the calculation is in a range where one or two additional base differences affect it markedly. When calculated (*6*) for best fit, there is 0.22 hit per site on 54 variable sites, which is discordant with the snake-bird comparison, but one more two-base change would bring the computations into line for 0.55 hit per site on 25 variable sites, with one-fourth as many REHC for turtle as for snake (0.14 versus 0.58). The statistical variance in the estimate of the number of variable sites is thus large and very strongly dependent on the sample of sequences available. This large variance is not, however, a property of the estimate of the number of REHC. In fact, it can be shown (*11*) that the observed difference in the number of amino acid substitutions between the snapping turtle-chicken and rattlesnake-chicken exceeds chance fluctuations by a factor of more than 3.6.

A direct comparison of turtle and snake (Table 3) shows that the two reptiles have diverged widely, and we infer that most of this divergence is due to changes in snake. The four birds inter alia show very little divergence, even though the penguin is in a different superorder (*9*) from the other three. When snake, turtle, and chicken are compared for amino acid differences with the cytochromes c of seven nonreptilian and nonavian vertebrates [lamprey, dogfish, tuna, kangaroo, bovine, rabbit, and human (*2*)], in each case the snake differs more than either turtle or chicken. The average amino acid difference between snake and these seven species is 21.0 ± 4.5 (S.D.); the corresponding average for both turtle and chicken is 13.4 ± 4.2.

The Reptilia are a large and diverse class that includes both primitive and "modern" animals, so that it might be expected that turtle and rattlesnake would represent widely divergent lines. However, the difference between turtle and rattlesnake of 21 amino acid residues per 100 codons is notably larger than many differences between representatives of widely separated classes, for example, 17 between chicken and lamprey, or 16 between horse and dogfish, or even 15 between dog and screwworm fly in two different phyla.

The identification of the variable codons in rattlesnake cytochrome c is further supported by comparing it with bovine cytochrome c. All 20 of the sites differing in this comparison also differ in the snake-turtle comparison.

The differences in Tables 1, 2, and 3 suggest that the evolutionary rate of change of vertebrate cytochromes c is species-dependent as well as time-dependent.

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11. For the rattlesnake-bird comparison (Table 2), there is a total of 58 ($2.3 \times 25$) evolutionary hits. For the snapping turtle-bird comparison, there are 14 ($0.55 \times 25$) such hits. The standard deviation in observable number of base differences to be found between the two pairs of homologous structural genes which code for these cytochromes can be calculated from Eqs. 23 and 24 in (*7*) with $L = 25 \times 3 = 75$ and $X = 58$ and 14, respectively, and are S.D. rattlesnake-bird = 3.4 and S.D. snapping turtle-bird = 1.3. One can then expect an uncertainty in the number of amino acid differences of 2.6 ($= 3.4 \times 0.76$) and 1.0 ($= 1.3 \times 0.76$), respectively, because of codon degeneracy. If the difference in the number of amino acid differences is taken, the error in this difference is about $[(2.6)^2 + (1.0)^2]^{1/2} = 2.8$. The observed difference (Table 2) is $18.5 - 7.8 = 10.7$, well over 3 standard deviations away, and significant.
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## Rapid Light-Induced Decrease in Pineal Serotonin N-Acetyltransferase Activity

Abstract. *Light acting by way of the eye causes the dark-induced activity of serotonin* N-*acetyltransferase in the pineal gland of the rat to decrease with a halving time of about 3 minutes. This effect, which is one of the more rapid physiological changes known to occur in the activity of any enzyme that metabolizes biogenic amines, appears to explain the rapid increase in the concentration of pineal serotonin that is caused by light exposure at night.*

Serotonin *N*-acetyltransferase converts serotonin (5-hydroxytryptamine) to *N*-acetylserotonin (*N*-acetyl-5-hydroxytryptamine), the precursor of melatonin (5-methoxy-*N*-acetyltryptamine) (*1*). The activity of serotonin *N*-acetyltransferase in the pineal gland of the rat increases at night in the dark, when rats are active, to values that are 15 to 70 times greater than the day values (*2, 3*). It was not known if light could decrease the activity of this enzyme after it had been dark-induced. We now report that exposure to light at night causes a rapid decrease in the activity of pineal *N*-acetyltransferase.

Groups of male Osborne-Mendel (NIH strain) rats (200 to 225 g) were housed for 7 to 10 days in a room without windows, but with automatically regulated lighting that provided 14 hours of light and 10 hours of darkness. The lights were turned off at 7:00 p.m. At 11:30 p.m. the animals were removed from that dark room and transferred to a room where the lighting was about 100 lumen/m$^2$ (*4*). After being in the light for 0.25 to 10 minutes, the animals were stunned and immediately decapitated. The pineal glands were removed within 30 seconds of decapitation, were frozen on solid $CO_2$, and were stored for 12 hours at −20°C. Serotonin *N*-acetyltransferase activity in individual pineal glands was estimated as described (*2, 3*). When animals were placed in the lighted room the activity of pineal serotonin *N*-acetyltransferase was above 5000 units (Fig. 1). During a 10-minute exposure to light the activity fell to 400 units. The halving time ($t/2$) was slightly less than 3 minutes.

