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# Homeostatic Control of Plasma Calcium Concentration

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**ABSTRACT:** Due to the importance of $Ca^{2+}$ in the regulation of vital cellular and tissue functions, the concentration of $Ca^{2+}$ in body fluids is closely guarded by an efficient feedback control system. This system includes $Ca^{2+}$-transporting subsystems (bone, intestine, and kidney), $Ca^{2+}$ sensing, possibly by a calcium-sensing receptor, and calcium-regulating hormones (parathyroid hormone [PTH], calcitonin [CT], and 1,25-dihydroxyvitamin $D_3$ [$1,25(OH)_2D_3$]). In humans and birds, acute $Ca^{2+}$ perturbations are handled mainly by modulation of kidney $Ca^{2+}$ reabsorption and by bone $Ca^{2+}$ flow under PTH and possibly CT regulation, respectively. Chronic perturbations are also handled by the more sluggish but economic regulatory action of $1,25(OH)_2D_3$ on intestinal calcium absorption. Peptide hormone secretion is modulated by $Ca^{2+}$ and several secretagogues. The hormones' signal is produced by interaction with their respective receptors, which evokes the cAMP and phospholipase C-$IP_3$-$Ca^{2+}$ signal transduction pathways. $1,25(OH)_2D_3$ operates through a cytoplasmic receptor in controlling transcription and through a membrane receptor that activates the $Ca^{2+}$ and phospholipase C messenger system. The calciotropic hormones also influence processes not directly associated with $Ca^{2+}$ regulation, such as cell differentiation, and may thus affect the calcium-regulating subsystems also indirectly.

**KEY WORDS:** parathyroid, calcitonin, vitamin D, bone, kidney, absorption.

## I. INTRODUCTION

The role of $Ca^{2+}$ in controlling a variety of metabolic functions in the body, ranging from muscle contraction to blood coagulation, has been well established. $Ca^{2+}$ also participates in the modulation of hormone secretion and action as a part of the cellular signal transduction pathway (Downes and Mitchell, 1985), and in regulation of the cell cycle (Lu and Means, 1993). Due to the importance of calcium in normal function, maintenance of a steady concentration of $Ca^{2+}$ in cells and in the extracellular space is a main concern of the organism. In effect, $Ca^{2+}$ concentration is one of the most guarded qualities in land vertebrates. A long-term interest of the organism is also to maintain proper bone calcification in order to establish structural support and its $Ca^{2+}$ reservoir.

Regulation of the $Ca^{2+}$ concentration in body fluids is achieved through the action of a complex feedback-control system that includes several subsystems and regulating hormones. A perturbation in plasma cal-

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cium or in any of the control subsystems usually results in a cascade of events with an often obscure temporal hierarchy. Therefore, it is difficult to obtain detailed information on the behavior of subsystems of the Ca control system by *in vivo* experimentation. In the last 3 decades, *in vitro* techniques have been used widely to isolate single components of the calcium-regulating system. Initially, isolated organs such as everted gut sacs (Wilson and Wiseman, 1954), isolated bone (Raisz, 1963), and kidney slices (Chase and Aurbach, 1967) have been used. Isolated organs *in situ* such as intestinal loops (Wasserman, 1963) or perfused endocrine glands (Copp et al., 1972) were also studied. With the development of culture techniques, cellular and subcellular preparations have been used to study components of the calcium control system. These include bone cells (Rodan and Rodan, 1974), cartilage cells (Pines and Hurwitz, 1988), parathyroid cells (Brown et al., 1976), kidney cells (Bar et al., 1980), and intestinal cells or brush border vesicles (Rasmussen et al., 1979). Some information acquired by *in vitro* techniques, especially that related to diagnosis of pathological states, can be implemented as such. However, for full physiological comprehension of the mode of operation of the control system, the mosaic of information obtained by *in vitro* experimentation ought to be assembled and treated in the context of the entire organism. Simulation algorithms are useful tools for such integration, as is discussed further.

## II. THE CONTROLLED SIGNAL — PLASMA $CA^{2+}$ CONCENTRATION

### A. State of Plasma Calcium

The plasma calcium concentration of land vertebrates (except for female birds and reptiles during reproduction) is maintained at approximately 2.5 m*M* (10 mg/dl). In mammalian species, ionic $Ca^{2+}$ is close to one half of the total plasma calcium. Although most of the remainder is protein bound, some $Ca^{2+}$ forms complexes with small molecules (Table 1). Most of the protein-bound calcium is associated with plasma albumin, which binds approximately 0.84 mg of $Ca^{2+}$ per gram according to Rawson and Sunderman (1948), but only 0.34 mg/g according to Müller-Plathe and Lindemann (1983). As suggested in a pioneering study of McLean and Hastings (1935), ionization of $Ca^{2+}$ can be described, at least as a first approximation, by the mass law,

$$\frac{[Ca^{2+}][Prot^{2-}]}{[Ca\ \ Prot]} = K$$

This simple relationship is affected by factors such as plasma pH and bicarbonate concentrations.

In female birds during reproduction or in male estrogenized birds, the total plasma calcium concentration may exceed 7.5 m*M* due to the appearance in the circulation of $Ca^{2+}$ complexed to vitelogenin — a ≈500-kd lipophosphoprotein (Tata and Smith, 1979) that is the precursor of the main protein fractions of the egg yolk; it is not related to the formation of the calcified egg shell.

**TABLE 1**
**State of Plasma Calcium in Mammals**

| Fraction | m*M* |
|---|---|
| Free ions | 1.18 |
| Protein bound | 1.14 |
| Calcium phosphate | 0.04 |
| Calcium citrate | 0.04 |
| Unidentified complexes | 0.08 |

From Walser, M. 1961. *J. Clin. Invest.* 40: 723–730. With permission.

## B. Efficacy of Plasma Calcium Regulation

The efficacy of regulation of plasma calcium in normal individuals can be evaluated by introduction of either an acute or a steady-state perturbation. After an increase by a bolus intravenous injection of $Ca^{2+}$, the plasma calcium concentration in the growing bird falls exponentially and returns to normal after approximately 40 min (Hurwitz et al., 1983). Also in growing chicks, the steady-state concentration of calcium as a function of dietary calcium intake is reminiscent of buffer titration (Figure 1) within a wide range of dietary calcium intake. The regulatory capacity is overwhelmed at the very low or very high levels of dietary intake. The ability to maintain plasma calcium in the face of acute or steady-state perturbations is lost when essential parts of the regulatory machinery are compromised, for example by parathyroidectomy, vitamin D deficiency, or when the vitamin D control system is bypassed (Hurwitz et al., 1984). Malfunctions of parts of the calcium control mechanisms are at the basis of various metabolic disorders in which hyper- or hypocalcemia are the main pathological manifestations.

## C. Oscillatory Behavior of Plasma Calcium

Deviations from the "normal" value of plasma calcium of around 10 mg/100 ml have been considered as errors of the controlling system. However, temporal oscillations corresponding to circadian rhythms have been observed for plasma calcium and some of its controlling systems in growing rats (Staub et al., 1988), dogs (Wong and Klein, 1984), humans (Carruthers et al., 1964; Halloran et al., 1985; Jubitz et al., 1972; Markowitz et al., 1981), and chickens (Hurwitz et al., 1994; Miller and Norman, 1979).

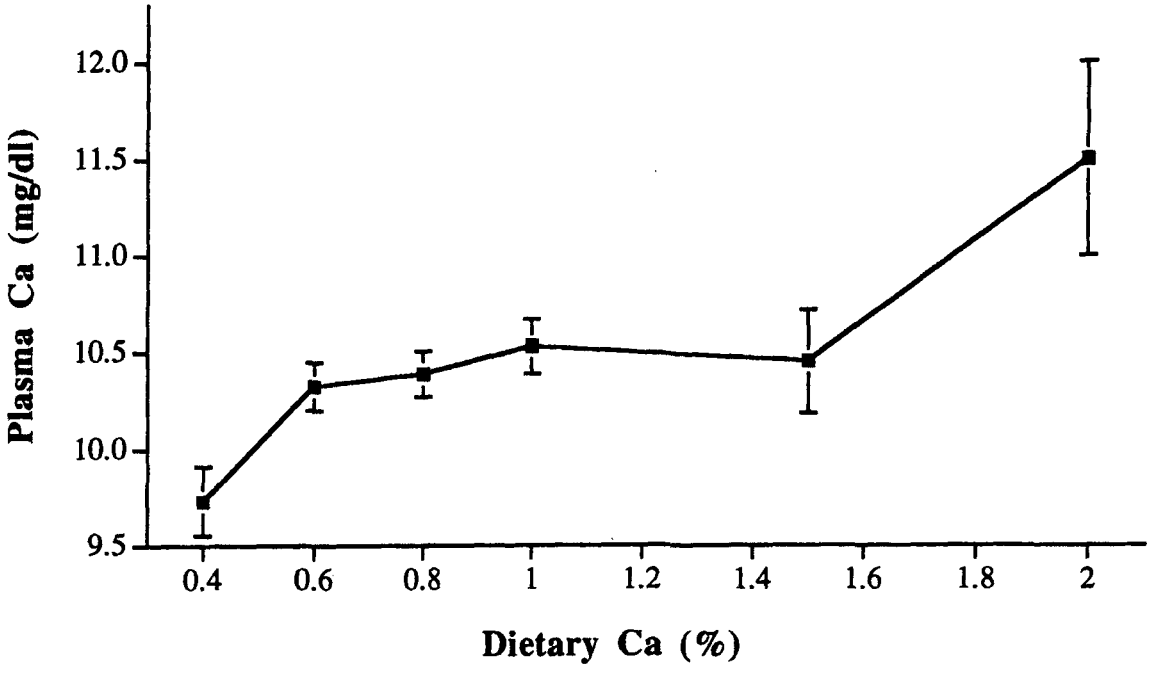



**FIGURE 1.** Plasma $Ca^{2+}$ concentration as a function of dietary Ca intake in chicks. (From Hurwitz, S., Plavnik, I., Shapiro, A., Wax, E., Talpaz, H., and Bar, A. 1995. *J. Nutr.* In Press. With permission.)

In many regulated biological systems, a spontaneous periodic behavior, rather than maintenance of constant values, is considered the normal mode of operation, conferring a functional advantage for the organism because it may be a part of the anticipatory (predictive) regulation mechanisms (Rapp, 1987; Moore-Ede, 1986). An advantage to calcium homeostasis provided by the circadian rhythm in the two major controlling systems, bone to blood calcium flow and the activity of 25-hydroxyvitamin $D_3$-1-hydroxylase, has been suggested (Hurwitz et al., 1994). Although opinions on the mechanism responsible for the generation of these oscillations differ, it is generally agreed that the oscillations are the consequence of the activity, rather than being errors of the calcium-regulating system. According to Staub et al. (1988), oscillations in plasma calcium are the manifestation of the self-organizing properties of the process of bone calcification. Hurwitz et al. (1987a), based on computer simulation, suggested that spontaneous oscillations in the Ca-regulating system were induced by the process of growth, and that the action of the major calcium-regulating hormone (i.e., parathyroid hormone, PTH) on several of the controlling systems was the determinant of their evolvement. The importance of intact parathyroid glands for inducing plasma calcium oscillations has been demonstrated experimentally in dogs (Wong and Klein, 1984).

Oscillations of a different nature in the Ca-regulating system in reproducing female birds result from rhythmic perturbations associated with the schedule of the egg shell. This type of oscillatory behavior is reflected by plasma calcium (Taylor and Hertelendy, 1961), calcium absorption (Hurwitz and Bar, 1965; Hurwitz et al., 1973), and uterine (shell gland) calcium transport and calbindin-$D_{28k}$ gene expression (Bar et al., 1992).

## III. REGULATION OF PLASMA CALCIUM

### A. The Ca Control System

Regulation of plasma $Ca^{2+}$ is schematically represented in Figure 2. The control system consists of three main subsystems: intestine, bone, and kidney. The net transport of $Ca^{2+}$ through the intestinal epithelium ($F_i$) is the only route of entry of $Ca^{2+}$ from the exterior. The net transport of $Ca^{2+}$ by the kidney ($F_k$) is the means of $Ca^{2+}$ removal to the exterior. Bone may remove $Ca^{2+}$ from the central plasma pool by the process loosely defined as bone formation, and return $Ca^{2+}$ to the system by resorption. $F_b$ defines the net $Ca^{2+}$ flow of bone. The change in total plasma $Ca^{2+}$ (M) is then given by the sum of the flows

$$dM/dt = F_i - F_k - F_b - k\Delta Ca \quad (1)$$

which, after integration and division by the blood volume ($V_b$), results in the plasma $Ca^{2+}$ concentration [Ca]. In addition, plasma $Ca^{2+}$ equilibrates rapidly (k) with extracellular $Ca^{2+}$ ($M_e$). The rate of equilibration is driven by the difference in $Ca^{2+}$ concentration between plasma and the extracellular fluid ($\Delta Ca$). When integrated and divided by the volume of extracellular fluid ($V_e$), the concentration of $Ca^{2+}$ in the extracellular fluid ($C_e$) is obtained.

The plasma $Ca^{2+}$ concentration is sensed by $Ca^{2+}$ receptors (CaR) probably. [The kidney responds directly to $Ca^{2+}$ by modulating its $Ca^{2+}$ excretion ($F_k$) (1) by the C-cells (ultimobranchial), which in response may vary calcitonin (CT) secretion, (2) by bone, which modifies its $Ca^{2+}$ flow ($F_b$), (3) by the parathyroid gland (PT) with the secretion of parathyroid hormone (PTH), (4) and by the kidney hydroxylase enzyme system (OH-ase), which is responsible for the pro-

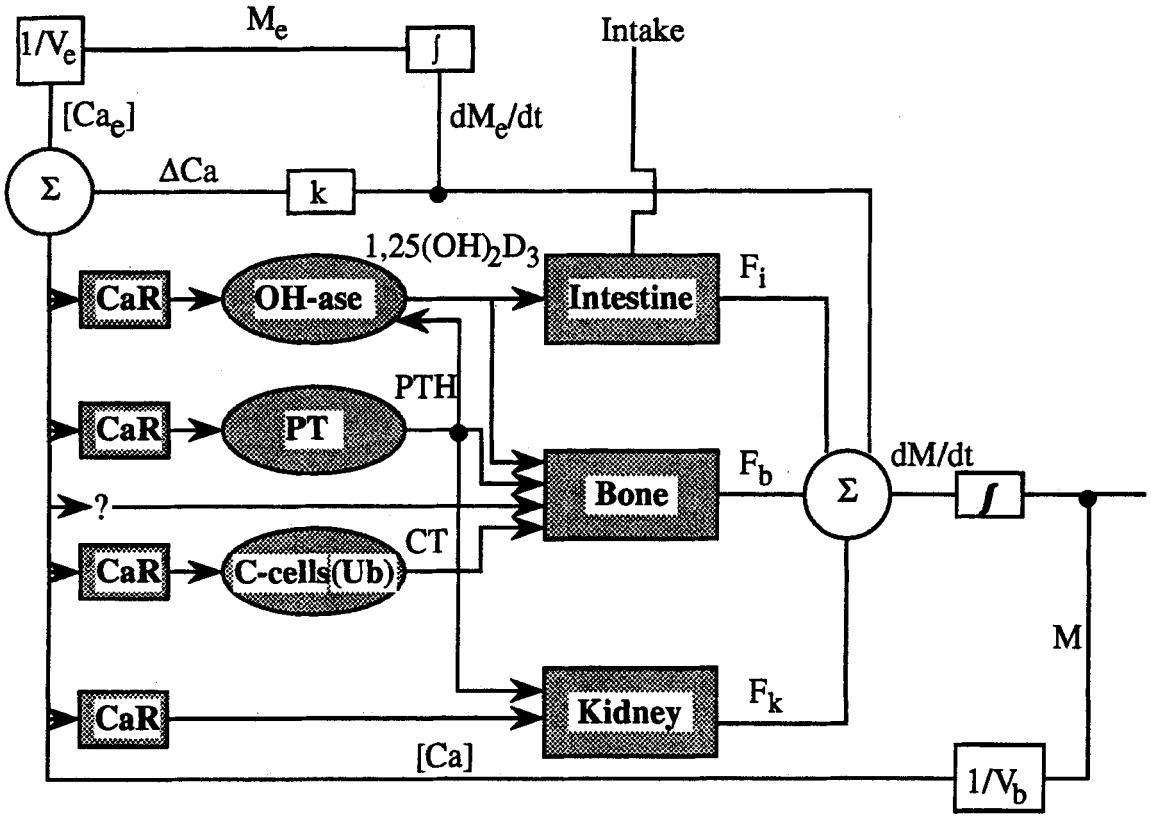



**FIGURE 2.** Schematic representation of regulation of plasma $Ca^{2+}$. The sum (Σ) of the net Ca flows from intestine ($F_i$), bone ($F_b$), and kidney ($F_k$) is the change in total plasma Ca, (dM/dt), which when added to existing plasma Ca and divided by the blood volume ($V_b$), yields the plasma Ca concentration (Ca). Plasma Ca exchanges rapidly with extracellular Ca ($M_e$); when divided by the volume ($V_e$), the extracellular Ca concentration ($Ca_e$) is obtained. Plasma Ca, after reacting with the Ca-sensing receptor (CaR), determines Ca excretion ($F_k$), bone Ca flow ($F_b$), calcitonin (CT) secretion by the ultimobranchial gland (UB), parathyroid hormone (PTH) secretion from the parathyroid gland (PT), and production of $1,25(OH)_2D_3$ by the kidney 25-hydroxyvitamin $D_3$-1-hydroxylase (OH-ase). Bone flow and $1,25(OH)_2D_3$ production are mainly controlled by PTH. (Modified from Hurwitz, S., Fishman, S., and Talpaz, H. 1987a. *Am. J. Physiol.* 252: R1173–R1181. With permission.)

duction of 1,25-dihydroxyvitamin $D_3$ [$1,25(OH)_2D_3$] (5). PTH regulates bone $Ca^{2+}$ flow ($F_b$), the production of $1,25(OH)_2D_3$, and the urinary $Ca^{2+}$ excretion. Bone $Ca^{2+}$ flow may also be influenced by calcitonin (CT). Intestinal $Ca^{2+}$ absorption is regulated by $1,25(OH)_2D_3$.

## B. Calcium Sensing

The sensing of extracellular $Ca^{2+}$ concentration is the first step in its feedback regulation. The relationship between the sensor output and the $Ca^{2+}$ concentration and the intensity of its produced signal are the main determinants of the so-called reference value or setpoint.

Initially it was theorized that modulation of the intracellular $Ca^{2+}$ concentration as a result of changes in extracellular $Ca^{2+}$ was the means of $Ca^{2+}$ sensing. However, the existence of a specialized $Ca^{2+}$-sensing molecule at the cell surface was predicted on the basis of a large volume of evidence (Brown, 1991). The Ca sensing receptor has been identified in bovine parathyroid cells

and has been cloned (Brown et al., 1993). The receptor is made of a large extracellular domain with several acidic amino acid residues that are probably involved in $Ca^{2+}$ binding. This domain is coupled to a seven-spanning membrane domain similar to other receptors of the G-protein-coupled receptor superfamily. The association of $Ca^{2+}$ with its receptor stimulates phosphoinoside-specific phospholipase C, resulting in the accumulation of inositol 1,4,5-triphosphate ($IP_3$) and diacylglycerol (DAG) (Kifor et al., 1992; Shoback et al., 1988). It also stimulates the release of $Ca^{2+}$ from intracellular stores and entry of $Ca^{2+}$ from the extracellular space (Brown et al., 1991), possibly by stimulating the activity of voltage-sensitive $Ca^{2+}$ channels (Muff et al., 1988).

Molecular techniques have been used to localize the receptor in kidney and brain (Brown et al., 1993). It has also been recently identified in the C cells of the thyroid gland (Garret et al., 1996). Indirect evidence also points to its presence in bone cells such as osteoclasts (Shankar et al., 1993), osteoblasts (Leis et al., 1994), and osteocytes (Kamioka et al., 1994), and in the human placenta (Lundgren et al., 1994).

Study of the regulation of the $Ca^{2+}$-sensing receptors has only begun. In a first study, Rogers et al. (1995) was unable to detect any relationship between the $Ca^{2+}$-sensing receptor level in parathyroid cells of vitamin D-deficient rats and the plasma Ca or $1,25(OH)_2D_3$ concentrations.

It is tempting to attribute the regulatory responses to $Ca^{2+}$, including PTH secretion, to the activity of the $Ca^{2+}$-sensing receptor. However, the steps linking between the receptor and the regulatory function remain to be elucidated.

## C. The Regulating Hormones

In the context of a feedback system, a quantitative relationship ought to exist between the rate of secretion of the regulating hormone and the controlled entity on the one hand, and between the response of the control system and the concentration of the regulating hormone on the other. The cellular response may also be modified by up- or downregulation of receptor number or affinity. Calcium metabolism is regulated by peptide and steroid hormones. Peptide hormone secretions (PTH and CT) respond to the plasma $Ca^{2+}$ concentration within minutes. This and the short half-life of the peptide hormones qualify them to deal with acute perturbations by transient actions (in the time range of minutes to hours), although they also may have long-term effects through activating other regulating hormones with slower response times. Bone is the immediate target for the rapid action of either PTH or CT. PTH stimulates bone resorption, thereby increasing $Ca^{2+}$ flow from bone to circulation. CT acts in the opposite direction by inhibiting bone resorption. The action of PTH may be considered noneconomical from a nutritional viewpoint because it involves a loss of calcium from bone. The reduction in bone resorption by CT also is not desirable because it can result in a decrease in bone turnover and a diminution of its calcium regulatory capacity, and in the decrease in the concentration of plasma phosphate, which in turn may impede bone formation. Under conditions of a sustained perturbation, production of the steroid hormone $1,25(OH)_2D_3$ is modulated by PTH, resulting in changes in intestinal calcium absorption and in bone balance. The physiological action of $1,25(OH)_2D_3$ becomes evident only after several hours, and its half-life is also in the range of hours (Hurwitz et al., 1983). Because $1,25(OH)_2D_3$ causes a change in the flow of $Ca^{2+}$ into circulation from the environment by controlling intestinal absorption, its action may be considered beneficial to the overall calcium economy of the organism.

## 1. Parathyroid Hormone (PTH)

### a. Source of PTH — The Parathyroid Glands

PTH is secreted from the chief cells of the parathyroid glands. It has been accepted that these glands develop from the third, fourth, and fifth embryonic pharyngeal pouches and migrate to a posterior position during embryonic development. Recent work, however, suggests that the glands in humans and chickens are actually of ectodermal placoidal origin (Mérida-Velasco, 1991). The anatomical location of the gland may vary among species and even within a single species. For example, in the human and rat, the glands are usually located on the thyroid gland. However, two pairs of parathyroid glands are found in the human, whereas only two single glands are found in the rat. In birds, two pairs of glands are found at the border of the thoracic cavity, just caudal to the thyroid gland. In humans, the location of the parathyroid glands may vary from the angle of the jaw to the heart and posterior mediastinum. In many cases, parathyroid gland localization requires highly specialized techniques (Eisenberg et al., 1989).

### b. Structure of PTH

The first active PTH preparation was obtained by Collip (1925) using HCl extraction. Full-length PTH has been isolated, and its amino acid sequence has been determined in the bovine (Brewer and Ronan, 1970), human (Keutman et al., 1978), and pig (Sauer et al., 1974). The amino acid sequence of the rat (Heinrich et al., 1984) and chicken preproPTH sequence was deduced from the nucleotide sequence (Khosla et al., 1988; Russell and Sherwood, 1988). The chain of the mammalian peptide is 84 amino acids long, whereas the avian hormone sequence includes 88 amino acids (Figure 3). A high degree of homology exists for the full-length amino acid sequence in the mammalian hormone. About 60% homology between the human and avian hormone can be found in the 1 to 34 amino terminal sequence, within which the 1 to 14 sequence contains only two substitutions.

An analog of PTH, parathyroid hormone-related protein (PTHrP), has been characterized recently as a cause of the hypercalcemia of malignancy (reviewed by Strewler and Nissenson, 1994). This protein was found in three isoforms of 139, 141, and 173 amino acids. The sequence homology of PTHrP with PTH is high at the amino terminus (1 to 13). Beyond this range, little homology exists even at the region of amino acids 18 to 34 that is considered to be important for receptor binding. Nevertheless, both peptides bind to the PTH receptor with a similar affinity (Orloff et al., 1989). The ability of PTHrP and also avian PTH to bind to PTH receptors, despite the limited homology of the primary sequence, hints at similarities among the peptides in their secondary structures. Indeed, computer algorithms predicted similar α-helices and β-turns for both PTH and PTHrP (Chorev and Rosenblatt, 1994). Due to the low circulating levels and the lack of relationship between its circulating level and plasma $Ca^{2+}$, PTHrP is not considered to be a calciotropic hormone, and therefore is not reviewed in the present context.

The sequence of 1 to 34 is believed to contain most of biological activity of PTH, although some effects of the midhormone and C-terminal regions have been reported (Somjen et al., 1990). However, full-length avian PTH was more effective in stimulating aldosterone secretion than the 1 to 34

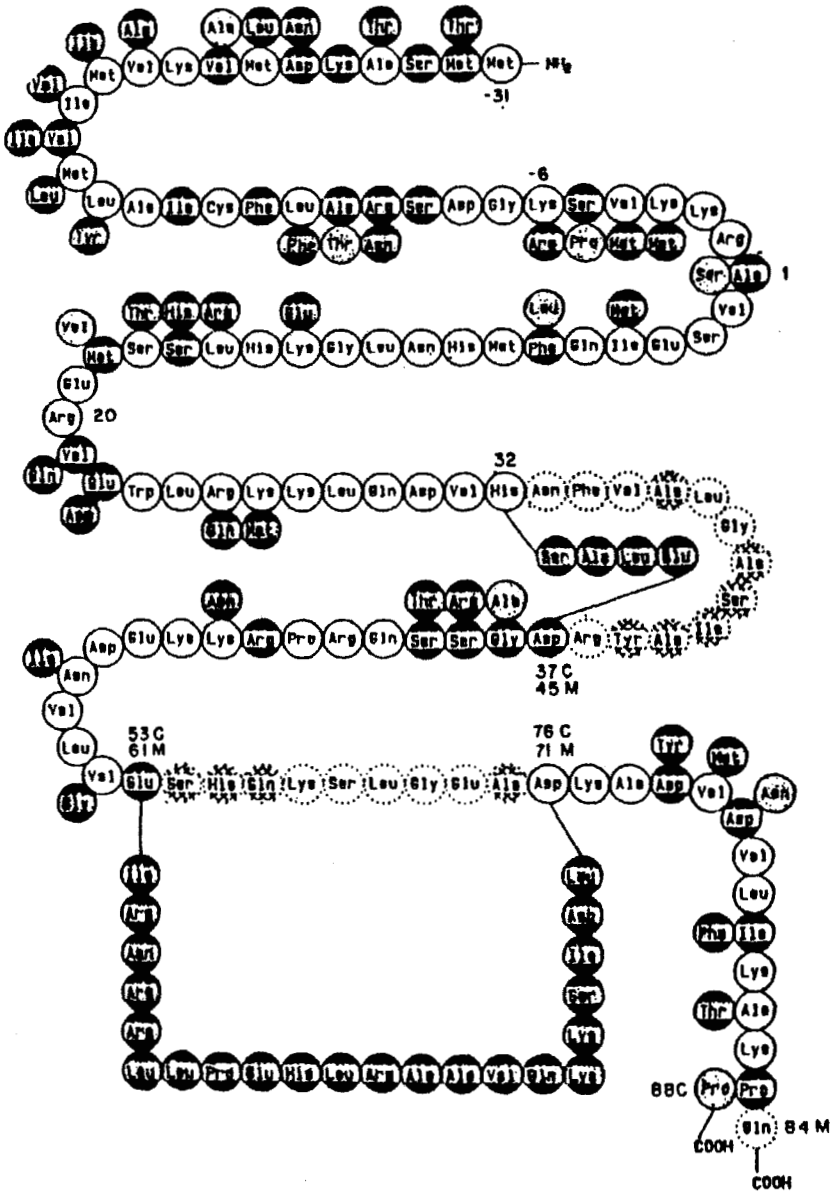


**FIGURE 3.** Comparative sequence of parathyroid hormone. The sequence of bovine parathyroid hormone is given in circles. A barred or hatched circle indicates a position at which the amino acid varies among the mammalian hormones (human, bovine, porcine, and rat); open circles indicate invariant mammalian residues. Stippled circles indicate sites where the sequence of chicken preproPTH is different from bovine but identical to one of the other mammalian hormones. Barred stippled circles indicate an amino acid unique to chicken preproPTH. Dotted circles represent amino acids that apparently have been deleted in the chicken sequence and replaced by unique peptides that are joined to the rest of the sequence by lines. 1, the first residue of mature PTH. M and C after numbers refer to mammalian and chicken sequences, respectively. (From Khosla, S., Demay, M., Pines, M., Hurwitz, S., Potts, J. T., Jr., and Kronenberg, H. M. 1988. *J. Bone Miner. Res.* 3: 689–698. With permission.)

peptide (Rosenberg et al., 1987). Three regions have been identified on the 1 to 34 peptide: the sequence of 1 to 6 is considered to be the activation domain, and the 7 to 34 is the inhibitory domain that contains the principal binding sequence of 25 to 34

(Mahaffey et al., 1979; Nussbaum et al., 1980). The sequence beyond amino acid 34 was found to be essential for proper intracellular processing of the hormone in the parathyroid cell (Lim et al., 1992).

