mune system. The amount of tissue culture interferon purified by affinity chromatography that was used in our experiments totaled about 5×10^6 units per mouse, which corresponds roughly to 2×10^5 units per gram of tissue. This represents at the most 0.5 μg of protein per gram of tissue, which, on a weight basis, makes interferon a highly active inhibitor of cellular immunity.

JAQUELINE DE MAEYER-GUIGNARD
ANNIE CACHARD, EDWARD DE MAEYER
Institut du Radium,
Université de Paris-Sud,
Bâtiment 110, 91405—Orsay, France

References and Notes

1. A. Isaacs and J. Lindenmann, *Proc. R. Soc. Lond. Ser. B Biol. Sci.* **147**, 258 (1957); T. J. Chester, K. Paucker, T. C. Merigan, *Nature (Lond.)* **246**, 92 (1973); L. E. Mobraaten, E. De Maeyer, J. De Maeyer-Guignard, *Transplantation* **16**, 415 (1973); E. De Maeyer, L. E. Mobraaten, J. De Maeyer-Guignard, *C. R. Hebd. Seances Acad. Sci. Ser. D Sci. Nat.* **277**, 2101 (1973); M. S. Hirsch, D. A. Ellis, P. H. Black, A. P. Monaco, M. L. Wood, *Transplantation* **17**, 234 (1974); B. R. Brodeur and T. C. Merigan, *J. Immunol.* **113**, 1319 (1974); R. H. Gisler, P. Lindahl, I. Gresser, *ibid.*, p. 438.
2. E. De Maeyer, J. De Maeyer-Guignard, M. Vandeputte, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1753 (1975).
3. C. von Pirquet, *Dtsch. Med. Wochenschr.* **34**, 1297 (1908); J. A. Brody and R. MacAlister, *Am. Rev. Respir. Dis.* **90**, 607 (1964); S. Starr and S. Berkovich, *N. Engl. J. Med.* **270**, 386 (1964); J. A. Brody, T. Overfield, L. M. Hammes, *ibid.* **271**, 1294 (1964); G. A. Lamb, *Am. J. Dis. Child.* **118**, 261 (1969); T. A. Kupers, J. M. Petrich, A. W. Holloway, J. W. St. Geme, Jr., *J. Pediatr.* **76**, 716 (1970); C. B. Hall and F. S. Kantor, *J. Immunol.* **108**, 81 (1972).
4. P. H. Lagrange, G. B. Mackaness, T. E. Miller, *J. Exp. Med.* **139**, 528 (1974).
5. All interferon titrations were performed in a plaque inhibition assay with Swiss mouse embryo fibroblasts (MEF) and vesicular stomatitis virus as challenge virus. One unit is the reciprocal of the dilution inhibiting 50 percent of the challenge virus plaques. Since MEF cultures are about ten times as sensitive to mouse interferon as are L cells, 1 MEF unit equals about 10 L cell units.
6. E. De Maeyer and J. De Maeyer-Guignard, *J. Virol.* **3**, 506 (1969); ———, D. W. Bailey, *Immunogenetics* **1**, 438 (1975).
7. H. K. Oie, A. F. Gazdar, C. E. Buckler, S. Baron, *J. Gen. Virol.* **17**, 107 (1972); M. G. Tovey, J. Begon-Lours, I. Gresser, *Proc. Soc. Exp. Biol. Med.* **146**, 809 (1974).
8. J. D. Sipe, J. De Maeyer-Guignard, B. Fauconnier, E. De Maeyer, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 1037 (1973).
9. D. M. V. Parrott and M. A. B. De Sousa, *Nature (Lond.)* **212**, 1316 (1966).
10. P. Jullien and J. De Maeyer-Guignard, *Int. J. Cancer* **7**, 468 (1971); P. Lindahl-Magnusson and I. Gresser, *Nat. New Biol.* **237**, 120 (1972); J. C. Cerottini, K. T. Brunner, P. Lindahl, I. Gresser, *ibid.* **242**, 152 (1973).
11. Some of the evidence for the activity of mRNA's in immune phenomena was reviewed by A. A. Gottlieb, *Prog. Nucleic Acid Res. Mol. Biol.* **13**, 409 (1973).
12. I. M. Kerr, J. A. Sonnabend, E. M. Martin, *J. Virol.* **5**, 132 (1970); I. M. Kerr, *ibid.* **7**, 448 (1971); E. Falcoff, R. Falcoff, B. Lebleu, M. Revel, *Nat. New Biol.* **240**, 145 (1972); *J. Virol.* **12**, 421 (1973); I. M. Kerr, R. M. Friedman, R. E. Brown, L. A. Ball, J. C. Brown, *ibid.* **13**, 9 (1974).
13. J. A. Green, S. R. Cooperband, S. Kibrick, *Science* **164**, 1415 (1969); L. B. Epstein, M. J. Cline, T. C. Merigan, *J. Clin. Invest.* **50**, 744 (1971); *Cell Immunol.* **2**, 602 (1971); L. B. Epstein, D. A. Stevens, T. C. Merigan, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2632 (1972); S. B. Salvin, J. S. Youngner, W. H. Lederer, *Infect. Immun.* **7**, 68 (1973); J. S. Youngner and S. B. Salvin, *J. Immunol.* **111**, 1914 (1973); H. S. Lawrence, *Transplant. Proc.* **1**, 49 (1973); D. L. Lodmell and A. L. Notkins, *J. Exp. Med.* **140**, 764 (1974).
14. We thank R. M. Fauve for helpful suggestions and stimulating discussions. Supported by the Centre National de la Recherche Scientifique and the Fondation pour la Recherche Médicale Française.

