MINIREVIEW

What Is Vitamin D Deficiency? (43371B)

MARIAN R. WALTERS,[1] UWE KOLLENKIRCHEN, AND JOHN FOX
Department of Physiology, Tulane University School of Medicine, New Orleans, Louisiana 70112

Vitamin D derived from the diet or produced in the skin is converted to its active form by two sequential hydroxylation reactions (1, 2). The first occurs in the liver, forming 25-hydroxyvitamin D_3 (25-(OH)D_3), and the second occurs in the kidney, forming the active hormonal form 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$). The activity of the renal 25-(OH)D_3-1α-hydroxylase enzyme (1-hydroxylase), which is tightly regulated according to the calcium and phosphorus requirements of the body, is stimulated predominantly by parathyroid hormone and low plasma calcium and phosphate concentrations. 1,25-Dihydroxyvitamin D_3 acts to increase intestinal active calcium transport and, in concert with parathyroid hormone, to stimulate renal tubular calcium reabsorption and bone mineral mobilization (1, 2). Vitamin D restriction by dietary deficiency and/or by reduced exposure to sunlight can lead to the development of vitamin D-deficiency rickets (2, 3).

The best recognized definition of vitamin D-deficiency, i.e., bony rickets, has its origin in the evolving understanding of this bone disease and the fact that vitamin D deficiency is the most frequent cause (Table I). In more recent times, many investigators have considered animals to be vitamin D deficient if they exhibit rickets, reduced intestinal calcium transport, hypocalcemia, or "undetectable" levels of 25-(OH)D_3 (Table II; 7–27). However, in a series of studies (28–30) directed toward establishing a normocalcemic rat model exhibiting vitamin D-deficiency, we realized that understanding or defining vitamin D deficiency is extremely complex. For example, most workers would probably agree that vitamin D deficiency should entail 1,25-$(OH)_2D_3$ deficiency. However, as described below, absence of 1,25-$(OH)_2D_3$ may not always be associated with absence of vitamin D or 25-(OH)D_3. Conversely, physiological levels of 1,25-$(OH)_2D_3$ can exist in the presence of extremely low levels of vitamin D or 25-(OH)D_3.

The purpose of this review was to explore these and other discrepancies in an effort to improve our awareness and understanding of this complex series of issues.

General Considerations

There are several simple points for which clarification is important at the outset. First, while many investigators seem to interchange the terms vitamin D "deficient" and "deplete," they are not synonymous. Thus, the term deficient is defined as lacking in some quality necessary for completeness, i.e., not up to standard (31), and deplete is defined as empty or exhausted (31, 32). "Replete," on the other hand, connotes fullness (32). Thus, any animal with reduced vitamin D is vitamin D deficient, only normal animals are vitamin D replete, and only animals totally lacking in vitamin D (or 1,25-$(OH)_2D_3$) are vitamin D deplete. Moreover, as illustrated in Table II, whether "undetectable" levels of vitamin D or its metabolites represent deficiency or depletion will depend in part on the sensitivity of the assay method.[2]

Another set of complications that will not be considered further is the issue of "free" versus "total" levels of the vitamin D metabolites (33), and the fact that alterations in the levels of the plasma-binding proteins, in particular, vitamin D-binding globulin, can significantly alter the biological activity of the compound (33). In a related way, alterations in 1,25-$(OH)_2D_3$ receptor levels, for example, by 1,25-$(OH)_2D_3$ (34, 35), calcium (35, 36), age (37), or cortisol (38), will also alter

[1] To whom requests for reprints should be addressed at Department of Physiology, Tulane University Medical School, 1430 Tulane Avenue, New Orleans, LA 70112.

P.S.E.B.M. 1992, Vol 199:385–393

0037-9727/92/1994-0385$3.00/0
Copyright © 1992 by the Society for Experimental Biology and Medicine

[2] The assay sensitivity in turn depends upon the volume of plasma extracted and the recovery of each vitamin D metabolite through the extraction and purification process. In particular, the limited sample volumes available from small animals tends to make the detection limit higher than with studies in humans in which sample volume is less of a problem.

sensitivity to the hormone. An extreme example of this relationship between 1,25-$(OH)_2D_3$ effects and receptor levels occurs in vitamin D-resistant rickets Type II (pseudovitamin D-deficiency rickets Type II), where hormone-binding or transcriptional activity is impaired due to alterations in the genes coding for the 1,25-$(OH)_2D_3$ receptor (39–41).

Finally, the recent advent of analogs of 1,25-$(OH)_2D_3$ that may differentiate between different hormonal effects, e.g., receptor-binding activity versus induction of intestinal calcium transport or elevation of plasma calcium (42, 43), will make precise definition of terms and experimental states essential.

Statement of the Problem

While "vitamin D deficiency" has been at various times defined experimentally by the presence of bone pathology, i.e., rickets, and/or substantial reductions in plasma 25-$(OH)D_3$ or calcium levels (Table II), the important functional hormone is 1,25-$(OH)_2D_3$. Furthermore, rickets is not always associated with the state of vitamin D-deficiency: (i) rickets can occur in disease states without vitamin D-deficiency, *e.g.*, hypophosphatemia (44); and (ii) vitamin D deficiency can occur without rickets, e.g., a vitamin D-deficient nonrachitogenic rat diet was developed many years ago (45). Moreover, there are many states in which alterations in the parameters of the vitamin D endocrine system occur in patterns that are either not consistent with the classical concept of vitamin D deficiency or in which altered 1,25-$(OH)_2D_3$ levels do not reflect pathologic states. These issues are discussed below and illustrated with examples from a recent series of experiments in our laboratory (28–30).

Normal Adaptive Responses Result in Significantly Decreased Plasma 1,25-$(OH)_2D_3$ Levels

Effect of Age. In normal rats, there are age-dependent reductions in plasma 1,25-$(OH)_2D_3$ levels (Fig. 1D) and 1,25-$(OH)_2D_3$-induced effects (duodenal active calcium transport, Fig. 1E) in the presence of constant plasma levels of 25-$(OH)D_3$ (Fig. 1C) and calcium (Fig. 1B). These effects, which have been noted previously in both rats (46, 47) and humans (48), seem to reflect reduced demand for calcium as growth rate slows (Fig. 1A) and may relate to the fact that middle-aged rats, in contrast to weanlings, can adapt to restriction of dietary vitamin D and calcium (49). Because of this normal age-related decrease in plasma 1,25-$(OH)_2D_3$, all evaluations of 1,25-$(OH)_2D_3$ deficiency must be compared with age-related controls, rather than by tracking reductions in 1,25-$(OH)_2D_3$ with time. A further complication results from the observation in many laboratories (see below) that vitamin D-deficient rats in some cases seem to have residual 1,25-$(OH)_2D_3$ levels of 10–20 pg/ml or more, a quite significant level when compared with normal adult rats with 1,25-$(OH)_2D_3$ levels of 25–50 pg/ml (Fig. 1D; 46, 47).