### c. Control of PTH Secretion

Overall hormone secretion is the sum of secretion of all glandular cells. Thus, secretion may be regulated by modulating the rate of secretion of the individual cell, or by changing cell number.

According to Parfitt (1994), regulation of the size of the parathyroid gland has been poorly investigated despite its utmost importance in calcium homeostasis. *In vitro,* a low $Ca^{2+}$ concentration causes an increase in parathyroid tissue growth (Raisz, 1963). In normal animals, a chronic calcium stress or growth and reproduction may lead to gland hypertrophy and hyperplasia. Conversely, a chronic excess of calcium in the system results in glandular involution, probably through apoptosis (Parfitt et al., 1994). In the rat (Luce, 1923) and chicken, either calcium (Hurwitz and Griminger, 1961) or vitamin D (Bar et al., 1972) deficiencies lead, within a few days, to large increases in parathyroid size. It is, however, not clear if the increase in size is due to hypertrophy or hyperplasia. Lee and Roth (1975) observed increased mitosis and DNA synthesis in response to low ambient $Ca^{2+}$. Other studies (Kremer et al., 1989; LeBoff et al., 1983) found no change in cell proliferation in parathyroid cells in response to ambient $Ca^{2+}$. Repletion with the deficient agent (vitamin D and/or calcium) leads to a rapid regression of gland size (Russell et al., 1993). *In vitro,* $1,25(OH)_2D_3$ treatment also resulted in the depression of induced parathyroid cell proliferation, by a mechanism apparently independent of ambient $Ca^{2+}$ (Kremer et al., 1989).

Only a fraction of parathyroid cells respond to stimulation at any time. The greater the drain of hormone, the more cells are recruited to release the hormone (Sun et al., 1993). This could also serve in the regulation of hormone release from the gland together with the response of the individual cell and the total number of cells.

The preproPTH is the complete translation product of the specific mRNA. As reviewed by Habener et al. (1984), Kemper (1986), and Hurwitz (1989), synthesis of the message is followed by transcriptional and posttranscriptional processing. The MET-MET residue of the N terminal of the preproPTH is cleaved off soon after the emergence on the ribosome. The leader sequence of 23 amino acids is cleaved, probably during insertion into the membrane of the endoplasmic reticulum, and the pro sequence of six amino acids is cleaved off after transport to the Golgi apparatus. The hormone is then enclosed within secretory granules and released into the cytoplasm, ready for exocytosis. According to Cohn and Elting (1983), proPTH appears in the endoplasmic reticulum within 1 min after initiation of synthesis, its arrival at the Golgi apparatus occurs after about 15 min, and secretion can commence within 30 min.

Exocytosis is initiated by the fusion of the secretory granule to the plasma membrane (DeLisle and Williams, 1986). The membrane is then disrupted by changes in the cellular microskeleton, possibly with the aid of changes in membrane potential (Bruce and Anderson, 1979) or by osmotic forces (Brown et al., 1978).

Ambient $Ca^{2+}$ is the classic modulator of PTH secretion, possibly operating through the calcium-sensing receptors discussed above. In isolated bovine parathyroid cells *in vitro* (Brown et al., 1976) and *in vivo* (Mayer et al., 1979), a sigmoidal relationship between PTH secretion and ambient $Ca^{2+}$ was observed. This relationship can be

represented by a four-compartment model (Brown, 1983),

$$V_s = D + \frac{A - D}{\left(1 + \frac{Ca}{C}\right)^B}$$

where Ca is the ambient Ca concentration, A is the minimal secretion rate not suppressible by ambient Ca, C is the midpoint of the Ca-suppressed component, and the exponent B determines the slope of the function. With the newly gained information on the interaction of $Ca^{2+}$ with its sensing receptor, and considering the shape of the function, this model can be replaced with another based on $Ca^{2+}$-binding kinetics. Because the plasma ionic $Ca^{2+}$ concentration is 1.2 to 1.5 m*M*, higher than the calculated midpoint of response of 0.95 m*M* of $Ca^{2+}$, the system does not operate symmetrically. A shift in the curve to the right was observed in parathyroid adenomas (Brown, 1983), in association with the exhibited hypercalcemia. In normal neonatal calves, the curve is shifted to the left with age, in association with a decrease in the normal plasma calcium concentration (Keaton et al., 1978).

Extracellular $Ca^{2+}$ affects several of the cellular messengers — $Ca^{2+}$ itself, diacylglycerol and phosphokinase C (Kobayashi et al., 1988), $IP_3$, and also cAMP (Brown 1991, 1994), probably as a result of its interaction with the calcium-sensing receptor. However, Miki et al. (1995) attributed regulatory responses also to promotion of the activity of $Ca^{2+}$ channels, leading to oscillations in intracellular $Ca^{2+}$. Because several responses of parathyroid cells to extracellular $Ca^{2+}$ are known, any of several of the cellular messengers may effect a different regulatory response.

$Ca^{2+}$ may inhibit PTH secretion using several mechanisms: (1) by interfering with PTH transcription (Russell et al., 1983, 1993), probably through a negative calcium-response element (nCaRE-PTH) that is located at the upstream flanking region of the PTH gene (Okazaki et al., 1992), (2) by inhibiting exocytosis via modulating membrane polarization by the $K^+$ channel activation (Kanazirska et al., 1995), or (3) modulation of intracellular degradation of the hormone (Hanley et al., 1978, 1986).

Several hormones such as catecholamines (Brown et al., 1977), dopamine (Attie et al., 1980), and secretin (Windeck et al., 1978) were found to stimulate PTH secretion, apparently through cAMP mediation. Cholera toxin, an activator of the ademylate cyclase enzyme system, stimulated cAMP production in bovine (Brown et al., 1979) and avian (Pines and Hurwitz, 1981) parathyroid cells, and augmented PTH secretion. This interaction of the PTH system with other membrane-active hormones may be part of a predictive control system that would protect the organism against anticipated hypo- or hypercalcemia. For example, Fischer et al. (1982) theorized that the response of the parathyroids to catecholamines may be related to elevated circulating PTH under some stress conditions, such as exercise (Vohra et al., 1983). However, the physiological significance of the interaction of these hormones has not been elucidated.

In view of the vitamin D-parathyroid interactions, a direct action of the $1,25(OH)_2D_3$ in the parathyroid gland could be expected. During vitamin D deficiency, the parathyroid glands of chicks become grossly hypertrophied (Bar et al., 1972). The hormone is present in the parathyroid gland (Henry and Norman, 1975), where a 3.3S receptor for $1,25(OH)_2D_3$ was identified (Pike et al., 1980); this suggests that the gland is a target organ for the hormone. In parathyroid cells treated with $1,25(OH)_2D_3$, PTH secretion decreases as a consequence of the reduction in specific mRNA synthesis (Cantly et al., 1985; Russell et al., 1986).

Suppression of preproPTH gene expression by $1,25(OH)_2D_3$ was demonstrated also in intact rats by Naveh-Many et al. (1990) and in the intact chick by Russell et al. (1993). A direct suppression of preproPTH gene expression by $1,25(OH)_2D_3$ could be a consequence of activation of sequences upstream of the PTH gene that bind to the vitamin D receptor (Demay et al., 1992). However, a significant portion of the effects attributed to $1,25(OH)_2D_3$ may in fact be secondary to the effects of the hormone on the cellular transport of $Ca^{2+}$, as suggested by Russell et al. (1993) and Brown et al. (1995), and as discussed in Section III.C.3.g.

Similarly to other peptide hormones, PTH is degraded and its fragments are excreted in the urine (reviewed by Arnaud and Pun, 1992). Some of the hormone degradation within the parathyroid cells is affected by ambient $Ca^{2+}$ (Habener et al., 1975; Mayer et al., 1979). Furthermore, secretion of the inactive C terminal is increased and that of the active hormone decreases in proportion to ambient $Ca^{2+}$ (Hanley and Ayer, 1986; D'Amour et al., 1992). Once secreted, the hormone is taken up by the liver, where it is broken down into C-terminal and N-terminal fragments, similar to those that are present in the circulation (Canterbury et al., 1975). The fragments are ultimately degraded by the renal tubular cells (Hesch et al., 1978) and cleared by filtration (Daugaard et al., 1994). Degradation of PTH fragments, and especially of the intact hormone by the liver and kidney, was greater at a high than at a low concentration of $Ca^{2+}$ (Daugaard et al., 1990). The half-life of the intact hormone is 10 min, and that of the 1 to 34 PTH is 2 min (Neuman et al., 1979; Schneider et al., 1980). The C-terminal fragments have longer half-lives than the intact hormone (Silverman and Yalow, 1973); this may explain differences in the relative distribution of the fragments under various physiological and pathological states.

### *d. The PTH Receptor*

Similarly to other peptide hormones, the action of PTH is initiated by its binding to specific membrane receptors (Kolakowski et al., 1991). Complementary DNA encoding the receptor for PTH and PTHrP from rat osteoblast-like cells, opossum kidney cells, and human osteoblast-like cells has been cloned (Abou-Samra et al., 1992; Jüppner et al., 1991; Schipani et al., 1993). The sequences of the receptor in kidney and bone of humans appeared identical (Schipani et al., 1989), although some binding characteristics were different in rat bone and kidney receptors (Muff et al., 1994). On the basis of nucleotide homology, Segre and Goldring (1993) have shown that the PTH receptor belongs to the category of seven membrane-spanning receptors within the G-protein-linked receptor superfamily, which includes secretin, calcitonin, vasoactive intestinal peptide (VIP), glucagon-like peptide 1, growth hormone-releasing hormone, and glucagon. The structure and functions of these receptors have been reviewed recently (Strader et al., 1994). PTH receptor was found in the classical target organs such as kidney and bone, but also in other tissues such as aorta, adrenal gland, bladder, brain, intestine, skeletal muscle, etc., where it is probably a component of the paracrine/autocrine system with PTHrP. Physiological responses to PTH in the vascular bed have been demonstrated in mammals (McCarron et al., 1984), birds, reptiles, amphibians, and lungfish (Pang et al., 1980).

The PTH receptor and receptor binding are downregulated in kidney cells (Abou-Samra et al., 1994) and osteoblast-like cells (Okano et al., 1994) by exposure to PTH. Because a profound hyperparathyroidism develops during vitamin D-deficiency hypocalcemia, the downregulation of PTH receptors (Carnes et al., 1980) by PTH may be the reason for the refractoriness to PTH

with regard to its calcemic (Harrison and Harrison, 1963; Gonnerman et al., 1975) or 25-hydroxyvitamin $D_3$-1-hydroxylase responses (Booth et al., 1985). However, according to Liang et al. (1984), the decrease in the number of receptors cannot explain the entire refractoriness of phosphate excretion to PTH during vitamin D deficiency. The upregulation of PTH receptors observed in the kidney of chickens during reproduction was attributed by Forte et al. (1983) to the action of estrogen. Changes in receptor abundance also occur in response to other physiological factors such as cell age and state of differentiation (Rouleau et al., 1988).

### *e. PTH Binding and Signal Transduction*

Nissenson and Arnaud (1979) and McKee and Murray (1985) have described a high-affinity PTH receptor coupled to adenylate cyclase. A low-affinity binding site has also been described (Muff et al., 1992; Murray et al., 1994). Early evidence (Aurbach and Heath, 1974; Aurbach, 1982; Chase and Aurbach, 1970) suggested that cAMP was the messenger of PTH action in target cells. The stimulation by PTH of cAMP production by the kidney tubular cells (Chase and Aurbach, 1967) results in excretion of this cyclic nucleotide in the urine of mammals (Brodaus, 1981) and chickens (Pines et al., 1983). Several actions of PTH on kidney can be mimicked by cAMP derivatives or promoters; this includes the stimulation of $Ca^{2+}$ reabsorption by the distal tubule (Boutiauy et al., 1991) and the downregulation of the PTH receptor (Abou-Samra et al., 1991). The sites along the nephron of cAMP production in response to PTH were found to be correlated well with sites of PTH action (Jand and Robert, 1974; Kawashima and Kurokawa, 1983). However, the $K_d$ for PTH inhibition of $Na^+$/phosphate cotransport in OK cells was considerably lower than for adenylate cyclase activation (Quamm et al., 1989), and PTH-induced inhibition of $Na^+$-$K^+$-ATPase activity was dissociated from cAMP generation (Ribiero and Mandel, 1992). Thus, it has been shown that in addition to cAMP, both inositol phosphate (Rappoport and Stern, 1986) and $Ca^{2+}$ (Filburn and Harrison, 1990), following activation of phospholipase C, are involved in PTH signal transduction in kidney and in bone cells (Civitelli et al., 1988; Dunlay and Hruska, 1990; Abou-Samra et al., 1992). It is of importance that the N-terminal sequence was found to be unique in receptor activation (Pines et al., 1994); the activation of the two signal transduction systems by PTH could be the result of coupling to two types of G proteins. The relative importance of either receptor-G protein complex in eliciting any of the physiological responses to PTH has not been determined. As shown by Kurokawa et al. (1992), the dose response curves for the various signals may be orders of magnitude apart. The DAG and the $IP_3$ signals show 50% response at picomolar concentrations of PTH, whereas 50% of the cAMP and $Ca^{2+}$ responses occurs at nanomolar concentrations. Although 50% inhibition of phosphate uptake is similar to the $IP_3$ signal, the PTH effect can be mimicked by cAMP analogs. PTH receptor downregulation cannot be produced by phorbol esters (Okano et al., 1994), although it can be modified by the action of phosphokinase C (Kitten et al., 1994). The effect of the hormone on tubular $Ca^{2+}$ reabsorption appears to require both signal transduction pathways (Friedman and Gesek, 1993). Thus, considerably more information on the molecular events that occur along the signal transduction pathways and the physiological responses should be gathered before the mechanism of the PTH signal transduction is understood.

### f. Physiological Actions of PTH

PTH is an essential component of the system of regulation of plasma calcium. On the one hand, PTH secretion responds rapidly in the reciprocal direction to changes in plasma calcium. On the other, the hormone causes a proportional increase in plasma calcium. Parathyroidectomy results in a rapid decline in plasma calcium in humans as well as other mammalian and avian species. Growing chicks can hardly survive parathyroidectomy due to the diminution of plasma calcium to levels lower than 4 mg/100 ml (Bar et al., 1972). The main actions of PTH within the context of extracellular calcium homeostasis are (1) stimulation of $Ca^{2+}$ flow from bone to blood, including osteoclastic bone resorption, and at low levels also stimulation of bone formation; (2) stimulation of $1,25(OH)_2D_3$ production, leading to the increase in intestinal absorption of $Ca^{2+}$; and (3) augmentation of the renal tubular $Ca^{2+}$ reabsorption. These are discussed in detail in the sections describing those systems.

Indirectly important in calcium homeostasis is the phosphaturia induced by PTH. PTH inhibits phosphate transport at the brush border of tubular cells, leading to a reduced renal tubular phosphate reabsorption (Kinoshita et al., 1986). A possible association between the handling of $Na^+$ and phosphate in the kidney has been inferred by the suppression by PTH of $Na^+$ and water reabsorption (Aurbach and Heath, 1974; Wideman and Youtz, 1985). Kumegawa et al. (1985) found inhibition of the $Na^+/H^+$ exchange, and Pollock et al. (1986) reported reduced $Na^+$-$H^+$ antiporter activity. PTH affected the $Na^+$/phosphate cotransport system at an apical site of cells of the proximal tubule (Muff and Fisher, 1992). The hormone also induces smooth muscle relaxation and consequently a reduction in blood pressure (Pang et al., 1980). In adrenocortical cells, PTH stimulates corticosterone and aldosterone secretion (Rosenberg et al., 1987, 1989). These actions are not reviewed in detail.

## 2. Calcitonin

The existence of "calcitonin", a peptide hormone that lowers plasma calcium, was first deduced by Copp et al. (1961), who later isolated the hormone from fish and chicken glands (Copp et al., 1967). Hirsch et al. (1964) established the thyroid origin of the hormone in the rat and termed the hormone "thyrocalcitonin".

### a. Source of Calcitonin

Pearse et al. (1966) discovered the C-cell origin of CT and showed that the C cells residing in the mammalian thyroid were of ultimobranchial origin (Pearse et al., 1967). Tauber (1967) identified the ultimobranchial origin of this substance in birds. The ultimobranchial glands are derived from the fifth endodermal pharyngeal pouch (Mérida-Velasco et al., 1989). The glands contain granular cells that produce CT (Isler, 1973). The cells respond by hypertrophy and hyperplasia to hypercalcemia and by a decrease in secretory activity during prolonged hypocalcemia (Bélanger, 1971). During hypocalcemia induced by vitamin D deficiency, the number of secretory cells decreased, but the remaining cells maintained CT biosynthetic activity, as indicated by CT gene expression (Eliam-Cisse et al., 1993).

### b. Structure and Biosynthesis

The hormone has been isolated and characterized in several mammalian species (Brewer et al., 1969; Potts et al., 1968; Sauer et al., 1974), in salmon (Niall et al., 1969), and in chicken (Nieto et al., 1973). The lat-

ter was sequenced by Homma et al. (1986) and by Lasmoles et al. (1985). As reviewed by Potts and Aurbach (1976) and Potts (1992), the peptide consists of a 32-amino acid sequence with proline amide at the carboxy terminus and a cystein at positions one and seven linked by a disulfide bond. The avian and salmon hormones show a high sequence homology (Lasmoles et al., 1985) and are more potent than mammalian CTs due to greater resistance to degradation and to greater affinity for the receptor.

The CT gene appears to express two mRNAs by tissue-specific alternate splicing, encoding CT and CT gene-related peptide (CGRP) in the ultimobranchial gland and central nervous system, respectively (Amra et al., 1982). CGRP appears to be a potent vasodilator (Brain et al., 1985).

### c. Calcitonin Secretion

CT secretion was found to be proportional to ambient $Ca^{2+}$ in both mammals (Care et al., 1968) and birds (Care and Bates, 1972; Ziegler et al., 1969). The importance of the ambient $Ca^{2+}$ concentration in the regulation of CT secretion was further demonstrated through the use of ionophores (Pento, 1986) and $Ca^{2+}$ channel agonists (Cooper et al., 1986). Ca-sensing receptors in thyroid C-cells (Garret et al., 1996) may be involved in the modulation of $Ca^{2+}$ sensitivity, possibly by association with calcium channels (Pento, 1986).

Similarly to PTH, CT secretion is also controlled by cAMP through activation of the adenylate cyclase system by β-agonists and glucagon (Care et al., 1970) and by various digestive hormones (Care et al., 1971b). CT may thus be secreted in anticipation of hypercalcemia associated with the ingestion of food (Swaminathan et al., 1973).

### d. Calcitonin Receptor and Signal Transduction

CT receptors have been cloned for the pig (Lin et al., 1991), human (Gorn et al., 1992), and rat (Sexton et al., 1993). Like other peptide hormone receptors, the CT receptor is a glycoprotein that belongs to the seven membrane-spanning G-protein-linked receptor superfamily (Housama et al., 1994).

Binding of CT to the receptor and its activation were first demonstrated in the kidney (Marx et al., 1973) and in the osteoclasts (Nicholson et al., 1986). cAMP was implicated in both kidney and bone as the second messenger (Heersche et al., 1974). More recent work, however, has shown that CT also promotes an increase in $Ca^{2+}$ in the target cell (Murphy et al., 1986; Zaidi et al., 1990), and activates the inositol phosphate signaling pathway (Chabre et al., 1992). Possibly, therefore, the CT receptor is coupled to either of two G proteins (Chakraborty et al., 1991; Zaidi et al., 1990). A single recombinant CT receptor expressed in HEK-293 cells has been shown to activate both signal transduction pathways (Chabre et al., 1992). Furthermore, different isoforms of the CT receptors produced by alternative splicing showed differences in ligand recognition and binding characteristics (Nussenzveig et al., 1994, 1995), but were equally effective in activating the two signal transduction systems.

As with PTH and other peptide hormone receptors, continuous exposure to the hormone leads to downregulation of receptor binding (Obie and Cooper, 1979). Internalization of the bound receptor (Raue et al., 1990) and suppression of the CT receptor gene expression (Wada et al., 1995) have been suggested as mechanisms for such downregulation. The downregulation of the

receptor may be responsible for the "escape" phenomenon (Tashijan et al., 1978) that characterizes a sustained exposure to CT. Another mechanism may involve suppression of the CT receptor by $1,25(OH)_2D_3$ (Minkin and Yu, 1991).

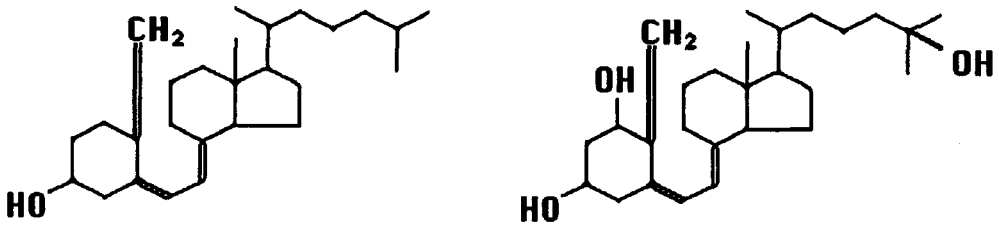
### e. Physiological Actions of Calcitonin

CT (thyrocalcitonin) lowers plasma calcium (and plasma phosphate) by inhibition of bone resorption (Raisz and Niemann, 1967). In the kidney, the hormone stimulates 25-hydroxyvitamin $D_3$-1-hydroxylase in locations that correspond to those of stimulated cAMP production (Kawashima and Kurakawa, 1983). These responses to CT are discussed further in other sections. CT inhibits tubular phosphate reabsorption and participates in the maintenance of a normal tubular reabsorption of $Ca^{2+}$.

The importance of CT in calcium homeostasis has not been established (Munson and Hirsch, 1992). Thyroidectomy results in a transient and small hypercalcemic response (Kalu et al., 1975), but Sammon et al. (1969) found no significant difference in steady-state plasma calcium and $^{45}Ca$ kinetics between normal and thyroidectomized rats fed diets with different calcium concentrations. Thyroidectomized rats, however, appeared to be less efficient than nonablated ones in handling a calcium bolus injection (Bronner et al. 1967). Munson and Hirsch (1992) concluded that "calcitonin could protect against hypercalcemia under extreme conditions, but under ordinary conditions this protection may not be called for". In birds, the importance of CT is even more obscure than in mammals. CT secretion in birds was found to be proportional to the plasma calcium level (Copp et al., 1972; Ziegler et al., 1969) or to ambient $Ca^{2+}$ *in vitro* (Feinblatt et al., 1974). In laying hens, CT secretion has been found to vary in the course of the daily reproductive cycle (Baimbridge and Taylor, 1981). On the other hand, ultimobranchiectomy and exogenous CT failed to affect plasma calcium (Kraintz and Intcher, 1969) or bone composition and turnover (Brown et al., 1970). With regard to its action on avian osteoclasts, reports are conflicting. Cao and Gay (1985) and de Vernejoul et al. (1988) observed inhibition of bone resorption by CT and the expected morphological changes in the osteoclast. In contrast, Nicholson et al. (1987) and Dempster et al. (1987) found that CT could neither bind to nor elicit a cAMP response in avian osteoclasts. Receptors to CT appear to be absent also in the chicken kidney, where the hormone failed to induce cAMP formation (Dousa, 1974) or to influence calcium or phosphorus excretion (Clark and Wideman, 1980). These findings are also in accord with the absence of any significant effect of ultimobranchiectomy in chickens on plasma calcium and bone calcification. Thus, although circulating levels of CT are even higher in birds than in mammals and respond to plasma calcium (Copp et al., 1972), CT does not appear to modulate calcium metabolism in birds.

## 3. 1,25-Dihydroxyvitamin $D_3$

Vitamin D has been regarded for many years as a fat-soluble nutritional factor that prevents or cures rickets. Following the discovery of the metabolic conversion of the vitamin to $1,25(OH)_2D_3$ (Figure 4), and the feedback relationships between calcium metabolism and $1,25(OH)_2D_3$, the metabolite has been classified as a seco-steroid hormone (Norman, 1994; Bouillon et al., 1995). In addition to its well-documented effect on intestinal calcium absorption and




Vitamin $D_3$ 1,25 dihydroxyvitamin $D_3$

**FIGURE 4.** Structure of vitamin $D_3$ and of 1,25 dihydroxyvitamin $D_3$ [1,25$(OH)_2D_3$].



thus on the supply of the mineral to bone, 1,25$(OH)_2D_3$ affects bone development directly by controlling differentiation of its cellular elements. More recently, this secosteroid has also been found to control differentiation and proliferation in other cells (Pols et al., 1990), notably the immune system, skin, cancer cells, and also the pancreatic β-cell (Lee et al., 1994). 1,25$(OH)_2D_3$ has also been implicated in control of the cell cycle (Godyn et al., 1994).

### *a. Natural Occurrence and Supply of Vitamin D*

Vitamin $D_3$ (cholecalciferol) is unique to the animal kingdom and not found in plants. The occurrence of the intact or glycosylated 1,25$(OH)_2D_3$ in some plants such as Solanum or Cestrum species (reviewed by Boland, 1986) is the exception. Several other forms of the vitamin have been identified. Of these, vitamin $D_2$ (ergocalciferol) is produced in yeast by ultraviolet irradiation and is an important supplement in human diets. Vitamin $D_2$ undergoes the same metabolic changes and functions equally well as vitamin $D_3$ in most mammalian species but is active neither in birds (Massengale and Nusmeier, 1930; Steenbock et al., 1923) nor in some New World primates.

Previtamin $D_3$ is synthesized from 7-dehydrocholesterol (provitamin D) in skin exposed to ultraviolet irradiation (Webb and Holick, 1988) and is then converted to vitamin $D_3$ by a temperature-dependent isomerization (Holick, 1989). Due to its limited water solubility, transport of vitamin D in circulation requires a specific $\alpha_1$-globulin-binding protein. Vitamin D may undergo metabolism in the liver, as is discussed later, or stored in adipose tissue from where it can be released only slowly. Vitamin $D_3$ in feed is absorbed in birds with an efficiency of about 60 to 70% (Bar et al., 1980).

### *b. Metabolism of Vitamin D*

The solubility of vitamin D in water is enhanced by hydroxylation at position 25, in the liver. 25-Hydroxyvitamin $D_3$ [25$(OH)D_3$] was first discovered by Blunt et al. (1968). Synthesis of 25$(OH)D_3$ is regulated by product inhibition (Omdahl and DeLuca, 1973) rather than by factors associated with calcium metabolism. Relative to other vitamin D metabolites, high concentrations of 25$(OH)D_3$ are found in the circulation, most of it bound to specific transport proteins (DeLuca et al., 1988). The compound is distributed in many tissues, most importantly in muscle, and therefore is considerably more available for further pro-

cessing than vitamin $D_3$ itself. $25(OH)D_3$ is further hydroxylated in the kidney to either $1,25(OH)_2D_3$ or $24,25(OH)_2D_3$ and other metabolites.

$1,25(OH)_2D_3$ was discovered in the early 1970s (Fraser and Kodicek, 1970; Norman et al., 1971; Holick et al., 1971), and, according to Brommage and DeLuca (1985), fulfills all body functions of vitamin D, including intestinal calcium absorption and bone development. Of the other metabolites produced in the kidney, $24,25(OH)_2D_3$ is the most important quantitatively, but a controversy exists with regard to its biological importance. $24,25(OH)_2D_3$ can elicit responses of the calcium-mobilizing system such as calcium absorption, but its affinity to intestinal receptors and its potency are considerably lower than those of $1,25(OH)_2D_3$ (Proscal et al., 1975). The metabolite has been found to be essential for the proper structural development of bone (Ornoy et al., 1978) and normal embryonic development in chickens (Henry and Norman, 1978). Brommage and DeLuca (1985), however, discount its biological importance. The importance of the other dihydroxy and trihydroxy metabolites has not been established, even though some appear to be biologically active.

### c. Regulation of $1,25(OH)_2D_3$ Synthesis

The regulatory step in the calcium control system is the synthesis of $1,25(OH)_2D_3$ from $25(OH)D_3$ in the proximal renal tubular cells (Akiba et al., 1980). The hydroxylation, carried out by the 25-hydroxyvitamin $D_3$-1-hydroxylase (1-hydroxylase) enzyme system, involves cytochrome P-450. As depicted in Figure 1, the synthesis and secretion of $1,25(OH)_2D_3$ is regulated by PTH (Fraser and Kodicek, 1973). A direct stimulation by PTH of 1-hydroxylase was observed in isolated renal tubular cells *in vitro* (Bar et al., 1980; Henry, 1981). Plasma calcium and plasma phosphate (reviewed by Omdahl and DeLuca, 1973) and the level of $1,25(OH)_2D_3$ (Omdahl et al., 1980) have also been implicated in the regulation of the enzyme activity. The separation between the direct control of 1-hydroxylase by $Ca^{2+}$ from that of PTH is rather difficult. However, Fox (1992) observed a different temporal pattern between hypocalcemia and PTH-stimulated flows of $1,25(OH)_2D_3$ from kidney to circulation.

Hormones such as growth hormone (Spanos et al., 1978), prolactin (Spanos et al., 1981), IGF-I (Nesbit and Drezner, 1993), or estrogen in birds (Tanaka et al., 1976) have been implicated as $1,25(OH)_2D_3$ secretagogues. However, as suggested by Bar and Hurwitz (1979), at least part of the response to those hormones could be secondary to an imposition of a calcium stress and the consequent increase in parathyroid activity.

