

The Influence of Light on Cone Disk Shedding in the Lizard, *Sceloporus occidentalis*

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ABSTRACT The lizard, *Sceloporus occidentalis* has an all-cone retina. In lizards maintained on a 12-h light:12-h dark (12L:12D) cycle, a burst of cone outer segment (COS) shedding occurs 2 h after light offset (1400 h circadian time) (Young, R. W., 1977, *J. Ultrastruct. Res.* 61:172-72). In this investigation, we studied the effect of different lighting regimes on the pattern of cone disk shedding in this species. When lizards entrained to a 12L:12D cycle are kept in constant darkness (DD), the shedding peak is advanced ~2 h and the magnitude of shedding is reduced to 30% of control. COS increased in mean length from 12 μm in controls to 14 μm after one cycle in DD and maintained this length during a second cycle in DD. In constant light (LL), disk shedding was damped to ~10% of control values. Shedding synchrony in LL was also perturbed and therefore cyclic shedding bursts could not be distinguished. During LL there was a much larger increase in COS mean length than in DD. After one cycle of LL, COS length was 15 μm and after two cycles COS length exceeded 17 μm . When lizards entrained to 12L:12D are shifted to a 6L:18D regimen, the first shedding cycle is biphasic. The first peak of 5% shedding occurs 2 h after light offset whereas a second larger peak (13%) occurs according to the entrained schedule (1400 h). This manipulation separates out a dark-triggered and circadian shedding component, which is normally superimposed in lizards entrained to a 12L:12D cycle. When entrained lizards are placed in 36 h of LL followed by light offset, the peak shedding response after light offset is double the control response (53% vs. 27%). After 30 h of LL (lights off 90° out of phase), there is a biphasic shedding response similar to the 6L:18D regimen although this time the dark-triggered shedding component is greater in magnitude than the circadian component.

COS turnover is estimated by extrapolating from COS mean length increases during LL. From this method we obtained a 2.7- μm increase in COS length during each day in LL. If COS growth is not augmented during LL, this would yield a 4-5-d turnover time for the average 12.5- μm COS.

Both cone and rod outer segments (COSs and ROSs)¹ periodically shed their most apical disks, which are then phagocytosed by the adjacent retinal pigment epithelium (RPE) (1). Both types of photoreceptors have been shown in several studies to shed their disks on a daily cycle (2-6, manuscript submitted for publication). In all species examined thus far however, rod shedding commences shortly after the onset of light reaching a maximum within 1 h or so (3-10). Cone shedding, however, has been shown to occur maximally early in the dark period in lizard (2), chicken (3), and goldfish (4),

¹ Abbreviations used in this paper: COS, cone outer segment; DD, constant dark; LL, constant light; ROS, rod outer segment; RPE, retinal pigment epithelium.

in the late dark period in the Eastern grey squirrel (5), and shortly after light onset in the cat (6) and tree shrew (manuscript submitted for publication). Thus, there are apparent differences in the way ROS and COS shedding relate to the daily lighting cycle.

In some species rod disk shedding is under circadian control, whereas in others it is triggered by light onset. In the rat (9, 11), mouse (12), and squirrel (13), shedding is apparently controlled by a circadian oscillator inasmuch as the cyclic shedding response persists under conditions of constant darkness. Shedding in *Rana pipiens* is triggered only by light onset (10, 14) whereas in *Xenopus laevis* it appears to have both a circadian and light-triggered component (15, 16).

We report here the effect of light and dark on cone disk shedding in the lizard, *Sceloporus occidentalis*. This species has an all-cone retina and previously has been shown to have a peak of disk shedding ~2 h after the beginning of darkness (2). There has been only one previous report on the effect of light on cone disk shedding. In the grey squirrel, cone shedding persists in darkness suggesting that it is circadian (5).

MATERIALS AND METHODS

Lizards, (*Sceloporus occidentalis*), maintained at ~25° C were entrained to a 12-h light:12 h dark cycle for at least 1 mo. For each experimental manipulation animals were maintained in a glass-bottomed aquarium to ensure proper exposure to the light cycles. Lighting was provided by a 100-W incandescent bulb ~20 cm from the aquarium top. This lighting condition is the same as was described by Young (2) in his study of disk shedding in *Sceloporus*. Animals were killed at various times during the light cycle or placed in different lighting regimes and then killed. During dark periods this procedure was carried out under illumination from a safelight fitted with a Kodak No. 2 red filter (Eastman Kodak Co., Rochester, NY) and a 15-W bulb at a distance of ~2 ft. Exposure to the safelight was brief inasmuch as time from sacrifice to time of immersion in fixative took ~30 s.

The eyes were enucleated, slit open at the equator with a razor blade, and fixed in 1% glutaraldehyde, 1% formaldehyde in a 0.086 M phosphate buffer (pH 7.2). After 15 min the anterior structures of the eye were removed and the eyecups were fixed for 4 h more. Eyecups were then rinsed in buffer and postfixed in 2% osmium tetroxide in the same phosphate buffer for 2 h, dehydrated in a graded series of ethanol, and embedded in Araldite 6005.

Phagosome Counts: Counts of phagosomes from different animals were done by light and electron microscopy.

For electron microscopy, ultrathin sections were cut on a Sorvall MT-2B microtome (DuPont Instruments, Sorvall Biomedical Div., Newtown, CT), and sections were placed on Formvar-coated slot grids. Only 1 of every 50 consecutive sections was counted to ensure that a phagosome would be counted only once. Grids were stained with 1% uranyl acetate and Reynolds lead citrate and examined with a JEOL 100U electron microscope (JEOL USA, Electron Optics Div., Peabody, MA). All phagosomes above well-aligned outer segments, whether fresh in appearance or showing signs of degradation, were counted.

Counts with the light microscope were made on 0.5- μ m-thick sections stained with *p*-phenylenediamine and toluidine blue with every fifth section counted. All deep-staining profiles in the RPE above well-aligned outer segments were counted as phagosomes. To ensure that these profiles were actually phagosomes, samples from three lizards were counted with both light and electron microscopy. In all three cases there was <3% variation in counts tabulated between the two techniques.

Phagosomes were tabulated as the number of phagosomes per 100 well-aligned COSs (expressed as percent). Between 400 and 500 COSs were counted for each animal.

Length Measurements: COS length measurements were done with a Zeiss Universal light microscope (Carl Zeiss, Inc., New York) using a \times 100 objective and an optical reticule. An average of 40 measurements were made per each sampling time.

All measurements were made in the mid-periphery of the retina.

RESULTS

The RPE-COS Interface

The RPE is a slender monolayer of cells into which the COSs project (Fig. 1). The average RPE cell is 12 μ m long and only 3–4 μ m in width excluding its apical processes. Within the RPE, the dominant structures are mitochondria, melanosomes, and myeloid bodies. Myeloid bodies are often round or convex in shape and composed of membranes similar to smooth endoplasmic reticulum. Melanosomes are found mainly in the apical portion of the RPE and mitochondria are most numerous in the mid and basal regions of the RPE. These structures have been described in greater detail for this species by Young (2).

