alanine, 0.90; cystine (½), 4.9; valine, 0; methionine, 0; isoleucine, 3.3; leucine, 3.1; tyrosine, 2.9; phenylalanine, 0.88. A sample of partially purified coherin subjected to electrophoresis at *p*H 8.7 on polyacrylamide gel (20 percent) gave a single weak band ($R_F = 0.485$) when stained with Coomassie blue.

Although complete homogeneity must still be demonstrated, our product gives a single spot on paper electrophoresis (*p*H 1.9, 6.0, 8.5, and 11.0) and represents a purification of about 200-fold, on the basis of intestinal inhibiting activity (Table 1).

Our results suggest that we have isolated from bovine posterior pituitary powder a new factor with unique biological activity and with chemical and physical properties distinct from those of the known hormones of the posterior pituitary gland. Although the identity of coherin as a hormone remains to be established, the potency of the preparation and the nature of its activity suggests such a role. If indeed it proves to be a hormone involved in the regulation of peristaltic function, our understanding of the mechanisms of gastrointestinal motor activity will be greatly enhanced.

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# Eyes Have a Role in Photoperiodic Control of Sexual Activity of Coturnix

*Abstract.* *In blinded Japanese quail* (Coturnix coturnix japonica) *encephalic photoreception of the stimulus from long photoperiods is sufficient to induce and maintain normal gonadal function in females (egg laying) and in males (enlargement of the cloacal gland). However, the termination of sexual activity by short days is dependent on these birds having experienced long days at the time of blinding.*

The importance of day length in controlling reproductive activity in many species of birds is well established. More than 35 years ago Benoit (*1*) reported that growth and development of testes of the domestic duck could be stimulated by direct photostimulation to the brain. In one of his experiments, severance of the optic nerve in immature male ducks decreased the photoresponsiveness to one-fifth of the normal. He concluded that if encephalic photoreception has any physiological role in avian reproduction the nature of its function would be supplemental to the eyes.

Recent studies have indicated that the eyes, or vision, are not necessary for photoperiodic induction of gonadal growth in the chicken (*Gallus domesticus*) (*2*), the common coturnix or Japanese quail (*Coturnix coturnix japonica*) (*3, 4*) and house sparrow (*Passer domesticus*) (*5*). Furthermore, after maturity, blinded and intact birds are equally responsive to changes in environmental light, as indicated by the testes weight in common coturnix (*6*), and by time of oviposition in the domestic fowl (*7*).

Both retinal and encephalic photoreception may be functional in the control of reproduction, but their interaction has not been determined. We have found that the eyes are involved in the termination of reproduction in birds or at least in Japanese quail. We now present a hypothesis that assigns different roles of ocular and encephalic photoreceptors in photoperiodic control of avian reproduction.

Independent studies with the common coturnix or Japanese quail (*Coturnix coturnix japonica*) at the University of Tokyo and the University of California at Davis were sufficiently similar in design so that it is possible to consider the data complementary.

The experimental Japanese quail used in Tokyo were derived from the stock that had been maintained at the University of Nagoya. Birds were first reared as a group under continuous light (LL). They were kept on short days (light : dark, LD 8 : 16, 20 lux, incandescent light) from 3 weeks of age and then were transferred to individual cages. Ten birds were blinded at 5 weeks of age, when the ovary was still undeveloped. When the birds were 8 weeks of age, the light regimen was changed to constant light (LL) and continued for 40 days when it was changed to LD 8 : 16. The time from onset of LL to sexual maturity (first egg), and ovipositing time of each egg laid, were recorded. Except during the summer season, the room temperature was controlled at 18° to 22°C. At 8 weeks of age half of the blinded birds and intact controls of the same hatch were subcutaneously injected around the skull with 0.1 to 0.3 ml of India ink (Pelikan) in an attempt to insulate the brain from the impinging light. The ink had been passed through a Sephadex G-25 column to remove toxic materials. In a preliminary test, ink injection reduced the amount of light to the brain indefinitely to less than 1/100 of that in the untreated controls.

