the control mothers do (Table 1). The ratio of males to females increases with the severity of the toxemia, as determined by the daily urinary excretion of protein or by the diastolic blood pressure (Table 2).

Information with respect to parity and blood groups (ABO and Rh with anti-D) of the mother and the newborn was available for 585 (55 percent) toxemic pregnancies, all of which were full-term (*6*). The ratio of males to females (1.22) in this group, which is not different from that for the entire group of toxemic patients (Table 1), indicates that the increased sex ratio is not related to prematurity. Parity had no effect on the sex ratio, because the ratio was the same in the case of primiparas as in that of multiparas. Distribution of the 16 different combinations of ABO groups and the four combinations of Rh groups within the toxemic group was similar to that within the 6096 controls (*7*).

In 1955, Salzmann reported an increased sex ratio in 287 babies born to toxemic mothers less than 30 years old (*8*). He put forward a theory about the role of male fetal hormones in the pathogenesis of toxemia. Today, the existence of a weak histocompatibility antigen (or antigens) determined by the Y chromosome is well established in the mouse (*9*), and there is considerable evidence for the same phenomenon in man. This evidence is based on the mother's ability to recognize the male fetus, as indicated by the following: (i) the fact that the ratio of males to females in live births decreases with increasing parity, whereas this ratio increases in stillbirths; and (ii) the fact that the number of preceding boys influences both the sex ratio of subsequent children and the intervals between births (*10*). Operation of an antigen (or antigens) dependent on the Y chromosome as a histocompatibility system in man is supported also by our recent data suggesting that the effect of feto-maternal ABO incompatibility and Y-chromosome-linked incompatibility on placental weight is cumulative (*11*).

We believe that it is possible to explain the increased ratio of males to females born to toxemic mothers as an expression of histoincompatibility between mother and fetus. Mothers with toxemia more often have HL-A antibodies (induced by cells from the conceptus) than healthy parturients do (*12*). We propose that toxemia of pregnancy has a partial immunological basis. Like other paternal antigens in all placentas (*13*), the potential Y-chromosome-dependent antigen (or antigens) must be present in the placenta of the male fetus. Histocompatibility antigens may potentiate the immunogenicity of other placental antigens known to be shared by the kidney (*14*), and this immunization may lead to renal lesions and other hallmarks of toxemia.

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Adenosine 3′,5′-Monophosphate in Rat Pineal Gland: Increase Induced by Light

Abstract. *Rats were maintained in alternating periods of 12 hours of light and 12 hours of darkness. The concentration of adenosine 3′,5′-monophosphate in pineal gland was six times higher at the end of the light period than at the end of the period of darkness. This effect of light was abolished in blinded animals.*

The concentration of adenosine 3′,5′-monophosphate (cyclic AMP) (*1*) and the activity of the adenyl cyclase (*2*) are particularly high in the pineal gland when compared with other areas of the brain. Norepinephrine increases pineal adenyl cyclase activity (*3*), and some of the effects of this catecholamine on the pineal gland are mediated through the formation of cyclic AMP (*4*). Because adenyl cyclase activity (*5*) as well as the concentration of norepinephrine in the pineal (*6*) are influenced by light, we investigated whether light also changes the content of cyclic AMP in this gland. Our experiments show that the concentration of cyclic AMP in rat pineal is six times higher after 10 hours of light than after 10 hours of darkness; moreover, the increased content of cyclic AMP induced by light is abolished in blinded rats.

Groups of 16 to 32 male Sprague-Dawley rats (180 to 220 g) were placed in two separate air-conditioned rooms and were kept in alternating photoperiods of 12 hours of light and 12 hours of darkness for at least 3 weeks. Light sources were white fluorescent lamps (General Electric) yielding 1070 to 1600 lu/m^2. A small blackened corridor separated the two rooms. Ten hours after exposure to light or darkness, the rats were decapitated and the pineal glands were removed and frozen within 30 seconds. For the rats in darkness, these operations were carried out with the aid of a lamp with a General Electric red soft bulb (BAS, 25 watts, 120 volts).

