synthesis which accumulates in PbI and FeD. The defect in EPP is different and allows the earlier intermediate PP to accumulate.

Finally, we wish to point out a clinical application of our observations. We and Joselow (*25*) have shown that measurement of the fluorescence, at 594 nm, of blood directly (without any extraction steps) can serve as the simplest and most specific screening test for PbI.

A. A. LAMOLA
T. YAMANE

Bell Laboratories,
Murray Hill, New Jersey 07974

References and Notes

1. A. A. H. Van Den Bergh and W. Grotepass, *Klin. Wochenschr.* **12**, 586 (1933).
2. R. J. Watson, E. Decker, H. C. Lichtman, *Pediatrics* **21**, 40 (1958).
3. H. C. Lichtman and F. Feldman, *J. Clin. Invest.* **42**, 830 (1963).
4. C. J. Watson, *A.M.A. Arch. Intern. Med.* **99**, 325 (1957).
5. S. Granick, S. Sassa, J. L. Granick, R. D. Levere, A. Kappas, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2381 (1972).
6. L. P. Kammholz, L. G. Thatcher, F. M. Blodgett, T. A. Good, *Pediatrics* **50**, 625 (1972).
7. J. J. Chisolm, Jr., *J. Pediatr.* **64**, 174 (1964).
8. S. Piomelli, B. Davidow, V. F. Guinee, P. Young, O. Gay, *Pediatrics* **51**, 254 (1973).
9. S. Schwartz, M. H. Berg, I. Bossenmaier, H. Dinsmore, in *Methods of Biochemical Analysis*, D. Glick, Ed. (Interscience, New York, 1960), vol. 8, pp. 221–294.
10. L. Wranne, *Acta Pediatr. Scand.* **49**, 1 (1960).
11. J. A. Whitaker and T. J. Vietti, *Pediatrics* **24**, 734 (1959).
12. A. A. Lamola, T. Yamane, S. Piomelli, M. Poh-Fitzpatrick, unpublished data.
13. S. Piomelli, A. A. Lamola, M. Poh-Fitzpatrick, E. Carlos, C. Seaman, L. Harber, unpublished data.
14. J. J. Leonard, T. Yonetani, J. B. Callis, *Biochemistry* **13**, 1460 (1974).
15. J. E. Falk, *Porphyrins and Metalloporphyrins* (Elsevier, New York, 1964), pp. 85–87.
16. R. S. Becker, *Theory and Interpretation of Fluorescence and Phosphorescence* (Wiley-Interscience, New York, 1969), pp. 190–197.
17. E. J. Underwood, *Trace Elements in Human and Animal Nutrition* (Academic Press, New York, ed. 2, 1962), p. 157.
18. M. Grinstein, R. M. Bannerman, C. V. Moore, *Blood* **14**, 476 (1959).
19. H. C. Schwartz, G. E. Cartwright, E. L. Smith, M. M. Wintrobe, *ibid.*, p. 486.
20. See G. H. Tait, in *Porphyrins and Related Compounds*, T. W. Goodwin, Ed. (Academic Press, New York, 1968), pp. 31–33.
21. See G. S. Marks, *Heme and Chlorophyll* (Van Nostrand, London, 1969), pp. 146–147.
22. H. Oyama, K. Sugita, Y. Yoneyama, H. Yoshikaya, *Biochim. Biophys. Acta* **47**, 413 (1961).
23. R. F. Labbe and N. Nubbard, *ibid.* **52**, 130 (1961).
24. J. N. Phillips, in *Comprehensive Biochemistry*, M. Florkin and E. H. Stotz, Eds. (Elsevier, Amsterdam, 1963), vol. 9, p. 34.
25. A. A. Lamola, M. Joselow, T. Yamane, *Clin. Chem.*, in press.
26. We are indebted to Dr. Alan Adler and Ms. V. Veradi for their gift of zinc protoporphyrin and magnesium protoporphyrin, to Dr. T. Kometani for performing the atomic absorption spectrophotometric analyses, and to Dr. M. Joselow for specimens of blood from PbI patients. This work was stimulated by investigations of EPP initiated at the Department of Dermatology, Columbia College of Physicians and Surgeons. A.A.L. is indebted to M. Poh and L. Harber for their collaboration in this regard.

14 August 1974

Arginine Vasotocin: Effects on Development of Reproductive Organs

Abstract. *Immature 25-day-old mice were injected daily with 1 microgram of arginine vasotocin for 3 or 4 days and killed 24 hours after the last injection. The ovaries were 30 percent smaller in treated females than in controls. The ventral prostates and accessory organs (seminal vesicles and coagulating glands) were less than half the size of these structures in control males. Similar results were observed when 15-day-old mice were given similar injections and killed 2 weeks after the last injection; furthermore, testis weights were 28 percent smaller than those of controls. It is speculated that arginine vasotocin, which has been found in mammalian pineal glands, might mediate effects of the pineal gland on normal sexual development.*

Arginine vasotocin (AVT), an octapeptide, has been identified in the mammalian pineal gland and proposed to be the antigonadotrophic product of that gland (*1*). Pavel *et al.* (*2*) reported that this cyclic peptide is a million times more potent than melatonin, the putative pineal hormone, in inhibiting the compensatory ovarian hypertrophy that occurs in adult female mice after unilateral ovariectomy. In this report we present experimental evidence that treatment of normal immature mice with AVT results in smaller reproductive organs in both males and females.