At the University of California at Davis (UCD), the experimental birds were from the UCD random-bred line of common coturnix. They were held under long daily photoperiods (LD 16 : 8, white fluorescent light, 200 lux) to 4 weeks of age then transferred to individual cages in two bioclimatic chambers in which temperature was controlled at 23°C (relative humidity 55 percent) and short photoperiods (LD 6 : 18) were provided. The birds were kept on short days until 15 weeks of age. Thereafter they were exposed to alternate periods of long (LD 16 : 8) and short (LD 6 : 18) days, each of which continued for several weeks. In addition to the periods of short days, further periods of complete darkness for 1 or 2 weeks were given to hasten gonadal regression. At 6 weeks of age, half of the birds in each chamber were pinealectomized, and at 7 weeks of age all of the birds in one chamber were blinded. The endocrine responses of the testes were assessed, indirectly, by the width of the cloacal gland and by the amount of its foamy secretion. A close relation between this gland and testicu-

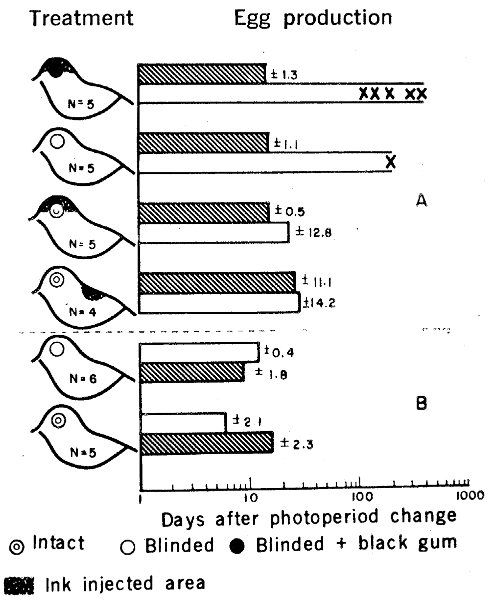


Fig. 1. Onset and duration of egg production of bilaterally blinded, India ink-injected (in head or back regions), and intact groups of Japanese quail in response to changes in photoperiods. Open bars give the mean duration of laying after change from continuous light to a light-dark period LD 8:16. Hatched bars give mean time in days to initiation of lay after LD 8:16 was changed to LL. Each treatment is shown with two bars, the upper indicates which photoperiod was given first. Two of the four groups in A were blinded before sexual maturity while on short days. X, time of last egg or autopsy of laying; ±, standard error in days.

lar activity has been detailed by several investigators (*8*). In the two experiments at Tokyo and at Davis, the birds were blinded by bilateral orbital enucleation under pentobarbital anesthesia. The empty orbits of a group of the blinded birds were filled with black gum arabic to block the entrance of light to the brain by this route.

Results of the experiments at the University of Tokyo are presented in Fig. 1A. During the first short-day period, all birds in the first four groups had completed their somatic growth but remained sexually immature. On subsequent exposure to continuous light (LL), the ovary was stimulated and egg laying occurred in both blinded and intact birds. The combination of injection of India ink around the skull and insertion of black gum in the orbit did not retard the time of gonadal maturation. This indicates a very low threshold value for encephalic photoreception and agrees well with our earlier data (*3*).

The egg production initiated in blind birds by long days was not affected by the second exposure to short days. In the two blinded groups, one with and the other without ink injection, there was no sign of decline in the rate of egg production (78 to 80 percent) for at least 130 and 196 days, respectively. All birds of the latter group were autopsied at 225 days. The observation period of the ink injected, blinded group was further extended to examine persistence of egg production. For this group, the mean age of the hen at last egg laying was 264 ± 64 days, but one female on LD 8 : 16 continued egg production for 457 days. However, the egg production by intact birds terminated after the transfer to short days within several weeks. On this photoperiodic regimen the intact birds failed to resume laying for about 6 months.

