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7-1-1985

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Vitamin D-Endocrine System

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Introduction

There is growing evidence that the vitamin D-endocrine system is important not only in the regulation of bone and mineral metabolism but in modulation of other systems as well. As will become evident, this is particularly true with regard to 1,25-dihydroxyvitamin D [1,25(OH)$_2$D][1].

Metabolism of vitamin D

The metabolism of vitamin D is outlined schematically in Fig. 1. Dietary vitamin D$_2$ (irradiated ergosterol) is absorbed in the proximal small intestine via the intestinal lymphatics; this process requires bile acids (1). Vitamin D$_3$ is produced in the skin from 7-dehydrocholesterol. Previtamin D$_3$ is synthesized photochemically from 7-dehydrocholesterol and over a period of several days undergoes a temperature-dependent isomerization to vitamin D$_3$ (2). The vitamin is selectively removed by way of the dermal capillaries by vitamin D-binding protein, which has a high affinity for the vitamin. Because vitamin D-binding protein has a low affinity for previtamin D$_3$, this metabolite remains in the dermis for eventual conversion to vitamin D$_3$.

Vitamin D is hydroxylated in the liver by the enzyme vitamin D-25-hydroxylase to form 25-hydroxyvitamin D (25-OHD) (3), and 25-OHD is further hydroxylated in the kidney either by 25-hydroxyvitamin D-1a-hydroxylase to form 1,25(OH)$_2$D (4), or by 25-hydroxyvitamin D-24-hydroxylase to form 24,25-dihydroxyvitamin D [24,25(OH)$_2$D] (5). Both 1,25(OH)$_2$D and 24,25(OH)$_2$D may undergo an additional hydroxylation to form 1,24,25-trihydroxyvitamin D (6). Upon oxidative cleavage of the side chain, 1,25(OH)$_2$D forms a C-23 carboxylic acid, calcitroic acid (7), which is biologically inactive (8). Production of calcitroic acid in target tissues may be an important mechanism for inactivation of the biologically potent 1,25(OH)$_2$D. In addition, 1,25(OH)$_2$D undergoes an enterohepatic circulation, as shown in rat (9) and humans (10).

Regulation of vitamin D metabolism

There is evidence in animal studies that 25-hydroxylation of vitamin D is inhibited by vitamin D (11), 25-OHD (12), and 1,25(OH)$_2$D (12), and in clinical studies that inhibition

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Received for publication 7 March 1985.

1. *Abbreviations used in this paper:* 1,25(OH)$_2$D, 1,25-dihydroxyvitamin D; 24,25(OH)$_2$D, 24,25-dihydroxyvitamin D; 25-OHD, 25-hydroxyvitamin D; cAMP, cyclic AMP; PTH, parathyroid hormone.

is produced by vitamin D (13) and 1,25(OH)$_2$D$_3$ (14). Whether 25-OHD plays a physiologic role in regulating its own synthesis has not been established. Inhibition of 25-hydroxylation by vitamin D seems to be a relatively ineffective control mechanism in view of the fact that marked increases in circulating 25-OHD are responsible for the abnormal mineral metabolism in vitamin D intoxication (15). In contrast, feedback regulation of 25-OHD production by 1,25(OH)$_2$D appears to be effective (Fig. 1). In small but pharmacologic doses, 1,25(OH)$_2$D$_3$ completely inhibits the increase in serum 25-OHD produced by vitamin D challenge in normal subjects (14), and serum 25-OHD may be reduced in a number of diseases in which serum 1,25(OH)$_2$D is increased including obesity (16), vitamin D-dependent rickets type II (17), sarcoidosis (18), hyperphosphatemic tumoral calcinosis (19), and tuberculosis (Bell, N. H., unpublished observations). Further, the renal production of 1,25(OH)$_2$D in normal children is regulated loosely (20). As a result, vitamin D challenge produces modest increments in serum 1,25(OH)$_2$D that prevent more substantial increases in serum 25-OHD. Feedback regulation of hepatic production of the precursor 25-OHD by 1,25(OH)$_2$D, therefore, serves to prevent abnormal increases in circulating 1,25(OH)$_2$D in normal children in whom 1,25(OH)$_2$D production is regulated loosely. The mechanism by which the hepatic production of 25-OHD is modulated by 1,25(OH)$_2$ is unknown. It does not seem to be a receptor-mediated event since receptors for 1,25(OH)$_2$D have not been shown in hepatic tissue (21).

