ly divided at the beginning of the experiment. Groups of 15-day-old male and female mice were given daily intraperitoneal injections of 1 μg of AVT or diluent for 3 days and necropsied 2 weeks after the last injection. Ovarian growth was significantly retarded ($P < .01$) in AVT-treated female mice compared to diluent-treated animals (Table 1). In male mice receiving AVT, growth of the testes ($P < .001$), ventral prostate ($P < .001$), and accessory organs ($P < .01$) was significantly depressed compared to that in control mice (Table 1).

The effects of AVT reported here appear to be due to a rather specific inhibition of normal developmental growth of some reproductive organs because body weights were not altered by the treatment. In addition, two other neurohypophyseal polypeptides, arginine vasopressin and oxytocin, are ineffective in blocking growth of the reproductive organs in these animals (*5*). Secretion of AVT by cultured human fetal pineal tissue has been reported (*6*). Furthermore, this compound has been tentatively identified in the cerebrospinal fluid of normal adult men (*7*). These findings, together with those reported here, prompt us to speculate that AVT might be an active principle in the remarkable alterations in sexual development which are associated with pathologic conditions of the pineal gland in young humans (*8*). Since the pineal gland controls photic regulation of reproductive function in rodents (*9*), there may also be a physiological role for pineal vasotocin in the regulation of reproduction in mammals in general, as well as in sexual development. However, AVT reproduced the classical effects of light deprivation on testes (which is mediated by the pineal gland) in only one of our experiments. It may be that AVT can retard testicular growth only if administered early in sexual development, when growth of these organs is most rapid.

MARY K. VAUGHAN*
GEORGE M. VAUGHAN*
DAVID C. KLEIN
Section on Physiological Controls, Laboratory of Biomedical Sciences, National Institute of Child Health and Human Development, Bethesda, Maryland 20014

References and Notes

1. S. Pavel, *Endocrinology* **77**, 812 (1965); ——— and S. Petrescu, *Nature (Lond.)* **212**, 1954 (1966); D. W. Cheesman, *Biochim. Biophys. Acta* **207**, 247 (1970).
2. S. Pavel, M. Petrescu, N. Vicoleanu, *Neuroendocrinology* **11**, 370 (1973).
3. Synthetic AVT was obtained from Schwarz/Mann, Orangeburg, New York. This material (lot Y-2046) was described by the manufacturer as having 0.70 μmole of peptide per milligram and a potency of 117 ± 20 units per milligram in United States Pharmacopaeia rat pressor test No. 17 [*Handbook of Experimental Pharmacology*, vol. 23, *Neurohypophysial Hormones and Similar Polypeptides*, E. Berde and O. Eichler, Eds. (Springer-Verlag, Berlin, 1968), pp. 163–173]. In unpublished studies from our laboratory, this material was identical in potency to that reported by Pavel for another preparation of synthetic AVT in the compensatory ovarian hypertrophy test (*2*). The material was dissolved (10 μg/ml) in distilled water just before intraperitoneal administration.
4. The mean ovarian weight for 25-day-old untreated female mice was 4.35 ± 0.18 mg, whereas the mean ovarian weight for 28-day-old diluent-treated mice was 5.29 ± 0.25 mg.
5. M. Vaughan, R. J. Reiter, T. McKinney, G. M. Vaughan, *Int. J. Fertil.*, in press.
6. S. Pavel, *Science* **181**, 1252 (1973).
7. ———, *J. Clin. Endocrinol.* **31**, 369 (1970).
8. For review, see J. I. Kitay and M. D. Altschule, *The Pineal Gland* (Harvard Univ. Press, Cambridge, Mass., 1954).
9. R. J. Reiter, in *Reproductive Biology*, H. Balin and S. Glasser, Eds. (Excerpta Medica, Amsterdam, 1972), pp. 71–114.

* Present address: Department of Anatomy, University of Texas Health Science Center, San Antonio 78284. Address reprint requests to M.K.A.

