PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 170, 53–58 (1982)

Biological Activity of 1,24(R)-Dihydroxyvitamin D_3 and 1,24(S)-Dihydroxyvitamin D_3 in the Rat[1] (41396)

C. M. SMITH, Y. TANAKA, AND H. F. DeLUCA[2]

Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin–Madison, Madison, Wisconsin 53706

Abstract. The activities of 1,24(*R*)-dihydroxyvitamin D_3 and 1,24(*S*)-dihydroxyvitamin D_3 have been compared with that of 1,25-dihydroxyvitamin D_3 in the stimulation of intestinal calcium transport, mobilization of bone calcium, elevation of serum inorganic phosphorus concentration, and in healing rickets in vitamin D-deficient rats. The 1α-hydroxyvitamin D_3 was also compared with the 1,24-dihydroxyvitamin D_3 compounds in antirachitic activity. In all parameters 1,25-dihydroxyvitamin D_3 is about 10 times more active than either 1,24(*R*)-dihydroxyvitamin D_3 and 1,24(*S*)-dihydroxyvitamin D_3. In antirachitic activity 1,25-dihydroxyvitamin D_3 is about two times more active than 1α-hydroxyvitamin D_3 which in turn is two times more active than 1,24(*R*)-dihydroxyvitamin D_3. The 1,24(*R*)-dihydroxyvitamin D_3 and 1,24(*S*)-dihydroxyvitamin D_3 compounds are equally active except in the healing of rickets where the 1,24(*S*)-dihydroxyvitamin D_3 elicits weak biological activity.

The physiological significance of 24-hydroxylation of vitamin D compounds is not known, while it is well established that physiologically 1-hydroxylation of the vitamin in the kidney is the essential structural modification for expression of biological activity (1). The most important analog of 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$)[3] is 1α-hydroxyvitamin D_3 (1α-OH-D_3) (2) that must be hydroxylated at C-25 for expression of biological activity (3). The activity of 1α-OH-D_3 was determined as one-half that of 1,25-$(OH)_2D_3$ in stimulating intestinal calcium transport and bone mineral mobilization (4). Another analog of significance is 1,24-dihydroxyvitamin D_3 (1,24-$(OH)_2D_3$) (5). As expected from results with 1,25-$(OH)_2D_3$, 1α-OH-D_3, and 1,24,25-trihydroxy vitamin D_3 (1,24,25-$(OH)_3D_3$), 1,24-$(OH)_2D_3$ stimulates intestinal calcium transport in anephric animals (6). It has been reported that 1,24-$(OH)_2D_3$ is as active as 1α-OH-D_3 and is less toxic (5–7). In this report we have compared the biological activity of 1,25-$(OH)_2D_3$ with that of 1,24(*R*)-$(OH)_2D_3$ and 1,24(*S*)-$(OH)_2D_3$.

Materials and Methods. *Animals.* Weanling male rats were purchased from the Holtzman Company (Madison, Wisc.) and were fed either a low-calcium (0.02%)–adequate phosphorus (0.3%), vitamin D-deficient diet (8) or a high-calcium (1.2%)–low-phosphorus (0.1%), vitamin D-deficient diet (9). Three weeks after they had been fed the low-calcium diet they developed hypocalcemia, and in the case of the high-calcium–low-phosphorus diet, they developed hypophosphatemia and severe rickets.

Vitamin D compounds. The 1,25-$(OH)_2D_3$ and 1α-OH-D_3 were gifts from Hoffman–La Roche (Nutley, N.J.) and Leo Pharmaceuticals (Ballerup, Denmark), respectively. The stereochemical isomers

[1] This work was supported in part by Program Project Grant AM-14881 from the National Institutes of Health, U.S.–Japan Cooperative Grant INT-8016902 from the National Science Foundation, and by the Harry Steenbock Research Fund of the Wisconsin Alumni Research Foundation.

[2] No reprints of this article will be available from the authors. Send all correspondence to H. F. DeLuca, Department of Biochemistry, 420 Henry Mall, University of Wisconsin–Madison, Madison, Wisc. 53706.

