could readily explain the occurrence of fibrin at sites where the endothelium is nominally intact.

The thrombogenic potential of tissue factor is illustrated by the high concentrations found in human atheromata. Ulceration of a plaque would then place tissue factor in contact with factor VII, thereby allowing initiation of the extrinsic system.

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10. Supported by grant HE-09057 from the National Institutes of Health and by a grant from the American Heart Association. Y.N. is an established investigator of the American Heart Association.

19 August 1971; revised 28 October 1971 ■

1,25-Dihydroxycholecalciferol: A Potent Stimulator of Bone Resorption in Tissue Culture

Abstract. *1,25-Dihydroxycholecalciferol (DHCC), isolated from kidney homogenates incubated with 25-hydroxycholecalciferol (HCC), stimulated the release of previously incorporated ^{45}Ca from fetal rat bones in organ culture, at concentrations of 10^{-10} to 10^{-8}M. The dose response curves for 1,25-DHCC and 25-HCC, the parent compound, are parallel, but 1,25-DHCC is about 100 times as potent on a weight basis. Brief exposure to maximum doses of either agent leads to prolonged bone resorption.*

Vitamin D_3 is hydroxylated in the liver to produce 25-hydroxycholecalciferol (HCC), which then enters the circulation and is taken up in target tissues (*1*). The active form of vitamin D was considered to be 25-HCC because it could stimulate intestinal transport of calcium and bone resorption directly in isolated systems (*2*). However, 25-HCC is further transformed to other active metabolites, one of which has now been identified as 1,25-dihydroxycholecalciferol (DHCC) (*3*). The second hydroxylation is carried out largely in the kidney (*4*). On the assumption that it is the active component of the more polar products than 25-HCC previously identified as peaks V, 4-B, or P (*5*), 1,25-DHCC appears to act on the gut more rapidly than 25-HCC but is less effective in curing rickets in rats. The experiments reported here were designed to test whether the second hydroxylation affects direct stimulation of bone resorption in organ culture.

Table 1. Relative potency of 1,25-dihydroxycholecalciferol (DHCC) and 25-hydroxycholecalciferol (HCC). Values for potency ratios (with 95 percent fiducial limits in parentheses) and for the index of precision were obtained with the use of a computer program for parallel line bioassay (*14*).

Assay	Potency ratio DHCC/HCC	Index of precision
3 × 3	103 (42–266)	0.45
2 × 2	332 (157–790)	0.30

The culture methods have been described (*6*). Bone shafts from 19-day rat fetuses labeled with ^{45}Ca in vivo were first incubated for 24 hours in a chemically defined medium supplemented with 5 percent human serum inactivated at 60°C for 30 minutes. Paired bones were transferred to vessels containing the same medium with or without 1,25-DHCC or 25-HCC dissolved in ethanol. Equal amounts of ethanol were added to control cultures. The final concentration was less than 0.5 percent.

1,25-DHCC was prepared by incubation of 25-[26,27-^{3}H]HCC (320 dpm/ng) with kidney homogenates from rachitic chicks (*4*). The products were isolated by Sephadex LH-20 chromatography (*7*). 25-HCC was prepared by chemical synthesis (*8*).

1,25-DHCC produced a graded increase in ^{45}Ca release from fetal bones in culture at concentrations of 0.025 to 10 ng/ml, or 10^{-10} to 10^{-8}*M* (Fig. 1). The dose response curves for pooled data indicate that a much higher concentration of 25-HCC was required to produce the same response. In two individual bioassays, the mean potency ratios for 1,25-DHCC to 25 HCC were 103 and 332 (Table 1). The higher ratio in the second assay was ascribable to a diminished response to 25-HCC.

1,25-DHCC shares with parathyroid hormone (PTH) and 25-HCC the ability to produce a prolonged increase in bone resorption, which we have termed induction (*9*). Significant stimulation of 48-hour ^{45}Ca release was obtained with as little as 30-minute exposure, although the response was less than to continuous exposure (Table 2). Six-hour exposure to either 1,25-DHCC at 0.025 μg/ml or 25-HCC at 80 times the concentration gave nearly maximum resorption.

The greater potency of 1,25-DHCC as compared to that of 25-HCC in mobilizing calcium in vitro, but not in vivo, could be due to differences in the concentrative uptake by bone cells. Labeled 25-HCC is taken up by bone

Table 2. Effect of brief exposure to 25-hydroxycholecalciferol (HCC) or 1,25-dihydroxycholecalciferol (DHCC) on subsequent release of ^{45}Ca from fetal rat bones in 48-hour culture. Paired bones were incubated with or without the 25-HCC or 1,25-DHCC for the indicated times and then transferred to fresh medium. Values are mean ± S.E. for ratio of treated to control cultures for the cumulative 48-hour ^{45}Ca release from four pairs of cultures.

