Both Rod and Cone Disc Shedding are Related to Light Onset in the Cat

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Nineteen domestic cats were entrained to a 12-hr light/12-hr dark lighting cycle and killed at different times of the cycle. The numbers of rod and cone phagosomes in the retinal pigment epithelium (RPE) were quantified by light microscopy. The number of both rod and cone phagosomes shows a large increase within 2 hrs after light onset. Thereafter, the phagosome count remains low until the latter part of the dark period when the number of rod and cone phagosomes increases slightly. In a few cases, substantial variability in phagosome numbers was found between animals killed at the same times in the lighting cycle. Small (0.4–0.8 μm), dense, membrane-bound granules, that have the ultrastructural features of lysosomes, occur in large numbers in the tapetal RPE cells. The granules may contain only a homogeneous matrix or matrix plus membrane fragments or melanin. Variations in the number of such granules in different animals shows no clear diurnal pattern. Phagosome number, size, and length measurements of outer segments are used to provide estimates of cone outer segment turnover time in the cat. Invest Ophthalmol Vis Sci 24:844–856, 1983

In a wide variety of vertebrate species, rod outer segments shed discs in response to light onset or at the beginning of the light part of the daily light-dark cycle.[1-7] In some species cone disc shedding occurs predominantly at the beginning of the dark cycle,[5,8] while in others, it reaches a maximum somewhat later in the photoperiod.[4,6] It has been suggested that the temporal relationship between cone disc shedding and the onset of darkness is analogous to the link between rod disc shedding and the onset of light.[9]

We investigated the daily cycle of photoreceptor disc shedding in domestic cats entrained to a 12-hr light/12-hr dark cycle. Cats have a duplex retina dominated by rod photoreceptors, but with a moderate population of cones[10] that are distinguished easily from rods because they are shorter and do not reach the apical border of the RPE.[11,12] The cones are contacted by specialized sheet-like RPE processes that fill the space between the tips of the cones and the apical surface of the RPE.[11,12] Thus, disc packets shed by cones in this species are particularly easy to distinguish from those shed by rods, since they remain within the processes of the cone sheath for some time before reaching the RPE cell bodies.[12,13] In the cat retina, our results clearly show that both rods and cones shed discs predominately during the first 2 hr after light onset.

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Supported by National Eye Institute research grants EY-00888 and EY-02082 and NEI Research Career Development Award EY-00174 to SKF.

Submitted for publication July 8, 1982.

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Materials and Methods

Nineteen domestic cats were kept on a 12-hr light/12-hr dark cycle for 2 weeks to several months. Temperature and humidity were also kept constant. Room lighting (270 lux) was provided by overhead fluorescent lamps. Water and cat chow were available at all times. Animals were killed at the following times (0 hr = lights on, 12 hr = lights off): 0, 1 (two cats), 2 (two cats), 3, 6, 9, 13, 14, 18 (three cats), 19, 20, 21, 22 (two cats), 23 hr.

Fixation

Cats were anesthetized by an initial intramuscular dose of ketamine HCl (Bristol); once sedation occurred (within a few minutes) they were given a lethal dose of sodium pentobarbital (Nembutal®, Abbott). For animals fixed during the dark period, anesthesia was done with illumination from a flashlight equipped with a red-transmitting filter or a safelight equipped with a Wratten No. 25 filter. For the animals fixed at 13, 14, 18 (two of three cats), 19, 20, 22 hr (one of two cats), enucleation and dissection of the eyes were done under red light. For those cats killed at 18 (one of three cats), 21, 22 (one of two cats), and 23 hr, anesthesia was administered under red light and,

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when respiration had stopped, the lights were turned on and enucleation of the eyes had begun. In all cases, enucleation and dissection of the eyes took less than 10 min.