Parathyroid hormone (PTH) is the principal regulator of the renal synthesis of 1,25(OH)$_2$D. Serum 1,25(OH)$_2$D is increased in primary hyperparathyroidism and is reduced in hypoparathyroidism (22, 23). Circulating 1,25(OH)$_2$D is increased by administration of parathyroid extract in normal subjects and in patients with hypoparathyroidism (23). The primacy of PTH in the regulation of synthesis of 1,25(OH)$_2$D is also well documented by both in vivo and in vitro studies using a wide variety of laboratory animals (21, 24–26).

In intact animals (27) and in normal adults (18, 20), the renal production of 1,25(OH)$_2$D is tightly regulated, and serum 1,25(OH)$_2$D changes very little in response to vitamin D challenge. This occurs because of feedback regulation of circulating PTH by the serum ionized calcium. Thus, increases in serum calcium produced by increases in circulating 1,25(OH)$_2$D inhibit PTH secretion and the renal production of 1,25(OH)$_2$D. In children, the loose regulation of 1,25(OH)$_2$D production may account in part for their high serum values, which are required for growth and development of the skeleton (20). On the other hand, the fact that serum 1,25(OH)$_2$D varies with the concentration of serum 25-OHD in patients with hypoparathyroidism

skin   liver   kidney

7-DHC ⟶ vitamin D ⟶ 25-OHD ⟶ 24,25(OH)$_2$D

$\ominus$ ↑   ↗ ↓ kidney   ↑ $\oplus$
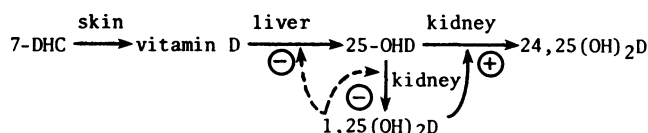
$\ominus$ ↓

1,25(OH)$_2$D

Figure 1. Metabolism of vitamin D depicted schematically. 1,25(OH)$_2$D (*a*) inhibits the hepatic enzyme vitamin D-25-hydroxylase and the renal enzyme 25-hydroxyvitamin D-1a-hydroxylase, thus reducing the synthesis of its precursor 25-OHD and of 1,25(OH)$_2$D itself, respectively, and (*b*) induces the renal enzyme 25-hydroxyvitamin D-24-hydroxylase and stimulates the production of 24,25(OH)$_2$D.

(28) suggests that in the absence of PTH, synthesis of 1,25(OH)$_2$D is determined by substrate concentration.

Increases in cyclic AMP (cAMP) and changes in phosphate concentration within the renal tubule are required for the stimulation of synthesis of 1,25(OH)$_2$D by PTH. The necessity for these biochemical alterations is underscored by the observation that urinary cAMP and phosphate excretion change very little and serum 1,25(OH)$_2$D remains low in response to parathyroid extract in patients with pseudohypoparathyroidism (23). The demonstration that serum 1,25(OH)$_2$D rises briskly and that the increases are associated with a reduction in serum phosphate in response to dibutyryl cAMP in such patients (29) indicates that impaired renal production of cAMP in response to PTH rather than lack or an abnormal form of 25-hydroxyvitamin D-1a-hydroxylase is responsible for diminished production of 1,25(OH)$_2$D.

Two other diseases, hypophosphatemic rickets and hyperphosphatemic tumoral calcinosis, in which abnormal metabolism of vitamin D and phosphate occur, provide strong evidence that the renal tubular transport of phosphate and renal production of 1,25(OH)$_2$D are closely linked. Whereas hypophosphatemic rickets is characterized by hypophosphatemia, impaired tubular reabsorption of phosphate, inappropriately low serum.1,25(OH)$_2$D for the serum phosphate (30, 31), and diminished increase in circulating 1,25(OH)$_2$D in response to PTH as compared with normal subjects (32), tumoral calcinosis is characterized by hyperphosphatemia, impaired renal excretion of phosphate and increased serum 1,25(OH)$_2$D (19). The disorders seem to be mirror images of each other. It is likely that elucidation of the mechanisms for abnormal phosphate transport in these two diseases will lead to a better understanding of the biochemical events concerning regulation of 1,25(OH)$_2$D production by phosphate.

