weeks after implantation. The test hosts were mice of the inbred strains C57BL/6Ks (a C57 black subline) and C57BR/cdJax (a C57 brown subline). These strains are not related to the A strain. The recipient mice were about equally divided by sex, and ranged in age from 2 to 4 months at the start of the experiment. All appeared healthy and vigorous.

Liver, kidney, and spleen were taken from rats, hamsters, and guinea pigs. The tissues were secured under sterile conditions, frozen over dry ice within 1 hr after being excised, and immediately thereafter subjected to freeze-drying. The lyophilized tissues were powdered and stored in ampoules sealed under vacuum. For purposes of injection, the tissues were ground in glass homogenizers in a vehicle of sterile double-distilled water. Injections of the resultant suspensions were given intraperitoneally twice weekly in the amount of 0.5 ml/injection, containing 10 mg dry weight of tissue. Control groups of mice received injections of lyophilized homologous tissue or saline.

One week after the last injection, single inoculations of bits of minced live tumor were made by trocar, under sterile conditions. The grafts were placed subcutaneously in the suprascapular region. The subsequent course of growth of the implants was followed by palpation. Animals were classified as "takes" if they died with progressively growing tumors, and as "negatives" if there was no sign of a graft for at least two consecutive months.

The data are presented in Table 1. It is apparent that only the prior injections of the lyophilized homologous mouse tumor tissue abrogated the resistance of the hosts to the homoiografts. The numbers of "takes" in the groups injected with lyophilized guinea pig kidney or spleen are not statistically significant, as compared with the saline-injected controls.

TABLE 1

EFFECT OF PRIOR INJECTIONS OF LYOPHILIZED TISSUES ON THE GROWTH OF TUMOR HOMOIOGRAFTS IN MICE

Substance injected	Total mg/mouse (dry wt)*	Mice dying with tumors†	
		C57BL/6Ks	C57BR/cdJax
Rat tissue			
Liver	100	—	0/17
Kidney	100	—	0/19
Spleen	100	—	0/10
Saline (control)	(5 ml)	—	0/20
Hamster tissue			
Liver	100	0/19	—
Kidney	100	0/20	—
Spleen	100	0/5	—
Saline (control)	(5 ml)	0/10	—
Guinea pig tissue			
Liver	50	0/23	0/23
	100	0/22	0/15
Kidney	50	1/22	0/18
	100	0/19	2/9
Spleen	100	1/6	—
Saline (control)	(2.5 or 5 ml)	0/24	1/25
Homologous tumor	50	9/10	7/10

* Injections given intraperitoneally twice weekly in 10 mg/injection.

† Numbers in numerators are the numbers dying in each experimental group; numbers in denominators are the total numbers of mice in each group.

The failure of the out-of-species tissues to abolish the resistance of the host to the homoiografts indicates that we are dealing with a specific reaction evoked by substances, present in both the lyophilized mouse tissues and the fresh tumor inoculum, which must have moieties in common. This supposition is further supported by the predominant absence of cross reactions (*3, 7, 8, 10*) when tissues from inbred mouse strains not homologous to the tumor inoculum are used.

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The Role of Darkness in Sexual Activity of the Quail[1]

Charles M. Kirkpatrick and A. Carl Leopold[2]

Department of Forestry and Conservation
and *Department of Horticulture,*
Purdue University, Lafayette, Indiana

Studies with plants have demonstrated that the duration of the night or dark period is an essential factor in photoperiodism (*1*). The requirements of some plants for short day lengths for flowering were shown to be constituted of two parts: (*a*) a requirement for a specific minimum amount of light followed by (*b*) a specific minimum of darkness (*2*). The possible role that periods of darkness may play in the photoperiodic responses of higher animals has not been conclusively shown.

Evidence that the dark period is critical to the photoperiodic responses of lower animals has been obtained in two instances. Shull (*3*) has shown that wing production by aphids is dependent upon a requirement for a light period followed by a long dark period, entirely comparable to the plant requirements mentioned above. Jenner (*4*) has found that reproduction in the pulmonate snail (*Lymnaea palustris*) requires long photoperiods, but short photoperiods with brief light interruptions of the night will bring on reproduction.

