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Pineal Organ as a Possible Photoreceptor in Photoperiodic Testicular Response in Japanese Quail

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ABSTRACT In male Japanese quail illumination of the pineal organ with luminous paint emitting orange light causes maintenance of testicular size for two weeks after transfer from continuous light (LL) to 8-hour daily photoperiods (8L16D). Non-treated controls and subjects treated with luminous paint emitting green light, submitted to the same change in day length, underwent extensive testicular regression. Although pinealectomy did not affect testicular size in birds subjected to LL, pinealectomized birds treated with luminous paint underwent testicular regression when changed to 8L16D. This suggests that the pineal organ may serve as a receptor for long wave lengths in the photoperiodic testicular response in this species.

Introduction

There have been very few investigations of the photoreception mechanism in photoperiodically induced testicular development in birds (Farner, 1959) since Benoit and his colleagues suggested that light could stimulate the hypothalamus directly through the orbit in the duck (Benoit and Assenmacher, 1959). In recent studies, evidence was obtained that suggests the existence of photoreceptors in the brain of the Japanese quail (Oishi *et al.*, 1966). Although Oksche and Vaupel-von Harnack (1965) found no typical photoreceptor structure in the birds studied by them, Bischoff and Richter (1966) found that the pineal cells of the quail had numerous micro-villi and membranous lamellar complexes suggestive of a photoreceptor function. Quay and Renzoni (1963) also suggested microscopically the presence of sensory cells in the pineal organ of birds. Should this be the case, there is the possibility of the phylogenetic persistence of a photoreceptor now involved in testicular development, since the pineal is thought to have a function as a photoreceptor of reptiles and amphibians (Bagnara, 1965; Clausen and Poris, 1937; van de Kamer, 1965). Also possibly pertinent is the report that the mammalian pineal, which has no direct role of photoreceptor,

secretes melatonin that inhibits light-induced gonadal growth (Wurtman and Axelrod, 1965). For these reasons, experiments were carried out to confirm the preliminary studies (Kato *et al.*, 1967) that have shown an effect of local illumination of pineal organ on testicular activity.

Materials and Methods

Experiments were carried out with mature male Japanese quail of 11 weeks of age, which had been reared under continuous light from hatching. Day-light fluorescent tubes were used as the light source. Light intensity at the floor of aviary was 100-1000 lux during light fraction and less than 0.01 lux in dark fraction of the 24-hour cycle. Room temperature was held at 25.5± 0.2°C throughout the experiments. Commercially prepared food and water were provided *ad libitum*.

To illuminate the pineal organ, radioluminous pigments were painted locally on the adjacent region of the skull. Two kinds of radioluminous pigments (LC-RIA and LC-GIH) made by Dai Nippon Sinleuch Co., Ltd. were used. LC-RIA emits orange light (maximum 600 mμ) and LC-GIH emits green light (maximum 520 mμ). The characteristic of these paints have been described previously (Kato *et al.*, 1967).

Pinealectomy was effected as follows. The skin on the midline of the occipital region of the head was cut open with a surgical scissors and a small rectangular flap of the skull (about 15 mm^2) was cut out with a hand drill. The pineal organ was carefully removed through an opening in the meninges. As soon as the pineal organ was taken out, a gelform sponge was placed on the wound for hemostasis. The survival rate in the operation was about 80% ; an anatomical examination of the pinealectomy was made each individual at autopsy. In the shamoperated controls all procedures were the same as for the pinealectomized birds except for the actual removal of the pineal organ. These operations were made four days before the beginning of the experiments.

Body weights and testicular weights were recorded at the beginning and the end of the experiments respectively. Brains and testes were fixed in Bouin's fluid for histological observation. The details of the experimental lighting regimens were shown in Fig.-1. Experimental conditions were maintained for two weeks in all cases.