### d. Kinetics of $1,25(OH)_2D_3$

Following a single intravenous administration of $1,25(OH)_2D_3$ in chicks, peak concentrations of the hormone in the intestinal mucosa are reached 1 to 2 h later (Hurwitz et al., 1983). At that time, the concentration of the hormone in the intestinal mucosa is about three times as high as that in blood plasma. After entry into the cell, the hormone becomes associated with the nuclear receptor (Wecksler and Norman, 1980; Fishman et al., 1986). Catabolism of $1,25(OH)_2D_3$ probably occurs through 24-hydroxylation, with ultimate elimination by biliary excretion. The half-life of $1,25(OH)_2D_3$ in the chick is 14 h (Hurwitz et al., 1983).

### e. The Vitamin D Receptor (VDR) and Signal Transduction

Receptors for $1,25(OH)_2D_3$ (VDR) have been found in several cellular constituents

of the calcium control system such as the epithelial cells of intestine and kidney (Haussler, 1986). VDR is also found in cartilage cells (Iwamoto et al., 1989), osteocytes and osteoblasts (Boivin et al., 1987), the shell gland of birds, parathyroid cells (Coty, 1980), and several tissues not associated with the calcium control system such as skin, skeletal muscle, testes, ovary, pituitary gland, pancreas, lymphocytes, and thymocytes (Haussler, 1986; Reichel et al., 1989).

VDR has been cloned in the case of the chicken (McDonnell et al., 1987), human (Baker et al., 1988), and rat (Burmester et al., 1988). On the basis of its structure, the receptor belongs to the superfamily of steroid hormone receptors (Evans, 1988; Moudgil, 1994). Functionally, the steroid receptors contain a short, strongly conserved cystein-rich DNA-binding domain and a well-conserved C-terminal ligand-binding domain (Evans, 1988). The cystein residues in the DNA-binding domain are bound to $Zn^{2+}$, forming two DNA-binding "Zinc Fingers" (Freedman and Towers, 1991). VDR appears to act as a homodimer or as a heterodimer with retinoic acid (Schräder et al., 1993; Whitfield et al., 1995). The manner of the interaction with retinoic acid has not been clearly elucidated, although functional interactions between vitamin D and retinoic acids have been documented in osteoblasts (Chentoufi and Marie, 1994) and osteoclasts (O'Niel et al., 1992). Similarly to other steroid receptors, VDR can undergo phosphorylation (Jones et al., 1991), but the functional significance of this step remains to be elucidated.

Receptor affinity for various vitamin D metabolites has been found to correlate well with their biological potency, $1,25(OH)_2D_3$ being the most potent (Proscal et al., 1975). The appearance of the intestinal receptor in the developing embryo also coincides with temporal changes in its $Ca^{2+}$ transport capacity (Seino et al., 1982).

### f. Genomic Action of Vitamin D

The dependence of the physiological action of vitamin D on protein synthesis was suggested by early studies that had showed a lag period of several hours in the response to vitamin D, and by the suppression of its action by inhibitors of protein synthesis such as actinomycin D (Zull et al., 1956). Direct evidence of the genomic action of the hormone was provided by the identification of response elements for VDR at the 5′ region of some genes affected by $1,25(OH)_2D_3$. Such response elements have been found on the preproPTH and VDR genes (Demay et al., 1992), 25-hydroxyvitamin $D_3$-24-hydroxylase gene (Ohyama et al., 1994), calbindin-$D_{9K}$ gene (Darwish and DeLuca, 1992), osteocalcin gene (Ozono et al., 1990), and several others. Of the additional proteins apparently induced by $1,25(OH)_2D_3$ such as alkaline phosphatase (Haussler et al., 1970), actin (Wilson et al., 1977), and tubulin (Nemere et al., 1987), Calbindin-$D_{28k}$ (calcium-binding protein, CaBP) (Christakos et al., 1992) has been studied most extensively. The protein can be induced by $1,25(OH)_2D_3$ *in vivo* (Wasserman and Taylor, 1966) in embryonic intestinal organ (Corradino and Fullmer, 1991) and in cell cultures (Ferrari et al., 1992). The protein has a molecular weight of 28,000 Da (Wasserman and Fullmer, 1983) and contains four calcium-binding domains with an apparent $K_a$ of $2 \times 10^6$ *M*. Its amino acid sequence was established from the cDNA sequence and chemical mapping (Hunziker, 1986; Wilson et al., 1985, 1988). The protein occurs in tissues concerned with $Ca^{2+}$ transport

such as the intestine (Wasserman and Taylor, 1966), kidney, chicken egg shell gland (Bar et al., 1984), rat incisor (Berdall et al., 1993), embryonic chicken yolk sac (Ono and Tuan, 1991), and also in tissues, the function of which is largely dependent on $Ca^{2+}$ movement such as pancreatic β-cells (Lee et al., 1994) and brain (Christakos et al., 1992). In general, the protein exists in the cytoplasm in a soluble form. However, some association was found between the protein and cellular organelles such as microtubules (Nemere and Norman, 1990; Nemere et al., 1992). In mammalian intestine, calbindin-$D_{9k}$, a 9000-Da cytosolic protein, has been identified (Desplan et al., 1983). This protein appears to play a role in the transcellular intestinal transport of $Ca^{2+}$ in mammalian species (Bronner et al., 1986). Recently, calbindin-$D_{9K}$ also has been detected in several avian tissues (Zanello et al., 1995).

Early after their discovery, calbindins were considered as the $Ca^{2+}$ carrier molecules participating in active transport of the cation or in its facilitated diffusion. It became clear, however, that calbindin-$D_{28k}$ was localized in the intestinal cytoplasm rather than in the brush border and could therefore not act as a membrane carrier. Bronner et al. (1986) hypothesized that calbindin acted to ferry $Ca^{2+}$ across the cell because, according to their calculation, the mobility of $Ca^{2+}$ was too slow to explain its flux across the entire length of the epithelial cell. This hypothesis has been supported by Feher (1983) and Feher et al. (1992), who were able to augment $Ca^{2+}$ diffusion through two membranes by adding calbindin-$D_{28k}$ to the central compartment. On the basis of considerations of the temporal, locational, and physiological responses to $1,25(OH)_2D_3$, several investigators such as Wasserman and Fullmer (1983) and Bikle (1990) favor the theory that calbindin sequesters $Ca^{2+}$ in the epithelial cell in order to avoid toxic intracellular $Ca^{2+}$ concentration and uptake of $Ca^{2+}$ by cellular organelles. This hypothesis is supported by the results of Chard et al. (1993), who found that calbindin $D_{28k}$ could buffer excess calcium in kidney and neurons, and in bodies of the avian intestinal nerves (Cai et al., 1994).

$1,25(OH)_2D_3$, when bound to VDR, participates in control of the cell cycle and of differentiation in several cells. The mechanism of $1,25(OH)_2D_3$ action on differentiation and other genomic actions is not unlike that of other steroid hormones (Moudgil, 1994; Schuchard et al., 1989). As mentioned, VDR binds to VDRE sequences on the respective gene. As discussed by Ozono et al. (1991), unbound VDR exhibits at least *in vitro* some binding affinity to VDRE. However, this affinity is markedly enhanced by binding to $1,25(OH)_2D_3$. According to Lian and Stein (1992), the VDR-VDRE complex functions primarily as a transcription enhancer. The enhancing activity is controlled by diverse and integrated cellular signaling pathways, acting synergistically and/or antagonistically with a series of basal regulatory elements and other hormone-regulated sequences (Stein and Lian, 1993). The vitamin D receptor complex may affect DNA stability, probably by association with heat-shock proteins (Moore et al., 1992). Other nuclear mechanisms of response to $1,25(OH)_2D_3$ may involve expression of various (proto)oncogenes such as c-*myc*, c-*myb*, c-*fms*, and c-*fos* in both cancer (Reitsma et al., 1983; Simpson et al., 1989) and normal (Matsumoto et al., 1990; Minghetti and Norman, 1988) cells; it may also involve differential stimulation of *fos* and *jun* family members (Candeliere et al., 1991) and of *raf* in osteoblasts (Lissoos et al., 1993). The specific action of vitamin D on the differentiation of bone cells is discussed in a later section.

### g. Nongenomic Actions of $1,25(OH)_2D_3$

It is commonly stated that $1,25(OH)_2D_3$ exerts its biological actions in target tissues via interaction with a specific, high-affinity intracellular receptor molecule (Lowe et al., 1992; Haussler, 1986; Walters, 1992; Ozono et al., 1991) and promotion of specific protein synthesis. This concept was questioned by Bikle et al. (1978), who found that cycloheximide or actinomycin D treatment did not block the early responses of calcium absorption in chicks to vitamin D, while synthesis of calbindin-$D_{28k}$ and alkaline phosphatase were significantly inhibited. Early responses of calcium absorption to $1,25(OH)_2D_3$ preceded those on protein synthesis (Wasserman and Fullmer, 1983), and the $1,25(OH)_2D_3$-induced increase in $Ca^{2+}$ transport was maintained in membranes of brush border vesicles isolated from intestinal mucosa (Rasmussen et al., 1979), possibly by changes in their lipid moiety. A nongenomic rapid stimulation by $1,25(OH)_2D_3$ of $Ca^{2+}$ transport was observed in the intestine (Nemere and Norman, 1990) and in other tissues (Walters, 1992; Selles and Boland, 1991). In the bone cell, cytoplasmatic and nuclear $Ca^{2+}$ concentrations (Khouri et al., 1995; Sorensen et al., 1993) were stimulated by $1,25(OH)_2D_3$, apparently together with the activation of voltage-dependent calcium channels (Farach-Carson et al., 1991). $1,25(OH)_2D_3$ also caused a rapid increase in intracellular $Ca^{2+}$ in the parathyroid cell (Sugimoto et al., 1988) and even induced oscillations in the intracellular $Ca^{2+}$ in pancreatic β-cells (Sergeev and Rhoten, 1995). The nongenomic suppression of 25-hydroxyvitamin $D_3$-24-hydroxylase by the hormone (Dick et al., 1990) also argued against the exclusive genomic thesis. The existence of an interaction of $1,25(OH)_2D_3$ with the cell membrane, rather than only with an intracellular receptor, has been inferred from activation by the hormone of the phospholipase C and the $Ca^{2+}$/PKC signal transduction system (Gross et al., 1993). It is, therefore, not surprising that a receptor for the hormone has been discovered on the basolateral membrane of the chicken intestinal epithelium (Baran et al., 1994; Nemere, 1995; Nemere et al., 1994). The receptor with a high binding affinity for $1,25(OH)_2D_3$ ($k_d$ = 0.72 n*M*) was downregulated by exposure to high levels of the hormone, and its affinity to various analogs of $1,25(OH)_2D_3$ correlated well with the rapid $Ca^{2+}$ absorption response of the intestine (Norman et al., 1993). In an analogous system, aldosterone had been considered to produce most of its effect through genomic mechanisms (Rossier and Palmer, 1992). The thesis that these mechanisms were not compatible with the observed rapid nongenomic responses to aldosterone of $Na^+$ transport in leukocytes and kidney cells led to the discovery of a membrane receptor for aldosterone (Wehling et al., 1992) with a $K_d$ of 0.1 n*M*, similar to that reported for $1,25(OH)_2D_3$ (Nemere et al., 1994). The existence of the membrane receptors, even by itself, would suggest the possibility of responses that do not involve interaction of the hormone with chromatin material. Interaction of the hormone with a membrane receptor could provide the initial stimulus for the increase in the transport of $Ca^{2+}$ into the cell.

The stimulation of $Ca^{2+}$ entry into the cell by $1,25(OH)_2D_3$ may also modify or effect some of the genomic actions attributed to the hormone. Calbindin-$D_{28k}$ is believed to be induced and regulated by $1,25(OH)_2D_3$ mainly on the basis of results in the intestine, where a positive correlation exists between the two and with $Ca^{2+}$ absorption (Feher and Wasserman, 1979). However, in other tissues such as the chicken

shell gland (Bar et al., 1984, 1992) or kidney *in vivo* (Bar et al., 1975; Rosenberg et al., 1986) and *in vitro* (Cravisio et al., 1987; Enomoto et al., 1992), synthesis of the protein appears to be associated with $Ca^{2+}$ influx rather than with $1,25(OH)_2D_3$ concentration. In embryonic intestine, verapamil, a $Ca^{2+}$ channel antagonist, suppressed the induction of calbindin-$D_{28k}$ by $1,25(OH)_2D_3$ (Corradino, 1985). Following a single dose of $1,25(OH)_2D_3$, an increase in intestinal calcium absorption preceded the appearance of calbindin-$D_{28k}$ and even calbindin-$D_{28k}$ mRNA by several hours (Spencer et al., 1978). It is thus not unlikely that at least part of the genomic actions attributed to $1,25(OH)_2D_3$ are secondary to those of $Ca^{2+}$, which has been implicated in the control of gene expression and other functions at the DNA level (Jones et al., 1989; Morgan and Curran, 1986), and most importantly in the secretion of several peptide hormones (Morgan and Curran, 1986; Preston et al., 1990; Vandenplas et al., 1990).

Due to a direct genomic effect of $1,25(OH)_2D_3$, on the one hand, and its indirect effects through stimulation of $Ca^{2+}$ flux on the other, interactions between the hormone and $Ca^{2+}$ could be expected in the stimulation/suppression of synthesis of various proteins. Such interactions were observed with regard to suppression of PTH and stimulation of VDR gene expression in the avian parathyroid glands (Russell et al., 1993) and osteoblast-like cells (van Leeuwen et al., 1990).

As mentioned, some of the nongenomic responses to $1,25(OH)_2D_3$ could be mediated by the phospholipase C system. In the parathyroid cell, Bourdeau et al. (1990) observed a rapid effect of $1,25(OH)_2D_3$ on the phospholipase C signal transduction pathway. In the kidney, Koyama et al. (1994) found that increased expression of the $25(OH)D_3$-24-hydroxylase gene by $1,25(OH)_2D_3$ could be prevented by blocking phosphokinase C, and be stimulated by PKC activators. Phosphokinase C was also found to be activated by $1,25(OH)_2D_3$ (Slater et al., 1995; Wali et al., 1992). Furthermore, a synergism exists between $1,25(OH)_2D_3$ and phorbol esters in regulating VDR gene expression in osteoblastic cells (Reinhardt and Horst, 1994). Such a mechanism was also suggested for the stimulation of keratinocyte differentiation (Su et al., 1994) and upregulation of VDR in osteoblast-like cells (van Leeuwen et al., 1990) by $1,25(OH)_2D_3$.

### *h. Vitamin D and Calcium Homeostasis*

The capacity of the control system of chicks to maintain normal plasma calcium concentration is overcome in the absence of the vitamin, resulting in a decline in plasma calcium from 10 to about 5 mg/dl within 18 d of consumption of a diet free of the vitamin or 3 to 4 d after the disappearance of $25(OH)D_3$ from circulation (Bar and Hurwitz, unpublished). $1,25(OH)_2D_3$ is also the active agent in the process of adaptation to low dietary calcium intakes toward a more economic utilization of dietary calcium (Edelstein et al., 1975) and prevention of steady-state hypocalcemia.

When the feedback response of $1,25(OH)_2D_3$ synthesis is bypassed by feeding $1\alpha(OH)D_3$ (a synthetic precursor of $1,25(OH)_2D_3$), the normal homeostatic decrease in calcium absorption during challenge with a high calcium intake is impeded (Hurwitz et al., 1984). The plasma calcium concentration then sharply increases. Thus, vitamin D is essential in avoiding both hypo- or hypercalcemia, and effects the adaptation

of intestinal calcium absorption to variations in calcium intake.

Some pathological states are associated with point mutations in genetic components of the vitamin D system. For example, some forms of vitamin D-resistant rickets in humans are due to molecular defects in the kidney 1-hydroxylase enzyme system. Defects in the $1,25(OH)_2D_3$ receptor in humans lead to various forms of vitamin D-resistant rickets (Feldman and Malloy, 1990).

The main actions of $1,25(OH)_2D_3$ within the calcium regulatory system, which is described further below, are (1) control of intestinal calcium absorption and (2) control of bone resorption and formation. Some evidence exists as to its importance in tubular reabsorption of calcium.

## D. The Controlling Systems

### 1. Bone

#### a. Morphological Organization

In macroscopic terms, the skeleton contains elements of cartilage located mostly in the growth plate, trabecular or spongy material found in the epiphyses of the long bones, and compact or cortical bone that populates the shafts. Bones of female birds during reproduction also include a gonadal hormone-dependent medullary bone with a distinct morphology. Calcium turnover rate, as measured with the aid of $^{45}Ca$, varies widely among the various bone types, with half-lives ranging from several months in cortical bone through several weeks in trabecular bone, to 1 to 2 d in medullary bone (Hurwitz, 1965).

#### b. Cellular Populations

Bone contains five important cell populations (not including bone marrow): (1) cartilage cells, (2) bone-forming cells (osteoblasts), (3) bone-resorbing cells (osteoclasts), (4) mature bone cells (osteocytes), and (5) bone-lining cells.

As reviewed by Howell (1992) and Leach and Gay (1987), chondrocytes are the main cellular component of the growth plate. Longitudinal bone growth is determined by chondrocyte proliferation and hypertrophy. The rate of proliferation of chondrocytes is enhanced by somatomedins and other growth factors (Isaksson et al., 1987; Centrella and Canalis, 1985), and cAMP activators such as PTH, and suppressed by cGMP (Pines and Hurwitz, 1988). During bone growth, endochondral calcification and cell atrophy precede the invasion of cartilage by bone cells and formation of osseous tissues (Leach and Gay, 1987) within the volume occupied by cartilage.

Osteoblasts originate from mesenchymal stem cells (Grigoriades et al., 1988) and are found in the growth plate, and on periosteal and endosteal surfaces. During the various stages of development and differentiation, the cells vary with regard to the expression of cell growth and bone-specific genes (Stein and Lian, 1993). Osteoblasts are equipped with receptors for both PTH and $1,25(OH)_2D_3$ (Rodan and Rodan, 1983), which modulate the activities of several enzyme systems such as alkaline phosphatase and probably also modify some $Ca^{2+}$ transport characteristics. Osteoblasts differentiate into osteocytes once embedded in osteoid, where they occupy lacunar spaces that are interconnected to each other, to cells on the bone surface and to blood vessels.

Bone resorption results from the action of the multinucleated osteoclasts, which exhibit tartarate-resistant phosphatase, bear calcitonin receptors, and resorb bone in culture (Rodan, 1992). These cells probably originate from monocyte precursors of the reticuloendothelial system (Hattersley et al., 1991; Scheven et al., 1986; Suda et al.,

1993). Cells such as macrophages may differentiate into osteoclasts under the influence of $1,25(OH)_2D_3$, losing their $1,25(OH)_2D_3$ receptors at the end of differentiation (Miyaura et al., 1986; Haussler, 1986). Osteoclast differentiation involves the interaction with osteoblasts, $PGE_1$ and $PGE_2$, and several growth factors and cytokines, acting during the different stages of osteoclast differentiation (Suda et al., 1992).

Osteoclast may migrate over bone surface using their podosomes. In the process of resorption, osteoclasts attach to the surface of bone, following the fusion of their podosomes by rearrangement of the cytoskeleton (Lakkaorpi and Väänänen, 1991) to form a broad ring termed the "sealing zone". Within the sealing zone, the osteoclast cell membrane is convoluted to form the "ruffled border". The enclosed space adjacent to the ruffled border contains lysosomal enzymes, and its pH is 4 (Baron et al., 1985). The low pH and the enzymes dissolve and remove the entire osseous mass, including both organic matrix and mineral (Hall and Kenny, 1987; Sundqvist et al., 1990; Teti et al., 1989). $Ca^{2+}$ released by dissolution is taken up by the osteoclast. This results in an increase in cellular $Ca^{2+}$ concentration, which in turn inhibits osteoclast function (Miyauchi et al., 1990).

Flat, elongated bone-lining cells cover the nonremodeling endosteal surfaces of bone. These cells, however, were implicated in the control of bone ionic fluxes (Bowman and Miller, 1986), including those involved in calcium homeostasis (Parfitt, 1987). Lining cells may also serve as progenitors to osteoblasts or limit bone resorption geographically (Rodan and Martin, 1981; Marks and Popoff, 1988).

### *c. Bone Mineralized Matrix*

Extracellular space in bone contains a calcified organic matrix that is made up of highly organized cross-linked fibers of triple helix collagen type I, proteoglycans, and several other proteins such as osteopontin, osteocalcin, and osteonectin. Details of the structure and molecular biology of these proteins can be found in Gehron-Robey et al. (1992). Bone mineral is made primarily of calcium and phosphate. The association between these two main ionic species may produce a variety of different salts with variable solubilities and stability, along a complex phase diagram, depending on the conditions prevailing during precipitation such as $Ca^{2+}$ and phosphate concentrations, and that of other cations and anions, pH, $pCO_2$, temperature, etc. Hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$ is the major bone salt, but other less-stable calcium phosphates may precipitate during early calcification. These calcium phosphates may be stabilized under some conditions (Parfitt, 1987). As reviewed by Glimcher (1992), bone mineral is highly organized and is laid down in association with the collagen fibers, apparently via bridges of acidic proteins. Weiner and Addadi (1991) found a common structural motif on the surfaces of several biological crystals, including both calcium phosphate and calcium carbonate; this motif allows the association of the crystal with the acidic protein. Nucleation may be initiated at such a binding site, leading to growth and the development of plate-shaped crystals (Bagambisa and Schilli, 1993; Weiner, 1986). Gorsky (1992) has suggested that the acidic phosphoproteins osteopontin, bone sialoprotein, and bone acidic glycoprotein may function as the binding molecules. As reviewed by Williams and Frolik (1991), an alternative theory for the initiation of calcification by nucleation postulates that the osteoblast, as well as the hypertrophied chondrocyte, secrete matrix vesicles (Anderson et al., 1969, 1989). These vesicles are made of a plasma mem-

brane enveloping specific enzymes, proteins, calcium, and phosphate.

### d. Action of PTH on Bone

Osteoclastic bone resorption is controlled by PTH both *in vivo* and *in vitro* (Raisz, 1976). Mammalian osteoclasts apparently lack PTH receptors as well as PTH-sensitive adenylate cyclase (Vaes, 1988). Furthermore, PTH does not act directly on osteoclast motility or bone resorption in culture (Chambers et al., 1985). The presence of other cells such as osteoblasts that bear PTH receptors (Rodan and Rodan, 1983; Hermann-Erlee et al., 1983) is required for activation of the osteoclasts (McSheehy and Chambers, 1986a, b). The action of interleukin-1 (IL-1) on bone resorption also appears to require mediation by osteoblasts. This may explain the synergism between PTH and IL-1 in bone resorption (Dewhirst et al., 1987). The identity of the factor(s) responsible for this mode of communication between the osteoblast and the osteoclast has not been clarified. Raisz (1976) identified prostaglandins as a paracrine activator of bone resorption. Perry et al. (1989) characterized two proteins as possible candidates, and Fuller et al. (1991) showed that stimulation of osteoclast resorption is associated with a membrane or matrix-bound factor.

PTH elicits in birds an *in vivo* calcium response within minutes (Candlish and Taylor, 1970). This rapid *in vivo* response of birds may be related to the more rapid resorption response of the avian compared with the mammalian osteoclast (Jones et al., 1986; Miller et al., 1984) resulting from the presence of PTH receptors (Agarwala and Gay, 1992) and PTH-dependent adenylate cyclase (Wong, 1984). The rapid $Ca^{2+}$ response to PTH in either mammals or birds may also involve mechanisms other than osteoclast activation, and that osteoblasts and osteocytes must be capable of $Ca^{2+}$ transport (Talmage et al., 1975). This suggestion is supported by Marcus and Orner (1980), who observed a stimulation of $Ca^{2+}$ uptake by PTH in bone cells, apparently by activating calcium channels (Hruska et al., 1991).

Receptors for PTH are also found in chondrocytes or chondoroprogenitor cells (Pines and Hurwitz, 1988), where PTH activates adenylate cyclase (Kawashima et al., 1980) and stimulates cell proliferation (Chin et al., 1986; Pines and Hurwitz, 1988). The presence of PTH receptors on the bone-forming cells (osteoblasts and chondrocytes) rater than on the bone-resorbing cells is paradoxical in view of the classic role of PTH as a stimulant of bone resorption. However, their presence in osteoblasts can explain the stimulation of bone formation (Tam et al., 1982) and the increase in bone mass by PTH (Guiness-Hey and Hock, 1984), as suggested by early studies of Albright and co-workers (reviewed by Dempster et al., 1993). Furthermore, these studies provide the basis for the concept of "coupling" between bone formation and bone resorption (Howard et al., 1981; Jarowski, 1984).

Stimulation of proliferation by PTH was observed in osteoblasts by MacDonald et al. (1986) and in chondrocytes by Pines and Hurwitz (1988). As reviewed by Dempster et al. (1993), PTH interacts with growth factors such as IGF-I and TGFβ in both chondrocytes and osteoblasts; it also interacts with EGF (Halevy et al., 1991; Ohta et al., 1989) in controlling bone cell proliferation, differentiation, and synthetic activity. The apparent antidifferentiating effect (Bellows et al., 1990) of PTH may involve early genes of the c-*fos* and c-*jun* family (Clohisy et al., 1992). The contradictory responses of bone cells to PTH may be related to different dose responses or to the activation of the two systems of signal trans-

duction, and further interaction with the $Ca^{2+}$-sensing system. The precise mechanism, however, remains to be elucidated.

### e. Action of Calcitonin in Bone

CT is known to be a potent inhibitor of osteoclastic bone resorption in mammalian bone (Friedman and Raisz, 1965). Activation of adenylate cyclase appears to be the mode of signal transduction for CT. *In vitro,* calcitonin causes the disappearance of the ruffled border of the osteoclasts and inhibits their motility, thereby inhibiting their bone-resorbing activity (Arnett and Dempster, 1987). The inhibitory effect of CT on bone resorption appears to be transient (Werner et al., 1972), possibly due to downregulation of CT receptors (Tashijan et al., 1978).

In addition to the well-documented effects on bone resorption, CT also appears to stimulate bone formation directly (Farley et al., 1988), apparently through stimulation of the proliferation and synthetic activity of osteoblasts (Farley et al., 1991).

### f. Actions of Vitamin D in Bone

Vitamin D affects both bone formation and bone resorption by controlling differentiation, in addition to its utmost importance in regulating the calcium (and phosphate) supply from the intestine. Both $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ have been found to be essential for normal growth-plate chondrocyte differentiation (Ornoy et al., 1978) and expression of alkaline phosphatase (Schwartz et al., 1988). According to Gerstenfeld et al. (1990), the dihydroxyvitamin $D_3$ metabolites act on chondrocyte maturation through specific genes that promote chondrocyte differentiation toward the morphologically hypertrophic phenotype. Although some authors have suggested that $1,25(OH)_2D_3$ also promotes DNA synthesis (Binderman and Somjen, 1984) and stimulates growth (Burch et al., 1988), others have shown that $1,25(OH)_2D_3$ inhibits DNA synthesis (Silbermann et al., 1987) and reduces cartilage cell proliferation (Grigoriadis et al., 1988). $1,25(OH)_2D_3$ specifically stimulates the transcription of collagen type II and increases morphological chondrogenesis in limb bud mesenchymal cells (Tsonis, 1991).

Similarly to its action on cartilage cells, $1,25(OH)_2D_3$ enhances differentiation of bone cells (Pols et al., 1986, 1990), inhibits bone cell proliferation (Beresford et al., 1986), and enhances the synthetic activity of several typical protein markers such as alkaline phosphatase (Beresford et al., 1986; Kyeyune-Nyombi et al., 1989; Majeska and Rodan, 1982), collagen type-I (Beresford et al., 1986; Harrison and Clark, 1986), osteocalcin (Yoon et al., 1988), and osteopontin (Stein and Lian, 1993). The response of bone cells to the hormone is dependent on their state of differentiation (Owen et al., 1991).

$1,25(OH)_2D_3$ stimulates bone resorption *in vivo* (Tanaka and DeLuca, 1971) and *in vitro* (Raisz et al., 1972). Because osteoclasts are devoid of vitamin D receptors, the stimulatory effects of the hormone on resorption must be mediated by other bone cells similarly to PTH, through a secretion of a cytokine (McSheehy and Chambers, 1986), possibly IL-6 (Lowik et al., 1989).

On a long-term basis, $1,25(OH)_2D_3$ promotes osteoclast recruitment (MacDonald et al., 1987) and resorption *in vivo* (Holtrop et al., 1981). It does so also in bone culture (Roodman et al., 1985) by acting on progenitor cell differentiation, stimulating the differentiation of mononuclear phagocytes (Suda et al., 1992). $24,25(OH)_2D_3$, on the other hand, appears to depress PTH-stimulated bone resorption (Matumoto et al., 1992; Yamato et al., 1993).

### g. Function of Bone in Ca Homeostasis

The skeleton may be considered as a large internal reservoir from which $Ca^{2+}$ can be extracted and deposited according to need. In the rat, bone was considered to be the most important calcium-control system because the kidney handled only a minor fraction of a calcium load (Bronner and Aubert, 1965). The existence of $Ca^{2+}$-binding sites in bone has been postulated by Bronner and Stein (1992) to explain the rapid disappearance of a $Ca^{2+}$ bolus in rats. In the chicken and human, the kidney handles a significant part of any calcium load, and the relative importance of bone in controlling hypercalcemia is not as great as in the rat. Bone, however, remains of utmost importance in supplying calcium during periods of deficiency.

Bone undergoes constant turnover due to formation and resorption, which constitute the remodeling process. Because bone flows are modulated by plasma calcium and phosphate and regulated by hormones, the normal turnover may be part of the calcium control system. Parfitt (1987), however, considered the system participating in calcium homeostasis to be independent of bone formation and resorption and carried out by different bone cell populations.

