

## An ocular capacity to promote photoperiodic testicular growth in the quail

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### SUMMARY

Disk-shaped, 0.6 × 0.2 mm pellets of radioluminous material were inserted in the anterior chamber of the eyes of sexually immature male quail on short daylengths. Two weeks later testicular weights and plasma testosterone levels were significantly increased compared with controls. The effectiveness of the ocular implants varied according to the spectrum of emission of the radioluminous material. Sectioning of the optic nerve suppressed all gonadal response to retinal photostimulation.

### INTRODUCTION

Gonadal activity in many avian species in the temperate zone is stimulated by visible radiations. Daylength constitutes the major component of the environmental cues involved in the control of the reproductive cycle. Since the pioneer work of Rowan (1926) much evidence has accumulated to elucidate this phenomenon. The system for the entrainment of the hypothalamic-pituitary axis must be connected to some photosensitive system. It was first suggested (Bissonnette, 1930) that in house sparrows and European starlings the photoreceptors for the photoperiodic gonadal response were located in the testes and in the skin of the foot. However, artificial lighting of the whole body, except for the head which was masked under a black hood, did not elicit the photosexual response in ducks (Benoit, 1935a) or sparrows (Ivanova, 1935). Consequently it was concluded that the eyes contain the photoreceptors responsible for gonadostimulation (Benoit, 1935a).

Photoperiodic determination of the breeding season in mammals depends upon retinal signals and there is a direct neural projection from the ganglion cells of the retina to the anterior hypothalamus (*nucleus suprachiasmaticus*) (Moore, 1978). The situation is more complicated in birds since blinding does not prevent environmental light from stimulating testicular growth in drakes (Benoit, 1935b), chickens (Hutt, 1943; Ookawa, 1970), quail (Oishi, Konishi & Kato, 1966; Saylor & Wolfson, 1966; Oliver & Baylé, 1973), sparrows (Underwood & Menaker, 1970), canaries (Munns, 1971) and white- and golden-crowned sparrows (Gwinner, Turek & Smith, 1971; Turek, 1975). The eyes are therefore unnecessary for the photoperiodic response to long days and the occurrence of extraretinal photoreceptors has been demonstrated in a variety of avian species (see Farner, 1980; Oliver & Baylé, 1982 for reviews). In fact, the problem of the participation of retinal photoreceptors in photoinduced testicular growth in birds is a much debated question, notwithstanding the fact that most authors in the last decade seemed to favour the opinion that 'the eyes do not participate in photoperiodic photoreception', according to evidence from the house sparrow (Menaker, Roberts, Elliott & Underwood, 1970; McMillan, Underwood, Elliott, Stetson & Menaker, 1975; Underwood, 1975).

The purpose of this paper is to reconsider the problem of the participation of ocular photoreceptors in photoinduced testicular development by insertion of radioluminous material (RLM) in the anterior chamber of the eyes of immature Japanese quail which is a



photosensitive species. The effect of retinal photostimulation on gonadal growth was compared with the gonadal responses to local infundibular illumination and to photoperiod.

#### MATERIALS AND METHODS

##### *Animals*

Male quail were reared in individual cages under 6 h light:18 h darkness (6L:18D) at a constant temperature of  $26 \pm 1^\circ\text{C}$ . Surgical procedures which included laparotomy, implantation of RLM and sectioning of the optic nerves were carried out under Equi-Thesin (Jensen, Salsbery, Missouri, U.S.A.) anaesthesia (0.2 ml/100 g body weight). Testicular development was ascertained at 6 weeks of age by intercostal laparotomy and animals with quiescent testes were allocated at random to eight groups of ten birds. The following treatment groups were established: groups IA and IB served as controls and were exposed to short (6L:18D) and long (18L:6D) days respectively. Groups IIA and IIB were exposed to short days and were implanted in the anterior chamber of the eye with green-emitting (530 nm) RLM and red-emitting (620 nm) RLM respectively. Groups IIIA and IIIB were exposed to short days and, after section of the optic nerves, were implanted with green- and red-emitting RLM in the anterior chamber of the eye respectively. Groups IVA and IVB were also exposed to short days but were implanted respectively with green- and red-emitting RLM in the infundibular complex.