Table I. A Chronology of Selected Developments in Defining Vitamin D Deficiency

Date	Event[a]
1645, 1650	Rickets first described
1921	Rickets cured by exposure to sunlight
1921–1922	Absence of vitamin D (structure undefined) proposed as cause of rickets
1936	Structure of vitamin D defined
1959	Bioassay developed for vitamin D (detection limit: 250 ng[b]).
1971	1,25-$(OH)_2D_3$ recognized as the active form of vitamin D
1971	Assay for 25-$(OH)D_3$ first developed (detection limit: 20 ng/ml[b])
1974	Assay for 1,25-$(OH)_2D_3$ first developed (detection limit: 80 pg[b])
	Current assays developed for:
1974–1976	Vitamin D (detection limit: 5 ng[b])
1985	25-$(OH)D_3$ (detection limit: 3 pg/tube[b]) (4[c])
1983, 1984	1,25-$(OH)_2D_3$ (detection limit: 1 pg/tube[b]) (5, 6)

[a] Reference citations are given in parentheses. Except as noted, further details and references can be found in Ref. 3.
[b] Concentration depends on the volume extracted.
[c] Kit marketed by INCSTAR, Stillwater, MN.

Effect of Increased Dietary Calcium. When weanling rats are fed vitamin D-deficient diets with a relatively low calcium content (Fig. 2, open bars), 25-$(OH)D_3$ levels fall rapidly (Fig. 2B). However, as long as adequate 25-$(OH)D_3$ levels remain (i.e., within the same order of magnitude as 1,25-$(OH)_2D_3$, see below), 1,25-$(OH)_2D_3$ levels (Fig. 2C) and effects (Fig. 2D) remain normal in order to maintain normal plasma calcium levels (Fig. 2A). In contrast, when the rats are fed a vitamin D-deficient diet with high calcium content (Fig. 2, stippled bars), there is a physiologic adaptation to the high calcium intake such that 1,25-$(OH)_2D_3$ levels (Fig. 2C) and effects (Fig. 2D) are diminished in spite of the presence of 25-$(OH)D_3$ levels similar to those in the latter group (Fig. 2B). These results reinforce several important concepts: (i) a wide range of physiologic adaptive mechanisms can alter 1-hydroxylase activity and reduce plasma 1,25-$(OH)_2D_3$ levels (for review, see Refs. 50 and 51); (ii) substantial reductions in 25-$(OH)D_3$ levels (97% in Fig. 2B) can occur without effects on 1,25-$(OH)_2D_3$ levels; and (iii) reduced 1,25-$(OH)_2D_3$ alone does not provide an adequate assessment of functional vitamin D deficiency.

Deficiency of Vitamin D is Not Always Associated with Bone Pathology or Hypocalcemia

In contrast to hypocalcemic vitamin D-deficient rats, by substantially increasing dietary calcium content and availability (see Ref. 29 for details), one can achieve

Table II. A Selection of Referenced Criteria for Establishing Vitamin D Deficiency (−D) in Rats

Reference[a]	Year	Criteria for vitamin D deficiency		
		Parameter	Definition −D	Assay sensitivity
Bronner and Freund (7)	1975	Plasma calcium	<6.5 mg/dl	—
		CaBP-D9K	Undetectable	8 nmol Ca bound/mg protein
Forte *et al.* (8)	1977	Plasma calcium	Low (5.0 vs 9.8 mg/dl)	—
		Body weight	Low	—
Boass *et al.* (9)	1977	Plasma 1,25-$(OH)_2D_3$	Undetectable	17 pg
Thomas and Forte (10)	1981	Plasma calcium	Low (4.6 vs 10 mg/dl)	—
Holtrop *et al.* (11)	1981	Serum 25-$(OH)D_3$	Undetectable	2–5 ng/ml
Harrison *et al.* (12)	1982	Serum 25-$(OH)D_3$	Undetectable	<0.5 ng/ml
		Serum 1,25-$(OH)_2D_3$	Low (19 vs 44 pg/ml)	—
Lester *et al.* (13)	1982	Serum 1,25-$(OH)_2D_3$	Undetectable	<0.6 pg/ml
Talmage and VanderWiel (14)	1983	Serum 1,25-$(OH)_2D_3$	Undetectable	<0.6 pg/ml
Underwood *et al.* (15)	1984	Plasma 25-$(OH)D_3$	Undetectable	<3 ng/ml
		Plasma 1,25-$(OH)_2D_3$	Undetectable	<7 pg/ml
Favus and Angeid-Backman	1985	Calcium flux	Reduced	—
		Plasma calcium	Reduced (9.2 vs 11.2 mg/dl)	—
Warner and Tenenhouse (17)	1985	Plasma calcium	Reduced (3.8 vs 10.4 mg/dl)	—
Gallagher *et al.* (18)	1986	Plasma 25-$(OH)D_3$	Undetectable	<0.5 ng/ml
Holtrop *et al.* (19)	1986	Plasma 25-$(OH)D_3$	Undetectable	<3 ng/ml
		Plasma 1,25-$(OH)_2D_3$	Undetectable	<10 pg/ml
Cade and Norman (20)	1986	Plasma calcium	Reduced (4.8 vs 10.3 mg/dl)	—
		Plasma 1,25-$(OH)_2D_3$	Undetectable	5 pg/ml
		Plasma 25-$(OH)D_3$	Undetectable	0.5 ng/ml
Tam *et al.* (21)	1986	Bone mineral apposition	Reduced (0.042 vs 0.068 μm/hr)	—
		Plasma 25-$(OH)D_3$	Low (1.1 vs 41 ng/ml)	—
Sonnenberg *et al.* (22)	1986	Plasma calcium	Low (7.4 mg/dl)	—
		Plasma 25-$(OH)D_3$	Undetectable	<5 ng/ml
Weishaar and Simpson (23)	1987	Serum calcium	Low (5–7 vs 10–12 mg/dl)	—
		Serum 25-$(OH)D_3$	Undetectable	0.1 ng/ml
		Serum 1,25-$(OH)_2D_3$	Undetectable	10 pg/ml
Weishaar and Simpson (24)	1987	Serum calcium	Low (4–5 vs 9–10 mg/dl)	—
		Serum 25-$(OH)D_3$	Undetectable	0.2 ng/ml
		Serum 1,25-$(OH)_2D_3$	Undetectable	1.0 pg/ml
Miller *et al.* (25)	1988	Serum 25-$(OH)D_3$	Undetectable	?
		Serum 1,25-$(OH)_2D_3$	Undetectable	?
Schaafsma *et al.* (26)	1988	Serum 25-$(OH)D_3$	Reduced (4–5 vs 20 ng/ml)	—
		Serum 1,25-$(OH)_2D_3$	Reduced (50–70 vs 178 pg/ml)	?
Kwiecinski *et al.* (27)	1989	Serum calcium	Reduced (5.1 vs 9.9 mg/dl)	—
		Serum 25-$(OH)D_3$	Undetectable	<0.5 ng/ml
		Serum 1,25-$(OH)_2D_3$	Reduced (10 vs 29 pg/ml)	10 pg/ml