30 June 1975

Synthesis of [6-^{3}H]-1α-Hydroxyvitamin D_3 and Its Metabolism in vivo to [^{3}H]-1α,25-Dihydroxyvitamin D_3

Abstract. *[6-^{3}H]-1α-Hydroxyvitamin D_3 was chemically synthesized and its full biological activity and radiochemical purity were demonstrated. With the use of this preparation it has been possible to demonstrate in vivo that in rats the [6-^{3}H]-1α-hydroxyvitamin D_3 is converted to [6-^{3}H]-1α,25-dihydroxyvitamin D_3, the natural hormone. In fact, in the intestine and bone of rats given 32 picomoles of [6-^{3}H]-1α-hydroxyvitamin D_3 each day for 6 days, more than 80 percent of the lipid-soluble radioactivity exists as [6-^{3}H]-1α,25-dihydroxyvitamin D_3, a finding that suggests that much of the biological effectiveness of 1α-hydroxyvitamin D_3 is due to its conversion to 1α,25-dihydroxyvitamin D_3.*

In order for vitamin D to carry out its physiological role in stimulating the mobilization of calcium from the intestine and bone, it must first be hydroxylated on C-25 in the liver and on C-1 in the kidney to 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$] (*1*). The fact that the kidney is essential in the synthesis of this hormone has led to the idea that renal osteodystrophy may be directly related to depressed production of 1,25-$(OH)_2D_3$ and that replacement therapy with the hormone would be useful in correcting these lesions (*2*). The first reported chemical synthesis of 1,25-$(OH)_2D_3$ suggested that synthesis of this hormone might be difficult and expensive (*3*). This prompted the synthesis of 1,25-$(OH)_2D_3$ analogs that were easier and less expensive to prepare (*4*). The most promising analog appeared to be 1α-hydroxyvitamin D_3 (1α-OH-D_3) (Fig. 1A), which was prepared as a model for the synthesis of 1α,25-$(OH)_2D_3$ (*5*). Holick *et al.* (*5*) showed that 1α-OH-D_3 is capable of stimulating intestinal calcium transport and bone calcium mobilization in normal and anephric rats. Furthermore, this analog is about 20 to 50 percent as active as 1,25-$(OH)_2D_3$ on a weight basis in the rat bioassay systems (*6*) and equally as active in eliciting biological responses in the chicken (*7*).

