per continental rise of Antarctica, similar to that in areas to the east (*2, 5, 11*). When it comes into contact with the north-south trending Kerguelen Plateau, the Antarctic Bottom Water will be diverted first to the north and then to the east (*13*), where our evidence indicates strong erosive activity in northern parts of the South Indian Basin.

The South Indian Basin is separated from the South Australian Basin to the north by the Southeast Indian Ridge (Fig. 1). A major problem in mapping deep bottom water circulation in the Southeast Indian Ocean is locating the supply route for bottom waters northward to the western sector of the South Australian and Wharton Basins, where the geologic evidence shows major erosive activity by deep bottom waters. The two major basins in the northern part of the Indian Ocean, the Bengal and Wharton Basins, are divided by the continuous north-south trending Ninetyeast Ridge and hence must receive separate branches of Antarctic Bottom Water from the south. The temperature of the Bengal Basin water, which is nearly a degree warmer then the Wharton Basin water (*13*), confirms this point. There appear to be two possible supply routes to the South Australian and Wharton Basins (see Fig. 1). The first is via a broad sector of the mid-ocean ridge northeast of the Kerguelen Plateau, where water depths range from about 3000 to 5000 m. The second is northward through some sector of the east-west trending Southeast Indian Ridge. None of our data suggest any major bottom water transport northeast of the Kerguelen Plateau. On the contrary, this area has experienced relatively quiescent bottom conditions as far westward as 110°E. Evidence for strong bottom current activity does occur, however, in narrow zones on the Southeast Indian Ridge at 110°E and at 120°E, suggesting that the supply route of bottom water to the South Australian Basin may occur in this area (Fig. 1). This interpretation differs from that of Burckle *et al.* (*14*) who, on the basis of the distribution of Antarctic diatoms apparently dispersed northward by Antarctic Bottom Water, postulated a major northward flow between 95°E and 100°E. All of the bottom photographs and piston cores we have analyzed from this area, however, show no evidence of major bottom current activity. On the other hand, if the proposed conduit is very narrow, its effect may not be detectable by our methods.

In the western part of the South Australian Basin, Antarctic Bottom Water flows in two directions. A major eastward flow through the basin eventually supplied highly active bottom waters to the South Tasman Basin south and east of Tasmania (*2*). Another major current flows northward through the western sector, creating substantial erosion and the development of the Southeast Indian Ocean Manganese Pavement. This northward flow continues to the Wharton Basin through a gap between the east-west trending Broken Ridge and Naturaliste Plateau (Fig. 1), which themselves form major barriers to any northward deep flow. Within this basin and on the continental margin of Western Australia, the presence of highly condensed Cenozoic sequences or major Cenozoic disconformities (*15, 16*) indicates that northward-flowing erosive bottom waters have been a major long-term feature of the southeast Indian Ocean.

In conclusion, we have demonstrated that sediment of the deeper parts of the southeast Indian Ocean and the Kerguelen Plateau have been subjected to long-term erosion or diminished accumulation rates. In the former regions this dynamic activity has been intimately associated with development of a major manganese nodule field.

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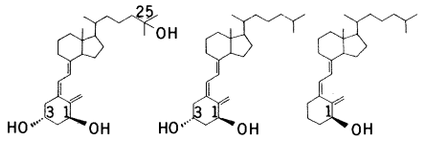
Vitamin D: 3-Deoxy-1α-Hydroxyvitamin D_3, Biologically Active Analog of 1α,25-Dihydroxyvitamin D_3

Abstract. *The ability of chemically synthesized 3-deoxy-1α-hydroxyvitamin D_3, an analog of the biologically active form of vitamin D_3 (1α,25-dihydroxyvitamin D_3), to stimulate intestinal calcium transport was assessed. The 3-deoxy analog acted significantly more rapidly than vitamin D_3 and only slightly slower than 1α,25-dihydroxyvitamin D_3. Comparison of the dose-response curves of these three vitamin D derivatives emphasizes the importance of the 3β-hydroxyl group to biological activity.*

For vitamin D to carry out its classical biological functions in stimulating intestinal calcium transport and mobilizing bone calcium, it must first be hydroxylated on C-25 (*1*) in the liver and then on C-1 by the kidney (*2*). The metabolite produced, 1α,25-dihydroxyvitamin D_3 [1α,25-$(OH)_2$-D_3], is considered to be the hormonally active form of vitamin D_3 (*3*); it is the most rapid and highly biologically active form of the vitamin in terms of stimulating intestinal calcium transport (*4*) or bone calcium mobilization (*5*). The mode of action of 1α,25-$(OH)_2$-D_3 in the intestine is believed to be analogous to that of other steroid hormones: it first associates with a cytoplasmic receptor (*6*), then is transferred to the nucleus where it associates with the chromatin (*7*), which results ultimately in synthesis of calcium-binding protein (*8*) and enhancement of calcium transport.