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Through the manipulation of daily light duration a photoperiodic control of sexual activity has been found in birds (*5*). Although much has been said of light as a stimulus, little or no importance has been accorded dark as a discrete factor in the photoperiodic responses of birds. Marshall (*6*) partially reviewed the literature of photoperiodism in birds and found many points of criticism of experimental methods. He states, "Before it can be unquestionably accepted that light fluctuation causes . . . prenuptial recrudescence, far more elegant experimental techniques than those used in the past will have to be devised."

Reproduction in bobwhite quail (*Colinus virginianus*) is brought on by long days and is entirely prevented if day lengths are kept short (*7*). The object of the present study was to determine whether sexual activity could be induced by changing the length of the dark period without changing the hours of light given.

Bobwhite quail hatched in August 1951 were transferred to indoor cages on January 22, 1952. Five groups of 6 pairs each were given artificial light treatments, each pair being subjected to light from a 40-w incandescent lamp. One group received a long day (17 hr), one group a short day (10 hr), and three groups were on a short day of 9 hr with a 1 hr interruption during the dark period. These interruptions, given at different times for each of the three groups, provided for maximum dark periods of 7, 10½, and 12½ hr. Temperatures in the cages ranged from 75° to 100° F.

After 37 days the birds were sacrificed. Full sexual activity, evidenced both by ovulation (or ripe follicles) and free spermatozoa, was obtained in all groups receiving either long days or short days with night interruptions. In contrast the group receiving short days with no interruption of the dark period showed no reproductive development. Average gonad and oviduct weights are presented in Fig. 1. In other experiments

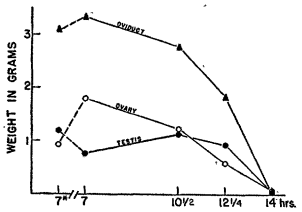

FIG. 1. Influence of length of dark period on weights of reproductive organs of the quail. Asterisk indicates 17-hr day. All other treatments received 10 hr of light/day.

(which will be described more fully later) we have obtained full sexual activity in both sexes with 30 min interruption and in the male with 15 min interruption when the basic light ration was 10 hr.

From the present experiment it is clear that 10 hr of light including a night interruption can evoke full sexual activity in the bobwhite quail, whereas the same light ration as a single daily exposure cannot. It can be concluded that both the light period and the dark period are involved in sexual activation of this species.

Fig. 1 reveals the role of the dark period. In the four short-day groups, each receiving 10 hr of light in each 24-hr cycle, the tissue weights are approximately inversely related to the length of the longest dark period. Since full sexual activity or no sexual activity could be obtained with the same number of light hours by varying the length of the dark period, it is evident that the dark period is an inhibiting factor.

This situation is comparable to the behavior of long-day plants. Under normal environmental conditions, reproductive responses both in quail and long-day plants are inhibited by long nights. If a short-day, long-night is given, a negative response results, but reproductive activity begins if the night is interrupted. It is apparent that the duration of the dark period is a major controlling factor of photoperiodic responses of plants, some lower animals, and of at least one higher animal—the bobwhite quail.

We believe that the demonstrations by Shull (*3*) and by Jenner (*4*) are the only reliable demonstrations of the role of the dark period in animals. Hart (*8*) was not conclusive in attempting to show that estrus is produced in ferrets by breaking the dark period, since his sexually activated group received both a night interruption and extra artificial light, neither of which was applied to the unactivated controls. It is highly probable that the ferret *is* responsive to the night interruption, like quail, but this was not unequivocally shown.

For the male starling, Burger (*9*) reported 12½ hr as the minimum light quantity for complete spermatogenesis and, with co-workers, later (*10*) obtained spermatogenesis with flashing light when the total light quantity ranged from 7.3 to 10 hr, but these investigators did not observe the implication that the length of the dark period is critical to the photoperiodic stimulus.

In fowls, increased egg production is obtained when a light period is given in the middle of the night (*11*), even when the light is of very short duration but of great intensity (*12*), but no attempt has been made to initiate sexual activity by these means, nor to clarify the role of the dark period per se.

It would appear that the present demonstration with bobwhite quail is the first clarification of the importance of the dark period in the photoperiodic response of any higher animal.

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