Similar to other steady-state systems in the body, the estimation of bone turnover is difficult. The *in vivo* kinetic approach considers inflow and outflow of $Ca^{2+}$ from bone as governed by physicochemical transport among different pools that can be studied with the aid of calcium isotopes. This approach uses compartmental analysis, which describes $Ca^{2+}$ movements among the compartments as either linear (Aubert et al., 1963) or nonlinear (Staub et al., 1988) processes. Furthermore, $Ca^{2+}$ was considered to move to bone mineral directly from the extracellular space, which is an "extension" of the central calcium pool. In contrast to the physicochemical approach, some have described bone extracellular space as separated from the circulation by bone cells (Talmage et al., 1975). $Ca^{2+}$ movement in and out of the mineral phase of the bone is then governed by the metabolism of bone cells, which in turn is affected by the calcium-regulating hormones. This process may be sufficiently rapid to account for the uptake of $Ca^{2+}$ by bone after a bolus Ca injection.

Morphological labels such as tetracycline (Frost et al., 1969) have also been used to estimate bone formation. Urinary excretion of hydroxyproline, an amino acid specific to collagen, has been used to estimate bone resorption. More recently, the excretion of specific cross-linkers has been used to estimate bone resorption (Rosen et al., 1994). Histological techniques and various markers of bone cellular activities have also been used to estimate bone formation and bone resorption activities (Nijweide et al., 1986).

One of the consequences of bone remodeling is the formation of the Haversian system in mammalian systems. Resorptive processes in bone result in formation of elongated cavities parallel to the long axis of the primary lamellar bone deposited during growth. These are refilled with secondary bone, made of units of Haversian osteons, formed around central blood capillaries. The remodeling process occurs throughout bone, is important in maintaining the mechanical integrity of bone and provides the means for effecting a finely tuned calcium homeostasis system.

## 2. Intestinal Absorption

Early disagreements concerning the mechanism of calcium absorption and its

control by vitamin D have still not been resolved. On the basis of *in vitro* experiments with the rat duodenum, Schachter and Rosen (1959) suggested that vitamin D promoted active calcium transport. Harrison and Harrison (1960, 1963) suggested that vitamin D increased the "diffusibility" of $Ca^{2+}$ across the intestinal mucosa. Wasserman (1963) concluded that vitamin D promoted passive diffusion of $Ca^{2+}$ in the chick intestine *in situ,* at the higher range of $Ca^{2+}$ concentrations. This hypothesis was supported by the analysis of *in vivo* calcium absorption in chicks (Hurwitz and Bar, 1972). Bronner (1987) concluded on the basis of experimentation with intestinal loops *in situ* and *in vitro* that in the rat, $1,25(OH)_2D_3$ regulated the saturable $Ca^{2+}$ translocation, consistent with the active transport hypothesis.

The disadvantage of the *in vitro* techniques is that they utilize artificially defined media, do not include functional circulation, and ignore other processes that may be important *in vivo* determinants of calcium absorption such as concentration of ionic $Ca^{2+}$ in the intestinal contents and differences in sites of absorption and transit time along the intestine. Although a significant active *in vitro* transport of $Ca^{2+}$ was observed in the duodenum of the rat, the rapid transit of digesta results in a small contribution of this intestinal segment to the overall absorption of calcium (reviewed by Nellans, 1990), casting doubt as to the relevance of many *in vitro* observations to the *in vivo* process.

Dietary calcium is typically in the form of poorly soluble salts such as calcium carbonate and calcium phosphates. These are solubilized in the stomach and made available for absorption. However, after entry of the digesta into the duodenum and titration of the acidity by digestive juice bicarbonate, $Ca^{2+}$ reprecipitates, leaving in solution only a fraction of the amount that had entered. The activity of $Ca^{2+}$ in the duodenum depends on the dietary calcium concentration and accompanying anion (Hurwitz and Bar, 1968). However, within a wide range of calcium intake, the activity of ionic calcium in the intestinal contents is sufficiently high to maintain a positive electrochemical potential gradient of $Ca^{2+}$ and to allow transmembrane diffusion. A need for active calcium transport arises only under extreme conditions of calcium deficiency (Hurwitz and Bar, 1968).

Calcium absorption may occur through paracellular or cellular routes (Warner and Colemen, 1976). Paracellular transport proceeds through the intercellular junctions, apparently by diffusion. Because paracellular transport is technically defined as the transfer of $Ca^{2+}$ without mixing with intracellular calcium, it may also include vesicular transport (Nellans, 1990; Nemere, 1991). On the basis of results with rat intestine, Nellans (1990), in agreement with Bronner (1990), concluded that paracellular transport is concentration dependent and can account for the entire serosal mucosal transfer of $Ca^{2+}$, and that under normal conditions, can also account for most of the mucosal-serosal movement. Karbach (1992) showed that 60 to 70% of the mucosal-serosal flux across the short-circuited rat duodenum, jejunum, or ileum was paracellular and that the paracellular $Ca^{2+}$ flux in both directions was stimulated by $1,25(OH)_2D_3$. The importance of the paracellular route in nutrient absorption, in general, has been discussed by Pappenheimer and Reiss (1986) and Madra and Pappenheimer (1987).

$Ca^{2+}$ transfer through the brush border membrane may be common to both transcellular and paracellular routes, if vesicular transport is included in the latter. Anatomically, the paracellular space extends beyond the basal membrane down to the tight junction, and $Ca^{2+}$ may be transported to this

space in close proximity to its transport across the brush border, without any need to move across the length of the cell, and be extruded from the space by hydrostatic pressure. The importance of osmotic water movement to $Ca^{2+}$ transport was pointed out by Karbach (1992) and may explain the early observations of Hurwitz et al. (1967) on the relationship of $Ca^{2+}$ transport to the concentration of monovalent ions.

Although $Ca^{2+}$ entry into the mucosal cell occurs down an electrochemical gradient apparently by a passive process, it may also involve regulation by vitamin D (Fullmer, 1992). Experiments with intestinal brush-border vesicles (Bikle, 1990), which isolate the $Ca^{2+}$ entry process, have shown that $1,25(OH)_2D_3$ stimulates calcium uptake (Rasmussen et al., 1979; Bikle et al., 1983; Kaune et al., 1992). Some evidence, reviewed by Bikle (1990), suggested that such an increase in $Ca^{2+}$ uptake preceded stimulation of calbindin-$D_{28k}$ synthesis and can be linked to changes in lipid composition, membrane fluidity, and a calmodulin-binding protein responsible for linking the cytoskeleton of the microvillus to the membrane. An association between membrane fluidity and $Ca^{2+}$ uptake by brush border vesicles was observed by Schedl et al. (1995). Bikle (1990) also suggested that the calmodulin-binding protein might act as the calcium channel protein, the importance of which in brush-border $Ca^{2+}$ transport was demonstrated later by Kune et al. (1992). Changes in microtubular proteins due to $1,25(OH)_2D_3$, with a temporal relationship similar to the stimulation of $Ca^{2+}$ transport, have been reported by Nemere et al. (1987, 1991).

Through the use of calcium imaging techniques, Chandra et al. (1990) showed that transport away from the brush border region is augmented by vitamin D. According to Bronner et al. (1986), this process may be facilitated by calbindin-$D_{9k}$ in mammals or calbindin-$D_{28k}$ in birds, which may act to ferry $Ca^{2+}$, and hence is also controlled by $1,25(OH)_2D_3$. Finally, $Ca^{2+}$ is transported uphill across the basolateral membrane. The basolateral transport is mediated by an ATP-activated calcium pump that is probably identical to the high-affinity, Ca-dependent ATP-ase (as reviewed by Wasserman and Fullmer, 1983). Wasserman et al. (1992) found that $1,25(OH)_2D_3$ stimulated a saturable ATP-dependent $Ca^{2+}$ uptake by intestinal basolateral membrane vesicles by increasing $V_{max}$ without affecting $K_m$. The presence of a $Ca^{2+}$ pump epitope, stimulated by $1,25(OH)_2D_3$, was also demonstrated by immunological techniques. The basolateral membrane also exhibits $Na^+/Ca^{2+}$ exchange characteristics and calcium channels. However, the participation of these mechanisms in the regulation of intestinal calcium transport has not been elucidated.

The effect of vitamin D on intestinal absorption of phosphate has been studied less intensively than that on calcium absorption. Harrison and Harrison (1963) observed that vitamin D augmented phosphorus permeability in rat intestine *in vitro*. Vitamin D increased the permeability to phosphate in chick intestine *in vivo* independently of its effect on calcium absorption (Hurwitz and Bar, 1972; Wasserman and Taylor, 1973).

Some results suggest that $1,25(OH)_2D_3$ also affects intestinal cell differentiation. In vitamin D-deficient animals, the intestinal villus is considerably shorter than normal. Through promoting proliferation by activating polyamine synthesis, $1,25(OH)_2D_3$ restores the normal length of the villus (reviewed by Suda et al., 1990).

### *a. Regulation of Intestinal Absorption and Calcium Homeostasis*

Intestinal absorption is the means for the entry of calcium into the body from the

environment, and is determined by the supply of calcium in the diet. Bronner and Aubert (1965) considered intestinal absorption as a "disturbing signal" in the context of calcium homeostasis. However, Nicolaysen et al. (1954) had previously observed an adaptational increase in calcium absorption of rats, to satisfy the increased demands during low calcium intakes. The adaptation of animals to the low calcium intakes was later linked to an increased synthesis of $1,25(OH)_2D_3$ (Edelstein et al., 1975; Ribovich and DeLuca, 1976), providing the feedback link between intestinal calcium absorption and calcium homeostasis. A large diurnal increase in calcium absorption was observed in laying hens during hours of egg shell formation and a subsequent return to low levels once calcification of the egg shell had been completed (Hurwitz and Bar, 1965; Hurwitz et al., 1973). Thus, the intestine is not only a simple gateway for calcium entry but also an important control system in calcium homeostasis.

In the normal young chicken, about 70% of calcium absorption is vitamin D dependent. The proportion is similar in the human. As mentioned above, interference with the vitamin D-intestinal axis results in loss of the ability to regulate plasma $Ca^{2+}$ concentration within the normal range (Hurwitz et al., 1984). Furthermore, in the human, hypercalcemia resulting from hyperabsorption of calcium is well recognized. In comparison, the vitamin D dependence of calcium absorption in the rat under normal feeding conditions is less than 10% of the total calcium absorbed (Hurwitz et al., 1969). In agreement, Lee et al. (1990) concluded that in contrast to the consensus that $1,25(OH)_2D_3$ is an important regulator of Ca and P absorption, the intestine is insensitive to vitamin D in the rapidly growing neonatal rat. Similarly, changes in Ca and P absorption due to pregnancy and lactation are also independent of vitamin D. The limited participation of the vitamin D-intestinal axis in calcium homeostasis of the rat is common and even more extreme in other nocturnal-vegetarian rodents such as the Damra mole-rat, which appears to possess the vitamin D "machinery", but its effect, if evoked, may be disadvantageous (Skinner et al., 1991).

## 3. The Renal $Ca^{2+}$ Flow

Following glomerular filtration, 96 to 99% of the filtered calcium load is reabsorbed by the renal tubule. Although responsible for only about 10% of $Ca^{2+}$ reabsorption, the distal tubule is the main site of its hormonal regulation, as reviewed by Friedman and Gesek (1993).

In general, the pattern of $Ca^{2+}$ reabsorption in the kidney is similar to that in the intestine (Kumar, 1995). $Ca^{2+}$ appears to be reabsorbed in the proximal tubule by transcellular and paracellular passive pathways, because the $Ca^{2+}$ concentration in the blood ultrafiltrate passing through this segment remains similar to that of blood (Yanagawa and Lee, 1992). However, in the distal tubule, the $Ca^{2+}$ concentration may fall to 0.1 m*M*, indicating uphill $Ca^{2+}$ transport, especially when the electrical potential gradient is brought into account. In the loop of Henle, which is responsible for about 20% of the total reabsorption of calcium, it has been estimated (Friedman and Gesek, 1993) that calcium transport is divided equally between transcellular and paracellular pathways. Paracellular absorption of $Ca^{2+}$ is dependent on that of $Na^+$ and $Cl^-$ (Bomsztyk et al., 1984) and responds to apical membrane hyperpolarization.

At the distal convoluted tubule, where hormonal control of calcium reabsorption occurs, transcellular absorption appears to be the more common mechanism of $Ca^{2+}$

transport. According to present concepts (Friedman and Gesek, 1993), the transport of $Ca^{2+}$ through either the apical or basolateral membrane is accomplished primarily through $Ca^{2+}$ channels. By analogy to the intestine and on the basis of the similarity of the distribution of calbindin and the active $Ca^{2+}$ reabsorption, Bronner (1989) hypothesized that the transport of $Ca^{2+}$ across the cell was facilitated by calbindin-$D_{28k}$. This protein may act only to sequester $Ca^{2+}$ and thus aid in the transcellular movement of $Ca^{2+}$ (Boutiauy et al., 1994). If calbindin does facilitate tubular transport, its concentration should increase when the reabsorption of calcium increased. However, in the chicken, the calbindin concentration increased when urinary excretion increased, in the opposite direction to calcium reabsorption and with an inverse relationship to $1,25(OH)_2D_3$ production and concentration (Edelstein et al., 1975; Rosenberg et al., 1986).

The extrusion of $Ca^{2+}$ from the intracellular to the extracellular space occurs against an electrochemical difference and must be, by definition, active; it may involve Ca-ATPase pump (Carafoli, 1991) or $Na^+/Ca^{2+}$ exchange mechanisms.

### *a. Hormonal Regulation of Tubular Reabsorption*

PTH improves Ca reabsorption at the distal tubule by activating $Ca^{2+}$ channels (Bacskai and Friedman, 1990; Wideman and Youtz, 1985). At physiological concentrations, calcitonin action appears to act similarly to PTH in that it reduces $Ca^{2+}$ excretion by promoting its tubular reabsorption (Quam, 1980), membrane hyperpolarization, and activation of $Ca^{2+}$ entry through dihydropyridine-sensitive calcium channels (Gesek and Friedman, 1993). There may be however, some differences in the site of action on $Ca^{2+}$ transport and in the mode of signal transduction between the two hormones (Kurokawa et al., 1992).

Receptors for $1,25(OH)_2D_3$ have been found along the entire nephron (Ilda et al., 1993). Direct action of the hormone on net $Ca^{2+}$ absorption in the collecting ducts has been observed by Bindles et al. (1991). Suzuki et al. (1990) found that $1,25(OH)_2D_3$ induced an increase in $Ca^{2+}$ concentration in rabbit renal proximal straight tubular cells. However, as reviewed by Friedman and Gesek (1993), most of the evidence suggests that $1,25(OH)_2D_3$ acts on kidney $Ca^{2+}$ transport through interaction with PTH.

### *b. Renal $Ca^{2+}$ Excretion and Calcium Homeostasis*

The degree of participation of renal calcium transport in calcium homeostasis varies widely among animals. In the growing rat, only a small amount of the absorbed calcium is normally excreted in the urine (Hurwitz et al., 1969). In nongrowing adult animals with a reduced net bone formation, the fraction of urinary calcium may increase considerably. In the human, urinary Ca excretion varies parabolically as a function of plasma calcium (Nordin et al., 1972). The entire curve is shifted to the right in hyperparathyroidism and to the left in hypoparathyroidism. Also in the human and in the growing chicken, a large fraction of the absorbed calcium is excreted in the urine, and calcium excretion is proportional to calcium intake (Fussell, 1960). Computer simulation (Hurwitz et al., 1983) has shown a rapid response of urinary calcium excretion in the growing chick to a bolus injection of calcium or EDTA.

## IV. PERTURBATIONS AND RESPONSES OF THE CA-REGULATING SYSTEM

Perturbations of the calcium-regulating system are commonly associated in medical literature with pathological situations. However, the calcium regulatory system evolved for the protection of the organism against the day to day perturbations that characterize life, such as those related to growth, reproduction, dietary intake of calcium and probably other minerals such as phosphate.

### A. Growth

Growth leads to two major perturbations in the system of calcium homeostasis. First, it causes volume expansion, which may result in a reduction in the plasma calcium concentration. Second, bone growth driven by genetic factors involves a significant drain of calcium from the central pool. It is therefore not surprising that the calcium requirements along with the activity of the calcium-regulating systems such as intestinal absorption and kidney 1-hydroxylase increase with the growth rate (Bar and Hurwitz, 1981; Hurwitz et al., 1995). Furthermore, computer simulation has shown that the periodicity of oscillations in the Ca-regulating systems (Figure 5) gradually increased and the magnitude of the oscillations was diminished as growth was reduced; oscillations virtually disappeared when growth was made equal to zero (Hurwitz et al., 1987a). The average plasma calcium concentration increased as growth was reduced, in agreement with experimental results (Bar and Hurwitz, 1981).

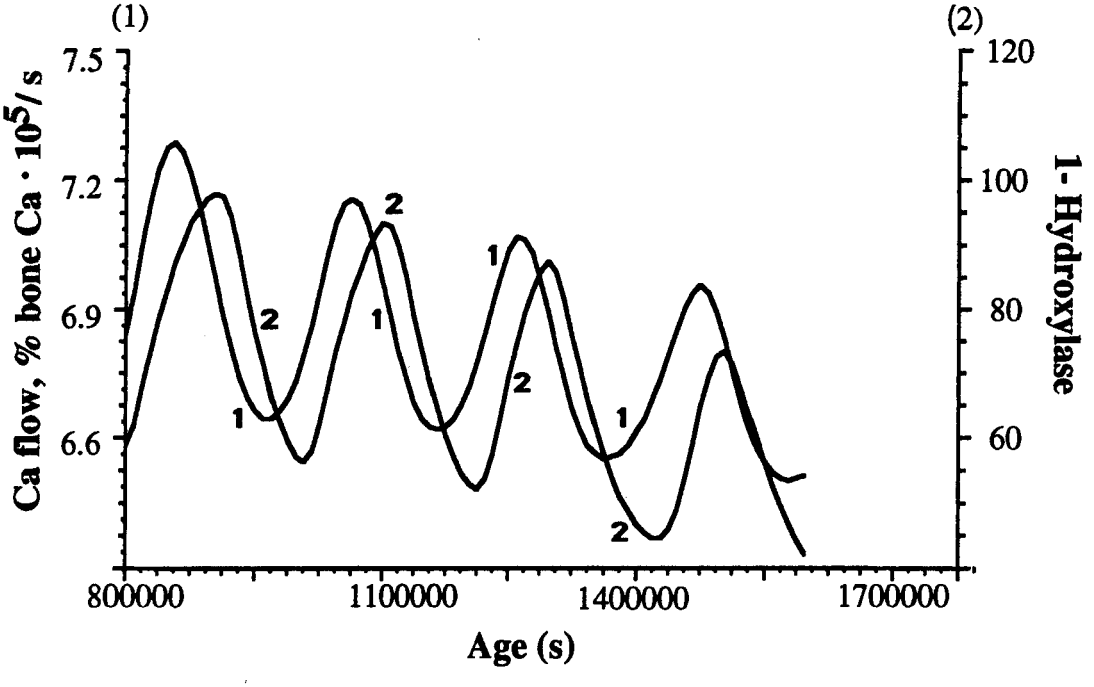



**FIGURE 5.** Simulated oscillations in the $Ca^{2+}$ flow from bone and 1-hydroxylase activity in chicken. (From Hurwitz, S., Miller, B., and Norman, A. W. 1994. *J. Cell. Biochem.* 56: 236–244. With permission.)

## B. Reproduction

Pregnancy and lactation in mammalian species is associated with increased needs for calcium to satisfy the calcium needs of the developing embryo and for milk production. In birds, the calcium needs associated with the formation of a calcified egg shell are huge. In the chicken (*Gallus domesticus*), a single egg shell may contain as much as 10% of the total body calcium. As reviewed by Garel (1987), the increased calcium needs during pregnancy and lactation are met by increases in calcium absorption (Thomas, 1991), mediated by an increase in the circulating $1,25(OH)_2D_3$ (Lobaugh et al., 1990, 1992), which in turn are initiated by changes in circulating PTH (Verhaeghe and Bouillon, 1992). The increase in activity of the calcium-regulating systems, however, is not sufficient to cover calcium losses to the product, so that some skeletal calcium is lost during pregnancy and lactation (Garel, 1987; Kohlmeier and Marcus, 1995). Furthermore, the homeostatic mechanisms may not adjust sufficiently rapidly to the increased demands during parturition and the onset of lactation, resulting in a temporary hypocalcemia in mammalian females. In some highly producing cows, the hypocalcemia during parturition may be so severe that partial paralysis occurs — a syndrome termed parturient paresis or "Milk Fever". The etiology of this syndrome is not entirely understood. According to Horst et al. (1994), the plasma levels of calciotropic hormones (PTH and $1,25(OH)_2D_3$) appear normal in the afflicted cows during parturition. However, the VDR number on target cells decreases dramatically, as does the target tissue response to $1,25(OH)_2D_3$. This defect in the response of bone and intestine to the hormone may lead to a severe calcium deficiency and the observed hypocalcemia.

In female birds at the onset of reproduction, synthesis of $1,25(OH)_2D_3$ and calcium absorption increase (Bar et al., 1978, 1992; Castillo et al., 1979) to accommodate the increased calcium needs for egg shell deposition. In addition, birds are faced with another challenge — the noncontinuous nature of the $Ca^{2+}$ needs. Within the diurnal reproductive cycle of the laying chicken, calcium flow into the shell occurs only during 15 h. To prevent a calcium catastrophe, the systems that supply the needed $Ca^{2+}$ must be switched on and off to accommodate these oscillating needs. Two special homeostatic systems have developed in birds to accommodate the discontinuity in the calcium needs. First, the calcium absorption rate increases considerably during shell formation and drops rapidly after oviposition (Hurwitz et al., 1973), independently of $1,25(OH)_2D_3$ production and its plasma levels (Bar et al., 1976, 1992). Second, medullary bone appears in the marrow cavity of the long bones. This osseous material is rich in blood supply and in cellular components, especially osteoclasts; its calcium is turned over extremely rapidly, with a half-life of less than 2 d compared with a half-life of months characteristic of cortical bone (Hurwitz, 1965). Medullary bone can thus operate as a "buffer" to supply or remove $Ca^{2+}$ rapidly and thus correct for acute errors in plasma $Ca^{2+}$.

# V. ALGORITHM OF CALCIUM HOMEOSTASIS

Simulation algorithms provide a means for integration of the detailed information collected by both *in vivo* and *in vitro* experimentation. Such models can be constructed at different levels of detail, ranging from the molecular through the subcellular and

cellular, up to the level of the entire organism. The selection of the appropriate level of detail depends on the available information and on the required answers, which in turn are functions of the expected inputs and outputs. The scope of a metabolic model depends on its level of detail and the number of factors included. The efficacy of the model is tested by its ability to predict measurable responses in tests that are independent of the experiments involved in parameter estimation during modeling. After testing, the algorithm can be used to predict the responses of the control subsystems to various perturbations.

Early attempts to formulate models that simulate calcium metabolism were made by Bonnier and Cabanac (1970) for the analog computer and by Jaros et al. (1979). The calcium metabolism of growing birds has been described within the framework of a more recent simulation model (Hurwitz et al., 1983, 1987a, b). The model as formulated included most of the interactions given in Figure 1. The concentration of a hormone (H) and other biochemicals that participate in regulating calcium movement is assumed to be governed by first-order kinetics: the synthesis of each [f(S)] is a function of the activity of the stimulant, while its transfer, including its disappearance, is determined by its own concentration and the decay rate ($\beta$):

$$\frac{dH}{dt} = f(S) - \beta \cdot H$$

The equation becomes more complex when the body contains specific hormone pools. This is true, for example, with $1,25(OH)_2D_3$, which is found in organs such as the intestine at concentrations higher than in blood plasma. A transfer term can then be added to the equations to account for transfer into and out of this pool. The simulation algorithm (Hurwitz et al., 1983, 1987a) utilizes numerical procedures for the simultaneous integration of the several differential constituent equations.

The parameters for the respective equations of the model have been estimated using results obtained by *in vivo* and *in vitro* experimentation or obtained by fitting the entire model to experimental observations (Hurwitz et al., 1983). Several known processes and regulating agents are still not included in the model due to insufficient quantitative information. Regulation at the level of the receptor falls into this category. Thus, the model is at present still limited in scope and hence in predictive capacity and practical use. Additional information can be included when made available.

The simulated responses of some of the important controlling systems to changes in calcium intake are shown in Figure 6 in order to demonstrate the predictive capacity of the model. When dietary calcium intake is changed, shifts can be noted in the activity of the major components of the regulatory system. The model demonstrates a rapid approach toward maximal or minimal levels in urinary Ca excretion and in bone resorption due to the rapid decay of components that control these rates, notably PTH. A very slow approach toward maximal values is predicted for the plasma $1,25(OH)_2D_3$ level and calcium absorption machinery due to the slow decay rate of kidney 1-hydroxylase activity, plasma $1,25(OH)_2D_3$, and the Ca absorption machinery. It is also of interest to note the generation, by the algorithm of the long (approximately 10 h) lag time of calcium absorption following the change in calcium intake, brought about by the long chain of events starting with plasma $Ca^{2+}$ and continuing through the PTH level, production and destruction of the 1-hydroxylase complex in the kidney and release of $1,25(OH)_2D_3$ into the circulation, uptake of

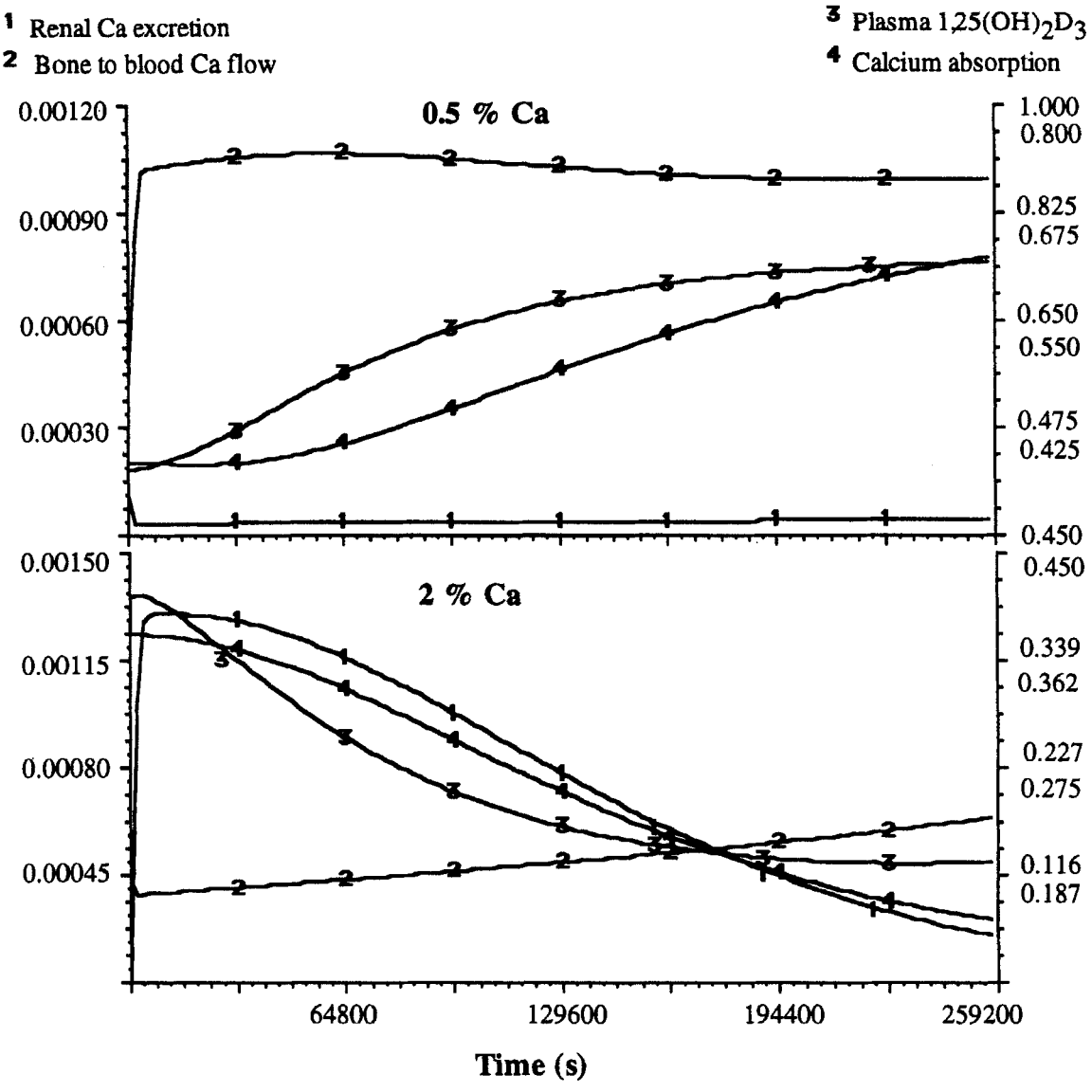



**FIGURE 6.** (1) Simulated responses of renal $Ca^{2+}$ excretion rate in moles per second, (2) bone to blood $Ca^{2+}$ flow in moles per second, (3) plasma $1,25(OH)_2D_3$ in picomoles per milliliter, and (4) fractional intestinal $Ca^{2+}$ absorption to intake of a low calcium diet (upper graph) on a high calcium diet (lower graph).

the hormone by the intestinal mucosa, and the building and destruction of the calcium absorption machinery. Results of simulation also demonstrate the change in the hierarchy of the activity of two main controlling system — bone and kidney. The increase in kidney flow exceeds the decrease in bone flow during the initial phase of exposure to a high calcium intake, decreasing later to a level similar to that of bone flow, as calcium absorption decreases due to the reduction in $1,25(OH)_2D_3$. During the exposure to a very low calcium intake, the change in bone flow of calcium exceeds the decrease in urinary exertion.