Phagosomes, when observed in the electron microscope, were found in all states of degradation in the RPE—from those still retaining a lamellar appearance and presumably

recently shed to those having a more disorganized and compact appearance indicative of later stages of digestion (Fig. 2). In the light microscope, newly shed phagosomes usually appear rectangular whereas older phagosomes are rounded in appearance (Fig. 1). Some phagosomes seem to be complex in structure and appear to contain material of autophagic origin (perhaps from myeloid bodies) as well as outer segment disks (Fig. 3).

Shedding of Disks

12 h LIGHT:12 h DARK: As previously reported by Young (2) for this species, COS shedding is confined to the dark period. During the entire light period, the RPE is completely devoid of phagosomes (Fig. 4). At light offset, the shedding process begins and after 1 h 12% of all COSs have fresh phagosomes above their tips (Fig. 5, solid line). By 2 h after light offset, shedding reaches its maximum of 27%. Throughout the remainder of the dark period, shedding levels decline, reaching zero shortly after light onset.²

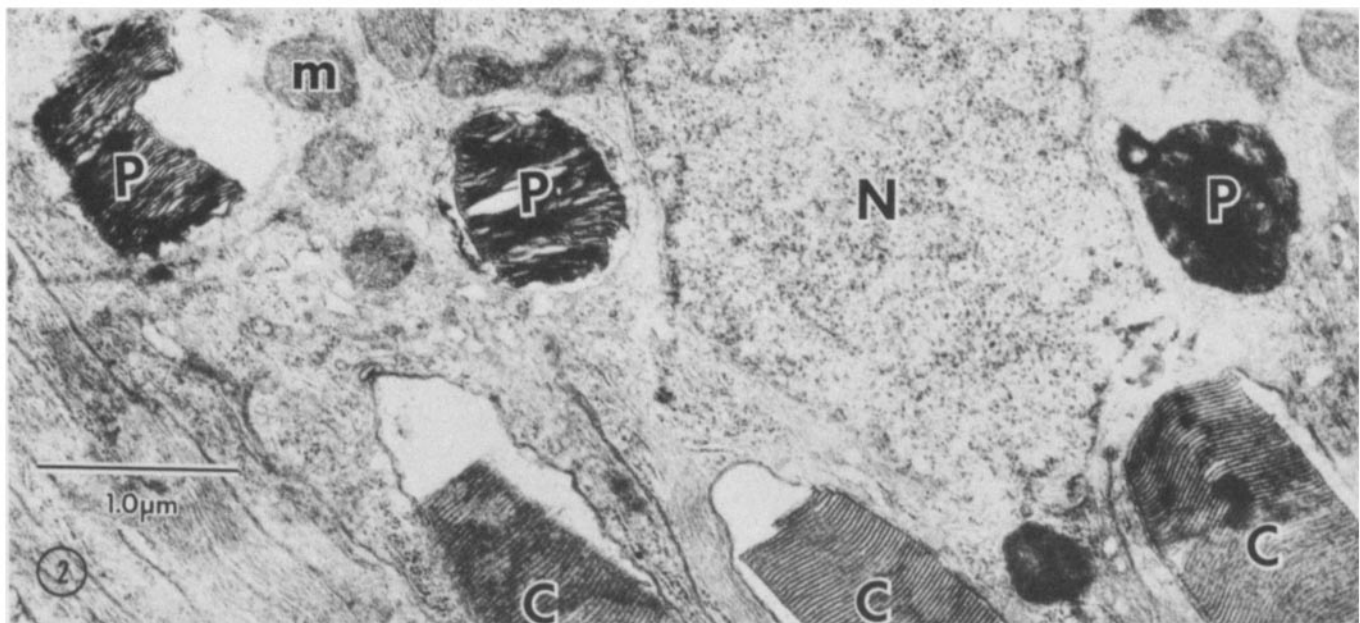
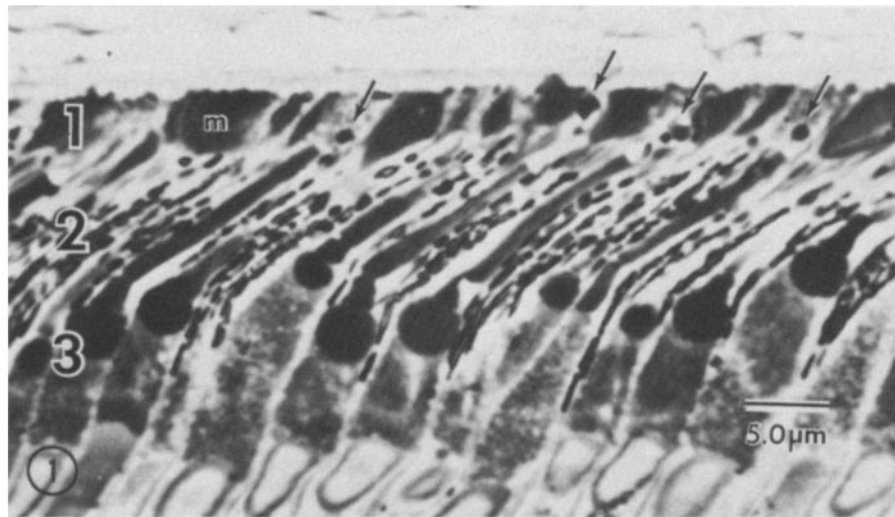
During the early dark period (1200–1400 h), more phagosomes with a lamellar structure are present (Fig. 5, solid line). After 3 h in the dark (1500 h), however, the condensed type becomes more prevalent and remains more numerous throughout the rest of the dark period (Fig. 5, dotted line). Fresh phagosomes are observed as late as 2 h before the onset of light.

CONSTANT DARK (DD): For this experiment, lizards were killed at regular intervals during a period of 60 h in DD. Inasmuch as the lights were turned off at the normal time for the entrainment cycle, the first 12 h of DD represents the normal dark period for the animal (Fig. 6). The later two peaks of shedding occur with no prior light period and are reached shortly after the subjective times of light offset. Both are slightly displaced with the first peak occurring at 16 h and the second beginning at ~8 h and reaching a peak at 15 h. The maximum amplitude of the two DD shedding events reached only 35% of the 12-h light:12-h dark (12L:12D) peak. The total area beneath each DD shedding curve was also 35% of that beneath the curve for the 12L:12D shedding response. During DD, phagosomes were occasionally found within the RPE during the subjective light period, although never at a level greater than 3.0%. A Poisson analysis used to compare the shedding responses in DD to a random distribution yielded results that suggests shedding is nonrandom and a discontinuous event ($P < 0.001$).

Fig. 7 shows the change in mean outer segment length during the two cycles of DD. COS mean length changed during the two cycles in DD. During the first 24 h, COSs increased their average length by 2 μ m. However, during the second 24-h period, no further increase in COS lengths was detected. The length change during the first 24-h period is statistically significant when evaluated by a Student's *t*-test ($P < 0.01$). The length variation between the first and second 24-h period of DD is not significant.

Between 2000 and 2400 h of the first DD cycle a greater number of lysosomes (defined as a membrane-bound, electron-dense inclusion of amorphous composition) was observed in the RPE than occurs in animals on a 12L:12D cycle.