Cyclic AMP was determined by a method described by Ebadi *et al.* (*7*). Briefly, the frozen pineals were homogenized in a Duall glass tissue grinder with 300 μl of $ZnSO_4$ (0.3M) containing 1×10^{-18} mole of cyclic [^{3}H]AMP (2.35 c/mmole) to monitor recovery. The homogenates were frozen and thawed several times to release the bound cyclic AMP. A portion (20 μl)

was stored for protein determination (*8*). Then, 100 μl of $HClO_4$ (0.4*N*) was added to the remainder, and the samples were rehomogenized. Perchloric acid was neutralized with 100 μl of $KHCO_3$ (0.4*M*). Nucleotides other than cyclic AMP were precipitated with 280 μl of $Ba(OH)_2$ (0.25*M*). After centrifugation, 50 μl each of $ZnSO_4$ and $Ba(OH)_2$ was added, and the mixture was recentrifuged. The supernatant fluid obtained after the second treatment with $Ba(OH)_2$ and $ZnSO_4$ was further purified with a cation exchange resin (AG 50W-X8; 200 to 400 mesh); the cyclic AMP was eluted with water (*9*).

The cyclic AMP fraction was collected in scintillation counting vials, divided into equal portions (blank and test), and lyophilized. Cyclic AMP was converted to ATP by a combined system of phosphodiesterase, myokinase, and pyruvate kinase (*10*). The tissue blanks received a mixture containing myokinase, pyruvate kinase, and either boiled or no phosphodiesterase. The reactions were terminated by adding 20 μl of 6 percent H_2O_2, and the vials were frozen. The frozen reaction mixtures containing the ATP were thawed, and 500 μl of 50 m*M* tris-(hydroxymethyl) aminomethane-hydrochloride buffer (*p*H 7.5) containing 3 m*M* $MgSO_4$ was added. At zero time, 300 to 600 μg of reconstituted firefly lantern extract (Sigma, St. Louis) was added, and the vials were mixed and placed rapidly (within 5 seconds) into the counting well of a Beckman liquid scintillation spectrometer. Twenty seconds after the firefly lantern extract was added, the samples were counted for 30 seconds. The number of counts accumulated in that time is the criterion employed to measure light emission produced by utilization of ATP (*11*). Fifteen milliliters of liquid scintillation fluid was then added, and the samples were recounted to calculate the recovery of cyclic [^{3}H]AMP. The counts from the standard cyclic AMP samples were plotted on full logarithmic paper, and the ATP concentration in the blank and test samples were individually determined.

The concentration of cyclic AMP in pineal (expressed as the number of picomoles per milligram of protein) was about six times higher in animals killed during light as compared with rats killed in darkness (Table 1). The difference in cyclic AMP concentrations was significant ($P < .001$) whether computed per pineal or per milligram of protein. Under the same lighting conditions, no differences were found in the cyclic AMP concentration in adrenal glands in three experiments (light—3.3, 3.7, and 4.1 pmole per milligram of tissue; darkness—2.4, 1.9, and 4.2 pmole per milligram of tissue).

Table 1. Effect of environmental lighting on the concentration of cyclic AMP in pineal gland of normal and blinded rats. Male rats were conditioned to an alternating cycle of 12 hours of light and 12 hours of darkness for at least 3 weeks. After the rats were exposed to either dark or light for 10 hours, they were decapitated; the pineal glands were removed and assayed for cyclic AMP (see text). The animals were blinded, under ether anesthesia, by bilateral orbital enucleation and then subjected to similar alternating cycles of light and darkness.

Experimental condition for 10 hours	*N*	Protein (mg/pineal)	Cyclic AMP	
			pmole/pineal	pmole/mg protein
		Normal rats		
Darkness	12	0.24 ± 0.08	6.5 ± 0.4	32 ± 4
Light	16	0.19 ± 0.02	36 ± 3.3*	185 ± 24*
		Blinded rats		
Darkness	5	0.27 ± 0.06	5.3 ± 0.6	19 ± 2
Light	5	0.16 ± 0.03	5.1 ± 0.3	35 ± 6

* $P < .001$ compared with 10 hours of darkness.