The algorithm also predicted the existence in chickens of growth- and calcium intake-dependent oscillatory behavior of

plasma calcium (amplitude of about 0.5 mg/100 ml) as well as of other components of the calcium-regulating system such as bone Ca flow and activity of the 1-hydroxylase enzyme (Figure 5). The existence of such oscillations and the length of the phase between the two variables have recently been verified experimentally (Hurwitz et al., 1994).

## REFERENCES


Abou-Samra, A. B., Goldsmith, P. K., Xie, L. Y., Jüppner, H., Spiegel, A. M., and Segre, G. V. 1994. Down-regulation of parathyroid (PTH)/PTHrP-related peptide receptor immunoreactivity and PTH binding in opossum kidney cells by PTH and dexamethasone. *Endocrinology* **135:** 2588–2594.

Abou-Samra, A. B., Jüppner, H., Force, T., Freeman, M. W., Kong, X. F., Schipani, E., Urena, P., Richards, J., Bonventre, J. V., Kronenberg, H. M., and Segre, G. V. 1992. Expression cloning of a common receptor for parathyroid hormone and parathyroid-hormone related peptide from rat osteoblast-like cells: a single receptor stimulates intracellular accumulation of both cAMP and inositol phosphates and increases intracellular free calcium. *Proc. Natl. Acad. Sci. U.S.A.* **89:** 2732–2736.

Abou-Samra, A. B., Zajac, J. D., Schiffer-Alberts, D., Skurat, R., Kearns, A., Segre, G. V., and Bringhurst, F. R. 1991. Cyclic adenosine 3′,5′-monophosphate (cAMP)-dependent and cAMP-independent regulation of parathyroid hormone receptors on UMR 106-01 osteoblastic osteosarcoma cells. *Endocrinology* **129:** 2547–2554.

Agarwala, N. and Gay, C. V. 1992. Specific binding of parathyroid hormone to living osteoclasts. *J. Bone Miner. Res.* **7:** 531–539.

Akiba, T., Endou, H., Koseki, C., Sakai, F., Horiuchi, N., and Suda, T. 1980. Localization of 25-hydroxyvitamin $D_3$-1-hydroxylase activity in mammalian kidney. *Biochem. Biophys. Res. Commun.* **94:** 313–318.

Amara, S. G., Jonas, V., Rosenfeld, M. G., Ong, E. S., and Evans, R. M. 1982. Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. *Nature* **298:** 240–244.

Anderson, H. C. 1969. Vesicles associated with calcification in the matrix of epiphyseal cartilage. *J. Cell. Biol.* **41:** 59–72.

Anderson, H. C. 1989. Biology of disease: mechanism of mineral formation in bone. *Lab. Invest.* **60:** 320–330.

Arnaud, C. D. and Pun, K.-K. 1992. Metabolism and assay of parathyroid hormone. In: *Disorders of Bone and Mineral Metabolism,* pp. 107–122 (Coe, F. L. and Favus, M. J., Eds.) New York: Raven Press.

Arnett, T. R. and Dempster, D. W. 1987. A comparative study of disaggregated chick and rat osteoclasts *in vitro:* effects of calcitonin and prostaglandins. *Endocrinology* **120:** 602–608.

Attie, M. F., Brown, E. M., Gardner, D. G., Spiegel, A. M., and Aurbach, G. D. 1980. Characterization of the dopamine-responsive adenylate cyclase of bovine parathyroid cells and its relationship to parathyroid hormone secretion. *Endocrinology* **107:** 1776–1781.

Aubert, J.-P., Bronner, F., and Richelle, L. 1963. Quantitation of calcium metabolism theory. *J. Clin. Invest.* **42:** 885–897.

Aurbach, G. D. and Heath, D. A. 1974. Parathyroid hormone and calcitonin regulation of renal function. *Kidney Int.* **6:** 331–345.

Aurbach, G. D. 1982. Polypeptide and amine hormone regulation of adenylate cyclase. *Annu. Rev. Physiol.* **44:** 653–666.

Bacskai, B. G. and Friedman, P. A. 1990. Activation of latent $Ca^{2+}$ channels in renal epithelial cells by parathyroid hormone. *Nature (London)* **347:** 388–391.

Bagambisa, F. B. and Schilli, U. J. W. 1993. A scanning electron microscope study of the ultrastructural organization of bone mineral. *Cells Mater.* **3:** 93–102.

Baimbridge, K. G. and Taylor, T. G. 1981. The role of calcitonin in controlling hypercalcemia in the domestic fowl (*Gallus domesticus*). *Comp. Biochem. Physiol. A* **68:** 647–651.

Baker, A. R., McDonnell, D. P., Hughs, M., Crisp, T. M., Mangelsdorf, D. J., Haussler, M. R., Pike, J. W., Shine, J., and O'Mally, B. W. 1988. Cloning and expression of full-length cDNA encoding the human vitamin D receptor. *Proc. Natl. Acad. Sci. U.S.A.* **85:** 3294–3298.

Bar, A., Cohen, A., Edelstein, S., Shemesh, M., Montecuccoli, G., and Hurwitz, S. 1978. Involvement of cholecalciferol metabolism in birds in the adaptation of calcium absorption to the needs during reproduction. *Comp. Biochem. Physiol. B* **59:** 245–249.

Bar, A., Eisner, U., Montecuccoli, G., and Hurwitz, S. 1976. Regulation of intestinal calcium absorption in the laying quail: independent of kidney vitamin D hydroxylation. *J. Nutr.* **106:** 1336–1342.

Bar, A. and Hurwitz, S. 1979. The interaction between dietary calcium and gonadal hormones in their effect on plasma calcium, bone, 25-hydroxycholecalciferol-1-hydroxylase, and duodenal calcium-binding protein, measured by a radioimmunoassay in chicks. *Endocrinology* **104:** 1455–1460.

Bar, A. and Hurwitz, S. 1981. Relationship between cholecalciferol metabolism and growth in chicks as modified by age, breed and diet. *J. Nutr.* **111:** 399–404.

Bar, A., Hurwitz, S., and Edelstein, S. 1975. Response of renal calcium-binding protein: independence of kidney vitamin D hydroxylation. *Biochim. Biophys. Acta* **411:** 106–112.

Bar, A., Hurwitz, S., and Cohen, I. 1972. Relationship between duodenal calcium-binding protein, parathyroid activity and various parameters of mineral metabolism in the rachitic and vitamin D-treated chick. *Comp. Biochem. Physiol. A* **43:** 519–526.

Bar, A., Hurwitz, S., and Maoz, A. 1980. The 25-hydroxycholecalciferol-1-hydroxylase activity of chicks' kidney cells: direct effect of parathyroid. *FEBS Lett.* **113:** 328–330.

Bar, A., Rosenberg, J., and Hurwitz, S. 1984. The lack of relationship between vitamin $D_3$ metabolites and calcium-binding protein in the egg shell gland of laying birds. *Comp. Biochem. Physiol. B* **78:** 75–79.

Bar, A., Sharvit, M., Noff, D., Edelstein, S., and Hurwitz, S. 1980. Absorption of cholecalciferol and 25-hydroxycholecalciferol and metabolites in birds. *J. Nutr.* **110:** 1930–1934.

Bar, A., Stream, S., Vax, E., Talpaz, H., and Hurwitz, S. 1992. Regulation of calbindin mRNA and calbindin turnover in the intestine and shell gland of the chicken. *Am. J. Physiol.* **262:** R800–R805.

Baran, D. T., Ray, R., Sorensen, A. M., Honeyman, T., and Holick, M. F. 1994. Binding characteristics of a membrane receptor that recognizes 1 alpha, 25-dihydroxyvitamin D-3 and its epimer, 1 beta, 25 dihydroxyvitamin D-3. *J. Cell. Biochem.* **46:** 510–517.

Baron, R., Neff, L., Louvard, D., and Courtoy, P. J. 1985. Cell mediated extracellular acidification and bone resorption: evidence for a low pH in resorbing lacunae and localization of 100 kD lysosomal membrane protein at the osteoclast ruffled border. *J. Cell Biol.* **101:** 2210–2222.

Bélanger, L. F. 1971. The ultimobranchial gland of birds and the effect of nutritional variations. *J. Exp. Zool.* **178:** 125–138.

Bellows, C. G., Ishida, H., Aubin, J. E., and Heersche, J. N. M. 1990. Parathyroid hormone reversibly suppresses the differentiation of osteoprogenitor cells into functional osteoblasts. *Endocrinology* **126:** 3111–3116.

Berdal, A., Hotton, D., Pike, J. W., Mathieu, H., and Dupret, J. M. 1993. Cell- and stage-specific expression of vitamin D receptor and calbindin in rat incisor: regulation by 1,25-dihydroxyvitamin $D_3$. *Dev. Histol.* **155:** 172–179.

Beresford, J. N., Gallagher, J. A., and Russell, R. G. G. 1986. 1,25-Dihydroxyvitamin $D_3$ and human bone-derived cells *in vitro:* effects on alkaline phosphatase, type I collagen and proliferation. *Endocrinology* **119:** 1776–1785.

Bikle, D. D. 1990. Regulation of intestinal calcium transport by vitamin D [$1,25(OH)_2D$]: role of membrane structure. *Membrane Transport and*

*Information Storage*, pp. 191–219. New York: Allan R. Liss.

Bikle, D. D., Munson, S., and Zolok, D. T. 1983. Calcium flux across chick duodenal brush border membrane vesicles: regulation by 1,25-dihydroxyvitamin D. *Endocrinology* **113:** 2072–2080.

Bikle, D. D., Zolok, D. T., Morrissey, R. L., and Herman, R. H. 1978. Independence of 1,25-dihydroxyvitamin $D_3$-mediated calcium transport from *de novo* RNA and protein synthesis. *J. Biol. Chem.* **256:** 3354–3360.

Binderman, I. and Somjen, D. 1984. 24,25-Dihydroxycholecalciferol induces the growth of chick cartilage *in vitro*. *Endocrinology*, **115:** 430–432.

Bindles, R. J. M., Hartog, A., Timmermans, J. A. H., and van Os, C. H. 1991. Active $Ca^{2+}$ transport in primary cultures of rat kidney CCD: stimulation by 1,25-dihydroxyvitamin $D_3$ and PTH. *Am. J. Physiol.* **261:** F799–F807.

Blunt, J. W., DeLuca, H. F., and Schmoes, H. K. 1968. 25-Hydroxycholecalciferol. A biologically active metabolite of vitamin $D_3$. *Biochemistry* **6:** 3317–3322.

Boivin, G., Mesguich, P., Pike, J. W., Bouillon, R., Meunier, P. J., Haussler, M. R., Bubois, P. M., and Morel, G. 1987. Ultrastructural immunocytochemical localization of endogenous 1,25-dihydroxyvitamin $D_3$ and its receptors in osteoblasts and osteocytes from neonatal mouse and rat calvaria. *Bone Miner.* **3:** 125–136.

Boland, R. L. B. 1986. Plants as a source of vitamin $D_3$ metabolites. *Nutr. Rev.* **44:** 1–8.

Bomsztyk, K., George, J. P., and Write, F. S. 1984. Effects of luminal fluid anions on calcium transport by proximal tubule. *Am. J. Physiol.* **246:** F600–F608.

Bonnier, G. and Cabanac, M. 1970. Régulation de la calcémie. Étude analogique. *Rev. Eur. Étud. Clin. Biol.* **15:** 551–557.

Booth, B. E., Tsai, H. C., and Morris, R. C., Jr. 1985. Vitamin D status regulates 25-hydroxyvitamin $D_3$-1$\alpha$-hydroxylase and its responsiveness to parathyroid hormone in the chick. *J. Clin. Invest.* **75:** 155–161.

Bouillon, R., Okamura, W. H., and Norman, A. W. 1995. Structure-function relationships in the vitamin D endocrine system. *Endocr. Rev.* **16:** 200–257.

Bourdeau, A., Atmain, F., Grosse, B., and Lieberherr, M. 1990. Rapid effects of 1,25-dihydroxyvitamin $D_3$ and extracellular $Ca^{2+}$ on phospholipid metabolism in dispersed porcine parathyroid cells. *Endocrinology* **127:** 2738–2743.

Bouhtiauy, I., Lajeunesse, D., and Brunette, M. G. 1991. The mechanism of parathyroid hormone action on calcium reabsorption by the distal tubule. *Endocrinology* **128:** 251–258.

Bouhtiauy, I., Lajeunesse, D., Christakos, S., and Brunette, M. 1994. Two vitamin $D_3$-dependent calcium binding proteins increase calcium reabsorption by different mechanisms. I. Effect of $CaBP_{28k}$. *Kidney Int.* **45:** 461–468.

Bowman, B. M. and Miller, S. C. 1986. The proliferation and differentiation of the bone-lining cells in estrogen induced osteogenesis. *Bone* **6:** 351–357.

Brain, S. D., Williams, T. J., Tippins, J. R., Morris, J. R., and McIntyre, I. 1985. Calcitonin gene-related peptide is a potent vasodilator. *Nature (London)* **313:** 54–56.

Brewer, H. B., Jr. and Ronan, R. 1969. Amino acid sequence of bovine thyrocalcitonin. *Proc. Natl. Acad. Sci. U.S.A.* **63:** 940–947.

Broadus, A. E. 1981. Nephrogenous cyclic AMP. *Recent Prog. Horm. Res.* **36:** 667–701.

Brommage, R. and DeLuca, H. F. 1985. Evidence that 1,25-dihydroxyvitamin $D_3$ is the physiologically active metabolite of vitamin $D_3$. *Endocr. Rev.* **6:** 491–511.

Bronner, F. 1987. Intestinal calcium absorption: mechanism and applications. *J. Nutr.* **116:** 1347–1352.

Bronner, F. 1989. Renal calcium transport: mechanisms and regulation — an overview. *Am. J. Physiol.* **257:** F707–F711.

Bronner, F. 1990. Intestinal transport: the cellular pathway. *Miner. Electrolyte Metab.* **16:** 94–100.

Bronner, F. and Aubert, J. P. 1965. Bone metabolism and regulation of blood calcium level in rats. *Am. J. Physiol.* **209:** 887–890.

Bronner, F., Pansu, D., and Stein, W. D. 1986. An analysis of intestinal calcium transport across the rat intestine. *Am. J. Physiol.* **250:** G561–G569.

Bronner, F., Sammon, P. J., Nichols, C., Stacey, R. E., and Shah, B. G. 1967. Thyrocalcitonin and plasma calcium homeostasis in the rat. *Excerpta Med. Int. Congr. Ser.* **159:** 353–369.

Bronner, F. and Stein, W. D. 1992. Modulation of bone calcium-binding sites regulates plasma calcium: an hypothesis. *Calcif. Tissue Int.* **50:** 483–489.

Brown, A. J., Zhong, M., Finch, J., Ritter, C., and Slatopolski, E. 1995. The roles of calcium and 1,25-dihydroxyvitamin $D_3$ in the regulation of vitamin D receptor expression by rat parathyroid glands. *Endocrinology* **136:** 1419–1425.

Brown, E. M. 1983. Four-parameter model of the sigmoidal relationship between parathyroid hormone release and extracellular calcium concentration in normal and abnormal parathyroid tissue. *J. Clin. Endocrinol. Metab.* **56:** 572–581.

Brown, E. M. 1991. Extracellular $Ca^{2+}$ sensing, regulation of parathyroid cell function, and role of $Ca^{2+}$ and other ions as extracellular (first) messengers. *Physiol. Rev.* **71:** 371–411.

Brown, E. M. 1994. Homeostatic mechanisms regulating extracellular and intracellular calcium metabolism. In: *The Parathyroids,* pp. 15–54. (Bilezikian, J. P. and Levine, M. A., Eds.) New York: Raven Press.

Brown, E. M., Gamba, G., Riccardi, D., Lombard, M., Butters, R., Kifor, O., Sun, A., Hediger, M. A., Lytton, J., and Hebert, S. C. 1993. Cloning and characterization of an extracellular $Ca^{2+}$-sensing receptor from bovine parathyroid. *Nature (London)* **366:** 575–580.

Brown, E. M., Gardner, D. G., Windeck, R. A., and Aurbach, G. D. 1979. Cholera toxin stimulates 3′,5′-adenosine monophosphate accumulation and parathyroid hormone release from dispersed bovine parathyroid cells. *Endocrinology* **104:** 218–225.

Brown, E. M., Hurwitz, S., and Aurbach, G. D. 1976. Preparation of viable isolated bovine parathyroid cells. *Endocrinology* **99:** 1582–1588.

Brown, E. M., Hurwitz, S., and Aurbach, G. D. 1977. Beta adrenergic stimulation of cyclic AMP content and parathyroid hormone release from isolated parathyroid cells. *Endocrinology* **100:** 1696–1702.

Brown, E. M., Pazoles, C. V. J., Creutz, C. E., Aurbach, G. D., and Pollard, H. B. 1978. Role of anions in parathyroid hormone release from bovine parathyroid cells. *Proc. Natl. Acad. Sci. U.S.A.* **75:** 876–880.

Brown, M. D., Perey, D. Y. E., and Jowsey, J. 1970. Effects of ultimobranchialectomy on bone composition and mineral metabolism in the chicken. *Endocrinology* **87:** 1282–1291.

Bruce, B. R. and Anderson, N. C., Jr. 1979. Hyperpolarization in mouse parathyroid cells by low calcium. *Am. J. Physiol.* **236:** C15–C21.

Burch, W. M., Lopez-Carlos, M., Uskokovic, M. R., and Drezner, M. K. 1988. 1,25-Dihydroxyvitamin $D_3$ stimulates avian and mammalian cartilage growth *in vitro*. *J. Bone Miner. Res.* **3:** 87–91.

Burmester, J. K., Maeda, N., and DeLuca, H. F. 1988. Isolation and expression of rat 1,25-dihydroxyvitamin $D_3$ receptor cDNA. *Proc. Natl. Acad. Sci. U.S.A.* **85:** 1005–1009.

Cai, Q., Tapper, D. N., Gilmour, R. F., Jr., deTalamoni, N., Aloia, R. C., and Wasserman, R. H. 1994. Modulation of the excitability of avian peripheral nerves by vitamin D: relation to calbindin-$D_{28k}$, calcium status and lipid composition. *Cell. Ca* **15:** 401–410.

Candeliere, G. A., Prud'homme, J., and St-Arnaud, R. 1991. Differential stimulation of *fos* and *jun* family members by calcitriol in osteoblastic cells. *Mol. Endocrinol.* **5:** 1780–1788.

Candlish, J. K. and Taylor, T. G. 1970. The response-time to parathyroid hormone in the laying fowl. *J. Endocrinol.* **48:** 143–144.

Canterbury, J. M., Bricker, L. A., Levey, G. S., Kozlovskis, P. L., Ruiz, E., Zull, J. E., and Reiss, E. 1975. Metabolism of bovine par-

athyroid hormone: immunological and biological characteristics of fragments generated by liver perfusion. *J. Clin. Invest.* **55:** 1245–1253.

Cantly, L. K., Russell, J., Lettieri, D., and Sherwood, L. M. 1985. 1,25-Dihydroxyvitamin $D_3$ suppresses parathyroid hormone secretion from bovine parathyroid cells in tissue culture. *Endocrinology* **116:** 2114–2119.

Cao, H. and Gay, C. V. 1985. Effects of parathyroid hormone and calcitonin on carbonic anhydrase location in osteoclasts of cultured embryonic chick bone. *Experientia* **41:** 1472–1474.

Carafoli, E. 1991. Calcium pump of the plasma membrane. *Physiol. Rev.* **71:** 129–153.

Care, A. D., Cooper, C. W., Duncan, T., and Orimo, H. 1968. A study of thyrocalcitonin secretion by direct measurement of *in vivo* secretion rates in pigs. *Endocrinology* **83:** 161–169.

Care, A. D. and Bates, R. F. L. 1972. Control of secretion of parathyroid hormone and calcitonin in mammals and birds. *Gen. Comp. Endocrinol.* **3:** 448–458.

Care, A. D., Bates, R. F. L., and Gitelman, H. J. 1970. A possible role for the adenyl cyclase system in calcitonin release. *J. Endocrinol.* **48:** 115.

Care, A. D., Bruce, J. B., Boelkins, J., Kenny, A. D., Conaway, H., and Anast, C. S. 1971. The role of pancreozymin-cholecystokinin and structurally related compounds as calcitonin secretagogues. *Endocrinology* **89:** 262–271.

Carnes, D. L., Nickols, G. A., Anast, C. S., and Forte, L. R. 1980. Regulation of renal adenylate cyclase by parathyroid hormone. *Am. J. Physiol.* **239:** E396–E400.

Carruthers, B. M., Copp, D. H., McIntosh, H. W., Uhlemann, I., Kore, R., and Stokoe, N. 1964. Diurnal variation in urinary excretion of calcium and phosphate and its relation to blood levels. *J. Lab. Clin. Med.* **63:** 959–968.

Castillo, L., Tanaka, Y., Wineland, M. J., Jowsey, J. O., and DeLuca, H. F. 1979. Production of 1,25-dihydroxyvitamin $D_3$ and formation of medullary bone in the egg laying hen. *Endocrinology* **104:** 1598–1601.

Centrella, M. and Canalis, E. 1985. Local regulators of skeletal growth: a perspective. *Endocr. Rev.* **6:** 544–551.

Chabre, O., Conklin, B. R., Lin, H. Y., Lodish, H. F., Wilson, E., Ives, H. E., Catanzariti, L., Hemmings, B. A., and Bourne, H. R. 1992. A recombinant calcitonin receptor independently stimulates 3′,5′-cyclic adenosine monophosphate and $Ca^{2+}$/inositol phosphate signaling pathways. *Mol. Endocrinol.* **6:** 551–556.

Chakraborty, M., Chatterjee, D., Kellokumpu, S., Rasmussen, H., and Baron, R. 1991. Cell cycle-dependent coupling of the calcitonin receptor to different G proteins. *Science* **261:** 1978–1982.

Chambers, T. J., McSheehy, P. M. J., Thomson, B. M., and Fuller, K. 1985. The effect of calcium-regulating hormones and prostaglandins on the bone resorption by osteoclasts disaggregated from neonatal rabbit bone. *Endocrinology* **116:** 234–239.

Chandra, S., Fullmer, C. S., Smith, C. A., Wasserman, R. H., and Morrison, G. H. 1990. Ion microscopy imaging of calcium transport in the intestinal tissue of vitamin D-deficient and vitamin D-replete chickens: a $^{44}Ca$ isotope study. *Proc. Natl. Acad. Sci. U.S.A.* **87:** 5715–5719.

Chard, P. S., Bleakman, D., Christakos, S., Fullmer, C. S., and Miller, R. J. 1993. Calcium buffering properties of calbindin $D_{28k}$ and parvalbumin in rat sensory neurons. *J. Physiol.* **472:** 341–357.

Chase, L. R. and Aurbach, G. D. 1967. Parathyroid function of the renal excretion of 3′,5′-adenylic acid. *Proc. Natl. Acad. Sci. U.S.A.* **58:** 518–525.

Chase, L. R. and Aurbach, G. D. 1970. The effect of parathyroid hormone on the concentration of adenosine 3′,5′-monophosphate in skeletal tissue, *in vitro*. *J. Biol. Chem.* **245:** 1520–1526.

Chentoufi, J. and Marie, P. J. 1994. Interactions between retinoic acid and $1,25(OH)_2D$ in mouse immortalized osteoblastic C1 cells. *Am. J. Physiol.* **266:** C1247–C1256.

Chin, J. E., Schalk, E. M., Kemic, M. L. S., and Wuthier, R. E. 1986. Effect of synthetic hu-

man parathyroid hormone on the levels of alkaline phosphatase activity and formation of alkaline phosphatase-rich matrix vesicles by primary cultures of chicken epiphyseal growth plate chondrocytes. *Bone Miner.* **1:** 421–436.

Chorev, M. and Rosenblatt, M. 1994. Structure-function analysis of parathyroid hormone and parathyroid hormone-related protein. In: *The Parathyroids,* pp. 139–156. (Bilezikian, J. P., Levine, M. A., and Marcus, R., Eds.) New York: Raven Press.

Christakos, S., Gabrielides, C., and Rhoten, W. B. 1989. Vitamin D-dependent calcium-binding proteins: chemistry, distribution, functional considerations and molecular biology. *Endocr. Rev.* **10:** 3–26.

Christakos, S., Gill, R., Lee, S., and Li, H. 1992. Molecular aspects of calbindins. *J. Nutr.* **122:** 678–682.

Civitelli, R., Reid, I. R., Westbrook, S., Avioli, L. V., and Hruska, K. A. 1988. PTH elevates inositol polyphosphates and diacylglycerol in a rat osteoblast-like cell line. *Am. J. Physiol.* **255:** E660–E667.

Clark, N. B. and Wideman, R. F., Jr. 1980. Calcitonin stimulation of urine flow and sodium excretion in the starling. *Am. J. Physiol.* **238:** R406–R412.

Clohisy, J. C., Scott, D. K., Brakenhoff, K. D., Quinn, C. O., and Partridge, N. C. 1992. Parathyroid hormone induces c-*fos* and c-*jun* messenger RNA in rat osteoblastic cells. *Mol. Endocrinol.* **6:** 1834–1842.

Cohn, D. V. and Elting, J. 1983. Biosynthesis, processing and secretion of parahormone and secretory protein-I. *Recent Prog. Horm. Res.* **39:** 181–209.

Collip, J. B. 1925. The extraction of a parathyroid hormone which will prevent or control parathyroid tetany and which regulates the level of blood calcium. *J. Biol. Chem.* **63:** 395–438.

Cooper, C. W., Borowsky, S. A., Farrell, P. E., and Steinsland, O. S. 1986. Effects of calcium channel activator Bay-K8644 on *in vivo* secretion of calcitonin and parathyroid hormone. *Endocrinology* **118:** 545–549.

Cooper, C. W., McPherson, M. B., Seitz, P. K., Greely, G. H., Abbas, S. K., Pickard, D. W., and Care, A. D. 1991. Stimulation of calcitonin secretion in the pig by calcitonin gene-related peptide. *Bone Miner.* **12:** 73–79.

Copp, D. H., Cockcroft, D. W., and Kuch, Y. 1967. Calcitonin from ultimobranchial glands of dogfish and chickens. *Science* **158:** 924–926.

Copp, H. D., Byfield, P. G. H., Kerr, C. R., Newsome, F., Walker, V., and Watts, E. G. 1972. Calcitonin and ultimobranchial function in fishes and birds. In: *Calcium, Parathyroid Hormone and the Calcitonins,* pp. 12–20. (Talmage, R. Y. and Munson, P. L., Eds.) Int. Congr. Ser. No. 243. Amsterdam: Excerpta Medical Foundation.

Copp, D. H., Davidson, A. G. F., and Cheney, B. A. 1961. Evidence for a new parathyroid hormone which lowers plasma calcium. *Proc. Can. Fed. Biol. Soc.* **4:** 17.

Corradino, R. A. 1985. Effects of verapamil and dexamethasone on the 1,25-dihydroxyvitamin $D_3$-mediated calcium absorptive mechanism in the organ cultured embryonic chick duodenum. *Biochem. Pharmacol.* **34:** 1971–1974.

Corradino, R. A. and Fullmer, C. S. 1991. Positive cotranscriptional regulation of intestinal calbindin-$D_{28K}$ gene expression by 1,25-dihydroxyvitamin $D_3$ and glucocorticoids. *Endocrinology* **128:** 944–950.

Coty, W. A. 1980. A specific, high-affinity binding protein for 1α,25 dihydroxy vitamin D in the chick oviduct shell gland. *Biochem. Biophys. Res. Commun.* **93:** 285–292.

Craviso, G. L., Garrett, K. P., and Clemens, T. L. 1987. 1,25-Dihydroxyvitamin $D_3$ induces the synthesis of vitamin D-dependent calcium-binding protein in cultured chick kidney cells. *Endocrinology* **120:** 894–902.

D'Amour, P., Plardy, J., Bahsali, G., Mallette, L. E., DeLélan, A., and Lepage, R. 1992. The modulation of circulating parathyroid hormone immunoheterogeneity in man by ionized calcium concentration. *Endocrinology* **74:** 525–532.

Darwish, H. M. and DeLuca, H. F. 1992. Identification of a 1,25-dihydroxyvitamin $D_3$-response

element in the 5′ flanking region of the rat calbindin D-9K gene. *Proc. Natl. Acad. Sci. U.S.A.* **89:** 603–607.

Daugaard, H., Egfjord, M., and Olgaard, K. 1990. Influence of calcium on metabolism of intact parathyroid hormone by isolated perfused rat kidney and liver. *Endocrinology* **126:** 1813–1820.

Daugaard, H., Egfjord, M., Lewin, E., and Olgaard, K. 1994. Metabolism of N-terminal and C-terminal parathyroid hormone fragments by isolated rat kidney and liver. *Endocrinology* **134:** 1373–1381.

DeLisle, R. C. and Williams, J. A. 1986. Regulation of membrane fusion in secretory exocytosis. *Annu. Rev. Physiol.* **48:** 225–238.

DeLuca, H. F., Nakada, M., Tanaka, Y., Scinski, R., and Phelp, M. 1988. The plasma binding protein for vitamin D is a site of discrimination against vitamin D-2 in the chick. *Biochim. Biophys. Acta* **965:** 16–21.

Demay, M. B., Kiernan, M. S., DeLuca, H. F., and Kronenberg, H. M. 1992. Sequences in the human parathyroid hormone gene that bind the 1,25-dihydroxyvitamin-$D_3$ receptor and mediate transcriptional repression in response to 1,25-dihydroxyvitamin-$D_3$. *Proc. Natl. Acad. Sci. U.S.A.* **17:** 8097–8101.

