gives R as defined in Eq. 2. If the quantity B differs from R and can be estimated by independent means, then

$$R = f/[12\pi\eta - (f/B)]$$

The latter equation clearly indicates the effective radius R for a friction bead is reduced for increased values of B.

28. Recent data suggest that the core particle is "disklike" with a radius of 50 Å and a height of 50 Å (J. C. Wooley and J. Pardon, personal communication). The radius of the equivalent spherical volume, that is, $\pi r^2 L = (4\pi/3)R^3$, is estimated to be 45.4 Å for the dehydrated nucleosome. It is unlikely that the 74-Å radius computed from the contiguous sphere model is due to hydration alone. The value of R (50 Å) used in the calculation of B (236 Å) is representative of the hydrated nucleosome (lower limit) since this value was estimated from hydrodynamic properties of the monomer (*1*). It could be argued that the asymmetric shape of the "disklike" structure of the core particle contributes significantly to the frictional properties of the dimer. This seems unlikely, however, since f/f_0 is about 1.1 for the monomer, suggesting almost spherical symmetry (*1*). In addition, Perrin's equations require an axial ratio a/b be greater than 5 to account for a 20 percent increase in the frictional properties of either prolate or oblate spheroids. The disklike model suggests $a/b \cong 2$, in which there is only a 4 percent increase in the friction factor compared to that of an equivalent sphere ($f/f_0 = 1.04$) [K. E. Van Holde, *Physical Biochemistry* (Prentice-Hall, Englewood Cliffs, N.J., 1971), p. 81]. The equivalent sphere model can, therefore, adequately represent the hydrodynamic properties of the nucleosome.
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1,25-Dihydroxycholecalciferol and Parathormone: Effects on Isolated Osteoclast-Like and Osteoblast-Like Cells

Abstract. *The actions of 1,25-dihydroxycholecalciferol [1,25-$(OH)_2D_3$] and parathormone, both effective bone-resorptive agents in vivo and in vitro, were tested on CT (osteoclast-like) and PT (osteoblast-like) bone cells maintained in culture. Both agents stimulated acid phosphatase activity and hyaluronate synthesis in the CT cells and decreased alkaline phosphatase, citrate decarboxylation, and collagen synthesis in the PT cells. Calcitonin inhibited the changes induced in the CT but not in the PT cells. The activity of 1,25-$(OH)_2D_3$ differed from that of parathormone in one key respect: it did not increase cellular cyclic adenosine monophosphate, whereas parathormone did. Prior incubation of the bone cells with 1,25-$(OH)_2D_3$ for 6 to 24 hours made the cells refractory to the effect of parathormone on cyclic adenosine monophosphate formation. These data suggest that 1,25-$(OH)_2D_3$ and parathormone induce bone resorption by affecting the same cell types (osteoblasts and osteoclasts) although at different cellular sites.*

Vitamin D is involved in at least two major aspects of bone metabolism—mineralization and resorption (*1–3*). Its physiologically active polar metabolite, 1,25-dihydroxycholecaliciferol [1,25-$(OH)_2D_3$] indirectly promotes mineralization of bone organic matrix by stimulating calcium and phosphate transport across the intestine to increase the concentrations of these ions at calcification sites (*3, 4*). In addition, this metabolite directly affects bone resorption in vivo (*5*) and in vitro (*6, 7*) leading to mineral release (*8*), degradation of organic matrix, and unique metabolic changes correlated with bone resorption, including decreased citrate decarboxylation (*9*) and increased lactate production (*6*).

The cellular basis for the direct effect of vitamin D_3 metabolites on bone is not known. Bone contains several different cell types. These vary from relatively undifferentiated osteoprogenitor cells to highly differentiated osteoblasts, osteocytes, and osteoclasts. Net gain or loss of bone is a result of the relative actions of these cells (*10*). Conceivably, 1,25-$(OH)_2D_3$ could affect one or all of these cells to exert its biochemical effect on bone resorption.

