Medical Progress

The Clinical Physiology of Calcium Homeostasis, Parathyroid Hormone, and Calcitonin

Part I

CHARLES R. KLEEMAN, M.D., SHAUL G. MASSRY, M.D., AND
JACK W. COBURN, M.D., *Los Angeles*

THE CONCENTRATIONS OF CALCIUM ion (Ca++) in the intracellular and extracellular fluids and in cellular and sub-cellular membranes are maintained with remarkable constancy, a reflection of the critical role of Ca++ in many fundamental biologic processes. The integrity, electrical properties, and permeability of these cellular and sub-cellular structures are critically dependent on calcium ion. Membranes depleted of calcium ion become increasingly porous and lose their selective permeability characteristics. Calcium ion is also an essential coupling factor or "biologic transducer" in the depolarization of cell membranes and conversion of electrical activity into contraction of skeletal, cardiac, and smooth muscles and in stimulus secretion coupling for glands of internal and external secretion. Calcium ion also plays an important role in the process of blood coagulation and in the activation of many intracellular enzyme systems.

In this report, the homeostatic regulation of Ca++ concentration of the body fluids and the role of the skeleton in this regulation will be discussed; this will lay the foundation upon which a discussion of the clinical physiology of parathyroid hormone and calcitonin is built. The contribution of the other major divalent ions, magnesium and phosphorus, will also be presented.

Physicochemical State of Calcium, Magnesium and Inorganic Phosphorus in the Plasma

Total plasma calcium is composed of two major fractions: (1) The protein-bound (non-diffusible or non-ultrafilterable) fraction which constitutes 40 ± 5% of the total plasma calcium concentration, and (2) The non-protein-bound (diffusible or ultrafilterable) fraction which constitutes 60 ± 5% of the total calcium level; 6 ± 5% of the diffusable fraction is present in the form of complexed calcium, and 94 ± 5% is in the form of calcium ion.[1] Therefore, the concentration of Ca++ in the plasma is about 50 percent of the total plasma level, and it is this concentration of Ca++ which is critically controlled by the homeostatic mechanisms.

The relationship between calcium ion and the concentration of protein in the blood is represented by a simple mass action expression:

$$\frac{(Ca++)\ (Proteinate)}{Calcium\ Proteinate} = K$$

where proteinate = concentration of plasma protein, primarily albumin.

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From the Departments of Medicine, Cedars-Sinai Medical Center, Wadsworth Veterans Administration Hospital and University of California, Los Angeles, School of Medicine.

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Dr. Massry is an Established Investigator of the American Heart Association.

Reprint requests to: Director, Divisions of Medicine, Cedars-Sinai Medical Center, 8720 Beverly Boulevard, Los Angeles, Ca. 90048 (Dr. C. R. Kleeman).

Since K is constant, the numerator and denominator must change proportionately in any physiologic or pathologic state. A change in the concentration of total plasma calcium will occur following a change in the concentration of serum proteins or alterations in their binding properties and after a primary change in the concentration of calcium ion. A fall in serum albumin reduces the proteinate and the calcium proteinate proportionately, resulting in a fall in total plasma calcium level with the free calcium ion concentration remaining normal. Therefore the diffusible fraction will constitute a larger portion of the total plasma calcium. A decrease in the concentration of serum albumin by 1 gram per 100 ml is usually associated with a fall of 1 mg per 100 ml in the concentration of total serum calcium.

Such hypocalcemia represents no basic disorder in the regulation of the concentration of calcium ion. It is apparent that a proper interpretation of a total plasma calcium cannot be made without a knowledge of simultaneous concentration of plasma albumin. Since only a small amount of calcium is bound to globulin, it is unusual to see a change in the total concentration of plasma calcium as a result of alteration in the level of plasma globulin. However, on rare occasions, when the globulin concentration in the plasma is extremely high (greater than 6 grams per 100 ml), a mild-to-moderate hypercalcemia may be seen due to an elevation of the globulin-bound calcium. In these circumstances, the concentration of free calcium ion is normal, and, therefore, this kind of hypercalcemia would not necessitate specific treatment. Therefore, in paients with elevation in total serum calcium and hyperglobulinemia, one should always determine the level of the diffusible fraction of serum calcium in order to differentiate between true hypercalcemia (elevation in the Ca++ concentration) and hypercalcemia secondary to increased binding of calcium by the high levels of globulins.

The concentration of plasma sodium may also affect the binding of calcium by serum albumin, resulting in changes in total serum calcium concentration. Walser[2] demonstrated that severe hypo- and hypernatremia (less than 120 mEq per liter and greater than 155 mEq per liter) could cause a predictable change in total plasma calcium concentration, due to a change in the calcium binding property of plasma proteins. Hyponatremia caused an increase in protein-bound calcium and therefore slight hypercalcemia, while hypernatremia caused a decrease in the protein-bound calcium and therefore slight hypocalcemia. In neither case was the concentration of Ca++ altered. If a tourniquet is left on the arm for 2 to 3 minutes before obtaining a blood specimen, transudation of protein-free fluid out of the capillary blood will result in concentration of the plasma proteins and subsequently lead to a rise in the protein-bound calcium;[3] this phenomenon might cause an elevation of 0.5 to 1.5 mg per 100 ml in the level of total serum calcium. Therefore, every effort should be made to obtain a blood specimen for the measurement of total serum calcium from free-flowing blood.

If for some physiological or pathological reason the concentration of free calcium ion changes, the calcium proteinate component will also change proportionately; this will result in a proportionate change in the level of total, bound, and diffusible calcium. Therefore, in all hypo- and hypercalcemic disorders due to primary changes in the concentration of calcium ion, the ratio of the diffusible to non-diffusible fractions remains constant, or normal. This is in contrast to altered ratios between the diffusible and bound fractions of calcium in cases where the change in total serum calcium is due to alterations in the concentrations of serum protein or in their binding properties.

In summary, the concentration of total plasma calcium is determined by the mass action relationship of Ca++ with the protein, while the concentration of Ca++ is controlled by the dynamic equilibrium between the metabolically active component of the skeleton and the extracellular fluid bathing this component.

The normal concentration of magnesium in the plasma is between 1.7 and 2.0 mg/100 ml; magnesium in the plasma is also present in two forms, a diffusible and a non-diffusible fraction. As in the case of calcium, the non-diffusible fraction is bound to proteins, and the relationship between the diffusable and the non-diffusable fraction follows the simple mass action equilibrium. The normal concentration of the inorganic phosphate in plasma of adults is 2.5 to 4.5 mg per 100

ml; it usually varies with age and sex[4] (Chart 1). The level of plasma phosphorus is 1 mg per 100 ml higher in children below the age of puberty; this higher level is possibly due to increased action of growth hormone, which elevates the plasma phosphorus. For practical purposes one can consider that all of the inorganic phosphorus is in diffusible form. At the pH of the body fluid, 80 percent of inorganic phosphorus is in the form of HPO_4 and 20 percent in the form of H_2PO_4.

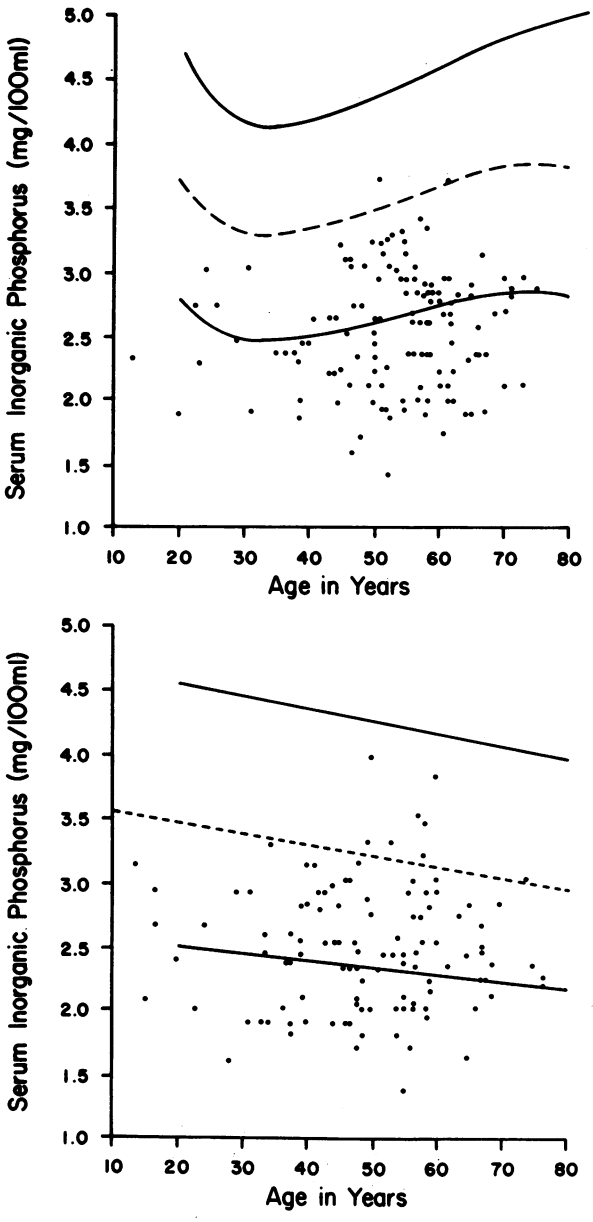


Chart 1.—Mean preoperative serum phosphorus values in patients with primary hyperparathyroidism; upper, men; lower, women. Broken lines represent normal mean expressed as regression with age; solid lines represent 2.5 and 97.5 percentile limits of normal distribution. Each dot represents the mean preoperative value for a patient. Individual preoperative values would show a larger proportion of patients having one or more values within the normal range. (Redrawn from Keating, F. R., Jr.[4])

Homeostatic Regulation of the Concentration of Calcium Ion Of the Plasma

The concentration of calcium ion in the plasma is maintained constant despite pronounced changes in external balance of calcium. If the fundamental factors regulating the calcium content of the body fluid are intact, a patient may lose 25 to 30 percent of the total body content of calcium without a change in the concentration of calcium ion of the plasma. Also, after the administration of a large oral or parenteral calcium load, serum calcium rapidly returns to normal after a brief period of disequilibrium. This rapid buffering of hypo- or hypercalcemic stress is illustrated in Chart 2.[5] This buffering capacity is fundamentally due to the dynamic equilibrium between the metabolically active part of the skeleton and the extracellular fluids. While variations in intestinal absorption and renal excretion of calcium will contribute to the concentration of calcium ion in the plasma, most clinically known hyper- or hypocalcemic disorders are not due,

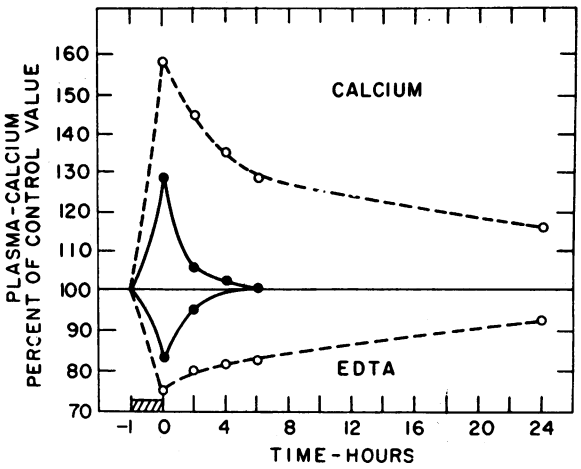


Chart 2.—The change in plasma calcium concentration induced by calcium or EDTA infusions into dogs before (closed circles) and after (open circles) thyroparathyroidectomy. The normal animal can rapidly restore the calcium concentration to normal; the thyroparathyroidectomized animal is unable to compensate quickly for either the hypocalcemia or hypercalcemia. (Redrawn by Potts & Deftos from Sanderson, P.H., et al.[5])

TABLE 1.—*Distribution of Calcium (Ca), Phosphorus (P) and Magnesium (Mg) in Body Tissues**

	Total body composition (gm./kg. fat free tissue)		
	Ca	*P*	*Mg*
	20-25	11-14	0.5
	Relative Distribution (Percent)		
Specific tissue			
Skeleton	99	85	66
Muscle	0.3	6	19
Other tissues	0.7	9	12

*Modified from S. M. Krane. (From Potts & Deftos[6])

per se, to an abnormality in the intestinal absorption or the renal excretion of calcium. Therefore, the basic cause of almost all physiologic and pathologic changes in the concentration of calcium ion in the plasma is an alteration in dynamic equilibrium between the bone and the extracellular fluid.

The Skeleton and the Homeostatic Control of the Concentration of Calcium Ion in the Plasma

The total calcium content of normal adult humans is 20 to 25 grams per kilogram of fat-free tissue, with 99 percent in the skeleton (Table 1)[6] and the remainder in the extracellular fluid. Studies utilizing calcium-45 and calcium-47 showed that 1 percent of skeletal calcium is freely exchangeable with that in the extracellular fluid, and these fractions constitute the miscible pool of calcium;[6] and a dynamic equilibrium is maintained between these two components of the miscible pool. The continuous bone remodeling involving the process of bone resorption and accretion, brought about by the metabolic activity of the skeleton, guarantees the maintenance of the readily exchangeable component.

To understand clearly the role of the different factors which may control or effect the processes of bone resorption and accretion, it is essential to visualize the functional anatomy of the skeleton. Figure 1[6] portrays the structure of mature bone and demonstrates the pattern of the vascular supply which is fundamental to support the metabolic functions of the skeleton.

