The pineal gland does not mediate phase shifts in the disc shedding rhythm of the rat retina. Arnold I. Goldman.

Albino rats were subjected to pinealectomy, superior cervical ganglionectomy, or the appropriate sham preparation and were placed in lighting conditions so that light onset was advanced by 10 hr. After 6 days of this regimen, all animals exhibited a complete shift in their outer segment disc shedding rhythm, indicating that the pineal gland is not a factor in mediating such a shift. (Invest Ophthalmol Vis Sci 22:111-113, 1982.)

When vertebrates are maintained in conditions of carefully controlled cyclic lighting, a characteristic burst of rod outer segment disc shedding and phagocytosis is generally observed between 1 and 2 hr of light onset.1-4 Because this diurnal rhythm resembles the rhythm of melatonin production,5 studies were conducted to determine whether the pineal gland, the major source of circulating melatonin,6 was responsible for maintaining this rhythm. In the frog, which has a light-mediated rhythm resembling the rhythm of melatonin production,7, 8 no effect on either light-induced or light-inhibited shedding was noted after pinealectomy.9 In rats receiving either pinealectomy (PX) or superior cervical ganglionectomy (SCG), the shedding rhythm persisted in animals kept in cyclic light10 and in those examined after 1 week of cyclic light and 24 hr of darkness.11

Although the shedding rhythm was inferred to be totally independent of the pineal gland by these studies, recent experiments have raised the possibility of certain types of dependence. Rats in which the optic nerves were severed intracranially maintained their shedding peak for up to 2 weeks after surgery but were unable to reset the rhythm when the light cycle was advanced,4 implying that shifts in the shedding rhythm required some form of central nervous system input via the optic nerves. Therefore the possibility existed that the pineal gland might be involved in this resetting of the shedding rhythm. The experiments in this report were designed to answer this question.

Materials and methods. Weanling Sprague-Dawley rats were subjected to PX, SCG, or the equivalent sham operations at Zivic Miller Laboratories, Allison Park, Pa.: the effectiveness of the surgery was judged as previously documented.10, 11 The rats were raised in a lighting schedule of 6AM-6PM, L:D (central daylight time) at Zivic Miller, held for 1 week after surgery before shipment to the Medical College of Wisconsin, and maintained on the same schedule for an additional week after shipment. At that time, animals were exposed to lighting that was advanced by 10 hr (8PM:5AM) according to the following regimen: The rats were placed in ventilated chambers in which maximum light levels were 20 ft.-cd. On the first day the lights were extinguished at 6 P.M. and turned back on at 8 P.M. On days 2 to 6 the lights were turned off at 8 A.M. and on at 8 P.M. On day 7 the lights were turned off at 8 A.M. and remained off until sacrifice, either at 9:30 P.M. or at 7:30 A.M. the next morning.

Four to five animals were used for each experimental point. They were sacrificed by overdose of sodium pentobarbital, the eyes were fixed by immersion in 1% formaldehyde, 1% glutaraldehyde, and were processed for light and electron microscopy.12 Phagosomes, defined as densely staining bodies at least one outer segment diameter, were counted and analyzed as previously described.3 Data were processed by an Apple II computer, equipped with software developed by the author.

Measurements of shedding in unshifted animals at 7:30 A.M. and 9:30 P.M. were not made in this study, since other studies in my present laboratory13 have produced shedding patterns identical to those previously reported.3-4

Results. The results of the experiments are summarized in Fig. 1. All animals observed at 9:30 P.M. displayed a burst of outer segment disc shedding and phagocytosis, indicating that a 10 hr phase advance in the shedding rhythm had occurred. This conclusion was further strengthened by observations of animals sacrificed at 7:30 A.M., the time of the original unshifted shedding peak. These animals displayed baseline levels of shedding regardless of treatment.

The extent of the baseline shedding at 7:30 A.M. was statistically indistinguishable between treatments (either by Student's t test or one-way analysis of variance), but in animals observed at 9:30 P.M. there was a significant difference between those receiving SCG and all other treatments at that time (p = 0.01 by analysis of variance). Nevertheless, the 9:30 P.M. shedding in the SCG animals was significantly higher than that in the SCG animals observed at 7:30 A.M. (p = 0.02 by
Fig. 1. Effect of PX and SCG on 10 hr phase advance. Animals raised on 6AM-6PM L:D cycle received PX or SCG, and were placed in 8PM-8AM lighting for 6 days. O, Operated animals; S, sham-operated animals. Each point represents four to five animals, and the error bars represent the S.E.M. Animals were sacrificed at 9:30 P.M. or 7:30 A.M. ALL. All animals regardless of treatment group except to time of sacrifice.

Student's t test), suggesting that at least a partial shift had been achieved.

When all animals sacrificed at 9:30 P.M. are compared with all animals sacrificed at 7:30 A.M., the difference in shedding is found to be highly significant (p < 10^-6 by Student's t test).

Discussion. These results extend and confirm the results of Tamai et al.14 and LaVail and Ward15 in which the pineal gland was found not to influence the rhythm of outer segment disc shedding in the rat. In those studies, it was demonstrated that the shedding rhythm is maintained for 2 to 3 weeks after PX or SCG. The present study demonstrates that the shedding rhythm will respond to a change in the lighting cycle even 3 weeks after either of these procedures.

It is interesting to note that the 9:30 P.M. peak was diminished in SCG animals, although this level was still significantly above the 7:30 A.M. baseline. No such change was noted in earlier studies, indicating that this procedure does not interfere with the shedding but might tend to delay the shift to a new rhythm by a slight degree. Since SCG cuts the innervation to the pineal gland without removing the gland itself, it is possible that such denervation might result in high serum levels of melatonin, which could delay the shift in the shedding rhythm.