Dose (μg/ml)	Time (hours)	^{45}Ca release (treated/control)
	25-HCC	
2	6	2.60 ± 0.29*
	1,25-DHCC	
0.025	½	1.65 ± 0.15*†
0.025	2	1.84 ± 0.13*†
0.025	6	2.11 ± 0.15*
0.025	48	2.82 ± 0.20*

* Significantly different from 1.0; $P < .02$. † Significantly different from response in 48-hour continuous culture; $P < .05$.

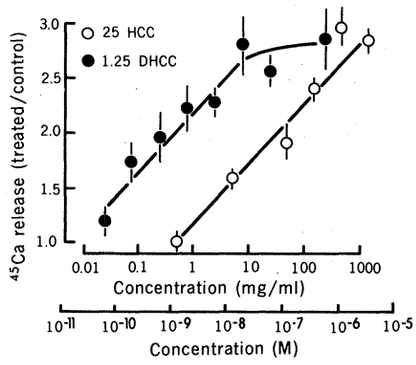

Fig. 1. Comparison of response to 1,25-dihydroxycholecalciferol (DHCC) and 25-hydroxycholecalciferol (HCC). Bones were first cultured for 24 hours, and then paired cultures were treated with or without 1,25-DHCC or 25-HCC at the indicated doses. Points indicate the means and vertical lines one standard error for the ratio of treated to control cultures of 48-hour ^{45}Ca release in 4 to 16 pairs of cultures. Data are pooled from five culture experiments.

in vivo, but is not concentrated by bone in culture (*10*). The specific activity of the 1,25-DHCC used in our experiments was sufficient to have detected a tenfold or greater concentrative uptake in bone cells. However, the radioactivity in chloroform-methanol extracts of bones treated with 1,25-DHCC was no greater than would be expected for an equivalent amount of medium. We cannot rule out a higher concentrative uptake in a small proportion of the bone cells.

The increased potency of 1,25-DHCC is specific to the extent that 21,25-DHCC, the only other dihydroxy metabolite tested in this system, is considerably less potent than 25-HCC (*11*). Moreover, the second hydroxylation does not appear to be required for 25-HCC to act. When labeled 25-HCC is incubated with fetal bones, there is no 1,25-DHCC detectable in the tissue or the medium (*12*).

Our studies do not tell us whether 1,25-DHCC acts on bone in vivo. Although the final level of mobilization achieved is no greater, 1,25-DHCC acts more rapidly than 25-HCC in mobilizing bone mineral in vivo in vitamin D–deficient rats (*13*). Because 25-HCC is present in plasma at much higher concentrations than 1,25-DHCC and probably has a longer half-life, it is possible that the physiologic or pathologic effects of vitamin D on bone resorption are actually mediated by the less potent metabolite. A low concentration in serum or short half-life could explain the low potency of 1,25-DHCC in curing rickets or mobilizing calcium in vitamin D–deficient rats. Whatever physiological role of 1,25-DHCC, the discovery of such a potent stimulator of bone resorption is of great interest.

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28 July 1971; revised 24 September 1971 ■

Spermatogenesis in Cultured Testes of the Cynthia Silkworm: Effects of Ecdysone and of Prothoracic Glands

Abstract. *In vitro spermatogenesis takes place when intact testes are cultured in blood plasma containing ecdysone or certain other steroids possessing ecdysone activity. The ecdysone requirement can be satisfied by culturing the testes in the presence of living, active prothoracic glands. The most likely explanation of these results is that the prothoracic glands constitute the principal source of ecdysone.*

Insect molting and metamorphosis depend on a hormone secreted by the prothoracic glands (*1*). In the absence of this hormone virtually all aspects of postembryonic development come to an abrupt halt. The hormone in question is thought to be ecdysone—a polyhydroxylated steroid first isolated in 1954 (*2*). By the injection of ecdysone, one can provoke all the developmental reactions that would otherwise require the presence of active prothoracic glands (*3*).

On the basis of this convincing but nonetheless circumstantial evidence, a long-standing principle of insect endocrinology is that ecdysone is synthesized and secreted by the prothoracic glands when the latter are activated by brain hormone (*4*). Manifestly one must allow for more complicated possibilities. For example, the very same results would be observed if the prothoracic glands secrete a hormone that promotes the release of ecdysone from some other endocrine organ. A further possibility is that the prothoracic glands secrete an inactive precursor of ecdysone, and that the precursor is transformed into active hormone elsewhere in the insect body. These prospects

Table 1. Spermatogenesis in vitro in intact testes of diapausing cynthia pupae cultured in blood plasma plus graded concentrations of α-ecdysone. After 7 days, the testes were torn open, and the percentages of developing germinal cysts were ascertained.

α-Ecdysone (μg/100 μl)	Testis cultures (No.)	Developed cultures (No.)	Developing cysts in responding testes (%)
8	2	2	60–90
4	4	4	30–70
2	4	4	40–60
0.4	2	2	15–50
0.16	2	2	25–40
0.08	3	3	15–40
0.04	2	2	15–40
0.01	4	4	10–40
0.005	4	2	10–30
0.001	4	0	0