² Time in this paper is expressed as circadian time, which is measured in hours after light onset (0000 h). Thus, 1200 = 12 h after light onset and marks the beginning of darkness in the 12L:12D entrainment cycle.



FIGURES 1 and 2 Fig. 1: Light micrograph showing typical photoreceptor. RPE interface in the mid-periphery of the retina. Animal was killed 2 h after light offset (1400 h). Arrows point to phagosomes in the RPE; the phagosome at middle left still retains its rectangular shape while the phagosome at far right is rounded. 1, retinal pigment epithelial layer; 2, COS layer; 3, cone inner segment layer; *m*, myeloid body $\times 2,000$. Fig. 2: Electron micrograph showing three phagosomes in various states of degradation. The phagosome at left still retains its lamellar appearance while the phagosome in the middle shows lamellae that are disorganized. The phagosome at far right shows compression and lamellae can no longer be recognized. C, COS; P, phagosomes; N, nucleus; *m*, mitochondria. $\times 26,000$.

When tabulated in the same manner as phagosomes, lysosome counts reached a peak of 30 per 100 COS at 2200 h of the first DD cycle. These lysosomes were often found adjacent to melanosomes and myeloid bodies but were never observed fused to phagosomes (Fig. 8). By the beginning of the subjective light period (0000 h of first DD cycle) all lysosomes had disappeared from the RPE. This is striking because lysosome numbers of this magnitude were not detected in the second cycle of constant dark or in the 12L:12D regime.

CONSTANT LIGHT (LL): For this experiment (Fig. 9) the lights were kept on for a period of 44 h from the time of light onset in the entrainment cycle; lizards were killed during the two periods of subjective dark and the second period of subjective light encompassed by this period. During the first and second subjective dark period (12–24 h of cycles 2 and 3), the shedding process was severely damped. No phagosomes

were observed in several of the animals killed when shedding is normally high. Different animals killed at single time points yielded varied results. In some animals phagosome counts were comparable to those in lizards kept in DD whereas in others no phagosomes were identified in the RPE. Phagosomes were found in two animals killed during the subjective light period (0 and 4 h of cycle 3) whereas none were found in two others (8 and 10 h of cycle 3). Shedding for the entire LL period did not differ significantly from random ($0.25 > P > 0.50$) when evaluated by Poisson analysis. The total phagosome quantity for cycles 2 and 3 declined to only 5–10% of the 12L:12D regime.

In that LL diminished the shedding response, outer segment mean length was measured for one animal at each experimental time point. Outer segment mean length increased from 12.5 μm in 12L:12D animals to over 17 μm in animals



FIGURE 3 Electron micrograph of an inclusion found within the RPE. This inclusion contains both disk material (phagosomal) and material similar to myeloid body membrane (autosomal). *My*, myeloid body; *D*, outer segment disk material. $\times 36,000$.

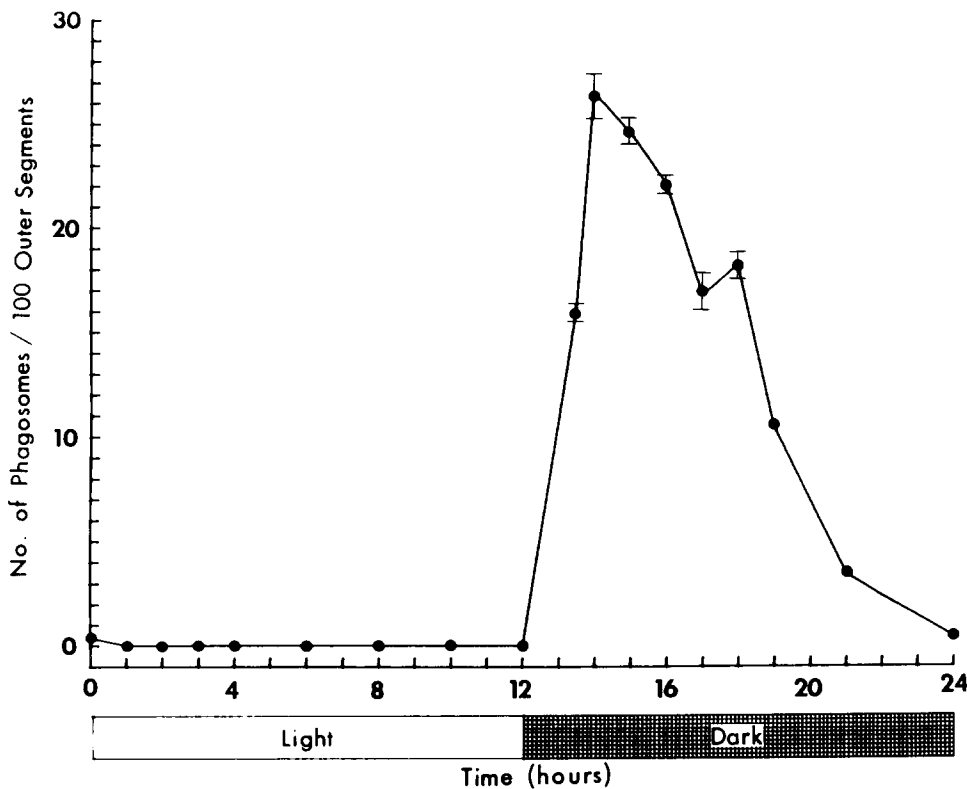


FIGURE 4 COS shedding during a 24-h period on 12L:12D. One animal was done per time point except for those with error bars. Time points with error bars are a mean of two animals with the bars indicating 1 SEM.

exposed to 36–44 hours of LL (Figs. 10–12). This change in mean length is statistically significant ($P < 0.001$) when evaluated by a Student's *t*-test.

SHORTENING THE LIGHT CYCLE: To determine whether the onset of darkness will trigger a shedding response in this all-cone system we shortened the light portion of one cycle to 6 h. Thus, light offset began at 0600 h for this experiment. At 2 h after light offset (0800 h), there was a small reproducible 5% shedding peak (Fig. 13, solid line).

This small peak was followed by a decline in phagosome number between 0900 h and 1000 h. At 1200 h (normal light offset time), phagosomes within the RPE started to increase again reaching a second larger peak of 13% at 1400 h (Fig. 5, solid line). The second shedding response has a similar time course to the 12L:12D control response (Fig. 13, dotted line) although the peak shedding magnitude is ~50% lower.

LL FOLLOWED BY LIGHT OFFSET: Because LL causes an increase in outer segment length while diminishing the

magnitude of shedding, the shedding response after light offset after an extended LL period, was examined. This experiment was done in two ways: by turning off the lights in phase with the entrainment cycle after 36 h of LL and by turning off the

light after 30 h of LL (90° off-phase from entrainment).