As controls, normal animals (A) were divided into two groups at the age of 11 weeks and were maintained under continuous light (LL) and 8 hours light 16 hours darkness (8L 16D), respectively. At the beginning of the experimental illumination, sham-operated birds (B) were divided to four groups. One group was maintained under LL, the other three groups were placed on under 8L 16D; the first and second groups were without radioluminous paint, whereas the birds in the third group were painted with the LC-RIA on a small surface area (about 15 mm^2) above the pineal organ; the fourth group was treated similarly with the LC-GIH. The pinealectomized birds (C) were also divided in four groups

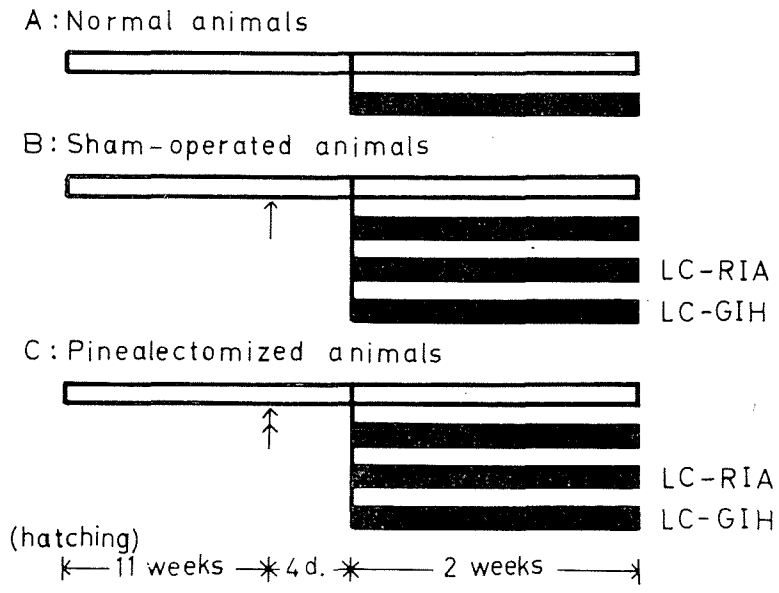

Fig. 1. : Experimental schedule
white line : continuous light (LL)
black line : 8-hours daily photoperiods (8L 16D)
↑ : sham operation
↕ : pinealectomy
LC-RIA : treatment with a radioluminous paint (orange light)
LC-GIH : treatment with a radioluminous paint (green light)

each treated identically with its corresponding control group.

Results and Discussion

The results obtained are summarized in Table I. Normal animals (group A), two weeks after change from LL to 8L 16D had a mean weight of 152 ± 45 mg. Whereas normal controls held continuously on LL to this time had a mean testicular weight of 1,328 ± 144 mg. The difference ie highly significant ($p<0.01$).

For the sham-operated birds on LL the mean testicular weight was 1, 352 ± 31 mg whereas it was 421 ± 159 mg for those changed to 8L 16D; the difference is significant ($p<0.05$). This difference between LL and 8L 16D indicates that the sham operation has no influence on the testicular development. The testes of the birds treated with LC-RIA (orange light) maintained their developed state (1,005 ± 58 mg) for at least two weeks on 8L16D whereas those treated with LC-GIH (green light) regressed considerably (475 ± 97 mg). The difference between these values was statistically significant ($p<0.05$).

Table 1. Experimental Results

Experiment	No. of Birds	Mean ± S. E.	
		Body Weight (gm)	Testes Weight (mg)
A : Normal animals			
Start	5	104.8± 3.8	1,396±112
LL	5	93 7± 3.2	1,328±144
8L16D	10	104.7±10.4	152± 45
B : Sham-operated animals			
Start	5	88.4± 4.6	1,490± 83
LL	5	99.8± 2.4	1,352± 31
8L16D	5	92.0± 6.3	421±159
8L16D (LC-RIA)	6	99.7± 2.6	1,005± 58
8L16D (LC-GIH)	6	99.5± 2.3	475± 97
C : Pinealectomizedanimals			
Start	5	85.6± 4.4	1,459± 66
LL	8	101.0± 3.9	1,670± 87
8L16D	6	102.0± 3.1	273±194
8L16D (LC-RIA)	6	91.6± 3.5	438±162
8L16D (LC-GIH)	10	103.6± 1.4	441±139