Although Havers described in 1691 the canal which bears his name, he failed to describe the concentric lamellae around these vascular canals; therefore the term *osteon* rather than Haversian system may be used more appropriately to describe the morphologic unit of compact bone. Cooper, Milgram, and Robinson[7] have defined an osteon as "an irregular, branching and anastomos-

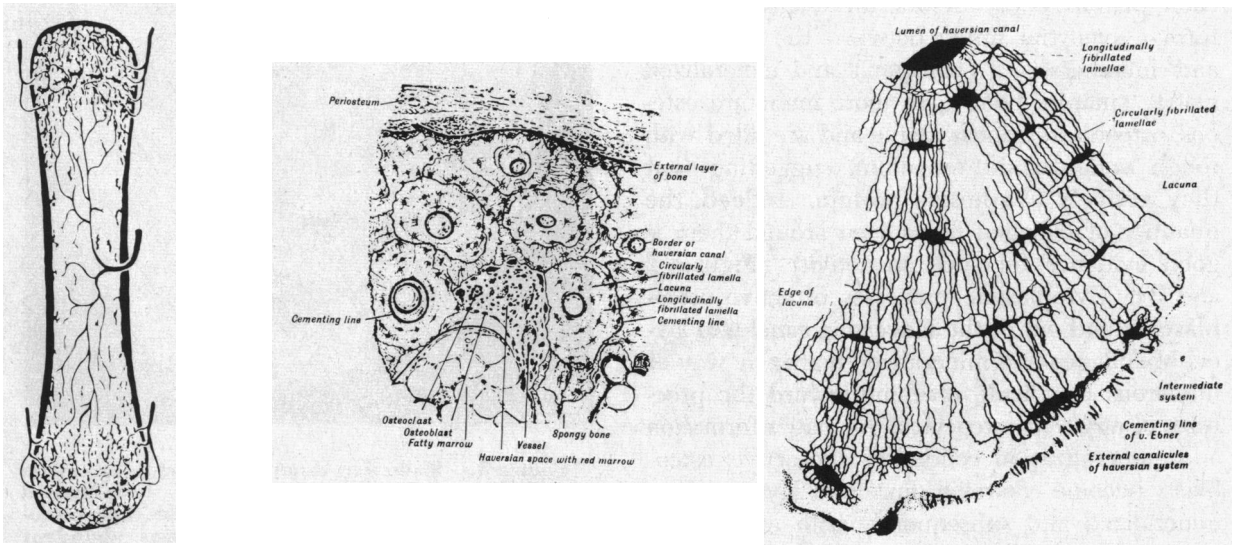


Figure 1.—Composite figure (left to right) of vascular supply of fully developed bone (phalange); a histologic cross-section of same illustrating cellular elements and details of numerous osteons with concentric lamellae, lacunae and central Haversian canals; and a higher magnification of a cross-section of a Haversian system. In the latter (magnification 520) the cavities and canalicules are filled with a dye which illustrates the connection via canalicules of the Haversian system and the lacunae (which *in vivo* contain osteocytes). (After A. A. Maximow. From Bloom, W., and Fawcett, D. W.: A Textbook of Histology, 9th ed., 1968, as reproduced from Ref. 6.)

ing cylinder composed of a more or less centrally placed cell-containing neurovascular canal surrounded by concentric, cell-permeated lamellae of bone matrix." They expand upon the definition: "For descriptive purposes, osteons and their enclosed Haversian canals can be divided into three types: developing, mature and resorbing, depending on the types of cell in the canal and the variations in the matrix around the canal. These categories cannot be sharply delineated since there are gradation of osteons, from the early developing to the mature; nevertheless, in any one ultra-thin section, one of these types predominates even though a different type of activity might be seen at another level of the same osteon." At one level of the osteon, usually near the periphery of the Haversian canal, osteoclasts are the predominant cells and bone resorption is the prevailing process. At another level the predominant cell may be osteoblasts, which are actively involved in the formation and deposition of collagen fibrils, in and around which mineralization is proceeding (Figure 2). Resorption of mineral and matrix may also occur around the osteocytes in their lacunae.

The cellular relationships around a capillary in Haversian canal, as visualized by electronmicroscopy, are shown in Figure 3. Osteoblasts and their primitive precursors, mesenchymal cells, form a syncytial lining between the capillaries and unmineralized (collagen) and mineralized matrix (mature bone). In more immature osteons, osteoblasts appear active and are filled with rough endoplasmic reticulum, suggesting that they actively synthesize protein. Indeed, the quantity of collagen fibers seen around them is good evidence for such an activity (Figures 2 and 3). Cytoplasmic processes of active osteoblasts extend out of the Haversian canal into layers of collagen and mineralized matrix by way of numerous canaliculi, reaching toward the processes of adjacent osteocytes. As matrix formation and mineralization continues, the active osteoblasts become encircled, first with layers of unmineralized and subsequently with mineralized matrix; they then become osteocytes lying within their lacunae. (Figure 2).

Osteoclasts, which are large multinucleated cells with abundant mitochondria containing dense granules of calcium-phosphate salts, are

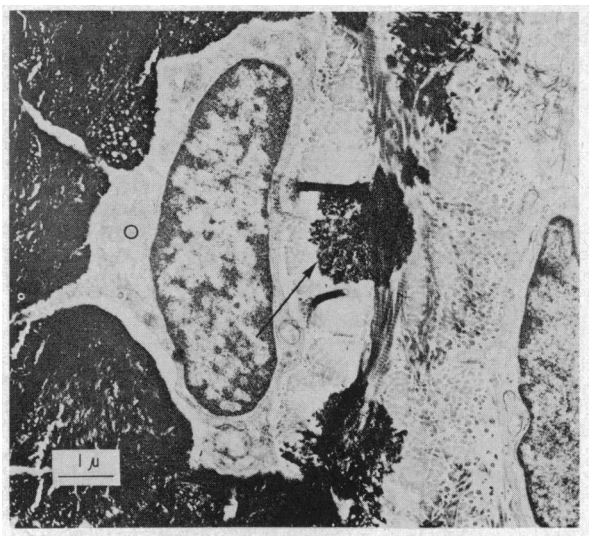


Figure 2.—The edge of an Haversian canal in a developing osteon showing an osteoblast (O) being buried by matrix which is mineralizing, thereby converting the cell to an osteocyte. Mineral is deposited in relation to the collagen fibrils irrespective of their direction. In the fibrils cut in cross-section, it can be seen that the mineral is within the fibrils (arrow) (lead citrate).[7]

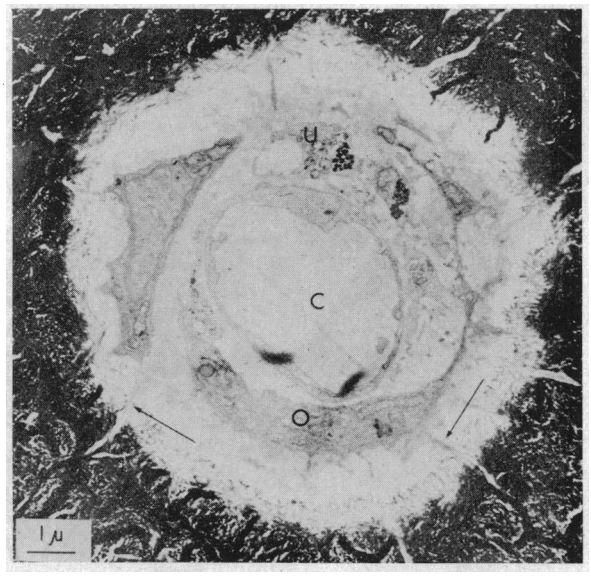


Figure 3.—Haversian canal in a developing osteon of a puppy. The central capillary (C) is composed of portions of several endothelial cells. The osteoblast (O) below the capillary contains abundant rough-surfaced endoplasmic reticulum. Osteoblast processes (arrows) leave the Haversian canal via canaliculi and extend into the surrounding matrix. The undifferentiated mesenchymal cells (U) contain dark clumps which probably represent glycogen. At the periphery of the canal irregular mineralization can be seen in relation to the white, negative images of collagen fibrils. The inner-most concentric mineralized lamellae surround the canal (lead citrate).[7]

seen along the edge of the Haversian canal in areas where bone resorption is active. These cells lack rough surface endoplasmic reticulum but contain smooth vesicles in their cytoplasm and have a ruffled border adjacent to the bone (Figure 4). Numerous bone crystals and remnants of collagen fibrils are seen between the cytoplasmic extensions of these cells.

The cells lining a mature Haversian canal appear to be inactive osteoblasts which are compressed against the walls of the mineralized matrix in the small space between the matrix and the Haversian capillary. These cells are extremely attenuated in places with the plasma membrane of one side of the cell in close contact to that of the other. There appear to be gaps between some of these cells, creating discontinuity in the cellular lining of the Haversian canal and allowing direct contact between extracellular or interstitial fluid and the fluid within the canaliculae and lacunae.

The crystal surfaces exposed to modified extracellular fluid along the walls of the lacunae, canaliculi and Haversian canals is immense; Robinson[8] estimated this to be 1500 to 5000 square meters in the average man. Bone crystal surfaces exposed in these areas could afford access to 3,120 square meters of surface on about 15.6 grams of bone crystals. Thus, an exchange of mineral ions may constantly occur via a water bridge which extends from the inside of the Haversian vessels to the crystal surfaces of bone on the walls of the Haversian canals, canaliculi, and lacunae.

In 1955, at the Ciba Foundation Symposium on Bone Structure and Metabolism, Dr. John E. Howard[9] made the following intuitive and perceptive statement: ". . . . The bones have been shown, beyond reasonable doubt to be the site of operation for a buffer system which stabilizes the concentration of calcium in the body fluids. One conceives of the bone crystal as a gigantic pile of mineral materials, controlled locally by an active barrier; this barrier having an inherent or basic level of operation. The parathyroid hormone is one force which effects it and alters its basic rate. Structurally, the barrier could be a membrane derived from endosteal cells or their proliferatives; and, therefore, changes in its properties would be due to cellular activity of the bone cells. In this visualization the bone cell has been given still further duties—those of the porter and the janitor, in addition to being the architect, the builder and the demolition squad of the skeleton mansion." Howard's hypothesis has gained increasing support and it seems probable that osteoblasts and certain osteoblast percursors constitute an effective active transport barrier that regulate the exchange of ions between bulk extracellular fluid and mineralizing bone collagen.[8,10-13]

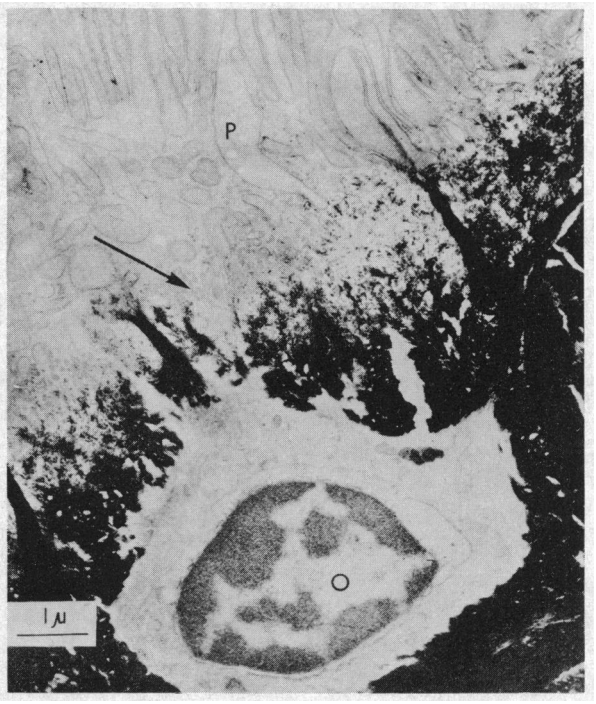


Figure 4.—In the upper portion of the micrograph the ruffled border of the osteoclast is seen. Between the cell processes (P) are numerous mineral crystals. Beneath the processes are portions of collagen fibrils (arrow) among which are many crystals. An osteocyte (O) is seen lying in its lacuna in the mineralized matrix (lead citrate).[7]

On the bony side of the osteoblasts, there exists the bone extracellular fluid flowing within the canicular and periosteocytic lacunar spaces and directly associated with mineral-like tissues; the ionic concentrations of calcium and phosphate of this fluid are determined by the solubility product of hydroxyl apatite and the levels of these ions are approximately one-third as much as their concentrations in extracellular fluid.[10,14] On the other side of the osteoblasts there exist the central Haversian canal, capillary and bulk extracellular and interstitial fluids which have a divalent ion composition identical to that of the general body interstitial or bulk extracellular

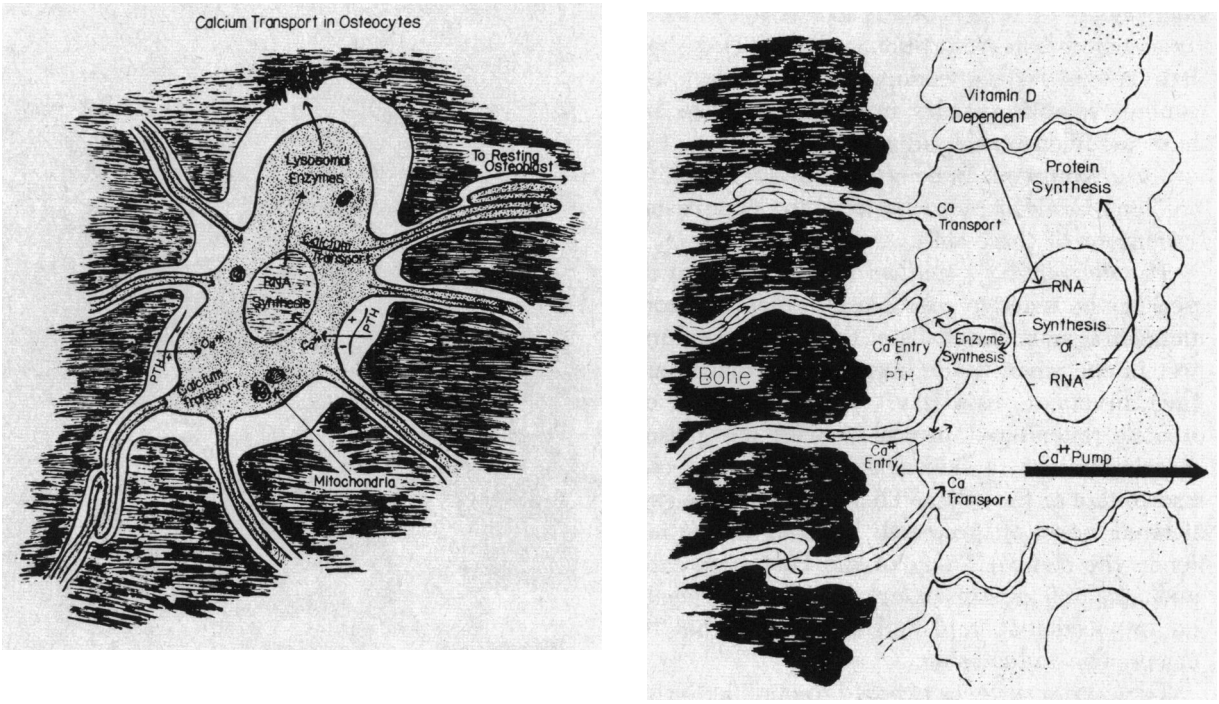


Figure 5.—Composite reproduction of diagrams from Talmage, R. V.[10] On the right, transcellular calcium transport through the "osteoblast" layer of bone is represented, while on the left is diagramed the osteocyte, "communicating" with the osteoblast through the canalicular system, with the various factors influencing calcium transport to and from the cell.

fluid. Thus, calcium and phosphate ions in bulk extracellular fluid may flow down their concentration gradients in the gaps (400 Å to 2 mμ) between the osteoblastic lining cells (Figure 5). This provides a mechanism for inward movement of Ca++ and $PO_4^{=}$ for mineralization of new matrix (bone accretion); thus, there is a continuous tendency for Ca++ and $PO_4^{=}$ to leave extracellular fluid and be deposited as new or growing crystals of hydroxyl apatite in or on the organic matrix of the skeleton. Calcium ion can also diffuse "downhill" into the lining cells for the intracellular Ca++ concentration of all cells is maintained at an extremely low level (10^{-7} to 10^{-6} molar which is less than one-thousandth the concentration of Ca++ in extracellular fluid). This low intracellular Ca++ concentration is maintained by low membrane permeability and the presence of an active transport pump capable of removing Ca++ from the cell into the extracellular fluid surrounding it.[10,11,15] It has been suggested that the actual active transport pump in the osteoblast lies on the side of the cell facing the Haversian capillary and that this surface is also less permeable to the inward diffusion of calcium ion.[10] Thus, there also may be net movement of large amounts of calcium ion by passive inward diffusion from bone through bone extracellular fluid into the cells lining the Haversian canal; from these cells calcium is actively transported into bulk extracellular space (Figure 5); control of this transport is determined by various hormonal and non-hormonal factors regulating the metabolic activity of these cells.

