INHIBITION WITH PROLACTIN OF LIGHT-INDUCED GONAD INCREASE IN WHITE-CROWNED SPARROWS

By ROBERT E. BAILEY

The ability of light to stimulate the gonads in many birds is now well known (see reviews of Burger, 1949; Bissonnette, 1936 and 1937; Rowan, 1938). Practically all experiments concerned with the role of light have been focused upon the ability of birds to respond to increased day length. Few data related to the return to a non-breeding condition have been reported. The demonstration of a refractory period (Riley, 1936; Wolfson, 1945; Miller, 1948) for a time following the breeding activities indicates that a mechanism is in operation which prevents the bird from responding to light and thus permits a "rest" before the bird returns to breeding condition. The stages in the annual cycle in birds may be briefly and tentatively outlined as follows: (1) Following the breeding season, adults and young (the latter questionably in some species) are refractory to light; that is, the normal action or release of gonadotropins by the pituitary is in some way blocked. (2) At a certain time in the autumn the refractoriness ends. The gonads, however, remain quiescent since the days are not long enough to stimulate the pituitary. (3) With increased day length in the late winter and early spring the pituitaries are again stimulated and by spring are releasing gonadotropins sufficient to bring the birds into full breeding condition.

Little explanation of the physiological basis for refractoriness has been presented. Miller (1949) has suggested that it is the pituitary which is refractory, since he was able to stimulate the testes of male Golden-crowned Sparrows (*Zonotrichia coronata*) during the refractory period with injections of pregnant mare serum (gonadin). It is possible that the failure of the pituitary to respond to light during this period may be explained on a hormonal basis, and for this reason the following experiments were undertaken to test to what degree injection of prolactin is able to duplicate the conditions existing during the refractoriness. The choice of this hormone is based upon experiments showing that prolactin causes gonadal regression in adult pigeons and fowl (Riddle and Bates, 1933; Bates, Lahr, and Riddle, 1935).

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METHODS

Two experiments were undertaken, the second primarily because no injected controls were used in the first. The methods in both were essentially the same. Wintering White-crowned Sparrows (*Zonotrichia leucophrys pugetensis*) were trapped on the University of California campus and confined, in groups of 8 to 10, in cages two feet by two feet by three feet. The birds were illuminated continuously with incandescent light and provided with ample food (bird seed, pablum, and fresh vegetables) and water. The experimentals were injected with 15 I. U. (as 0.075 ml of an aqueous solution) of prolactin. The injected controls were given 0.075 ml of a 2 per cent aqueous solution of albumin daily. In both groups the injections were subcutaneous. The normal controls were subjected to the same light and environmental conditions as the other birds but were not injected.

From time to time, in both experiments, birds were sacrificed to determine the progress made by the gonads, and on the day following the last injections the remaining birds were killed and autopsied. The dimensions of the testes were taken and then these, along with the ovaries, were fixed in Bouin's fluid or formol-sublimate. The gonads of all the birds used in the first experiment and about half of those in the second were sectioned at 10 micra and stained with Delafield's hematoxylin and eosin.

The first experiment was started on December 23, 1949, using 8 experimentals and 10 normal controls. All these birds had been captured on either December 17 or 20. The injections were continued until January 15, 1950, and the birds were sacrificed on January 16. The duration of the experiment was 25 days.

The second experiment began on February 3, 1950. On this date 37 birds were captured; 10 were immediately examined, 9 were used as experimentals, 9 as injected controls, and 9 as normal controls. This experiment was concluded on the 17th day, February 20, 1950.

The majority of the birds used were males and consequently emphasis has been placed on testis size to indicate the results of the experiments. The volume of each testis was computed from the formula for the volume of an ellipsoid, $V = 4/3\pi ab^2$, where $a = \frac{1}{2}$ the longest diameter and $b = \frac{1}{2}$ the shortest diameter. The mean testis volume for each group was also determined.

RESULTS

Testis volume.—In the first experiment 4 controls and 6 experimentals were males. The testis volume of the two controls remaining at the end of the experiment were 106.8 mm^3 and 44.39 mm^3 (avg. 75.6). The mean testis volume of the 5 remaining prolactin-injected birds was 6.31 mm^3, one-twelfth that of the controls (see fig. 42, left).

The average testis volume of the 7 males collected in the field at the beginning of the second experiment was 1.237 $mm.^3$ At the end of 17 days, the average volume for the experimentals was 6.21 mm^3; for the albumin-injected controls, 26.4 mm^3; and for the normal controls, 23.9 $mm.^3$ The testis volume of the prolactin-injected birds was 5 times greater than the original size and the albumin-injected and normal controls were about 20 times greater. The testis volume of the two types of controls was not significantly different. Together these showed an increase of about 4 times over the prolactin-injected birds (fig. 42, right).