After enucleation, the cornea, lens, and as much of the vitreous as possible were dissected away, and the eye was immersed in a fixative composed of glutaraldehyde and formaldehyde (1% each) in 0.086 M sodium phosphate buffer (pH 7.2) with 20 mM $CaCl_2$ added.[14] They were kept in fixative for 12 to 24 hrs at 4 C and then washed several times in phosphate buffer with 4.5% sucrose and 20 mM $CaCl_2$ at pH 7.2. During the buffer wash, a notch was cut in the superior pole of the eye cup to allow orientation after osmication. The eyes were post-fixed in 2% osmium tetroxide (in veronal acetate buffer with 4.5% sucrose and 20 mM $CaCl_2$ at pH 7.1) for 2 to 2½ hr at 4 C, rinsed in distilled H_2O, and stained overnight in 0.5% aqueous uranyl acetate. After a brief wash in distilled H_2O, the tissue was dehydrated in a cold ethanol-H_2O series, passed through propylene oxide, and embedded in Araldite 6005® (Cargille). While in absolute ethanol, the eyecup was divided into superior and inferior halves and the superior half further divided into two quadrants with the more temporal portion including the area centralis. Only this quadrant was used in our study.

Microscopy

One micrometer thick sections were taken from the superior-temporal quadrant of one eye from each cat; the tapetal region just peripheral to the area centralis was used because in the area centralis itself, there is little or no space between cone outer segment tips and the apical border of the RPE (unpublished observations). One micrometer thick sections for phagosome counts were taken with a Sorvall MT2-B ultramicrotome, placed on glass slides, and stained either with a solution of 0.1% toluidine blue and 0.1% azure II in 0.5% aqueous sodium tetraborate or with saturated, aqueous p-phenylenediamine.

Thin sections (50–70 nm thick) were stained with uranyl acetate and lead citrate and examined by electron microscopy.

Quantitative Data

Phagosomes were identified by light microscopy on the basis of their size and staining characteristics (see results). Phagosome counts were made using a Zeiss Universal Research Microscope fitted with a 63× planapochromat objective at a total magnification of 787.5 times. A mean number of phagosomes/mm of RPE was obtained by counting a total of 20 to 40 mm of RPE in areas of well-aligned photoreceptors. This involved counting 10 to 20 sections for each specimen. The mean number of phagosomes/mm is derived from counts of each of several sections, and the standard error was used as a measure of variability between the different sections. Each section used for counting was separated from its nearest neighbor by at least 5 μm to avoid duplication of phagosome counts. Phagosomes within the cone sheaths were not counted in the "RPE phagosome" category but were counted separately as cone phagosomes. Because of the relatively low number of cones, the cone phagosomes were tabulated as number of phagosomes/100 well-aligned cone sheaths. Since phagosomes tend to stay in the center of the cone sheath as they ascend to the RPE, a 1-μm-thick section through a well-aligned cone outer segment and sheath will almost certainly reveal any phagosomes within that sheath. All slides were coded so that the two individuals doing counts did not know the time of fixation.

The diameter of RPE phagosomes was measured, using an optical reticule, for cats killed at 1, 2, and 6 hr after light onset and for phagosomes in the cone sheaths of cats killed at 1 and 2 hr after light onset. Small (0.4–0.8 μm diameter), homogeneous granules (distinct from phagosomes) were also counted in 1 mm of RPE for each animal.

Measurements of outer segment length from photomicrographs and of the areas under the curves constructed from phagosome frequency data were made using a Zeiss digital image processor (MOP-3).

Results

Cat Tapetal Retinal Pigment Epithelium

The RPE cells of the posterior tapetal region are irregularly shaped because large choroidal capillaries indent their basal surfaces (see Fig. 5A for example). In longitudinal sections, the RPE cell depth, as measured from the apical to basal surfaces, varies from 2 to 9 μm; their width from one lateral border to the next is between 14 and 17 μm when the section plane passes through the center of the nucleus.

Three major cytoplasmic inclusions can be recognized by light microscopy in these cells: phagosomes (phagocytosed packets of discs shed from underlying photoreceptors), melanin granules, and a large population of homogeneously staining dense granules measuring 0.4 to 0.8 μm in diameter. Mature melanin granules are found in all of our samples, but their frequency is low as would be expected from retina overlying a tapetum. They are distinguished easily from phagosomes by their staining properties, size, and shape.