The osteocyte, which can produce large amounts of lactic acid and form considerable quantities of hydrolytic enzymes and collagenases, which play critical roles in mineral and matrix resorption, is also capable of active calcium transport.[8,12] During active osteocytic bone resorption (osteolysis), Ca++ and $PO_4^{=}$ ions are transported from the osteocytes to the osteoblasts along the protoplasmic extensions of these cells, which make contact within the canaliculi and in the fluid flowing in the lacunar-canalicular system (Figures 1 and 5). Thus, mechanisms are available whereby these ions may be delivered into the circulation from the depths of mature or fully mineralized bone. Osteolysis, rather than osteoclastic bone resorption, is considered by

many to be the primary metabolic activity of the skeleton responsible for maintaining the normal Ca++ concentration in the blood.[6,10-13] Although the osteoclast is also capable of causing very active bone resorption, its contribution to the normal control of blood Ca++ has recently been minimized.[6,10,11,12,13] Although parathyroid hormone affects the number and activity of osteoclasts, Talmage[10,17] has shown that this action of the hormone cannot satisfactorily explain the regulation of serum calcium levels by parathyroid hormone. However, the osteoclast is considered to have an important role in skeletal homeostasis or bone remodeling. Two clinical examples of hypocalcemia despite marked osteoclastic bone resorption are found in cases with osteitis fibrosa of renal osteodystrophy and occasionally in patients with classical pseudohypoparathyroidism.[16]

Neuman and Neuman[14] have shown that the mineral phase of inert bone mineral comes into equilibrium with Ca++ and $PO_4^=$ at an ion product of about one-third of that normally found in plasma and extracellular fluid; thus, there is supersaturation of Ca++ and $PO_4^=$ in extracellular fluid with respect to bone mineral. In the absence of metabolic activity of bone cells or the cellular barrier interposed between bulk extracellular fluid and the mineralized matrix, the Ca++ and $PO_4^=$ concentrations in the plasma would rapidly fall as these ions are deposited in the skeleton. Therefore, the maintenance of normal Ca++ concentrations and, in part, the $PO_4^=$ concentration in plasma are dependent upon the cellular processes of bone resorption and "uphill" transport of these ions against their physicochemical gradient. It is apparent that these processes must be continuous, and the importance of parathyroid hormone in their maintenance is clearly evident from the rapid fall in plasma Ca++ concentration which occurs when the parathyroid glands are removed.

Hormonal and Non-Hormonal Regulators of Mineral Homeostasis

When bone growth, *per se*, has ceased and a normal calcium and phosphorus balance is present in healthy adults, the urinary excretion of these ions is approximately equal to their net absorption from the gastrointestinal tract. Bone remodeling takes place at all times, and, therefore, there should be a continuous balance between the processes of bone accretion and bone resorption. Such a balance is brought about by several hormonal and non-hormonal factors which continuously regulate the activity of the osteoblasts, osteocytes and osteoclasts which are involved in these two processes. These factors include inorganic phosphate, calcium ion, magnesium ion, vitamin D, adrenal glucocorticoids, parathyroid hormone, and calcitonin.

Inorganic Phosphate ($PO_4^=$). Experimental and clinical evidence indicates that inorganic phosphate plays an important role in calcium homeostasis and bone metabolism; the rate of net flux of calcium into and out of the skeleton under the influence of parathyroid hormone and calcitonin is intimately dependent on the level of inorganic phosphate bathing the internal and external environment of the metabolically active bone cells.

A positive external balance of inorganic phosphate or a rise in the concentration of this ion in the extracellular fluid shifts the skeletal dynamic equilibrium toward a net movement of calcium into the skeleton; this is associated with a fall in the concentration of Ca++ in the plasma and a reduction in the excretion of calcium in the urine. Conversely, a negative external balance of inorganic phosphate is accompanied by hypophosphatemia, a tendency for plasma Ca++ concentration to rise, marked hypercalciuria, and a net negative calcium balance.

Albright, et al,[18] almost 40 years ago, were the first to demonstrate that high phosphate intake could reverse the biochemical picture of primary hyperparathyroidism. Recently, inorganic phosphate has again been used as an important adjunct in the treatment of many hypercalcemic disorders, which are characterized by relative or absolute excesses of bone resorption.[19,20] The administration of inorganic phosphate to these patients with hypercalcemic disorders is associated with a fall in serum calcium, a reduction in urinary calcium, and a positive calcium balance. These effects of inorganic phosphate are probably primarily due to movement of calcium and phosphate ions into the skeleton. Whether phosphate administration to such patients may lead to an increase in the soft tissue content of these ions is as yet an unanswered question. Soft tissue deposition of these ions during phosphate administration may be minimal unless hyper-

phosphatemia is produced in the face of sustained hypercalcemia.

Raisz[21] evaluated the effects of inorganic phosphate on the metabolism of embryonic bone studied in tissue culture; he found that an increase in the concentration of inorganic phosphate in the incubation fluid directly antagonizes the calcium mobilizing effect of parathyroid hormone and enhances the ability of calcitonin to inhibit bone resorption. Finally, a high phosphate diet fed to humans, rats or rabbits for many days can lead to a state of nutritional or physiological hyperparathyroidism with hyperplasia of the parathyroid glands, changes in the skeleton consistent with mild osteitis fibrosa, hypophosphatemia, a high renal clearance of phosphate and low urinary calcium.[13,22-25] A possible explanation for the hypophosphatemia is the creation of a new steady state in which the mildly hyperplastic parathyroid glands require a slightly higher than normal level of plasma Ca++ to suppress their increased secretory activity. Under these circumstances a higher rate of $PO_4^=$ clearance will be maintained and hypophosphatemia will ensue.

Phosphate depletion and hypophosphatemia shift the skeletal dynamic equilibrium with a marked net movement of calcium out of the skeleton; this state is associated with a rapid loss of bone mineral, progressive hypercalciuria, a negative calcium balance, and virtual absence of phosphate from the urine. A bone lesion indistinguishable from rickets and osteomalacia can be seen in humans, rats and dogs after long periods of phosphate depletion.[26,27,28,29] The hypercalciuria of phosphate depletion is due to a decrease in the tubular reabsorption of calcium but the exact mechanism causing the change in the renal handling of calcium is as yet unknown.[30] Phosphate depletion in humans and animals produces a state of physiologic hypoparathyroidism.[27,28,30] In the phosphate depleted state, both in humans and experimental animals, the parathyroid glands are not required for the maintenance of a normal level of serum calcium.[27,28,30] Thus, serum calcium may be normal or even elevated in phosphate depleted parathyroidectomized humans or experimental animals (Chart 3); the administration of small amounts of inorganic phosphate under these circumstances will lead to a rise in serum phosphorus and a marked

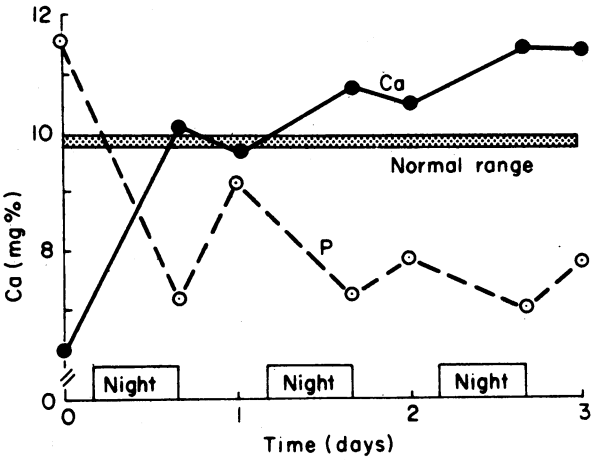


Chart 3.—Effect of low phosphate diet on plasma calcium and phosphorus in a young parathyroidectomized rat. (From Copp, D. H.[28])

fall in serum calcium.[28,30] In addition, the skeleton of phosphate depleted rats is resistant to the calcium mobilizing effect of parathyroid hormone.[28] It is hard to explain how an animal or human with minimal secretion of parathyroid hormone and the skeleton that is probably resistant to the action of the hormone can maintain a normal or even elevated serum calcium. Baylink et al[29] have shown in the rat that phosphate depletion prevents maturation and mineralization of osteoid while simultaneously causing a threefold increase in osteoclastic bone resorption. One might expect that the latter could be blocked by calcitonin, but Copp[28] and Kennedy et al[31] have shown that phosphate depletion and hypophosphatemia impair the ability of calcitonin to inhibit bone resorption or lower serum calcium.

The exact mechanisms by which changes in the body stores of phosphate exert their affects on bone are unknown. It has been suggested that changes in the Ca++ x $PO_4^=$ product of the extracellular fluid, brought about by changes in the level of blood $PO_4^=$ favor the movement of these ions into or out of the bone by simple physicochemical equilibrium.[11] However, it is most probable that inorganic phosphate has a direct effect on the intermediary metabolism of all bone cells, possibly by bringing about an alteration in the intracellular calcium. The net effect is inhibition of bone resorption and stimulation of new bone formation.[11-13,21,23] Glimcher[32] suggested that the conversion of inorganic phosphate to organic phospho-proteins in the collagen is essential for its initial mineralization. Also, Raisz[11]

and Nichols[12] have found that the presence of inorganic phosphate in an *in vitro* bone cellular system enhances collagen synthesis, while the removal of inorganic phosphate enhances efflux of calcium and bone resorption.

Phosphate depletion in humans is associated with certain clinical symptoms which have been described by Lotz et al.[27] This syndrome is not frequent and is usually caused by the large intake of phosphate binding antacid, such as aluminum hydroxide gel, used in the treatment of various forms of gastrointestinal diseases.

Calcium Ion. Raisz[11] found that a low concentration of calcium ion in the media bathing a tissue culture of fetal rat bone can definitely block the calcium mobilizing effects of both parathyroid hormone and the active metabolite of vitamin D, 25-hydroxycholecalciferol. In thyroparathyroidectomized rats, Nichols[12] has shown that parathyroid hormone increases the uptake of calcium-45 into osteocytes and that the maximal rate of calcium efflux from the skeleton is dependent on the cellular content of calcium. He proposed that the serum calcium directly reflects the calcium content of the osteocyte. Borle noted that physiologic concentrations of parathyroid hormone can increase the intracellular concentration of calcium by augmenting the permeability of the cell membrane and that calcitonin alters the cellular transport of calcium by inhibiting its active efflux from the cells.[15,33] As discussed later, almost all the effects of parathyroid hormone can be attributed to its ability to increase the entry of calcium into the cell; it has, therefore, been postulated that intracellular calcium ion, itself, is the central regulator[10-13,15,32] either by directly affecting cell metabolism through changes in ion activity or by binding to specific nuclear or cytoplasmic proteins that control calcium transport or cell differentiation. This formulation is presented in the theoretical model proposed by Raisz,[34] Chart 4.

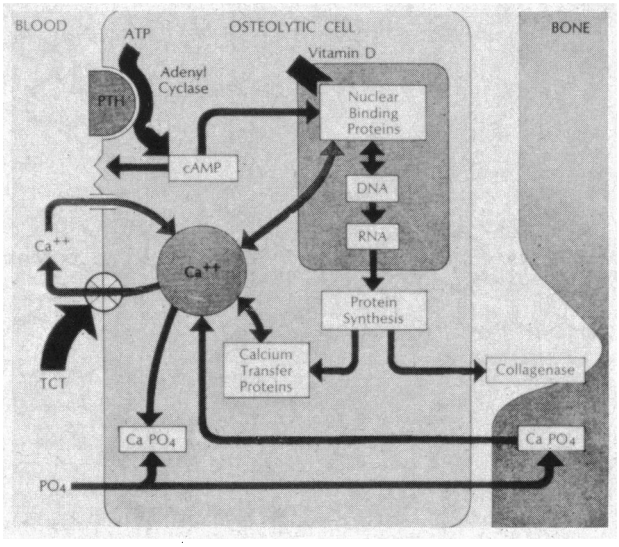


Chart 4.—Theoretical model of an osteolytic cell: Parathyroid hormone (PTH) is assumed to bind to cell membrane, activating adenyl cyclase to produce a local increased in cyclic AMP (cAMP) concentration. Calcium entry into cell is by passive diffusion along a concentration gradient and is controlled by changes in membrane permeability; cAMP may alter this permeability and could also act on nuclear transcription or other metabolic processes in the cell. Vitamin D may act at nucleus to increase calcium entry or by binding directly to the chromatin to alter nuclear transcription of DNA. It could also synergize with other effects of PTH to increase intracellular Ca++ or stimulate synthesis of proteins involved in resorption (*e.g.*, collagenase) or calcium transport. Ca++ is removed from cell by active transport. The transporting pump may be blocked by calcitonin (TCT), producing a rapid drop in resorption. Phosphate could act by enhancing Ca deposition in bone. Since enzymes cannot work on fully mineralized matrix, this would also prevent matrix removal.[34]

Magnesium Ion. The exact role of magnesium ion (Mg++) in the regulation of bone metabolism and the control of Ca++ concentration in body fluids is unknown. Magnesium is not an integral part of the hydroxyapatite crystal, but a large amount of magnesium is present in the inorganic phase of the skeleton. This is probably located within the crystal lattice and hydration shell of hydroxyapatite. It is probable that the Mg++ content of the bone cells is similar to that of the other tissues and that Mg++ is involved in similar pathways of intermediary metabolism.