36-h GROUP: Shedding in the 36 h group (Fig. 14, solid line) showed a similar time course to that in the 12L:12D shedding response (Fig. 14, broken line). Phagosomes first

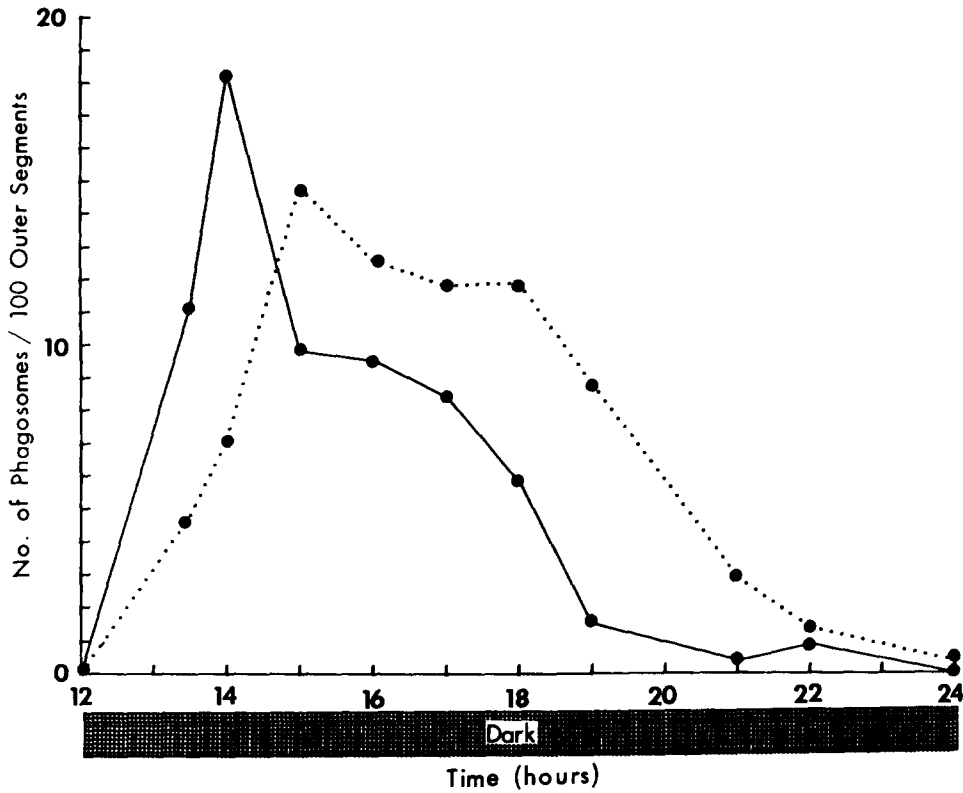


FIGURE 5 COS shedding during the dark portion of a 12L:12D cycle with phagosomes divided into two classes; lamellar (solid line) and condensed (broken line).

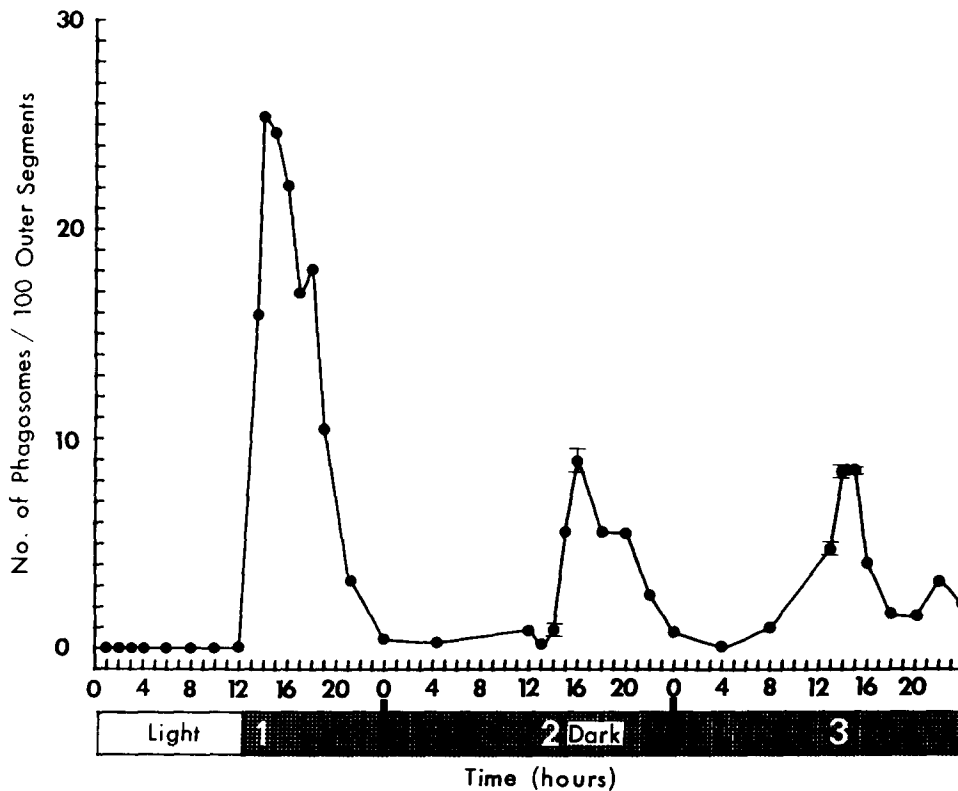


FIGURE 6 Effect of DD on shedding. Cycle 1 is data replotted from the dark portion of the 12L:12D cycle. Cycles 2 and 3 demonstrate shedding without a prior light cue. One animal was done per time point except where error bars are indicated. Time points with error bars are a mean of two animals.

appeared in the RPE shortly after light offset and reached a peak 2 h later (1400 h). The magnitude of the response was much greater than the 12L:12D maximum, reaching 53% at its peak.

30-h GROUP: Animals exposed to the darkness after a period of 30 h in constant light (Fig. 15) showed a biphasic shedding response similar to those in the 6L:18D light regime (Fig. 13, solid line). Shedding began shortly after lights-out reaching a peak of 33% after 1 h (0700 h) of darkness. By 2 h (0800 h), shedding was declining, and by 5 h (1100 h) no

phagosomes were found in the RPE. A second brief shedding peak of 23% was found at 3700 h which declined to lower levels (~5%) 1 h later where it remained for the rest of the experiment.

INCREASES IN COS MEAN LENGTH IN LL: Since COS mean length increases with time in LL (Fig. 10), outer segment lengths from all time points were measured and graphed against time. A graph of this data is shown in Fig. 16, a line was fit to the data by a linear regression and shows a length increase of 2.7 μm for each 24 h period in LL ($r = 0.92$).

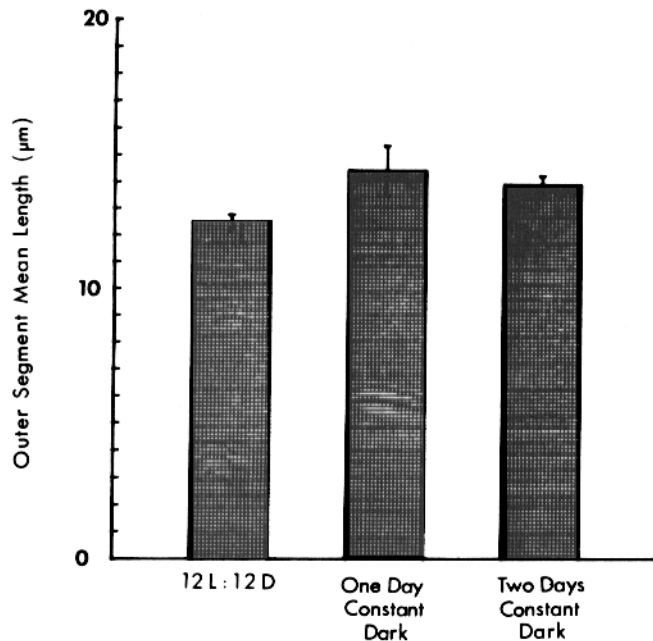


FIGURE 7 Increase in mean COS length during two cycles of DD. Each bar is an average of 12 animals with over 40 COS lengths measured from each. Error bars indicate 1 SEM.