[3] Abbreviations used: 1,25-$(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; 1α-OH-D_3, 1α-hydroxyvitamin D_3; 1,24-$(OH)_2D_3$, 1,24-dihydroxyvitamin D_3; 1,24,25-$(OH)_3D_3$, 1,24,25-trihydroxyvitamin D_3; 24,25-$(OH)_2D_3$, 24,25-dihydroxyvitamin D_3.

0037-9727/82/050053-06$01.00/0

of 1,24-$(OH)_2D_3$ were synthesized by Ochi *et al.* (10). They were dissolved in ethanol and adjusted at concentrations so that each dose was given in 0.05 ml ethanol. A molar extinction coefficient of 18,200 at 264 nm was used for all compounds. In the case of subcutaneous injections, the samples were dissolved in propylene glycol/ethanol (95/5).

Intestinal calcium transport measurement. Rats were decapitated and blood was collected. Their duodena were removed immediately after decapitation and used for the measurement of calcium transport activity as described by Martin and DeLuca (11).

Serum calcium and inorganic phosphorus determination. Blood was centrifuged to yield serum. The calcium concentration was measured in the presence of 0.1% lanthanum chloride by means of a Perkin–Elmer atomic absorption spectrometer (Model 403). Inorganic phosphorus was determined by the method of Chen *et al.* (12).

Antirachitic activity measurement. The radii and ulnae of rats fed the high-calcium–low-phosphorus diet were removed, cleaned of adhering tissue, split lengthwise, and stained in 1.5% silver nitrate as described in the U.S. Pharmacopoeia (13).

Statistical evaluation. This was calculated by Student's *t* test.

Determination of biopotency. The method outlined previously (14, 15) using a daily graded dosage regime for 7 days was followed. The rats were killed and the parameter in question was measured within 12 hr of the last dose. The amount of compound required to give equivalent target organ activity was used as the basis for comparison.

Results. The data shown in Tables I through III are biological responses of rats to a single almost saturating dose of either 1,24-$(OH)_2D_3$ or 1,25-$(OH)_2D_3$ administered intrajugularly. As shown in Table I, a single dose of 65 pmole of either compound in question stimulated intestinal calcium transport significantly as early as 6 hr postadministration though the 24-hydroxy isomers showed lower activity. The *S* isomer appears less active than the *R* isomer. At 168 hr the activity of the 1,24-$(OH)_2D_3$ compounds had disappeared in contrast to that of 1,25-$(OH)_2D_3$. The data in Table

TABLE I. INTESTINAL CALCIUM TRANSPORT IN RESPONSE TO A SINGLE DOSE OF 1,25-$(OH)_2D_3$, 1,24(*R*)-$(OH)_2D_3$, OR 1,24(*S*)-$(OH)_2D_3$

Compound given	^{45}Ca serosal/^{45}Ca mucosal		
	6 hr	24 hr	168 hr
Control	$1.5 \pm 0.5^{*,a}$	1.9 ± 0.3^{a}	1.5 ± 0.2^{a}
1,25-$(OH)_2D_3$	5.6 ± 0.8^{b}	4.0 ± 0.2^{b}	2.7 ± 0.5^{b}
1,24(*R*)-$(OH)_2D_3$	3.9 ± 0.6^{c}	3.3 ± 0.4^{b}	1.9 ± 0.2^{c}
1,24(*S*)-$(OH)_2D_3$	2.6 ± 0.5^{d}	3.5 ± 0.4^{b}	1.7 ± 0.4^{d}
Significance of difference	*b,c* from *a* $P < 0.001$ *d* from *a* $P < 0.01$ *b* from *c* $P < 0.01$ *b* from *d* $P < 0.001$ *c* from *d* $P < 0.005$	*b* from *a* $P < 0.001$	*b* from *a* $P < 0.001$ *c* from *a* $P < 0.01$ *d* from *a* NS

* Standard deviation from the mean. Rats were fed a low-calcium, vitamin D-deficient diet for 3 weeks. They were then given 65 pmole of either 1,25-$(OH)_2D_3$, 1,24(*R*)-$(OH)_2D_3$, or 1,24(*S*)-$(OH)_2D_3$ dissolved in 0.05 ml ethanol intrajugularly. Rats in control group were given ethanol. Intestinal calcium transport activity was measured either 6, 24, or 168 hr after a single dose as described in text. Each group has five or six rats.

