quart, but this is added as a public health measure and is not naturally present in milk. Second, humans exposed to sufficient sunlight (1 h at noon on the back of the hand is sufficient) have no *dietary* requirement for vitamin D.

Hormones are secreted into the blood by endocrine glands in response to some stimulus and travel by the blood to some distant target where they have specific effects. Vitamin D meets some of these criteria. As we will see later, vitamin D affects intestinal absorptive cells by a mechanism entirely analogous to that used by the steroid hormones. Yet vitamin D is not secreted by a gland, circumscribed or not. Instead, its precursor is formed in the deep layers of the skin and then absorbed into the blood where it binds to a carrier protein.

Making the distinction between vitamin D as a hormone or a vitamin is equivalent to defining a natural environment for humans. Primitive peoples, who live out of doors and regulate their activities by the sun and moon, have no requirement for vitamin D and are seldom afflicted by rickets even though their diet may contain gross deficiencies. Civilized people, who live indoors and regulate their activities by clocks and electric lighting, require dietary vitamin D even though their diet may otherwise contain no errors.

THE LIVER ACTIVATES VITAMIN D BY 25-HYDROXYLATION: THE KIDNEY ACTIVATES IT BY 1-HYDROXYLATION

Vitamin D synthesized in the skin is transported in blood by a 52-kDa globulin also called **transcalciferin**. Vitamin D may also be absorbed efficiently (80%) from the diet and then transported to the liver in chylomicrons. In the liver, vitamin D is converted to 25(OH) vitamin D by a microsomal enzyme. This reaction is inhibited by its product, 25(OH) vitamin D.

25(OH) vitamin D is further hydroxylated in the kidney proximal tubule by a mitochondrial enzyme, 25(OH) 1α hydroxylase. The reaction produces **1,25 $(OH)_2$vitamin D.** This is the most active form of vitamin D with regard to Ca^{2+} and Pi homeostasis. It is referred to as **calcitriol** because it has three hydroxyl groups: one at the 3 position, originally present in 7-dehydrocholesterol, and the ones at the 25 position and 1 position added by hydroxylation in the liver and kidney, respectively. The 1α hydroxylase enzyme is regulated by PTH, plasma [Ca^{2+}] and [Pi] and by fibroblast growth factor 23 (FGF23). Figure 9.7.6 summarizes vitamin D metabolism.

VITAMIN D INACTIVATION BEGINS WITH 24-HYDROXYLATION

Kidney mitochondria also contain another hydroxylase that converts 25(OH) vitamin D to 24,25$(OH)_2$ vitamin D. The product, another triol, may have some biological activity or it may represent the beginning of degradation. This reaction, and that of the 1α hydroxylase, is regulated.

PTH CONTROLS METABOLISM OF VITAMIN D

Figure 9.7.7 shows that conversion of 25(OH) vitamin D to 1,25$(OH)_2$ vitamin D or 24,25$(OH)_2$ vitamin D depends critically on the plasma [Ca^{2+}]. At just about the normal plasma [Ca^{2+}], production of 1,25$(OH)_2$ vitamin D turns on when plasma [Ca^{2+}] falls, and the falling [Ca^{2+}] turns off production of 24,25$(OH)_2$. This metabolic switch makes sure the active form of vitamin D helps raise plasma [Ca^{2+}] back toward normal. These effects are mediated through changes in the amounts of 1α hydroxylase present in the kidney.

PTH and low plasma [Pi] stimulate the 1α hydroxylation reaction that converts 25(OH)D to 1,25$(OH)_2$D. The product 1,25$(OH)_2$D weakly inhibits further formation of 1.25$(OH)_2D_3$. Plasma [Ca^{2+}] also directly controls the 1α hydroxylase enzyme, probably through CaSR present in the kidney. Thus the 1α hydroxylase appears to be under the control of plasma [Ca^{2+}] both directly and indirectly through PTH: when plasma [Ca^{2+}] falls near the normal plasma [Ca^{2+}], it activates PTH secretion (see Figure 9.7.3). PTH then stimulates the 1α hydroxylase enzyme, forming the active form of vitamin D, 1,25$(OH)_2$D. Because PTH