In order to understand the direct action of 1,25-$(OH)_2D_3$ on bone, it would seem essential to identify the cells responsive to this agent and to define the biochemical changes elicited. We have recently developed a technique that allows us to study in cell culture separate bone cell populations enriched in cells which express biochemical markers generally associated with either osteoblasts (parathormone-sensitive, or PT cells) or osteoclasts (calcitonin- and parathormone-sensitive, or CT cells) (*11–13*). We have reported (*13*) that the CT cells have high basal levels of acid phosphatase and hyaluronate-synthesizing capacity; these activities are stimulated by parathormone, and calcitonin blocks this stimulation. In contrast, the PT cells exhibit high levels of prolyl hydroxylase (*13*), collagen synthesis (*14*), citrate decarboxylation, and alkaline phosphatase

Table 1. Responses of CT and PT cells to 1,25-$(OH)_2D_3$, parathormone, calcitonin, and 24,25-$(OH)_2D_3$. Primary cultures (6 days old) of CT cells (populations 2 and 3) and PT cells (populations 5 and 6) were subdivided into multiwell tissue culture dishes containing minimum essential medium (Gibco) supplemented with 10 percent fetal calf serum (FCS) at a density of 1×10^5 cells per well. After the cells became attached to the wells overnight, 1,25-$(OH)_2D_3$ ($10^{-9}M$), parathormone ($4 \times 10^{-9}M$), calcitonin ($2 \times 10^{-9}M$), or 24,25-$(OH)_2D_3$ ($10^{-9}M$) was added in fresh FCS-supplemented medium, except when collagen synthesis was measured, in which instance the FCS was omitted. Incubation was continued for 5 minutes only for cyclic AMP formation, 44 hours for collagen synthesis, and 48 hours for the remainder of the tests. For the analysis of hyaluronate synthesis, [^{3}H]glucosamine (20 μc/ml) was present from hours 44 to 48. [^{14}C]Citrate (0.1 μc/ml) was present from hours 46 to 48 in order to measure citrate decarboxylation ($^{14}CO_2$ evolved). For the analysis of collagen synthesis, ascorbic acid (0.1 mg/ml) and [^{14}C]proline (0.4 μc/ml) were present for the entire 44-hour period. Total collagen (cells and medium) was assayed as collagenase-digestible radioactive protein. When FCS was omitted the cells ceased to divide but retained their characteristic morphology and continued to synthesize protein. These experiments were performed on five different preparations of primary cell cultures, with similar results each time. Results are expressed as disintegrations per minute per 10^5 cells for triplicate or quadruplicate samples (± standard deviation).

Cells	Control	1,25-$(OH)_2D_3$	Parathormone	Calcitonin	24,25-$(OH)_2D_3$
			Hyaluronate synthesis		
CT	13,000 ± 500	18,000 ± 1,400*	22,000 ± 2,200*	12,000 ± 570	14,000 ± 1,000
PT	15,000 ± 1,800	14,100 ± 2,000	13,000 ± 2,300	12,000 ± 1,800	(Not done)
			^{14}C-labeled collagen synthesized		
CT	54,000 ± 2,700	48,000 ± 4,600	51,700 ± 5,000	49,700 ± 6,500	51,000 ± 8,500
PT	117,000 ± 9,800	69,800 ± 800*	63,800 ± 4,900*	107,000 ± 12,000	108,700 ± 9,100
			Citrate decarboxylation		
CT	5,000 ± 500	3,500 ± 800	4,000 ± 600	5,500 ± 300	6,000 ± 1,200
PT	39,000 ± 1,400	16,000 ± 700*	18,000 ± 1,200*	33,600 ± 1,100	41,000 ± 4,800

* $P < .02$.

(*13*); parathormone inhibits these activities and calcitonin has no effect on this inhibition. This culture system has enabled us to determine whether 1,25-$(OH)_2D_3$ directly affects either or both types of bone cells in the absence of the matrix. We report here that 1,25-$(OH)_2D_3$ acts on both CT and PT cells in an identical manner to parathormone in terms of altering several biochemical markers that are believed to be associated with the total process of bone resorption.