[a] Reference citations are given in parentheses.

substantial reductions in plasma 25-$(OH)D_3$ (Fig. 3B, stippled bars) and 1,25-$(OH)_2D_3$ levels (Fig. 3C) and its hormonal effects (Fig. 3D), but without influencing plasma calcium (Fig. 3A), bone mineral content (Fig. 3E), or bone biochemical activity (Fig. 3F). Importantly, although this dissociation between deficiency of vitamin D metabolites and plasma Ca and/or bone pathology (see above) has been reported previously (52), the long-term nature of this study reinforces the validity of the earlier observation.

Significant 1,25-$(OH)_2D_3$ Levels Can Exist in the Presence of "Undetectable" 25-$(OH)D_3$ Levels

As noted in the Introduction, "undetectable" 25-$(OH)D_3$ is frequently used as a determinant of vitamin D deficiency (Table II). However, our studies have elucidated several conditions in which significant 1,25-$(OH)_2D_3$ levels are accompanied by 25-$(OH)D_3$ levels well below the 3–5 ng/ml limit of detectability of the commonly used competitive protein-binding assay using vitamin D-binding globulin (3). Our ability to accurately measure such substantially diminished 25-$(OH)D_3$ levels is due to our development of a radioimmunoassay for 25-$(OH)D_3$ with a detection limit of 10–12 pg/ml[2] (53). Importantly, this experimental observation has clinical correlates in which individuals exhibit low 25-$(OH)D_3$ levels, but normal or high 1,25-$(OH)_2D_3$ levels (54–60)

Developing Vitamin D Deficiency: Deficiency of 1,25-$(OH)_2D_3$ Lags Behind Reductions in 25-$(OH)D_3$. As rats are fed the vitamin D-deficient low calcium diet, 25-$(OH)D_3$ levels decrease much more rapidly

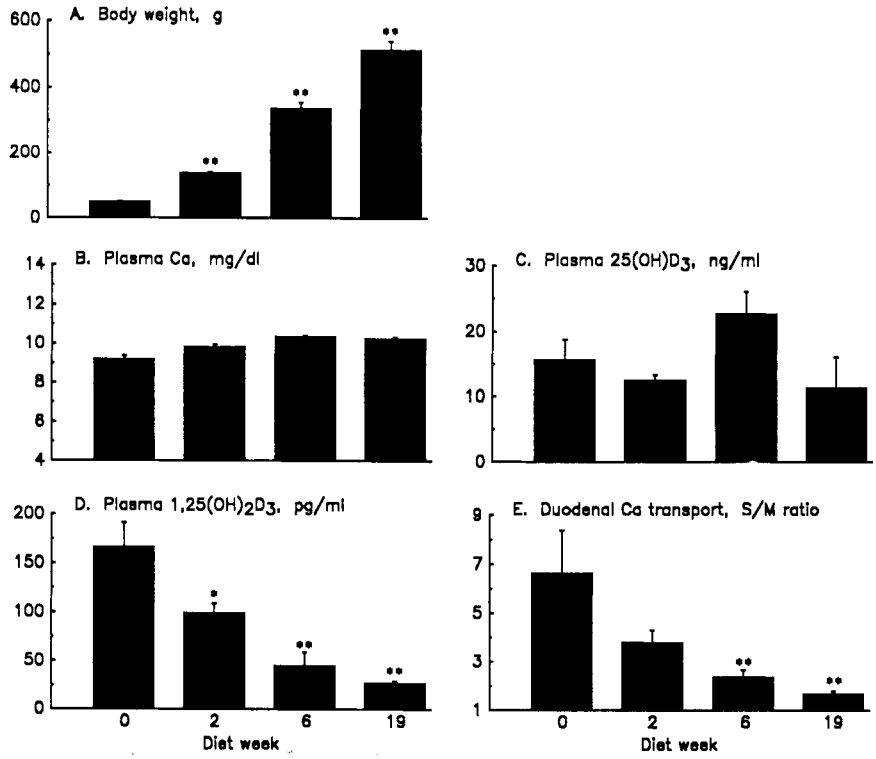

Figure 1. Changes in $1,25\text{-}(OH)_2D_3$ and other parameters in normal vitamin D-replete rats from 3 to 22 weeks of age (0–19 diet weeks). Normal male weanling rats were placed on a vitamin D-replete diet containing 0.8% calcium and 0.5% phosphorus. At the indicated intervals, rats were anesthetized, blood was collected by cardiac puncture, and the indicated parameters were determined (29). Data are expressed as mean ± SE for $n = 5$ individual rats. * $P < 0.05$, ** $P < 0.01$ vs diet week 0 (analysis of variance and Dunnett's test).