##### *Insertion of radioluminous material*

Two kinds of RLM were provided by Lumina Laboratories (Colombes, France). Green RLM had its maximum emission at 530 nm and its brightness was  $0.029 \text{ cd/m}^2$  after mixing with the binding agent. Red RLM had its maximum emission at 620 nm and its brightness was  $0.015 \text{ cd/m}^2$ . The pellets were disk-shaped, 0.6 mm in diameter and 0.2 mm thick. For implantation into the infundibular complex the pellet was secured at the tip of a varnished stainless steel tube, 0.3 mm in diameter. The head of the quail was fixed in the stereotaxic frame (David Kopf 900). The skin of the head was incised and a hole, 0.8 mm in diameter, was drilled into the skull at the anterior and lateral coordinates (Baylé, Ramade & Oliver, 1974) of the infundibular complex (A+4.2; L+0.3). The tube supporting the pellet of RLM was mounted on the stereotaxic holder and lowered to the vertical coordinate (+0.2) of the infundibular complex. The tube was cemented to the bone and secured outside the skull. For implantation into the anterior chamber of the eye the birds were fixed in lateral decubitus. The cornea was incised at the upper level of the iris and the pellet introduced within the anterior chamber tangentially to the cornea and gently slipped toward the pupil. It was necessary to check that the pellet was maintained in position 1 and 2 days after implantation and eventually to put it back to its central pupillar position.

Optic nerve section was performed bilaterally. The skin was incised dorsally to the eyeball. A small curved hook was slipped behind the eyeball and passed under the optic nerve which was cut with sash-scissors.

Quail implanted with RLM were fed forcibly through an oesophageal cannula for 4 days after the operation to avoid any possible malnutrition.

##### *Photoperiodic treatments*

The birds were photostimulated either by exposure to long days (18L:6D) by means of incandescent bulbs (150 lux at floor level) for 2 weeks or by exposure to RLM for 2 weeks whilst remaining on a short photoperiod (6L:18D). After 2 weeks of treatment all the birds, including the non-photostimulated controls, were killed by decapitation between 09.00 and 11.00 h. Blood was collected in tubes containing solid heparin and the plasma separated by centrifugation and stored at  $-28^\circ\text{C}$  for assay of testosterone. The combined

weight of pairs of testes was recorded and body weight determinations were made at the time of laparotomy and at the time of death.

#### Assay of testosterone

Testosterone was measured after solvent (diethyl ether GR, Merck) extraction of plasma samples (300–500  $\mu$ l) in duplicate at one dilution using commercial radioimmunoassay kits (Bio-Mérieux, Paris). The minimum detectable amount of testosterone was 15 pg per assay tube. Intra- and interassay coefficients of variation for samples were 5.6 and 9.8% respectively ( $n = 9$ ). Testosterone titres are given as nmol/l (means  $\pm$  s.e.m.).

Student's *t*-test was used for statistical comparisons.

### RESULTS

Body weight was not affected significantly in experimental groups II, III and IV and ranged from  $155 \pm 5$  to  $164 \pm 9$  g with respect to control values of group I ( $154 \pm 6$  and  $159 \pm 3$  g). It increased by 14–22 g during the experimental period, i.e. between 6 weeks (laparotomy) and 9 weeks (autopsy) of age.

Small testes ( $33.6 \pm 4.5$  mg) and low testosterone values ( $0.46 \pm 0.03$  nmol/l) were found in intact quail maintained under short days (group IA) while exposure to 14 long days (18L:6D, group IB) led to markedly increased testicular weight ( $2540 \pm 243$  mg) and increased concentrations of testosterone in plasma ( $7.89 \pm 1.11$  nmol/l).

The effectiveness of the RLM used in this experiment was checked by the effects of placing the RLM in the infundibular complex of unphotostimulated quail (group IV). Radioluminous material emitting both green and red light stimulated a significant release of gonadotrophin as judged by the weight of the testes although the testicular response to red light (testicular weight =  $2509 \pm 241$  mg; plasma testosterone level =  $6.23 \pm 0.59$  nmol/l) was greater than the response to green light ( $1461 \pm 35$  mg;  $1.96 \pm 0.12$  nmol/l).

The presence of a radioluminescent pellet in the anterior chamber of the eye also promoted significant gonadotrophic stimulation. However, the effects that could be noted were weaker than after infundibular lighting, even using RLM emitting red light (testicular weight =  $822 \pm 96$  mg; plasma testosterone level =  $2.91 \pm 0.09$  nmol/l). The response to stimulation of the retina with green light was much less ( $179 \pm 22$  mg;  $1.10 \pm 0.16$  nmol/l) but still greater than in controls.

No gonadal stimulation was observed after insertion of RLM pellets into the eyes of quail with sectioned optic nerves. Testicular weights remained very small ( $37.1 \pm 3.7$  and  $40.3 \pm 9.2$  mg in groups IIIA and B respectively) and testosterone levels were low (group IIIA,  $0.52 \pm 0.04$  nmol/l; group IIIB,  $0.56 \pm 0.06$  nmol/l).