Changes in the level of Mg++ in the blood and/or in body tissues may affect bone metabolism either by affecting the activity of the parathyroid glands[35,36] or by altering the responses of the skeleton to the action of parathyroid hormone (PTH).[38,39] The most notable effect of Mg++ on bone metabolism in humans is the hypocalcemia of Mg++ deficiency; a similar effect has been reported in dogs, pigs and calves.[37] Such hypocalcemia has been attributed to resistance of the skeleton to the action of PTH.[37,38] The exact cause of the failure of the bone to respond to PTH is unknown. Raisz has shown in tissue culture that PTH-stimulated bone resorption is impaired when the Mg++ concentration in the incubation medium is low.[39] Magnesium

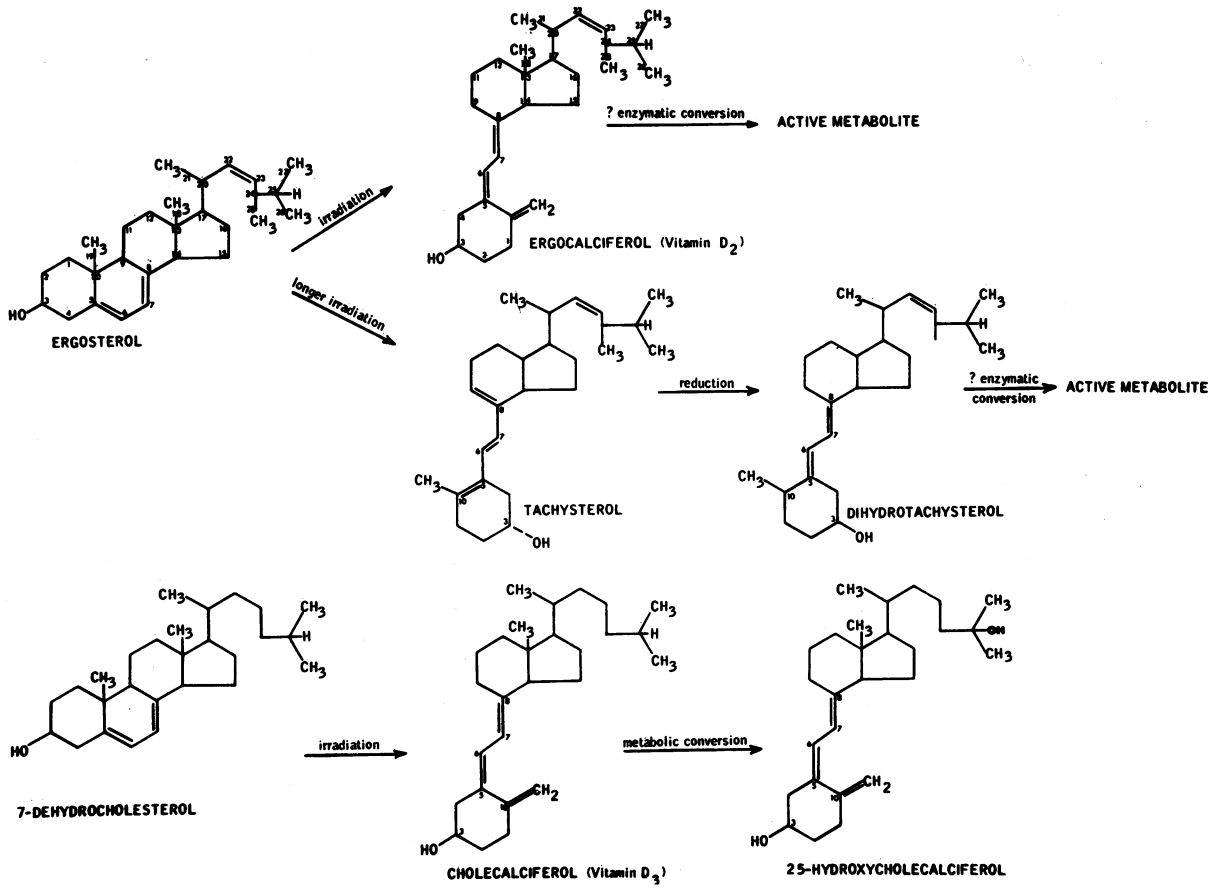


Chart 5.—Schematic illustrating structure, origin and active metabolic products of vitamins D_2 and D_3. The formation in vivo of 25-hydroxycholecalciferol, the active metabolite of vitamin D_3, has been established. It is likely, but has yet to be proved, that 25-hydroxylation may be a common mechanism of metabolic conversion for the antirachitic compounds in vivo; hence, this is shown as probable for dihydrotachysterol and ergocalciferol. Products other than tachysterol are formed after irradiation of ergosterol; hence, tachysterol is usually purified from the irradiation mixture before reduction. Double bonds at carbons 5, 6 and 7, 8 are characteristic of vitamin D compounds that are biologically active. (Data provided through the courtesy of Dr. H. F. DeLuca).[6]

deficient patients do not show the expected increase in cyclic 3′5′ AMP excretion after PTH administration.[38] Raisz[11] suggested that PTH unresponsiveness is related to a Mg++ requirement for PTH activated adenyl cyclase which may represent a fundamental initial step in the cellular action of PTH. It is apparent, therefore, that extracellular and/or intracellular Mg++ concentration must be normal in order to maintain Ca++ and $PO_4^{=}$ homeostasis.

Vitamin D. Vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol) are produced by the ultraviolet irradiation of ergosterol and 7-dehydrocholesterol, respectively. The former is present in plants and plant products, while the latter is of animal origin. A growing body of evidence[40] indicates that these steroids are hydroxylated at the 25-position before they can exert a biological effect (Chart 5). This conversion of vitamin D_3 to 25-hydroxycholecalciferol (25-OH-CC) occurs within the liver.[41] The direct addition of 250-H-CC to transport models of intestine and bone, *in vitro,* produces actions similar to those produced when vitamin D is administered *in vivo.*[42,43] The parent vitamin D, *per se,* lacks such an *in vitro* effect. Evidence for further metabolic conversion of the 25-OH-CC to other metabolites which may be more active in tissues has also been advanced.[44-46]

It has been shown that vitamin D or its metabolites act directly to enhance the gastrointestinal transport of calcium and to augment the resorption of bone; however, there is no unequivocal

evidence for a direct effect of the vitamin on renal handling of divalent ions.[6,47] Although the exact mechanism by which vitamin D acts to enhance intestinal calcium transport remains speculative, there is evidence to indicate that 25-OH-CC enters the intestinal cell to be converted to another metabolite which becomes bound to the nucleus.[45,46] The result is enhanced DNA-dependent RNA synthesis, with consequent stimulation of protein synthesis. In the intestinal cell, two proteins, a specific calcium-binding protein[48] and a calcium-activated ATPASE,[40] are produced along the brush border following administration of 25-OH-CC. Although the matter is as yet unresolved, either calcium-binding protein or the calcium-dependent ATPase may be important in enhancing the energy-dependent calcium transport which is stimulated by vitamin D. The increased efficiency of intestinal absorption of calcium in time of need requires the combined action of vitamin D and PTH, although the former seems to be more important. Thus, hypoparathyroid animals receiving a normal intake of vitamin D have defective intestinal absorption of calcium[49,50] and a large dose of vitamin can correct this abnormality. Moreover, vitamin D deficient animals have reduced absorption of calcium despite hypersecretion of PTH.[6,47,49] The action of vitamin D on bone has been less extensively delineated. Twenty-five hydroxycholecalciferol stimulates bone resorption in tissue cultures in a manner quite similar to that produced by parathyroid hormone,[43] and in such a model 25-OH-CC and PTH act synergistically. Both are dependent on the presence of Ca++ at the plasma membrane or within the bone cell.[11] While there is increasing evidence that PTH exerts its action on bone by activating adenylcyclase, vitamin D has no effect on this system. Raisz[11] suggested that the two agents are "physiologic synergists that act not at the same receptor site in the bone resorbing cells but at separate sites linked so that the effects of one can enhance the response to the other (Chart 4)." He postulated that the synergism between 25-OH-CC and PTH could be explained if PTH controlled Ca++ entry into the cell and 25-OH-CC controlled Ca++ entry into the nucleus, with the latter controlling transcription and cellular transformation (Chart 4). In the absence of vitamin D, much larger doses of PTH would be required to enhance cellular transport of Ca++ and nuclear transcription.

A vitamin D deficient or resistant state causes classic biochemical and clinical syndromes: rickets in the child and osteomalacia in the adult. They are characterized by: (1) generalized demineralization of skeleton with deformities of weight-bearing bones and pseudofractures, which are symmetrical linear areas of bone resorption at sites where nutrient arteries penetrate the bone, and with histologically defective mineralization of newly formed matrix and relative impairment of the normal bone resorption;[51] (2) hypocalcemia in dogs and rats and either hypocalcemia or normocalcemia in humans, and in all species, the hypocalcemia is relatively unresponsive to PTH; (3) marked impairment of Ca++ and, secondarily, $PO_4^{=}$ absorption from the gastrointestinal tract; (4) pronounced hypocalciuria; (5) hypophosphatemia, with a high renal clearance of $PO_4^{=}$; (6) parathyroid hyperplasia with hypersecretion of PTH.[6,11] These characteristics may all be explained by the basic defects in the bone and gut caused by vitamin D deficiency, *per se,* and the pronounced secondary hyperparathyroidism induced by hypocalcemia.

Conversely, pharmacologic doses of vitamin D cause enhanced bone resorption and increased gastrointestinal absorption of calcium, leading to hypercalciuria and, with greater doses of the vitamin, to frank hypercalcemia. Parfitt[52] has shown that the delay in the return of plasma CA++ to normal following the acute hypocalcemic stress of an EDTA infusion, which is characteristic of hypoparathyroid humans (Charts 2 and 6), can be completely corrected by adequate therapeutic doses of vitamin D. Thus, not only can vitamin D replace PTH in the maintenance of a normal plasma Ca++ concentration under steady-state conditions, it can replace the hormone in producing a normal skeletal response to a hypocalcemic stress.

Adrenal Steroids. Mild-to-moderate hypercalcemia has been noted in patients with adrenal insufficiency,[2] and cortisol or its analogues have been used in the treatment of hypercalcemia. Although the chronic administration of these steroids may inhibit the gastrointestinal absorption of calcium and increase its urinary excretion, it is most likely that the ability of these drugs to lower plasma calcium Ca++ is due to a direct effect upon the skeleton. In hypopara-

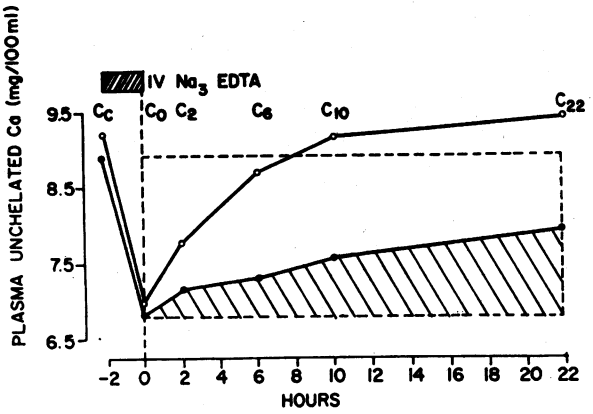


Chart 6.—Response to EDTA infusion. Open circles: mean changes in two normal subjects; closed circles: mean changes in two patients with severe post-surgical hypoparathyroidism. Area under curve of recovery is shaded, area corresponding to instantaneous recovery enclosed by broken line rectangle. (From Parfitt, A. M.[52])

thyroid dogs (unpublished observations) and rats,[53] adrenalectomy causes a definite increase in plasma Ca++ concentration, even to hypercalcemic levels. Although Walser[2] suggested that the hypercalcemia of adrenal insufficiency is due to an increase in protein-bound and complexed Ca++, we have observed that free Ca++ concentration rises in adrenalectomized animals (unpublished observations). Furthermore, when pharmacologic doses of glucocorticoids are administered to hypoparathyroid patients, a significant decrease in plasma calcium is seen.[54] Although the exact mechanism whereby steroids exert such an effect on serum calcium is unknown, it may be postulated that glucocorticoids may exert a "tonic suppressive" effect on bone cells responsible for the maintenance of a normal Ca++ concentration in extracellular fluids. When added to cultures of embryonic rat bone, glucocorticoids can completely inhibit the PTH-induced release of Ca^{45} from bone.[55] These steroids are known to be inhibitors of cellular lysosome enzymes. Lysosomes are intracellular membrane-bound vesicles containing potent proteolytic enzymes. During active bone resorption, these enzymes are released from both osteocytes and osteoclasts into the lacunar-canalicular space, and the bone content of these enzymes correlates quantitatively with the magnitude of bone resorption.[56] Glucocorticoids may stabilize lysosome membranes to inhibit release of these proteolytic enzymes, thereby reducing the magnitude of bone resorption.

Parathyroid Hormone. Parathyroid hormone is the single most important regulator of Ca++ metabolism in mammals. Although the parathyroid glands were first described more than a hundred years ago, their physiologic significance was not appreciated until the early 1900's. The major landmarks in the history of parathyroid hormone are listed in Table 2.

Parathyroid hormone is a single chain polypeptide with a molecular weight of 9,000; its complete structure has not yet been elucidated, but dilute acid cleavage of the purified hormone produces a fragment of about 35 amino acids, which is both biologically and immunologically active.[57] The hormone represents only .004 percent of the wet weight of the parathyroid glands. The currently used assay unit for PTH is the USP unit, and 100 USP units are the amount of hormone which, when injected subcutaneously into a dog weighing 10 to 12 kg, increase the plasma calcium concentration by 1.0 mg per 100 ml within 16 hours. Pure bovine PTH has a potency of 2500 to 3500 USP units per mg, and 1 ml of commercial parathyroid extract (Eli Lilly), contains 100 USP units. The hormone can be measured by bioassay and radioimmunoassay; the rat bioassay can detect 2 μg of the pure hormone while a sensitive radioimmunoassay can measure as little as 1×10^{-5} μg.[6,57] The availability of radioimmunoassay has allowed: (1) quantitation of the dynamic turnover of PTH in the blood; (2) direct investigation of the factors that control PTH secretion; and (3) the development of a newer concept about the pathogenesis of certain disorders.

All actions of PTH tend to promote an increased movement of Ca++ ion into the extracellular fluid. This is primarily achieved by actions of the hormone to (1) induce bone resorption, (2) enhance renal tubular reabsorption of calcium, and (3) augment intestinal transport of this ion. Another effect of the hormone, that of stimulating urinary phosphate excretion, produces hypophosphatemia and, thereby, secondarily causes plasma Ca++ to rise.

Parathyroid hormone appears to have a dual action on bone. First, it promotes the release of Ca++ and $PO_4^{=}$ from bone mineral into extra cellular fluids within minutes, possibly through

TABLE 2.—*Landmarks in the History of Parathyroid Hormone

1. 1835—Raynaud described the onset of tetany in dogs following removal of the thyroid gland which involved removal of the parathyroids as well.
2. 1850—Owen described the parathyroid glands in the rhinoceros.
3. 1880—Sandström found the glands in several species and correctly identified and located the four glands in man.
4. 1895—Kohn showed that the parathyroid developed in the third and fourth branchial arches.
5. 1900—Vassale and Generali were able to produce tetany by parathyroidectomy in cats and dogs even though the thyroid gland was left intact.
6. 1901—Loeb showed that intravenous injections of oxylate, which removed calcium from the blood by precipitation, resulted in tetany.
7. 1908—MacCallum and Voegtlin showed that parathyroidectomized animals had a low blood calcium.
8. 1911—Greenwald and Gross showed that in experimental parathyroid deficiency, the urinary excretion of phosphorous was greatly diminished and the concentration in blood was greatly increased.
9. 1923-1925—Hanson and Collip each prepared crude, hydrochloric acid, active extracts of the parathyroids which were subsequently used in systematic studies of the physiological effects of the parathyroid hormone in animals and man. This is the commercially available preparation, parathyroid extract, Eli Lilly.
10. 1891—von Recklinghausen described osteitis fibrosa and distinguished it from other demineralizing diseases of the bone.
11. 1926—The first successful identification of hyperparathyroidism with surgical removal of a parathyroid adenoma by Mandl. Another patient, Captain Charles Martell, was studied about the same time in New York and Boston and, after a number of unsuccessful operations, a parathyroid adenoma was removed.
12. 1930—Beginning in the 1930's, Albright and his colleagues pioneered many of the basic investigations of the clinical picture of deficiency and excess of parathyroid hormone.
13. 1942—Patt and Luckhardt demonstrated the role of the blood calcium in the control of the secretion of PTH.
14. 1948—Barnicot and Chang showed that transplantation of the parathyroids into the brains of mice led to direct resorption of the adjacent bone of the skull.
15. 1950—Gaillard obtained similar results with combined explants of parathyroids and bone and tissue culture.
16. 1956—Talmadge and Elliot used the technique of peritoneal lavage in nephrectomized animals showed the parathyroid hormone had a direct action on bone.
17. 1955—Munson showed that pure parathyroid hormone had a direct phosphoturic effect on the kidney.
18. 1959—Aurbach and Rasmussen and their associates prepared highly purified preparations of PTH and established that there was only one hormonal product and it had actions on both bone and kidney.
19. 1963—Berson, Yalow, Aurbach and Potts developed the first successful radioimmunoassay of bovine and human PTH.
20. 1967—Chase and Aurbach demonstrated for the first time that the same enzyme systems (adenylcyclase→cAMP) are affected directly by PTH in bone and kidney and that this represents an important early step in the biochemical mode of action of the hormone.

