J. Biosci., Vol. 19, Number 4, October 1994, pp 479-484. © Printed in India.

Formoguanamine-induced blindness and photoperiodic responses in the Japanese quail, *Coturnix coturnix japonica*

T OISHI† and Y OBARA*
Department of Biology, Faculty of Science, Nara Women's University, Nara 630, Japan
*Department of Agricultural Chemistry, Faculty of Agriculture, Meijo University, Nagoya 468, Japan

Abstract. We administered a blindness-inducing substance (formoguanamine hydrochloride) to Japanese quail in order to find whether it is effective to induce retinal degeneration in avian species other than the chicken. We also investigated its effects on the photoperiodic response of various organs including gonads and the entrainment of circadian locomotor activity rhythms. Histological observation revealed conspicuous degeneration of the photoreceptor outer segments and pigment epithelium. Behavioural responses of formoguanamine hydrochloride-treated birds to visual stimuli were completely abolished. These results proved that this chemical substance is effective to induce blindness in avian species other than the chicken. In formoguanamine hydrochloride-treated birds, the locomotor activity rhythm was entrained to light-dark cycles and the photoperiodic gonadal response was almost normal, suggesting that the extraretinal photoreceptors remained intact even after the formoguanamine hydrochloride-treatment.

Keywords. Formoguanamine; blindness; photoperiod; gonad; circadian activity rhythm; extraocular photoreception.

1. Introduction

Obara *et al* (1985) found that formoguanamine hydrochloride (FG) was a potent chemical to induce blindness in chickens by disrupting the pigment epithelium and photoreceptor cells in the retina. FG was also effective in preventing the eye-enlargement induced by suturing eye-lids of chicks (Oishi and Lauber 1988), as well as the characteristic eye-enlargement of light-induced avian glaucoma (Lauber and Oishi 1990). In this report, we administered this substance to Japanese quail in order to find whether the retinal degeneration is also induced in avian species other than chickens. We also investigated its effects on the photoperiodic responses of gonads and other organs and on the entrainment of locomotor activity rhythms by light-dark cycles in an attempt to find whether formoguanamine has any effect on the extraocular photoreceptors as well as the retina, since the extraocular photoreception has been known to play an important role for the photoperiodic gonadal response in birds (Benoit 1935; Oishi and Kato 1968; Oishi and Lauber 1973a,b; Oliver and Bayle 1976; Glass and Lauber 1981).

2. Materials and methods

One day old male Japanese quail (*Coturnix coturnix japonica*) were obtained from

†Corresponding author.

