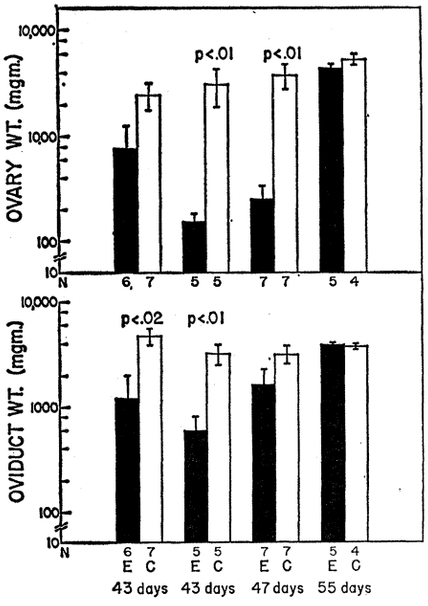

Fig. 2. Comparison of ovary and oviduct weights of *Coturnix coturnix japonica* from 43 days of age to sexual maturity. *E*, experimentals (pinealectomized); *C*, controls (sham-operated). Bars on columns represent one standard error.

2435.6 ± 747.9 mg; $P < .02$. In the normal development of the ovary, there is a period (from approximately 40 to 45 days of age) during which there is a rapid accumulation of yolk in maturing ova preceding the onset of the laying period. Pinealectomized birds failed to show this response, and egg production was delayed. By 55 days, both the experimental and control groups showed equivalent ovarian weights and oviposition.

The response of the oviducts reflected the differences in ovarian weight at 43 days (Fig. 2), but differences in oviducal weights were no longer evident by 47 days of age. Concomitant differences (± S.E.) in adenohypophyseal weights were also present at 43 days: experimentals, 1.292 ± 0.086 mg; controls, 1.638 ± 0.090 mg; $P < .02$ (combined data from both 43-day groups). There were no differences in adenohypophyseal weight at earlier or later ages. Body weights (body weight minus ovary and oviduct weight) were not significantly different after 30 days of age. In the males, there were no differences in body weight, testicular weight, or adenohypophyseal weight.

Obviously, pinealectomy in juvenile female *Coturnix* delays maturation of the ovaries and oviducts and hence delays the onset of egg production. The simultaneous occurrence of decreased ovarian, oviducal, and adenohypophyseal weights suggests that the pineal may be exerting an influence on the pituitary by possibly altering pituitary gonadotropin secretion. Whatever the relation between the pineal and the adenohypophysis may be, the effects are obviously transitory and occur for only a brief period preceding the onset of sexual maturity. Thus, the pineal appears to have a progonadotropic influence on ovarian development during a narrow phase of growth in birds exposed to a stimulatory photoperiod.

Other studies of birds suggest that the pineal exerts an age-dependent, transitory, and possibly sex-dependent influence on gonadal function. Shellabarger (*7*) found that the effect of the pineal in male White Leghorns is progonadotropic during the first 20 days and antigonadotropic between 40 and 60 days of age. The progonadotropic differences were much smaller than those that we found in the *Coturnix* females. Bovine pineal extracts reversed the effects of pinealectomy in the two age groups, thereby confirming an age-dependent effect (*7*). Removal of the superior cervical ganglia in mature female *Coturnix* resulted in cessation of egg laying for up to 13.2 days, but it failed to produce an effect on the testes in mature males (*5*). Identical treatment in 7-week-old females delayed the onset of laying. Hence these ganglia probably have a regulatory influence on the female avian pineal, and its role appears to be stimulatory with respect to reproductive activity. In contrast, studies of mammals (*1*) indicate an inhibitory influence on reproductive function.

The finding (*8*) that pinealectomy in Japanese quail exposed to stimulatory photoperiods (14L : 10D) did not affect ovarian or testicular weight at 4 weeks of age agrees with our results for this age group in both sexes. Because Homma *et al.* made no observations after day 28, the effects of pinealectomy in birds older than 28 days, as in our experiments, were not observed.

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References and Notes

1. R. J. Wurtman, M. D. Altschule, U. Holmgren, *Amer. J. Physiol.* **197**, 108 (1959); E. W. Chu, R. J. Wurtman, J. Axelrod, *Endocrinology* **75**, 238 (1964); R. A. Hoffman and R. J. Reiter, *Science* **148**, 1609 (1965).
2. W. O. Wilson, H. Abplanalp, L. Arrington, *Brit. Poultry Sci.* **3**, 105 (1962); K. Tanaka, F. B. Mather, W. O. Wilson, L. Z. McFarland, *Poultry Sci.* **44**, 662 (1965).
3. J. Axelrod, R. J. Wurtman, C. M. Winget, *Nature* **201**, 1134 (1964).
4. A. Wolfson, in *Recent Progress in Hormone Research*, G. Pincus, Ed. (Academic Press, New York, 1966), pp. 177–239.
5. L. Z. McFarland, K. Homma, W. O. Wilson, *Anat. Rec.* **154**, 386 (1966).
6. R. J. Wurtman, J. Axelrod, J. E. Fischer, *Science* **143**, 1328 (1964).
7. C. J. Shellabarger and W. R. Breneman, *Proc. Indiana Acad. Sci.* **59**, 299 (1949); C. J. Shellabarger, *Endocrinology* **51**, 151 (1952); *Poultry Sci.* **32**, 189 (1953).
8. K. Homma, L. Z. McFarland, W. O. Wilson, *Poultry Sci.* **46**, 314 (1967).
9. Supported by a research grant (GB-1041) from NSF and the Research Committee of Northwestern University to A.W. and by a PHS predoctoral fellowship (1-F1-GM-32,499-01) from the Institute of General Medical Science to A.S. We thank A. Dowd, B. Alexander, and M. Salmon for assistance.