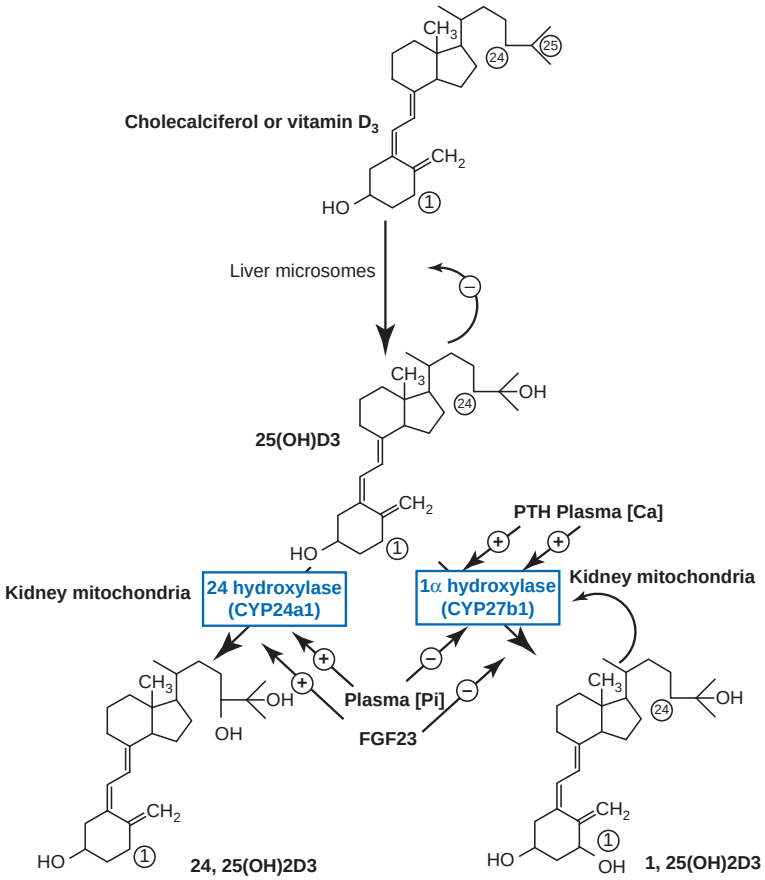

FIGURE 9.7.6 Summary of the control of vitamin D metabolism. Cholecalciferol that is made in the skin (see Figure 9.7.5) travels through the blood bound to a carrier protein. It is hydroxylated first in the liver at the 25 position to form 25(OH)D_3. The kidneys then add another hydroxyl group at the 1 position to make 1,25$(OH)_2D_3$, the most potent form of the vitamin. The kidney 1α hydroxylase enzyme is regulated by plasma [Pi], [Ca^{2+}] and FGF23. Inactivation of vitamin D also begins in the kidney by hydroxylation at the 24 position.

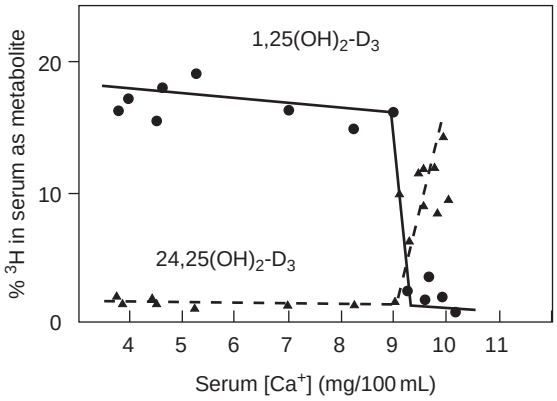

FIGURE 9.7.7 Switch of vitamin D metabolism from 1,25$(OH)_2D$ to 24,25 $(OH)_2D$ with increasing plasma [Ca^{2+}] above normal.

is the major stimulant of the 1α hydroxylase enzyme, it is the major determinant of circulating levels of 1,25 $(OH)_2D_3$. FGF23 inhibits synthesis of the 1α hydroxylase enzyme.

The 24-hydroxylase enzyme is stimulated by normal plasma Pi levels and by 1,25$(OH)_2D$.