17 January 1974; revised 21 June 1974

Vitamin D in Solution: Conformations of Vitamin D_3, 1α,25-Dihydroxyvitamin D_3, and Dihydrotachysterol$_3$

Abstract. *Solution conformations of the A and seco B rings of vitamin D_3, 1α,25-dihydroxyvitamin D_3, 1α-hydroxyvitamin D_3, and dihydrotachysterol$_3$ have been established by high resolution, 300-megahertz proton magnetic resonance spectroscopy. The A ring of these steroids is dynamically equilibrated between two chair conformers. For vitamin D_3, 1α-hydroxyvitamin D_3, and 1α,25-dihydroxyvitamin D_3 the relative proportions of the two conformers are 1 : 1, whereas dihydrotachysterol$_3$ exists principally as only one conformer. Thus, the substituent groups on the A ring may be either equatorially or axially oriented, and suggests a refinement of the existing topological model for vitamin D hormonal activity.*

Structural studies of vitamin D, its metabolites, and analogs in solution have been undertaken for the purpose of deriving structure-function relationships which will be of utility in a parallel program directed toward synthesis of useful analogs. Our first studies have centered on vitamin D_3 (D_3) and its analog dihydrotachysterol$_3$ (DHT_3). Both compounds have been

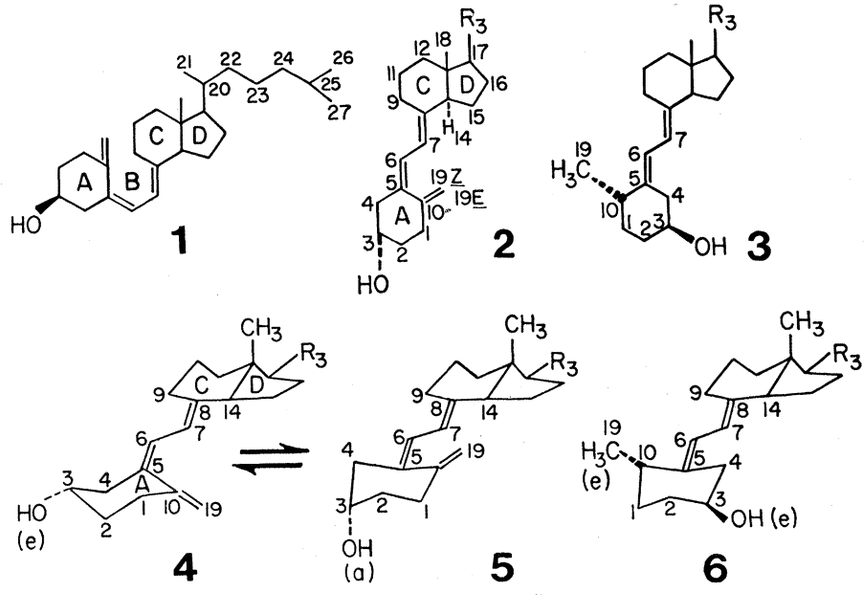

Fig. 1. Structural representations of vitamin D_3 and dihydrotachysterol$_3$. For vitamin D_3: structure **1**, classic folded steroid version; structure **2**, extended version derived from x-ray crystallographic analysis; structures **4** and **5**, dynamic equilibrium between chair conformers derived from high resolution PMR analysis. For DHT_3 structure **3**, extended version; structure **6**, predominant solution conformer. R_3 is the side chain, given in **1**, for carbons 20 to 27. In 1α,25-$(OH)_2$-D_3, the 25- and 1α-hydrogen atoms of structures **1**, **2**, **4**, and **5** are replaced by hydrogen groups. Thus, the A ring hydroxyls are *trans* to one another. In 1α-OH-D_3, only the A ring is similarly modified; *a*, axial conformation; *e*, equatorial conformation.