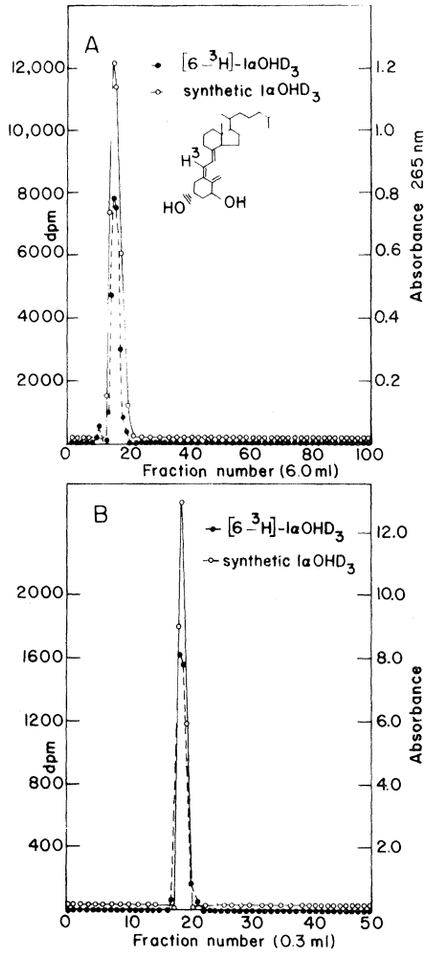

Fig. 1. Cochromatography of [6-^{3}H]-1α-OH-D_3 with crystalline synthetic 1α-OH-D_3. (A) Sephadex LH-20 column (1 by 60 cm) packed and developed with $CHCl_3$ and Skellysolve B (1 : 1). (B) High pressure liquid chromatography (2.5 cm by 2.0 mm) on Zorbax-Sil (du Pont de Nemours), developed with isopropanol and Skellysolve B (1 : 9). Abbreviation: *dpm*, disintegrations per minute.

The question as to whether 1α-OH-D_3 is hydroxylated on C-25 before it can function has been raised (*6–8*). Zerwekh *et al.* (*8*), using their chromatin-associated 1,25-$(OH)_2D_3$ receptor assay for 1,25-$(OH)_2D_3$, have suggested that the intestine of the chicken rapidly converts 1α-OH-D_3 to its 25-hydroxy derivative and that this hydroxylation can be demonstrated in vitro. However, as Zerwekh *et al.* stated, the metabolism of 1α-OH-D_3 to 1,25-$(OH)_2D_3$ can only be established with radioactive 1α-OH-D_3.

Using an intermediate available through our earlier work on the synthesis of 1,25-$(OH)_2D_3$ and 1α-OH-D_3 (*3*, *5*), we have prepared [6-^{3}H]-1α-OH-D_3 and have demonstrated that it is metabolized to 1,25-$(OH)_2D_3$. The introduction of a tritium at C-6 was accomplished by reducing 1α,3β-diacetoxy-5α-cholestan-6-one (*9*) with $NaBT_4$ (New England Nuclear) in isopropanol. The resulting [6-^{3}H]-6β-alcohol was dehydrated in pyridine with $POCl_3$ to yield [6-^{3}H]-1α,3β-diacetoxycholest-5-ene. Allylic bromination with *N,N'*-dibromodimethylhydantoin and dehydrobromination with trimethyl phosphite yielded the corresponding $\Delta^{5,7}$-intermediate (λ_{max} 295, 282, 271 nm), which on irradiation yielded 1α-[6-^{3}H] hydroxy-previtamin D_3-1α,3β-

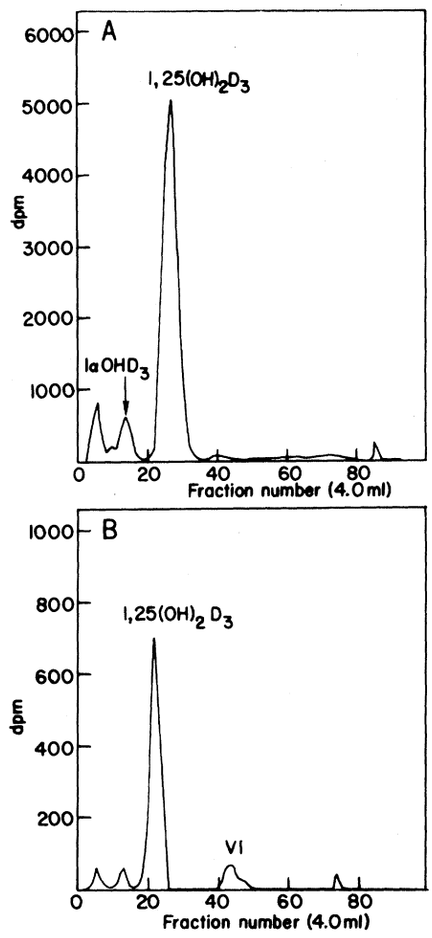

Fig. 2. Profiles of chromatographed lipid extracts of (A) intestine and (B) bone from rats that received 32 pmole of [6-^{3}H]-1α-OH-D_3 orally for 6 days. Sephadex LH-20 column (1 by 60 cm) packed and developed with $CHCl_3$ and Skellysolve (15 : 25).