TABLE II. INCREASE IN SERUM CALCIUM IN RATS ON A LOW-CALCIUM DIET IN RESPONSE TO A SINGLE DOSE OF 1,25-$(OH)_2D_3$, 1,24(*R*)-$(OH)_2D_3$ OR 1,24(*S*)-$(OH)_2D_3$

Compound given	Serum calcium (mg/100 ml)	
	24 hr	120 hr
Control	$4.2 \pm 0.1^{*,a}$	3.9 ± 0.2^{a}
1,25-$(OH)_2D_3$	5.7 ± 0.1^{b}	4.3 ± 0.2^{b}
1,24(*R*)-$(OH)_2D_3$	4.9 ± 0.2^{c}	4.3 ± 0.2^{b}
1,24(*S*)-$(OH)_2D_3$	4.7 ± 0.1^{d}	4.1 ± 0.2^{b}
Significance of difference	*b,c,d* from *a* $P < 0.001$ *c,d* from *b* $P < 0.001$ *d* from *c* NS	*b* from *a* NS

* Standard deviation from the mean. Rats were fed a low-calcium, vitamin D-deficient diet for 3 weeks. They were then given 650 pmole of either 1,25-$(OH)_2D_3$, 1,24(*R*)-$(OH)_2D_3$, or 1,24(*S*)-$(OH)_2D_3$ dissolved in 0.05 ml ethanol intrajugularly. Rats in control group received ethanol vehicle. Twenty-four or 120 hr after a single dose of either compound, they were killed and blood was collected. Blood was centrifuged to yield serum. The serum calcium concentration was determined as described in the text. Each group had five rats.

I for 1,25-$(OH)_2D_3$ are in agreement with the previous observation that the stimulation of intestinal calcium transport by a single dose of 1,25-$(OH)_2D_3$ is highest at 6–7 hr after the dose, then subsides but to a level which is significantly higher than control (16) and sustained at that level for more than a week (15). However, the 1,24-$(OH)_2D_3$ compounds had a shorter duration of action. Thus to estimate biological potency this property must be kept in mind. Table II shows bone calcium mobilization obtained by administration of either compound at a dose level of 650 pmole, 24 hr prior to sacrifice. A rise in serum calcium in rats fed a low-calcium diet reflects mobilization of calcium from bone since absorption of calcium by intestine from the diet is negligible. All compounds show significant stimulation at 24 hr postadministration, though the 1,24-$(OH)_2D_3$ compounds appeared less active. The response to all three compounds had diminished by 120 hr after the injection.

The response of intestinal calcium transport and the elevation of serum inorganic

TABLE III. INCREASE OF INTESTINAL CALCIUM TRANSPORT AND SERUM INORGANIC PHOSPHORUS IN RESPONSE TO A SINGLE DOSE OF 1,25-$(OH)_2D_3$, 1,24(*R*)-$(OH)_2D_3$, OR 1,24(*S*)-$(OH)_2D_3$

Compound given	Intestinal Ca transport (^{45}Ca serosal/^{45}Ca mucosal)	Serum inorganic phosphorus (mg/100 ml)
Control	$1.9 \pm 0.2^{*,a}$	1.9 ± 0.3^{a}
1,25-$(OH)_2D_3$	3.6 ± 0.4^{b}	4.8 ± 0.5^{b}
1,24(*R*)-$(OH)_2D_3$	3.4 ± 0.8^{c}	4.3 ± 0.3^{c}
1,24(*S*)-$(OH)_2D_3$	3.2 ± 0.9^{d}	4.0 ± 0.2^{d}
Significance of difference	*b* from *a*, $P < 0.001$ *c* from *a*, $P < 0.005$ *d* from *a*, $P < 0.025$ *c,d* from *b*, NS	*b,c,d* from *a*, $P < 0.001$ *b* from *c*, $P < 0.05$ *b* from *d*, $P < 0.005$ *c* from *d*, $P < 0.025$

* Standard deviation from the mean. Rats were fed high-calcium–low-phosphorus, vitamin D-deficient diet for 3 weeks. They were then given 650 pmole of either 1,25-$(OH)_2D_3$, 1,24(*R*)-$(OH)_2D_3$, or 1,24(*S*)-$(OH)_2D_3$ dissolved in 0.05 ml ethanol intrajugularly. Rats in the control group received the vehicle only. Twenty hours after the administration of either compound, rats were killed and intestinal calcium transport and elevation of serum inorganic phosphorus were measured as described in the text. Each group had five or six rats.