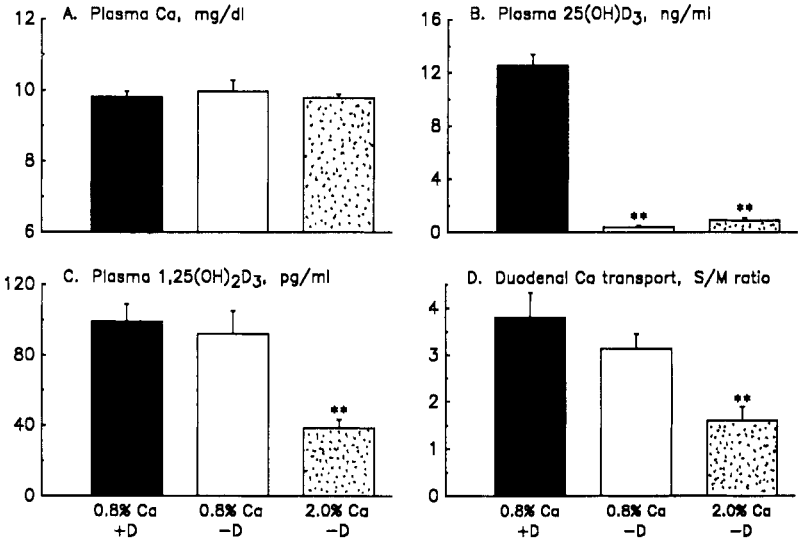

Figure 2. Comparison of plasma $25\text{-}(OH)D_3$ and $1,25\text{-}(OH)_2D_3$ levels in rats fed vitamin D-deficient (-D) diets with low versus high calcium content. Prior to sacrifice (as in Fig. 1), weanling rats were fed the following diets for 2 weeks: ■ vitamin D-replete (+D) diet with 0.8% calcium and 0.5% phosphorus; □ -D diet with 0.8% calcium and 0.5% phosphorus; and ▨ -D diet with 2% calcium, 1.25% phosphorus, and 20% lactose (see Ref. 29 for details). Data are expressed as mean ± SE for $n = 5$ individual rats. * $P < 0.05$; ** $P < 0.01$ vs +D controls.

than do $1,25\text{-}(OH)_2D_3$ levels (Fig. 4). Thus, it is not unusual to see "normal" $1,25\text{-}(OH)_2D_3$ levels (Fig. 4, 2 weeks) in the presence of $25\text{-}(OH)D_3$ levels so substantially reduced (0.4 ng/ml in Fig. 4) as to be "undetectable" by usual criteria.

Re-Exposure to Vitamin D: Very Efficient Conversion of $25\text{-}(OH)D_3$ to $1,25\text{-}(OH)D_2D_3$. When the hypocalcemic vitamin D-deficient rats shown in Figure 3 received a brief exposure to vitamin D (Fig. 5, open bars), there were substantial elevations in plasma levels of calcium (Fig. 5A), $25\text{-}(OH)D_3$ (Fig. 5B), and $1,25\text{-}(OH)_2D_3$ (Fig. 5C). Thus, in spite of the fact that $25\text{-}(OH)D_3$ levels remained substantially below the usual detection limit of 3–5 ng/ml, $1,25\text{-}(OH)_2D_3$ levels were fully twice that of the normal vitamin D-replete rats (53.7 vs 21.6 pg/ml) and were sufficient to normalize

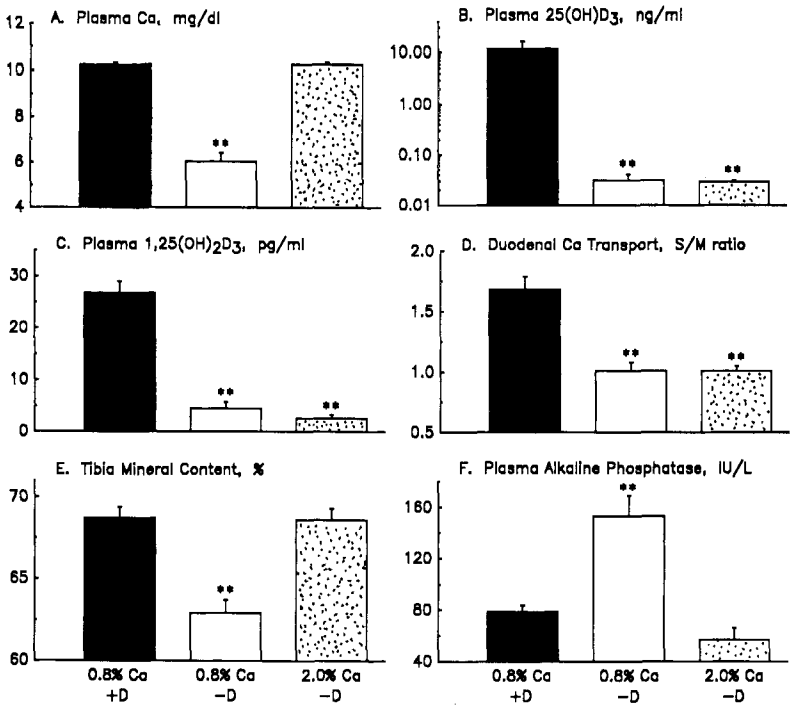

Figure 3. Comparison of long-term effects on plasma calcium, vitamin D metabolite levels, and bone parameters in rats given vitamin D-deficient diets with low (□) versus high (□) calcium content. Weanlings were fed the diets as described in Figure 2 for 19 weeks prior to sacrifice. Data are expressed as mean ± SE for $n = 5$ individual rats. * $P < 0.05$; ** $P < 0.01$ vs +D (■).

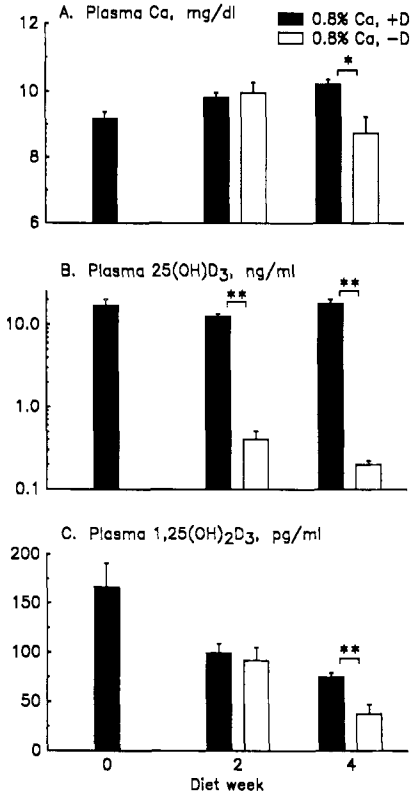

Figure 4. Comparison of 25-$(OH)D_3$ and 1,25-$(OH)_2D_3$ levels in rats fed vitamin D-replete (■) and low calcium vitamin D-deficient (□) diets. Weanling rats were given the indicated diets as in Figure 2 prior to sacrifice. Data are expressed as mean ± SE for $n = 5$ individual rats. * $P < 0.05$; ** $P < 0.01$ vs +D as indicated.

plasma calcium (Fig. 5A) and duodenal calcium transport (Fig. 5D). The ability to achieve normal 1,25-$(OH)_2D_3$ levels under these conditions is due to the substantially increased 1-hydroxylase activity as vitamin D deficiency progresses. For example, we have shown that the activity of this enzyme can increase more than 100-fold in hypocalcemic vitamin D-deficient rats (28). Thus, in this and other conditions, the 1-hydroxylase enzyme can be so efficient as to maintain plasma 1,25-$(OH)_2D_3$ levels within an order of magnitude of 25-$(OH)D_3$ levels (Table III), in spite of the far more rapid clearance rate of 1,25-$(OH)_2D_3$ (61).