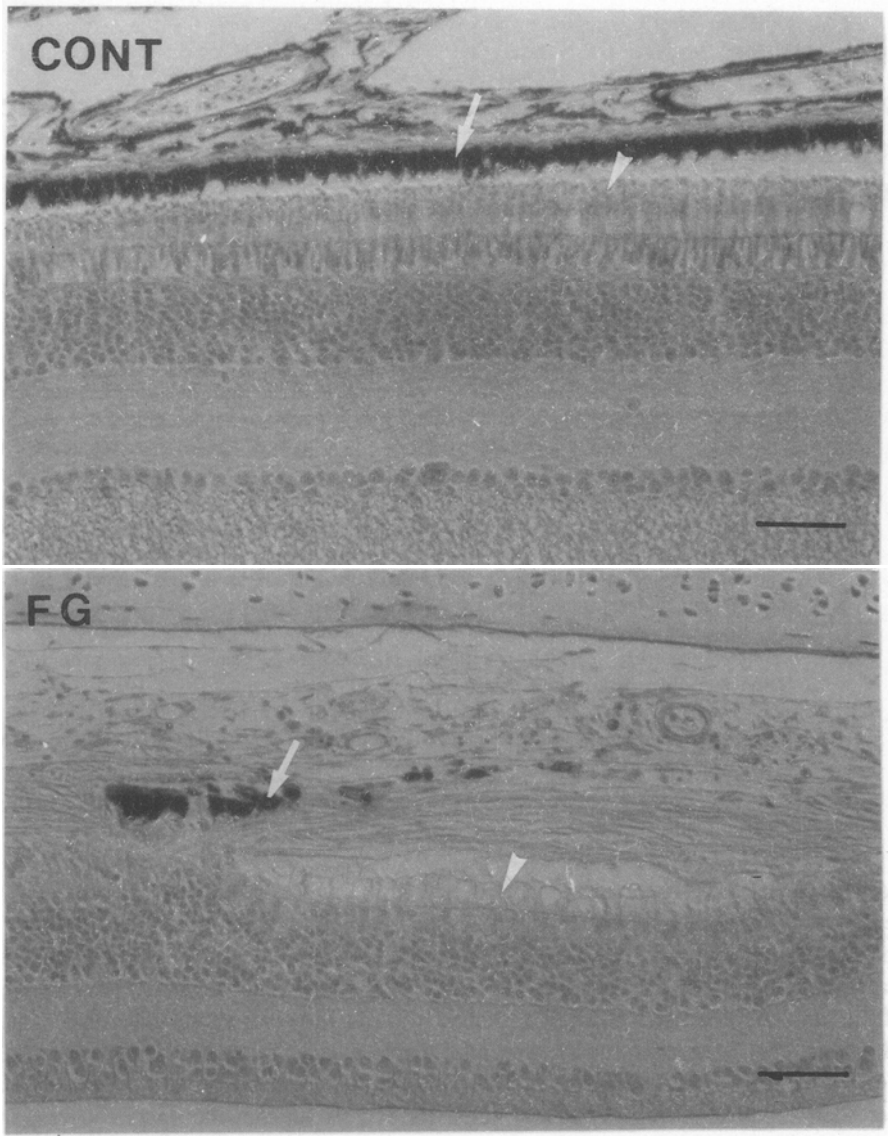

Figure 1. Retina in control and FG-treated quail. Note the conspicuous degeneration of the pigment epithelium (arrow) and the outer segments (arrow head) of photoreceptor cells in the retina of FG-treated quail. Bars indicate 100 μm.

a local breeder. Fifty birds were divided into a group treated with FG and a control group. FG (1·5 mg) dissolved in 0·1 ml of saline was injected subcutaneously in the experimental group and 0·1 ml of saline in the control group. Injection was done two days after hatching. These two groups were maintained in continuous light (LL) at 35°C for 2 weeks and, then, transferred to a short photoperiod (LD 8 : 16; light on from 10:00 to 18:00) at 25°C. After one additional week, each group was divided further into two groups, and one group was kept in LD 8 : 16 and another in a long photoperiod (LD 16 : 8; light on from 6:00 to 22:00) for 3 weeks at 25°C. Light

Table 1. Effects of photoperiods on various organ weights in FG-treated quail.

	Control		Formoguanamine	
	16L	8L	16L	8L
No.	11	11	11	11
Body wt. (g)	93·1 ± 2·8	103·0 ± 1·9**	78·6 ± 2·5$^{\#\#}$	98·4 ± 3·3***
Cloacal gland size (cm^2)	2·16 ± 0·18	0·18 ± 0·02***	1·82 ± 0·33	0·20 ± 0·01***
Relative organ wt. (mg/g body wt.)				
Testes	29·2 ± 2·8	0·39 ± 0·1***	20·0 ± 3·3$^{\#}$	1·10 ± 0·4***
Adrenal	1·55 ± 0·09	1·27 ± 0·08*	1·52 ± 0·08	1·10 ± 0·08**
Bursa of F.	0·99 ± 0·10	1·28 ± 0·12	0·89 ± 0·09	1·02 ± 0·12
Thymus	1·40 ± 0·03	2·93 ± 0·29**	1·88 ± 0·40	3·95 ± 0·33**$^{\#}$
Liver	2·15 ± 0·10	2·25 ± 0·10	1·88 ± 0·11	1·97 ± 0·07$^{\#}$

Values are mean ± SE. Significant difference: *P<0·05, **P<0·01, ***P<0·001 (16L vs 8L), $^{\#}P$<0·05, $^{\#\#}P$<0·01 (Control vs FG).

intensity was kept at 200-500 lux with incandescent lamps. At the end of the experiment, body weight and weights of the adrenal, bursa of Fabricius, thymus and liver were recorded. The size of the cloacal gland (a typical secondary sex character of Japanese quail) was measured with a caliper. The eyes were dissected out and fixed in Bouin's solution. Paraffin sections (4 μm) were made and stained with eosin and hematoxylin to check the extent of retinal degeneration by the FG-treatment.

Behavioural tests were performed to check the blindness of the FG-treated birds by moving a pencil in front of them or by observing whether they can find food in a transparent sealed plastic petri dish.