DISCUSSION

Shedding during a 12L:12D Cycle

Our initial experiments confirm the findings of Young (2) that shedding in this species is confined to the dark portion of the daily cycle. There are essentially no phagosomes in the RPE during the light portion of the cycle. The peak of shedding that occurs consistently within 2 h of darkness and the lack of background shedding during the day make this a convenient species in which to study the effect of varying light conditions on cone disk shedding.

Circadian Shedding as Demonstrated in Constant Darkness

The ability of a physiological event to persist for a period of ~24 h while the organism is isolated from external cues is one indicator of circadian control (17). This criterion (termed "free running") has been met by ROS in rat (9, 11), mouse (12), and *Xenopus* (15) with data from tree squirrel (13), showing persistence for at least one cycle of DD. In all of these species the amplitude of shedding is reduced during DD. Cones in the lizard studied here also continue shedding disks cyclicly through two cycles of DD; the peak of shedding occurs daily but is delayed ~2 h and is substantially reduced in amplitude.

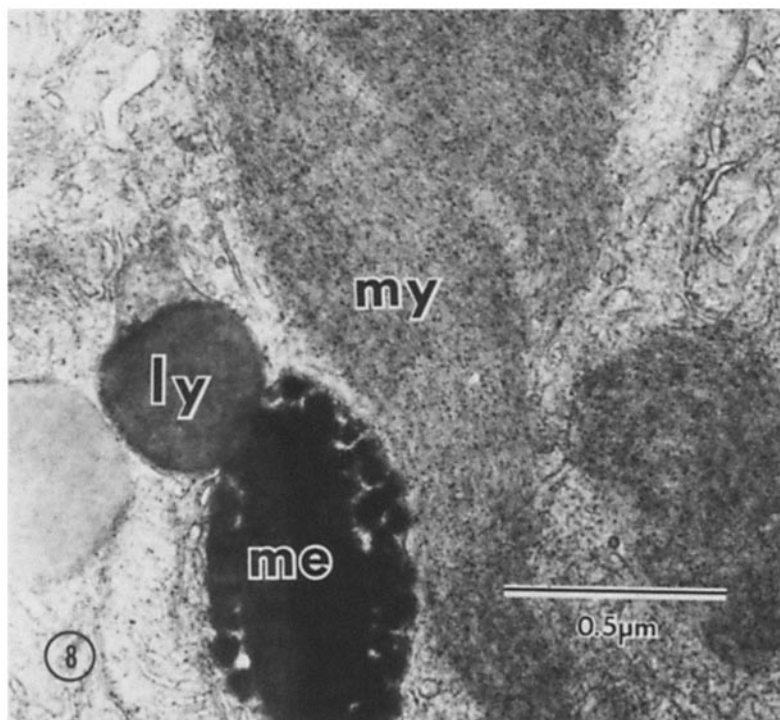
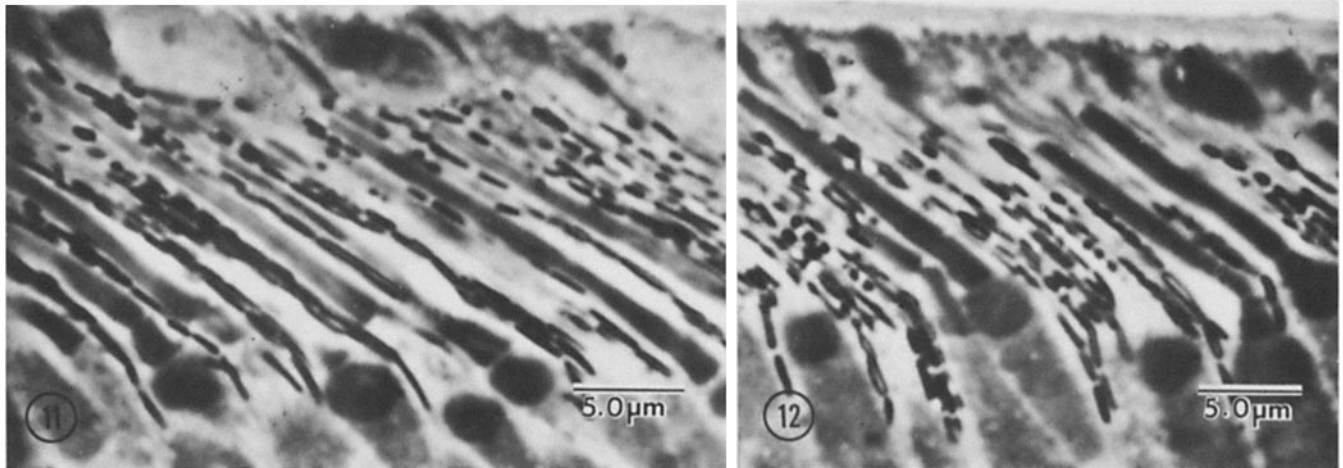


FIGURE 8 Electron micrograph of a lysosome found within the RPE. *my*, myeloid body; *me*, melanosome; *ly*, lysosome. $\times 60,000$.



FIGURES 11-12 Light micrographs demonstrating increases in COS length during LL. Fig. 11 shows COS length after a 36-h exposure to LL. Fig. 12 shows COS length during a 12L:12D cycle. Both examples were taken from the retinal mid-periphery. $\times 3,000$.