To determine whether we were actually observing an effect of light acting by way of the eye or if handling of the animals was causing the decrease in enzyme activity, we used blinded animals in a second study. The rats were blinded by bilateral enucleation while under ether anesthesia 36 hours prior to decapitation. They were housed in cages with control animals that had been

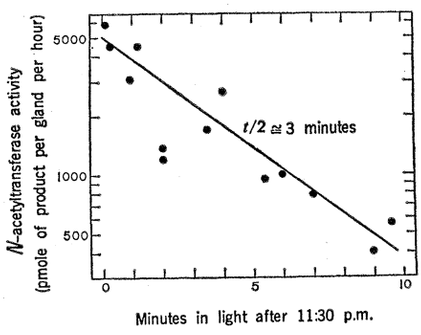


Fig. 1. Effect of light exposure on pineal *N*-acetyltransferase activity in animals at night in the dark. Each point represents enzyme activity in an individual rat pineal gland. Halving time, $t/2$. The line was drawn by the least-squares method.

anesthetized but not blinded at the time of surgery. The response of five control and six blinded animals to light exposure was determined over a 15-minute period in the same type of experiment as above (Fig. 2). The $t/2$ for the disappearance of enzyme activity in controls was again about 3 minutes. The correlation coefficient of enzyme activity and the time in light was 0.93 for the control group; that of the blinded group was 0.28. This lack of any effect of light in blinded animals indicates that light is acting by way of the eye, and that the decrease is not due to handling or to light acting by way of a nonretinal photoreceptor. This is consistent with the observation of a 24-hour rhythm in pineal *N*-acetyltransferase activity in blinded animals kept in continuous lighting (*2*).

Illnerová has observed that the concentration of serotonin in the rat pineal gland increases by about twofold from night values to day values within 14 minutes (*5*). The mechanism of this rapid effect was unknown, but the explanation may lie in the rapid change in *N*-acetyltransferase activity. Based on the available evidence, it has been proposed that the circadian rhythm of serotonin in the pineal gland is regulated by the inverse rhythm of *N*-acetyltransferase, which causes an increased removal of serotonin by *N*-acetylation at night and a decreased rate of removal during the hours of daylight (*2*). It appears reasonable to extend the *N*-acetylation explanation to include the rapid effect of light on pineal serotonin.

The physiological importance of a rapid change in *N*-acetylation of serotonin may be related to the secretion of melatonin, the putative antigonadotrophic hormone of the pineal gland (*5*). The findings of organ culture experiments indicate that large changes in the production and "secretion" of melatonin by the pineal gland are regulated by the activity of *N*-acetyltransferase (*2, 3, 7*). Perhaps the rapid effect in vivo of light on the activity of *N*-acetyltransferase would be transferred into a similar large and abrupt decrease in melatonin production and secretion, thus increasing the precision and accuracy of the pineal gland acting as an "endocrine transducer" that changes information about the daily dark period into an endocrine signal, that is, the duration of melatonin secretion (*8*). This precision may be most important to seasonal breeders, such as the ferret, which become reproductively active when the daily dark period is shortened several hours (*9*). Shorter nights would be expected to produce shorter daily periods of melatonin secretion. The accumulative effect of this may be gradual changes in reproductive activity. Alternatively, the rapid change in *N*-acetyltransferase activity may be related to daily changes in reproductive or other behavior patterns.

The mechanism through which light acts to decrease pineal *N*-acetyltransferase activity may involve only the turning off of the system that is responsible for the transsynaptic norepinephrine–adenosine 3′,5′-monophosphate induction of enzyme activity by darkness. This neural-biochemical pathway appears to include the retina, central neural structures, the superior cervical ganglia, the release of norepinephrine from sympathetic nerve endings, the activation of adenylate cyclase, and the stimulation of pineal adenosine 3′,5′-monophosphate (*7, 10*). Alternative hypotheses include: (i) the nonadrenergic neural transmission of a visual signal; (ii) the release of a second transmitter from nerve endings in response to the net uptake of norepinephrine, which would occur at the termination of the dark-induced norepinephrine release (*11*); and (iii) electrochemical changes in the pinealocyte, which may be associated with termination of neural stimulation. The rapid nature of the change in enzyme activity fits better with a model for enzyme inactivation, rather than with one including enzyme degradation. Perhaps the rapid change in activity depends on the rapid conversion of an *N*-acetyltransferase stabilizing compound to a nonfunctional form that allows the spontaneous thermal inactivation of the unstabilized enzyme.

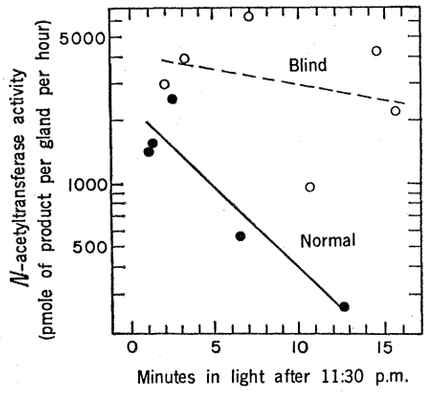


Fig. 2. Effect of blinding on the light-induced disappearance of pineal serotonin *N*-acetyltransferase activity at night. Lines were drawn by the method of least squares.

The light-induced drop in the activity of pineal serotonin *N*-acetyltransferase is one of the more rapid physiological changes known to occur in the activity of any enzyme that metabolizes biogenic amines. Serotonin *N*-acetyltransferase activity has been detected in several areas of the brain (*3*). Large, rapid, and localized changes in the activity of this enzyme in the brain may occur and may be involved in the mediation of rapid changes in behavioral states that seem to involve serotonin, such as sleep (*12*).

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