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Diurnal Rhythm and Photoperiodism in Testicular Recrudescence of the House Finch

Abstract. *A circadian rhythm in house finches appears to control the timing of the photoperiodic response of testicular recrudescence. A 6-hour light period coupled with dark periods of varying duration does not stimulate spermatogenesis in cycle lengths of 24, 48, and 72 hours, but initiates spermatogenesis in cycles of 12, 36, and 60 hours.*

It is well established that the photoperiod influences the reproductive cycle of birds in temperate zones (*1*). Since the discovery of the avian photoperiodic response by Rowan (*2*) in 1925, many investigators have sought to explain how birds make the critical distinction between long days and short days. Rowan himself thought that the increasing length of the day in spring was the important factor. Later investigators postulated that the absolute length of the light period is critical (*3*); some believed that the light period is rate limiting but with a "carry-over period" into the darkness (*1*). Others postulated that there is a dark-dependent phase (*4*), and some believed that darkness has an inhibitory role (*5*). Another hypothesis (*6*), originally advanced to explain the photoperiodic mechanisms of plants, has since been extended to animals (*7*) and postulates that an endogenous diurnal rhythm is involved in the photoperiodic response. Experiments have been performed for the specific purpose of testing this hypothesis in animals (*8*), but the evidence for all those hypotheses still is subject to controversy. The experiments described in this paper were designed to test the endogenous rhythm hypothesis by utilizing a technique that has proved useful in examining the photoperiodic responses of short-day plants (*9*).

Adult house finches, *Carpodacus mexicanus*, a common nonmigratory fringillid of western North America, were trapped in the vicinity of this university and placed in groups of 10 (experiment 1) and 6 (experiment 2) in cages measuring approximately 70 × 30 × 30 cm. Commercial canary seed, water, and gravel were available at all times, and fresh fruit was supplied once a week. The cages were placed in the open drawers of "photocyclers" (*10*) and all were exposed to the same intensity of light (4400 to 11,000 lu/m^2) from GE Cool White fluorescent tubes. When the drawers were closed the cages were in complete darkness. The temperature varied from 24° to 32°C.

All the birds in both experiments were given a similar preliminary treatment. For 2 months they were subjected to short days of 6 hours light and 18 hours darkness (6L/18D). After this 2-month period, some of the birds, selected at random from the stock, were placed on long-days (18L/6D) for 25 days. These birds responded positively by showing complete testicular recrudescence. The testes of four birds castrated before both experiments served as preexperimental controls; their testes were all in an immature condition. The experimental birds subjected to a 6L/18D schedule (identical to the initial 2-month treatment) served as short-day controls. In both experiments a 6-hour light period was coupled with a variety of dark periods to give cycles of 12, 24, 36, 48, 60, and 72 hours.

In experiment 1, the surviving finches were killed with chloroform after 33 days; the left testis was removed, weighed, and preserved in Bouin's solution. The testes were sectioned at 10μ and stained with Harris' haematoxylin and eosin. In experiment 2, the birds were assayed after 22 cycles of treatment (except that the birds subjected to the 6L/6D regimen received 44 cycles). These birds were anesthetized, unilaterally castrated, and subsequently released. The left testis was weighed, preserved, sectioned, and stained as above. The degree of spermatogenesis in both experiments was categorized after Bartholomew (*11*).

Data on the weight of the testes of birds in both experiments are presented in Fig. 1. Enlargement and maturation of the testes did not occur in the house

Table 1. The stages of spermatogenesis in testes of birds in experiment 1 (x) and experiment 2 (o). Stage I, spermatogonia only; stage VI, many sperm in tubules.

Stage	6L/6D	6L/18D	6L/30D	6L/42D	6L/54D	6L/66D
VI	ooooo		o			
V	xxxxo		xxoo		xo	
IV	x		xoo		x	
III					xxx	
II					ooo	o
I		xxxxxxo		xooooo		xxxxoooooo

finches subjected to a short day regimen (6L/18D), or in those subjected to cycles of 48 hours (6L/42D) or 72 hours (6L/66D). The birds responded to these cycles as though they had received short-day, nonstimulatory treatment. In contrast, the birds subjected to cycles of 12 (6L/6D), 36 (6L/30D), and 60 hours (6L/54D) responded as though they had been subjected to long days. Their testes increased in weight and spermatogenesis began (Table 1). Spermatogenesis was not initiated in birds subjected to 24-, 48-, and 72-hour cycles except for one bird from the 72-hour cycle in which spermatogonia and a few spermatocytes were present.