VITAMIN D HAS TWO FORMS OF EQUAL POTENCY IN HUMANS

Cholecalciferol is known as vitamin D_3 for historical reasons. The chemical structure of vitamin D_2 was worked out earlier than that of D_3 because of the availability of ergosterol, a sterol derived from fungus. UV light converts ergosterol to **ergocalciferol**, an analogue of cholecalciferol. It is known as vitamin D_2 and equals vitamin D_3 in preventing rickets. One international unit of vitamin D = 0.025 μg; 400 IU or 10 μg of irradiated ergosterol are typically added per quart of milk. The US RDA (recommended dietary allowance) for vitamin D is 400 IU day^{-1}. The structures of ergosterol and ergocalciferol are shown in Figure 9.7.8. Ergocalciferol differs from cholecalciferol by a double bond and an extra methyl group.

25$(OH)_2D$ IS THE MAJOR CIRCULATING FORM OF VITAMIN D

Table 9.7.1 summarizes the half-lives and circulating levels of vitamin D, 25(OH)D and 1,25$(OH)_2D$. The circulating levels of vitamin D depend on exposure to

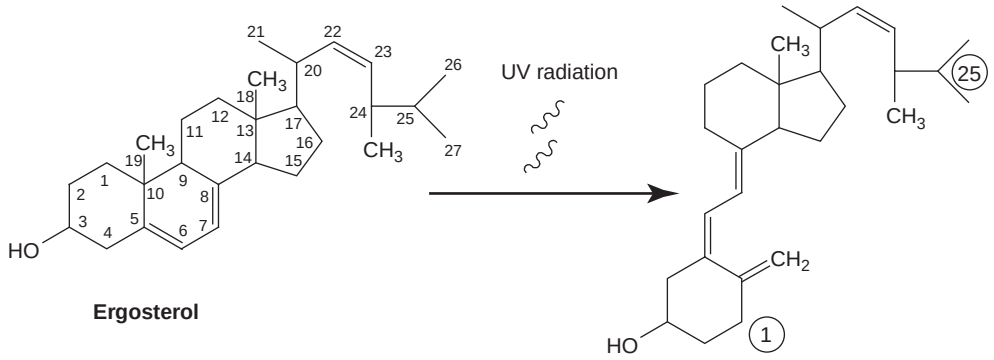

FIGURE 9.7.8 Structure of ergosterol and vitamin D_2, ergocalciferol.

TABLE 9.7.1 Half-Life and Circulating Levels of Vitamin D Metabolites

Metabolite	Half-Life	Plasma Concentration
Vitamin D	~1 day	0–220 μg/L = 0–310 nM
25(OH)D	~3 weeks	8–60 μg/L = 20–150 nM
$1,25(OH)_2D$	~5 h	16–60 ng/L = 40–150 pM

sunlight and dietary content of vitamin D; the circulating levels of 25(OH)D integrate the circulating vitamin D levels and so it serves as a long-term index of vitamin D status. The levels of $1,25(OH)_2D$ are adjusted more quickly and so it reflects shorter term regulation of Ca^{2+} and Pi homeostasis.

VITAMIN D MAINTAINS CONDITIONS FOR BONE MINERALIZATION

The mechanism of action of vitamin D on its target tissues will be discussed in Chapter 9.8. In summary:

- Vitamin D increases Ca^{2+} and Pi absorption from the intestine.
- Vitamin D increases Ca^{2+} and Pi reabsorption from the renal tubules.
- Vitamin D promotes bone resorption.
- Vitamin D increases FGF23 release from bone cells.

The "goal" of vitamin D differs from that of PTH. PTH responds to hypocalcemia and its effects correct the condition by resorbing bone and discarding the Pi generated from the resorption. Vitamin D also resorbs bone, but it also promotes bone mineralization by raising both plasma [Ca^{2+}] and [Pi]. The actions of vitamin D on both intestine and kidney are to raise both [Ca^{2+}] and [Pi].

BONE CELLS RELEASE FGF23 IN RESPONSE TO A VARIETY OF SIGNALS

FGF23, fibroblast growth factor 23, primarily regulates phosphate metabolism, but in the course of doing so also regulates calcium balance. Bone cells, particularly osteocytes, mainly release FGF23 in response to PTH, $1,25(OH)_2D_3$, P_i and sympathetic nervous stimulation. Osteocytes synthesize FGF23 as a 251-amino acid protein with a M_r of 32 kDa due to several sites of O-glycosylation. The N-terminal 24 amino acids comprise the signal peptide that directs the protein to the endoplasmic reticulum. FGF23 interacts with **α-Klotho (KL)** on the surface membrane of target tissues. However, some KL is also secreted into the circulation, forming soluble KL or sKL. Klotho is a coreceptor that interacts with fibroblast growth factor receptor to bind FGF23. In some tissues, FGF23 may have effects independent of Klotho.