Ultrastructurally, the most prominent features of these cells are a centrally located nucleus, large quan-

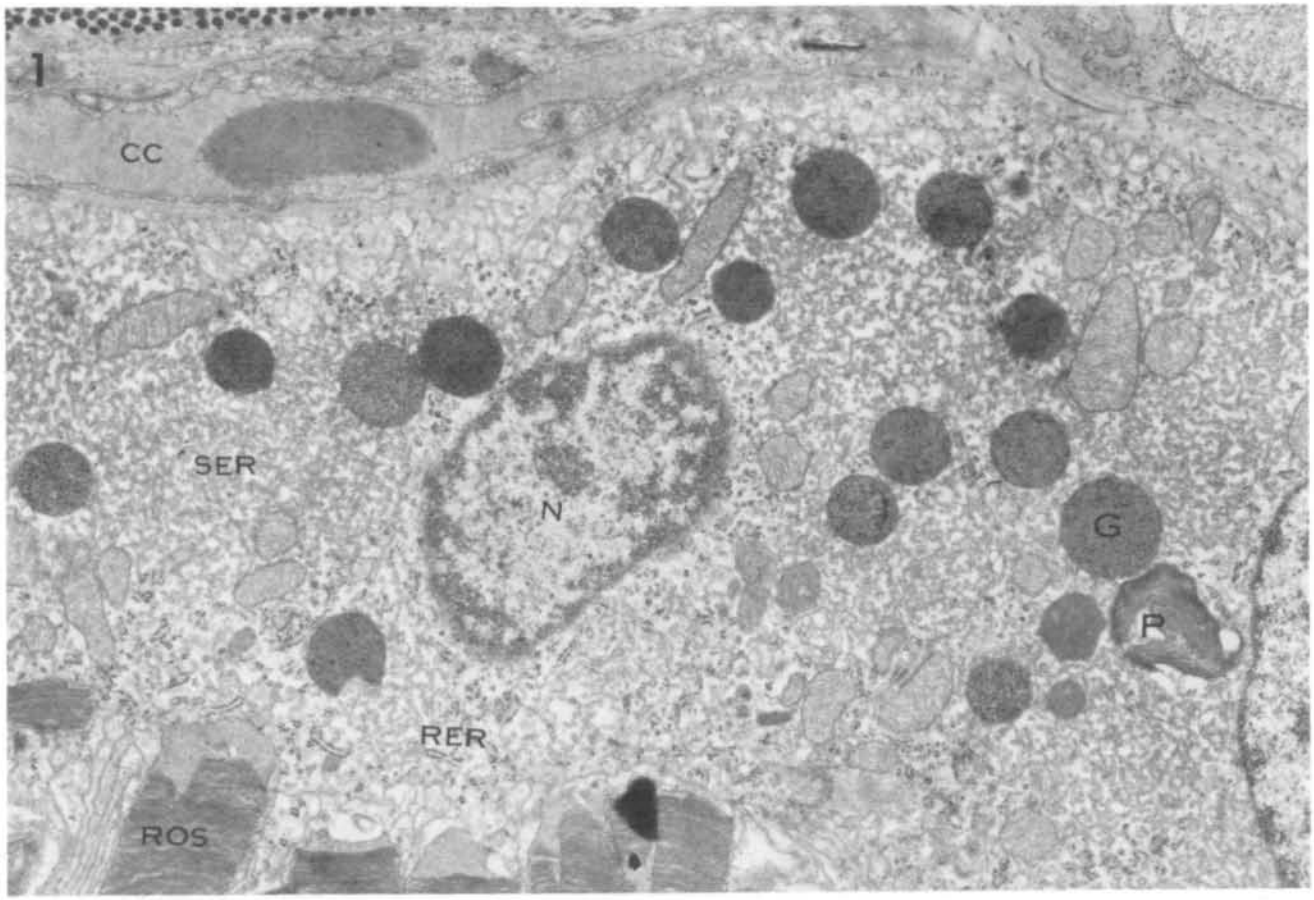

Fig. 1. An electron micrograph of an RPE cell from the tapetal region of the cat's retina. A choroidal capillary (CC) lies adjacent to the basal infoldings of the cell. The most prominent organelles are the centrally located nucleus (N), the dense granules (G), and extensive tubules of smooth endoplasmic reticulum (SER). There is also a network of rough endoplasmic reticulum and free ribosomes (RER) extending along the basal and apical borders and around the nucleus. Scattered mitochondria and a phagosome (P) are also present. The tips of a few rod outer segments (ROS) indent the apical surface (×11,000).