Immature Swiss-Webster mice were obtained from Hilltop Lab Animals, Inc. (Scottdale, Pennsylvania) or bred from stock derived from that source. In these animals, vaginal patency occurs at 30 to 35 days of age and reproductive competence for both males and females is attained by 60 days of age. The animals were maintained in an environment with automatically controlled lighting that provided 14 hours of light per day. In the first experiment, groups of 25-day-old female and male mice received daily intraperitoneal injections for 3 and 4 days, respectively, either of 1 μg of AVT in 0.1 ml distilled water or of diluent alone (*3*). Their reproductive organs were examined 24 hours after the last injection. In addition, two groups of normal male and female mice were necropsied at 25 days of age. Compared to diluent treatment of age-matched mice, AVT treatment decreased the weight of the ovaries ($P < .001$) in female mice and accessory organs (seminal vesicles and coagulating glands) ($P < .01$) and ventral prostates ($P < .001$) in male mice (Fig. 1). In the period between days 25 and 28, ovaries of control mice increased in weight by 22 percent whereas AVT treatment during this period prevented this normal developmental growth (*4*).

In the second experiment, five litters of mice were reduced to eight pups per mother on the day of birth and random-

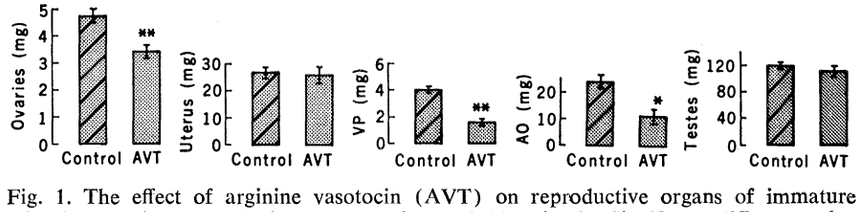

Fig. 1. The effect of arginine vasotocin (AVT) on reproductive organs of immature mice in experiment 1. Each group consisted of 11 animals. Significant differences between AVT groups (gray bars) and controls (striped bars) are indicated as follows: * $P < .01$; ** $P < .001$. *VP*, ventral prostate; *AO*, accessory organs.

Table 1. Weights of reproductive organs of immature mice treated with AVT in experiment 2. Data for each group are means ± standard error for eight to ten mice.

Female group	*Body weight (g)*	*Ovaries (mg)*	*Uterus (mg)*	
Control	20.3 ± 0.3	4.93 ± 0.20	17.9 ± 2.1	
AVT	18.5 ± 0.6	3.44 ± 0.23*	15.2 ± 0.8	
Male group	*Body weight (g)*	*Testes (mg)*	*Ventral prostate (mg)*	*Accessory organs (mg)*
Control	24.3 ± 0.5	166.5 ± 3.5	4.15 ± 0.09	22.4 ± 1.1
AVT	23.3 ± 0.5	120.5 ± 4.1†	2.34 ± 0.19†	15.0 ± 1.0*

* $P < .01$. † $P < .001$.

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

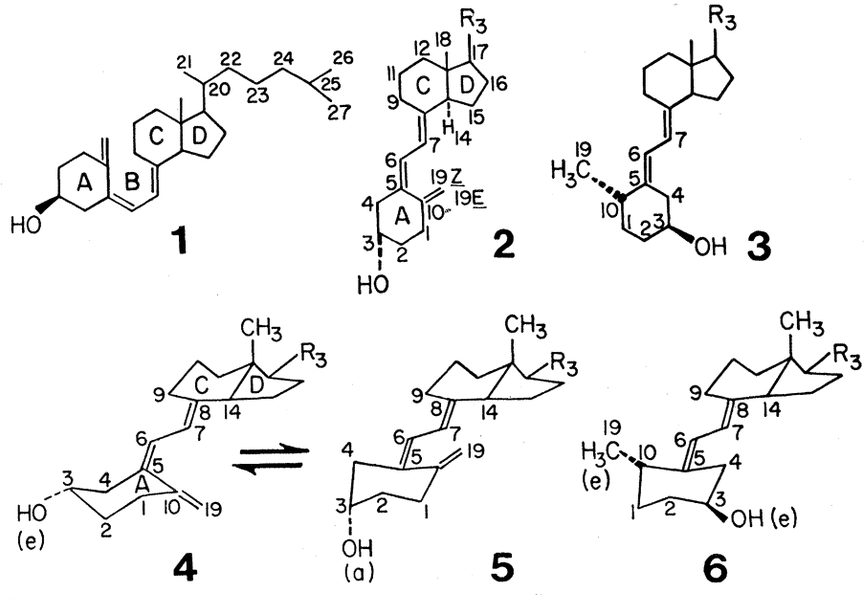

Fig. 1. Structural representations of vitamin D_3 and dihydrotachysterol$_3$. For vitamin D_3: structure **1**, classic folded steroid version; structure **2**, extended version derived from x-ray crystallographic analysis; structures **4** and **5**, dynamic equilibrium between chair conformers derived 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.