of photoproducts. Retinas were exposed first to a saturating white flash and then, at 10-second intervals, to two near-ultraviolet flashes produced by interposing a Corning CS 7-51 filter between the xenon flash gun and the preparation. The photoproduct difference spectra in such experiments showed significantly less fading of the 380-nm photoproduct, whereas the fading of the 465- to 475-nm peak was greater than that in those experiments in which retinas were exposed to a single white flash.

These observations agree with those of Matthews *et al.* (*5*), who showed that a flash of intense near-ultraviolet light rapidly converts metarhodopsin II to a mixture of rhodopsin, isorhodopsin, and the 467-nm intermediate. This procedure differentiates metarhodopsin II from a mixture of free retinal and opsin, which has a similar absorption spectrum but does not undergo rapid spectral shifts immediately after near-ultraviolet irradiation.

Our results are also consistent with recent studies of the early receptor potential (ERP) in the rat eye (*12*). These studies correlated components of the ERP with the presence of metarhodopsins I and II and the 467-nm intermediate, and determined the time courses of formation and decay of these photoproducts. In the rat, metarhodopsin I is formed almost immediately after a saturating flash and decays in a few milliseconds at physiological temperatures. This explains why we did not observe this intermediate in our experiments. On the other hand, at room temperature in the rat or rabbit eye, metarhodopsin II forms with a half-time of 1 msec and decays over several minutes, whereas the 467-nm intermediate forms and decays even more slowly (*11, 12*).

Our results demonstrate that the presence of metarhodopsin II and the 467-nm intermediate after a bleaching exposure does not affect scotopic visual sensitivity in the rat retina. This conclusion differs from that of Donner and Reuter (*7*), who have proposed that metarhodopsin II affects scotopic sensitivity in the frog. Our results suggest that, after the completion of neural adaptation, the rod threshold is simply determined by the fraction of intact rhodopsin in the retina.

It may be argued that neural adaptation is somehow related to photoproduct decay [see, for example, (*7*)], but our results make this suggestion unlikely. Although most of the neural recovery takes place almost immediately upon cessation of the adapting light, it usually continues for some 4 to 5 minutes (*1, 8*). The photoproducts seen in our experiments are still present and decaying long after neural adaptation has ended. On the other hand, earlier photoproducts—prelumirhodopsin, lumirhodopsin, and metarhodopsin I—disappear in the rat retina within milliseconds after the flash at physiological temperatures (*4, 11, 12*), long before neural recovery is complete. In addition, neural adaptation may change electroretinographic thresholds by several logarithmic units even though negligible rhodopsin has been bleached (*1, 8*). In such a situation, the concentration of intermediates must be extremely small, yet neural adaptation can be as extensive as when considerable bleaching has occurred.

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Enzymatic Synthesis of Melatonin in Avian Pineal Body: Extraretinal Response to Light

Abstract. *In the chick pineal body, activity of the melatonin-forming enzyme hydroxyindole-O-methyl transferase is greater in the light than in darkness. Neither bilateral enucleation of the eyes nor sympathetic denervation prevented this light-induced elevation of enzyme activity. This fact indicates that in the bird, in contrast to mammals, neither the retinas nor sympathetic innervation of the pineal body are essential for environmental control of melatonin formation.*

Enzymatic synthesis of melatonin by hydroxyindole-*O*-methyl transferase (HIOMT) in the pineal organs of birds is enhanced in the light and suppressed by darkness (*1*). In the rat, on the other hand, pineal activity is greater in the dark than in the light (*2*). Information about lighting appears to reach the pineal body via retinal receptors and sympathetic nerves in the rat (*3*). We here report experiments with birds indicating that this light-influenced pineal HIOMT activity is independent of the eyes and of sympathetic innervation.

Male White Rock chicks were reared from the day of hatching under a 14-hour photoperiod (LD 14:10), given free access to food (chick starter) and water, and provided with heat without light during the brooding period, according to standard rearing practice. Pineal bodies were denervated by removal of both superior cervical sympathetic ganglia within the first 4 days after hatching (*4*). Birds were anesthetized with Combuthal (*5*). Removal of the ganglion could be readily confirmed by the development of permanent ipsilateral ptosis; transitory erection of the head feathers sometimes occurred during the first hours after ganglionectomy (*6*). In some controls both ganglia were exposed but not removed. Both eyes were removed from other chicks (1 to 3 days old). In some cases, the second eye was removed 1 day after the first; in others, both eyes were removed during the same operation. After enucleation, the orbits were packed with gelatin foam, and the eyelids were sutured shut. Survival was good after both operations.