diacetate (λ_{max} 260 nm). Saponification of the previtamin with KOH in methanolic at 80° C for 2 hours yielded [6-^{3}H]-1α-OH-D_3 (λ_{max} 265 nm, λ_{min} 228 nm). The final product cochromatographed with crystalline synthetic 1α-OH-D_3 on Sephadex LH-20 ($CHCl_3$ and Skellysolve B, 1 : 1) and on high pressure liquid chromatography with 10 percent isopropanol in Skellysolve B, a procedure that separates all known metabolites of vitamin D_3 (*10*) (Fig. 1).

For bioassay and metabolism studies, male weanling rats (Holtzman) were fed a vitamin D–deficient rachitogenic diet (*11*) for 2 weeks and groups of nine animals were given 32 pmole of either [6-^{3}H]-1α-OH-D_3 or crystalline 1α-OH-D_3 (*6*) orally in 0.1 ml of propylene glycol daily for the next 6 days while the control group received only 0.1 ml of propylene glycol. On day 7 the animals were killed, their radii and ulnae were collected for antirachitic assay (*12*), and their small intestines were used for intestinal calcium transport studies (*13*). A group of three animals receiving the tritiated compound had their small intestines, femurs, tibias, and fibulas removed and extracted with $CHCl_3$, MeOH, and H_2O (2 : 2 : 1) for chromatography. Intestinal and bone extracts were chromatographed on a Sephadex LH-20 column (1 by 60 cm) slurried and developed with $CHCl_3$ and Skellysolve B (75 : 25) (*14*). Cochromatography was done on Sephadex LH-20 slurried and developed with either $CHCl_3$ and Skellysolve B (65 : 35) or $CHCl_3$ and Skellysolve B (1 : 1) (*15*) and on high pressure liquid chromatography with 10 percent isopropanol in Skellysolve B. The [26,27-^{14}C]-1,25-$(OH)_2D_3$ was prepared with chicken kidney homogenates (*16*) in vitro from [26,27-^{14}C]-25-$OH-D_3$ synthesized as described (*17*).

When rats were dosed orally for 6 days with 32 pmole of [6-^{3}H]-1α-OH-D_3, the major component of lipid-soluble radioactivity in both the bone and intestine was a metabolite that migrated in the region of the Sephadex chromatogram where 1,25-$(OH)_2D_3$ normally elutes (Fig. 2). This polar metabolite of 1α-OH-D_3 isolated from the intestine cochromatographs identically with [26,27-^{14}C]-1,25-$(OH)_2D_3$ on Sephadex LH-20 ($CHCl_3$ and Skellysolve B, 65 : 35) and with synthetic 1α,25-$(OH)_2D_3$ and [26,27-^{14}C]-1,25-$(OH)_2D_3$ on high pressure liquid chromatography (Fig. 3). Similarly the polar metabolite from the bone also cochromatographed with [26,27-^{14}C]-1,25-$(OH)_2D_3$ in both systems.

The calcification response for the animals receiving the [6-^{3}H]-1α-OH-D_3 was identical to that observed with crystalline synthetic 1α-OH-D_3 (both gave antirachitic activity values of 150 units per microgram; vitamin D_3 gave values of 40 units per microgram). Similarly the intestinal calcium transport as determined by the everted gut sac technique in the same animals showed ratios of serosal to mucosal ^{45}Ca of 4.0 $\pm$ 0.2 and 3.5 $\pm$ 0.2 for the labeled and unlabeled materials, while that from vitamin D–deficient controls was 1.8 $\pm$ 0.2. These results demonstrate that the [6-^{3}H]-1α-OH-D_3 is fully as biologically active as crystalline 1α-OH-D_3.