Dempster, D. W., Cosman, F., Parisien, M., Shen, V., and Lindsey, R. 1993. Anabolic actions of parathyroid hormone on bone. *Endocr. Rev.* **14:** 690–709.

Dempster, D. W., Murills, R. J., Herbert, W. R., and Arnett, T. R. 1987. Biological activity of chicken calcitonin: effects on neonatal rat and embryonic chick osteoclasts. *J. Bone Miner. Res.* **2:** 443–448.

Desplan, C., Thomasset, M., and Moukhtar, M. 1983. Synthesis, molecular cloning and restriction analysis of DNA complementary to vitamin D-dependent calcium-binding protein mRNA from rat duodenum. *J. Biol. Chem.* **258:** 2762–2765.

Dewhirst, F. E., Ago, J. M., Peros, W. J., and Stashenko, P. 1987. Synergism between parathyroid hormone and interleukin I in stimulating bone resorption in organ culture. *J. Bone Miner. Res.* **2:** 127–133.

Dick, I. M., Retallack, R., and Prince, R. L. 1990. Rapid nongenomic inhibition of 25-hydroxyvitamin $D_3$ 1-hydroxylase by 1,25-dihydroxyvitamin $D_3$. *Am. J. Physiol.* **259:** E272–E277.

Dousa, T. P. 1974. Effects of hormones on cyclic AMP formation in kidneys of nonmammalian vertebrates. *Am. J. Physiol.* **226:** 1193–1197.

Downes, P. and Mitchell, R. 1985. Inositol phospholipid breakdown as receptor controlled generator of second messengers. In: *Molecular Mechanisms of Transmembrane Signaling*, pp. 3–56. (Cohen, P. and Houslay, M. D., Eds.) New York: Elsevier.

Dunlay, R. and Hruska, K. 1990. PTH receptor coupling to phospholipase C is an alternative pathway of signal transduction in bone and kidney. *Am. J. Physiol.* **258:** F223–F231.

Edelstein, S., Harell, A., Bar, A., and Hurwitz, S. 1975. The functional metabolism of vitamin D in chicks fed low calcium and low phosphorus diets. *Biochim. Biophys. Acta* **385:** 438–444.

Eisenberg, H., Pallotta, J., Sacks, B., and Brickman, A. S. 1989. Parathyroid localization, three-dimensional modeling, and percutaneous ablation techniques. *Endocr. Metab. Clin. North Am.* **18:** 659–700.

Eliam-Cisse, M. C., Taboulet, J., Bielakoff, J., Lasmoles, F., de Vernejoul, M. C., and Treilhou-Lahille, F. 1993. Influence of calcium and vitamin D-deficient diet on calcitonin gene expression in the ultimobranchial cells of the developing chicken. *Gen. Comp. Endocrinol.* **89:** 195–205.

Enomoto, H., Hendy, G. N., Andrews, G. K., and Clemens, T. L., 1992. Regulation of avian calbindin-$D_{28K}$ gene expression in primary chick kidney cells: importance of posttranscriptional mechanisms and calcium ion concentration. *Endocrinology* **130:** 3467–3474.

Evans, R. M. 1988. The steroid and thyroid hormone receptor superfamily. *Science* **240:** 889–895.

Farach-Carson, M. C., Sergeev, I., and Norman, A. W. 1991. Nongenomic actions of 1,25-dihydroxyvitamin $D_3$ in rat osteosarcoma cells:

structure-function studies using ligand analogs. *Endocrinology* **129:** 1876–1884.

Barley, J. R., Tarbaux, N. M., Hall, S. L., Linkhart, T. A., and Baylink, D. J. 1988. The antibone-resorptive agent calcitonin also acts *in vitro* to directly increase bone formation and bone cell proliferation. *Endocrinology* **123:** 159–167.

Farley, J. R., Wergedal, J. E., Hall, S. L., Herring, S., and Tarbaux, N. M. 1991. Calcitonin has direct effect on $^3$[H]-thymidine incorporation and alkaline phosphatase activity in human osteoblast-line cells. *Calcif. Tissue Int.* **48:** 297–301.

Feher, J. J. 1983. Facilitated calcium diffusion by intestinal calcium-binding protein. *Am. J. Physiol.* **244:** C303–C307.

Feher, J. J., Fullmer, C. S., and Wasserman, R. H. 1992. Role of facilitated diffusion of calcium by calbindin in intestinal calcium absorption. *Am. J. Physiol.* **262:** C517–C526.

Feher, J. J. and Wasserman, R. H. 1979. Calcium absorption and intestinal calcium-binding protein: quantitative relationship. *Am. J. Physiol.* **236:** E556–E561.

Feinblatt, J. D., Raisz, L. G., and Kenny, A. D. 1973. Secretion of avian ultimobranchial calcitonin in organ culture. *Endocrinology* 93: 277–284.

Feldman, D. and Malloy, P. J. 1990. Hereditary 1,25-dihydroxyvitamin D resistant rickets: molecular basis and implications for the role of $1,25(OH)_2D_3$ in normal physiology. *Mol. Cell. Endocrinol.* **72:** C57–C62.

Ferrari, S., Molinari, S., Battini, R., Cossu, G., and Lamon-Fava, S. 1992. Induction of calbindin-$D_{28k}$ by 1,25-dihydroxyvitamin $D_3$ in cultured chicken intestinal cells. *Exp. Cell Res.* **200:** 528–531.

Filburn, C. R. and Harrison, S. 1990. Parathyroid hormone regulation of cytosolic $Ca^{2+}$ in rat proximal tubules. *Am. J. Physiol.* **258:** F545–F552.

Fishman, S., Talpaz, H., Bar, A., and Hurwitz, S. 1986. Parameter estimation for ligand binding system kinetics applied to 1,25-dihydroxycholecalciferol. *Anal. Biochem.* **154:** 144–151.

Fischer, J. A., Blum, J. W., Born, M. A., and Dempster, D. W. 1982. Regulation of parathyroid hormone secretion *in vitro* and *in vivo*. *Calcif. Tissue Int.* **34:** 313–316.

Forte, L. R., Langeluttig, S. G., Bieler, H. V., Poelling, R. E., Magiola, L., and Thomas, M. L. 1983. Upregulation of kidney adenylate cyclase in the egg-laying hen: role of estrogen. *Am. J. Physiol.* **245:** E273–E280.

Fox, J. 1992. Hypocalcemia, but not PTH or hypophosphatemia, induces a rapid increase in $1,25(OH)_2D_3$ levels in rats. *Am. J. Physiol.* **262:** E211–E215.

Fraser, D. R. and Kodicek, E. 1970. Unique biosynthesis by kidney of a biologically active vitamin D metabolite. *Nature (London)* **228:** 764–766.

Fraser, D. and Kodicek, E. S. 1973. Regulation of 25-hydroxycholecalciferol-1-hydroxylase by parathyroid hormone. *Nature New Biol.* **241:** 163–166.

Freedman, L. P. and Towers, T. L. 1991. DNA binding properties of the vitamin $D_3$ receptor zinc finger region. *Mol. Endocrinol.* **5:** 1815–1826.

Friedman, P. A. and Gesek, F. A. 1993. Calcium transport in renal epithelial cells. *Am. J. Physiol.* **264:** F181–F198.

Friedman, J. and Raisz, L. G. 1965. Thyrocalcitonin: inhibitor of bone resorption in tissue culture. *Science* **150:** 1465–1467.

Frost, H. M., Villanueva, A. R., Jett, S., and Eyring, E. 1969. Tetracycline-based analysis of bone remodelling in osteoporosis. *Clin. Orthoped.* **65:** 203–217.

Fuller, K., Gallagher, A. C., and Chambers, T. J. 1991. Osteoclast resorption stimulating activity is associated with osteoblast surface and/or the extracellular matrix. *Biochim. Biophys. Res. Commun.* **181:** 67–73.

Fullmer, C. S. 1992. Intestinal calcium absorption: calcium entry. *J. Nutr.* **122:** 644–650.

Fussell, M. H. 1960. Studies on the Calcium and Phosphorus Metabolism in the Hen with Particular Reference to Absorption and Excre-

tion. Ph.D. dissertation, Cambridge University.

Garel, J.-M. 1987. Hormonal control of calcium metabolism during reproductive cycle in mammals. *Physiol. Rev.* **66:** 1–66.

Garret, J. E., Tamir, H., Kifor, O., Simin, R. T., Rogers, K. V., Gagel, R. F., and Brown, E. M. 1996. Calcitonin-secreting cells of the thyroid express extracellular calcium-sensing receptor gene. *Endocrinology* In press.

Gehron Robey, P., Bianco, P., and Termine, J. D. 1992. The cellular biology and molecular biochemistry of bone formation. In *Disorders of Bone and Mineral Metabolism,* pp. 241–263. (Coe, F. I. and Favus, M. J., Eds.) New York: Raven Press.

Gerstenfeld, L. C., Kelly, C. M., von Deck, M., and Lian, J. B. 1990. Effect of 1,25-dihydroxyvitamin $D_3$ on induction of chondrocyte maturation in culture: extracellular matrix gene expression and morphology. *Endocrinology* **126:** 1599–1609.

Gesek, F. A. and Friedman, P. A. 1993. Calcitonin stimulates calcium transport in distal convoluted tubule cells. *Am. J. Physiol.* **264:** F744–F751.

Glimcher, M. J. 1992. The nature of the mineral component of bone and the mechanism of calcification. In: *Disorders of Bone and Mineral Metabolism,* pp. 265–286. (Coe, F. L. and Favus, M. J., Eds.) New York: Raven Press.

Godyn, J. J., Xu, H., Zhang, F., Kolla, S., and Studzinski, G. P. 1994. A dual block to cell cycle progression in HL60 cells exposed to analogues of vitamin $D_3$. *Cell Prolif.* **27:** 37–46.

Gonnerman, W. A., Ramp, W. K., and Toverud, S. U. 1975. Vitamin D, dietary calcium and parathyroid interactions in chicks. *Endocrinology* **96:** 275–281.

Gorn, A. H., Lin, H. Y., Yamin, M., Auron, P. E., Flannery, M. R., Tapp, D. R., Manning, C. A., Lodish, H. F., Krane, S. M., and Goldring, S. R. 1992. Cloning, characterization, and expression of human calcitonin receptor from an ovarian carcinoma cell line. *J. Clin. Invest.* **90:** 1726–1735.

Gorski, J. P. 1992. Acidic phosphoproteins from bone matrix: a structural rationalization of their role in biomineralization. *Calcif. Tissue Int.* **50:** 391–396.

Grigoriadis, A. E., Heersche, J. N. M., and Aubin, J. E. 1988. Differentiation of muscle, fat, cartilage and bone from progenitor cells present in a bone-derived clonal cell population: effect of dexamethasone. *J. Cell. Biol.* **106:** 2139–2151.

Grosse, B., Bordeau, A., and Lieberherr, M. 1993. Oscillations in inositol 1,4,5-triphosphate and diacylglycerol induced by vitamin D metabolites in confluent mouse osteoblasts. *J. Bone Miner. Res.* **8:** 1059–1069.

Guiness-Hey, M. and Hock, J. M. 1984. Increased trabecular bone mass in rats treated with human synthetic parathyroid hormone. *Metab. Bone Dis. Relat. Res.* **5:** 177–181.

Habener, J., Kemper, B., and Potts, J. T., Jr. 1975. Calcium-dependent intracellular degradation of parathyroid hormone: a possible mechanism for regulation of hormone stores. *Endocrinology* **97:** 431–441.

Habener, J., Rosenblatt, M., and Potts, J. T., Jr. 1984. Parathyroid hormone: biochemical aspects of biosynthesis, secretion, action and metabolism. *Physiol. Rev.* **64:** 985–1053.

Halevy, O., Schindler, D., Hurwitz, S., and Pines, M. 1991. Epidermal growth factor receptor gene expression in avian epiphyseal growth-plate cartilage cells: effect of serum, parathyroid hormone and atrial natriuretic peptide. *Mol. Endocrinol.* **75:** 229–235.

Hall, G. E. and Kenny, A. D. 1987. Role of carbonic anhydrase in bone resorption: effect of acetazolamide on basal and parathyroid hormone-induced bone metabolism. *Calcif. Tissue Int.* **40:** 212–218.

Halloran, B. P., Portale, A. A., Castro, M., Morris, R. C., Jr., and Goldsmith, R. S. 1985. Serum concentration of 1,25-dihydroxyvitamin D in the human: diurnal variation. *J. Clin. Endocrinol. Metab.* **60:** 1104–1110.

Hanley, D. A. and Ayer, L. 1986. Calcium-dependent release of carboxyl-terminal fragments of parathyroid hormone by hyperplastic human

parathyroid tissue *in vitro*. *J. Clin. Endocrinol. Metab.* **63:** 1075–1079.

Hanley, D. A., Takatsuki, K., Sultan, J. M., Schneider, A. B., and Sherwood, L. M. 1978. Direct release of parathyroid hormone fragments from functioning bovine parathyroid glands *in vitro*. *J. Clin. Invest.* **62:** 1247–1254.

Harrison, H. E. and Harrison, H. C. 1963. Theories of vitamin D action. In: *Transfer of Calcium and Strontium Across Biological Membranes,* pp. 229–251. (Wasserman, R. H., Ed.) New York: Academic Press.

Harrison, H. E. and Harrison, H. C. 1960. Transfer of $Ca^{45}$ across intestinal wall *in vitro* in relation to vitamin D and cortisol. *Am. J. Physiol.* **199:** 265–271.

Harrison, J. R. and Clark, N. B. 1986. Avian medullary bone in organ culture: effect of vitamin D metabolites on collagen synthesis. *Calcif. Tissue Int.* **39:** 35–43.

Haussler, M. R. 1986. Vitamin D receptors: nature and function. *Annu. Rev. Nutr.* **6:** 527–562.

Haussler, M. R., Nagode, L. A., and Rasmussen, H. 1970. Induction of intestinal brush border alkaline phosphatase by vitamin D and its identity with CaATPase. *Nature (London)* **228:** 1199–1201.

Hattersley, G., Kergy, J. A., and Chambers, T. J. 1991. Identification of osteoclast precursors in multilineage hemopoietic colonies. *Endocrinology* **128:** 259–262.

Heersche, J. N. M., Marcus, R., and Aurbach, G. D. 1974. Calcitonin and the formation of 3′,5′,-AMP in bone and kidney. *Endocrinology* **94:** 251–257.

Heinrich, G., Kronenberg, H. M., Potts, J. T., Jr., and Habener, J. F. 1984. Gene encoding parathyroid hormone: nucleotide sequence of the rat gene and deduced amino acid sequence of rat preproparathyroid hormone. *J. Biol. Chem.* **259:** 3320–3329.

Henry, H. 1981. $25(OH)D_3$ metabolism in kidney cell culture: lack of direct effect of estradiol. *Am. J. Physiol.* **240:** E119–E124.

Henry, H. L. and Norman, A. W. 1975. Studies on the mechanism of action of calciferol. VII. Localization of $1,25\text{-}(OH)_2D_3$ in chicken parathyroid glands. *Biochem. Biophys. Res. Commun.* **67:** 781–788.

Henry, H. L. and Norman, A. W. 1978. Vitamin D: two dihydroxylated metabolites are required for normal chicken egg hatchability. *Science* **201:** 835–837.

Hermann-Erlee, M. P. M., Nijweide, P. J., van der Meer, J. M., and Ooms, M. A. C. 1983. Action of bPTH and bPTH fragments on embryonic bone *in vitro:* dissociation of the cyclic AMP and the bone-resorbing response. *Calcif. Tissue Int.* **35:** 70–77.

Hesch, R. D., Ebel, H., Hermann, R., and Jüppner, H. 1978. Endocrinological aspects of PTH metabolism in the kidney. *Cont. Nephrol.* **13:** 104–114.

Hirsch, P. F., Voelkel, E. F., and Munson, P. L. 1964. Thyrocalcitonin:hypocalcemic, hypophosphatemic principle of the thyroid gland. *Science* **146:** 412–414.

Holick, M. F. 1989. Phylogenetic and evolutionary aspects of vitamin D from phytoplankton to humans. In: *Vertebrate Endocrinology: Fundamentals and Biomedical Implications,* Vol. 3, pp. 7–44. (Pang, P. T. and Schreibman, Eds.) New York: Academic Press.

Holick, M. F., Schnoes, H. K., and DeLuca, H. F. 1971. Identification of 1,25-dehydroxycholecalciferol. A metabolite of vitamin $D_3$ metabolically active in the intestine. *Proc. Natl. Acad. Sci. U.S.A.* **68:** 803–804.

Holtrop, M. E., Cox, K. A., Clark, M. B., Holick, M. F., and Anast, C. S. 1981. 1,25-Dihydroxycholecalciferol stimulates osteoclasts in rat bones in the absence of parathyroid hormone. *Endocrinology* **108:** 2293–2301.

Homma, T., Watanabe, M., Hirose, S., Kanai, A., Kangawa, K., and Matsuo, H. 1986. Isolation and determination of the amino acid sequence of chicken calcitonin I from chicken ultimobranchial glands. *J. Biochem.* **100:** 459–467.

Horst, R. L., Goff, J. P., and Reinhardt, T. A. 1994. Calcium and vitamin D metabolism in the dairy cow. *J. Dairy Sci.* **77:** 1936–1951.

Housama, S., Findlay, D. M., Brady, C. L., Myers, D. E., Martin, T. J., and Sexton, P. M. 1994.

Isoforms of the rat calcitonin receptor: consequences for ligand binding and signal transduction. *Endocrinology* **135:** 183–190.

Howard, G. A., Bottemiller, B. L., Turner, R. T., Rader, J. I., and Baylink, D. 1981. Parathyroid hormone stimulates bone formation and resorption in organ culture: evidence for a coupling mechanism. *Proc. Natl. Acad. Sci. U.S.A.* **78:** 3204–3208.

Howell, D. S. 1992. The biology, chemistry, and biochemistry of the mammalian growth plate. In: *Disorders of Bone and Mineral Metabolism,* pp. 313–353. (Coe, F. L. and Favus, M. J., Eds.) New York: Raven Press.

Hruska, K. A., Civitelli, R., Duncan, R., and Avioli, L. V. 1991. Regulation of skeletal remodelling by parathyroid hormone. In: *Calcium Regulating Hormones. II. Calcium Transport, Bone Metabolism, and New Drugs,* pp. 38–42. (Mori, H., Ed.) Basel: S. Karger.

Hunziker, W. 1986. The 28-kDa vitamin D-dependent calcium-binding protein has a six-domain structure. *Proc. Natl. Acad. Sci. U.S.A.* **83:** 7578–7582.

Hurwitz, S. 1965. Calcium turnover in different bone segments of laying fowl. *Am. J. Physiol.* **208:** 203–207.

Hurwitz, S. 1989a. Parathyroid hormone. In: *Vertebrate Endocrinology, Fundamentals and Biomedical Implications,* Vol. 3, *Regulation of Calcium and Phosphate,* pp. 45–77. (Pang, P. K. T. and Schreibman, M. P., Eds.) New York: Academic Press.

Hurwitz, S. 1989b. Calcium homeostasis in birds. *Vitam. Horm.* **45:** 173–221.

Hurwitz, S. and Bar, A. 1965. Absorption of calcium and phosphorus along the gastrointestinal tract of the laying fowl as influenced by dietary calcium and egg shell formation. *J. Nutr.* **86:** 433–438.

Hurwitz. S. and Bar, A. 1968. Activity, concentration, and lumen-blood electrochemical potential difference of calcium in the intestine of laying hens. *J. Nutr.* **95:** 647–654.

Hurwitz, S. and Bar, A. 1972. Site of vitamin D action in chick intestine. *Am. J. Physiol.* **222:** 761–767.

Hurwitz, S., Bar, A., and Cohen, I. 1973. Regulation of calcium absorption by fowl intestine. *Am. J. Physiol.* **225:** 150–154.

Hurwitz, S., Fishman, S., Bar, A., Pines, M., Risenfeld, G., and Talpaz, H. 1983. Simulation of calcium homeostasis: modeling and parameter estimation. *Am. J. Physiol.* **245:** R664–R672.

Hurwitz, S., Fishman, S., Bar, A., and Talpaz, H. 1984. Role of the 1,25-dihydroxycholecalciferol-regulated component of calcium absorption in calcium homeostasis. In: *Epithelial Calcium and Phosphate Transport. Molecular and Cellular Aspects,* pp. 357–362. (Bronner, F. and Peterlik, M. Eds.) New York: Alan R. Liss.

Hurwitz, S., Fishman, S., and Talpaz, H. 1987a. Model of plasma calcium regulation: system oscillations induced by growth. *Am. J. Physiol.* **252:** R1173–R1181.

Hurwitz, S., Fishman, S., and Talpaz, H. 1987b. Calcium dynamics: a model system approach. *J. Nutr.* **73:** 177–185.

Hurwitz, S. and Griminger, P. 1961. The response of plasma alkaline phosphatase, parathyroids and blood and bone minerals to calcium intake in the fowl. *J. Nutr.* **73:** 177–185.

Hurwitz, S., Harrison, H. C., and Harrison, H. E. 1967. The effect of vitamin $D_3$ on the *in vitro* transport of calcium by the chick intestine. *J. Nutr.* **91:** 319–323.

Hurwitz, S., Miller, B., and Norman, A. W. 1994. Oscillatory behavior of control-systems of calcium homeostasis in chickens. *J. Cell. Biochem.* **56:** 236–244.

Hurwitz, S., Plavnik, I., Shapiro, A., Wax, E., Talpaz, H., and Bar, A. 1995. Calcium metabolism and requirements of chickens as affected by growth. *J. Nutr.* In press.

Hurwitz, S., Stacy, R. E., and Bronner, F. 1969. Role of vitamin D in plasma calcium regulation, *Am. J. Physiol.* **216:** 254–262.

Iida, K., Taniguchi, S., and Kurokawa, K. 1993. Distribution of 1,25-dihydroxyvitamin $D_3$ receptor and 25-hydroxyvitamin $D_3$-24-hydroxylase mRNA expression along rat nephron seg-

ments. *Biochim. Biophys. Res. Commun.* **194:** 659–664.

Isaksson, O. G. P., Lindahl, A., Nilsson, A., and Isgaard, J. 1987. Mechanism of the stimulatory effect of growth hormone on longitudinal bone growth. *Endocr. Rev.* **8:** 426–438.

Isler, H. 1973. The structure of the ultimobranchial body of the chick. *Anat. Rec.* **177:** 441–460.

Iwamoto, M., Sato, K., Nakashima, K., Shimazu, A., and Kato, Y. 1989. Hypertrophy and calcification of rabbit permanent chondrocytes in pellet cultures: synthesis of alkaline phosphatase and 1,25-dihydrocholecalciferol receptor. *Dev. Biol.* **136:** 500–508.

Jande, S. S. and Robert, P. 1974. Cytochemical localization of parathyroid hormone-activated adenyl cyclase in rat kidney. *Histochemistry* 40: 323–327.

Jaros, G. G., Coleman, T. G., and Guyton, A. C. 1979. Model of short-term regulation of plasma ionic calcium concentration. *Simulation* **32:** 193–204.

Jarowski, Z. F. G. 1984. Coupling of bone formation to bone resorption: a broader view. *Calcif. Tissue Int.* **36:** 531–535.

Jones, B. B., Jurutka, P. W., Haussler, C. A., Haussler, M. R., and Whitfield, G. K. 1991. Vitamin D receptor phosphorylation in transfected ROS 17/2.8 cells is localized to the N-terminal region of the hormone-binding domain. *Mol. Endocrinol.* **5:** 1137–1146.

Jones, D. P., McConkey, D. J., Nicotera, P., and Orrentius, S. 1989. Calcium-activated DNA fragmentation in liver nuclei. *J. Biol. Chem.* **264:** 6398–6403.

Jones, S. J., Ali, N. N., and Boyde, A. 1986. Survival and resorptive activity of chick osteoblasts in culture. *Anat. Embryol.* **174:** 265–275.

Jubitz, W., Canterbury, J. M., Reiss, E., and Tyler, F. 1972. Circadian rhythms in serum parathyroid hormone concentration in human subjects: correlation with serum calcium, phosphate, albumen and growth hormone levels. *J. Clin. Invest.* **52:** 2040–2046.

Jüppner, H. J., Abou-Samra, A.-B., Freeman, M., Kong, X. F., Schipani, E., Richards, J., Kolakowski, L. F., Jr., Hock, J., Potts, J. T., Jr., Kronenberg, H. M., and Segre, G. V. 1991. A G protein-linked receptor for parathyroid hormone and parathyroid hormone-related peptide. *Science* **254:** 1024–1026.

Kalu, D. N., Hadji-Georgopoulus, A., and Foster, G. V. 1975. Evidence for physiological importance of calcitonin in the regulation of plasma calcium in rats. *J. Clin. Invest.* **55:** 722–727.

Kamioka, H., Sumitani, K., Tagami, K., Miki, Y., Terai, K., Hakeda, Y., and Kawata, T. 1994. Divalent cations elevate cytosolic calcium of chick osteocytes. *Biochem. Biophys. Res. Commun.* **204:** 519–524.

Kaazirska, M. P. V., Vassilev, P. M., Ye., C. P., Francis, J. E., and Brown, E. M. 1955. Intracellular $Ca^{2+}$ activated $K^+$ channels modulated by variations in extracellular $Ca^{2+}$ in dispersed bovine parathyroid cells. *Endocrinology* **136:** 2238–2243.

Karbach, U. 1992. Paracellular calcium transport across the small intestine. *J. Nutr.* **122:** 672–677.

Kaune, R., Kassianoff, I., Schröder, B., and Harmeyer, J. 1992. The effect of 1,25-dihydroxyvitamin D-3 deficiency on $Ca^{2+}$-transport and $Ca^{2+}$-uptake into brush-border membrane vesicles from pig small intestine. *Biochim. Biophys. Acta* **1109:** 187–194.

Kawashima, H. and Kurokawa, K. 1983. Unique hormonal regulation of vitamin D metabolism in mammalian kidney. *Miner. Electrolyte Metab.* **9:** 227–235.

Kawashima, K., Iwata, S., and Endo, H. 1980. Selective activation of diaphyseal chondrocytes by parathyroid hormone, calcitonin and $N^6$:$O^2$-dibutyryl adenosine 3′,5′-cyclic monophosphoric acid in proteoglycan synthesis in chick embryonic femur cultivated *in vitro*. *Endocr. Jpn.* **26:** 351–361.

Keaton, J. A., Barto, J. A., Moore, M. P., Gruel, J. B., and Mayer, G. P. 1978. Altered parathyroid response to calcium in hypercalcemic

neonatal calves. *Endocrinology* **103:** 2161–2167.

Keutman, H. T., Sauer, M. M., Hendy, G. N., O'Riordan, J. L. H., and Potts, J. T., Jr. 1978. Complete amino acid sequence of human parathyroid hormone. *Biochemistry* **17:** 5723–5729.

Kemper, B. 1986. Molecular biology of parathyroid hormone. *CRC Crit. Rev. Biochem.* **19:** 353–379.

Khosla, S., Demay, M., Pines, M., Hurwitz, S., Potts, J. T., Jr., and Kronenberg, H. M. 1988. Nucleotide sequence of cloned cDNAs encoding chicken preproparathyroid hormone. *J. Bone Miner. Res.* **3:** 689–698.

Khouri, R. S., Weber, J., and Farach-Carson, M. C. 1995. Vitamin D metabolites modulate osteoblast activity by $Ca^{2+}$ influx-independent genomic and $Ca^{2+}$ influx-dependent nongenomic pathways. *J. Nutr.* **125:** 1699S–1703S.

Kifor, O., Kifor, I., and Brown, E. M. 1992. Effect of high extracellular calcium concentrations on phosphoinositide turnover and inositol phosphate metabolism in dispersed bovine parathyroid cells. *J. Bone Miner. Res.* **7:** 1327–1336.

Kinoshita, Y., Fukase, M., Miyauchi, A., Takenaka, M., Nakada, M., and Fujita, T. 1986. Establishment of a parathyroid hormone-responsive phosphate transport system *in vitro* using cultured renal cells. *Endocrinology* **119:** 1954–1963.

Kitten, A. M., Hymer, T. K., and Katz, M. S. 1994. Bidirectional modulation of parathyroid hormone-responsive adenylyl cyclase by protein kinase C. *Am. J. Physiol.* **266:** E897–E904.

Kobayashi, N., Russell, J., Lettieri, D., and Shrewood, L. M. 1988. Regulation of protein kinase C by extracellular calcium in bovine parathyroid cells. *Proc. Natl. Acad. Sci. U.S.A.* **85:** 4857–4860.

Kohlmeier, L. and Marcus, R. 1995. Calcium disorders of pregnancy. *Endocrinol. Metab. Clin. North Am.* **24:** 15–39.

Kolakowski, J., Jr., Hock, J., Potts, J. T., Jr., Kronenberg, H. M., and Segre, G. V. 1991. A G protein-linked receptor for parathyroid hormone and parathyroid-hormone related peptide. *Science* **254:** 1024–1026.

Koyama, H., Inaba, M., Nishizawa, Y., Ohno, S., and Morii, H. 1994. Protein kinase C is involved in 24-hydroxylase gene expression induced by $1,25(OH)_2D_3$ in rat intestinal epithelial cells. *J. Cell. Biochem.* **55:** 230–240.

Kraintz, L. and Intcher, K. 1969. Effect of calcitonin on the domestic fowl. *Can. J. Physiol. Pharmacol.* **46:** 313–315.