1 2 3

Fig. 1. Structures of 1α,25-$(OH)_2$-D_3 (**1**), 1α-OH-D_3 (**2**), and 3-D-1α-OH-D_3 (**3**).

Because of the number of pathological conditions that might be due to abnormalities in vitamin D metabolism, it is of interest to determine the relationships between the chemical structure of 1α,25-$(OH)_2$-D_3 and its biological function in the target organ. The osteomalacia and hypocalcemia associated with uremia, vitamin D–resistant rickets, or antagonism of vitamin D action by anticonvulsant drugs have all been related in some degree to abnormalities in vitamin D metabolism (*9*). Norman, Coburn, and co-workers (*10*)

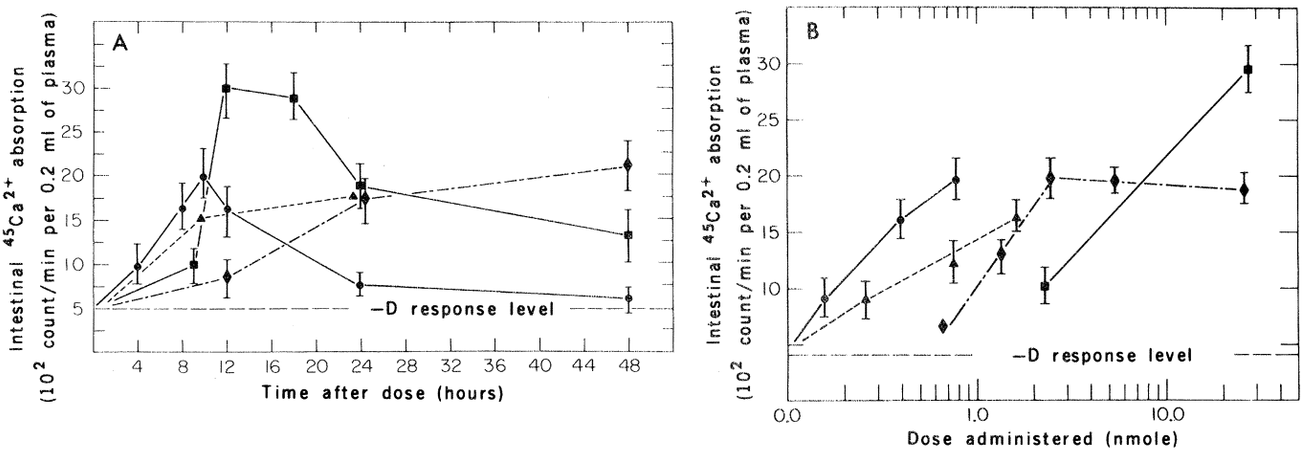


Fig. 2. (A) Intestinal calcium transport in vitamin D–deficient chicks in response to intraperitoneal injection of 3.25 nmole of vitamin D_3 (◆), 0.65 nmole of 1α,25-$(OH)_2$-D_3 (●), and 32.5 nmole of 3-D-1α-OH-D_3 (■). The response to 1.2 nmole of 1α-OH-D_3 (▲) is also indicated at 9 and 24 hours. The vertical bars represent the standard errors of the means (S.E.M.); eight to ten birds were used for each measurement. The –D response level indicates calcium absorption by vitamin D–deficient, rachitic chicks. (B) Stimulation of intestinal calcium transport by increasing doses of 1α,25-$(OH_2$-D_3 (●) and the analogs 1α-OH-D_3 (▲), vitamin D_3 (◆), and 3-D-1α-OH-D_3 (■). Drugs were given intraperitoneally to groups of eight to ten rachitic birds. The interval between injection and assay was 10 hours for 1α-OH-D_3 and 1α,25-$(OH)_2$-D_3, 14 hours for 3-D-1α-OH-D_3, and 24 hours for vitamin D_3. Values are the mean ± S.E.M. Tsai *et al.* (*20*) reported that a dose of 0.05 nmole of 1α,25-$(OH)_2$-D_3 to rachitic chicks saturates the intestinal chromatin receptors and also produces a maximum stimulation of intestinal calcium transport.

have reported that administration of small amounts of 1α,25-$(OH)_2$-D_3 can effectively bypass the renal impairment of vitamin D metabolism associated with chronic renal failure and can stimulate intestinal calcium transport.