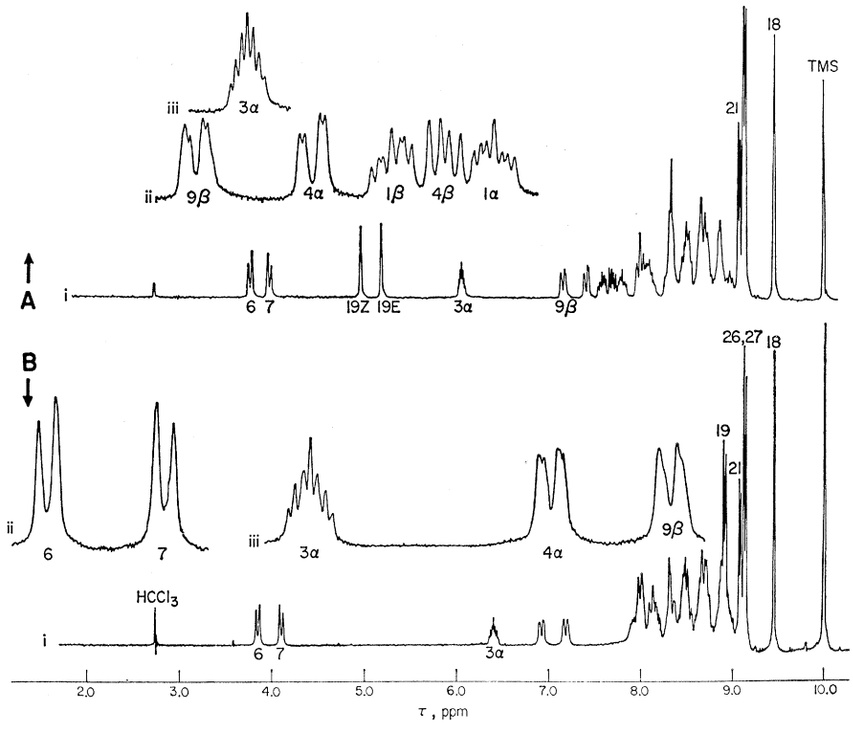

Fig. 2. Proton magnetic resonance at 300 megahertz of (A) D_3 and (B) DHT_3 in deuterochloroform with tetramethylsilane (TMS) and chloroform ($HCCl_3$) as internal standards. The proton assignments follow the labels given in Fig. 1. $HCCl_3$ is 2180-hertz downfield of TMS; expansions are fivefold.

shown to be useful for mediating normal calcium metabolism (*1*) and for treating clinical disorders such as hypoparathyroidism, chronic renal failure, and vitamin D–resistant rickets (*2*).

The structure of D_3 was originally viewed in a conformation relating it to its precursor steroid (Fig. 1, structure **1**) (*3*). Hodgkin *et al.* (*4*) established the structure by x-ray crystallographic analysis of an ester of vitamin D_2. The extended nature of the seco B ring was immediately accepted as being an important topological feature of D_3 (Fig. 1, structure **2**) and its biologically active analog DHT_3 (Fig. 1, structure **3**). A feature of the crystallographic structure (*4, 5*) which has been less emphasized is the chair nature of the A ring (Fig. 1, structure **4**). We now report that the A ring of these secosteroids exists in solution as a mixture of two chairlike conformers (see Fig. 1, structures **4** and **5**) in dynamic equilibrium with one another.

Proton magnetic resonance (PMR) spectra of D_3 and DHT_3 are presented in Fig. 2. Analysis of the spectra, with the aid of dipolar shifts induced by tris(dipivalomethanoto)europium(III), $Eu(dpm)_3$ (*6*), leads to the assignments indicated in Fig. 2 and confirms the essential stereochemical features of both D_3 and DHT_3 to be as reported (*4, 5, 7*). In particular, the conformations of the seco B rings seem assured for both molecules.

Study of the PMR spectrum of D_3 reveals that a conformational equilibration of the A ring between two chair forms (*8*) must be occurring. It is well established that conformations of six-membered rings may be derived from the couplings between suitably placed protons (*9*). *Trans*-vicinal protons are ideal in this respect, since in one chair form a 180° dihedral angle with couplings of about 11 hertz is extant, whereas in the inverted chair the same dihedral angle is now 60° and the coupling is about 3 hertz (*10*). Thus an averaging of two chair conformations will result in an average value for this coupling.