*This table is constructed from material in the chapter of Potts and Deftos.[6]

stimulation of osteocytic bone resorption (osteolysis), and, second, it produces extensive bone remodeling under the influence of osteoclastic bone resorption. While the latter process must also liberate $Ca++$ and $PO_4^{=}$ ions into the neighboring extracellular fluid, the role of the osteoclastic bone resorption in maintaining plasma $Ca++$ under normal conditions is not clearly defined.[10] However, in clinical or experimental situations in which an abnormally high circulating level of PTH is sustained, excessive osteoclastic bone resorption may well contribute to the degree of hypercalcemia. A number of the observed effects of PTH on the skeleton are listed in Table 3, the net result of these effects must be a shift in the equilibrium toward increased bone resorption with the transfer of $Ca++$ and $PO_4^{=}$ ions into extracellular fluid. These cellular activities must be continuously maintained to counteract the physicochemical forces which tend to drive $Ca++$ and $PO_4^{=}$ ions from extracellular fluid into bone. Therefore, the maintenance of a normal concentration of $Ca++$ and $PO_4^{=}$ in the extracellular fluid requires a given level of PTH to be present in the circulation at all times. Indeed, recent measurements by radioimmunoassay of the concentrations of PTH in

TABLE 3.—*Effects of PTH on the Skeleton*[6]

1. An increase in the number and resorptive activity of osteoclasts.
2. Increased periosteocytic bone resorption (osteocytic osteolysis).
3. In association with 1 and 2
   (a) Enhanced lysosomal activity and hydrolytic enzyme formation in osteoclasts and osteocytes
   (b) Enhanced collagenase activity
   (c) Enhanced organic acid production by osteoclasts and osteocytes
4. Inhibition of the differentiation and activity of osteoblasts leading to a decrease in the rate of collagen or matrix synthesis.

the plasma have shown that this is the case.[6,58,59]

In the kidney, PTH enhances the tubular reabsorption of calcium and therefore decreases its renal clearance. In their earliest studies, Albright and co-workers[60] noted that an increase in serum calcium produced by the administration of parathyroid extract was associated with a minimal increase in urinary calcium. Three decades later, Talmadge and Kraintz[61] observed in rats that parathyroidectomy caused immediate hypercalciuria which persisted until significant hypocalcemia ensued; the administration of parathyroid extract corrected the hypercalciuria. Studies in humans from our laboratory demonstrated that for any given level of serum Ca++ and filtered load of this ion, the renal clearance of calcium is lower in the presence of PTH and higher when the hormone is absent;[62,63] these studies are most consistent with the conclusion that PTH increases the renal tubular reabsorption of calcium. This action of PTH would explain the frequent finding of a normal or only slightly elevated renal clearance of calcium in patients with primary hyperparathyroidism, the high clearance of this ion in other disorders with comparable degrees of hypercalcemia (malignant tumors with osteolytic metastases, Boeck's sarcoid, and vitamin D intoxication),[62,64] and the hypercalciuria observed in hypoparathyroid patients made normocalcemic with vitamin D or calcium supplementation[63,65] (Chart 7). The relationships between calcium excretion and the level of serum calcium in normal, hypoparathyroid and hyperparathroid subjects are illustrated in Chart 8; it is evident that low calcium excretion is present in hypoparathyroidism only when the patient is hypocalcemic.[65]

The diffusable fraction of calcium in the blood is filtered at the glomerulus, and 97 to 99 percent of this filtered calcium is actively reabsorbed along the entire length of the nephron. A major portion (65 to 80 percent) of filtered calcium is reabsorbed in the proximal tubule while only 10 precent is transported by the distal convoluted tubule and the collecting duct.[67] Evidence to date strongly suggests that PTH acts on the distal reabsorption of calcium.[68,69] Since the renal handling of calcium is closely related to that of sodium and magnesium, one should always consider the excretory rates of sodium and magnesium in the evaluation of the renal clearance of calcium.

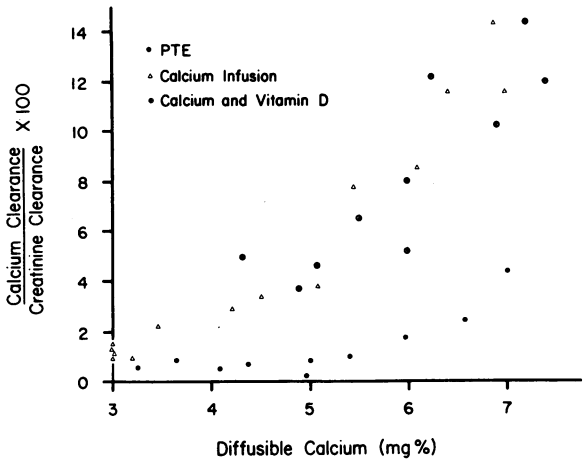


Chart 7.—The effect of calcium infusion, oral calcium and vitamin D, and parathyroid extract on the renal clearance of diffusible calcium at increasing concentrations of plasma diffusible calcium in a patient with hypoparathyroidism. Note that for any given level of plasma calcium the clearance is significantly lower while the patient is receiving parathyroid extract (PTE).[63]

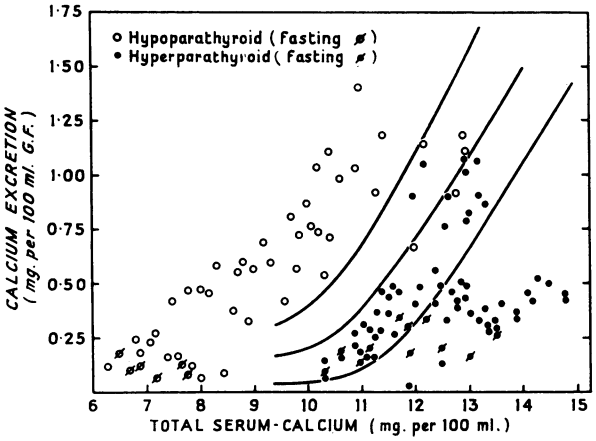


Chart 8.—Serum and urine calcium data in hyperparathyroidism and hypoparathyroidism.[66]

It has also been demonstrated that parathyroid hormone may enhance the renal tubular reabsorption of magnesium in rats,[70] dogs,[71] and man.[72] However, the exact role of PTH in the homeostatic regulation of Mg++ concentration in the plasma is unclear. During rigid dietary restriction or with excessive gastrointestinal losses of magnesium, the plasma level of this ion falls and the urine becomes free of magnesium. Since hypomagnesemia may stimulate the parathyroid glands,[35,36] increased levels of circulating PTH may be responsible, at least in

part, for the renal retention of magnesium under these circumstances.

In 1911, Greenwald and Gross,[73] demonstrated that the urinary excretion of $PO_4^=$ falls and the plasma $PO_4^=$ level increases following parathyroidectomy (Table 2). Conversely, augmented renal excretion of $PO_4^=$ was one of the earliest effects noted following the administration of parathyroid extract.[74] The renal handling of $PO_4^=$ involves filtration at the glomerulus and subsequent active tubular reabsorption: little definitive evidence exists to support the concept of tubular secretion. Under normal conditions, 80 to 90 percent of filtered $PO_4^=$ is reabsorbed; thus, the amount of $PO_4^=$ cleared is 10 to 20 percent of that filtered at the glomerulus. Parathyroid hormone enhances $PO_4^=$ excretion by directly inhibiting tubular reabsorption of this ion. This PTH-induced phosphaturia causes a fall in serum $PO_4^=$ level; and this decrease in plasma $PO_4^=$ concentration alters the dynamic equilibrium between bone and extracellular fluid in a manner promoting the movement of Ca++ and $PO_4^=$ out of bone. An increase in the plasma concentration of $PO_4^=$ will antagonize the calcium mobilizing effect of PTH on bone. Thus, the mechanism whereby PTH enhances the renal clearance of $PO_4^=$ from extracellular fluid may be of importance in permitting PTH to continuously maintain or slightly increase plasma Ca++ concentration. In patients with severe renal failure and overt secondary hyperparathyroidism, $PO_4^=$ clearance cannot be enhanced further when PTH mobilizes Ca++ and $PO_4^=$ from the skeleton; therefore, both plasma $PO_4^=$ and Ca++ concentrations increase.[75] Under these circumstances the correction of secondary hyperparathyroidism by subtotal parathyroidectomy causes both Ca++ and $PO_4^=$ concentrations to fall (Chart 9).

Many factors other than parathyroid hormone affect the renal handling of $PO_4^=$; these factors include: (1) dietary intake of phosphate, (2) plasma $PO_4^=$ concentration, (3) filtered load of $PO_4^=$, (4) Serum Ca++ level, (5) the renal handling of sodium, (6) growth hormone, and (7) adrenal glucocorticoids. An increased quantity of $PO_4^=$ in the diet can augment clearance of this ion in hypoparathyroid, normal, and hyperparathyroid subjects.[19,20,22,75,77] In the latter group, a high phosphorus intake may result in the renal excretion of 50 to 75 percent of the filtered load

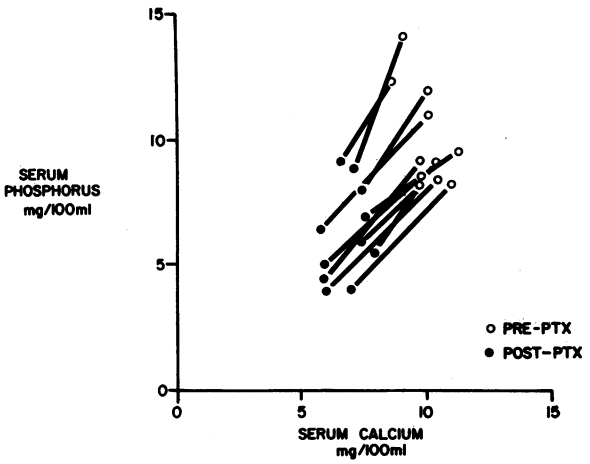


Chart 9.—Changes in total serum calcium and inorganic phosphorus observed in 11 uremic patients before and following subtotal parathyroidectomy (PTX) for severe secondary hyperparathyroidism.[75]

while plasma $PO_4^=$ concentration is unchanged or even decreased.[20,75] Conversely, with rigid dietary restriction of $PO_4^=$ or the excessive use of phosphate-binding antacids, phosphate clearance falls markedly and $PO_4^=$ may actually disappear from the urine.[76,77,78] Although the plasma concentration of $PO_4^=$ may certainly affect the fractional reabsorption of this ion, it is possible that the intracellular content of $PO_4^=$ in the kidney may also affect the renal handling of $PO_4^=$.

The concentration of Ca++ in plasma, *per se*, may also influence the renal handling of $PO_4^=$. Eisenberg[79] demonstrated that the hyperphosphatemia and hypophosphaturia of hypocalcemic hypoparathyroid humans could be completely corrected when normocalcemia was achieved by a prolonged calcium infusion. Furthermore, Schussler and his associates[80,81] noted a correlation between hypercalcemia and hypophosphatemia in patients with breast carcinoma and skeletal metastasis; the low plasma $PO_4^=$ concentration was attributed to a high renal clearance of $PO_4^=$. They concluded that the hypercalcemia, *per se*, may have been the cause of the phosphaturia and subsequent hypophosphatemia.

Several recent studies have indicated that the reabsorption of $PO_4^=$ is closely linked to that of sodium, particularly under conditions of massive saline infusion with expansion of extracellular fluid volume.[82,83] Puschett and coworkers[84] con-

cluded from micropuncture studies that this effect was secondary to the proportional inhibition of the proximal reabsorption of both sodium and phosphate. They found that the infusion of saline solution, parathyroid extract and dibutyl-cyclic AMP each inhibited the tubular reabsorption of $PO_4^=$ to a similar extent; and they concluded that PTH-induced phosphaturia is dependent on both cyclic AMP and sodium reabsorption.

In both normal and hypoparathyroid humans and animals, the administration of growth hormone produces renal effects which are opposite to those of parathyroid hormone. For example, the clearance of $PO_4^=$ falls and that of Ca++ increases. Furthermore, growth hormone can antagonize the renal effect of PTH.[85] The clinical significance of these observations is unclear. However, it is likely that the high $PO_4^=$ concentration of plasma and the low $PO_4^=$ creatinine clearance ratios observed in acromegaly and in pubertal children are due to the increased action of growth hormone. Cortisol and other glucocorticoids are capable of increasing the renal clearance of $PO_4^=$[86]; this action may be due to a direct effect of steroids on the renal tubule. It should be emphasized that although $PO_4^=$ clearance is frequently used clinically to indicate the activity of the parathyroid glands, it is clear from the foregoing discussion that several other factors must be considered before alterations in the rate of $PO_4^=$ excretion can be completely attributed to changes in the blood levels of PTH.

The effects of PTH to enhance the tubular reabsorption of calcium and to inhibit the reabsorption of $PO_4^=$ have been shown to be prompt and are closely related to the levels of PTH in the circulation.[62,87,88] In the gastrointestinal tract, the evidence to date, while not conclusive, strongly suggests that PTH enhances the intestinal absorption of calcium.[6,50] This effect cannot be demonstrated within a short period of time but can only be detected hours or days after the administration of PTH.