### DISCUSSION

The response of the gonadotrophic axis of intact quail to photoperiodic treatments has been discussed previously (Follett & Farner, 1966; Follett & Robinson, 1980) as has the effects of local light stimulation within the infundibular complex (Oliver & Baylé, 1982). In the present experiments we found a somewhat poorer testicular response than was obtained in our previous experiments on quail with infundibular implants which emitted green light (testicular weight = 1460 v. 1840 mg; Oliver & Baylé, 1976; Oliver, Herbuté & Baylé, 1977) or red light (testicular weight = 2500 v. 3300 mg; plasma testosterone level = 6.23 v. 7.98 nmol/l; Oliver, Jallageas & Baylé, 1979). Perhaps the progressive reduction in brightness of RLM with time after its preparation may account for these differences.

Benoit (1938a, 1964) and Benoit, Walter & Assenmacher (1950) reported that when light was introduced directly to the hypothalamus of ducks by means of a quartz rod, all visible wavelengths could promote testicular growth. However, in quail (Oliver & Baylé, 1976; Oliver *et al.* 1977, 1979; present data) red RLM (620 nm, 0.015 cd/m<sup>2</sup>) was more effective than green RLM (530 nm, 0.029 cd/m<sup>2</sup>) in stimulating infundibular photoreceptors. In the same species, Homma & Sakakibara (1971) failed to obtain any testicular response to blue (440 nm) RLM inserted in the *fissura longitudinalis cerebri* in 12 out of 13 cases. Possibly these facts can be explained by the better tissue penetration of light of longer wavelengths (Benoit, Assenmacher & Manuel, 1953).

As pointed out in the introduction, the results of a series of investigations (Menaker & Keatts, 1968; Menaker *et al.* 1970; McMillan *et al.* 1975; Underwood, 1975) make it extremely improbable that retinal signals participate in the photosexual response of the house sparrow. Amongst other convincing evidence is the fact that the gonads did not respond to long days when Indian ink was injected beneath the skin of the head even if the eyes were intact. However, other workers think that information from retinal receptors does participate in photoperiodically induced testicular growth and regression in quail (Homma, Wilson & Siopes, 1972; Homma, Ohta & Sakakibara, 1979) and white- and golden-crowned sparrows (Gwinner *et al.* 1971).

Another series of experiments is important in relation to the present study. Benoit (1938b) isolated the retinal photoreceptors of ducks by means of slats of opaque rubber and blackened paraffin wax deposited in the posterior part of the orbit. A black hood was placed over the head of the duck, with a hole at the level of the eye. Intact ducks showed approximately twice the testicular development of the blinded (optic nerve-severed) ducks. Testicular growth was also found to be significantly greater in intact than enucleated mallards submitted to weak illumination (Benoit & Assenmacher, 1953; Benoit, Assenmacher & Walter, 1953) and similar data were reported from studies on the quail (Bons, Jallageas & Assenmacher, 1975).

The present results provide more direct evidence that retinal photoreceptors of quail are able to induce, by themselves, the stimulation of testicular growth when they are illuminated selectively. In the same species, Homma & Sakakibara (1971) and Homma *et al.* (1980) failed to elicit any testicular growth after intraocular orange RLM implantation. The main difference with the present experiment seems to lie in the exact location of the RLM implants, i.e. the posterior chamber of the eye. Insertion of rather large (3 × 2 × 0.8 mm) plates of RLM may possibly have resulted in severe intraocular disturbance and in retinal alteration. In our experiment the gentle insertion of a small (0.6 × 0.2 mm) radioluminous pellet in the anterior chamber of the eye avoided such alterations in retinal function. This was corroborated by the negative results which occurred when a surgical mistake resulted in the opening of the posterior chamber, behind the lens. Moreover, the mere displacement of the pellet sliding beside the iris instead of remaining over the pupil led to a markedly reduced gonadal increment.

Comparing the spectral sensitivity of the duck retina, Benoit *et al.* (1950) observed that the 'visual' retina was most sensitive to yellow light whereas the 'photosexual' retina had a maximum sensitivity to orange-red rays and did not respond to blue-yellow light. In this respect, it may seem surprising that RLM which emitted green light was able to stimulate the photosexual retina of the quail significantly. Green RLM has its peak of emission at 530 nm but its spectrum ranges between 480 and 600 nm (P. Caro, personal communication); the upper part of this spectrum reaches the level of sensitivity of the photosexual retina.

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