These experiments show that [6-^{3}H]-1α-OH-D_3 given each day for 6 days was hydroxylated on C-25 to produce the natural hormone 1,25-$(OH)_2D_3$. Furthermore 24 hours after the last dose of 1α-OH-D_3, more than 80 percent of the lipid-soluble radioactivity in the intestine and bone was in 1,25-$(OH)_2D_3$. The fact that the polar metabolite was 1,25-$(OH)_2D_3$ is demonstrated by its ability to comigrate with [26,27-^{14}C]-1,25-$(OH)_2D_3$ and synthetic 1α,25-$(OH)_2D_3$ on Sephadex LH-20 and high pressure liquid chromatography, the latter being capable of separating 25,26-dihydroxyvitamin D_3 [25,26-$(OH)_2D_3$] from 1,25-$(OH)_2D_3$ and 1,25-$(OH)_2D_3$ from 1,25-$(OH)_2D_2$ (*9*).

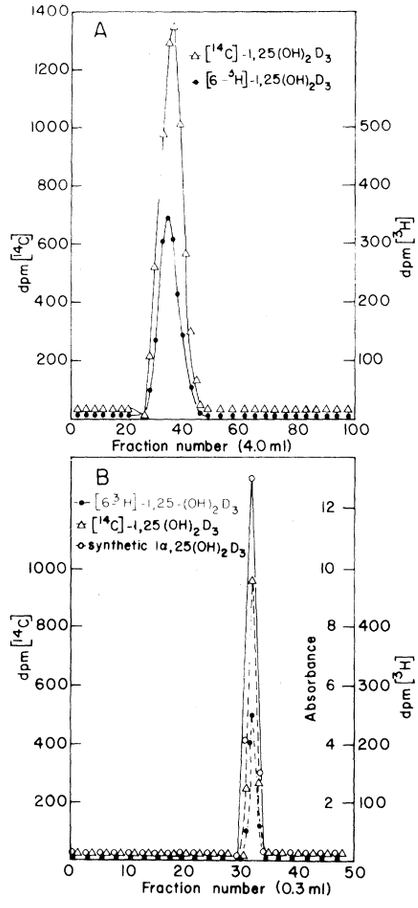

Fig. 3. (A) Cochromatography of [6-^{3}H]-1α,25-$(OH)_2D_3$ with [^{14}C]-1α,25-$(OH)_2D_3$ on Sephadex LH-20 (1 by 60 cm packed and developed with $CHCl_3$ and Skellysolve B, 65 : 35); (B) cochromatography of [6-^{3}H]-1α,25$(OH)_2D_3$ with crystalline 1α,25-$(OH)_2D_3$ and [26,27-^{14}C]-1,25-$(OH)_2D_3$ by high pressure liquid chromatography (25 cm by 2.1 mm) on Zorbax-Sil developed with isopropanol and Skellysolve B (1 : 9).

The question as to whether 1α-OH-D_3 requires hydroxylation on C-25 before it can function cannot be determined from the data presented. Although 1,25-$(OH)_2D_3$ is generated from 1α-OH-D_3 in vivo in rats, it is not yet known whether the hydroxylation occurs rapidly enough to account for the early responses reported for this compound in chicks and rats (*6, 7*). Furthermore, it is unknown whether the 25-hydroxylation occurs in the liver as in the case with vitamin D_3 (*1*) or in the intestine as suggested by Zerwekh *et al.* (*8*). These and other questions relating to the use of 1α-OH-D_3 can now be approached with the use of [6-^{3}H]-1α-OH-D_3.

M. F. HOLICK*, S. A. HOLICK†
T. TAVELA, B. GALLAGHER
H. K. SCHNOES, H. F. DELUCA
Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin–Madison, Madison 53706