A second major regulator of the renal production of 1,25(OH)$_2$D is the vitamin D status of the animal or tissue or 1,25(OH)$_2$D itself (Fig. 1). In the vitamin D-deficient state, renal synthesis of 1,25(OH)$_2$D is maximal, and synthesis is inhibited by addition of 1,25(OH)$_2$D (24, 33, 34). In vitamin D-replete chicks, both basal and PTH-stimulated renal 1a-hydroxylase activity are diminished (35). In contrast, basal and PTH-stimulated activities increase with deprivation of vitamin D, and basal renal 25-hydroxyvitamin D-1a-hydroxylase varies inversely with serum 1,25(OH)$_2$D (35). These findings provide evidence that 1,25(OH)$_2$D modulates its own synthesis both in the basal state and in response to PTH.

Parathyroid hormone inhibits the renal synthesis of

24,25(OH)$_2$D (24, 36), and 25-hydroxyvitamin D-24-hydroxylase activity is enhanced by 1,25(OH)$_2$D (33, 34). In the vitamin D-deficient state, 24,25(OH)$_2$D production is markedly diminished both in vivo and in vitro, and 24-hydroxylase activity is induced by 1,25(OH)$_2$D (34, 36, 37). In the vitamin D-replete state, 24,25(OH)$_2$D production is enhanced.

Extrarenal production of 1,25(OH)$_2$D

Hypercalcemia in sarcoidosis is caused by increased circulating 1,25(OH)$_2$D, and the characteristic increased sensitivity to vitamin D is caused by lack of regulation of serum 1,25(OH)$_2$D (18, 38). Indeed, impaired regulation of serum 1,25(OH)$_2$D in response to vitamin D challenge can be shown in patients with normal calcium metabolism (39). Defective regulation of circulating 1,25(OH)$_2$D in sarcoid is also evident by the lack of change in serum 1,25(OH)$_2$D in response to increased calcium intake as compared with the reduction of circulating 1,25(OH)$_2$D that occurs in normal subjects (40). In sarcoid, the production of 1,25(OH)$_2$D is extrarenal. Hypercalcemia caused by increased serum 1,25(OH)$_2$D, which was corrected by prednisone in a patient with sarcoid, was the first evidence for extrarenal production of the metabolite (41). Conversion of ^{3}H-25-OHD$_3$ to ^{3}H-1,25(OH)$_2$D$_3$ was shown in cultures of alveolar macrophages from a patient with sarcoid, elevated serum 1,25(OH)$_2$D, and hypercalcemia (42) and in the homogenate of a lymph node from another patient who was normocalcemic (43). The mass spectrum of the putative 1,25(OH)$_2$D produced by sarcoid tissue in vitro was confirmed (44).

Hypercalcemia associated with abnormal elevation of serum 1,25(OH)$_2$D was described in tuberculosis (45, 46), lymphoma (47, 48), disseminated candidiasis (49), and silicon-induced granuloma (50). Modest impairment in the regulation of circulating 1,25(OH)$_2$D in response to vitamin D challenge is present in patients with active pulmonary tuberculosis and normal calcium metabolism (45). In view of the demonstration of synthesis of 1,25(OH)$_2$D by granulomatous tissue in sarcoid (42–44), it is likely but not established that the production of 1,25(OH)$_2$D in these diseases is also extrarenal.

Production of 1,25(OH)$_2$D by rat placenta (51), human decidua (52), chick calvarial cells (53), and human bone and osteosarcoma cells (54) was shown. The identity of 1,25(OH)$_2$D synthesized by rat placenta (51) and chick bone cells (55) was confirmed by mass spectral analysis.

The demonstration of low but detectable values for serum 1,25(OH)$_2$D by bioassay and receptor assay in anephric patients who were on chronic hemodialysis provides evidence for extrarenal production of 1,25(OH)$_2$D (56). Serum 1,25(OH)$_2$D varied with the serum 25-OHD in the patients, indicating that production was not regulated. The mass spectrum of the hormone has not been confirmed, and the site of synthesis is not known. However, inflammation in the liver and other organs and granulomatous hepatitis as a consequence of spalled particles of silicone from blood-pump tubing are reported in patients on chronic hemodialysis (57). This raises the possibility that 1,25(OH)$_2$D synthesized in the inflammatory lesions produced by silicone may account for the presence of 1,25(OH)$_2$D in anephric individuals.