The mechanisms whereby PTH exerts its effect on the end-organs have received considerable attention, and evidence indicates that the adenylcyclase 3′5′-adenylmonophosphate (cyclic-AMP) system[6,11,49,89,90,91] may be the mediator of the physiologic action of PTH on bone, kidney and gut. Adenylcyclase, an enzyme located on the plasma membrane, is activated when the hormone is combined with the plasma membrane; the result is accelerated production of cyclic-AMP from ATP. This system may be common to the stimulus-secretion and secretion-action coupling of all endocrine glands. Thus, the acute effect of PTH on bone (Table 3) and kidney are accompanied by the enhanced activity of adenylcyclase and increased intracellular production of cyclic-AMP (Chart 10). In normal persons an increase in the urinary excretion of cyclic-AMP occurs after PTH administration.[92] It is of considerable interest that in one clinical disorder, pseudo-hypoparathyroidism, where the end-organs are refractory to the action of PTH, the administration of the PTH fails to cause the normal rise in the renal excretion of cyclic-AMP (Chart 11).[92]

As yet, there are no definitive data concerning specific metabolic systems which are subsequently stimulated intracellularly by the increased quantities of cyclic-AMP. However, in view of the strong evidence indicating that intracellular concentration of calcium is changed by parathyroid hormone, it may well be that cyclic-AMP in some way regulates the intracellular Ca++ content. The latter would then be the final transducer between the binding of the hormone to its receptor site and final cellular activity (Chart 4). The adenylcyclase hypothesis of PTH action has permitted certain predictions with respect to the type of drugs and agents which may significantly affect calcium metabolism.[90] Thus, an increase in cyclic-AMP content of the bone should accelerate Ca++ mobilization and cause an increase in blood Ca++ concentration, while a decrease should cause a fall in the level of blood Ca++. Phosphodiesterase is the intracellular enzyme which is responsible for the breakdown of cyclic-AMP. Imidazole, which activates phosphodiesterase, causes a pronounced and prolonged fall of plasma Ca++ and $Po_4^=$ as well as inhibiting the hypercalcemic effect of PTH.[93] Other drugs which inhibit adenylcyclase, such as 2-thiophene, carboxylic acid and 5-methyl carboxylic acid, result in effects opposite to PTH and cause pronounced hypocalcemic and hypophosphatemic responses.[94] If it can be shown that these drugs can correct hypercalcemia without causing serious physiologic dysfunction in other organ systems, they will provide a useful addition to our therapeutic armamentarium.

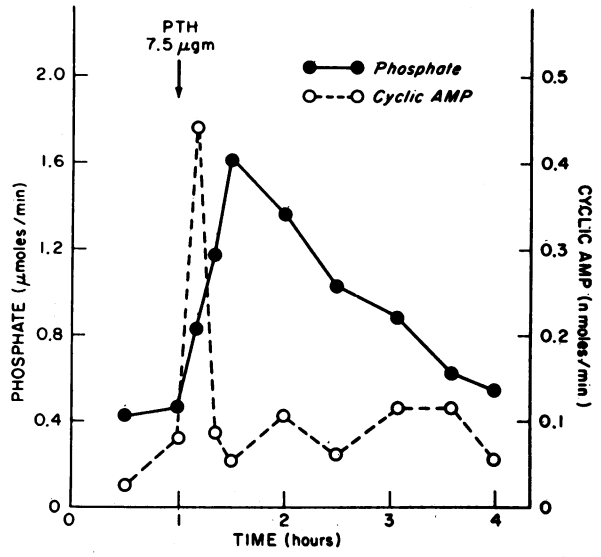
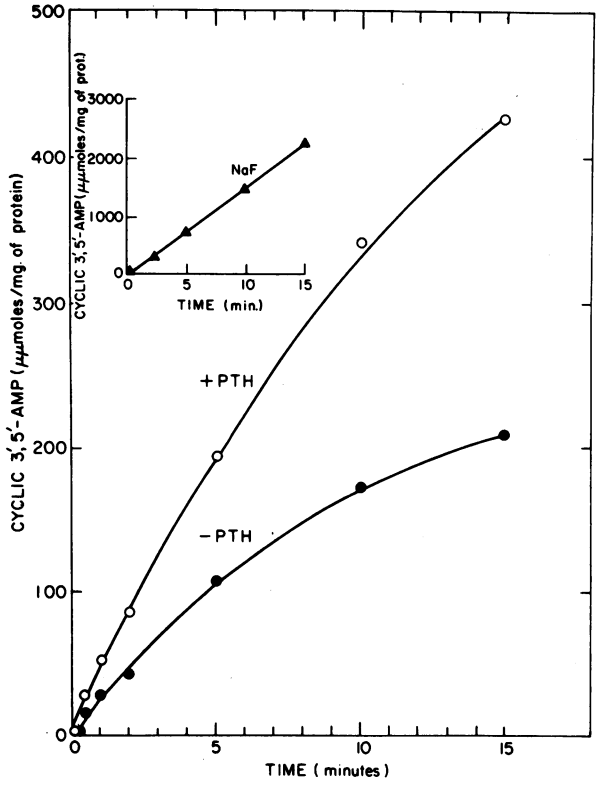


Chart 10.—Chart at top shows effect of an injection of parathyroid hormone (arrow) on the urinary excretion of phosphate and 3′5′ cyclic AMP in a parathyroidectomized rat. (From Chase, L. R. and Aurbach, G. D., Proc. Natl. Acad. Sci. (Wash.) 58: 518-525, 1967.)

The lower chart shows effect of parathyroid hormone on adenyl cyclase activity and production of 3′5′ cyclic AMP in suspensions of bone cells prepared from rat calvaria *in vitro*. A stimulation by parathyroid hormone is evident within one minute. The insert depicts the maximal stimulation of adenyl cyclase system induced by sodium fluoride. (Courtesy of Drs. L. Chase and G. D. Aurbach).[6]



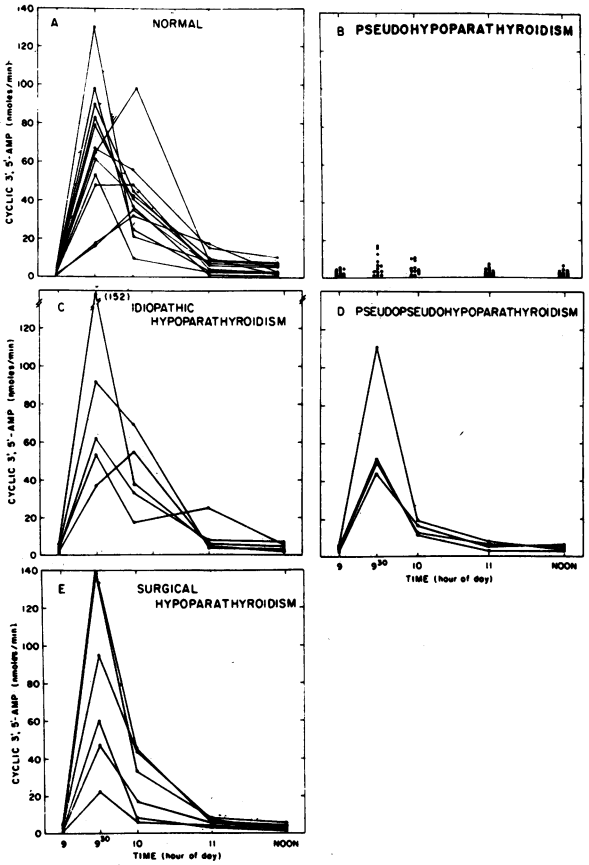


Chart 11.—Effect of parathyroid hormone on the urinary excretion of cyclic 3′,5′-AMP. 300 U of parathyroid hormone were infused from 9:00 to 9:15 a.m. and urine collected at intervals of 30 minutes to one hour until noon. Results represent the rate of excretion of cyclic 3′,5′-AMP for each interval and are plotted to coincide with the end of the period. Each continuous line represents the pattern of excretion for one subject. Individual patterns of excretion are not shown in B, where each point represents the result for one subject.[92]

Ever since *in vivo* perfusion of the parathyroid glands was performed by Patt and Luckhardt,[95] evidence has progressively accumulated to indicate the fundamental role of Ca++ in the control of the secretion of PTH. Chart 12 presents the results of a study with an *in vivo* perfusion of the parathyroid glands carried out in our laboratory. Perfusion of the intact parathyroid glands of dogs with hypocalcemic blood (6 to 7 mg per 100 ml) caused an elevation of Ca++ concentration in peripheral blood in less than an hour and almost immediate rise in renal clearance of $PO_4^=$; perfusion of these glands with hypercalcemic blood (12 to 14 mg per 100 ml) produced a fall in Ca++ concentration in the

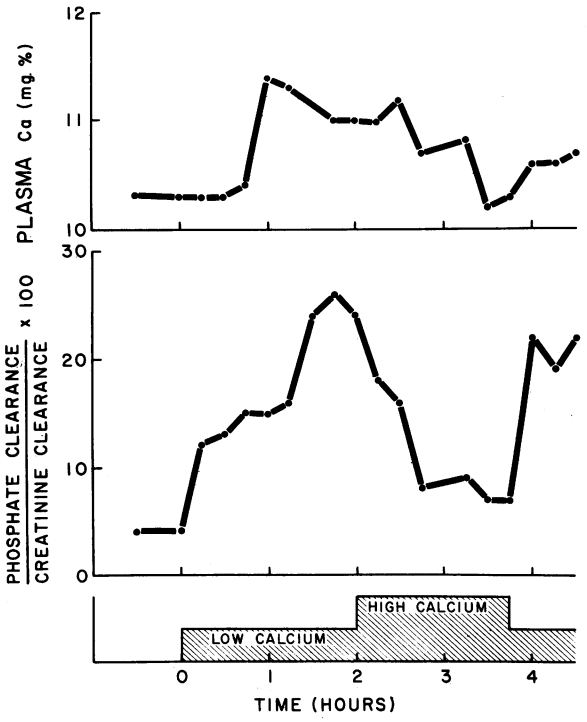


Chart 12.—Response of plasma calcium and phosphate clearance to bilateral thyroparathyroid block perfusion with hypo- and hypercalcemic blood.

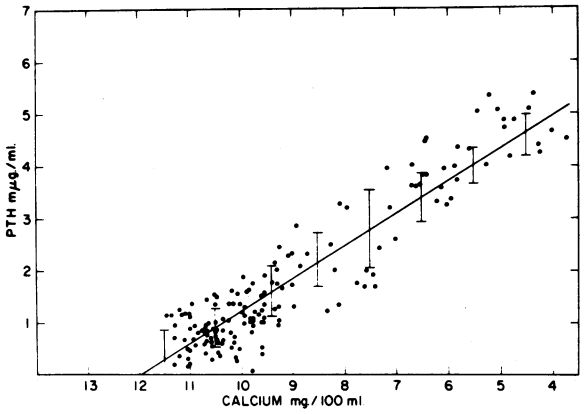


Chart 13.—Relation between concentrations of blood calcium (treated as independent variable) and parathyroid hormone (dependent variable). Linear relation is derived from treating data as a simple regression by the least-squares method. Vertical lines and horizontal bars give standard deviation of observed from predicted hormone concentrations over each interval of the linear function.[6]

systemic blood and a decrease in $PO_4^=$ clearance. Parson and Robinson[96,97] demonstrated that when the isolated tibia of a cat is perfused with blood containing PTH, an increase in the venous Ca++ concentration of blood leaving the tibia occurs within 10 to 15 minutes. These studies illustrate the rapid biologic effect of the hormone on the skeleton.

The development of a sensitive radioimmunoassay for PTH has permitted systematic studies of factors regulating the secretion of PTH in normal and diseased states. Hypercalcemia of 12 mg per 100 ml, produced by the intravenous infusion of calcium salts, causes a fall of plasma PTH to undetectable levels. Conversely, the production of hypocalcemia by the infusion of EDTA causes a 5-to 10-fold rise in the hormone level in the blood.[59] Indeed, available studies indicate that the relationship between the levels of circulating PTH and the concentration of Ca++ in blood is inverse and linear (Chart 13).[6,57,58,59,98]

Evidence also exists indicating that the concentration of Mg++ in plasma is also important in the regulation of PTH secretion. Hypomagnesemia or magnesium depletion may stimulate the parathyroid glands, and hypermagnesemia may inhibit their activity.[35,99,100,101] In a recent study, Massry et al[99] summarized the available data regarding the effect of Mg++ on PTH secretion and demonstrated that an increase in plasma Mg++ concentration of 1.7 to 2.0 mg per 100 ml was adequate to suppress the activity of the parathyroid glands. When these investigators evaluated the simultaneous effects of modest hypocalcemia and hypermagnesemia, they found that a decrease in the level of plasma Ca++ is more potent than an increase in plasma Mg++ concentration in the regulation of parathyroid activity. Sherwood et al[100] evaluated the effect of changes in concentrations of Ca++ and Mg++ on the in vitro rate of synthesis and secretion of PTH of bovine parathyroid glands incubated in organ culture. They observed parallel changes in synthesis and secretion of the hormone in response to variation in the concentration of these cations in the incubating media. They concluded that Ca++ and Mg++ are equipotent in blocking hormone release, and that the rate of secretion depends only on the sum of the two ions. Recently, Massry et al[102] presented evidence suggesting that the infusion of strontium chloride to dogs also suppresses the activity of the parathyroid glands. These studies indicate that the activity of these glands may be in-

fluenced by a divalent cation which is not normally present in the body.

The reported levels of circulating PTH from subjects with normal Ca++ concentration in the blood have varied from one laboratory to another. Buckle et al[103] found levels of 0.1 to 0.2 mμg per ml[103] while Potts and Deftos reported values of 0.5 to 1.0 mμg per ml.[6] These values are equivalent to 0.3 to 3.0 USP m units per ml. In the steady state, the amount of hormone destroyed or secreted is equal to that secreted, and it is generally agreed that changes in hormone concentration in the blood accurately reflect changes in its secretion rate.[104]

The apparent volume of distribution of the PTH is about 20 percent of body water, and its biologic half-life is about 30 minutes.[104] Using the normal levels of the hormone reported by Potts and Deftos,[6] a 70 kg man will secrete approximately 240 mμg per min or 0.5 mg per 24 hours under normal conditions; and the amount of the hormone will increase during acute stimulation of the glands to 1750 mμg per min or 2.5 mg per 24 hours; this is an increase from 1500 to 7500 USP units a day. If one uses the values reported by Buckle et al,[103] the calculated normal secretion rate of the hormone would be equivalent to 300 to 600 USP units a day. That the latter value may be more correct is suggested from our observations that intramuscular injection of 200 USP units 4 to 5 times a day in a normal adult will cause mild hypercalcemia; therefore, 800 to 1000 USP units a day represents a "hyperparathyroid" rate of secretion.

The parathyroid glands do not store large quantities of PTH, and the hormone content of the glands is only .004 percent of their wet weight. The rate of hormone secretion, therefore, depends on the rate of hormone synthesis. As it is most likely that each cell has a maximal capacity to synthesize new hormone, parathyroid hyperplasia may begin when the increased demand for PTH exceeds the secretory or synthetic capacity of the normal number of cells.[105] This is probably the process underlying all forms of secondary hyperplasia—for example, chronic low calcium-high phosphate diet, vitamin D deficient and resistant states, chronic renal failure, and steatorrhea.