Four birds at 34 days of age taken from control and FG-treated groups, respectively, were transferred into individual cages and kept in LD 12 : 12 (light on from 6:00 to 18:00). The locomotor activity rhythm of these birds were recorded for 10 days from 34 to 43 days of age with a photo-transistor system (PL-3, Hokuyo Denki Co., Osaka) and an event recorder (R9H1 2T, Fuji Denki Seizo Co., Tokyo).

Statistical analysis was done by ANOVA, Student's *t* test and Duncan's multiple range test using SAS computer program.

3. Results

Histological examination of the retina in FG-treated quail revealed conspicuous degeneration of the outer and inner segments of photoreceptor cells and the pigment epithelium (figure 1), and this clearly indicated that retinal degeneration can be induced in avian species other than the chicken. The inner and outer nuclear layers and ganglion cells seem to have remained intact. Since the FG-treated birds did not respond to a moving object in front of them and could not find food in a

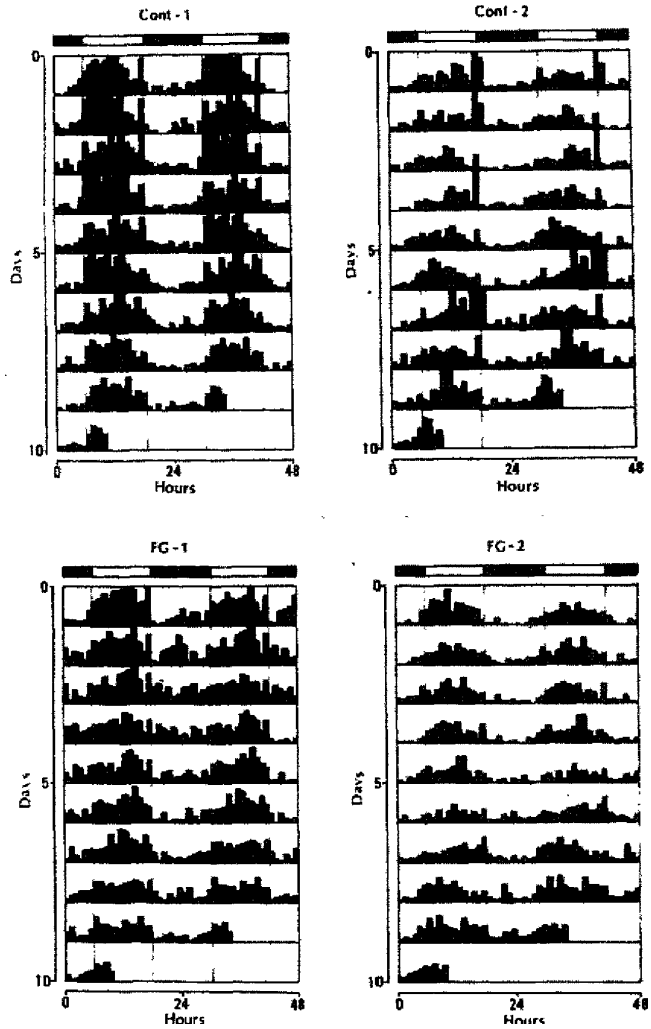

Figure 2. Locomotor activity rhythms of quail under LD 12:12. The activity records for 10 days are double plotted. Distinct diurnal rhythms entrained to light-dark cycles are observed in both control and FG-treated birds.

transparent sealed petri dish, they were considered to be blind. Electroretinogram also showed that blindness was induced in FG-treated quail (Sasaki M, Obara Y and Oishi T, unpublished).

As it is shown in table 1, testicular weight and cloacal gland size of control birds were greater in the long photoperiod (LD 16 : 8) than in the short photoperiod (LD 6: 18), thus displaying the expected photoperiodic gonadal response. In FG-treated quail, the testes and cloacal gland size were also significantly enlarged under the long photoperiod in comparison with those under the short photoperiod. Although the testicular weight was slightly smaller than that in control birds under LD 16:8, this is probably due to significantly retarded body growth in the FG-treated birds. Relative adrenal weight was significantly larger in LD 16:8 than in LD 8 : 16 similar to the results of the testes weight in both control and FG-treated birds.

In contrast, relative thymus weight was larger in LD 8 : 16 than in LD 16 : 8 in both control and FG-treated birds. Relative liver and bursa of Fabricius weights were not significantly different between LD 16 : 8 and LD 8 : 16.