Start : at the beginning of the experiments (11 weeks of age)
LL : continuous light
8L16D : 8 hours light : 16 hours darkness
LC-RIA : radioluminous paint (orange light)
LC-GIH : radioluminous paint (green light)

Important in Group B is not that a slight difference occured between the sham-operated birds on LL and LC-RIA-treated group but that gonadal development was maintained on the local illumination with LC-RIA. It suggests strongly that the pineal organ has some photoreceptive function. It is also clear that the maintenance of maturity was not due to the contamination of tritium radiation from the radioluminous paint, because the two substances (IC-RIA and LC-GIH) caused different testicular responses (see Table I). These results also suggest that the pineal organ is involved in reception of long wave lengths such as orange but not with short wave lengths like green.

In the pinealectomized birds on LL, testicular weight was maintained (1,670 ± 87 mg), Whereas there was significant regression (273 ± 194 mg; $p<0.01$) in these changed to 8L16D. These testicular responses were the same as those of normal animals. However the testicular weight of the birds treated with LC-RIA and IC-GIH were lower than those of birds held on LL ($p<0.05$) and showed no significant difference from those changed to 8L16D.

Despite a short-day treatment gonadal activity was maintained in the intact birds painted with LC-RIA (B-fourth group), but not in the pinealectomized birds (C-fourth group). This suggests that the latter had lost a part of the photo-

receptor system in the pinealectomy. However in pinealectomized birds (C-second group), the testicular responses were perfectly the same as that of normal. It is thought that the system that induces gonadal activity can also be activated through a retinal pathway alone.

The pineal organ has not been reported previously to have a photoreceptor function in birds. It was suggested by preliminary study (Kato *et al.*, 1967) and now confirmed in the experiment reported here. The results coincide well the observation that the orange-red light had a tendency to be most effective for the development of gonads in normal birds (Konishi and Kato, unpublished; Benoit and Ott, 1938). The same tendency can also be observed in enucleated quail (Oishi and Kato, unpublished). It is also significant that a substantial amount of light can reach the pineal through the skull. Ganong *et al.* (1963) have reported that light penetrates into the brain in mammals. Similarly we also observed in quail that a considerable amount of light penetrated through the skull into the brain.

In our preliminary work, it was found that the testes could consequently respond photoperiodically to the local illumination of eyes; the retina functions as one of the photoreceptors in the photoperiodic testicular responses (Oishi and Kato, unpublished). Thus, it is suggested that quail has, at least, two sets of photoreceptor elements for photoperiodic testicular response, one in eye and the other pineal organ. We must also consider the report that light stimulates the hypothalamus directly thereby causes gonadal growth (Benoit, 1959, in ducks; and Lisk and Kannwisher, 1964, in rats). Thus, it is clear that non-retinal photoreceptor in the photoperiodic responses still require further study. Recently, pineal organ has been shown to be one of the endocrine glands involved in the control of gonadal function in mammals (Thieblot, 1965). In hamsters it was found that pinealectomy prevents the atrophy of gonad in short day (Hoffman, 1965); administration of pineal extracts produces gonadal atrophy in rats (Ifft, 1962; Kitay, 1954). Similar results had already been seen in white leghorn chickens as that mammals (Shellabarger, 1953). In more recent experiments pinealectomy seems to give no effect on the photoperiodically induced testicular development not only in young quail (Homma *et al.*, 1967, Sayler and Wolfson, 1967) but also in adults (present work). On the other hand, it has been reported that melatonin synthesis in the hen pineal and serotonin content of pigeon pineal are controlled by light (Axelrod *et al.*, 1964; Quay, 1966). Consequently it is conceivable that the pineal organ of birds utilizes environmental light energy, particularly for testicular development in male quail. In this conjunction it is important to note again that the quail pineal has a possible photoreceptable fine structure that suggests a photoreceptor function. This may represent a new phylogenetic aspect of pineal functions in consideration of its role in reptiles and amphibians.

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