In rats, nerve impulses generated by light reach the pineal gland by a pathway involving the retina, inferior accessory optic tract, median forebrain bundle, superior cervical ganglion, and nervi conarii (*12*). To study the influence of the retinal receptors on the rise of pineal cyclic AMP concentrations, rats were blinded by bilateral orbital enucleation and were then subjected to alternating cycles of 12 hours of light and darkness for 3 weeks. Blinding abolished the increase of cyclic AMP concentration induced by light in each pineal gland (Table 1). There were, however, still slight differences in the concentration of cyclic AMP when calculated on the basis of milligrams of protein. But this change apparently was due to the light-induced decrease in the total proteins in the pineal gland during darkness, which appear to be significant in blinded but not in normal rats. We conclude that blinding abolished the rise in cyclic AMP concentrations caused by light in rat pineal.

The light-induced elevation in the concentration of cyclic AMP could be due to increased synthesis, decreased destruction, or reduced utilization of this nucleotide. It is unlikely that light caused a decreased destruction of cyclic AMP since direct measurement of phosphodiesterase activity of pineal gland showed no differences between enzyme activity of rats killed in the light (1.8 ± 0.2 nmole/mg protein per minute) and that of animals killed in darkness (1.6 ± 0.2). However, measurement of pineal adenyl cyclase activity in rats kept in alternating light and dark cycles showed that the basal as well as the norepinephrine-stimulated enzyme activity increased during light and decreased during darkness (*13*). These changes occurred without a concomitant alteration of the concentration of ATP or in the uptake of phosphate (*14*). Thus, although we do not as yet have data concerning the effects of light on the release or efflux of cyclic AMP from the pineal gland, the available information suggests as a working hypothesis that the light-induced increase of the pineal concentrations of this nucleotide is due to a synthesis rate of cyclic AMP faster during light than during darkness, without changing the rate of cyclic AMP metabolism.

The sequence of events leading to an increase in the concentrations of cyclic AMP in the pineal gland are still uncertain. Light may be acting by way of the sympathetic nervous system causing the release of norepinephrine from sympathetic nerve terminals in the pineal gland. Since catecholamines increase pineal adenyl cyclase activity (*3*), this would result in an increased concentration of cyclic AMP. However, it has been reported that light may decrease neuronal activity afferent to the pineal (*15*). Another possibility is that the effects are mediated by way of the endocrine system. Indeed, female sex hormones can inhibit the activity of pineal adenyl cyclase (*16*), but we have no data supporting the view that light-induced increase in the pineal concentration of cyclic AMP is concomitant with changes of concentrations of a given hormone in the blood and does not occur when this hormonal secretion is curtailed.

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Extraretinal Light Perception: Entrainment of the Biological Clock Controlling Lizard Locomotor Activity

Abstract. *The circadian activity rhythm of the iguanid lizard* Sceloporus olivaceus *can be entrained by light cycles whether or not the animals have eyes. Removal of the pineal organ and parietal eye in blinded lizards does not prevent entrainment. Our data demonstrate the existence of an extraretinal photoreceptor which can mediate entrainment of a biological clock in reptiles.*

In the absence of any external cues, activity rhythms of many vertebrates exhibit periodicities which are approximately, but rarely exactly, 24 hours. Such circadian rhythms reflect the functioning of the animal's endogenous biological clock. Without exception, circadian rhythms can be readily entrained (synchronized) to light cycles with periods of exactly 24 hours. Recently several investigators have demonstrated that the photoreceptors involved in mediating entrainment of activity rhythms in at least two classes of vertebrates are, in part, extraretinal. The perching activity of the house sparrow *Passer domesticus* can be entrained via extraretinal receptors located in the brain (*1*). The locomotor rhythms of both the slimy salamander *Plethodon glutinosus* and the green frog *Rana clamitans* can be entrained by light cycles after the removal of the eyes (*2*). Extraretinal receptors also participate in time-compensated celestial orientation of the southern cricket frog *Acris gryllus* and thus by inference in the entrainment of its biological clock (*3*). Data are presented here which show that the activity rhythms of a third class of vertebrates, the reptiles, can be entrained by light cycles in the absence of all known photoreceptive structures.