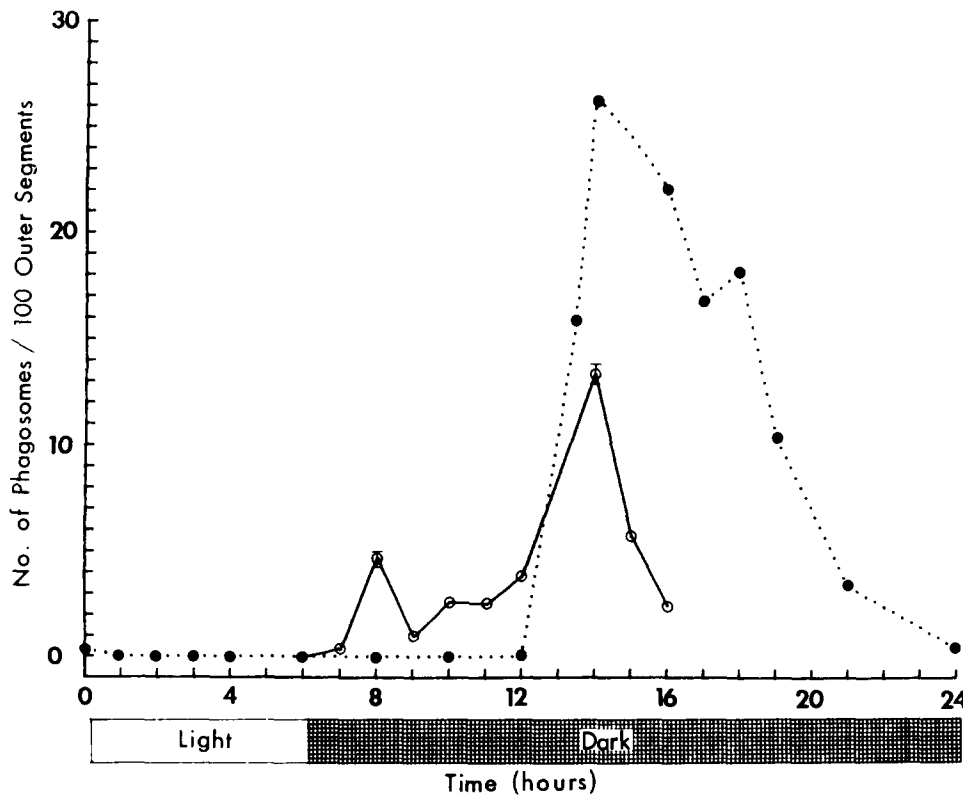


FIGURE 13 Immediate effects of shortening the light cycle to 6L:18D (solid line). The dotted line is the shedding response during the 12L:12D regimen for comparison. Data points with error bars are a mean of two animals.

kept in DD, an increase in ROS length of 2% per day has been reported (14). With *Rana* ROS addition rates during DD can be monitored directly by length increases in that shedding approaches zero in DD and is not a factor that influences ROS length.

During DD, disk addition rates vary from species to species. In *Xenopus* (15) and *Rana* (14), disk addition is reduced up to 50% of control values. In the Ozark cave salamander (19), disk addition is reduced by 17% and in pigmented mouse (12) by only 7%. Our results suggest that disk addition is near normal during the first day of DD but is considerably reduced during the second day of DD. In this experiment the COS increased in length by an average of 2 μm during the first day

of DD whereas during the second day no further increase was observed. In that the amount of shedding was at a similar level on both days of DD the growth rate would have to slow for outer segment length to remain the same. A growth of $\sim 2 \mu\text{m}/\text{day}$ for the first day in DD is probably near the normal level of COS growth. We estimate a growth rate of $\sim 2.7 \mu\text{m}/\text{day}$ from the LL data (see below) but average daily outer segment length increase will be less than this because of shedding. In *Xenopus* ROS there also is evidence that disk addition changes with time in DD. Besharse et al. (15) showed that disk addition to *Xenopus* ROS is reduced by 50% after 1 d of DD, but by day 3, no disk addition could be measured by band displacement in autoradiograms.

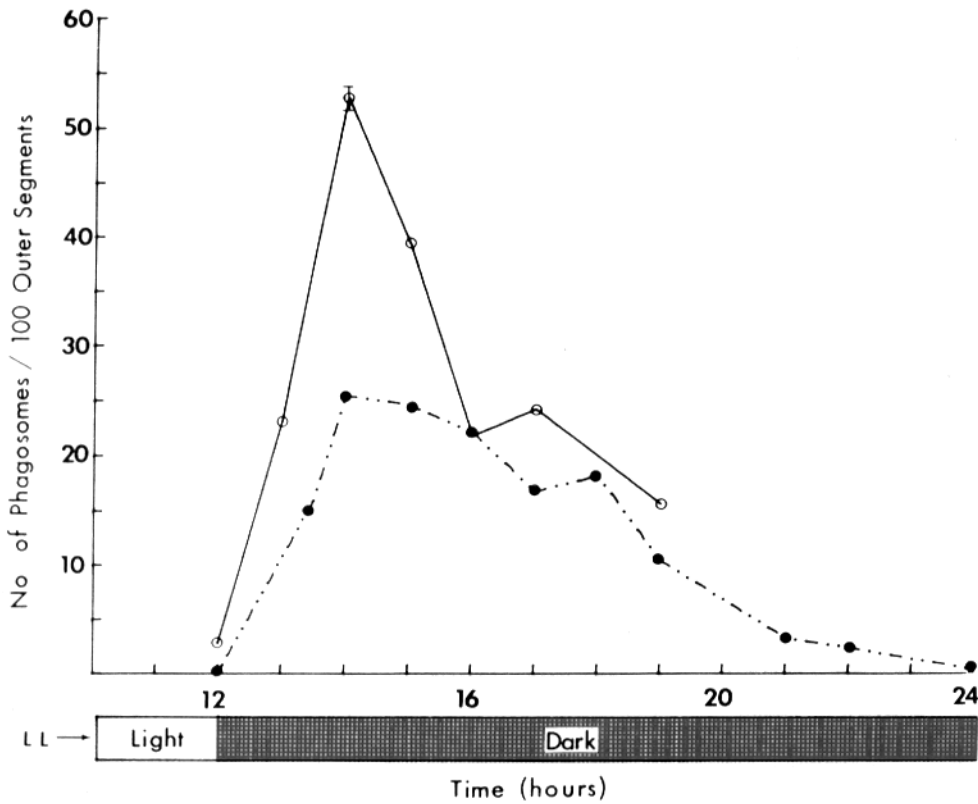


FIGURE 14 Shedding after 36 h of LL followed by light offset. Shedding during this period (solid line) has a similar time course to the 12L:12D shedding response (dashed line).

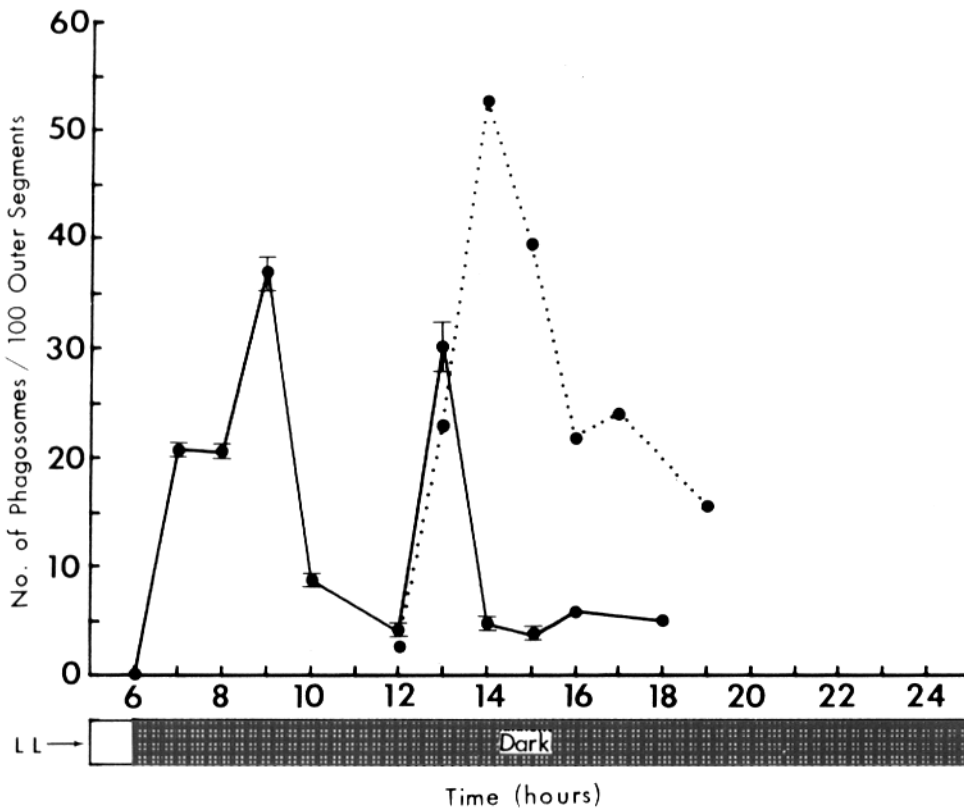


FIGURE 15 Shedding after 30 h of LL followed by light offset (solid line). The dotted line is a plot of the previous figure's data for comparison.