An attractive approach to the structure-activity problem is to assess the contribution of the key functional groups (the 1-, 3-, and 25-hydroxyl groups) and the topology of the carbon skeleton of 1α,25-$(OH)_2$-D_3 to its biological activity in the intestine (Fig. 1). In view of the results of Okamura and colleagues (*11*), particular emphasis should be focused on the A ring of vitamin D. This ring exists in solution in two different chair conformations so that the key 1α-hydroxyl may be either equatorial or axial. Previous reports have assessed the contribution of the 25-hydroxyl group [25-hydroxyvitamin D_3 (25-OH-D_3) (*12*); 5,6-*trans*-25-OH-D_3 (*13*)], the positioning of the C-19 methyl group [5,6-*trans*-vitamin D_3 and 5,6-*trans*-25-OH-D_3 (*13*)], and the prime importance of the 1-hydroxyl group [1α-hydroxyvitamin D_3 (1α-OH-D_3) (*14*)] to the biological activity of various analogs. In fact, 1α-OH-D_3 already appears to be of clinical value in treatment of calcium disorders of uremia, as it too can bypass the kidney and stimulate intestinal calcium absorption. However, no information is available concerning the contribution of the 3β-hydroxyl group to the biological activity of 1α,25-$(OH)_2$-D_3.

We recently completed the synthesis of 3-deoxy-1α-hydroxyvitamin D_3 (3-D-1α-OH-D_3) (*15*) and report here on its ability to stimulate intestinal calcium transport relative to other vitamin D metabolites and analogs. The biological activity of 3-D-1α-OH-D_3, 1α,25-$(OH)_2$-D_3, 1α-OH-D_3, and vitamin D_3 were examined by the in vivo intestinal calcium transport assay of Coates and Holdsworth (*16*), as modified by Hibberd and Norman (*17*). In this assay, at varying times after oral or intraperitoneal administration of the compound being tested, a 2.0-mg portion of a mixture of radioactive and nonradioactive Ca^{2+} (10 μc) is placed in an exposed duodenal loop. The appearance of $^{45}Ca^{2+}$ in the plasma is determined 30 minutes later. All compounds examined were dissolved in 0.2 ml of 1,2-propanediol and administered intraperitoneally. Controls received 0.2 ml of 1,2-propanediol.

As shown in Fig. 2A, 3-D-1α-OH-D_3 is biologically active. With this compound, the onset of stimulation of intestinal calcium transport was significantly more rapid than that mediated by vitamin D_3. Maximum stimulation of intestinal calcium transport occurred at 9 to 11 hours for 1α,25-$(OH)_2$-D_3, 12 to 16 hours for both 3-D-1α-OH-D_3 and 1α-OH-D_3, and 36 to 48 hours for vitamin D_3. These results are consistent with the view that time is required for the successive two-step hydroxylation in the liver and kidney. Thus, 1α,25-$(OH)_2$-D_3 is capable of interacting directly in the intestine without further metabolism, while both 1α-OH-D_3 and 3-D-1α-OH-D_3 may require some time to become hydroxylated at C-25 in the liver (*18*).

Figure 2B presents dose-response curves for stimulation of intestinal calcium transport by the four compounds. Each compound was tested at the time (Fig. 2A) of maximum stimulation of calcium transport. The doses that produced 50 percent of maximum response were 0.23 nmole for 1α,25-$(OH)_2$-D_3, 0.56 nmole for 1α-OH-D_3, 1.1 nmole for vitamin D_3, and 8.0 nmole for 3-D-1α-OH-D_3. Thus the ratio of amounts of these steroids required to produce 50 percent of maximum response in the intact animal was 1 : 2 : 5 : 35. Since it is known that vitamin D_3 is hydroxylated at both C-1 and C-25 and it is suspected that 1α-OH-D_3 is hydroxylated at C-25 (*18*), then the activities of 1α-OH-D_3 and vitamin D_3 relative to that of 1α,25-$(OH)_2$-D_3 reflect, in one sense, the efficiency of metabolism of the two former compounds to 1α,25-$(OH)_2$-D_3. Thus it is difficult in the intact animal to quantitatively assess the effect of the absence of the 25-hydroxyl groups on biological activity. This can only be done in a system, such as the embryonic chick intestinal culture system described by Corradino (*19*), in which a biological response can be produced but no significant amount of hydroxylation at C-25 is believed to occur. However, there are no reports of the existence of steroid 3β-hydroxylases in higher animals. Thus the 14 : 1 ratio of biological activities of 1α-OH-D_3 and 3-D-1α-OH-D_3 is probably a reflection of the essential contribution of the 3β-hydroxyl group of 1α,25-$(OH)_2$-D_3 for binding to intestinal receptors or for efficient hydroxylation at C-25.