Figure 2 shows the responses of blinded and intact males at Davis when subjected to alternate periods of short and long day lengths. The birds that were blinded while on short days prior to sexual maturity were more advanced in sexual development compared to the intact controls. A similar tendency was also observed in the females (93 as compared to 101 days), but was not statistically significant. After sexual maturity, during the successive periods of alternating short and long days, the blinded males maintained an active cloacal gland regardless of the light regimen, indicating functional endocrine testes. At the end of the first cycle of short days, regression of the cloacal gland was incomplete in the intact males, but after the second cycle regression was rapid and complete. The resistance of some *Junco hyemalis* males in the early stages of development to the inhibitory effects of continuous darkness (DD) for 18 days observed by Wolfson (*9*). He associated a long-day activity pattern, which persisted in darkness, with the results obtained. Blinded birds were not used in his experiments.

Pinealectomy is known to affect the locomotor activity pattern of the house sparrow (*5*), but knowledge of the pineal's role in the reproduction of birds is ambiguous. In our study, pinealectomy failed to affect the basic patterns of gonadal responses in either the blinded or intact males.

Although the experiments at Tokyo and Davis were carried out independently, the data agree well on an important point, that is, the short days did not inhibit the gonadal function of the blinded birds, whereas the gonads of intact birds responded to periods of long and short days with proliferative and regressive changes, respectively. In most instances, blinding tended to hasten sexual maturity. Our results, which demonstrate clearly that the eyes are related to gonadal regression, tempted us to assume that the eyes may be the critical component in the system in which decreased day length is the information that results in decreased gonadotropic activity and regression of the gonads.

In another experiment we exposed females to continuous light from the time of hatching through sexual maturation. These birds became sexually ma-

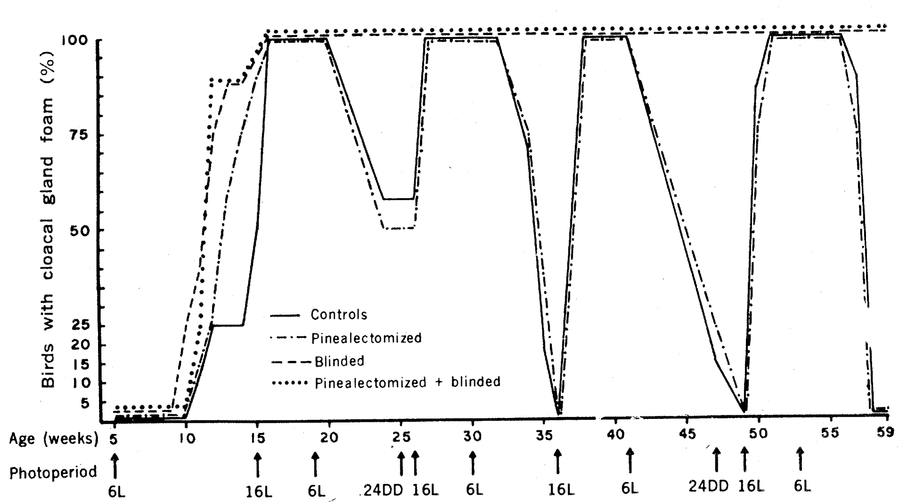


Fig. 2. Photoperiodic response of the cloacal gland to long and short daily photoperiods. Each of the four groups consisted of eight males. The notation *6L* and *16L*, for example, refers to the hours of light provided per 24-hour period. Arrow indicates the start of the photoperiod which continued until the next arrow on the right.

ture at about 7 weeks of age. Six laying birds were blinded at 17 weeks of age, and five intact birds of the same age were used as controls (Fig. 1B). When laying females were blinded under long days there was a brief transient pause in laying which lasted for about 4 days, and then the birds resumed a normal pattern of egg laying. On subsequent exposure to short days (LD 8 : 16), these blinded birds soon terminated egg production as did the intact controls of the same age and the same photoperiodic history. After the second exposure to long days both blinded and intact birds resumed egg production within 3 weeks. This agrees with the report of testicular regression in blinded male Japanese quail exposed to short days by Oishi *et al.* (*6*). Their birds were reared under continuous (LL) daylight-type fluorescent light (100 to 1000 lux) until they were enucleated at either 8 or 17 weeks of age and then they were exposed to short days.