Therefore, observation of the coupling between $H_{3\alpha}$-$H_{4\beta}$ to be 7.6 hertz in D_3 implies an approximate equimolar mixture, with about half of the 3β-hydroxy groups equatorial as in the crystal structure (*4*). In DHT_3, however, the equilibrium mixture favors equatorial over axial 3β-hydroxy groups by a ratio of 9 : 1 ($J_{3\alpha,4\beta} = 10.0$ hertz, where J is the spin-spin coupling constant). The pattern of the resonances observed for the A ring protons of 1α,25-dihydroxyvitamin D_3 [1α,25-$(OH)_2$-D_3] and of 1α-hydroxyvitamin D_3 (1α-OH-D_3) are identical, and this shows that the nature of the side chain has no effect on the A ring population. However, the $J_{3\alpha,4\beta}$ splitting (6.5 hertz) for these latter substances is slightly smaller than that for D_3. This implies that there is now a slight bias toward the A ring chair with an axial 3β-OH (that is, there is a bias in favor of equatorial 1α-hydroxy groups). If the cyclohexanol coupling constants (3 and 11 hertz) accurately apply here (*10*), the observed coupling constant $J_{3\alpha,4\beta}$ actually gives estimates of the 3β-hydroxy equatorial conformer population (Fig. 1, structures **4** and **6**) as 88 percent for DHT_3, 57 percent for D_3, and 44 percent for 1α,25-$(OH)_2$-D_3 and 1α-OH-D_3. Conformational averaging in solution of this type is expected to be a feature of all vitamin D–like secosteroids, and other putative seco B or seco C steroids.

These results, when considered in conjunction with reports of biological assays (*11*), have led us to propose a refined topological model for vitamin D_3 activity (*12*). It was proposed that the 1α-hydroxyl of 1α,25-$(OH)_2$-D_3 or its geometric equivalent in analogs must occupy the equatorial as opposed to the axial orientation for optimization of biological activity. This model suggests the synthesis of putative new analogs. One such analog, 3-deoxy-1α-hydroxyvitamin D_3, has been synthesized and bioassayed (*13*). Its ability to elicit a greater maximal intestinal calcium response than 1α,25-$(OH)_2$-D_3 is consistent with this model (*12*). The bioassay of the deoxy analog (*13*) has been described.

RICHARD M. WING
WILLIAM H. OKAMURA
MARCEL R. PIRIO
STEVEN M. SINE
ANTHONY W. NORMAN

Departments of Chemistry and Biochemistry, University of California, Riverside 92509

References and Notes

1. A. W. Norman and H. Henry, *Recent Prog. Horm. Res.* **30**, 431 (1974); A. W. Norman, *Biol. Rev.* **43**, 97 (1968); J. Omdahl and H. F. DeLuca, *Physiol. Rev.* **53**, 327 (1973); M. Kaye, G. Chatterjee, G. F. Cohen, *Ann. Int. Med.* **73**, 225 (1970).
2. R. F. Fletchers, J. H. Jones, D. B. Morgan, *Q. J. Med.* **32**, 321 (1963); R. W. Winters, J. B. Graham, T. F. Williams, V. W. McFalls, C. H. Burnett, *Medicine* **37**, 97 (1958); A. Richens and D. J. F. Rowe, *Br. Med. J.* **4**, 73 (1970); A. W. Norman and H. Henry, *Clin. Orthop. Related Res.* **98**, 258 (1974); R. Gaynor, G. W. Ogden, G. Just, M. Kaye, *Can. J.*