The main effects of FGF23 are:

- reduce synthesis of 1α vitamin D hydroxylase in the kidney and increase 24 hydroxylase
- increase phosphate excretion by the kidney
- inhibit PTH secretion.

SUMMARY

The body regulates free plasma calcium concentration within narrow limits. Total plasma [Ca^{2+}] is about 10 mg%, but only about half of this is free in the plasma with the remainder being bound primarily by albumin. Plasma [Ca^{2+}] is linked to plasma [phosphate] because when bone is resorbed both Ca^{2+} and phosphate are released into the plasma, and mineralization of the organic matrix of bone removes both from plasma. All body Ca^{2+} and Pi derives ultimately from maternal stores or from the diet. Youngsters exhibit a

positive calcium balance in which calcium is stored in the bone. Adults often make small but regular withdrawals from this store of mineral in order to stabilize blood levels of Ca^{2+} and Pi. The resulting long term but small negative calcium balance over many years can lead to **osteoporosis**.

Four hormones regulate calcium and Pi balance: **PTH**, **CT**, **vitamin D** and FGF23. These affect mineral transport in three organs: the **intestine**, the **bone**, and the **kidney**.

The parathyroid glands secrete an 84 amino acid protein hormone, PTH, in response to low plasma $[Ca^{2+}]$. The relationship between secretion and plasma $[Ca^{2+}]$ is steep and depends on the rate of fall of plasma $[Ca^{2+}]$ as well as the level of plasma $[Ca^{2+}]$. PTH increases the resorption of mineral from bone, increases renal excretion of Pi and retention of Ca^{2+}, and activates vitamin D. These actions highlight the "goals" of PTH, which is to keep plasma $[Ca^{2+}]$ within its normal limits.

Parafollicular cells in the thyroid gland secrete a 32 amino acid polypeptide, CT, in response to hypercalcemia. CT stops bone resorption by direct action on bone cells called osteoclasts. Its importance in adult humans is not established, because there are no diseases associated with over- or underproduction of CT.

Vitamin D is inappropriately named because free-living humans in a natural, outdoor environment have no dietary requirement for it. UV radiation from the sun converts 7-dehydrocholesterol in the skin to pre-vitamin D, which nonenzymatically converts to cholecalciferol, or vitamin D_3. Cholecalciferol undergoes two hydroxylation reactions: the liver converts it to $25(OH)D_3$ and the kidney converts $25(OH)_2D_3$ to $1,25(OH)_2D_3$. PTH hormone stimulates the 1α hydroxylase enzyme in the kidney, whereas high plasma [Pi] inhibits the reaction. The active form of vitamin D, $1,25(OH)_2D_3$ increases Ca and Pi absorption from the intestine and kidney and has multiple effects on bone.

FSG23 is a protein hormone produced by osteocytes in response to PTH, increased plasma Pi, and $1,25(OH)_2D_3$. Its major effects are to inhibit PTH secretion, inhibit production of $1,25(OH)_2D_3$, and increase loss of phosphate in the urine.

REVIEW QUESTIONS

1. What is meant by positive calcium balance? Negative calcium balance? Where is most of the body's calcium found?
2. Why is total plasma $[Ca^{2+}]$ dependent on plasma proteins? How does plasma pH change free plasma $[Ca^{2+}]$? What is regulated?
3. Why is Ca^{2+} homeostasis linked to Pi homeostasis? What is the normal plasma free $[Ca^{2+}]$? What is normal total plasma $[Ca^{2+}]$? What is normal plasma P? Why is the normal plasma [Pi] given as phosphorus instead of phosphate?
4. What is PTH? Where is it secreted? What cells secrete it? What is the stimulation for secretion? What does PTH do (in general terms)?
5. What is CT? Where is it secreted? What cells secrete it? What is the stimulation for secretion? What does CT do?
6. What is vitamin D? Where is it secreted? What is the stimulation for secretion? How is it activated? Where does activation occur? What stimulates activation? What inhibits it?