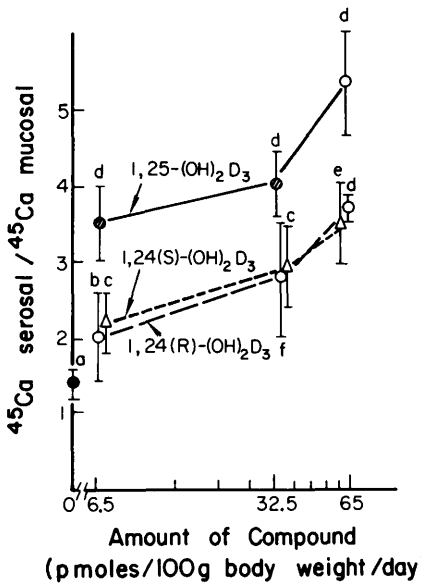

FIG. 1. Intestinal calcium transport of rats given a daily dose of 1,25-$(OH)_2D_3$, 1,24(*R*)-$(OH)_2D_3$, or 1,24(*S*)-$(OH)_2D_3$. The rats had been fed a low-calcium diet for 3 weeks and were given subcutaneously either 1,25-$(OH)_2D_3$, 1,24(*R*)-$(OH)_2D_3$, or 1,24(*S*)-$(OH)_2D_3$ dissolved in propylene glycol/ethanol (95/5, v/v) daily for 7 days. Rats in the control group received the vehicle in the same manner. Twenty-four hours after the last dose, rats were killed and their duodena were removed to measure intestinal calcium transport as described in the text. Each point represents the mean of values from five or six rats in a group, while the vertical bars at the point represent standard deviation from the mean. Significance of difference: *a* from *b*, NS; *a* from *c*, $P < 0.005$; *a* from *d*, $P < 0.001$; *a* from *e*, $P < 0.0025$; *a* from *f*, $P < 0.01$.

phosphorus of rats on a low-phosphorus rachitogenic diet is shown in Table III. All compounds produced a marked response. 1,25-$(OH)_2D_3$ appeared to produce a greater elevation in serum phosphorus.

In order to compare the biopotency of the isomers with that of 1,25-$(OH)_2D_3$ or with 1α-OH-D_3 the compounds were given each day in small doses for one week and a variety of parameters known to be responsive to vitamin D were measured before the effect of the last dose of the shortest acting compound could diminish. Figure 1 shows the intestinal calcium transport activity supported by daily dosing with 1,25-$(OH)_2D_3$, 1,24(*R*)-$(OH)_2D_3$, or 1,24(*S*)-$(OH)_2D_3$. It appears that the 24 isomers are barely active at low levels though marked stimulation is observed at higher dose levels. In contrast, the response to 1,25-$(OH)_2D_3$ is very significant even at the lowest level of the dosage tested. Since 65 pmole of the 1,24-$(OH)_2D_3$ compounds were required to produce a response almost equal to that produced by 6.5 pmole 1,25-$(OH)_2D_3$, it appears that 1,25-$(OH)_2D_3$ is 10 times more effective than 1,24-$(OH)_2D_3$ in the stimulation of intestinal calcium transport. The bone calcium mobilization activity of 1,25-$(OH)_2D_3$ and 1,24-$(OH)_2D_3$ compounds are compared in Fig. 2. All compounds in question increased serum calcium levels significantly at all dose levels; however, the same level of activity is given by 6.5 pmole of 1,25-$(OH)_2D_3$ and 65 pmole 1,24$(OH)_2D_3$. Similarly, 1,25-$(OH)_2D_3$ is 10 times more active in increasing serum inorganic phosphorus than are the 1,24-$(OH)_2D_3$ compounds (Fig. 3). Figure 4 illustrates that 1,25-$(OH)_2D_3$ gives 4 units of antirachitic activity/32.5 pmole while 1α-OH-D_3 gave 2.5 units/32.0 pmole and 1,24(*R*)-$(OH)_2D_3$