Regulation of bone and mineral metabolism

The major determinant of intestinal absorption of calcium is circulating $1,25(OH)_2D$ (58). Compensatory changes in calcium absorption in response to alterations in dietary calcium are mediated through PTH and $1,25(OH)_2D$ (58, 59). It is also well established that PTH modulates the tubular reabsorption of calcium (60). Thus, in response to calcium restriction PTH acts directly on the kidney to prevent calcium loss and indirectly on the intestine to promote calcium absorption to maintain the serum calcium.

Indications are accumulating that sensitivity of the skeleton to PTH may be important in determining bone mass. Changes in skeletal sensitivity to PTH occur in response to hormones and possibly to physical strain. There is evidence that estrogens may alter the vitamin D-endocrine system. In normal women, balance studies show that intestinal absorption of calcium declines and urinary calcium increases after the menopause and that these changes are corrected by estrogen replacement (61). These results are consistent with the hypothesis that estrogen deficiency in postmenopausal women increases the sensitivity of the skeleton to PTH (58, 61). The ensuing decline in serum PTH is followed by decreases in the renal production of $1,25(OH)_2D$ and intestinal absorption of calcium and to increases in urinary calcium resulting from diminished tubular reabsorption (58, 61). Estrogen replacement decreases the sensitivity of the skeleton to PTH, increases serum PTH, and reverses the changes in intestinal absorption and renal excretion of calcium.

Increased strain on the skeleton produced by obesity also seems to modify the skeletal response to PTH. Obesity is associated with secondary hyperparathyroidism, which is reversible with weight loss (62). Moreover, the decline in serum immunoreactive PTH varies in proportion to the amount of weight lost. In ongoing studies, we found that urinary cAMP is increased in obese white subjects and correlates with ideal body weight (63). Further, obese individuals have a significantly higher serum $1,25(OH)_2D$ and significantly lower serum 25-OHD and urinary calcium than age-matched nonobese white individuals. Serum calcium, serum ionized calcium, and serum phosphate in the two groups are the same. The increased serum $1,25(OH)_2D$ and decreased urinary calcium are attributed to secondary hyperparathyroidism. The low serum 25-OHD that occurs in obese subjects (16, 62, 63) is attributed to feedback inhibition of hepatic production of 25-OHD by $1,25(OH)_2D$ (see above).

Blacks are known to have an increased skeletal mass (64, 65) and decreased urinary calcium (66) as compared with values in whites. The greater skeletal mass in blacks is attributed to a greater muscle mass (65). We found biochemical changes in normal black nonobese subjects similar to those described above in obese white individuals (67). The increased skeletal mass in blacks is therefore attributed to alteration of the vitamin D-endocrine system and the reduced urinary calcium to enhanced tubular reabsorption caused by secondary hyperparathyroidism (67). In this view, increased strain on the skeleton imposed by the greater muscle mass may be the initiating event. The biochemical changes by which estrogen and physical forces modify the skeletal response to PTH are not known.

PTH also plays an important physiologic role in the regulation of body fluid balance. In dogs, expansion of plasma volume and dilution of serum ionized calcium lead to increases in serum immunoreactive PTH and enhanced renal excretion of sodium by inhibition of proximal tubular reabsorption (68). Thus, the parathyroids provide a means for feedback regulation of sodium excretion. In normal subjects but not in patients with hypoparathyroidism, serum immunoreactive PTH, serum $1,25(OH)_2D$, and fractional intestinal absorption of calcium increase in response to sodium loading and decrease in response to sodium restriction (69). $1,25(OH)_2D$, however, does not influence sodium excretion indirectly by inhibiting renin production since administration of $1,25(OH)_2D_3$ to normal subjects does not alter plasma renin activity (70).

Although $1,25(OH)_2D_3$ produces bone resorption (71), the mechanism by which this occurs is not known. $1,25(OH)_2D_3$ increases the number of osteoclasts (72), and receptors for $1,25(OH)_2D$ were shown in bone (73, 74). However, autoradiographic studies show uptake of $[^3H]1,25(OH)_2D_3$ by osteoprogenitor cells and osteoblasts but not by osteoclasts (75). If bone resorption produced by $1,25(OH)_2D$ is mediated by osteoclasts, it is unlikely to be a direct effect in view of the absence of a receptor for the hormone.