A number of conclusions may be drawn from these data. First, those hypotheses that stress the necessity for a given duration of light to trigger the photoperiodic response are inapplicable to house finches. All the birds received 6 hours of light per cycle; thus, no treatment constituted a long day in the usual photoperiodic sense. Yet the birds on the 36-hour cycle showed testicular enlargement despite the fact that they were given only 6 hours of light every day and a half. As the source of energy input in a photodependent system, light is necessarily important, but in these experiments the length of the light period was not critical; nor was it the length of the dark period which initiated testicular recrudescence. The six experimental treatments differed in their durations of darkness. There is no seeming reason why, if darkness itself is important, the birds subjected to 36-hour cycles should respond while those subjected to 24- and 48-hour cycles should not.

These results indicate that there is within the bird an endogenous rhythm with a periodicity of about 24 hours. When light is given at the proper phase of this rhythm recrudescence occurs, and when light is given at the incorrect phase of the rhythm no response is elicited. This circadian rhythm may therefore serve as a biological clock to time the photoperiodic response of testis maturation in the house finch (*12*).

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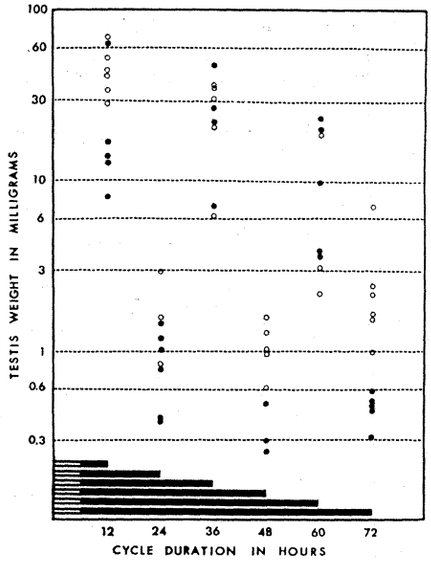

Fig. 1. Data on the weights of the testes from birds in experiment 1 (solid circles) and experiment 2 (open circles). Solid black bars represent different durations of darkness; open boxes represent standard light period of 6 hours.

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Oxygen Uptake from a Reservoir of Limited Volume by the Human Cornea in vivo

Abstract. *Oxygen flux across the anterior surface of the human cornea from a closed reservoir of air-saturated, physiological, isotonic saline was measured in vivo as a function of time. The rate of oxygen consumption calculated from this flux compares favorably with estimates given in the literature, which were based on studies in which the microrespirometer technique of Warburg was used.*

The consumption of oxygen by the cornea directly from the atmosphere has been known for some time (*1*). Although the atmosphere is the major source of oxygen for the cornea, no direct measurements of the flux across the cornea-atmosphere interface have been reported previously.

Rates of oxygen uptake by the cornea of a variety of animals have been published (*2–6*) (see Table 1), but the results were obtained by methods which differ considerably from our technique. Previous workers macerated the corneas and suspended the cells in saline. The oxygen uptake of the resultant suspension was measured by the microrespirometer technique developed by Warburg. By our method, the flux of oxygen across the cornea-atmosphere boundary can be measured in vivo. This flux is known to be only a part of the total supply of oxygen to the eye, but is probably the major source for the cornea.

Our equipment is shown schematically in Fig. 1. A Lucite tube was cemented to a scleral contact lens made of acrylic plastic, and the material directly under the tube was cut away. An oxygen electrode covered with a polyethylene membrane (of the Clark type) (*7*, *8*) fitted tightly into the Lucite tube so as to exclude passage of liquid between the tube and the electrode. By moving the electrode assembly the volume in the reservoir could be changed as desired. The exit and inlet tubes cemented to the Lucite tube enabled the reservoir to be flushed rapidly with a solution of known oxygen tension, so that we could make a calibration while the lens was on the subject's eye (*9*).

The oxygen consumption of the electrode was negligible, as shown by the decline in oxygen tension when the membrane-covered electrode was pressed against a soft surface impermeable to oxygen (a synthetic leather cushion).

Table 1. Rates of oxygen uptake by the corneas of different animals, recalculated on the assumption that all the cornea's oxygen requirements are met by the atmosphere.

Animal	$\mu l/cm^2$ per hour	Source of original data
Ox	7.6	de Roetth (*2*)
Rabbit	8.6	Langham (*3*)
Rabbit	4.6	Robbie (*4*)
Rat	24.9	Lee and Hart (*5*)
Rat	40	Bessey and Wolbach (*6*)