Distinct diurnal locomotor activity rhythms were observed in both control and FG-treated blind birds (figure 2). The locomotor activity increased significantly just after the light-on signal and decreased just after the light-off. High activity was observed during 1 h before light-off signal. There were no significant differences in the activity pattern between control and FG-treated blind quail.

4. Discussion

FG-treatment clearly induced blindness in Japanese quail due to degeneration of the photoreceptor cells and the pigment epithelium similar to the result in chickens reported by Obara *et al* (1985). We have reported previously that this chemical substance was potent to prevent the development of myopia which was induced by suturing eye-lids in chicks (Oishi and Lauber 1988) and the eye enlargement of light-induced avian glaucoma (Lauber and Oishi 1990). Our present study proved that FG is effective to induce blindness in avian species other than the chicken. However, the extraocular photoreceptors such as the pineal organ (Oishi and Kato 1968) and the hypothalamus (Benoit 1964; Oishi and Lauber 1973a, b; Oliver and Bayle 1976; Glass and Lauber 1981) seem to have kept intact, because the photoperiodic gonadal response and the entrainment of circadian locomotor activity rhythms were almost normal. The present study also indicates that the differences of adrenal and thymus weights between long and short photoperiods were statistically significant in FG-treated blind birds as well as control birds. The results of thymus weight in control birds confirmed our previous reports (Mase and Oishi 1986, 1991). Thus, although FG disrupted vision, it does not seem to affect the function of the extraocular photoreceptors. Since degeneration of the outer segments of photoreceptor cells and the pigment epithelium in the retina was induced by FG-treatment, it might have inhibited the synthesis or periodic renewal of disc membranes of the outer segments. The reason why FG did not affect the extraocular photoreceptors might be that the pinealocytes in the pineal of Japanese quail have the outer segments with already degenerated membrane structure (Oishi 1972) and that the cells in the hypothalamus do not seem to have special membrane structure comparable to the retina. Anyhow, FG could be a useful tool to study photobiological phenomena by differentiating the vision and the extraretinal photoreception.

References

Benoit J 1935 Stimulation par la lumière artificielle du developpement testiculaire chez des canards aveuglés par énucléation des globes oculaires; *C. R. Soc. Biol.* **120** 136-139

Benoit J 1964 The role of the eye and of the hypothalamus in the photo-stimulation of gonads in the duck; *Ann. N. Y. Acad. Sci.* **117** 204-216

Glass J D and Lauber J K 1981 Sites and action spectra for encephalic photoreception in the Japanese quail; *Am. J. Physiol.* **240** R220-R228

Lauber J K and Oishi T 1990 Kainic acid and formoguanamine effects on environmentally-induced eye lesions in chicks; *J. Ocular Pharmacol.* **6** 151-156

Mase Y and Oishi T 1986 Effects of photoperiods on the weights of bursa of Fabricius and thymus in Japanese quail; *Growth* **50** 317-324

Mase Y and Oishi T 1991 Effects of castration and testosterone treatment on the development and involution of the bursa of Fabricius and the thymus in the Japanese quail; *Gen. Comp. Endocrinol.* **84** 426-433

Obara Y, Matsuzawa T, Kuba N and Fujita K 1985 Retinal damage in hatched chicks induced by formoguanamine. Decrease in ornithine aminotranferase activity and vitamin B_6 content; *Exp. Eye Res.* **41** 519-526

Oishi T and Kato M 1968 Pineal organ as a possible photoreceptor in photoperiodic testicular response in Japanese quail; *Mem. Fat: Sci. Kyoto Univ. Ser. Biol.* **2** 12-18

Oishi T 1972 *Photoendocrine responses and photoreception in Japanese quail* (*Coturnix coturnix japonica*), Ph. D. dissertation, University of Alberta, Canada

Oishi T and Lauber J K 1973a Photoreception in the photosexual response of quail. I. Site of photoreceptor; *Am. J. Physiol.* **225** 155-158

Oishi T and Lauber J K 1973b Photoreception in the photosexual response of quail. II. Effects of intensity and wavelength; *Am J. Physiol.* **225** 880-886

Oishi T and Lauber J K 1988 Chicks blinded with formoguanamine do not develop lid suture myopia; *Curr. Eye Res.* **7** 69-73

Oliver J and Bayle J D 1976 The involvement of the preoptic suprachiasmatic region in the photosexual reflex in quail: Effects of selective lesions and photic stimulations; *J. Physiol.* (*Paris*) **72** 627-637