tities of smooth endoplasmic reticulum (SER) scattered throughout the cell, free ribosomes, and rough endoplasmic reticulum (RER) restricted to thin apical, basal, and perinuclear zones (Fig. 1). Freshly ingested phagosomes, lamellar in appearance, are enveloped by a membrane originating from the apical RPE membrane at the time of phagocytosis (Figs. 2A–B). The homogeneous, dense granules seen by light microscopy appear in a variety of forms by electron microscopy. The most common form consists of a homogeneous matrix separated from the surrounding membrane by an electron lucent space (Figs. 2B, 3A). In other granules of the same size and staining properties, we found a variety of inclusions including dense clumps of granular material (Figs. 1, 4B), membranous vesicles (Fig. 3D), groups of outer segment discs (Fig. 3B), or portions of melanin granules (Fig. 4C). In granules with these inclusions, there is also a lightly stained halo between the matrix and the surrounding membrane (Figs. 3C, 3D). We were able to find tubules of SER that appear to link these structures with each other and with phagosomes (Fig. 4A). These granules occur most prominently in the mid and basal RPE, tending to occur in clusters. Their number varied between 31/mm and 219/mm for the 19 animals. No clear temporal pattern in variation emerged from this data. Microperoxisomes[15] are also common in these cells (Fig. 4A).

None of these inclusions described above occur in the apical processes, except for cone phagosomes (see below).

Diurnal Variations

RPE phagosomes: We categorized RPE phagosomes as those located in the main body of the RPE but excluding those found in the cone sheaths. When the RPE is examined in cats killed at different times of the lighting cycle, striking changes in the number of RPE phagosomes are found. Fig. 5 shows light