27 September 1967

Pemoline Levels in Brain: Enhancement by Dimethyl Sulfoxide

Abstract. *Pemoline-C^{14} dissolved in dimethyl sulfoxide and injected intraperitoneally into rats was found in larger amounts in the brain than was a similar dose given in 0.3 percent tragacanth suspension. This appeared to be related to a partial breakdown of the blood-brain barrier in vivo by the dimethyl sulfoxide.*

A number of recent reports have presented conflicting data on the efficacy of magnesium pemoline (PMH) in facilitating learning in humans (*1*) and in rats (*2*). The original observation by Glasky and Simon (*3*) that PMH activates brain RNA-polymerase in vitro was not confirmed by others for the in vivo situation (*4*). In the behavioral studies cited, PMH was administered either orally or intraperitoneally (as a suspension in 0.3 percent tragacanth), yet virtually nothing is known of the relation between levels of the drug in the brain and behavioral performance. Blood levels of pemoline-2-C^{14} (PIO-C^{14}) have been reported to reach a value of 3 to 4 percent of the administered dose per liter of serum in 5 hours, when this compound was administered to humans by the oral route (*5*).

In view of the fact that PMH tends to be more soluble in dimethyl sulfoxide (DMSO) than in aqueous solution (*3*),

and that DMSO enhances absorption of drugs in vivo (*6*), we have administered PIO-C^{14} (*7*) dissolved in 100 percent DMSO to rats and measured blood and brain levels of the drug at different times. Under these conditions, we determined both facilitation of learning (*8*) and increases in blood and brain levels of this compound relative to the amounts found when 0.3 percent tragacanth was used as the vehicle.

Pemoline-2-C^{14} was dissolved in 100 percent DMSO (Baker reagent grade, 6.75 mg/ml) and injected intraperitoneally into Sprague-Dawley rats (400 to 450 g) at a dose of 4.5 mg/kg. With 0.3 percent tragacanth as the medium, PIO-C^{14} was dispersed at a concentration of 10 mg/ml, and the same dose was used as in the studies with DMSO. Rats were anesthetized with ether at 10, 20, 30, 60, and 120 minutes after receiving the drug, and samples of whole blood were obtained by rapidly opening the chest between the forepaws and severing the vena cava; care was taken to prevent rupture of the diaphragm. Blood was rapidly transferred by Pasteur pipette to calibrated centrifuge tubes containing 2 ml of potassium oxalate solution (10 mg/ml), and the amount collected was found by taking the difference between the final and initial volumes. The brain (including the cerebellum) was isolated as rapidly as possible; after removal of small adhering blood clots, it was weighed and frozen for subsequent analysis. Samples of blood were digested in 1 volume of 60 percent $HClO_4$ and 2 volumes of 30 percent H_2O_2 at 80°C for 30 minutes, by the method of Mahin and Lofberg (*9*), and then mixed with a PPO-toluene-cellosolve fluor (*10*) for counting in a Packard liquid scintillation counter. Brains were homogenized in an equal volume of H_2O, and samples of the 50 percent homogenate were processed for counting by the same procedure used for blood. The counts obtained in each sample were corrected for quenching and expressed as micrograms of PIO. The results shown in Fig. 1 indicate that DMSO facilitates the absorption of PIO-C^{14} into the blood by a factor of 2, compared to PIO-C^{14} in tragacanth. Values are expressed as the percentage of the administered dose present in the total blood volume of the rat (58 ml/kg). The levels of PIO-C^{14} in brain (Fig. 2) show a similar elevation induced by DMSO and may be attributed to a partial breakdown of the blood-brain barrier within the first 30 minutes. This is indicated by the ratio of drug levels in blood to those in brain being smaller for the DMSO-treated rats than in animals treated with tragacanth. No radioactivity could be detected in the brain 24 hours after injections of the drug.

