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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30 May 1972; revised 23 August 1972

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-

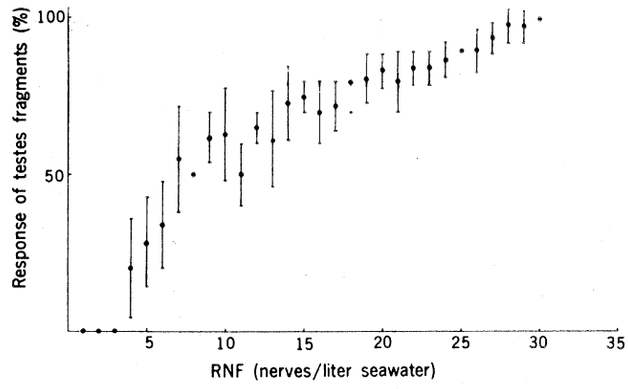

Fig. 1. Dose response curve of the radial nerve factor of *S. purpuratus*. A minimum of three groups of ten competent testes fragments was used for each concentration assayed. The extract preparation and assay system are described in the text. (Mean ± standard deviation.)

tion of radial nerve extract tested (Fig. 1).

Homogenates of the intestine, digestive diverticulum, tube feet, and lantern muscle did not elicit any response even though the mass of these tissues was greater than that of the radial nerves. A very slight spawning response was obtained by application of a large amount of homogenized ampullae. This was probably caused by a contamination of the ampullae with the small side branches of the radial nerves.

The concentration of the spawn-inducing substance in the radial nerves was examined during the course of 1 year. For this purpose, radial nerves from 20 animals were extracted as described above, at approximately weekly intervals. The supernatant liquids were stored at −25°C until they were bioassayed in January 1972. An annual fluctuation of the RNF concentration was found (Fig. 2), which approximately parallels the reproductive season of this species at the southern California coast (*7*). No such annual fluctuation of the RNF level has been found in *Asterias forbesi*, the only species for which a report is available (*8*). It should be noted, however, that seasonal variations in the stainability of neurosecretory cells in certain other asteroids have been reported (*5, 8, 9*). These cells may be implicated in the production of the RNF; however, no direct evidence for this is available.

As these data illustrate, some differences exist between the mechanisms of action described for asteroids and that for *S. purpuratus* described here. For example, sea urchin gonad fragments react within 1 minute, whereas starfish have a minimum 30-minute delay before the onset of spawning. This is not only true for *S. purpuratus*, but also for the sea urchins *Lytechinus pictus* and *S. franciscanus*, whose own radial nerve extracts induce an immediate response. On the other hand, the RNF of *S. purpuratus* induces spawning in the starfish *Pisaster ochraceous*, *Pisaster giganteа*, and *Patiria miniata* only after an average time lag of 45 minutes. Yet RNF from those starfish produced a response in *S. purpuratus* within 1 minute.

The time lag in spawning of asteroid females after administration of asteroid RNF is attributed to several factors. The RNF is known to cause the synthesis and release in the ovary of 1-methyladenine (*10*), which in turn induces oocyte maturation as well as the dissolution of cementing substances with which the ova are attached to the alveolar walls (*4*). Ova can then be released through ovarian contractions. However, this explanation does not suffice for the delayed spawning in male asteroids since the spermatozoa are free within the testes. In contrast to asteroids, both male and female echinoids have free gametes in their gonads, and release them within 1 minute. Possibly then, the difference between asteroids and echinoids lies in the time of onset of gonadal contraction (*8*). Whether this is indeed so, or whether additional factors account for the differences in response to RNF in asteroids and echinoids, remains to be determined (*11*).

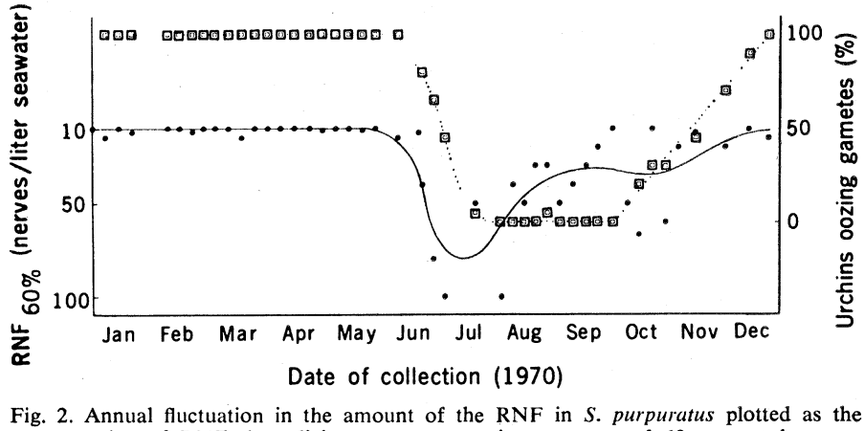

Fig. 2. Annual fluctuation in the amount of the RNF in *S. purpuratus* plotted as the concentration of RNF that elicits a mean spawning response of 60 percent by competent testes fragments (solid curve). A minimum of three groups of ten testes fragments was used for the assay on each date given. The fluctuation in concentration of the RNF parallels the reproductive season of this species, as measured by the percentage of animals that released gametes when opened for the removal of the radial nerves (dotted curve).

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11. The RNF that causes nearly instantaneous spawning in echinoids is not acetylcholine. Preliminary fractionations on Sephadex G-100, and DEAE-50 ion exchange chromatography have revealed that it is a heat-stable oligopeptide, digestible with Pronase, pepsin, and trypsin.

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