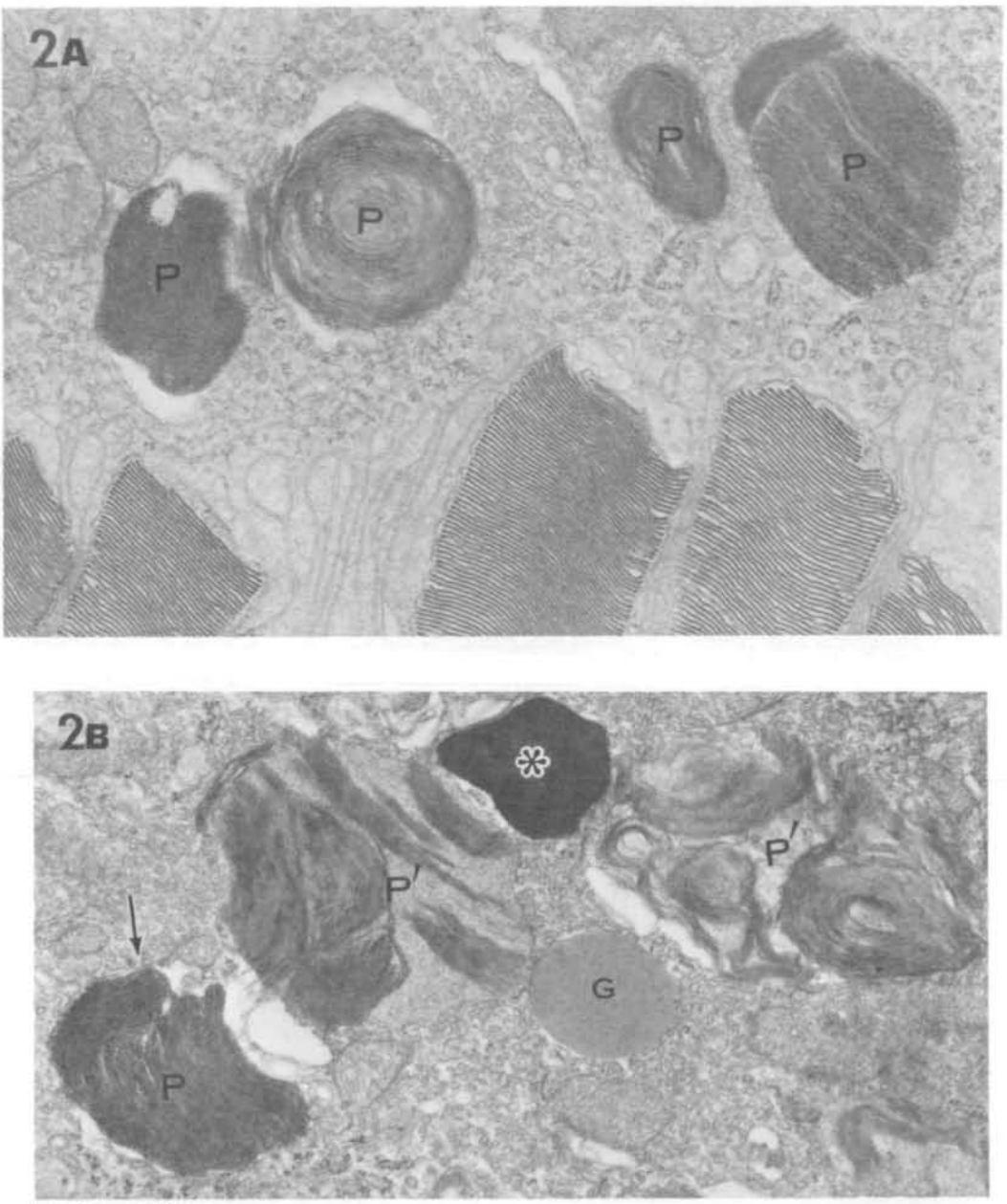

Fig. 2. A, four freshly-shed phagosomes (P) in the apical RPE from the cat killed 2 hr after light onset. The phagosome on the left is slightly more electron dense than the others (×16,600). **B**, Phagosomes in different stages of degradation from the cat killed 2 hr after light onset. The phagosome labeled "P" is slightly more electron dense than the rod outer segments (not shown), while the one labeled with the asterisk is greatly compacted and very dense. P′ designates two of the phagosomes assumed to be undergoing enzymatic digestion and are characterised by distorted discs, granular material, and a larger diameter than freshly-shed ones. The arrow indicates an area in which the RPE membrane enveloping the phagosome is evident. G = one of the prominent dense granules (×19,000).

micrographs taken from cats killed at light onset (0 hr), 1, 2, and 3 hrs after light onset and 1 hr after the beginning of the dark period (13 hr). At these times, there is a large number of RPE phagosomes only in the 1- and 2-hr animals (Figs. 5B–C); two freshly shed phagosomes are indicated by arrows in the 0 hr animal (Fig. 5A).

Fig. 6 shows the average number of RPE phagosomes/mm in 19 cats killed throughout the light-dark cycle. Except for one animal killed at 18 hr, the main trend of the data points toward a large increase in RPE phagosomes between 1 and 2 hr after the onset of light. The data show a slight increase in phagosomes beginning about half-way through the dark