3-Deoxy-1α-hydroxyvitamin D_3 produced a greater degree of stimulation of intestinal calcium transport than the other three compounds. This can be explained most simply in terms of our recent model

(*11*). We proposed that the conformations of the A ring of vitamin D steroids might be of critical importance to their biological activity. Thus, the predominant chair conformation of 3-D-1α-OH-D_3 present in solution would be predicted to have most of its 1α-hydroxyls in the equatorial conformer, while 1α-OH-D_3 would be anticipated to have its 1α-hydroxyl in a 1 : 1 equilibrium mixture of axial and equatorial orientations.

These results emphasize both the complexity of a precise determination of structure-activity relationships for analogs of 1α,25-$(OH)_2$-D_3 and the possibility of designing new molecular species that enhance or diminish facets of its activity. Not only is the presence or absence of hydroxyls on a putative analog of importance, but also consideration of their precise orientation or conformation must be respected.

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Corn Stunt Spiroplasma: Isolation, Cultivation, and Proof of Pathogenicity

Abstract. *A spiroplasma isolated from corn infected with corn stunt disease has been successfully cultivated in vitro. Acid formation was associated with its growth in liquid medium, and fried egg–shaped or granulated colonies developed on solid agar medium. Healthy leafhopper vectors injected with or allowed to feed on isolates from various serial passages through liquid medium were able to transmit the corn stunt agent to healthy corn plants, inducing typical corn stunt disease in the plants. The spiroplasma was reisolated and cultivated from such diseased plants and was indistinguishable morphologically from the original isolates. These results provide the first definite proof that the corn stunt disease is caused by the corn stunt spiroplasma.*

Corn stunt (CS) is a serious disorder of corn (*Zea mays* L.) occurring in the southern United States and in Central and South America. It is one of the many yellows-type plant diseases suspected to be caused by mycoplasma-like organisms. Numerous attempts have been made to cultivate the causal agents of these diseases in cell-free media. Chen and Granados (*1*) achieved a long-term maintenance of CS infectivity in a liquid medium. Although no colonies were obtained on agar, evidence from negative staining and ultrathin-section electron microscopy of mycoplasma-like organisms maintained in vitro, as well as infectivity tests, suggested that limited growth did take place in the primary cultures and that the organism in culture was the CS agent (*2*).

Davis *et al.* (*3*) reported that, unlike mycoplasmas, the presumed CS agent possesses a helical-filamentous morphology and exhibits a definite contractile motility. The trivial term "spiroplasma" was proposed for the microorganism (*4*). Subsequently, another spiroplasma was dis-

Table 1. Transmission of CS spiroplasmas from various successive subcultures in C-3 medium by *D. elimatus* either injected with or fed on the cultures; the primary culture was derived from corn plants infected with CS disease. Reisolations of CS spiroplasmas were made from inoculated corn plants showing typical CS symptoms. In experiments 1 to 4 insects were injected with cultured spiroplasma; in experiment 5 insects were fed with cultured spiroplasma in 6 percent sucrose solution. In all experiments the control insects (50 leafhoppers injected with sterile C-3 medium or allowed to feed on pure sucrose solution) were not infective.

Experiment	Isolate	Passage at bioassay	Insects injected (*N*)	Incubation period + inoculation test feed (days)	Insects surviving at end of test feed (*N*)	Plants infected/ plants inoculated	Positive reisolations/ diseased plants used
1	746	3	85	14+7	10	9/10	3/3
2	746	4	89	14+7	32	26/32	2/2
3	747	3	120	14+7	62	35/62	3/3
4	747	8	106	0+10	61	0/61	0/2
				10+5	46	28/46	2/2
				15+5	37	21/37	
				20+5	26	26/26	
5	746	5	30	0+7	23	0/23	0/1
				7+7	17	10/17	2/2
				14+7	11	8/11	
				21+7	8	6/8	