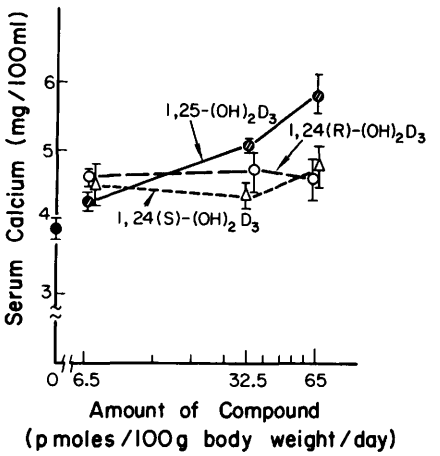

FIG. 2. Serum calcium concentration of rats given a daily dose of 1,25-$(OH)_2D_3$, 1,24(*R*)-$(OH)_2D_3$, or 1,24(*S*)-$(OH)_2D_3$. The rats were prepared and administered either compound as described in Fig. 1. Twenty-four hours after the last dose, they were killed and blood was collected. Serum calcium was determined as described in the text. Each point represents the mean of values from five or six rats in a group, while the vertical bars represent standard deviation from the mean. All points obtained by administration of any compound are significantly different from control level (0 dosage) $P < 0.001$.

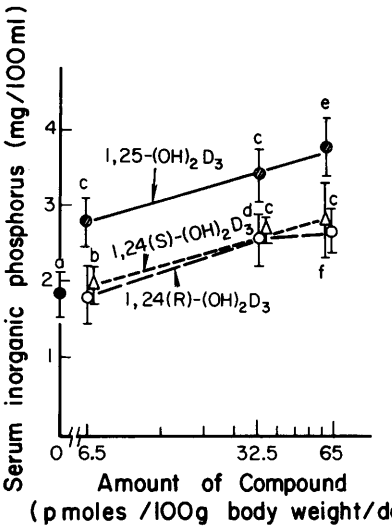

FIG. 3. Serum inorganic phosphorus concentration of rats given a daily dose of 1,25-$(OH)_2D_3$, 1,24(*R*)-$(OH)_2D_3$, or 1,24(*S*)-$(OH)_2D_3$. The rats which had been fed high-calcium–low-phosphorus, vitamin D-deficient diet for 3 weeks were given either compound as described in Fig. 1. Twenty-four hours after the last dose, rats were killed and their blood was collected. Inorganic phosphorus was determined on serum. Each point represents the mean of values from five or six rats in a group, while the vertical bars at the points represent standard deviation from the mean. Significance of difference: *a* from *b*, NS; *a* from *c*, $P < 0.005$; *a* from *d*, $P < 0.01$; *a* from *e*, $P < 0.001$; *a* from *f*, $P < 0.025$.

gave 1.5 units/32.5 pmole. The *S* isomer has little or no activity in this system at the dosage given.

Discussion. The present report demonstrates that 1,24(*R*)-$(OH)_2D_3$ and 1,24(*S*)-$(OH)_2D_3$ have significant biological activity in vitamin D-deficient rats, but they are considerably less active than 1,25-$(OH)_2D_3$ (10 times). This is not surprising since 1,24-$(OH)_2D_3$ would be expected to be metabolized to 1,24,25-$(OH)_3D_3$ (17,18) that is in turn about 1/10th as active as 1,25-$(OH)_2D_3$. Since 1α-OH-D_3 is metabolized to 1,25-$(OH)_2D_3$ (3) its biological activity is expected to be high. From previous work it seems that 1α-OH-D_3 is about one-half as active as 1,25-$(OH)_2D_3$ in rats (15), chicks (19), and man (20). The present data agree with these previous results, namely 1α-OH-D_3 is one-half as active as 1,25-$(OH)_2D_3$ in antirachitic activity (Fig. 4). 1α-OH-D_3 certainly appeared more active than 1,24-$(OH)_2D_3$ in antirachic activity (Fig. 4). By inference from the literature (4, 19, 20) it would appear that 1α-OH-D_3 is at least two times more active than 1,24(*R*)-$(OH)_2D_3$ in all systems measured. This would appear to be inconsistent with a previous claim that the two compounds are equal in biological activity (6, 7). Our results do not support that conclusion but we do agree that 1,24(*R*)-$(OH)_2D_3$ is a potent 1,25-$(OH)_2D_3$ analog.