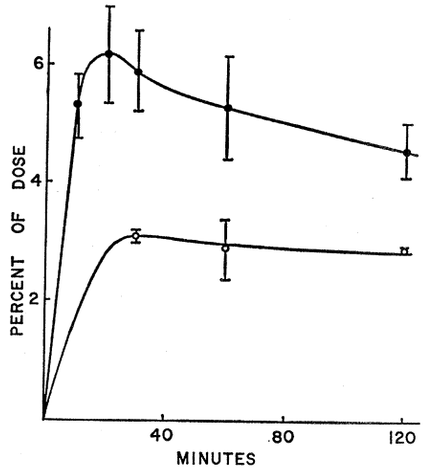

Fig. 1. Blood levels of pemoline-C^{14} after intraperitoneal administration in DMSO or in tragacanth suspension. Blood was collected from the thoracic cavity at different intervals after injection and analyzed for radioactivity. Each point represents the mean and range of separate analyses on two to three rats and is expressed as percentage of the total blood volume (58 ml/kg). Solid circles: DMSO solution. Open circles: tragacanth suspension.

The evidence that PMH markedly facilitates learning when administered in DMSO (*8*) supports our finding of PIO in the brain in larger quantities than in animals that did not receive the drug in DMSO. The pronounced effects of PMH versus PIO on behavioral performance, found by Plotnikoff and Meekma (*11*), may be attributed to the greater solubility of PMH in aqueous solution. Using a spectrophotometric procedure, we have measured the quantities of PIO and PMH in saturated water solutions of these two compounds. The molar extinction coefficient of PIO was calculated to be 2.56×10^4 at 216 mμ in 0.04*N* NH_4OH (*p*H 10.8), and corresponding concentrations of PIO and the chromophore of PMH in water were 1.4×10^{-3} and $2.1 \times 10^{-3}M$, respectively. In DMSO, however, the PIO component of PMH readily goes into solution, leaving the $Mg(OH)_2$ as an insoluble residue. The low levels of these two compounds in aqueous media could account for the lack of facilitative effects on learning found in humans after oral administration of the drugs (*1*). The high solubility of PIO in DMSO (132 mg/ml) is well suited for studies where low doses of DMSO together with large doses of PIO are desired, and may prove to be of value in study of the mechanism of action of this compound in influencing cognitive behavior.

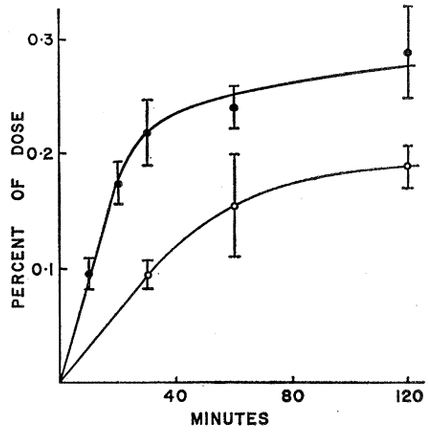

Fig. 2. Brain levels of pemoline-C^{14} after intraperitoneal administration in DMSO or in tragacanth. Brains were homogenized in water, and samples were analyzed for radioactivity. Points represent the means and ranges of individual analyses on two to three rats; they are expressed as percentages of the administered doses in whole brain. Solid circles: DMSO solution. Open circles: tragacanth suspension.

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References and Notes

1. J. T. Burns, R. F. House, F. C. Fench, J. G. Miller, *Science* **155**, 849 (1967); R. G. Smith, *ibid.*, p. 605; G. A. Talland, *Psychon. Sci.* **6**, 493 (1966); G. A. Talland and M. T. McGuire, *Psychopharmacologia* **10**, 445 (1967).
2. G. Beach and D. P. Kimble, *Science* **155**, 698 (1967); P. W. Frey and V. J. Polidora, *ibid.*, p. 1282; N. Plotnikoff, *ibid.* **151**, 703 (1966); N. Plotnikoff, *Life Sci.* **5**, 1695 (1966).
3. A. J. Glasky and L. N. Simon, *Science* **151**, 702 (1966).
4. N. R. Morris, G. K. Aghajanian, F. E. Bloom, *ibid.* **155**, 1125 (1967).
5. J. H. Nodine, J. M. Platt, J. Carranza, R. Dykyj, Y. Mapp, *Int. J. App. Rad. Isotopes* **15**, 263 (1964).
6. S. W. Jacob and D. C. Wood, *Curr. Ther. Res.* **9**, 229 (1967).
7. Pemoline-C^{14} (2-imino-5-phenyl-oxazolidinone-4-2-C^{14}) was a gift from Drs. H. G. Schoepke and J. C. Netwal of the Pharmacology Dept. of Abbott Laboratories and was diluted to a specific activity of 0.1 μc/μmole with recrystallized iminophenyloxazolidinone from the Aldrich Chemical Co.
8. D. G. Stein, J. J. Brink, A. H. Patterson, unpublished.
9. D. T. Mahin and R. T. Lofberg, *Anal. Biochem.* **16**, 500 (1967).
10. Fifteen milliliters of 0.38 percent 2,5-diphenyloxazole dissolved in methyl cellosolve–toluene (6 : 10 by volume).
11. N. Plotnikoff and P. Meekma, Jr., *J. Pharm. Sci.* **56**, 290 (1967).
12. Supported in part by grants MH 13576-01 to J.J.B. and MH 13705-01 to D.S., and the PHS biomedical sciences general research support program to Clark University.

14 August 1967