References and Notes

1. D. R. Fraser and E. Kodicek, *Nature (Lond.)* **228**, 764 (1970); J. L. Omdahl and H. F. DeLuca, *Physiol. Rev.* **53**, 327 (1973).
2. M. F. Holick and H. F. DeLuca, *Annu. Rev. Med.* **25**, 349 (1974); A. S. Brickman, J. W. Coburn, A. W. Norman, *N. Engl. J. Med.* **287**, 891 (1972).
3. E. J. Semmler, M. F. Holick, H. K. Schnoes, H. F. DeLuca, *Tetrahedron Lett.* **40**, 4147 (1972).
4. M. F. Holick and H. F. DeLuca, *Adv. Steroid Biochem. Pharmacol.* **4**, 111 (1974).
5. M. F. Holick, E. J. Semmler, H. K. Schnoes, H. F. DeLuca, *Science* **180**, 190 (1973); D. H. R. Barton, R. H. Hesse, M. M. Pechet, E. Rizzardo, *J. Am. Chem. Soc.* **95**, 2748 (1973).
6. M. F. Holick, P. Kasten-Schraufrogel, T. Tavela, H. F. DeLuca, *Arch. Biochem. Biophys.* **166**, 63 (1975).
7. M. R. Haussler, J. E. Zerwekh, R. H. Hesse, E. Rizzardo, M. M. Pechet, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2248 (1973).
8. J. E. Zerwekh, P. F. Brumbaugh, D. H. Haussler, D. J. Cork, M. R. Haussler, *Biochemistry* **13**, 4097 (1974).
9. This ketone was prepared as described in (*5*). Its physical properties are: m.p., 134.5° to 135.5°C; molecular weight, 502; NMR ($CDCl_3$), δ 4.99 (m, 2H, C-1, and C-3-H), 2.11 and 2.02 (s, 6-H, acetates), 0.65 (s, 3-H, C-19-H); elemental analysis $C_{31}H_{50}O_5$: calculated: C, 74.06 percent; H, 10.03 percent; found, C, 74.27 percent; H, 10.11 percent.
10. G. Jones and H. F. DeLuca, unpublished results.
11. T. Suda, H. F. DeLuca, Y. Tanaka, *J. Nutr.* **100**, 1049 (1970).
12. *U.S. Pharmacopeia* (Mack, Easton, Pa., 15th revision, 1955), p. 889.
13. D. L. Martin and H. F. DeLuca, *Arch. Biochem. Biophys.* **134**, 139 (1969).
14. M. F. Holick, A. Kleiner-Bossaller, H. K. Schnoes, P. M. Kasten, I. T. Boyle, H. F. DeLuca, *J. Biol. Chem.* **248**, 6691 (1973).
15. M. F. Holick and H. F. DeLuca, *J. Lipid Res.* **12**, 460 (1971).
16. R. W. Gray, J. L. Omdahl, J. G. Ghazarian, H. F. DeLuca, *J. Biol. Chem.* **247**, 7528 (1972).
17. T. Suda, H. F. DeLuca, R. B. Hallick, *Anal. Biochem.* **43**, 139 (1971).

18. Supported by PHS grant AM-14881 and contract 72-2226, and by AEC contract AT(11-1)-1668.

* Present address: Department of Medicine, Massachusetts General Hospital, Boston.
† Present address: Boston University, Department of Chemistry, Boston, Mass.

26 March 1975

Regulation of Serum 1α,25-Dihydroxyvitamin D_3 by Calcium and Phosphate in the Rat

Abstract. *A new radioreceptor assay was used to quantify changes in serum concentration of 1α,25-dihydroxyvitamin D_3 in rats with low calcium or low phosphate diets. Low availability of either ion elicits a fivefold increase in the circulating concentration of 1α,25-dihydroxyvitamin D_3. The enhancement of 1α,25-dihydroxyvitamin D_3 concentration in response to calcium deficiency is dependent on the presence of the parathyroid or thyroid glands (or both), suggesting that this effect is mediated by parathyroid hormone. In contrast, the response to phosphate deficiency is independent of these glands and may result from an action of low serum phosphate concentration or some factor associated with phosphate depletion on the renal synthesis of the 1α,25-dihydroxyvitamin D_3 hormone.*

Vitamin D_3 is metabolized to 25-hydroxyvitamin D_3 (25-OHD_3) (*1*) and subsequently to 1α,25-dihydroxyvitamin D_3 [1α,25-$(OH)_2D_3$] (*2*). The latter conversion occurs exclusively in the kidney (*3*) and produces what is considered to be the hormonal form of the vitamin. In stimulating both intestinal calcium transport and bone calcium mobilization, 1α,25-$(OH)_2D_3$ is the most active and the fastest acting metabolite of vitamin D (*4*).