Potts and associates called attention to the high degree of parathyroid adaptation which is observed in the chronic hypocalcemic syndrome of cows.[106] An unexplained resistance to the action of PTH develops in the late states of pregnancy, and, when these animals calve and then lactate, severe hypocalcemia develops. For any given decrease in blood Ca++ level in these animals, the amount of PTH secreted in the adapted state is several fold greater than that occurring in the normal state; however, hypercalcemia of 12 mg per 100 ml completely suppressed the secretion of PTH both in the normal and adapted animals. (Chart 14).

Patients with chronic renal failure provide a clinical situation in which the parathyroid glands are decidedly hyperplastic, and the circulating levels of PTH may be 20-fold more than normal.[6,107,108] In these patients, elevated levels of PTH in blood have been found even when serum calcium was elevated to normal or moderately hypercalcemic levels.[75,108] This apparent nonsuppressibility of the parathyroid glands may be explained by the considerable increase in the number of the secreting cells and is probably not due to secretory autonomy of the parathyroid glands.[75,105,107-109] Indeed, the cells of the parathyroid glands of a uremic patient may behave much as they do in normal persons. An elevation of Ca++ concentration in blood to greater than normal levels might cause a similar degree of suppression of PTH secretion in each cell in both uremic and normal subjects. However, the enormous number of cells in the hyperplastic glands of the uremic patient may provide for the release of large absolute quantities of hormone for any given level of hypercalcemia which is below the concentration necessary to inhibit all hormone secretion from the hyperplastic glands (Chart 15).[75] It is also possible that in some of these patients each cell may produce a greater quantity of hormone under the influence of an equal stimulus. With the same degree of suppression as occurs in normal cells following hypercalcemia, the effect of a large gland mass on total hormone production would be accentuated (Chart 15).[75]

Calcitonin (Thyrocalcitonin). A fascinating page in the story of recent research in calcium metabolism has been the discovery of calcitonin. In the ten years which followed the discovery

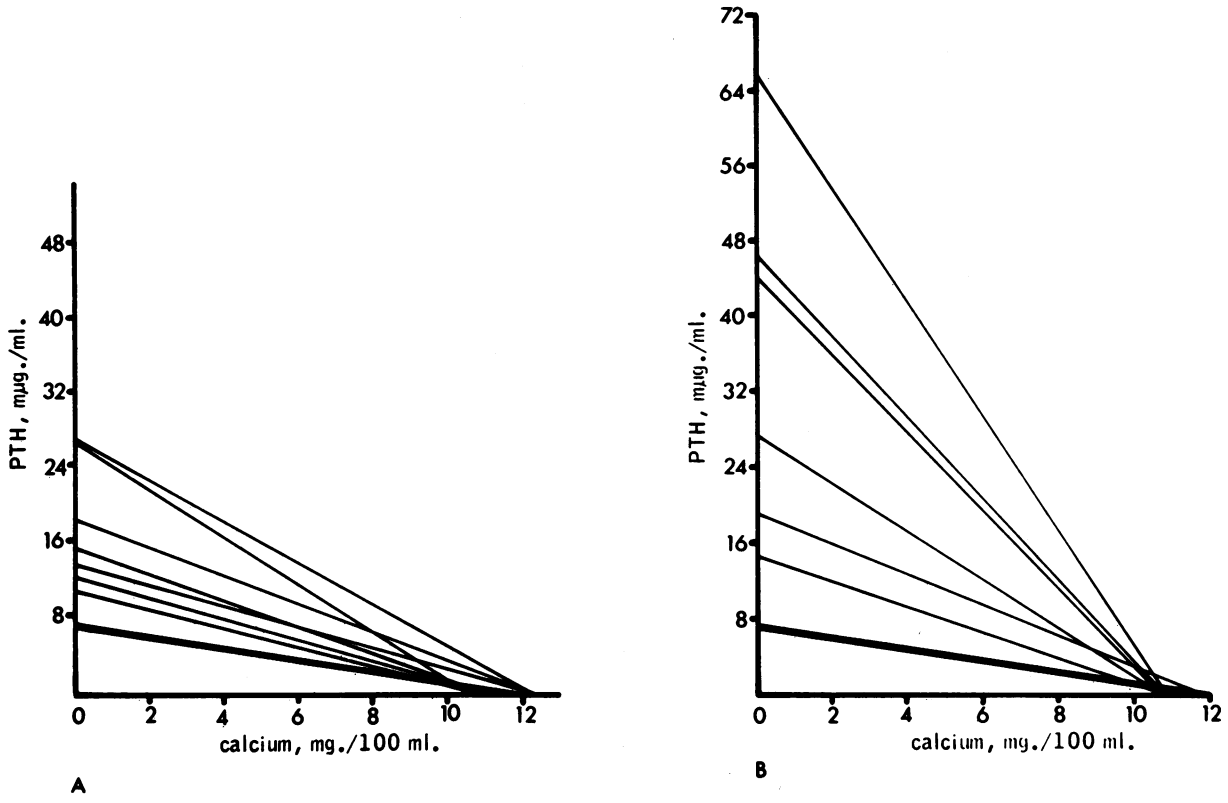


Chart 14.—Comparison of the relationship between blood calcium (independent variable) and blood parathyroid concentration (dependent variable) in a series of parturient cows with secondary hypoparathyroidism. A linear, inversely proportional relationship is evident in animals with secondary hyperparathyroidism as is seen in normal animals (Chart 13). Unlike the findings in normal animals, where pooled data from all animals could be treated simultaneously, it was evident that the response in secondary hyperparathyroidism is unique to each animal. The slope of the line relating hormone secretion as a function of blood calcium is steeper in all animals with secondary hyperparathyroidism than in normal animals (heavy line-data taken from normal animals, Chart 13, and replotted for comparison). The persistence of control by blood calcium of parathyroid hormone secretion, despite excessive rates of hormone production, in animals with parathyroid hyperplasia is evident from the fact that hormone secretion falls to zero in all animals at approximately 12 milligrams per 100 ml in blood calcium. In group B, those with more severe secondary hyperparathyroidism, the slope of the response line is two to eight times as great as the slope of the response line in normal animals.[106]

of this hormonal activity, the identification and isolation, the structural analysis, the synthesis and the characterization of the physiological mode of action of this hormone was achieved.[6] Because the discovery of calcitonin has been so recent, the events leading to and since its discovery will be described in some detail.

Before 1960, it was generally accepted that the precise regulation of plasma Ca++ concentration was dependent on the negative feedback relationship between blood Ca++ levels and the rate of secretion of PTH.[110] In 1960, Sanderson and co-workers[5] observed in thyroparathyroidectomized dogs that there was a delay in the correction of hypercalcemia after an intravenous calcium load (Chart 2). The significance of

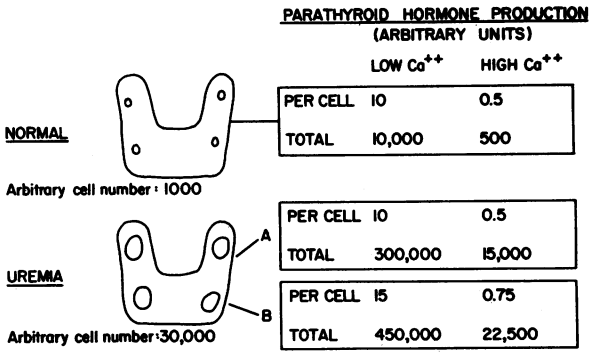


Chart 15.—Theoretical effect of the large mass of the gland in uremia on hormone production. In A, the cell response to calcium level is entirely normal; in B, hormone production per cell is increased but the percentage suppression by elevated Ca++ is normal. In both instances, total hormone production is high.[75]

these observations was not recognized at the time, but in 1961, Copp and associates[111,112] found that perfusion of the isolated thyroid-parathyroid glands with hypercalcemic blood caused more rapid development of systemic hypocalcemia than occurred following thyroparathyroidectomy (Chart 16). They also found that when the perfusate from the thyroparathyroid glands was given to another animal, hypocalcemia developed. From these results, they postulated the existence of a second calcium regulating hormone, which they termed, calcitonin. The thyroid glands and parathyroid glands could not be perfused separately in their experiments in dogs, but two years later the thyroid and parathyroid glands were perfused separately in goats[113] and the results indicated that the thyroid gland rather than the parathyroids was the source of the hypocalcemic substance. In 1963, Hirsch, Gauthier and Munson[114] noted that parathyroidectomy produced by electro-cautery, which injures the underlying thyroid gland as well, resulted in more rapid and marked hypocalcemia than that which was caused by simple, careful surgical parathyroidectomy or even total thyroparathyroidectomy (Chart 17). They suggested that the injured thyroids had released a calcium-lowering substance, which they were subsequently able to prepare from extracts of rat and pig thyroid tissue. These extracts caused both hypocalcemia and hypophosphatemia within an hour after injection into rats,[115] and they termed it *thyrocalcitonin* to indicate the gland of origin. Subsequent studies in rats, dogs, goats, guinea pigs, cows, monkeys and humans have confirmed that the thyroid was the major source of the hypocalcemic principle.[6,116] Utilizing histological and immunofluorescent techniques, Pearse and associates concluded that the production of calcitonin was confined to scattered parafollicular or "C" cells, (Figure 6) which proved to be of ultimo-branchial origin.[117-119] As is summarized in Chart 18, these cells originate phylogenetically along with the parathyroid glands, the thymus, and aortic and carotid bodies from the branchial pouches. In all lower vertebrates other than mammals, these ultimo-branchial glands exist as distinct bodies. The parathyroid glands, which develop later phylogenetically, are present in all air-breathing animals. Their development may be related to the shift away from the marine environment, with its low phosphorus and high calcium levels, to a terrestial state, where there is exposure to lower calcium and a higher phosphate.[116] Copp and associates[116] were able to find large quantities of calcitonin in the ultimo-branchial glands of chickens, dogs and fish in-

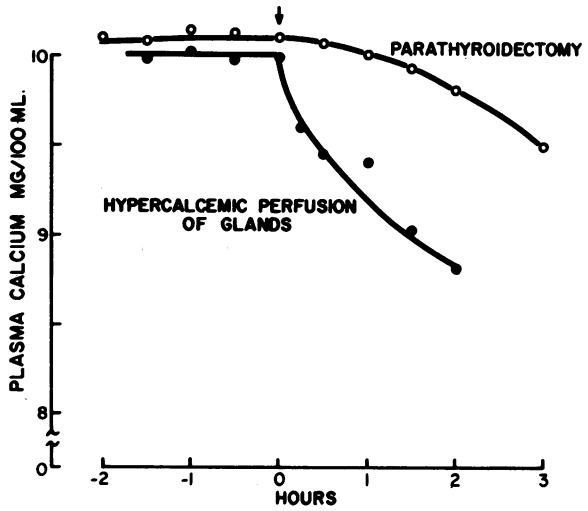


Chart 16.—Comparison of the rate of fall in systemic blood calcium in dogs induced by thyroparathyroidectomy versus regional perfusion of thyroparathyroid tissue with hypercalcemic blood. (From Munson, et al: Recent Progr. Hormone Res., 1968, 24:589-650.)

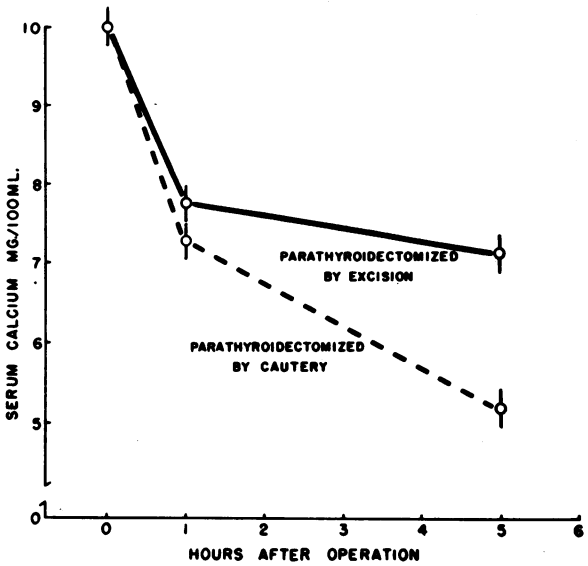


Chart 17.—Comparison of the rate of fall in blood calcium in rats subjected to parathyroidectomy by excision versus parathyroidectomy by cautery. (The latter damages the thyroid and releases calcitonin.) (From Munson, et al: Recent. Progr. Hormone Res., 1968, 24:589-650.)

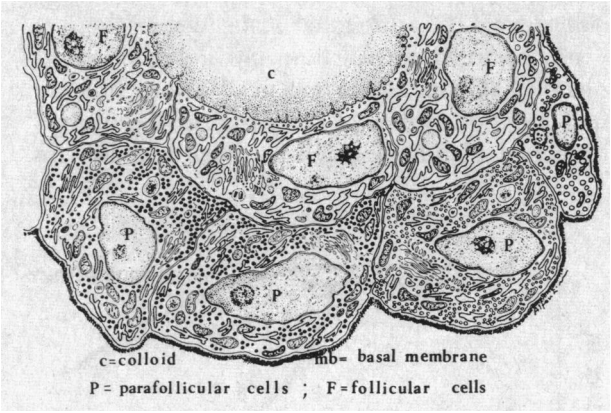


Figure 6.—Schematic representation of the parafollicular cells of the mammalian thyroid gland. (From Azzali, C.: In *Calcitonin*, S. Taylor, Ed. London, Heinemann Medical Publishing Company, 1968, pp. 152-166.)[6]

cluding sharks. Finally, calcitonin has been also clearly demonstrated to be present in the parathyroid glands and thymus of humans.[120] This would explain why total thyroidectomy may not remove all cells capable of producing calcitonin.[121,122]

The complete amino-acid sequences of porcine, bovine, human, and salmon calcitonin have now been determined, and porcine calcitonin has been synthesized.[116,122] It is composed of 32 amino acids with a molecular weight of 3570 to 3590. Although the basic structure of calcitonin is similar in various species, there are a number of differences in individual amino acids in the center of the molecule (Chart 19); the entire molecule seems to be necessary for biological activity. Of some interest, because of its greater biological activity in man, is salmon calcitonin.[116,122,123] Its biological activity is 20 to 200 times greater than that of either porcine or human calcitonin, and it has a longer duration of action (Chart 20).