Re-Exposure to Vitamin D in Vitamin D-Deficient Normocalcemic Rats: 1,25-$(OH)_2D_3$ Formation Ultimately Depends on the Need for Plasma Calcium. The complexity of the issue of defining vitamin D deficiency was further underscored when the vitamin D re-exposure experiment of Figure 5 was performed in vitamin D-deficient normocalcemic rats (Fig. 5; stippled bars). In this case, 25-$(OH)D_3$ levels also increased substantially, but there was no change in plasma calcium or 1,25-$(OH)_2D_3$ (2.6 vs 1.0 pg/ml) levels, or in duodenal calcium transport. Thus, given the ability to adapt to calcium demands, or the lack thereof, in normal animals (Figs. 1 and 2), one could probably argue whether or not these vitamin D-deficient normocalcemic animals do in fact exhibit functional vitamin D deficiency or whether their deficiency is in fact a potential vitamin D deficiency, i.e., that their vitamin D deficiency is not exhibited until stressed by reduced calcium availability. Thus, perhaps the most reliable

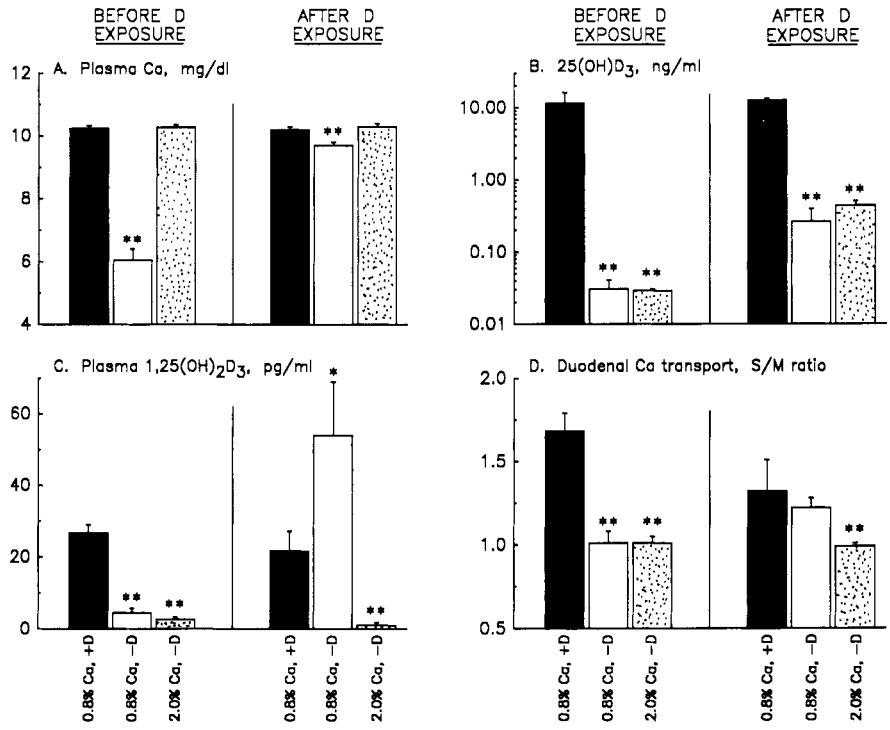

Figure 5. Effect of re-exposure to vitamin D on vitamin D-deficient hypocalcemic (□) and normocalcemic rats (▨). Rats maintained on the indicated diets as in Figure 2 received an inadvertant exposure to vitamin D prior to sacrifice (a classic serendipitous experiment). Data represent mean ± SE of $n = 5$ individual rats. * $P < 0.05$; ** $P < 0.01$ vs +D (■).

Table III. Conversion of 25-(OH)D_3 to 1,25-$(OH)_2D_3$ Can Be Very Efficient

Experiment	Rat status	Plasma levels (pg/ml)	
		25-(OH)D_3	1,25-$(OH)_2D_3$
A	−D diet with 1% Ca/0.6% P[a]	123 ± 8	68 ± 11
B	−D diet with 0.65% Ca/0.4% P[b]	107 ± 15	22 ± 2
C	−D diet with 0.8% Ca/0.5% P + refed vitamin D[c]	260 ± 130	54 ± 15
D	−D diet with 2% Ca/1.25% P + refed vitamin D[a]	180 ± 30	45 ± 16
	−D diet with varying Ca/P content[d]	30 ± 10	2.5 ± 0.7–4.5 ± 1.2

[a] J. Fox, U. Kollenkirchen, and M. R. Walters, unpublished.
[b] J. Fox, Ref. 63.
[c] M. R. Walters, U. Kollenkirchen, and J. Fox (see Fig. 5).
[d] U. Kollenkirchen, M. R. Walters, and J. Fox, Ref. 29.

test of functional vitamin D-deficiency in such normocalcemic animals (and possibly in humans with high levels of dietary calcium supplementation) would be to test for the ability to compensate for reduced calcium intake, i.e., a test analogous to the glucose tolerance test.

1,25-$(OH)_2D_3$ Infusion in Vitamin D-Deficient States. An experimental correlate of these situations arises when vitamin D-deficient rats are stimulated with 1,25-$(OH)_2D_3$ in the absence of other vitamin D metabolites. For example, we recently developed a protocol (62) in which normocalcemic vitamin D-deficient rats were infused with 1,25-$(OH)_2D_3$ to plasma levels (0–500 pg/ml) that spanned the physiologic range. Although this 1,25-$(OH)_2D_3$ stimulation resulted in significant elevations in intestinal calcium transport, normocalcemia could be maintained by reducing dietary calcium content (62), whereas normal dietary calcium resulted in hypercalcemia. Thus, this regimen provides a model of 1,25-$(OH)_2D_3$ repletion with normocalcemia, and is an important adjunct to the previous model of vitamin D deficiency with normocalcemia (29, 30).

How Low is Low 1,25-$(OH)_2D_3$?