Primary cultures of bone cells enriched for CT or PT cells were prepared by sequential trypsin-collagenase digestion of mouse calvaria as described (*12, 13*). Experiments were initiated on day 6, when the cells were treated with 1,25-$(OH)_2D_3$, parathormone, or calcitonin singly or in combination for 5 minutes to 24 hours, as indicated. Cholecalciferol and 24,25-dihydroxycholecalciferol [24,25-$(OH)_2D_3$] were used as control substances in some studies. All other procedures, test agents, and culture supplies were as previously described (*12, 13*).

Tables 1 and 2 show that in the CT cells, both the synthesis of hyaluronate and the activity of acid phosphatase were substantially increased by 1,25-$(OH)_2D_3$ and, in agreement with our earlier report (*13*), by parathormone. In the PT cells, both 1,25-$(OH)_2D_3$ and parathormone decreased citrate decarboxylation, alkaline phosphatase activity, and collagen synthesis (*14*). In separate studies (data not shown), we found that at a maximum concentration of 1,25-$(OH)_2D_3$, parathormone did not increase the magnitude of change in the parameters studied and vice versa. Calcitonin by itself did not affect these parameters in either the CT or PT cells (Tables 1 and 2) but inhibited the stimulatory actions of both parathormone and 1,25-$(OH)_2D_3$ on acid phosphatase in the CT cells (data not shown). The analog 24,25-$(OH)_2D_3$ at the same concentration of 1,25-$(OH)_2D_3$ did not affect any of the parameters studied in either cell population, although effects similar to those produced by 1,25-$(OH)_2D_3$ on acid and alkaline phosphatase activity and collagen synthesis were seen at concentrations of $10^{-7}M$, in agreement with the relative biological potency previously reported for 24,25-$(OH)_2D_3$ (*15*). Cholecalciferol was without effect on collagen synthesis at concentrations as high as $10^{-6}M$ (data not shown). Thus, 1,25-$(OH)_2D_3$ initiated a series of biochemical reactions similar to those seen with parathormone in these cultured bone cells. Nevertheless, the initial molecular intermediates utilized by these two hormones were different. The response to parathormone involved an increase in adenosine 3′,5′-monophosphate (cyclic AMP) content of both CT and PT cell populations (*12*), whereas 1,25-$(OH)_2D_3$ did not affect the cyclic AMP content of either cell type at any time (Table 3). Thus the vitamin D metabolite acted at a cellular site different from that at which parathormone acts and at a point in the metabolic pathway distal to that of cyclic AMP formation. This interpretation is in keeping with the reported binding of 1,25-$(OH)_2D_3$ to intracellular receptors (*16*) beyond the plasma membrane.

Prior exposure to 1,25-$(OH)_2D_3$ diminished the parathormone-induced increases in cyclic AMP in the CT and PT cells (Fig. 1). Partial refractoriness was induced within 6 hours of culture, and after 24 hours parathormone no longer elicited an increase in cyclic AMP. In contrast, prior treatment of the cells with 1,25-$(OH)_2D_3$ did not affect the capacity of calcitonin to stimulate cyclic AMP formation. The induction of refractoriness to parathormone in normally responsive cells is reminiscent of prior exposure to

Table 2. Responses of CT and PT cells measured in terms of acid and alkaline phosphatase activity. Conditions as in Table 1. Results expressed as nanomoles of substrate cleaved per 30 minutes for triplicate or quadruplicate samples (± standard deviation).

Cells	Control	1,25-$(OH)_2D_3$	Parathormone	Calcitonin	24,25-$(OH)_2D_3$
			Acid phosphatase		
CT	70 ± 10	180 ± 20*	160 ± 11*	100 ± 14	77 ± 7
PT	80 ± 20	90 ± 10	80 ± 14	80 ± 17	85 ± 9
			Alkaline phosphatase		
CT	126 ± 19	100 ± 15	108 ± 9	140 ± 10	110 ± 20
PT	360 ± 8	150 ± 17*	170 ± 20*	320 ± 21	340 ± 15

* $P < .02$.