The production of this hormone is influenced by the calcium and vitamin D status of the animal (*5, 6*). The primary regulated step appears to be the 1α-hydroxylation of 25-OHD_3 in the kidney (*6*); however, the mechanism and details of this endocrine regulation are poorly understood. DeLuca and his co-workers, on the basis of the in vivo conversion of radioactive 25-OHD_3 to 1α,25-$(OH)_2D_3$, have reported (*5*) that hypocalcemia in the rat stimulates the production of 1α,25-$(OH)_2D_3$, and that this stimulation is dependent on the presence of the parathyroid glands (*7*). In contrast to these findings, and also to those of Fraser and Kodicek (*8*), MacIntyre and associates have observed that in both rats (in vivo) (*9*) and chicks (in vitro) (*10*) parathyroid glands are not essential for the production of 1α,25-$(OH)_2D_3$. Moreover, Tanaka and DeLuca (*11*) have reported that phosphate deficiency also results in enhanced production of the 1α,25-$(OH)_2D_3$ hormone. To date, all experiments investigating the control of 1α,25-$(OH)_2D_3$ formation have used either the measurement of the renal 1α-hydroxylase in vitro, or the ability of the animal to metabolize 25-OHD_3 to dihydroxy derivatives. Since these methods measure the conversion of 25-OHD_3 to 1α,25-$(OH)_2D_3$ at any given time and are affected by the pool size of 25-OHD_3 precursor, they may not reflect the steady state concentration of this hormonal product in the blood. In any case, the regulation of the renal synthesis and secretion of 1α,25-$(OH)_2D_3$ appears to be a complex endocrine feedback system which will probably require the results of a number of experimental approaches before it can be adequately described.

Since the recent development of a specific and sensitive radioreceptor assay for 1α,25-$(OH)_2D_3$ (*12*), it has become possible to eliminate the effects of alterations in hormone degradation rates and determine the total serum activity of the hormone. Using this assay, it is possible to directly assess the effects of proposed regulators of 1α,25-$(OH)_2D_3$ serum concentration. The data obtained from the measurement of the circulating concentration of this hormone, combined with the data obtained by in vitro enzyme activity studies and in vivo conversion experiments, should clarify the control of 1α,25-$(OH)_2D_3$ formation as well as the relationships between vitamin D and mineral homeostasis.

In our study, the serum 1α,25-$(OH)_2D_3$ concentration was measured in response to changes in dietary calcium and phosphorus content and in response to surgical removal of the thyroid and parathyroid (TPTX) glands of rats that were subsequently given replacement thyroxine. Thus, the TPTX rats were deficient in calcitonin and parathyroid hormone. A total of 169 male weanling Holtzman rats, either intact or TPTX, were fed one of the following partially synthetic diets: (i) a normal diet (0.6 percent calcium, 0.6 percent phosphorus); (ii) a low calcium diet (0.01 percent calcium, 0.6 percent phosphorus); (iii) a low phosphorus diet (0.6 percent calcium, 0.04 percent phosphorus); or (iv) high calcium diet (1.8 percent calcium, 0.65 percent phosphorus, 20 percent lactose). The concentration of salts (potassium phosphate, calcium carbonate, and potassium chloride) were varied in these diets in order to achieve the desired levels of calcium and phosphorus; fibrin was used as the source of protein because of its low phosphorus content; and when lactose was used, it was substituted for sucrose. The constituents of these different diets (*13*) were essentially the same except for inorganic salt content. All diets contained vitamin D_3 (2 international units per gram of diet). After 2 weeks on the diets, blood was obtained from the rats by cardiac puncture for measurement of serum calcium, phosphorus, and 1α,25-$(OH)_2D_3$. The serum sample (*14*) for the 1α,25-$(OH)_2D_3$ assay was extracted with a mixture of methanol and chloroform (2 : 1, by volume), and the vitamin D hormone was isolated by successive Sephadex LH-20 and Celite column chromatography (*15*). A sensitive, competitive binding assay with a high affinity, saturable receptor system from the hormone's target tissue was used to measure the 1α,25-$(OH)_2D_3$ concentration in the serum (*12*). This technique has been shown to be a reliable and valid method for detecting hormone concentrations as low as 1.0 ng per 100 ml of serum (*12*), and recent modifications (*15, 16*) have improved the speed of this radioreceptor assay.

In intact animals (no surgery) on a normal calcium and phosphate diet, circulating 1α,25-$(OH)_2D_3$ was 17.3 ng/100 ml (Table 1). A marked increase of this hormone to 90 or 82 ng/100 ml occurs when the dietary intake of either calcium or