Avian Pineal Gland: Progonadotropic Response in the Japanese Quail

Abstract. *Pinealectomy in juvenile Japanese quail* (Coturnix coturnix japonica) *exposed to stimulatory photoperiods results in delay of ovarian and oviducal development and decreased pituitary weight. These effects are transitory and occur during a phase of growth immediately preceding the onset of sexual maturity. No effect was found in males. These results suggest that the pineal gland in females has a stimulatory effect on gonadal growth during maturation.*

Although the pineal gland has been found to be a mediator in the gonadal responses of rats and hamsters to light (*1*), there have been few studies of birds where the relation between light and the gonadal cycle is well known. In view of the fact that reproductive activity in the Japanese quail *Coturnix coturnix japonica* can be readily manipulated by photoperiod (*2*), we wanted to determine the influence of the pineal on the reproductive activity of this species.

We now report on experiments to determine the effect of pinealectomy on the development of sexual maturity in both males and females. The results show that the pineal in the females contributes to the sudden and rapid development of the ovaries to maturity during a brief interval before the onset of the laying period. In the absence of the pineal, this maturation and the onset of egg production is delayed for several days.

Axelrod *et al.* (*3*) found that the weight of the pineal and the activity of the melatonin-forming enzyme hydroxyindole-*O*-methyltransferase (HIOMT) were greater in chickens exposed to continuous light or to diurnal light than in those exposed to continuous darkness. Because light stimulates avian gonadal activity (*4*), melatonin production was probably induced during the long photoperiods that also accelerate gonadal development. McFarland *et al.* (*5*) reported that superior cervical ganglionectomy in Japanese quail caused a significant decrease in egg production and delayed the onset of the laying period. If the pineal gland in birds is innervated by the superior cervical ganglion, as it is in mammals (*6*), then the pineal is probably exerting a stimulatory influence, directly or indirectly, on gonadal activity.

Parental stocks of Japanese quail, obtained during the spring and summer of 1966 from the University of Wisconsin, Wisconsin State University, Beloit College, and Michigan State University, were mixed randomly in mating groups, and a breeding stock was developed over a period of several months. During the period of experimentation, from October 1966 to March 1967, eggs collected daily were stored and incubated weekly until hatching. After the hatching period, each group of chicks was placed in a heated brooder for 10 days under controlled conditions of light, each light-dark cycle consisting of 16 hours of light and 8 hours of darkness (16L : 8D). Heat was provided with an electric heater that glowed dimly during the dark period.

Pinealectomies (experimentals) and sham-operations (controls) were performed in both males and females between 7 and 9 days after hatching. We performed the pinealectomies by first removing a small piece of bone in the area immediately dorsal to the pineal organ and making a small incision in the dura mater immediately adjacent to the pineal stalk. The pineal and its stalk were removed with jeweler's forceps. In sham-operated birds, the identical procedure was followed, but the pineal was not removed. Electrocautery and Gelfoam were used to control bleeding. Absence of the pineal was verified at autopsy by examination under the dissecting microscope. Only animals with complete pinealectomies were used in the analysis of the results.

At 10 days of age, the birds were housed individually and maintained on the 16L : 8D cycle. Food (Purina Startena) and water were available continuously. Autopsies in the separate groups were performed at 25, 30, 43 (two groups), 47, and 55 days of age. At autopsy, we obtained data on weight of body, adenohypophysis, gonads, and oviducts. The results for each group were analyzed with both the *t*-test and Wilcoxon rank-sum test. The *P* values given are those from the *t*-tests.

In the female (Fig. 1), there were no differences in ovarian weight until 43 days of age. This difference was observed in the second group autopsied at 43 days and in the group autopsied at 47 days (Fig. 2). The ovarian weights of the first 43-day group were not statistically different because of one experimental ovary which weighed over 4 g (Fig. 1). If this ovary is excluded, the experimental and control groups differ significantly (mean ± S.E.): experimentals, 90.6 ± 25.3 mg; controls,

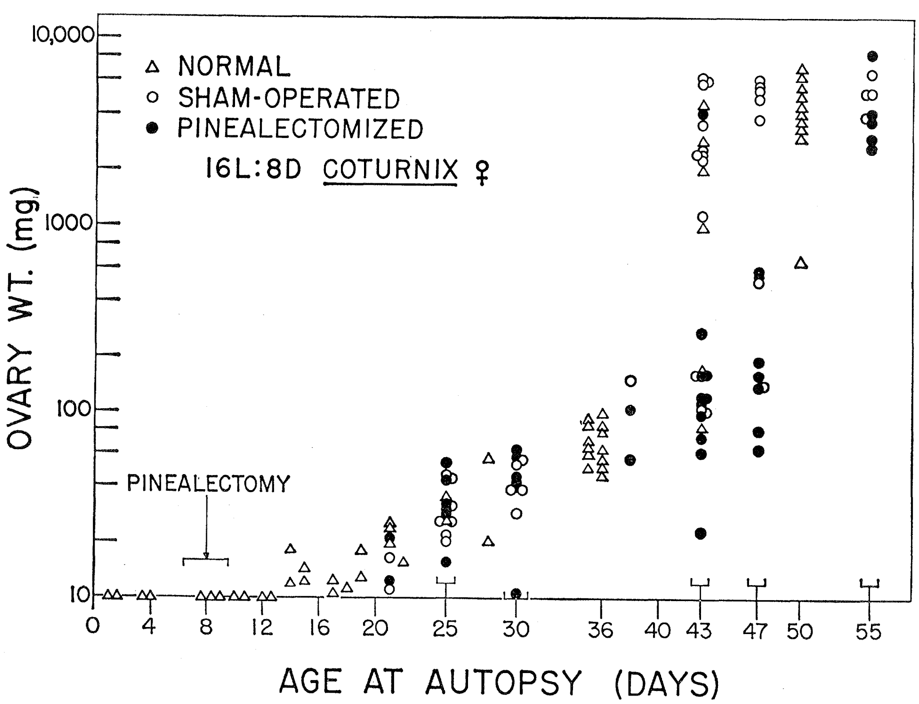

Fig. 1. Ovarian weights of *Coturnix coturnix japonica* from hatch to sexual maturity. Symbols on 10-mg line indicate that weights were 10 mg or less.

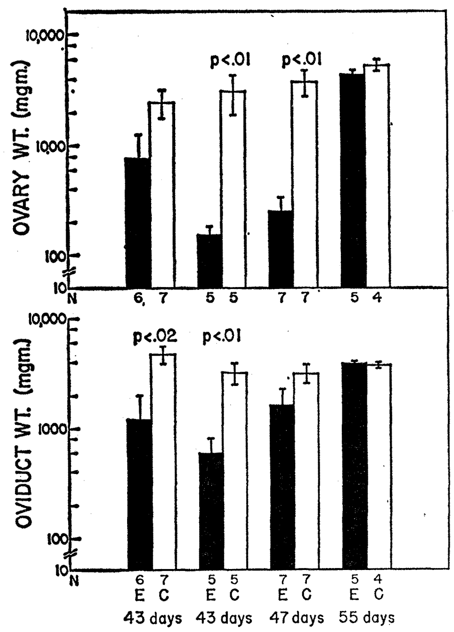

Fig. 2. Comparison of ovary and oviduct weights of *Coturnix coturnix japonica* from 43 days of age to sexual maturity. *E*, experimentals (pinealectomized); *C*, controls (sham-operated). Bars on columns represent one standard error.

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 ($\pm$ 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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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*),