While ideally one would prefer to study the effects of 1,25-$(OH)_2D_3$ readdition against a background of absolute vitamin D depletion, this goal is in reality

Table IV. Selected Reports of Significant $1,25\text{-}(OH)_2D_3$ Levels in Vitamin D Deficient (−D) Rats

Reference[a]	$1,25\text{-}(OH)_2D_3$ Levels (pg/ml)	
	+D	−D
Fox (63)	100 ± 7	22 ± 2
Underwood *et al.* (15)	—	34.1 ± 13.1
Harrison *et al.* (12)	43.5	19.3
Lester *et al.* (64)	—	30% of normal
Tam *et al.* (21)	30.8	71.9
Holtrop *et al.* (19)	183	<10 pg/ml[b]
Weishaar and Simpson (23)	97	<10 pg/ml[b]
Kwiecinski *et al.* (27)	29	10
S. C. Christakos[c]	90.5 ± 12.4	23.4 ± 1.5
M. E. Bruns[c,d]	72.9 ± 12.1	22.9 ± 4.6

[a] Reference citations are given in parentheses.
[b] Limit of assay sensitivity.
[c] Personal communication.
[d] Vitamin D-deficient pregnant mice.

undefinable and likely unachievable. Thus, it is important to establish criteria for an experimentally acceptable degree of vitamin D deficiency. In this regard, there are at least two additional relevant observations.

Vitamin D-Deficient Rats with 20 pg/ml (or more) $1,25\text{-}(OH)_2D_3$? In our initial studies of vitamin D-deficient animals, $1,25\text{-}(OH)_2D_3$ levels seemed to decrease to a plateau of approximately 20 pg/ml (Table III). While this level of $1,25\text{-}(OH)_2D_3$ was not observed in later experiments (29; Fig. 4), we found that it is not unusual for investigators to report $1,25\text{-}(OH)_2D_3$ levels of 10–20 pg/ml (or more) in vitamin D-deficient animals (Table IV), often with "undetectable" $25\text{-}(OH)D_3$. In some cases above, the levels may be artifacts of the assay sensitivity (compare with Table II), but this does not seem to explain all the data. Furthermore, since $1,25\text{-}(OH)_2D_3$ levels in normal adult rats decline to 25–50 pg/ml (Fig. 1), this residual $1,25\text{-}(OH)_2D_3$ could represent a very significant level of the hormone.

What Is/Are the Dose-Response Curves to $1,25\text{-}(OH)_2D_3$? In many of the experimental models listed in Table IV, the presence of attendant hypocalcemia or impaired active calcium transport documents that there is a functional vitamin D deficiency, at least with respect to these classical responses to the $1,25\text{-}(OH)_2D_3$ hormone. However, $1,25\text{-}(OH)_2D_3$ receptors and effects are now known to exist in a wide range of tissues (51, 65) for which *in vivo* dose-response curves are as yet unknown. Thus, a modest reduction in $1,25\text{-}(OH)_2D_3$ levels, while sufficient to reduce classical responses, may not affect nonclassical effects, particularly if they exhibit more potent responsiveness. In this regard, we have recently determined that the K_d for $1,25\text{-}(OH)_2D_3$ receptors in some nonclassical target tissues is an order of magnitude lower (i.e., the affinity is higher) than the K_d for the principal receptor sites in the kidney (66). While the ramifications of this new information are not yet fully understood, these observations raise the possibility that $1,25\text{-}(OH)_2D_3$ effects in at least some tissues may be approximately 10-fold more sensitive to circulating $1,25\text{-}(OH)_2D_3$ levels.

Conclusion

These observations clearly illustrate that vitamin D deficiency is a heterogenous collection of physiologic conditions. While it is tempting to propose a nomenclature (-D, Type I, Type II . . . Type N) for these states, such an approach rapidly becomes unmanageable due to the seemingly infinite number of possible combinations of the pertinent parameters. We, therefore, recommend that classical vitamin D deficiency (-D Type I, if one wishes) be defined as a state exhibiting the whole range of predicted parameters. All other permutations of vitamin D-deficient states (except those clinical states for which nomenclature is already in use) should be fully defined with respect to those parameters that differ from the classical picture. Given the wide

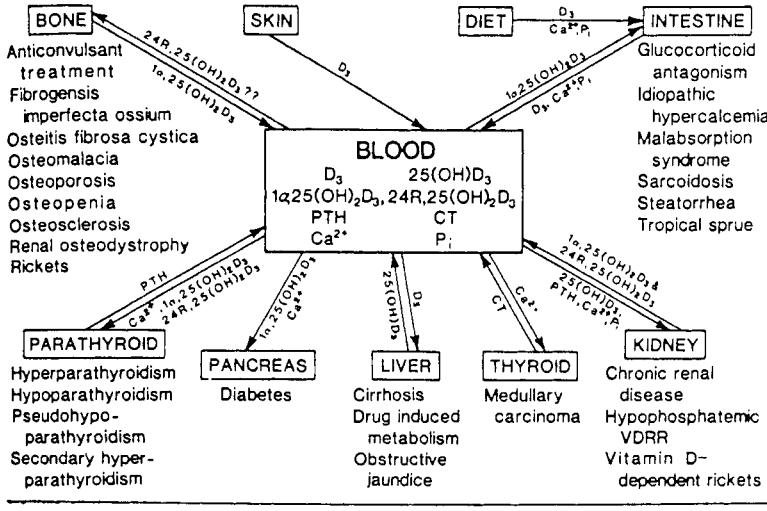

Figure 6. Disease states in humans related to vitamin D. Reproduced with permission from A. W. Norman (Ref. 67).

range of clinical conditions related to vitamin D (Fig. 6; 67), this careful definition of parameters is essential as we continue our efforts to understand normal and abnormal conditions associated with the vitamin D endocrine system.

These studies were supported by a grant-in-aid from the American Heart Association (M. R. W.), by NIH Grant DK-31847 (M. R. W.), and by a grant-in-aid from the American Heart Association-Louisiana (J. F.)