When LaVail1 gave rats intraperitoneal injections of reserpine, the morning shedding burst was inhibited. Since reserpine depletes norepinephrine in the afferent terminals of the pineal, he reasoned that the pineal gland might be involved with the maintenance of this rhythm. Recent experiments, however, have shown that when reserpine is administered to a single eye by intravitreal injection, the injected eye shows baseline levels of shedding 1 1/2 hr after light onset, whereas the control eye exhibits a full shedding burst.14 This means that the reserpine effect in LaVail's experiment was most likely confined to the eye and was not linked with the pineal gland.

Of course, ruling out the pineal gland in no way leads us to the pathway that controls the re-entrainment of the shedding rhythm. To date, no published studies have attempted to link the rhythm of disc shedding to the suprachiasmatic nucleus of the hypothalamus, even though this organ functions as a biological clock16 with respect to several other circadian rhythms.

It is now known that the optic nerve in the rat contains retinal efferents from the pretectal area.15 It remains to be demonstrated whether the signals that re-entrain the shedding rhythm use these efferents or travel along another pathway. Although it is possible that other humoral factors may mediate this entrainment, removal of the pineal and superior cervical ganglion from consideration lessens the likelihood of a non-neural pathway.

Special thanks are given to Ms. Theresa Davis for her technical assistance and to Ms. Kathleen Wichman for typing of the manuscript.

From the Departments of Ophthalmology and Anatomy, The Medical College of Wisconsin, Milwaukee. Supported by National Eye Institute grants EY02975 and EY01931, an unrestricted grant from Research to Prevent Blindness, Inc., and Institutional PHS-BRSG award No. 402. Submitted for publication June 29, 1981.

Reprint requests: Arnold I. Goldman, Ph.D., The Eye Institute, Room 818, Medical College of Wisconsin, 8700 West Wisconsin Ave., Milwaukee, Wisc. 53226.

Key words: disc shedding, phagocytosis, circadian rhythm, pinealectomy, superior cervical ganglioneuromy, phase shift, rat

REFERENCES
ATPase activities of human retina and pigment epithelium—choroid. BARRY S. WINKLER AND JAMES M. HOPKINS.

The Mg2+, the Na+·K+, and the HCO3−-stimulated ATPase activities have been measured in the retina and pigment epithelium of postmortem human donor eyes. Activities were measured between 24 and 72 hr after death, following storage of the eyes in moistened chambers kept in a refrigerator. There appeared to be no significant differences in the ATPase activities between the 24 and 72 hr tissues. The human retina demonstrated both Mg2+- and Na+·K+-stimulated ATPase activity, but not HCO3−-stimulated ATPase activity. The Na+·K+ ATPase activity was 1.54 μmol Pi/hr/mg protein, which amounted to 37% of the total ATP hydrolysis of the retina. The pigment epithelium—choroid showed significant Mg2+-, Na+·K+-, and HCO3−-stimulated ATPase activities, with the stimulation caused by HCO3− about 25% greater than the effect due to Na+·K+, 0.64 vs. 0.51 μmol Pi/hr/mg protein. These findings in human retina and pigment epithelium—choroid show that certain metabolic processes continue to operate for several days after death. (INVEST OPHTHALMOL. VIS SCI 22:113–115, 1982.)

In all tissues, active ion transport processes play an essential role in maintaining the intracellular concentrations of specific ions at functionally required levels. Active ion fluxes are energy dependent and are presumably mediated either directly or indirectly by enzymes, ATPases, which hydrolyze ATP. The retina and pigment epithelium from a variety of vertebrates have been reported to contain several ATPases whose activities are stimulated by Mg2+, Na+ and K+, HCO3− (or other anions), and Ca2+. Nothing is known, however, concerning these ATPase activities in the human retina and pigment epithelium. In the present communication, we report on the activities of the Mg2+, Na+·K+, and HCO3− ATPases in these human tissues obtained postmortem.

Materials and methods. Human eyes, used as donors for corneal transplants, were obtained from the Michigan Eye Bank, Ann Arbor. The eyes were stored in moistened chambers kept in a refrigerator and were dissected within 72 hr after death. Donor ages ranged from 5 to 67 years. Although the causes of death varied, none of the donors had a past history of ocular disease. The retina and pigment epithelium—choroid appeared normal on histologic examination.

Each human neural retina, isolated from other ocular tissues by procedures similar to those used previously for the rabbit retina,4 was homogenized in 10 ml of H2O, average protein concentration was 1.07 mg/ml. The pigment epithelium—choroid was separated from the sclera and homogenized in 3.5 ml of H2O, yielding an average protein concentration of 1.48 mg/ml. ATPase activity was measured by determining the phosphate liberated from ATP according to the method of Fiske and Subbarow,8 as modified by Winkler and Riley.2 ATPase activity of a 0.1 ml portion of homogenate was measured in a final volume of 0.5 ml containing 2 mM ATP, 2 mM MgSO4, 5 mM KCl, 130 mM NaCl, 30 mM Tris, and 0.3 mM EDTA (final concentrations). It has been reported that the activity of the Na+·K+ ATPase of the bullfrog pigment epithelium is highest with imidazole as the buffer and that it is inhibited by Tris. However, we find that the Na+·K+ ATPase activity of the rat retina is not significantly different in the presence of Tris or imidazole (25 mM, pH = 7.4). For this