We did not compare toxicity of 1α-OH-D_3 and the 1,24-$(OH)_2D_3$ analogs but if 1,24-$(OH)_2D_3$ compounds are less active than 1α-OH-D_3, lower toxicity would be expected.

The two 1,24-$(OH)_2D_3$ isomers were found approximately equal in biological activity except in antirachitic activity where 1,24(*S*)-$(OH)_2D_3$ proved to be much less active. These results are similar to the finding that 1,24(*R*),25-$(OH)_3D_3$ and 1,24-

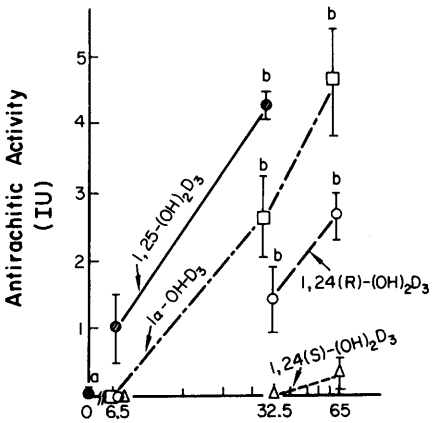

FIG. 4. Antirachitic activity of 1α-OH-D_3, 1,25-$(OH)_2D_3$, 1,24(*R*)-$(OH)_2D_3$, or 1,24(*S*)-$(OH)_2D_3$. Rats were prepared as described in Fig. 3, and administered either compound as described in Fig. 1. Twenty-four hours after the last dose, they were killed and their radii and ulnae were removed to determine epiphyseal plate calcification as described in the test. Each point represents the mean of values from five or six rats in a group, while the vertical bars at the points represent standard deviation from the mean. Significance of difference: *a* from *b*, $P < 0.001$.

(*S*),25-$(OH)_3D_3$ are equal in biological activity (18). On the other hand, 24(*R*),25-dihydroxyvitamin D_3 (24(*R*),25-$(OH)_2D_3$) and 24(*S*),25-$(OH)_2D_3$ are distinctly different in biological activity (21). This results from the 1α-hydroxylase system being more active on the 24(*R*)-25-$(OH)_2D_3$ than on the 24(*S*),25-$(OH)_2D_3$.

It should be noted that the data in Tables I–III were obtained to learn of a possible difference in the time course of response and decay of the response to the different analogs. These data cannot be used to assess biological potency *except* to obtain a rough estimate. We chose chronic administration of graded doses for 7 days and parameters were measured such that the decay of the response is not a factor. Biopotency was estimated from the amount of compound required to give an equal level of target organ activity. Thus 10 times more 1,24(*R*)-$(OH)_2D_3$ than 1,25-$(OH)_2D_3$ was required to give identical calcium transport activity or bone calcium mobilization level.