1. Reichel H, Koeffler HP, Norman AW. The role of the vitamin D endocrine system in health and disease. N Engl J Med **320**:980–991, 1989.
2. DeLuca HF, Krisinger J, Darwish H. The vitamin D system: 1990. Kidney Int **38**(suppl 29):S2–S8, 1990.
3. Norman AW. Vitamin D: The calcium homeostatic hormone. New York: Academic Press, 1979.
4. Hollis BW, Pittard WB. Improved radioimmunoassay for vitamin D and its use in assessing vitamin D status. Clin Chem **31**:1815–1819, 1985.
5. Fraher LJ, Adami S, Clemens TL, Jones G, O'Riordan JLH. Radioimmunoassay of 1,25-dihydroxyvitamin D_3: Studies on the metabolism of vitamin D_2 in man. Clin Endocrinol **19**:151–165, 1983.
6. Reinhardt TA, Horst RL, Orf JW, Hollis BW. A microassay for 1,25-dihydroxyvitamin D not requiring high performance liquid chromatography: Application to clinical studies. J Clin Endocrinol Metab **58**:91–98, 1984.
7. Bronner F, Freund T. Intestinal CaBP: A new quantitative index of vitamin D deficiency in the rat. Am J Physiol **229**:689–694, 1975.
8. Forte LR, Nickols GA, Anast CS. Renal adenylate cyclase and the interrelationship between parathyroid hormone and vitamin D in the regulation of urinary phosphate and adenosine cyclic 3′,5′-monophosphate excretion. J Clin Invest **57**:559–568, 1976.
9. Boass A, Toverud SU, McCain TA, Pike JW, Haussler MR. Elevated serum levels of 1α,25-dihydroxycholecalciferol in lactating rats. Nature **267**:630–632, 1977.
10. Thomas ML, Forte LR. Sex differences during the development of vitamin D deficiency in the rat: Serum parathyroid hormone, calcitonin, calcium, and phosphorus. Endocrinology **109**:1528–1532, 1981.
11. Holtrop ME, Cox KA, Clark MB, Holick MF, Anast CS. 1,25-Dihydroxycholecalciferol stimulates osteoclasts in rat bones in the absence of parathyroid hormone. Endocrinology **108**:2293–2301, 1981.
12. Harrison JE, Hitchman AJW, Jones G, Tam CS, Heersche JNM. Plasma vitamin D metabolite levels in phosphorus deficient rats during the development of vitamin D deficient rickets. Metabolism **31**:1121–1127, 1982.
13. Lester GE, VanderWiel CJ, Gray TK, Talmage RV. Vitamin D deficiency in rats with normal serum calcium concentrations. Proc Natl Acad Sci USA **79**:4791–4794, 1982.
14. Talmage RV, VanderWiel CJ. Influence of shifts in dietary calcium on postprandial renal calcium excretion in thyroid-intact and thyroidectomized rats as affected by vitamin D deficiency. Endocrinology **113**:69–76, 1983.
15. Underwood JL, Phelps ME, DeLuca HF. Complex carbohydrate diets are not capable of maintaining normal plasma calcium and phosphorus levels in vitamin D-deficient rats. Proc Natl Acad Sci USA **81**:2352–2353, 1984.
16. Favus MJ, Angeid-Backman E. Effects of $1,25(OH)_2D_3$ and calcium channel blockers on cecal calcium transport in the rat. Am J Physiol **248**:G676–G681, 1985.
17. Warner M, Tenenhouse A. Regulation of renal vitamin D hydroxylase activity in vitamin D deficient rats. Can J Physiol Pharmacol **63**:978–982, 1985.
18. Gallagher JA, Beneton M, Harvey L, Lawson DEM. Response of rachitic rat bones to 1,25-dihydroxyvitamin D_3: Biphasic effects on mineralization and lack of effect on bone resorption. Endocrinology **119**:1603–1609, 1986.
19. Holtrop ME, Cox KA, Carnes DL, Holick MF. Effects of serum calcium and phosphorus on skeletal mineralization in vitamin D-deficient rats. Am J Physiol **251**:E234–E240, 1986.
20. Cade C, Norman AW. Vitamin D_3 improves impaired glucose tolerance and insulin secretion in the vitamin D-deficient rat in vivo. Endocrinology **119**:84–90, 1986.
21. Tam CS, Heersche JNM, Jones G, Murray TM, Rasmussen H. The effect of vitamin D on bone in vivo. Endocrinology **118**:2217–2224, 1986.
22. Sonnenberg J, Luine VN, Krey LC, Christakos S. 1,25-Dihydroxyvitamin D_3 treatment results in increased choline acetyltransferase activity in specific brain nuclei. Endocrinology **118**:1433–1439, 1986.
23. Weishaar RE, Simpson RU. Vitamin D_3 and cardiovascular function in rats. J Clin Invest **79**:1706–1712, 1987.
24. Weishaar RE, Simpson RU. Involvement of vitamin D_3 with cardiovascular function II. Direct and indirect effects. Am J Physiol **253**:E675–E683, 1987.
25. Miller SC, Miller MA, Omura TH. Dietary lactose improves endochondral growth and bone development and mineralization in rats fed a vitamin D-deficient diet. J Nutr **118**:72–77, 1988.
26. Schaafsma G, Visser WJ, Dekker PR, Van Schaik M. Effect of dietary calcium supplementation with lactose on bone in vitamin D-deficient rats. Bone **8**:357–362, 1988.
27. Kwiecinski GG, Petrie GI, DeLuca HF. Vitamin D is necessary for reproductive functions of the male rat. J Nutr **119**:741–744, 1989.
28. Fox J, Kollenkirchen U, Walters MR. Deficiency of vitamin D metabolites directly stimulates renal 25-hydroxyvitamin D_3-1-hydroxylase activity in rats. Metabolism **40**:438–441, 1991.
29. Kollenkirchen U, Walters MR, Fox J. Plasma Ca influences vitamin D metabolite levels as rats develop vitamin D deficiency. Am J Physiol **260**:E447–E452, 1991.
30. Kollenkirchen U, Fox J, Walters MR. Normocalcemia without hyperparathyroidism in vitamin D-deficient rats. J Bone Miner Res **6**:273–281, 1991.
31. Gove PB (Ed). Webster's 3rd New International Dictionary. Springfield, IL: G & C Merriam Co., p605, 1969.
32. Friel JP (Ed). Dorland's Illustrated Medical Dictionary, 25th ed. Philadelphia: WB Saunders, p423, 1974.
33. Bikle DD, Gee E. Free, not total, 1,25-dihydroxyvitamin D regulates 25-hydroxyvitamin D metabolism by keratinocytes. Endocrinology **124**:649–654, 1989.
34. Reinhardt TA, Horst RL. Parathyroid hormone down-regulates 1,25-dihydroxyvitamin D receptors (VDR) and VDR messenger ribonucleic acid in vitro and blocks homologous upregulation of VDR in vivo. Endocrinology **127**:942–948, 1990.
35. Sandgren ME, DeLuca HF. Serum calcium and vitamin D regulate 1,25-dihydroxyvitamin D_3 receptor concentration in rat kidney in vivo. Proc Natl Acad Sci USA **87**:4312–4314, 1990.
36. Goff JP, Reinhardt TA, Beckman MJ, Horst RL. Contrasting effects of exogenous 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] versus endogenous $1,25(OH)_2D_3$, induced by dietary calcium restriction, on vitamin D receptors. Endocrinology **126**:1031–1035, 1990.
37. Horst RL, Goff JP, Reinhardt TA. Advancing age results in reduction of intestinal and bone 1,25-dihydroxyvitamin D receptor. Endocrinology **126**:1053–1057, 1990.
38. Hirst MA, Feldman D. Glucocorticoid regulation of $1,25\text{-}(OH)_2$ vitamin D_3 receptors: Divergent effects on mouse and rat intestine. Endocrinology **111**:12400–12405, 1982.