Why, in blinded birds of the same species, should short days sometimes cause regression of gonads and sometimes not? In the experimental conditions used, neither light intensity nor environmental temperature seems to be crucial. In the Japanese quail and possibly in other avian species, the photoperiod experience via the eyes determines the type of response of the blinded birds to future photoperiod information.

Gonadal growth of intact birds under short days has been induced by continuous illumination to the brain, along the *fissura longitudinalis cerebri* adjacent to the hypothalamic area, without exposing the eyes to long days (*3*). Even though the females were kept on short days, egg production continued normally for the 4-week period of observation from the time of the removal of the intracerebral light source until autopsy.

The effects described above indicate different functions of ocular and encephalic photoreception in the control of avian reproduction. The development that occurred possibly was dependent on age in addition to the previous photoperiodic experience. In other words, the integration of incoming photoperiodic information with retrospect to that which has been previously established through the eyes modifies the reproductive activity of birds.

It is often difficult to ascertain the photoperiod histories of wild birds prior to capture. Our hypothesis of control by both ocular and encephalic photoreception may offer a better insight for the future studies of photorefractoriness and other photoperiodic phenomena in avian reproduction that defies solution by hypotheses that involve single-factor control systems. An excellent review, on day length as information used by birds in controlling reproduction (*10*), admonishes not to generalize prematurely on our understanding of photoperiodic mechanism of birds. Therefore, future research will determine whether our results from experiments with the common coturnix may be applicable to other avian species.

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## Echinoid Spawning Induced by a Radial Nerve Factor

Abstract. *An extract of radial nerves of the sea urchin,* Strongylocentrotus purpuratus, *induces spawning within 1 minute in this species as well as in other echinoids, but a delayed spawning in several asteroid species. The concentration of the spawn-inducing factor in the radial nerves fluctuates annually correlated with the reproductive season of this species along the coast of southern California.*

Echinoids are known to release their gametes in response to nonphysiological stimuli, such as mechanical damage, electrical shock, and isotonic KCl (*1*). Asteroids, by comparison, do not readily respond to such stimuli. The discovery of a radial nerve factor (RNF), which induces spawning in asteroids (*2*), led to the elucidation of the mechanism of gamete maturation and release in this class of Echinodermata (*3*, *4*). The occurrence of an echinoid RNF has not been demonstrated despite the report that asteroid RNF can induce sea urchins to spawn (*5*, *6*). In the present report we give evidence for the existence of such an RNF in sea urchins as well.

Purple sea urchins, *Strongylocentrotus purpuratus*, which are common along the coast of southern California, were used in this study. Animals weighing 80 to 100 grams were cut open, and the radial nerves were stripped from the ambulacral groove with forceps. One hundred nerves were boiled in 10 ml of seawater for 10 minutes. Denatured proteins and debris were removed by centrifugation at 3000 rev/min for 10 minutes. The supernatant liquid was then available for use in the bioassay.

For the bioassay, testes from sexually mature males were diced into fragments approximately 2 mm in size, and washed with seawater until oozing of spermatozoa had stopped. Copious amounts of spermatozoa still remained in the follicles at this point. Ovarian fragments could also be used, but oozing eggs adhere to the tissue and cannot easily be washed off before application of the extract. Each testis fragment was then placed in a beaker containing 10 ml of seawater. An aliquot of radial nerve extract was added and mixed in thoroughly. The fragment was watched under a dissecting microscope for 1 minute. If the fragment did not respond, the spawning capability was checked by spraying the fragment directly with concentrated extract. The number of fragments spawning, divided by the number capable of spawning, is expressed as percentage responding. Although both male and female urchins spawn within 1 minute when radial nerve extract is injected into the coelom, this method of bioassay was adopted for ease of quantification. A dose response was established by using a minimum of three groups of ten testes fragments for each concentra-