1. DeLuca HF. The vitamin D system in the regulation of calcium and phosphorus metabolism. Nutr Rev **37**:161–193, 1979.
2. Holick MF, Semmler EJ, Schnoes HK, DeLuca HF. 1α-Hydroxy derivative of vitamin D_3: A highly potent analog of 1α,25-dihydroxyvitamin D_3. Science **180**:190–191, 1973.
3. Holick MF, Tavela TE, Holick SA, Schnoes HK, DeLuca HF, Gallagher BM. Synthesis of 1α-Hydroxy[6-^{3}H]vitamin D_3 and its metabolism to 1α,25-dihydroxy[6-^{3}H]vitamin D_3 in the rat. Biol Chem **251**:1020–1024, 1976.
4. Holick MF, Kasten-Schraufrogel P, Tavela T, DeLuca HF. Biological activity of 1α-hydroxyvitamin D_3 in the rat. Arch Biochem Biophys **166**:63–66, 1975.
5. Morisaki M, Koizumi N, Ikekawa N, Takeshita T, Ishimoto S. Synthesis of active forms of vitamin D. Part IV. Synthesis of 1α-24-dihydroxycholecalciferol. J Chem Soc Perkin Trans 1:1421–1424, 1975.
6. Kawashima H, Hosina K, Hashimoto Y, Takeshita T, Ishimoto S, Noguchi T, Ikekawa N, Morisaki M, Orimo H. Biological activity of 1α,24-dihydroxycholecalciferol; a new synthetic analog of the hormonal form of vitamin D. FEBS Lett **76**:177–181, 1977.
7. Kawashima H, Hoshina K, Ishizuka S, Hashimoto Y, Takeshita T, Ishimoto S, Noguchi T, Ikekawa N, Morisaki H, Orimo H. Biological activity of 1α,24-dihydroxyvitamin D_3; A new synthetic analog of the hormonal form of vitamin D. In: Normal AW, Coburn JW, DeLuca HF, Fraser D, Grigoleit HG, Herrath Dv, eds. Vitamin D: Biochemical, Chemical and Clinical Aspects Related to Calcium Metabolism. Berlin/New York, de Gruyter, p143, 1977.
8. Suda T, DeLuca HF, Tanaka Y. Biological activity of 25-hydroxyergocalciferol in rats. J Nutr **100**:1049–1052, 1970.
9. Tanaka Y, DeLuca HF. Role of 1,25-dihydroxyvitamin D_3 in maintaining serum phosphorus and curing rickets. Proc Nat Acad Sci USA **71**:1040–1044, 1974.
10. Ochi K, Matsunaga I, Shindo M, Kaneko C. Synthesis of 1α,24(R)- and 1α,24(S)-dihydrocholesterol from cholic acid derivatives. Chem Pharm Bull **26**:2386–2387, 1978.
11. Martin DL, DeLuca HF. Influence of sodium on calcium transport by the rat small intestine. Amer J Physiol **216**:1351–1359, 1969.
12. Chen PS Jr, Toribara TY, Warner H. Microdetermination of phosphorus. Anal Chem **28**:1756–1758, 1956.
13. United States Pharmacopoeia, 15th rev. ed. Easton, Pa, Marck Publ Co, p889, 1955.
14. Tanaka Y, Frank H, DeLuca HF. Biological activity of 1,25-dihydroxyvitamin D_3 in the rat. Endocrinology **92**:417–422, 1973.
15. Tanaka Y, DeLuca HF. Role of 1,25-dihydroxyvitamin D_3 in maintaining serum phosphorus and curing rickets. Proc Nat Acad Sci USA **71**:1040–1044, 1974.
16. Tanaka Y, DeLuca HF. Bone mobilization activity of 1,25-dihydroxycholecalciferol, a metabolite of vitamin D. Arch Biochem Biophys **146**:574–578, 1971.
17. Holick MF, Kleiner-Bossaller A, Schnoes HK, Kasten PM, Boyle IT, DeLuca HF. 1,24,25-Trihydroxyvitamin D_3: A metabolite of vitamin D_3 effective on intestine. J Biol Chem **248**:6691–6696, 1973.
18. Castillo L, Tanaka Y, DeLuca HF, Ikekawa N. On the physiological role of 1,24,25-trihydroxyvitamin D_3. Mineral Electrolyte Metab **1**:198–207, 1978.
19. Boris A, Hurley JF, Trmal T. Relative activities of some metabolites and analogs of cholecalciferol in stimulation of tibia ash weight in chicks otherwise deprived of vitamin D. J Nutr **107**:194–198, 1977.
20. Neer RM, Holick MF, DeLuca HF, Potts JT Jr. Effects of 1α-hydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_3 on calcium and phosphorus metabolism in hypoparathyroidism. Metabolism **24**:1403–1413, 1975.
21. Tanaka Y, DeLuca HF, Ikekawa N, Morisaki M, Koizumi N. Determination of stereochemical configuration of the 24-hydroxyl group of 24,25-dihydroxyvitamin D_3 and its biological importance. Arch Biochem Biophys **170**:620–626, 1975.
22. Tanaka Y, DeLuca HF, Akaiwa A, Morisaki M, Ikekawa N. Synthesis of 24S and 24R-hydroxy-[24-^{3}H]vitamin D_3 and their metabolism in rachitic rats. Arch Biochem Biophys **177**:615–621, 1976.

Received July 1, 1981. P.S.E.B.M. 1982, Vol. 170.