Table 3. Responses of CT and PT cells measured in terms of the amount of intracellular cyclic AMP present. Conditions as in Table 1. Results expressed as picomoles of cyclic AMP per 10^5 cells for triplicate or quadruplicate samples.

Cells	Control	1,25-$(OH)_2D_3$	Parathormone	Calcitonin	24,25-$(OH)_2D_3$
CT	2.0 ± 0.3	2.1 ± 0.2	3.6 ± 0.4*	3.2 ± 0.4*	1.9 ± 0.2
PT	2.8 ± 0.3	2.9 ± 0.4	14.0 ± 1.0*	2.8 ± 0.1	2.7 ± 0.2

* $P < .02$.

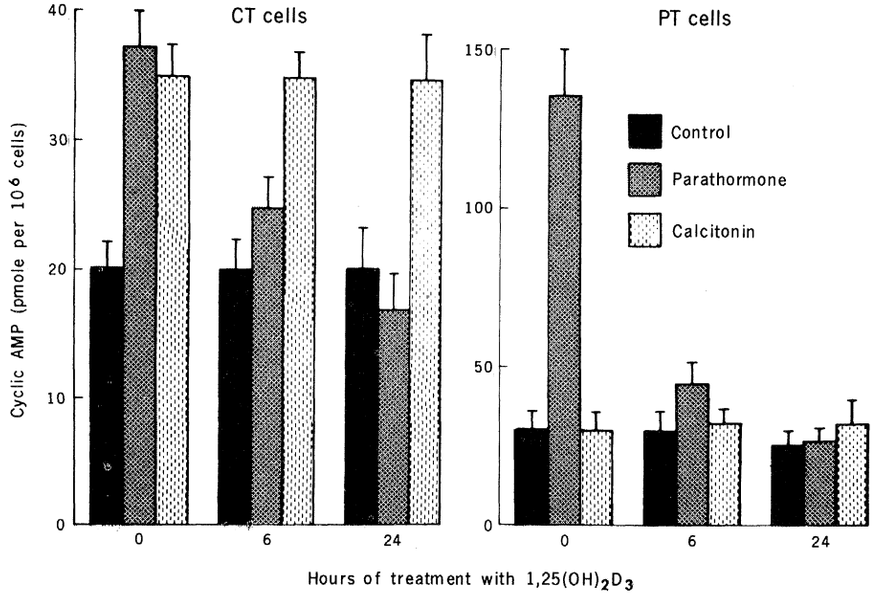

Fig. 1. Effect of treatment of bone cells with 1,25-$(OH)_2D_3$. Cells (1×10^5) were cultured in minimum essential medium (Gibco) supplemented with 10 percent FCS and 1,25-$(OH)_2D_3$ ($10^{-9}M$) for 0, 6, or 24 hours. Control cells received no 1,25-$(OH)_2D_3$. At the indicated times, cellular cyclic AMP increases were measured in treated and untreated cells after 5 minutes in the presence of parathormone ($4 \times 10^{-9}M$) or calcitonin ($2 \times 10^{-9}M$). Data are plotted as hours of treatment against picomoles of cyclic AMP released per 10^6 cells ± standard deviation. These experiments were performed in triplicate on five different primary cultures with similar results each time.

parathormone itself (*17*) and resembles similarly induced desensitizations in the cases of epinephrine and prostaglandin E_2 (*18*). This observation, and the fact that the various changes induced by the two agents were not additive at maximal concentrations of each, suggest that the same bone cells were the targets for both hormones and that generally the same metabolic pathways were used.