Kremer, R., Nolivar, I., Goltzman, D., and Hendy, G. N. 1989. Influence of calcium and 1,25-dihydroxycholecalciferol on proliferation and protooncogene expression in primary cultures of bovine parathyroid cells. *Endocrinology* **125:** 935–941.

Kumar, R. 1995. Calcium transport in epithelial cells of the intestine and kidney. *J. Cell. Biochem.* **57:** 392–398.

Kumegawa, M., Kahn, A. M., Dolson, G. M., Hise, M. K., Bennet, S. C., and Weinman, E. J. 1985. Parathyroid hormone and butyryl cAMP inhibit $Na^+/H^+$ exchange in renal brush border vesicles. *Am. J. Physiol.* **248:** F212–F218.

Kurokawa, K., Fukagawa, M., Hayashi, M., and Saruta, T. 1992. Renal receptors and cellular mechanisms of hormone action in the kidney. In: *The Kidney: Physiology and Pathophysiology,* 2nd ed., pp. 1339–1372. (Seldin, D. W. and Giebisch, G., Eds.) New York: Raven Press.

Kyeyune-Nyombi, E., Lau, K.-H. W., Baylink, D. J., and Strong, D. D. 1989. Stimulation of cellular alkaline phosphatase activity and its messenger RNA levels in a human osteosarcoma cell line by 1,25-dihydroxyvitamin $D_3$. *Arch. Biochem. Biophys.* **275:** 363–370.

Lakkakorpi, P. T. and Väänänen, H. K. 1991. Kinetics of the osteoclast cytoskeleton during the resorption cycle *in vitro*. *J. Bone Miner. Res.* **6:** 817–826.

Lasmoles, F., Jullienne, A., Desplan, C., Milhaud, G., and Moukhtar, M. S. 1985. Structure of chicken calcitonin predicted by partial nucleotide sequence of its precursor. *FEBS Lett.* **180:** 113–116.

Leach, R. M., Jr. and Gay, C. V. 1987. Role of epiphyseal cartilage in endochondral bone formation. *J. Nutr.* **116:** 784–790.

LeBoff, M. S., Rennke, H. G., and Brown, E. M. 1983. Abnormal regulation of parathyroid cell secretion and proliferation in primary cultures of bovine parathyroid cells. *Endocrinology* **113:** 277–284.

Lee, B. N., Hardwick, L. L., Hu, M.-S., and Jamgotchin, N. 1990. Vitamin D-independent regulation of calcium and phosphate absorption. *Miner. Electrolyte Metab.* **16:** 167–173.

Lee, M. J. and Roth, S. I. 1975. Effect of calcium and magnesium on deoxyribonucleic acid synthesis in rat parathyroid glands, *in vitro*. *Lab. Invest.* **1:** 72–79.

Lee, S., Clark, S. A., Gill, R. K., and Christakos, S. 1994. 1,25-Dihydroxyvitamin $D_3$ and pancreatic β-cell function: vitamin D receptors, gene expression, and insulin secretion. *Endocrinology* **134:** 1602–1610.

Leis, H. J., Zach, D., Huber, E., Ziermann, L., Gleispach, H., and Windischhofer, W. 1994. Extracellular $Ca^{2+}$ sensing by the osteoblast-like cell line, MC3T3-E1. *Cell Ca* **15:** 447–456.

Lian, J. B. and Stein, G. S. 1992. Transcriptional control of vitamin D-regulated proteins. *J. Cell. Biochem.* **49:** 37–45.

Liang, C. T., Balakir, R. A., Barnes, J., and Sacktor, B. 1984. Responses of chick renal cell to parathyroid hormone: effect of vitamin D. *Am. J. Physiol.* **246:** C401–C406.

Lieberherr, M. 1987. Effects of vitamin $D_3$ metabolites on cytosolic free calcium in confluent mouse osteoblasts. *J. Biol. Chem.* **262:** 13168–13173.

Lim, S. K., Gardella, T. J., Baba, H., Nussbaum, S. R., and Kronenberg, H. M. 1992. The carboxy-terminus of parathyroid hormone is essential for hormone processing and secretion. *Endocrinology* **131:** 2325–2330.

Lin, H. Y., Harris, T. L., Flannery, M. S., Kaji, E. H., Gorn, A., Kolakowski, L. F., Arufo, A., Lodish, H. F., and Goldring, S. G. 1991. Expression cloning of a calcitonin receptor, a novel adenyly cyclase-coupled receptor. *Science* **254:** 1023–1024.

Lissoos, T. W., Beno, D. W. A., and Davis, B. H. 1993. 1,25-Dihydroxyvitamin $D_3$ activates *raf* kinase and *raf* perinuclear translation via a protein kinase C-dependent pathway. *J. Biol. Chem.* **268:** 25132–25138.

Lobaugh, B., Boass, A., Garner, S. C., and Toverud, S. U. 1992. Intensity of lactation modulates renal 1 alpha-hydroxylase and serum 1,25 $(OH)_2D$ in rats. *Am. J. Physiol.* **262:** E840–E844.

Lobaugh, B., Boass, A., Lester, G. E., and Toverud, S. U. 1990. Regulation of 1,25-dihydroxyvitamin $D_3$ in lactating rats. *Am. J. Physiol.* **259:** E665–E671.

Lowe, K. E., Maiyar, A. C., and Norman, A. W. 1992. Vitamin D-mediated gene expression. *Crit. Rev. Eukary. Gene Exp.* **2:** 65–109.

Lowik, C. W. G. M., van der Pluijm, G., Bloys, H., Hoekman, K., Bijvoet, O. L. M., Aarden, L. A., and Papapoulus, S. E. 1989. Parathyroid hormone (PTH) and PTH-like protein (PLP) stimulate interleukin-6 production by osteogenic cells: a possible role of interleukin-6 in osteoclastogenesis. *Biochem. Biophys. Res. Commun.* **162:** 1546–1552.

Lu, K. P. and Means, A. R. 1993. Regulation of the cell cycle by calcium and calmodulin. *Endocr. Rev.* **14:** 40–58.

Luce, E. M. 1923. The size of the parathyroids in rats, and the effect of a diet deficiency in calcium. *J. Pathol.* **26:** 200–208.

Lundgren, S., Hjalm, G., Hellman, P., Ek, B., Juhlin, C., Rastad, J., Klareskog, L., Akerstrom, G., and Rask, L. 1994. A protein involved in calcium sensing of the human parathyroid and placental cytotrophoblast cells belongs to the LDL-receptor protein superfamily. *Exp. Cell Res.* **212:** 344–350.

MacDonald, B. R., Gallagher, J. A., and Russell, R. G. G. 1986. Parathyroid hormone stimulates the proliferation of cells derived from human bone. *Endocrinology* **118:** 2445–2449.

Madara, J. L. and Pappenheimer, J. R. 1987. Structural basis for physiological regulation of

paracellular pathways in intestinal epithelia. *J. Membr. Biol.* **100:** 149–164.

Mahaffey, J. E., Rosenblatt, M., Shepard, G. L., and Potts, J. T., Jr. 1979. Parathyroid hormone inhibitors. *J. Biol. Chem.* **254:** 6496–6498.

Majeska, R. J. and Rodan, G. A. 1982. The effects of 1,25-$(OH)_2D_3$ on alkaline phosphatase in osteoblastic osteosarcoma cells. *J. Biol. Chem.* **256:** 3362–3365.

Marcus, R. and Orner, F. B. 1980. Parathyroid hormone as a calcium ionophore in bone cells: test of specificity. *Calcif. Tissue. Int.* **32:** 207–211.

Markowitz, M., Rotkin, L., and Rosen, J. F. 1981. Circadian rhythms of blood minerals in humans. *Science* **213:** 672–674.

Marks, S. C. and Popoff, S. N. 1988. Bone cell biology, the regulation of development, structure and function in the skeleton. *Am. J. Anat.* **183:** 1–44.

Marx, S. J., Woodward, C. J., Aurbach, G. D., Glassman, H., and Keutman, H. J. 1973. Renal receptors for calcitonin: binding and degradation of the hormone. *J. Biol. Chem.* **248:** 4797–4802.

Massengale, O. N. and Nussmeier, M. 1930. The action of activated ergosterol in the chicken. *J. Biol. Chem.* **87:** 423–425.

Matsumoto, K., Hashimoto, K., Nishida, Y., Hashiro, M., and Yoshikawa, K. 1990. Growth inhibitory effects of 1,25-dihydroxyvitamin $D_3$ on normal human keratinocytes cultured in serum-free medium. *Biochem. Biophys. Res. Commun.* **166:** 916–923.

Matsumoto, T., Yamato, H., Okazaki, R., Kumegawa, M., and Ogata, E. 1992. Effect of 24,25-dyhydroxyvitamin $D_3$ in osteoclasts. *Proc. Soc. Exp. Biol. Med.* **200:** 161–164.

Mayer, G. P., Keaton, J. A., Hurst, J. G., and Habener, J. F. 1979. Effect of plasma calcium concentration on the relative proportion of hormone and carboxyl fragment in parathyroid venous blood. *Endocrinology* **104:** 1778–1784.

McCarron, D. A., Ellison, D. H., and Anderson, S. 1984. Vasodilation mediated by human PTH 1-34 in the spontaneously hypertensive rat. *Am. J. Physiol.* **246:** F96–F100.

McDonnell, D. P., Mangelsdorf, D. J., Pike, J. W., Haussler, M., and O'Malley, B. W. 1987. Molecular cloning of complementary DNA encoding the avian receptor for vitamin D. *Science* **235:** 1214–1217.

McKee, M. D. and Murray, T. M. 1985. Binding of intact parathyroid hormone to chicken renal plasma membranes: evidence for a second binding site with carboxyl-terminal specificity. *Endocrinology* **116:** 1930–1939.

McLean, F. C. and Hastings, A. B. 1935. The state of calcium in the fluids of the body. I. The conditions affecting ionization of calcium. *J. Biol. Chem.* **108:** 285–322.

McSheehy, P. M. J. and Chambers, T. J. 1986a. Osteoblastic cells mediate osteoclastic responsiveness to parathyroid hormone. *Endocrinology* **118:** 824–828.

McSheehy, P. M. J. and Chambers, T. J. 1986b. Osteoblast-like cells in the presence of parathyroid hormone release soluble factor that stimulates osteoclastic bone resorption. *Endocrinology* **119:** 1654–1659.

Mérida-Velasco, J. A. 1991. Experimental study of the origin of the parathyroid glands. *Acta Anat.* **141:** 163–169.

Mérida-Velasco, J. A., García-García, J. D., Espín-Ferra, J., and Linares, J. 1989. Origin of the ultimobranchial body and its colonizing cells in human embryos. *Acta Anat.* **136:** 325–330.

Miki, H., Maercklein, P. B., and Fitzpatrick, L. A. 1995. Spontaneous oscillations of intracellular calcium in single bovine parathyroid cells may be associated with the inhibition of parathyroid hormone secretion. *Endocrinology* **136:** 2954–2959.

Miller, B. and Norman, A. W. 1979. Studies on the metabolism of calciferol. XIV. Evidence for a circadian rhythm in the activity of the 25-hydroxycholecalciferol-1-hydroxylase. *Biochem. Biophys. Res. Commun.* **88:** 730–734.

Miller, S. C., Bowman, B. M., and Myers, R. L. 1984. Morphological and ultrastructural as-

pects of activation of avian medullary bone osteoclasts by parathyroid hormone. *Anat. Rec.* **208:** 223–231.

Minghetti, P. P. and Norman, A. W. 1988. $1,25(OH)_2$-vitamin $D_3$ receptors: gene regulation and genetic circuitry. *FASEB J.* **2:** 3043–3053.

Minkin, C. and Yu, X.-H. 1991. Calcitonin receptor expression and its regulation by $1\alpha,25$-dihydroxyvitamin $D_3$ during *de novo* osteoclast formation in organ culture of fetal mouse metatarsals. *Bone Miner.* **13:** 191–200.

Miyauchi, A., Hruska, K. A., Greenfield, E. M., Duncan, R., Alvarez, J., Barattolo, R., Colucci, S., Zambonin-Zallone, A., and Teitelbaum, S. L. 1990. Osteoclast cytosolic calcium, regulated by voltage-gated calcium channels and extracellular calcium, controls podosome assembly and bone resorption. *J. Cell Biol.* **111:** 2543–2552.

Miyaura, C., Segawa, A., Nagasawa, H., Abe, E., and Suda, T. 1986. Effects of retinoic acid on the activation and fusion of mouse alveolar macrophages induced by 1,25-dihydroxy vitamin $D_3$. *J. Bone Miner. Res.* **1:** 359–368.

Moore, D. C., Carter, D. L., and Stodzinski, G. P. 1992. Inhibition by 1,25-dihydroxyvitamin $D_3$ of c-*myc* down-regulation and DNA fragmentation in cytosine arabinoside-induced erythroid differentiation of K562 cells. *J. Cell. Physiol.* **151:** 539–548.

Moore-Ede, M. C. 1986. Physiology of the circadian timing system: predictive *versus* reactive homeostasis. *Am. J. Physiol.* **250:** R735–R762.

Morgan, J. I. and Curran, T. 1986. Role of ion flux in the control of c-*fos* expression. *Nature (London)* **322:** 552–556.

Moudgil, V. K. 1994. Steroid/nuclear receptor superfamily: recent advances in relation to health and disease. In: *Steroid Hormone Receptors,* pp. 3–44. (Moudgil, V. K., Ed.) Boston: Birkenhauser.

Muff, R., Born, W., Kaufmann, M., and Fischer, J. A. 1994. Parathyroid hormone and parathyroid hormone-related protein receptor update. *Mol. Cell. Endocrinol.* **100:** 35–38.

Muff, R., Fischer, J. A., Biber, J., and Murer, H. 1992. Parathyroid hormone receptors in control of proximal tubule function. *Ann. Rev. Physiol.* **54:** 67–79.

Muff, R., Nemeth, E. F., Haller-Brem, S., and Fischer, J. A. 1988. Regulation of hormone secretion and cytosolic $Ca^{2+}$ by extracellular $Ca^{2+}$ in parathyroid and C-cells: role of voltage sensitive $Ca^{2+}$ channels. *Arch. Biochem. Biophys.* **265:** 128–135.

Munson, P. L. and Hirsch, P. F. 1992. Importance of calcitonin in physiology, clinical pharmacology, and medicine. *Bone Miner.* **16:** 162–165.

Murray, T. M., Rao, L. G., and Rizzoli, R. E. 1994. Interactions of parathyroid hormone, parathyroid hormone related protein and their fragments with conventional and nonconventional receptor sites: In: *The Parathyroids,* pp. 185–211. (Bilezikian, J. P., Levine, M. A., and Marcus, R., Eds.) New York: Raven Press.

Müller-Plathe, O. and Lindemann, K. 1983. Ionized calcium *versus* total calcium. *Scand. J. Lab. Invest.* **43:** (Suppl. 165): 71–73.

Murphy, E., Chamberlin, M. E., and Mandel, L. J. 1986. Effects of calcitonin on cytosolic $Ca^{2+}$ in suspension of rabbit medullary thick ascending limb tubules. *Am. J. Physiol.* **251:** C491–C495.

Naveh-Many, T., Marx, R., Keshet, E., Pike, J. W., and Silver, J. 1990. Regulation of 1,25-dihydroxyvitamin $D_3$ receptor gene expression by 1,25-dihydroxvitamin $D_3$ in the parathyroid *in vivo. J. Clin. Invest.* **86:** 1968–1975.

Nellans, H. N. 1990. Intestinal calcium absorption. Interplay of paracellular and cellular pathways. *Miner. Electrolyte Metab.* **16:** 101–108.

Nemere, I. 1991. Vesicular calcium transport in chick intestine. *J. Nutr.* **122:** 657–661.

Nemere, I. 1995. Nongenomic effects of 1,25-dihydroxyvitamin $D_3$: potential relation of a plasmalemmal receptor to the acute enhancement of intestinal calcium transport in chick. *J. Nutr.* **125:** 1695S–1698S.

Nemere, I., Dormanen, M. C., Hammond, M. W., Okamura, W. H., and Norman, A. W. 1994.

Identification of a specific binding protein for 1 alpha,25-dihydroxyvitamin D-3 in basal-lateral membranes of chick intestinal epithelium and relationship to transcalthacia. *J. Biol. Chem.* **269:** 23750–23756.

Nemere, I., Feld, C., and Norman, A. W. 1991. 1,25-Dihydroxyvitamin $D_3$-mediated alterations in microtubule proteins isolated from chick intestinal epithelium: analysis by electric focusing. *J. Cell. Biochem.* **47:** 369–379.

Nemere, I. and Norman, A. W. 1987. The rapid, hormonally stimulated transport of calcium (transcalthacia). *J. Bone Miner. Res.* **2:** 167–169.

Nemere, I. and Norman, A. W. 1990. Transcalthacia, vesicular calcium transport, and microtubule-associated calbindin-$D_{28k}$: emerging views of 1,25-dihydroxyvitamin $D_3$-mediated intestinal calcium absorption. *Miner. Electrochem. Metab.* **16:** 109–114.

Nemere, I., Opperman, L. A., Ross, F. P., and Norman, A. W. 1992. Noncytoplasmic and filamentous appearance of calbindin-$D_{28k}$ and tubulin in double, indirect immunofluorescent staining of embryonic chick tissue. *Mol. Cell. Endocrinol.* **86:** 83–91.

Nemere, I., Theofan, G., and Norman, A. W. 1987. 1,25-Dihydroxyvitamin $D_3$ regulates tubulin expression in chick intestine. *Biochem. Biophys. Res. Commun.* **148:** 1270–1276.

Nesbit, T. and Drezner, M. K. 1993. Insulin-like growth factor-I regulation of renal 25-hydroxyvitamin D-1-hydroxylase activity. *Endocrinology* **132:** 133–138.

Neumann, M., Neumann, W. F., and Lane, K. 1979. Formation and serum disappearance of fragments of parathyroid hormone in the infused dog. *Calcif. Tissue Int.* **28:** 70–81.

Niall, H. D., Keutmann, H. T., Copp, D. H., and Potts, J. T., Jr. 1969. Amino acid sequence of salmon ultimobranchial calcitonin. *Proc. Natl. Acad. Sci. U.S.A.* **64:** 771–778.

Nicholson, G. C., Moseley, J. M., Sexton, P. M., and Martin, T. J. 1987. Chicken osteoclasts do not possess calcitonin receptors. *J. Bone Miner. Res.* **2:** 53–60.

Nicholson, G. C., Moseley, J. M., Sexton, P. M., Mendelsohn, F. A., and Martin, T. J. 1986. Abundant calcitonin receptors in isolated rat osteoclasts. *J. Clin. Invest.* **78:** 355–360.

Nicolayesen, R., Eeg-Larsen, N., and Malm, O. J. 1953. Physiology of calcium metabolism. *Physiol. Rev.* **33:** 424–444.

Nieto, A., Noya, F., and R-Candela, J. L. 1973. Isolation and properties of two calcitonins from chicken ultimobranchial gland. *Biochim. Biophys. Acta* **322:** 383–391.

Nijweide, P. J., Burger, E. H., and Feyen, J. H. M. 1986. Cells of bone: proliferation, differentiation, and hormonal regulation. *Physiol. Rev.* **66:** 855–886.

Nissenson, R. A. and Arnaud, C. D. 1979. Properties of the parathyroid hormone receptor adenylate cyclase system in chicken renal plasma membranes. *J. Biol. Chem.* **254:** 1469–1475.

Nordin, B. E. C., Peacock, M., and Wilkinson, R. 1972. Hypercalcemia and calcium stores disease. In: *Clinics in Endocrinology and Metabolism,* pp. 169–183. (McIntyre, I., Ed.) London: W. B. Saunders.

Norman, A. W. 1994. Editorial. The vitamin D endocrine system: identification of another piece of the puzzle. *Endocrinology* **134:** 1601A–1601C.

Norman, A. W., Myrtle, J. F., Midgett, R. J., Nowicki, H. G., Williams, V., and Popjak, G. 1971. 1,25-Dihydroxycholecalciferol: identification of the proposed active form of vitamin $D_3$ in the intestine. *Science* **173:** 51–54.

Norman, A. W., Okamura, W. E. H., Farach-Carson, M. C., Alleqert, K., Branisteanu, D., Nemere, I., Muralidharan, K. R., and Bouillon, R. 1993. Structure function studies of 1,25-dihydroxyvitamin $D_3$ and the vitamin D endocrine system. 1,25-Dihydroxy-pentadeuterio-previtamin $D_3$ (as a 6-*s*-*cis* analog) stimulates nongenomic but not genomic biological responses. *J. Biol. Chem.* **268:** 13811–13819.

Nussbaum, S. R., Rosenblatt, M., and Potts, J. T., Jr. 1980. Parathyroid hormone-renal receptor interactions. *J. Biol. Chem.* **255:** 10183–10187.

Nussenzveig, D. R., Matthew, S., and Gershengorn, M. C. 1995. Alternative splicing of a 48-nucleotide exon generates two isoforms of the human calcitonin receptor. *Endocrinology* **136:** 2047–2051.

Nussenzveig, D. R., Thaw, C. N., and Gershengorn, M. C. 1994. Inhibition of inositol phosphate second messenger formation by intracellular loop one of a human calcitonin receptor. Expression and mutational analysis of synthetic receptor genes. *J. Biol. Chem.* **269:** 28123–28129.

Obie, J. F. and Copper, C. W. 1979. Loss of calcemic effects of calcitonin and parathyroid hormone infused continuously into rats using the Alzet osmotic minipump. *J. Pharmacol. Exp. Ther.* **209:** 422–429.

Ohta, S., Shigeno, C., Yamamoto, I., Okumura, H., Lee, K., Uneno, S., Konishi, J., and Yamamuro, T. 1989. Parathyroid hormone down-regulates the epidermal growth factor receptors in clonal osteoblastic mouse calvarial cells, MC3T3-E1: possible mediation by adenosine 3′,5′-cyclic monophosphate. *Endocrinology* **124:** 2419–2426.

Ohyama, Y., Ozono, K., Uchida, M., Shinki, T., Kato, S., Suda, T., Yamamoto, O., Noshiro, M., and Kato, Y. 1994. Identification of vitamin D-responsive element in the 5′-flanking region of the rat 25-hydroxyvitamin $D_3$ 24-hydroxylase gene. *J. Biol. Chem.* **269:** 10545–10550.

Okano, K., Wu, S., Huang, X., Pirola, K., Jüppner, H., Abou-Samra, A.-B., Segre, G. V., Iwasaki, J., Fagin, J. A., and Clemens, T. L. 1994. Parathyroid hormone (PTH)/PTH-related protein (PTHrP) receptor and its messenger ribonucleic acid in rat aortic vascular smooth muscle cells and UMR osteoblast-like cells: cell specific regulation by angiotensin-II and PTHrP. *Endocrinology* **135:** 1093–1099.

Okazaki, T., Ando, K., Igarishi, T., Ogata, T., and Fujita, T. 1992. Parathyroid hormone gene *versus* atrial natriuretic polypeptide gene. *J. Clin. Invest.* **89:** 1268–1273.

Omdahl, J. L. and DeLuca, H. F. 1973. Regulation of vitamin D metabolism and function. *Physiol. Rev.* 53: 327–372.

Omdahl, J. L., Hunsaker, L. A., Evan, A. P., and Torrez, P. 1980. *In vitro* regulation of kidney 25-hydroxyvitamin $D_3$-hydroxylase enzyme activities by vitamin $D_3$ metabolites. *J. Biol. Chem.* **255:** 7460–7466.

O'Niel, R. P. J., Jones, S. J., Boyde, A., Taylor, M. L., and Arnett, T. R. 1992. Effect of retinoic acid on the resorptive activity of chick osteoclasts *in vitro*. *Bone* **13:** 23–27.

Ono, T. and Tuan, R. S. 1991. Vitamin D and chick embryonic yolk calcium mobilization: identification and regulation of expression of vitamin D-dependent $Ca^{2+}$-binding protein, calbindin-$D_{28k}$, in the yolk sac. *Dev. Biol.* **144:** 167–172.

Orloff, J. J., Wu, T. L., and Stewart, A. F. 1989. Parathyroid hormone-like proteins: biochemical responses and receptor interactions. *Endocr. Rev.* **10:** 476–495.

Ornoy, A., Goodwin, D., Noff, D., and Edelstein, S. 1978. 24,25-Dihydroxy vitamin D is a metabolite of vitamin D essential for bone formation. *Nature (London)* **276:** 517–519.

Owen, T., Aronow, M. S., Barone, L. M., Bettencourt, B., Stein, G. S., and Lian, J. B. 1991. Pleiotropic effects of vitamin D on osteoblast gene expression are related to the proliferative and differentiated state of bone cell phenotype: dependency upon basal levels of gene expression, duration of exposure, and bone matrix competency in normal rat osteoblast culture. *Endocrinology* **128:** 1496–1504.

Ozono, K., Sone, T., and Pike, J. W. 1991. The genomic mechanism of action of 1,25-dihydroxyvitamin $D_3$. *J. Bone Miner. Res.* **6:** 1021–1027.

Pang, P. K. T., Yang, M. C. M., Ogur, C., Phillips, J. G., and Yee, J. A. 1980. Hypotensive actions of parathyroid hormone preparations in vertebrates. *Gen. Comp. Endocrinol.* **41:** 135–138.

Pappenheimer, J. R. and Reiss, K. Z. 1987. Contribution of solvent drag through intercellular junctions to absorption of nutrients by the rat small intestine. *J. Membr. Biol.* **100:** 123–136.

Parfitt, A. M. 1987. Bone and plasma calcium homeostasis. *Bone* **8** (Suppl. 1): S1–S8.

Parfitt, A. M. 1994. Parathyroid growth, normal and abnormal. In: *The Parathyroids,* pp. 373–405. (Bilezikian, J. P., Levine, M. A., and Marcus, R., Eds.) New York: Raven Press.

Pearse, A. G. E. 1966. The cytochemistry of the thyroid C cells and their relationship to calcitonin. *Proc. R. Soc. London Ser. B* **170:** 71–80.

Pearse, A. G. E. and Cavalheira, A. F. 1967. Cytochemical evidence for an ultimobranchial origin of rodent thyroid C cells. *Nature (London)* **214:** 923–929.

Pento, J. T. 1986. Influence of the calcium ionophores A123187 and X537A on calcitonin secretion from the isolated perfused porcine thyroid. *Mol. Cell. Endocrinol.* **45:** 71–75.

Perry, H. M., Skogen, W., Chappel, J., Kahn, A. J., Wilner, G., and Teitelbaum, S. L. 1989. Partial characterization of a parathyroid hormone-stimulated resorption factor(s) from osteoblast-like cells. *Endocrinology* **125:** 2075–2082.

Pike, J. W., Gooze, L. L., and Haussler, M. R. 1980. Biochemical evidence for 1,25-dihydroxyvitamin D receptor macromolecules in parathyroid, pancreatic, pituitary and placental tissues. *Life Sci.* **26:** 407–414.

Pines, M., Adams, A. E., Stueckle, S., Bessale, R., Rashti-Behar, V., Chorev, M., Rosenblatt, M., and Suva, L. J. 1994. Generation and characterization of human kidney cell lines stably expressing recombinant human PTH/PTHrP receptor: lack of interaction with a C-terminal human PTH peptide. *Endocrinology* **135:** 1713–1715.

Pines, M. and Hurwitz, S. 1981. Cyclic-AMP activates and calcium inhibits protein kinase activity in avian parathyroid glands. *FEBS Lett.* **133:** 27–30.

Pines, M. and Hurwitz, S. 1988. The effect of parathyroid hormone and atrial natriuretic peptide on cyclic nucleotide production and proliferation of avian epiphyseal growth plate chondroprogenitor cells. *Endocrinology* **123:** 360–365.

Pines, M., Polin, D., and Hurwitz, S. 1983. Urinary cyclic AMP excretion in birds: dependence on parathyroid hormone activity. *Gen. Comp. Endocrinol.* **49:** 90–96.

Pollock, A. S., Warnok, D. G., and Strewler, G. J. 1986. Parathyroid hormone inhibition of $Na^+$-$H^+$ antiporter activity in cultured renal cell line. *Am. J. Physiol.* **250:** F217–F255.

Pols, H. A. P., Schilte, H. P., Herrmann-Erlee, N. M. P., Visser, T. J., and Birkenhager, J. C. 1986. The effect of 1,25 dihydroxyvitamin $D_3$ on growth, alkaline phosphatase and adenylate cyclase of rat osteoblast-like cells. *Bone Miner.* **1:** 397–405.

Pols, H. A. P., Birkenhager, J. C., Foekens, J. A., and Van Leeuwen, J. P. T. M. 1990. Vitamin D: a modulator of cell proliferation and differentiation. *J. Steroid Biochem. Mol. Biol.* **36:** 873–876.

Potts, J. T., Jr. 1992. Chemistry of calcitonin. *Bone Miner.* **16:** 169–173.

Potts, J. T., Jr. and Aurbach, G. D. 1976. Chemistry of calcitonins. In: *Handbook of Physiology,* Sect. 7, *Endocrinology,* Vol. 7, pp. 443–464. (Astwood, E. and Jeep, R., Eds.) Washington, D.C.: American Physiological Society.

Potts, J. T., Jr., Niall, H. D., Keutman, H. T., Brewer, H. B., Jr., and Deftos, L. J. 1968. The amino acid sequence of porcine thyrocalcitonin. *Proc. Natl. Acad. Sci. U.S.A.* **59:** 1321–1328.

Preston, G. M., Billis, W. M., and White, B. A. 1990. Transcriptional and posttranscriptional regulation of the rat prolactin gene by calcium. *Mol. Cell. Biol.* **10:** 442–448.