There is evidence that osteoclasts are derived from stem cells in the bone marrow and are transported to bone (76–78). Further, osteoclasts do not proliferate but become multinucleated by fusion of precursors with single nuclei (79, 80). Since peripheral monocytes also are derived from bone marrow, do not proliferate, and share some features of osteoclasts, they have been used as models to investigate mechanisms of bone resorption. The fact that peripheral monocytes have receptors for $1,25(OH)_2D$ (81) and osteoclasts do not (75) makes it unlikely that circulating monocytes are the precursors of osteoblasts. Nevertheless, human peripheral monocytes are attracted chemotactically by several constituents of organic matrix of bone including type I collagen peptides, a_2HS glycoprotein, and Gla protein (82), and resorb devitalized adult and fetal human bone (83). $1,25(OH)_2D_3$ induces differentiation of human promyelocytic leukemia cells (HL-60) into multinucleated macrophage-like cells that produce bone resorption (84). Cell maturation in response to $1,25(OH)_2D$ is associated with reversible reduction in the c-myc onchogen (85). In mice, vitamin D-deficiency produces abnormalities in both bone matrix and in peripheral monocytes, which diminish their attachment to each other to initiate the resorptive process (86). Further, the attachment of rat peritoneal macrophages to bone was shown to be mediated by saccharides (87). Finally, high dose $1,25(OH)_2D_3$ activated both osteoclasts and peripheral monocytes to produce bone resorption in a patient with malignant osteopetrosis, a disease characterized by impaired osteoclast function (88). The clinical status of the patient, however, did not change. These results provide additional indications that $1,25(OH)_2D$ may be important in regulating cellular differentiation in osseous tissue.

Mechanism of action of vitamin D

The biologic effects of $1,25(OH)_2D$ are initiated by binding of the hormone to a cytosol receptor in target organs. The $1,25(OH)_2D$-receptor complex becomes tightly associated with the cell nucleus and stimulates transcription of DNA to synthesize messenger RNA and translation of proteins that carry out the biologic action of the hormone. Messenger

RNA for calcium-binding protein in chick intestine is stimulated by 1,25(OH)$_2$D$_3$ (89, 90). Other proteins that are synthesized in response to 1,25(OH)$_2$D$_3$ include ornithine decarboxylase (91) and spermidine N'-acetyltransferase (92) in the chick intestine and alkaline phosphatase (93, 94) and Gla protein by bone and rat osteosarcoma cells (95, 96), which have many of the features of osteoblasts, and by human bone cells (97).

The receptor for 1,25(OH)$_2$D from chick intestine has been purified by two laboratories and has been shown to have a molecular weight of 64,000 D (98) and 67,000 D (99). Antibodies obtained from the rat and from hybridomas demonstrated the presence of the receptor in intestinal tissue from fish, frog, chick, and rat and in cultured cells from a variety of tissues including human breast and intestine (100). These results provide evidence for evolutionary conservation of the biochemical structure of the receptor.

The disease vitamin D-dependent rickets type II, which occurs in humans (101, 102), and in marmoset, a new world monkey (103), points to the importance of the 1,25(OH)$_2$D receptor in mediating the biologic effects of vitamin D. The disorder is characterized by rickets or osteomalacia, which is present despite marked increases in circulating 1,25(OH)$_2$D and results from abnormalities in the receptor for 1,25(OH)$_2$D (101, 102). Studies with cultured skin fibroblasts demonstrated that vitamin D-dependent rickets type II is a heterogeneous disorder (104). In one kindred, undetectable high affinity binding of [^{3}H]1,25(OH)$_2$D$_3$ by cytosol was associated with impaired induction of 25-hydroxyvitamin D-24-hydroxylase by 1,25(OH)$_2$D$_3$ (105). In a second kindred, a postreceptor defect was indicated by normal nuclear uptake of [^{3}H]1,25(OH)$_2$D$_3$ in association with defective induction of 25-hydroxyvitamin D-24-hydroxylase (106). In another patient, a decrease in binding of [^{3}H]1,25(OH)$_2$D$_3$ to ~10% of normal suggested a deficiency in the number of receptors (104). Radioimmunoassay of receptors in skin fibroblasts from a variety of patients with vitamin D-dependent rickets type II, however, indicated no quantitative deficiency of the receptor (107). In view of these findings, it is likely that structural abnormalities in the receptor may account for the defective response of target organs to 1,25(OH)$_2$D.