The young of the Puget Sound race of White-crowned Sparrows normally breed in the first spring following hatching and at that time are indistinguishable from the older birds (Blanchard, 1941). Since the present experiments were conducted before the prenuptial molt was completed, it was possible to distinguish the young birds, but no differences between the response of these and older birds were found.

Tubule size and spermatogenesis.—Sections of all the testes from the first experiment and some of those from the second were examined and revealed that the size of the seminiferous tubules and the progress of spermatogenesis were closely correlated with testis size. The tubules of the controls were considerably larger than those of the prolactin-injected birds (fig. 43, a and b). Spermatogenesis was also more advanced in the controls than in the experimentals. The average tubule size for the 6 experimentals in the first experiment was 78 micra. Most of these showed only a few primary spermatocytes and one showed a few secondary spermatocytes. The average tubule diameter of the two controls in the first experiment was 255 micra and both showed spermatids and one a few mature sperm.

Follicle size.—In both experiments prolactin inhibited almost completely the development of ovarian follicles (fig. 43, c and d). Follicles measuring 0.5 to 1.3 mm in diameter could be seen with the naked eye in the ovaries of all controls, but the ovaries of the prolactin-injected birds had to be sectioned to reveal the small and often atretic follicles. The average size of the largest follicle of the three injected females in the first experiment was 265 micra. The average largest follicle of the 5 controls was 1021 micra.

Interstitial cells.—Not enough is known concerning the interstitial cells of birds to permit any interpretation of the response of these elements. A few observations which may bear on the secretion of sex hormones by the gonads, however, were noted. After

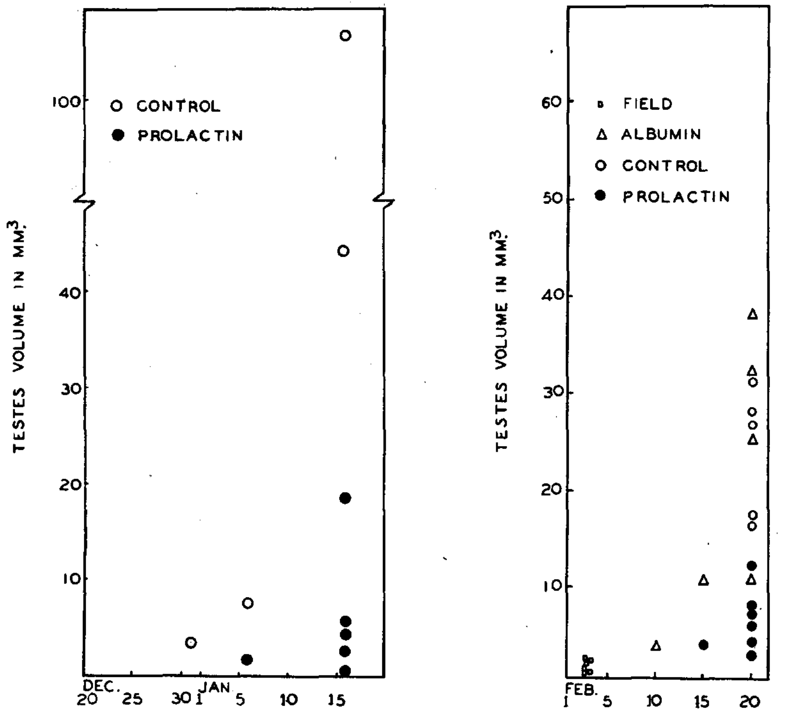

Fig. 42. Left, testis size of experimental and control White-crowned Sparrows in first experiment. All birds subjected to continuous artificial light from December 23, 1949, to January 15, 1950; experimentals received prolactin daily.

Right, testis size of experimental and control White-crowned Sparrows in second experiment. All birds except those denoted by the small squares (which were sacrificed immediately) subjected to continuous artificial light from February 3 to February 20, 1950. Normal controls untreated; injected controls received albumin daily; experimentals received prolactin daily.

about the thirteenth day of the first experiment and during the last few days of the second, loud singing was heard coming from the controls. Although this singing by the controls was noted on numerous occasions, it was never observed among the prolactin-injected birds. If the secretion of sex hormones is responsible for the loud and persistent singing of breeding birds, the absence of singing in the prolactin-injected group is possible evidence that the sex hormone-secreting elements were being inhibited. The oviducts of the female controls had increased considerably in size over those of the prolactin-injected birds. This would again suggest that prolactin is inhibiting, through some mechanism, the secretion of sex hormones. This latter response has previously been demonstrated by Bates, Lahr, and Riddle (1935) in chickens.