Physiol. Pharmacol. **52**, 272 (1974); A. S. Brickman, J. W. Coburn, S. G. Massry, A. W. Norman, *Ann. Int. Med.* **80**, 161 (1974).
3. L. F. Fieser and M. Fieser, *Steroids* (Reinhold, New York, 1959), pp. 127–130.
4. D. C. Hodgkin, B. M. Rimmer, J. D. Dunitz, K. N. T. Trueblood, *J. Chem. Soc.* (1963), p. 4945.
5. C. Knobler, C. Romers, P. B. Braun. J. Hornstra, *Acta Cryst.* **B28**, 2097 (197[illegible] E. Havinga, *Experientia* **29**, 1181 (1973). This paper alludes to the possible importance of conformational factors on the photochemical behavior of vitamin D.
6. R. M. Wing, J. J. Uebel, K. K. Anderson, *J. Am. Chem. Soc.* **95**, 6046 (1973); R. von Ammon and R. D. Fischer, *Angew. Chem. Int. Ed.* **11**, 675 (1972); A. F. Cockerill, G. L. O. Davies, R. C. Hardin, D. M. Rackham, *Chem. Rev.* **73**, 533 (1973).
7. J. L. J. van de Vliervoet, P. Westerhof, J. A. Keverling-Buisman, E. Havinga, *Rec. Trav. Chim. Pays-Bas* **75**, 1179 (1956). Our experiments described here are the first to definitively establish that the configuration at C-10 in DHT_3 is the alpha configuration.
8. E. L. Eliel, N. L. Allinger, S. J. Angyal, G. A. Morrison, *Conformational Analysis* (Interscience, New York, 1967), chap. 2.
9. F. A. L. Anet and R. Anet, in *Determination of Organic Structures by Physical Methods*, F. C. Nached and J. J. Zuckerman, Eds. (Academic Press, New York, 1971), vol. 3, chap. 7.
10. F. A. L. Anet, *J. Am. Chem. Soc.* **84**, 1053 (1962).
11. K. A. Hibberd and A. W. Norman, *Biochem. Pharmacol.* **18**, 2347 (1969); T. Suda, R. B. Hallick, H. F. DeLuca, H. F. Schnoes, *Biochemistry* **9**, 1651 (1970); F. von Werder, *Justus Liebigs Ann. Chem.* **603**, 15 (1957); P. Westerhof and J. A. Keverling-Buisman, *Rec. Trav. Chim. Pays-Bas* **76**, 679 (1957).
12. W. H. Okamura, M. N. Mitra, A. W. Norman, M. R. Pirio, S. M. Sine, R. M. Wing, *Fed. Proc.* **33**, 1574 (1974); W. H. Okamura, A. W. Norman, R. W. Wing, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
13. A. W. Norman, W. H. Okamura. M. N. Mitra, R. M. Wing, *Science*, in pr[illegible] W. H. Okamura, M. N. Mitra, R. M. Wing, A. W. Norman. *Biochem. Biophys. Res. Commun.* **60**, 179 (19[illegible] M. N. Mitra, A. W. Norman, W. H. Okamura, *J. Org. Chem.* **39**, 2931 (1974).
14. We thank Dr. D. R. Kearns for access to the 300-megahertz proton magnetic resonance facility. This work was supported by PHS grants AM-09012 and AM-16595 and a grant from the Intramural Research Fund, University of California, Riverside. We thank Drs. Andor Fürst (Basel) and Milan Uskoković (Nutley) of Hoffmann–La Roche for generous samples of 1α-OH-D_3 and $1\alpha,25$-$(OH)_2$-D_3.

8 July 1974; revised 27 September 1974

Ethanol Inhibition of Vitamin A Metabolism in the Testes: Possible Mechanism for Sterility in Alcoholics

Abstract. *Vitamin A (retinol) is essential for spermatogenesis. Alcohol dehydrogenase, the enzyme responsible for ethanol metabolism, is also required for the conversion of retinol to bioactive retinal at the end organ site. Ethanol inhibits the oxidation of retinol by testicular homogenates containing alcohol dehydrogenase. Thus, a possible biochemical mechanism for the sterility of chronic alcoholics is identified.*