Proscal, D. A., Okamura, W. H., and Norman, A. W. 1975. Structural requirements for the interaction of $1\alpha,25(OH)_2$-vitamin $D_3$ with its chick intestinal receptor system. *J. Biol. Chem.* **250:** 8382–8388.

Quamm, G. A. 1980. Effect of calcitonin on calcium and magnesium absorption in rat nephron. *Am. J. Physiol.* **238:** E573–E578.

Quamm, G. A., Pfeilschifter, J., and Murer, H. 1989. Parathyroid inhibition of $Na^+$/phosphate cotransport in OK cells: generation of second

messengers in the regulatory cascade. *Biochem. Biophys. Res. Commun.* **158:** 951–957.

Raisz, L. G. 1963. Stimulation of bone resorption by parathyroid hormone in tissue culture. *Nature (London)* **196:** 1015–1016.

Raisz, L. G. 1963. Regulation by calcium of parathyroid growth and secretion *in vitro*. *Nature (London)* **197:** 1115–1117.

Raisz, L. G. 1976. Mechanisms of bone resorption. In: *Handbook of Physiology,* Sect. 7, Vol. 7, pp. 117–136. (Aurbach, G. D., Ed.) Washington, D.C.: American Physiological Society.

Raisz, L. G. and Niemann, I. 1967. Early effects of parathyroid hormone and thyrocalcitonin on bone in organ culture. *Nature (London)* **214:** 486–487.

Raisz, L. G., Trummel, C. L., Holick, M. F., and DeLuca, H. F. 1972. 1,25-Dihydroxycholecalciferol: a potent stimulator of bone resorption in tissue culture. *Science* **175:** 768–769.

Rapp, P. E. 1987. Why are so many biological systems periodic? *Prog. Neurobiol.* **29:** 261–273.

Rappaport, M. S. and Stern, P. 1986. Parathyroid hormone and calcitonin modify phospholipid metabolism in rat limb bones. *J. Bone Miner. Res.* **1:** 173–179.

Rasmussen, H., Fontain, O., Max, E. E., and Goodman, D. B. P. 1979. The effect of 1-hydroxyvitamin $D_3$-administration on calcium transport in chick intestine brush border vesicles. *J. Biol. Chem.* **254:** 2993–2999.

Raue, F., Schneider, H.-G., and Ziegler, R. 1990. The calcitonin receptor: characterization and processing. In: *Molecular and Cellular Regulation of Calcium and Phosphate Metabolism,* pp. 67–79. New York: Alan R. Liss.

Rawson, A. J. and Sunderman, F. W. 1948. Studies on serum electrolytes. XV. The calcium-binding property of serum proteins (multiple myeloma, lymphorgranuloma venerum and sarcoidosis). *J. Clin. Invest.* **27:** 82–90.

Reichel, H., Koeffler, H. P., and Norman, A. W. 1989. The role of vitamin D endocrine system in health and disease. *N. Engl. J. Med.* **320:** 980–991.

Reinhardt, T. A. and Horst, R. L. 1994. Phorbol 12-myristate 13-acetate and 1,25-dihydroxyvitamin $D_3$ regulate 1,25-dihydroxyvitamin $D_3$ receptors synergistically in rat osteosarcoma cells. *Mol. Cell. Endocrinol.* **101:** 159–165.

Reitsma, P. H., Rothberg, P. G., Astrin, S. M., Trial, J., Bar-Shavit, Z., Hall, A., Teitlelbaum, S. L., and Kahn, A. J. 1983. Regulation of *myc* gene expression in HL-60 leukemia cells by a vitamin D metabolite. *Nature (London)* **306:** 492–494.

Ribiero, C. P. and Mandel, L. J. 1992. Parathyroid hormone inhibits tubule $Na^+$-$K^+$-ATPase activity. *Am. J. Physiol.* **262:** F209–F216.

Ribovich, M. L. and DeLuca, H. F. 1976. Intestinal calcium transport: parathyroid hormone and adaptation to dietary calcium. *Arch. Biochem. Biophys.* **175:** 256–261.

Rodan, G. A. 1992. Introduction to bone biology. *Bone* **13:** S3–S6.

Rodan, G. A. and Martin, T. J. 1981. Role of osteoblasts in hormonal control of bone resorption — a hypothesis. *Calcif. Tissue Int.* **33:** 349–351.

Rodan, S. B. and Rodan, G. A. 1974. The effect of parathyroid hormone and thyrocalcitonin on the accumulation of cyclic adenosine 3′,5′-monophosphate in freshly isolated bone cells. *J. Biol. Chem.* **249:** 3068–3074.

Rodan, G. A. and Rodan, S. B. 1983. Expression of the osteoblastic genotype. In: *Bone and Mineral Research Annual 2,* pp. 244–285. (Peck, W. A., Ed.) Amsterdam: Elsevier.

Roodman, G. D., Ibbotson, K. J., MacDonald, B. R., Kuehl, T. J., and Mundy, G. R. 1985. 1,25-Dihydroxy vitamin $D_3$ causes formation of multinucleated cells with several osteoclast characteristics in cultures of primate marrow. *Proc. Natl. Acad. Sci. U.S.A.* **82:** 8213–8217.

Rogers, K. V., Dunn, C. K., Conklin, R. L., Hadfield, S., Petty, B. A., Brown, E. M., Hebert, S. C., Nemeth, E. F., and Fox, J. 1995. Calcium receptor messenger ribonucleic acid levels in the parathyroid glands and kidney of

vitamin D-deficient rats are not regulated by plasma calcium or 1,25-dyhydroxyvitamin $D_3$. *Endocrinology* **136:** 499–504.

Rosen, H. N., Dresner-Pollak, R., Moses, A. C., Rosenblatt, M., Zeind, A. J., Clemens, J. D., and Greenspan, S. L. 1994. Specificity of urinary excretion of cross-linked *N*-telopeptides of type I collagen as a marker of bone turnover. *Calcif. Tissue Int.* **54:** 26–29.

Rosenberg, R., Hurwitz, S., and Bar, A. 1986. Regulation of kidney calcium-binding protein in the bird (*Gallus domesticus*). *Comp. Biochem. Physiol. A* **83:** 277–281.

Rosenberg, J., Pines, M., and Hurwitz, S. 1987. Response of kidney and adrenal cell cAMP and of steroid hormone secretion to parathyroid hormone. *J. Endocrinol.* **116:** 91–95.

Rosenberg, J., Pines, M., Sherwood, L., Rosenblatt, M., Russell, J., Caulfeld, M., and Hurwitz, S. 1989. Renal and adrenal cyclic AMP production and corticosteroid secretion in response to synthetic (1-34) chicken PTH. *Endocrinology* **125:** 1082–1089.

Rossier, B. C. and Palmer, L. G. 1992. Mechanism of aldosterone action on sodium and potassium transport. In: *The Kidney: Physiology and Pathophysiology,* 2nd ed., pp. 1373–1409. (Seldin, D. W. and Giebisch, G., Eds.) New York: Raven Press.

Rouleau, M. F., Mitchel, J., and Goltzman, D. 1988. *In vivo* distribution of parathyroid hormone receptors in bone: evidence that the predominant osseous target cell is not the mature osteoblast. *Endocrinology* **123:** 187–191.

Russell, J., Bar, A., Sherwood, L. M., and Hurwitz, S. 1993. Interaction between calcium and 1,25 dihydroxyvitamin $D_3$ in the regulation of preproparathyroid hormone and vitamin D receptor in avian parathyroids. *Endocrinology* **132:** 2639–2644.

Russell, J., Lettieri, D., and Sherwood, L. M. 1983. Direct regulation by calcium of cytoplasmatic mRNA coding for pre-proparathyroid hormone in bovine parathyroid cells. *Endocrinology* **72:** 1851–1855.

Russell, J., Lettieri, D., and Sherwood, L. M. 1986. Suppression by 1,25-dihydroxyvitamin $D_3$ of transcription of the parathyroid hormone gene. *Endocrinology* **119:** 2864–2866.

Russell, J. and Sherwood, L. M. 1989. Nucleotide sequence of the avian preproparathyroid hormone mRNA and the deduced amino acid sequence of the hormone precursor. *Mol. Endocrinol.* **3:** 325–331.

Sammon, P. J., Stacey, R. E., and Bronner, F. 1969. Further studies on the role of thyrocalcitonin in calcium homeostasis and metabolism. *Biochem. Med.* **3:** 252–270.

Sauer, R. T., Niall, H. D., Hogan, M. L., Keutmann, H. T., O'Riordan, J. L. H., and Potts, J. T., Jr. 1974. The amino acid sequence of porcine parathyroid hormone. *Biochemistry* **13:** 1994–1999.

Schachter, D. and Rosen, S. M. 1959. Active transport of $^{45}Ca$ by the small intestine and its dependence on vitamin D. *Am. J. Physiol.* **196:** 357–362.

Schedl, H. P., Christensen, K. K., Clark, E. D., and Buettner, G. R. 1995. Surface charge, fluidity, and calcium uptake by rat intestinal brush-border vesicles. *Biochim. Biophys. Acta* **1234:** 81–89.

Scheven, B. A. A., Visser, J. W. M., and Nijweide, P. J. 1986. *In vitro* osteoclast generation from different bone marrow fractions, including a highly enriched homeopoietic stem cell population. *Nature (London)* **321:** 79–81.

Schipani, E., Karga, H., Karaplis, A. C., Potts, J. T., Jr., Kronenberg, H. M., Segre, G. V., Abou-Samra, A.-B., and Jüppner, H. 1993. Identical complementary deoxyribonucleic acids encode a human renal and bone parathyroid hormone (PTH)/PTH-related peptide receptor. *Endocrinology* **132:** 2157–2165.

Schneider, N., Teitelbaum, A. P., and Neuman, W. F. 1980. Tissue deposition and metabolism of $^{125}I$-labeled synthetic amino-terminal parathyroid hormone bPTH 1-34. *Calcif. Tissue Int.* **30:** 147–150.

Schräder, M., Bendik, I., Becker-André, M., and Carlberg, C. 1993. Interaction between retinoic acid and vitamin D signaling pathways. *J. Biol. Chem.* **268:** 17830–17836.

Schuchard, M., Landers, J. P., Sandhu, N. P., and Spelsberg, T. C. 1993. Steroid hormone regulation of nuclear proto-oncogenes. *Endocr. Rev.* **14:** 659–669.

Schwartz, Z., Schlader, D. L., Swain, L. D., and Boyan, B. D. 1988. Direct effect of 1,25-dihydroxyvitamin $D_3$ and 24,25-dihydroxyvitamin $D_3$ on growth zone and resting zone chondrocyte membrane alkaline phosphatase and phospholipase-$A_2$ specific activities. *Endocrinology* **123:** 2878–2884.

Segre, G. V. and Goldring, S. R. 1993. Receptors for secretin, calcitonin, parathyroid hormone (PTH)/PTH-related peptide, glucagon like peptide 1, growth hormone-releasing hormone, and glucagon belong to a newly discovered G-protein-linked receptor family. *TEM* **4:** 309–314.

Seino, Y., Yamaoka, K., Ishida, M., Yabuuchi, H., and Ichkawa, M. 1982. Biochemical characterization of $1,25(OH)_2D_3$ receptors in chick embryonal duodenal cytosol. *Calcif. Tissue Int.* **34:** 265–269.

Selles, J. and Boland, R. 1991. Rapid stimulation of calcium uptake and protein phosphorylation in isolated cardiac muscle by 1,25-dihydroxyvitamin $D_3$. *Mol. Cell. Endocrinol.* **77:** 67–73.

Sergeev, I. N. and Rhoten, W. B. 1995. 1,25-Dihydroxyvitamin $D_3$ evokes oscillations of intracellular calcium in a pancreatic β-cell line. *Endocrinology* **136:** 2852–2861.

Sexton, P. M., Houssami, S., Hilton, J. M., O'Keefe, L. M., Center, R. J., Gillespie, M. T., Darcy, P., and Findlay, D. M. 1993. Identification of brain isoforms of rat calcitonin receptor. *Mol. Endocrinol.* **7:** 815–821.

Shankar, V. S., Bax, C. M. R., Alam, A. S. M. T., Bax, B. E., Huang, C. L. H., and Zaidi, M. 1993. The osteoclast $Ca^{2+}$ receptor is highly sensitive to activation by transition metal cations. *Biochem. Biophys. Res. Commun.* **187:** 913–918.

Shoback, D. M., Membreno, L. A., and McGhee, J. 1988. High calcium and other divalent cations increase inositol triphosphate in bovine parathyroid cells. *Endocrinology* **123:** 382–389.

Silbermann, M., von Der Mark, K., Mirsky, N., Van Menxel, M., and Lewinson, D. 1987. Effect of increased doses of 1,25-dihydroxyvitamin $D_3$ on matrix and DNA synthesis in condylar cartilage of suckling mice. *Calcif. Tissue Int.* **41:** 95–104.

Silverman, R. and Yalow, R. S. 1973. Heterogeneity of parathyroid hormone: clinical and physiological implications. *J. Clin. Invest.* **52:** 1958–1971.

Simpson, R. U., Hsu, T., Wendt, M. D., and Taylor, J. M. 1989. 1,25-Dihydroxyvitamin $D_3$ regulation of c-*myc* protooncogene transcription. Possible involvement of protein kinase C. *J. Biol. Chem.* **264:** 19710–19715.

Skinner, D. C., Moodley, G., and Buffenstein, R. 1991. Is vitamin $D_3$ essential for mineral metabolism in the Damra mole rat (*Cryptomys damarensis*)? *Gen. Comp. Endocrinol.* **81:** 500–505.

Slater, S. J., Kelly, M. B., Taddeo, F. J., Larkin, J. D., Yeager, M. D., McLane, J. A., Ho, C., and Stubbs, D. 1995. Direct activation of protein kinase C by 1α,25-dihydroxyvitamin $D_3$. *J. Biol. Chem.* **270:** 6639–6643.

Somjen, D., Harell, A., Jaccard, N., Weisman, Y., and Kaye, A. M. 1990. Reciprocal modulation by sex steroid and calciotrophic hormones of skeletal cell proliferation. *J. Steroid Biochem. Mol. Biol.* **36:** 491–499.

Sorensen, A. M., Bowman, D., and Baran, D. T. 1993. 1α,25-Dihydroxyvitamin $D_3$ rapidly increases calcium levels in rat osteosarcoma cells. *J. Cell. Biochem.* **52:** 237–242.

Spanos, E., Barrett, D., MacIntyre, I., Pike, J. W., Safilian, E. F., and Haussler, M. R. 1978. Effect of growth hormone on vitamin D metabolism. *Nature (London)* **273:** 246–247.

Spanos, E., Brown, D. J., Stevenson, J. C., and MacIntyre, I. 1981. Stimulation of 1,25-dihydroxycholecalciferol production by prolactin and related peptides in intact renal preparations *in vitro*. *Biochim. Biophys. Acta* **672:** 7–15.

Spencer, R., Chraman, M., and Lawson, D. E. M. 1978. Stimulation of intestinal calcium-binding protein mRNA synthesis in the nucleus of

vitamin D-deficient chicks by 1,25-dihydroxycholecalciferol. *Biochem. J.* **175:** 1089–1094.

Staub, J. F., Tracqui, P., Brezillon, P., Milhaud, G. and Perault-Staub, A. M. 1988. Calcium metabolism in the rat: a temporal self-organized model. *Am. J. Physiol.* **254:** R134–R149.

Stein, G. S. and Lian, J. B. 1993. Molecular mechanisms mediating proliferation/differentiation interrelationships during progressive development of the osteoblast phenotype. *Endocr. Rev.* **14:** 424–442.

Strader, C. D., Fong, T. M., Tota, M. R., Underwood, D., and Dixon, R. A. 1994. Structure and function of G protein-coupled receptors. *Annu. Rev. Biochem.* **63:** 101–132.

Strewler, G. J. and Nissenson, R. A. 1994. Skeletal and renal actions of parathyroid-hormone related protein. In: *The Parathyroids,* pp. 311–320. (Bilezikian, J. P., Levine, M. A., and Marcus, R., Eds.) New York: Raven Press.

Su, M. J., Bikle, D. D., Manchianti, M.-L., and Pillai, S. 1994. 1,25-Dihydroxyvitamin $D_3$ potentiates the keratinocyte response to calcium. *J. Biol. Chem.* **269:** 14723–14729.

Suda, T., Shinki, T., and Takahashi, N. 1990. The role of vitamin D in bone and intestinal cell differentiation. *Annu. Rev. Nutr.* **10:** 195–211.

Suda, T., Takahashi, N., and Abe, E. 1992. Role of vitamin D in bone resorption. *J. Cell. Biochem.* **49:** 53–58.

Suda, T., Takahashi, N., and Martin, T. J. 1993. Modulation of osteoclast differentiation. *Endocr. Rev.* **13:** 66–80.

Sugimoto, T., Ritter, C., Reid, I., Morrissey, J., and Slatopolsky, E. 1988. Effect of 1,25-dihydroxyvitamin $D_3$ on cytosolic calcium in dispersed parathyroid cells. *Kidney Int.* **33:** 850–854.

Sun, F., Ritchie, C. K., Hassager, C., Maercklein, P., and Fitzpatrick, L. A. 1993. Heterogeneous response to calcium by individual parathyroid cells. *J. Clin. Invest.* **91:** 595–601.

Sundqvist, K., Lakkakorpi, P., Wallmark, B., and Väänänen, K. 1990. Inhibition of osteoclast proton transport by bafilomycin abolishes bone resorption. *Biochim. Biophys. Res. Commun.* **168:** 309–313.

Suzuki, M., Kurihara, S., Kawaguchi, Y., and Sasaki, O. 1990. Vitamin $D_3$ metabolites increase $[Ca^{2+}]$ in rabbit renal proximal straight tubule cells. *Am. J. Physiol.* **258:** F690–F696.

Swaminathan, R., Bates, R. F. L., Bloom, S. R., Ganguili, P. C., and Care, A. D. 1973. The relationship between food gastrointestinal hormones, and calcitonin secretion. *J. Endocrinol.* **59:** 217–230.

Talmage, R. V., Roycroft, J. H., and Anderson, J. J. B. 1975. Morphological and physiological considerations in a new concept of calcium transport in bone. *Am. J. Anat.* **129:** 467–476.

Tam, C. S., Heersche, J. N. M., Murray, T. M., and Parsons, J. A. 1982. Parathyroid hormone stimulates the bone apposition rate independently of its resorptive action: differential effects of intermittent and continuous administration. *Endocrinology* **110:** 506–512.

Tanaka, Y. and DeLuca, H. F. 1971. Bone mineral mobilization activity of 1,25-dihydroxycholecalciferol, a metabolite of vitamin D. *Arch. Biochem. Biophys.* **146:** 574–578.

Tanaka, Y., Castillo, L., and DeLuca, H. F. 1976. Control of renal vitamin D hydroxylases in birds by sex hormones. *Proc. Natl. Acad. Sci. U.S.A.* **73:** 2701–2705.

Tashijan, A. H., Jr., Write, D. R., Ivey, J. L., and Pont, A. 1978. Calcitonin binding sites in bone: relationship to biological response and "escape". *Recent Prog. Horm. Res.* **34:** 285–334.

Tata, J. R. and Smith, D. F. 1979. Vitellogenesis: a versatile model for hormonal regulation of gene expression. *Recent Prog. Horm.* **35:** 47–90.

Tauber, S. D. 1967. The ultimobranchial origin of thyrocalcitonin. *Proc. Natl. Acad. Sci. U.S.A.* **58:** 1684–1687.

Taylor, T. G. and Hertelendy, F. 1961. Changes in the blood calcium associated with egg shell calcification in the domestic fowl. II. Changes in diffusible calcium. *Poult. Sci.* **40:** 115–123.

Teti, A., Blair, H. C., Teitelbaum, S. L., Kahn, A. J., Koziol, C., Konesk, J., Zambonin-Zallone, A., and Schlesinger, P. 1989. Cytoplasmic pH regulation and chloride/bicarbon-

ate exchange in avian osteoclasts. *J. Clin. Invest.* **83:** 229–233.

Thomas, M. L. 1991. Calcium uptake by duodenal epithelial cells is increased during lactation. *Proc. Soc. Exp. Biol. Med.* **196:** 214–217.

Tsonis, P. 1991. 1,25-Dihydroxyvitamin $D_3$ stimulates chondrogenesis of the chick limb bud mesenchymal cells. *Dev. Biol.* **143:** 130–134.

Vaes, G. 1988. Cellular biology and biochemical mechanism of bone resorption. A review of recent developments on the formation, activation and mode of action of osteoclasts. *Clin. Orthop.* **231:** 239–271.

van Leeuwen, J. P. T. M., Birkenhäger, J. C., Burman, C. J., Schilte, J. P., and Pols, H. A. P. 1990. Functional involvement of calcium in the homologous up-regulation of 1,25-dihydroxivitamin $D_3$ receptor in osteoblast-like cells. *FEBS Lett.* **270:** 165–167.

van Leeuwen, J. P. T. M., Birkenhäger, J. C., Schilte, J. P., Burman, C. J., and Pols, H. A. P. 1990. Role of calcium and cAMP in heterologous up-regulation of the 1,25-dihydroxyvitamin $D_3$ receptor in an osteoblast cell line. *Cell. Calcif.* **11:** 281–289.

Vandenplas, M. L., Mouton, W. L., Vandenplas, S., Bester, A. J., and Ricketts, M. H. 1990. Increased cellular $Ca^{2+}$ is necessary for maximal expression of the proto-oncogene c-*jun* in the jurkat T-cell line. *Biochem. J.* **267:** 349–351.

Verhaeghe, J. and Bouillon, R. 1992. Calciotropic hormones during reproduction. *J. Steroid Biochem. Mol. Biol.* **41:** 469–477.

de Vernejoul, M.-C., Horowitz, M., Demignon, J., Neff, L., and Baron, R. 1988. Bone resorption by isolated chick osteoclasts in culture is stimulated by murine spleen cell supernatant fluids (osteoclast activating factor) and inhibited by calcitonin and prostaglandin $E_2$. *J. Bone Miner. Res.* **3:** 69–80.

Vohra, N., Kukerja, S. C., York, P. A. J., Bowser, E. N., Hargis, G. K., and Williams, G. A. 1983. Effect of exercise on serum calcium and parathyroid hormone. *J. Clin. Endocrinol. Metab.* **57:** 1067–1069.

Wada, S., Martin, T. J., and Findlay, D. M. 1995. Homologous regulation of the calcitonin receptor in mouse osteoclast-like cells and human breast cancer T47D cells. *Endocrinology* **136:** 2611–2621.

Wali, R. K., Baum, C. L., Bolt, M. J. G., Brasitus, T. A., and Sitrin, M. D. 1992a. 1,25-Dihydroxyvitamin $D_3$ inhibits $Na^+/H^+$ exchange by stimulating membrane phosphoinositide turnover and increasing cytosolic calcium in CaCo-2 cells. *Endocrinology* **131:** 1125–1133.

Walser, M. 1961. Ion association. VI. Interactions between calcium, magnesium inorganic phosphate, citrate and protein in normal human plasma. *J. Clin. Invest.* **40:** 723–730.

Walters, M. N. 1992. Newly identified actions of the vitamin D endocrine system. *Endocr. Rev.* **13:** 719–764.

Warner, R. R. and Coleman, J. R. 1975. Electron probe analysis of calcium transport by small intestine. *J. Cell. Biol.* **64:** 54–74.

Wasserman, R. H. 1963. Vitamin D and the absorption of calcium and strontium *in vivo*. In: *The Transfer of Calcium and Strontium Across Biological Membranes,* pp. 197–228. (Wasserman, R. H., Ed.) New York: Academic Press.

Wasserman, R. H., Chandler, J. S., Meyer, S. A., Smith, C. A., Brindak, M. E., Fullmer, C. S., Penniston, J. T., and Kumar, R. 1992. Intestinal calcium transport and calcium extrusion processes at the basolateral membrane. *J. Nutr.* **122:** 662–671.

Wasserman, R. H. and Fullmer, C. S. 1983. Calcium transport proteins, calcium absorption, and vitamin D. *Annu. Rev. Physiol.* **45:** 375–390.

Wasserman, R. H. and Taylor, A. N. 1966. Vitamin D-induced calcium-binding protein in chick intestinal mucosa. *Science* **152:** 791–793.

Wasserman, R. H. and Taylor, A. N. 1973. Intestinal absorption of phosphate in the chick: effect of vitamin $D_3$ and other parameters. *J. Nutr.* **103:** 586–599.

Webb, A. R. and Holick, M. F. 1988. The role of sunlight in the cutaneous production of vitamin $D_3$. *Annu. Rev. Nutr.* **8:** 375–399.

Wecksler, W. R. and Norman, A. W. 1980. Biochemical properties of 1α,25-dihydroxyvitamin D receptors. *J. Steroid Biochem.* **13:** 977–989.

Weiner, S. 1986. Organization of extracellularly mineralized tissues: a comparative study of biological crystal growth. *CRC Crit. Rev. Biochem.* **20:** 365–408.

Weiner, S. and Addadi, L. 1991. Acidic macromolecules of mineralized tissues: the controllers of crystal formation. *TIBS* **16:** 252–256.

Werner, J. A., Gorton, S. J., and Raisz, L. G. 1972. Escape from inhibition of resorption in cultures of fetal bone treated with calcitonin and parathyroid hormone. *Endocrinology* **90:** 752–759.

Wheling, M., Christ, M., and Theisen, K. 1992. Membrane receptors for aldosterone: a novel pathway for mineralocorticoid action. *Am. J. Physiol.* **263:** E974–E979.

Whitfield, G. K., Hsieh, J.-C., Jurutka, P. W., Selznick, S. H., Haussler, C. A., MacDonald, P. N., and Haussler, M. R. 1995. Genomic actions of 1,25-dihydroxyvitamin $D_3$. *J. Nutr.* **125:** 1690S–1694S.

Wideman, R. F., Jr. 1987. Renal regulation of avian calcium and phosphorus metabolism. *J. Nutr.* **116:** 818–825.

Wideman, R. F., Jr. and Youtz, S. L. 1985. Comparison of avian renal responses to bovine parathyroid extract, synthetic bovine (1-34) parathyroid hormone, and synthetic human (1-34) parathyroid hormone. *Gen. Comp. Endocrinol.* **56:** 480–490.

Williams, D. C. and Frolik, C. A. 1991. Physiological and pharmacological regulation of biological calcification. *Int. Rev. Cytol.* **126:** 195–292.

Wilson, P. W., Harding, M., and Lawson, D. E. M. 1985. Putative amino acid sequence of chick calcium-binding protein deduced from a complementary DNA sequence. *Nucleic Acids Res.* **13:** 8867–8880.

Wilson, P. W. and Lawson, D. E. M. 1977. 1,25-Dihydroxyvitamin D stimulation of specific membrane proteins in chick intestine. *Biochem. Biophys. Acta* **496:** 805–811.

Wilson, P. W., Rogers, J., Harding, M., Pohl, V., Pattyn, G., and Lawson, D. E. M. 1988. Structure of chick chromosomal genes for calbindin and calretinin. *J. Mol. Biol.* **200:** 615–625.

Wilson, T. H. and Wiseman, G. 1954. The use of sacs of everted small intestine for the study of transference of substances from the mucosal to the serosal surface. *J. Physiol.* **123:** 116–125.

Windeck, R., Brown, E. M., Gardner, D. G., and Aurbach, G. D. 1978. Effect of gastrointestinal hormones on isolated bovine parathyroid cells. *Endocrinology* **103:** 2020–2026.

Wong, G. L. 1984. A comparison of the PTH-dependent cAMP responses in osteoclastic and osteoblastic bone cells. *Miner. Electrolyte Metab.* **10:** 77–81.

Wong, K. M. and Klein, L. 1984. Circadian variations in contributions of bone and intestine to plasma calcium in dogs. *Am. J. Physiol.* **246:** R688–R692.

Yamato, H., Okazaki, R., Ishii, T., Ogata, E., Sato, T., Kumegawa, M., Akaogi, K., Taniguchi, N., and Matsumoto, T. 1993. Effect of 24R,25-dihydroxyvitamin $D_3$ on the formation and function of osteoclastic cells. *Calcif. Tissue Int.* **52:** 255–260.

Yanagawa, N. and Lee, D. B. N. 1992. Renal handling of calcium and phosphorus. In: *Disorders of Bone and Mineral,* pp. 3–40. (Coe, F. L. and Favus, M. J., Eds.) New York: Raven Press.

Yoon, K., Rutledge, S. J. C., Buenaga, R. F. and Rodan, G. A. 1988. Characterization of the rat osteocalcin gene: stimulation of promoter activity by 1,25-dihydroxyvitamin $D_3$ *Biochemistry* **26:** 8521–8526.

Zaidi, M., Datta, H. K., Moonga, B. S., and McIntyre, I. 1990. Evidence that the action of calcitonin on rat osteoclasts is mediated by two G proteins acting via two post-receptor pathways. *J. Endocrinol.* **26:** 473–481.

Zanello, S. B., Boland, R. L., and Norman, A. W. 1995. cDNA sequence identity of a vitamin D-dependent calcium-binding protein in the chick to calbindin D-9K. *Endocrinology* **136:** 2784–2786.

Ziegler, T., Telib, M., and Pfifer, E. F. 1969. The secretion of calcitonin by the perfused ultimobranchial gland of the hen. *Horm. Metab. Res.* **1:** 39–40.

Zull, J. E., Czarnowska-Misztal, F., and DeLuca, H. F. 1956. On the relationship between vitamin D and actinomycin-sensitive processes. *Proc. Natl. Acad. Sci. U.S.A.* **55:** 177–184.