39. Pike JW. Vitamin D receptors: Structure and function in transcription. Annu Rev Nutr **11**:189–216, 1991.
40. Feldman D, Malloy PJ. Hereditary 1,25-dihydroxyvitamin D resistant rickets: Molecular basis and implications for the role of $1,25(OH)_2D_3$ in normal physiology. Mol Cell Endocrinol **72**:C57–C62, 1990.
41. Marx SJ, Barsony J. Tissue-selective 1,25-dihydroxyvitamin D_3 resistance: Novel applications of calciferols. J Bone Miner Res **5**:481–485, 1988.
42. Kanis JA, Drezner MK, Evans DB, Horst RL, Malluche HH, Norman AW, Thavarajah M, Uskokovic MR. Prospects for the modelling of vitamin D activity in man. In: Norman AW, Schaefer K, Grigoleit H-G, von Herrath D (Eds), Vitamin D Molecular, Cellular, and Clinical Endocrinology. Berlin: Walter de Gruyter and Co., pp739–748, 1988.
43. Brown AJ, Finch JL, Lopez-Hilker S, Dusso A, Ritter C, Pernalete N, Slatopolsky E. New active analogs of vitamin D with low calcemic activity. Kidney Int **38**(suppl 29):S22–S27, 1990.
44. Eicher EM, Southard JL, Scriver CR, Glorieux FH. Hypophosphatemia: Mouse model for human familial hypophosphatemic (vitamin D resistant) rickets. Proc Natl Acad Sci USA **73**:4667–4671, 1976.
45. Steenbock H, Herting DC. Vitamin D and growth. J Nutr **57**:449–468, 1955.
46. Armbrecht HJ, Forte LR, Halloran BP. Effect of age and dietary calcium on renal 25(OH)D metabolism, serum $1,25(OH)_2D$, and PTH. Am J Physiol **246**:E266–E270, 1984.
47. Fox J, Mathew MB. Heterogeneous response to PTH in aging rats: Evidence for skeletal PTH resistance. Am J Physiol **260**:E933–E937, 1991.
48. Epstein S, Bryce G, Hinman JW, Miller ON, Riggs BL, Hui SL, Johnston CC. The influence of age on bone mineral regulating hormones. Bone **7**:421–425, 1986.
49. Armbrecht HJ, Forte LR. Adaptation of middle-aged rats to long-term restriction of dietary vitamin D and calcium. Arch Biochem Biophys **242**:464–469, 1985.
50. Kumar R. Metabolism of 1,25-hydroxyvitamin D_3. Physiol Rev **64**:478–504, 1984.
51. Norman AW, Roth J, Orci L. The vitamin D endocrine system: Steroid metabolism, hormone receptors, and biological response. Endocrinol Rev **3**:331–355, 1982.
52. Underwood JL, DeLuca HF. Vitamin D is not directly necessary for bone growth and mineralization. Am J Physiol **246**:E493–E498, 1984.
53. Fox J, Della-Santina CP. Oral verapamil and Ca and vitamin D metabolism in rats: Effect of dietary Ca. Am J Physiol **257**:E632–E638, 1989.
54. Chesney RW, Zimmerman J, Hamstra A, DeLuca HF, Mazess RB. Vitamin D metabolite concentrations in vitamin D deficiency. Am J Dis Child **135**:1025–1028, 1981.
55. Eastwood JB, De Wardener HE, Gray RW, Lemann JL Jr. Normal plasma $1,25(OH)_2$ vitamin D concentrations in nutritional osteomalacia. Lancet **1**:1377–1378, 1979.
56. Garabedian M, Vainsel M, Mallet E, Guillozo H, Toppet M, Grimberg R, Nguyen H, Balsan S. Circulating vitamin D metabolite concentrations in children with nutritional rickets. J Pediatr**103**:381–386, 1983.
57. Mallet E, Nguyen TM, Garabedian M, Basuyau JP. Circulating parathyroid hormone and dihydroxylated vitamin D metabolites after oral 25-hydroxycholecalciferol in infantile rickets. Horm Metab Res **14**:503–506, 1982.
58. Steichen JJ, Tsang RC, Greer FR, Ho M, Hug G. Elevated serum 1,25-dihydroxyvitamin D concentrations in rickets of very low birthweight infants. J Pediatr **99**:293–297, 1981.
59. Rasmussen H, Baron R, Broadus A, De Fronzo R, Lang R, Horst R. $1,25\text{-}(OH)_2D_3$ is not the only D metabolite involved in the pathogenesis of osteomalacia. Am J Med **69**:360–368, 1980.
60. Kashiwa H, Nishi Y, Usui T, Seino Y. A case of rickets with a normal serum level of $1,25(OH)_2D$ and low 25OHD. Hiroshima J Med Sci **30**:61–63, 1981.
61. Halloran BP, Castro ME. Vitamin D kinetics in vivo: Effect of 1,25-dihydroxyvitamin D_3 administration. Am J Physiol **252**: E686–E691, 1989.
62. Fox J, Kollenkirchen U. Normal plasma calcium, phosphate and parathyroid hormone levels during $1,25(OH)_2D_3$ infusions in rats. Am J Physiol (in press).
63. Fox J. Verapamil induces PTH resistance but increases duodenal calcium absorption in rats. Am J Physiol **255**:E702–E707, 1988.
64. Lester GE, Lian JB, Talmage RV. Changes in bone and serum osteocalcin and bone density in vitamin D deficient rats. Proc 7th Workshop on Vitamin D, p181A, 1985.
65. Walters MR, Cuneo DM, Jamison AP. Possible significance of new target tissues for 1,25-dihydroxyvitamin D_3. J Steroid Biochem **19**:913–920, 1983.
66. Gensure RC, Riggle PC, Antrobus SD, Walters MR. Evidence for two classes of 1,25-dihydroxyvitamin D binding sites in classical vs nonclassical target tissues. Biochem Biophys Res Commun **180**:867–873, 1991.
67. Norman AW. The vitamin D endocrine system. Physiologist **28**:219–231, 1985.