One can speculate that, because an animal may often encounter periods of prolonged darkness (i.e., cave dwelling), there is an adaptive process for maintaining photoreceptors under such conditions. LL on the other hand, is a condition

that never occurs in a temperate animal's environment and therefore there may be no mechanism for maintaining the photoreceptors in that environment. Thus, LL leads to abnormal increases in ROS length in *Xenopus laevis* (15, 20),

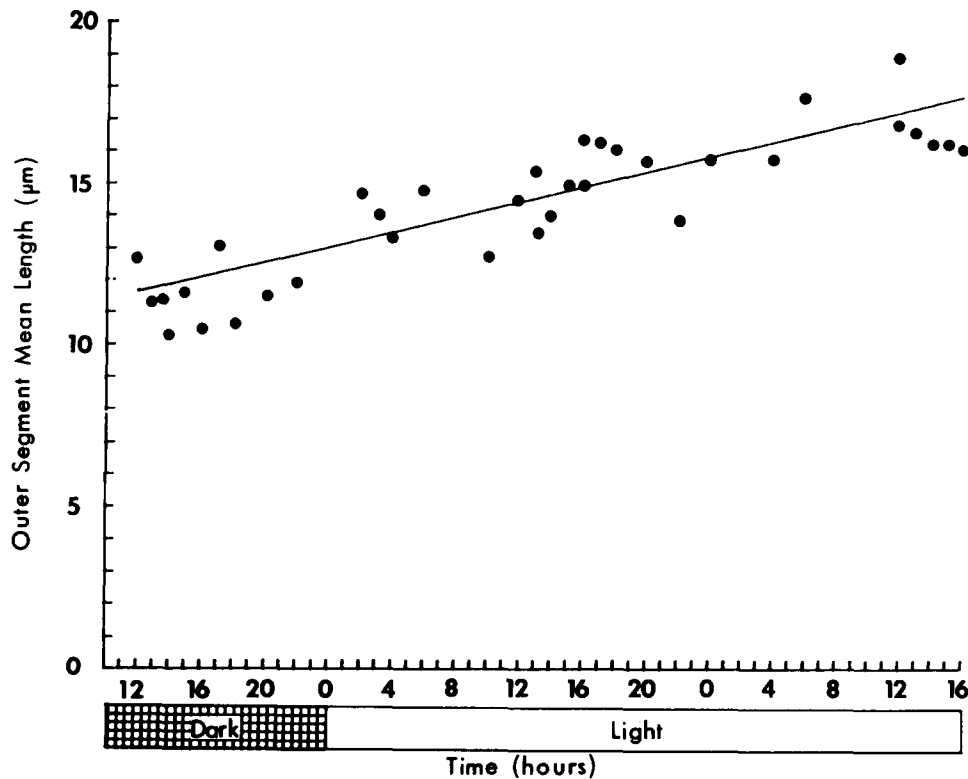


FIGURE 16 Increase of mean COS length over time in LL. Data points are a mean of 50 COS measurements. The line through the points is a linear regression of these points ($r = 0.092$).

Rana pipiens (14), and rat (9) and COS length in the lizard and has been shown to cause photoreceptor degeneration in several vertebrate species (21).

Dark-triggered Shedding

In mammals, ROS shedding is probably exclusively under endogenous (circadian) control—light activation only occurs under exceptional regimens (9). In amphibians, however, ROS shedding is apparently initiated by light onset (*Rana pipiens*) (10, 14) or a combination of light and an endogenous stimulus (*Xenopus laevis*) (15). The control of COS shedding in the lizard, *Sceloporus occidentalis*, may be analogous to the control for ROS shedding in *Xenopus* with the major difference that the external trigger appears to be dark onset in the lizard. The effect of both can be seen in the experiment where the day length was shortened to 6 h and the one in which the lizards were exposed to 30 h of light; in both experiments shedding occurred in two peaks—one about 2 h after light offset and another at the appropriate time for disk shedding in the entrainment cycle.

The biphasic response elicited by this light regime, demonstrates that the dark-triggered response and circadian response may act concurrently. Both signals may act simultaneously to stimulate shedding in lizards entrained to the 12L:12D regime. In lizards, dark-triggered shedding reaches its maximum 2 h after light offset whereas circadian shedding peaks at 1400 h. Because light offset is at 1200 h in a 12L:12D regime both shedding systems are superimposed. By shortening the light portion of the cycle, we have separated exogenous and circadian controls.

In the three vertebrate species studied by Young (2, 3) and O'Day and Young (4), cone disk shedding related temporally to the beginning of darkness. Our data shows that in one of these species, *Sceloporus*, the onset of darkness can actually

act as a trigger for cone disk shedding. There are no previous reports demonstrating that the offset of light acts as a stimulus in the renewal process. In the locust eye, however, the light to dark transition does apparently stimulate microvillar synthesis in the rhabdom (22).

Our evidence suggests that we are able to manipulate the relative effect of the two control mechanisms by altering the light cycle. When the day length was shortened to 6 h the first (dark-triggered) peak was smaller than the endogenous peak. When the day length was extended to 30 h (placing lights-off in phase with 6 h of light), the dark-triggered peak was much larger than the endogenous peak. Thus exposure to an extended light period abolished both the dark-triggered and circadian responses allowing the COSs to grow and perhaps the accumulation of some "priming signal" for the dark-triggered response, whereas the circadian response maintained the outer segments at $\sim 2 \mu\text{m}$ longer than those in the 12L:12D animals.

When the lizards were exposed to 36 h of light and then darkness (in phase with the 12L:12D cycle), there was a single very large shedding response that represents the additive dark-triggered and endogenous responses. The latter experiment also indicates that the extended light period did not reset the circadian oscillator and, thus, that the dampened, asynchronous shedding found in the LL animals represents perturbations of the link between the oscillator and the shedding mechanism, or disruption of the shedding process itself.

Methods for Estimating COS Renewal

In comparison to ROS, renewal or turnover time for COS is very difficult to estimate because the latter label diffusely with ^3H -amino acids (18). We can, however, estimate the daily growth of COS from our LL data. The linear regression of this data results in the average outer segment growth of

~2.7 μm per day. In that an average COS is ~12.5 μm long (in the mid-periphery), it would take 4–5 d to turn over an equivalent length of COS. This analysis will be inaccurate if LL augments the disk addition process. In ROS, however, disk addition during LL does not differ significantly from that in 12L:12D controls (14, 15).