The biological activity of calcitonin is determined by measuring the decrease in serum calcium level in a young rat given the hormone-containing test sample intravenously. The international bioassay reference standard, provided by the British Medical Research Council, is known as the MRC unit and is equivalent to 5 μg of purified porcine calcitonin. The most sensitive bioassay is capable of detecting as little as 1 mμg or 0.2 MRC milliunits of calcitonin[124] while a sensitive immunoassay can detect as little as 1 X 10^{-5} μg or .002 MRC milliunits of calcitonin.[125]

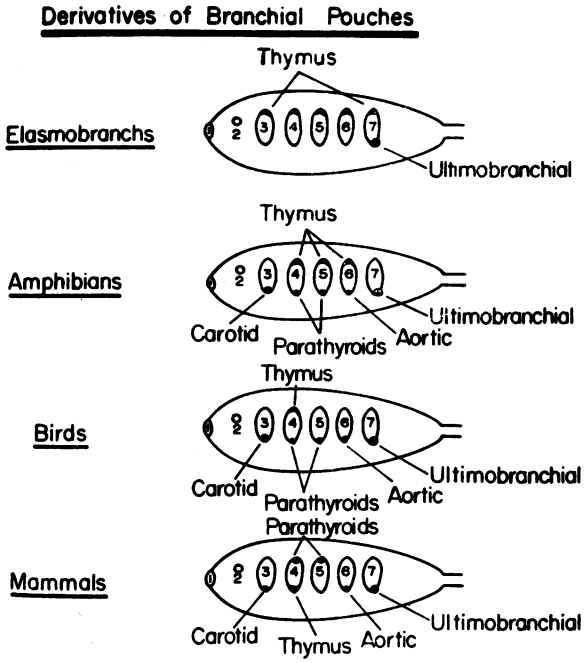


Chart 18.—Embryological development of glandular derivatives of the branchial pouches in various classes of vertebrates. Adapted from Figure 11.26, p 291, in: H. Smith, *Evolution of Chordate Structure*, Holt, Rinehart & Winston, New York, 1960.[116]

The most notable effect of calcitonin is to rapidly reduce the plasma concentrations of Ca++ and $PO_4^{=}$. This hypocalcemic action of calcitonin is most profound in young or growing animals and also in other conditions when bone remodeling is active. The effect decreases with age in all species, and the injection of calcitonin has almost no effect on plasma Ca++ concentration in normal adult subjects. The hypocalcemic action of calcitonin has been shown to occur in animals without parathyroid glands, without the intestinal tract or liver and without the kidneys; hence its action does not depend on the presence of these organs. Furthermore, the action of calcitonin is associated with no change in the soft tissue content of Ca++ or $PO_4^{=}$. The overwhelming evidence to date indicates that the most important action of calcitonin is to inhibit bone resorption.[116,122-124] This effect is significantly enhanced by a high $PO_4^{=}$ diet and hyperphosphatemia (Chart 21). Conversely $PO_4^{=}$ depletion and hypophosphatemia may decrease or prevent the hypocalcemic effect of calcitonin.[25,31] This effect of $PO_4^{=}$ may well have been predicted from our earlier discussion of the role of $PO_4^{=}$ on bone accretion and resorption. The action of calcitonin

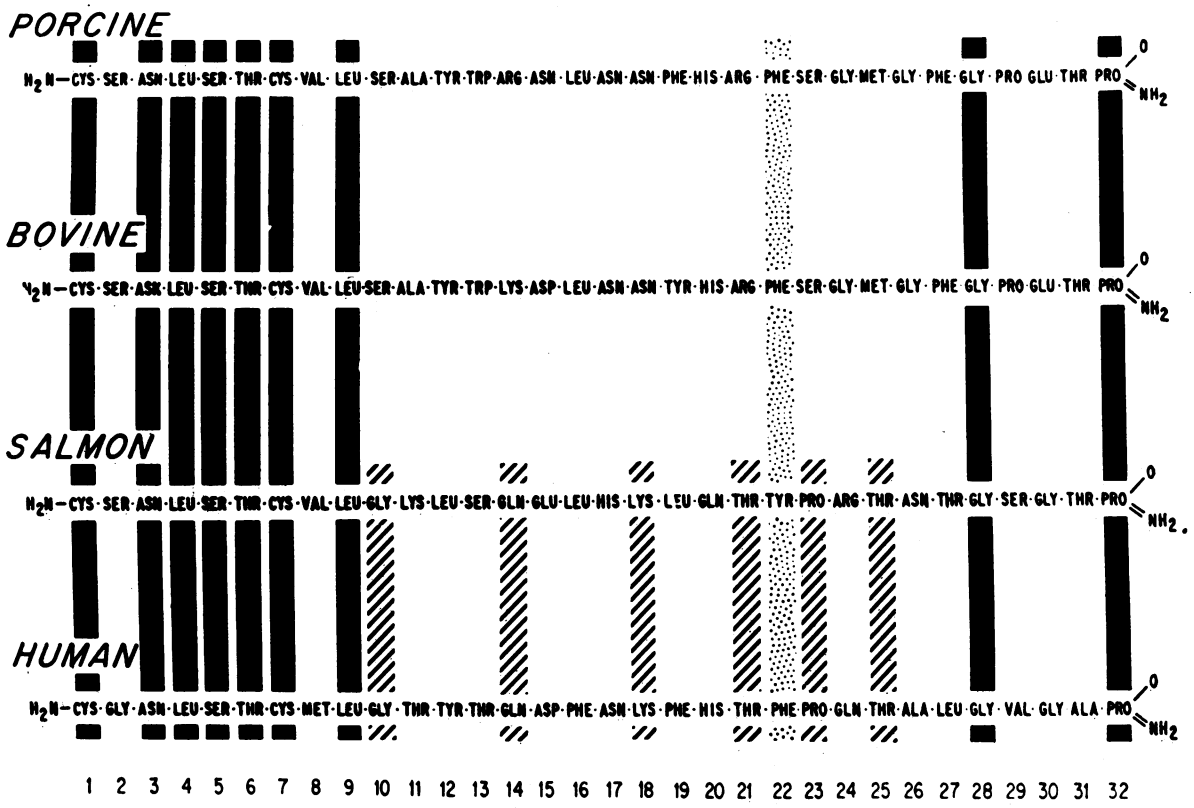


Chart 19.—Comparison of amino acid sequence of porcine, bovine, human and salmon calcitonins. Solid bars indicate sequence positions homologous among all four molecules. Cross-hatched bars indicate the additional positions of homology between salmon and human hormones; stippled bar indicates position where either phenylalanine or tryosine is found in each of the calcitonins. (Amino acid 27 in human calcitonin is isoleucine, and not leucine as shown in this figure.)[123]

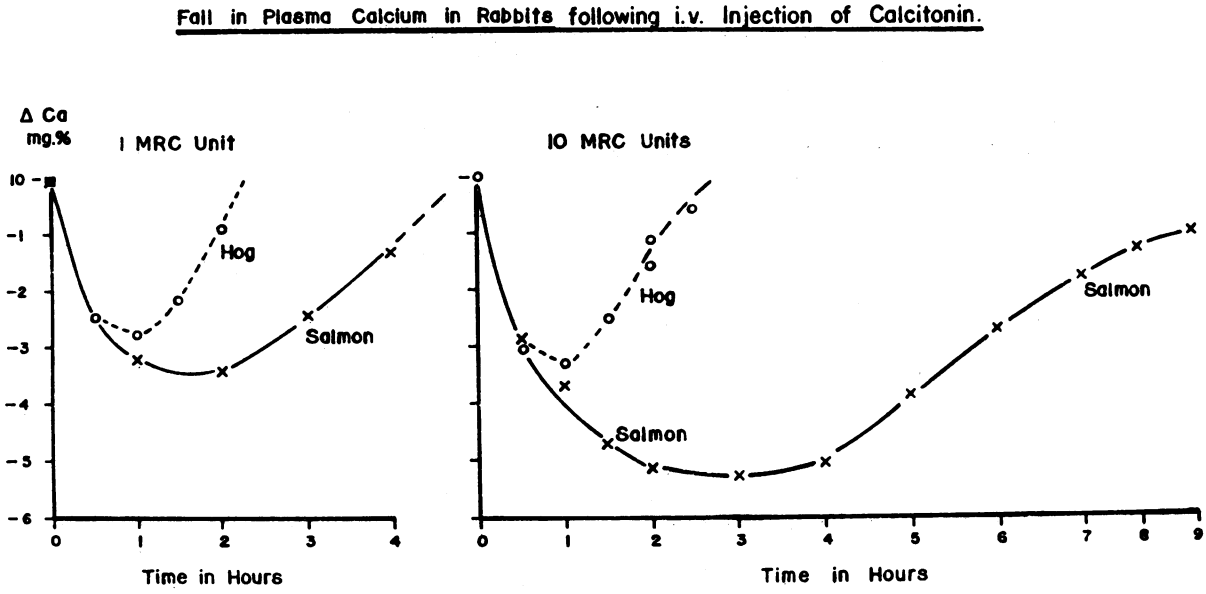


Chart 20.—Comparison of the response in young rabbits to equivalent doses (in MRC units) of salmon and porcine calcitonin.[116]

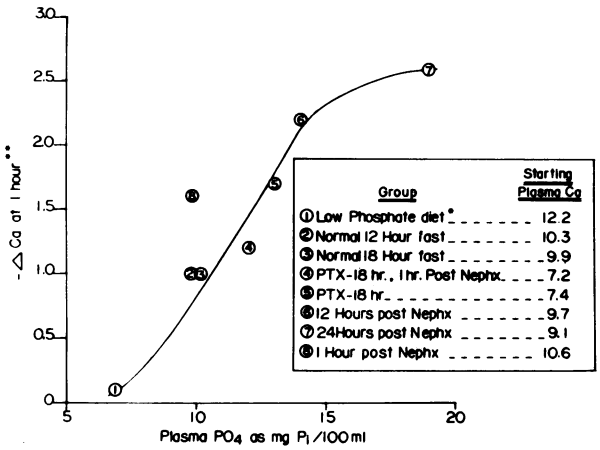


Chart 21.—Relationship of the effect of thyrocalcitonin to the starting plasma phosphate levels. (a) PTX=parathyroidectomized; (b) Nephx=nephrectomized; (c) *low phosphate diet from Nutritional Biochemical Corporation, Cleveland, Ohio, (d) **plasma changes in mg Ca/100 ml.[31]

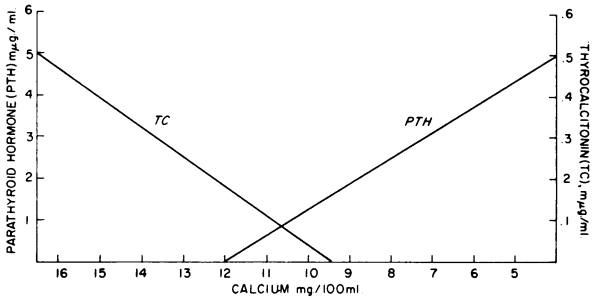


Chart 22.—Effects of changes in serum calcium on the concentration of parathyroid hormone and thyrocalcitonin in peripheral blood. The concentration of thyrocalcitonin is directly proportional to calcium concentration; the concentration of parathyroid hormone is inversely proportional to calcium concentration. (This model is formulated from data obtained from several different species.)[6]

on bone is associated with inhibition of both the number and activity of osteoclasts,[126] and, in addition, the osteocytic-osteolysis produced by injection of PTH is inhibited.[128] Thus, the administration of calcitonin acutely reduces the calcium mobilizing effect of PTH in numerous *in vivo* and *in vitro* experiments.[6,116,122,124,127] Following the long-term administration of calcitonin to rats over several weeks, Foster and associates[126] found a decrease in the osteoclast count and a remarkable increase in cortical and trabecular bone. Also, calcitonin was effective in preventing the stunting of growth and osteoporosis which are produced by toxic doses of vitamin A in rats.[129]

The biochemical mechanisms by which calcitonin affects bone resorption remain unknown. Its action does not require vitamin D,[130] and there is strong evidence against its having an effect on either protein synthesis or the adenylcylase system. Borle and co-workers[33] found that purified porcine calcitonin inhibits the active efflux of Ca^{45} from kidney cells in tissue culture, and Raisz[11,34] found a similar effect in tissue culture of resorbing bone cells (Chart 4).

There are important differences between the synthesis, storage, secretion and metabolism of calcitonin in contrast to that of PTH. Unlike the cells of the parathyroid glands, the parafollicular cells appear to be capable of storing large amounts of calcitonin. These stores are sufficient to support secretion for many hours without necessitating new hormone synthesis, and to permit several fold increases in secretion rates during a hypercalcemic stimulus.[6,122] Thus, the parafollicular cells are hyperplastic and filled with secretory granules in chronically hypocalcemic, parathyroidectomized rats,[131,132] while the calcitonin content of C cells decreases and remains at reduced levels after prolonged periods of hypercalcemia. In studies ultilizing both bioassay and radioimmunoassay,[125,133-136] calcitonin has been detected in the plasma of the rabbit[125,134] and man [133,135,136] when plasma calcium levels are normal. These observations indicate that calcitonin must be continuously secreted in the absence of a hypercalcemic stimulus. Utilizing *in vivo* physiologic studies of Ca++ released from the skeleton of rat, Klein and Talmage[137] reached similar conclusions that calcitonin is secreted during both normal and mild hypocalcemic states. The level of calcitonin in normal human plasma has been found to range from 150 to 350 milliunits per liter or 0.75 to 1.75 μg per liter.[125,134-136,138] Following a calcium infusion, the blood level of calcitonin may rise as much as five times, depending upon the magnitude of the hypercalcemia.[133-138] It seems clear that the rate of secretion of this hormone is under direct proportional control of the blood Ca++ concentration (Chart 22) while, as previously noted, PTH is under inverse proportional control. This implies that the regulation of Ca++ concentration is under dual hormonal control. The continued secretion of both hormones at a normal concentration of blood Ca++, the rapid increase in calcitonin secretion with hypercalcemia, and the rapid in-

crease in PTH secretion with hypocalcemia all assure extremely precise modulation of blood Ca++ concentration through the regulation of bone resorption, which controls the supply of calcium liberated from bone into extracellular fluid.[116,122] It is of interest that the two regression lines for the relationship between plasma Ca++ concentration and the levels of these two hormones intersect near the normal plasma Ca++ level[116,122] (Chart 22). Riggs and associates,[139] in studying the plasma kenetics of porcine calcitonin in man with radioimmunoassay, reported a metabolic clearance rate of 823 ± 47 ml per minute. This rate of disappearance is extremely high—3 to 50 times more rapid than that of other polypeptide hormones. If one assumes that the normal mean plasma level of calcitonin is that noted above, this would indicate a turnover or secretion rate of approximately 800 mμg or 160 MRC μU per minute.[139] Further studies regarding the turnover rate of this hormone are necessary to clarify this matter.

Although calcitonin may be of major physiologic importance in preventing hypercalcemia in marine vertebrates, which are exposed to high concentrations of calcium of sea water, and to certain terrestrial vertebrates, such as fowl and herbivores, which ingest high calicum diets, its homeostatic role in dog and man remains unclear.[120,140-142] Although total thyroidectomy in these species does not cause a significant abnormality in the regulation of plasma Ca++ concentration, this procedure may not remove all calcitonin secreting tissue[120,142]; indeed normal basal blood levels of calcitonin were found in three totally thyroidectomized humans.[142] Sherwood[143] called attention to the fact that endogenous calcitonin is ineffective in controlling the hypercalcemia of hyperparathyroidism. Since all experimental and clinical studies indicate that calcitonin is progressively ineffective with increasing age and since primary hyperparathyroidism usually does not occur before the age of 20 or 25 years, it is conceivable that calcitonin might prevent the manifestations of hypercalcemic-hyperparathyroidism during childhood or adolescence.

(End of Part I. Part II will appear in the next issue of CALIFORNIA MEDICINE.*)*

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