Our results demonstrate that 1,25-$(OH)_2D_3$ affects the metabolism of those cells of bone that may represent, respectively, the bone-resorbing (CT) and bone-forming (PT) cells of the tissue (*19*). If the changes in vitro that we observed are, at the concentration of both agents tested, representative of metabolic events that would occur in bone in vivo, then our results suggest that 1,25-$(OH)_2D_3$ induces bone resorption both by stimulating osteoclastic and by inhibiting osteoblastic activities. Moreover, these results would argue against one hormonal agent being a prerequisite for the other—a possibility often considered in the literature (*1*).

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Phenylketonuria: A New Method for the Simultaneous Determination of Plasma Phenylalanine and Tyrosine

Abstract. *This quantitative spectrophotometric method is based on the conversion of phenylalanine and tyrosine by phenylalanine ammonia-lyase to* trans-*cinnamic acid and* trans-*coumaric acid, respectively. Neither deproteinization nor prior incubation of the sample is required, and the entire procedure can be performed in 20 minutes. The method is sensitive to 1-micromolar concentrations of the two compounds, and only 20 microliters of plasma or serum is required to determine both phenylalanine and tyrosine simultaneously. These amino acids were determined between molar ratios (phenylalanine to tyrosine) of 0.1 to 40 in the serum or plasma of healthy individuals and plasma of phenylketonuric patients.*

Classic phenylketonuria, which is transmitted by an autosomal recessive gene, is a result of a defect in liver phenylalanine hydroxylase (*1*). This deficiency causes an excess of phenylalanine to accumulate in the blood and spinal fluid. Individuals with this disease usually develop significant irreversible brain damage and behavioral derangement unless they are placed on a diet low in phenylalanine within 1 to 3 months of age (*2*). The prompt use of dietary treatment is dependent upon an effective screening test. For this purpose, a semiquantitative assay based on the reversal by phenylalanine of β-2-thienylalanine inhibition of the growth of *Bacillus subtilis* was developed by Guthrie (*3*) and has been applied by health departments in most of the states in this country.

Table 1. Phenylalanine (Phe) and tyrosine (Tyr) concentrations in the serum of healthy individuals determined by enzymatic spectrophotometry and automated amino acid analysis. Serums were prepared from 20 healthy nonfasting individuals with a mean age of 24.7 ± 3.5 years [± standard deviation (S.D.)]. The Phe and Tyr determinations were performed by our enzyme method described in the text. For automated amino acid analysis, serums were deproteinized with four volumes of 3.75 percent sulfosalicylic acid in 0.3*N* lithium citrate buffer (*p*H 2.2), and the mixture was centrifuged. Amino acid concentrations were expressed as milligrams per 100 ml of serum. Values obtained by the enzyme method were the mean of triplicate determinations, and those obtained by the amino acid analyzer were the average of duplicate determinations.

Sample No.	Phenylalanine ammonia-lyase		Amino acid analyzer	
	Phe	Tyr	Phe	Tyr
1	0.99	0.89	1.00	0.51
2	0.93	0.73	0.86	0.45
3	1.25	1.03	1.08	0.79
4	1.11	0.97	1.07	0.58
5	1.48	0.89	1.46	0.89
6	0.93	0.68	0.85	0.39
7	0.76	1.05	0.99	0.86
8	0.90	1.22	1.21	1.15
9	0.93	1.35	1.14	1.23
10	1.61	0.95	1.15	0.84
11	1.15	0.77	0.74	0.60
12	1.24	0.76	1.22	0.80
13	1.87	1.04	1.29	0.88
14	1.30	0.72	0.84	0.75
15	1.27	0.90	1.11	0.82
16	1.44	1.10	1.22	1.18
17	1.17	0.74	1.11	0.71
18	1.23	0.91	1.29	0.74
19	0.96	1.33	1.28	1.04
20	1.08	1.08	0.60	0.56
Mean ± S.D.	1.18 ± 0.27*	0.96 ± 0.20‡	1.08 ± 0.21*	0.79 ± 0.24‡

*$P < .20$. ‡$.02 < P < .05$.