Testicular atrophy and aspermatogenesis are found in 50 to 75 percent of male chronic alcoholics with Laennec's cirrhosis (*1*). Histologic study of testicular biopsy and autopsy material has demonstrated marked seminiferous tubular atrophy with loss of germinal elements and, in more advanced cases, total germinal cell aplasia (*2*). The pathogenesis of this lesion is poorly defined, as are its rate of development and reversibility. In one autopsy review, the degree of testicular histologic damage appeared to correlate with liver disease activity, suggesting that testicular and liver injury were coincident and that both were partially reversible (*3*). Our own recent results establish that a high incidence of azoospermia is found not only in patients with Laennec's cirrhosis, but also in chronic alcoholics with relatively mild liver disease (*4*). Whatever the disease mechanism, it is estimated that 9 million adult Americans are alcoholics or alcohol abusers and that 10 percent of these may ultimately develop cirrhosis (*5*). These findings taken together make it almost certain that alcoholism is a common nonfunctional cause of male sterility in the United States, and suggest the possibility that alcohol per se (rather than or as well as liver disease) plays a significant role in the pathogenesis of azoospermia in male alcoholics.

Table 1. Alcohol dehydrogenase (ADH) activity of rat testes. Forty testes (20 animals) were used for each age. The results are expressed as mean ± standard error of the mean. The protein concentration in the reaction vessel varied for each homogenate but ranged between 30 and 200 μg/ml. The ADH activity is expressed as nanomoles of NADH per minute, retinol oxidation activity as nanomoles of retinal per minute per gram of tissue.

Age of rats	ADH activity			
	Total	Per gram of tissue	Per mg of protein	Retinol oxidation activity
20	966 ± 30	350	59	
43	4585 ± 96	170	30	34.1 ± 2.4
50	3944 ± 82	89	20	22.6 ± 2.6
61	5861 ± 90	120	23	15.0 ± 1.3

It is well established that vitamin A is essential for spermatogenesis (*6*). Rats develop germinal cell aplasia within 6 to 8 weeks of the initiation of a vitamin A–deficient diet, and full spermatogenesis is restored by replacement therapy. Similarly, vitamin A supplementation is required for the initiation of spermatogenesis in organ culture systems (*7*). Vitamin A is ingested as inactive retinol and is oxidized to active retinal by alcohol dehydrogenase (ADH), the enzyme which metabolizes ethanol to acetaldehyde. Recent studies have demonstrated ADH activity in the retina, and have shown that alcoholics experience night blindness because of the competitive inhibition of retinal formation by ethanol (*8, 9*). We now propose that ADH activity is demonstrable in testicular tissue, that retinol is converted to retinal by testicular ADH, that ethanol inhibits testicular retinal formation, and that "relative vitamin A deficiency" may be a factor in the pathogenesis of sterility in chronic alcoholics.

Testicular and hepatic tissue were obtained from 20 Wistar rats at each of the following ages: 20, 43, 50, and 61 days. Homogenates of both types of tissue were prepared in a 1:9 dilution with 0.32*M* sucrose in 10 m*M* tris(hydroxymethyl)aminomethane (tris) buffer, at *p*H 7.5. These homogenates were then made successively 35 and 60 percent saturated with $(NH_4)_2SO_4$. Essentially all the ADH activity was located in the fraction precipitated at 35 to 60 percent $(NH_4)_2SO_4$. This precipitate was redissolved in 40 m*M* tris buffer,

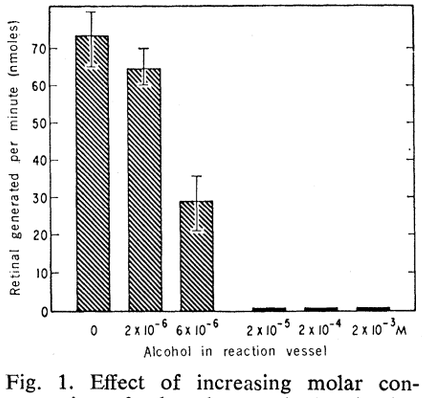

Fig. 1. Effect of increasing molar concentration of ethanol on retinol oxidation by testicular homogenates containing alcohol dehydrogenase activity. The retinol concentration and protein content in the incubation mixture were $6 \times 10^{-6}M$ and 100 μg, respectively. Results are expressed as mean ± standard error of the mean. Note complete inhibition of retinol oxidation at molar concentrations of ethanol equal to or greater than 2×10^{-5}.