A second method for determining COS membrane turnover time uses phagosome counts and the average phagosome size as a basis to estimate membrane addition. In this calculation, the area under the 12L:12D shedding curve is integrated using an estimate of degradation time for phagosome disappearance as the upper limit. A good estimate of phagosome degradation time is therefore essential. The degradation time we used initially was 1 h inasmuch as a similar analysis using cat rods was in agreement with turnover times already established by autoradiography (6).

By integration, the number of phagosomes shed each day is 138 for 100 lizard COSs. This suggests that some COSs shed more than once per day and that on an average each COS sheds two times every 3 d. Because the average size of each phagosome is ~1.2 μm and the average outer segment size is 12.5 μm , the amount of time it takes for membrane turnover is 7–8 d. This method of turnover estimation results in almost twice the turnover time as in the previous method.

If our estimate from the LL data is more accurate, then we must shorten the degradation time by 25% to 45 min to give a renewal time of 4–5 d. Unfortunately, there is no clear method to measure actual phagosome degradation rates. The best estimates come from the appearance and disappearance of the shedding peak using the response of several animals. The sharpest shedding peak we have encountered was during the 30L:18D light regime (Fig. 14) where there was a transient peak at 13 h with subsequent reductions to 4–5% by 14 h indicating that degradation time may in fact be <1 h.

Conclusions

In the past few years considerable progress has been made towards understanding the renewal process in COS. In the original autoradiographic studies, there was no evidence to suggest that cones undergo the familiar shedding–membrane production cycle described so elegantly in ROS (18). We now know, however, that not only do cones share this property with rods but that they shed disks according to a daily cycle (2–6; manuscript submitted for publication). In this study we have shown for the first time that rods and cones share even more properties: both not only shed disks cyclicly but can be under the control of lighting or endogenous cues and the endogenous control has properties of a circadian rhythm. The systems do differ, however, because, in all rod systems studied, shedding occurs primarily after light onset (3–10), whereas cones have been shown to shed maximally at light offset, later in the dark period, or at light onset depending on species (2–6; manuscript submitted for publication). Also, cones in the lizard can be stimulated to shed by light offset whereas rods

are stimulated to shed by light onset (15, 16). Whether or not this is a general property of those cone systems that shed during the night remain to be seen. It should be pointed out that *Sceloporus* possesses an all-cone retina and the control processes may be considerably different in duplex retinæ.

Estimates of COS renewal continue to be difficult to arrive at because there is no direct method of measurement. From our results, we believe the renewal time is most likely in the range of 4–5 d in *Sceloporus*. This is comparable with renewal times previously published for ROS of similar proportions (6).

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REFERENCES

- Anderson, D. H., and S. K. Fisher. 1976. The photoreceptors of diurnal squirrels: outer segment structure, disc shedding, and protein renewal. *J. Ultrastruct. Res.* 55:119–141.
- Young, R. W. 1977. The daily rhythm of shedding and degradation of cone outer segment membranes in the lizard retina. *J. Ultrastruct. Res.* 61:172–185.
- Young, R. W. 1978. The daily rhythm of shedding and degradation of rod and cone outer segment membranes in the chick retina. *Invest. Ophthalmol. Visual Sci.* 17:105–116.
- O'Day, W. T., and R. W. Young. 1978. Rhythmic daily shedding of outer segment membranes by visual cells in the goldfish. *J. Cell Biol.* 76:593–604.
- Tabor, G. A., S. K. Fisher, and D. H. Anderson. 1980. Rod and cone disc shedding in light-entrained tree squirrels. *Exp. Eye Res.* 30:545–557.
- Fisher, S. K., B. A. Pfeffer, and D. H. Anderson. 1983. Both rod and cone disc shedding are related to light onset in cat. *Invest. Ophthalmol. Visual Sci.* 24:844–856.
- LaVail, M. M. 1976. Rod outer segment disk shedding in rat retina: relationship to cyclic lighting. *Science (Wash. DC)*. 194:1071–1074.
- LaVail, M. M. 1976. Rod outer segment shedding in relation to cyclic lighting. *Exp. Eye Res.* 23:277–280.
- Goldman, A. I., P. S. Teirstein, and P. J. O'Brien. 1980. The role of ambient lighting in circadian disc shedding in the rod outer segment of the rat retina. *Invest. Ophthalmol. Visual Sci.* 19:1257–1267.
- Basinger, S. F., R. T. Hoffman, and M. Matthes. 1976. Photoreceptor shedding is initiated by light in the frog retina. *Science (Wash. DC)*. 194:1074–1076.
- LaVail, M. M. 1980. Circadian nature of rod outer segment disc shedding in rat. *Invest. Ophthalmol. Visual Sci.* 19:407–411.
- Besharse, J. C., and J. G. Hollyfield. 1979. Turnover of mouse photoreceptor outer segments in constant light and darkness. *Invest. Ophthalmol. Visual Sci.* 18:1019–1024.
- Tabor, G. A., D. H. Anderson, S. K. Fisher, and J. G. Hollyfield. 1982. Circadian rod and cone disc shedding in mammalian retina. In *The Structure of the Eye*, Vol. 4. J. G. Hollyfield, editor. Elsevier North-Holland, Amsterdam. 67–73.
- Basinger, S. F., R. T. Hoffman. 1982. Regulation of rod shedding in frog retina. In *The Structure of the Eye*, Vol. 4. J. G. Hollyfield, editor. Elsevier North-Holland, Amsterdam. 75–83.
- Besharse, J. C., J. G. Hollyfield, and M. E. Rayborn. 1977. Turnover of rod photoreceptor outer segments. II. Membrane addition and loss in relationship to light. *J. Cell Biol.* 75:507–527.
- Flannery, J. G., and S. K. Fisher. 1978. Light-triggered rod disc shedding in *Xenopus* retina in vitro. *Invest. Ophthalmol. Visual Sci.* 18:638–642.
- Pittendrigh, C. S. 1975. Circadian clocks: What are they? In *The Molecular Basis of Circadian Rhythms*. J. W. Hastings, and H. Schweiger, editors., Dahlem Konferenzen, Berlin. 11–48.
- Young, R. W. 1967. The renewal of photoreceptor cell outer segments. *J. Cell Biol.* 33:61–72.
- Besharse, J. C., and J. G. Hollyfield. 1976. Renewal of normal and degenerating photoreceptor outer segments in the Ozark cave salamander. *J. Exp. Zool.* 198:287–302.
- Currie, J., J. G. Hollyfield, and M. E. Rayborn. 1978. Rod outer segments elongate in constant light: darkness is required for normal shedding. *Vision Res.* 18:995–1003.
- Noell, W. K., V. S. Walker, B. S. Kang, and S. Berman. 1966. Retinal damage by light in rats. *Invest. Ophthalmol. Visual Sci.* 5:450–473.
- Williams, D. S. 1982. Ommatidial structure in relation to turnover of photoreceptor membrane in locust. *Cell Tissue Res.* 225:595–617.