Determining the structure of the 1,25(OH)$_2$D receptor in normal and disease states would be of great importance and could be accomplished either by direct peptide sequencing of the purified receptor or by determination of the gene sequence of the receptor through recombinant DNA technology. The latter method would be quite difficult technically in view of the fact that the receptor represents such a small fraction of the protein synthesized by the cell. This approach, therefore, may not be feasible.

Regulation of cell growth and differentiation

The finding of receptors for 1,25(OH)$_2$D in a number of cells and tissues other than those of established target organs, intestine, bone, and kidney (21) indicates that the hormone may have a number of biologic effects in addition to those related to mineral metabolism. Anemia and myelofibrosis are reported in children with vitamin D-deficient rickets and are corrected by vitamin D (108). Infections are also common (109), and leukocytes from such patients show impaired random motility (110) and defective ability

to phagocytose bacteria (111). Similarly, vitamin D-deficient mice have a depressed inflammatory response, macrophages which show impaired phagocytosis, and macrophages and leukocytes which exhibit defective spontaneous migration (112). The abnormalities in the mice are corrected by 1,25(OH)$_2$D$_3$.

1,25(OH)$_2$D regulates growth and differentiation of cells, particularly those derived from bone marrow. 1,25(OH)$_2$D receptors were demonstrated in promyelocytic leukemia cells (HL-60), and 1,25(OH)$_2$D$_3$ inhibited growth and induced differentiation of them into monocyte-macrophages (113–116). The importance of the receptor in mediating this action of 1,25(OH)$_2$D$_3$ was demonstrated by the finding of reduced cellular uptake of [^{3}H]1,25(OH)$_2$D$_3$ and diminished numbers of receptors in resistant clones of the HL-60 cells (113, 115, 116). 1,25(OH)$_2$D$_3$ inhibited cell growth and induced differentiation of mouse myeloid leukemia cells (M1) into macrophages in vitro (117) and prolonged survival time of nude mice inoculated with the M1 cells (118). 1,25(OH)$_2$D$_3$ and two fluorinated analogs of 1,25(OH)$_2$D$_3$ induced macrophage differentiation of normal human and leukemic stem cells (119, 120). These intriguing findings indicate the possible use of 1,25(OH)$_2$D$_3$ in the treatment of human leukemias.

1,25(OH)$_2$D receptors are not present in normal resting human T and B lymphocytes. However, they can be demonstrated in human T lymphocytes 24 hours after activation by phytohemagglutinin P or concanavalin, and in human B lymphocytes after infection with Epstein-Barr virus for 3 wk (81, 121). 1,25(OH)$_2$D$_3$ inhibits proliferation and suppresses interleukin-2 activity of phytohemagglutinin-stimulated human lymphocytes (122). Since interleukin-2 is a growth-promoting lymphokine for activated lymphocytes, inhibition of lymphocyte proliferation may be mediated by inhibition of interleukin-2. Similarly, 1,25(OH)$_2$D inhibits antigen-induced production of interleukin-2 by cloned Ia-restricted T cell hybridomas (123) and proliferation and production of immunoglobulins IgG and IgM by activated human peripheral mononuclear cells (124). 1,25(OH)$_2$D$_3$ induces maturation of the human monocyte cell line U937, which contains receptors for the hormone (125), and stimulates production of interleukin-1 and lymphocyte-activating factor when incubated with a factor from lectin-stimulated human T lymphocytes (126). These results support a role for 1,25(OH)$_2$D in the regulation of the immune system.

Conclusions

Technological advancements over the past 15 yr in development and application of assays for PTH, vitamin D and its metabolites, and the synthesis and availability of 25-OHD$_3$, 1,25-(OH)$_2$D$_3$, 1α-hydroxyvitamin D$_3$, and other metabolites of the vitamin in stable and [^{3}H]labeled forms for clinical and basic studies have permitted delineation of the role of the vitamin D-endocrine system in health and disease both in human and laboratory animals. The recent demonstration of receptors in a variety of tissues not previously regarded as targets for 1,25(OH)$_2$D has provided exciting new concepts and raised many important questions concerning the possible physiologic and pathophysiologic role of vitamin D and offered new approaches for the treatment of diseases heretofore not related to the vitamin. This, together with recent technical developments in cellular and molecular biology, should allow an even more rapid advance of knowledge in an already very active field.

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