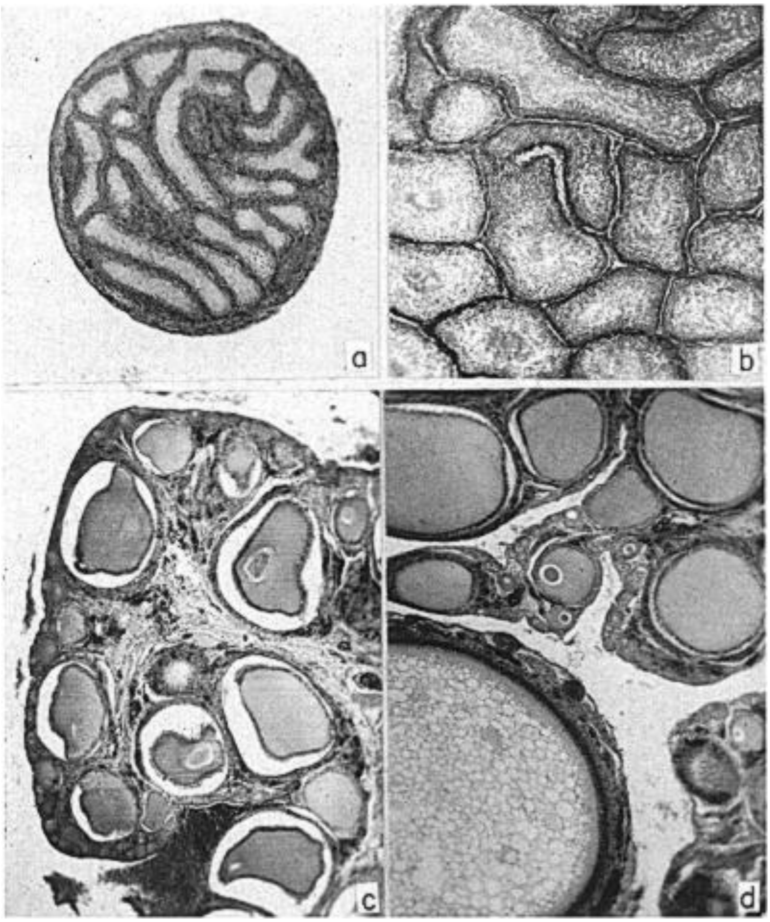

Fig. 43. (a) Testis of White-crowned Sparrow subjected to continuous light and injected with prolactin daily from December 23, 1949, to January 15, 1950. (b) Same as "a" without daily injections of prolactin. (c) Ovary of White-crowned Sparrow subjected to continuous light and injected with prolactin daily from December 23, 1949, to January 15, 1950. (d) Same as "c" without daily injections of prolactin. All figures 61×.

DISCUSSION AND CONCLUSIONS

In their work on the anti-gonadal activity of prolactin, Riddle and his associates used adult pigeons and fowls, forms which have lost to a considerable degree the cyclic reproduction characteristic of most wild birds. Their birds were in breeding condition at the time of the experiments and the response to prolactin was measured by the amount of gonadal regression. The testes of adult pigeons were reduced in size by about 90 per cent after injections for 10 days, and the formation of large follicles in the ovaries was stopped while those already formed were resorbed.

In the present experiment it was demonstrated that prolactin is capable of inhibiting light-induced recrudescence of the gonads of White-crowned Sparrows. The interest lies primarily in the ability of prolactin to exert its influence in the face of a supposedly strong stimulus to the pituitary, namely, light. Bates, Riddle, and Lahr (1937) showed that when follicle stimulating hormone was injected simultaneously with prolactin, the action of the latter on the gonads was overcome. This is strong evidence that prolactin, in some way, blocks the secretion of gonadotropins by the pituitary. This may be quite similar to the situation existing during the refractory period, for at this time it appears that it is the pituitary which is refractory and not the gonads (Miller, 1949).

While it has been shown that prolactin is probably physiologically capable of being the hormone responsible for refractoriness in birds, only a suggestion of this mechanism is possible at present. The prolactin content of the pituitary over the entire year must be determined and correlated with the events of the annual cycle, particularly the onset, duration, and termination of the refractory period.

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