When 4 weeks old, all chicks were transferred to either continuous light (LD 24:0) or constant darkness (LD 0:24) in environment chambers so designed that they could be completely

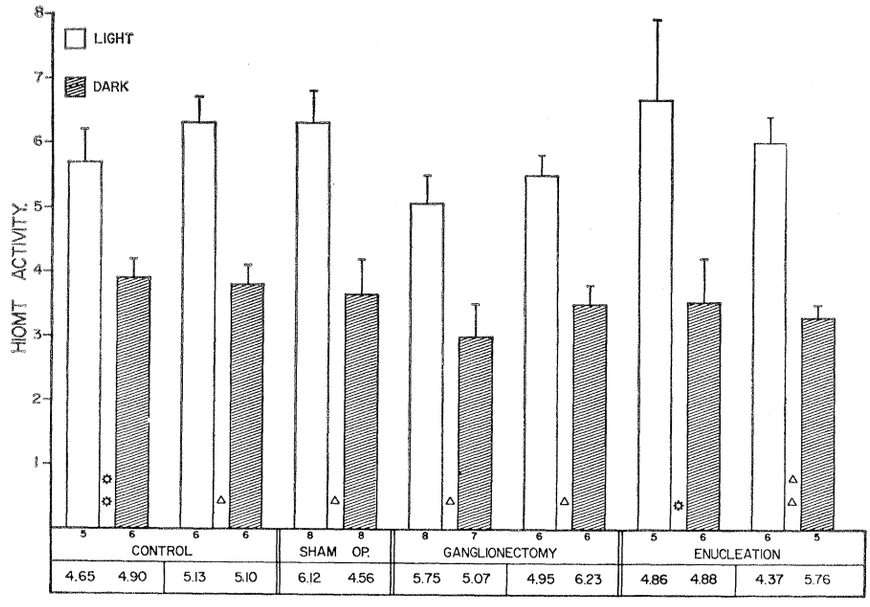

Fig. 1. Hydroxyindole-*O*-methyl transferase (HIOMT) activity in the chick pineal body under conditions of constant light or constant darkness, and after several surgical procedures. The HIOMT activity is expressed as millimicromoles of C^{14}-melatonin formed, per milligram of pineal body, from *N*-acetyl serotonin and *S*-adenosylmethionine-C^{14}. The small vertical line above each bar indicates standard error; △ △ between two bars signifies $P < .001$; △, $P < .01$; * *, $P < .02$; *, $P < .05$. Five to eight chicks were used, as indicated below each bar. Average pineal weights are given in milligrams at the bottom. *Sham op.*, sham operated.

serviced from the outside without use of a safelight. When 5½ weeks old, all chicks were decapitated in the light or dark environment to which they had been assigned. The pineal body was quickly removed, weighed, and frozen. The order in which chicks of various treatments were killed was rotated to facilitate detection of any diurnal pattern that might be superimposed on the experimental results. Hydroxyindole-*O*-methyl transferase was assayed by methods previously described (*7*).

Enzymatic synthesis of melatonin in the pineal body was almost twice as high in light as in darkness, in confirmation of our previous findings (*1*). This difference persisted after removal of the eyes and after interruption of sympathetic nerve impulses to the pineal body (Fig. 1).

In rats, pineal HIOMT varies with environmental lighting, and this response is abolished by enucleation (*2*). Pineal serotonin, however, apparently follows an endogenous diurnal rhythm which persists even in the absence of the eyes (*8*).

Our observation that the avian pineal body can respond to light even in the absence of the eyes suggests that the thin skull of the bird may allow light to penetrate. In addition, the pineal body, or some structure which can influence it, can apparently act as a photoreceptor. That physiologically active amounts of light can penetrate the skull has been suggested for mammals (*9*) and for birds (*10*). Benoit's experiments on gonadal stimulation in blinded ducks suggest that, in the absence of the eyes, the hypothalamus region can receive and respond to light (*11*). In view of the close developmental and evolutionary association of the pineal organ with the "third eye" of some reptiles, and the frontal organ of amphibians (*12*), it would not be surprising to find that in birds, as well, a derivative of the embryonic epiphysis could respond to light. Our results suggest that it might be of interest to shield the pineal organ of blinded chicks while exposing the rest of the organism to light.

The fact that removal of both superior cervical ganglia did not alter pineal HIOMT activity in the chick is in direct contrast to the situation in the rat, where intact sympathetic innervation is required for the characteristic pineal response to environmental lighting (*3*). Although the possibility cannot be excluded that other fibers may also innervate the avian pineal body, it is at least known that the follicular (parenchymal) portion of the chick's pineal has abundant sympathetic fibers (*13*).

The relationship of the pineal body to gonadal development and function has been the subject of numerous studies (*14*). In rats and several other mammals, the pineal body inhibits reproductive processes (*15*). In birds, most of the evidence suggests a progonadal role for the pineal: pinealectomy in quail temporarily delays ovarian maturation (*16*). Likewise, pinealectomy of newly hatched White Leghorn cockerels inhibited testis growth during the ensuing 20 days (*17*).

In an ingenious experiment in which radioluminous paint was applied to the top of the head, Kato *et al.* found that constant light from this source prevented the gonadal regression expected when mature male quail were transferred to a short environmental photoperiod (*18*). Removal of superior cervical ganglia temporarily interrupts egg production in female *Coturnix*, but has no effect on male gonads (*19*). Since several aspects of the reproductive process in birds are influenced by light, it seems reasonable to consider the possibility that photosensitive pineal events may in some way be related to gonadal or pituitary gonadotropic functions. However, the question of how information about lighting reaches the pineal remains open.

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