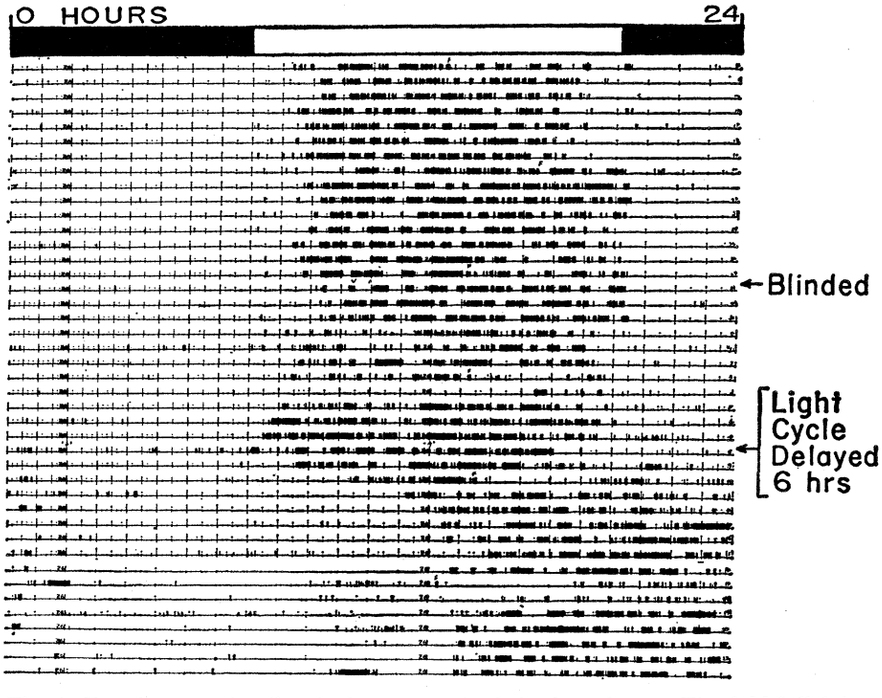

Fig. 1. Entrainment of a lizard after removal of the lateral eyes. The initial lighting regimen is diagramed at the top of the record [solid black, darkness; white, white light (30 lux)]. The lizard was blinded on the 16th day of the record. Eleven days days later the light cycle was delayed by 6 hours. Note entrainment to the new light cycle after several days of transients. Hour zero is at midnight Central Standard Time.

The activity rhythm of the iguanid lizard *Sceloporus olivaceus* is entrained by a cycle of 12 hours of fluorescent light (30 lux) and 12 hours of dark (*LD 12* : 12) after complete surgical removal of both eyes (Fig. 1) (*4*). All blind lizards which gave measurable amounts of activity (17 cases) clearly entrained to this stimulus.

In addition to their eyes, many lizards possess two organs embryologically derived from the roof of the diencephalon, which, on the basis of ultrastructural and electrophysiological evidence, are thought to be photoreceptive—the parietal eye and the pineal organ (*5, 6*). Among the lizards these two organs are diverse in morphology and location. Some lizards do not possess a parietal eye at all. Electron microscopy has shown that the pineal organ (or epiphysis proper) often contains cells with modified photoreceptive ultrastructure (*5*). The parietal eye contains well-organized photoreceptors which resemble the cones of the lateral eye (*5–7*). Electrophysiological responses to illumination have been recorded from the parietal eye of several lizard species, including members of the iguanids, and from the pineal organ of lacertid and iguanid lizards after removal of the parietal eye (*8*).

In spite of the strong evidence for a photoreceptive function of both the parietal eye and the pineal organ in some species of lizards, complete removal of both these organs in previously blinded animals did not prevent entrainment to a fluorescent light cycle