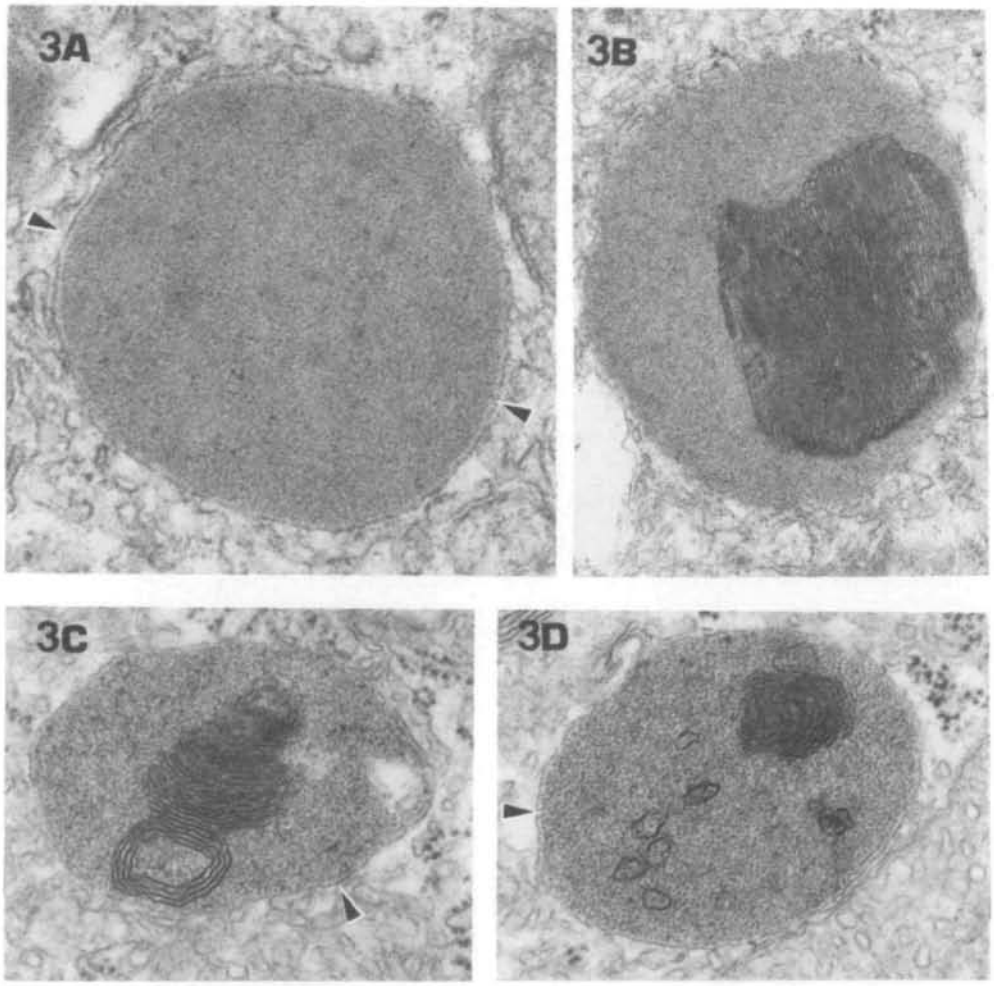

Fig. 3. Different morphologies of the dense granules found in cat RPE. The most common form is shown in **a**, where a granular matrix is separated from the limiting membrane by an electron-lucent halo (arrowheads). In **b**, **c**, and **d**, there are fragments of membrane and vesicles present within the matrix. In "**b**," the internal structure is apparently derived from a phagosome. The arrowheads in **c** and **d** indicate the halo between the matrix and membrane (**a** = ×60,000, **b** = ×35,000, **c** = ×45,500, **d** = ×53,500).

part of the cycle, leading up to the large increase shortly after light onset. During the last half of the dark cycle and on into the first 2 hr of light, there is a substantial degree of individual variability in the phagosome counts. This is shown most dramatically at 18 hr, where one animal has a very large number of RPE phagosomes (250/mm) while two others have relatively low numbers (72/mm, 25/mm). Similarly, at 1 and 2 hr after light onset, two animals had very large numbers of RPE phagosomes (225/mm and 265/mm) and two others had lower numbers (74/mm and 33/mm).

The RPE phagosomes in the three animals exhibiting maximal shedding rates (1, 2, and 18 hr) can be divided into two categories based on their structure. Phagosomes in the apical RPE generally appear compact and densely stained by light microscopy; their mean diameters in the 1 and 2 hour animals are 1.8 ± 0.4 μm and 1.5 ± 0.3 μm, respectively. By electron microscopy, these appear as compact packets of outer segment discs that may or may not be slightly more electron dense than underlying outer segments (Fig. 2). Otherwise, the structure of the discs is not particularly distorted in these phagosomes. Phagosomes in the mid and basal RPE usually appear vacuolated in the light microscope with areas of both darkly and lightly stained material. In the cat with the greatest number of RPE phagosomes (2 hr), their diameter is 2.3 ± 0.4 μm. By electron microscopy, they appear as complex assemblages of ingested discs, granular material, and vacuoles (Fig. 2B). It was much more likely to find dense, but otherwise undegraded, phagosomes in mid and basal RPE than it was to find the vacuolated, complex phagosomes in the apical RPE. Beneath the choroidal capillaries, where the RPE depth is narrowest, both types are compressed into a single row.

Cone phagosomes: Phagosomes within the cone sheath (Figs. 5C, 7), that is, in the space between the cone tip and the apical RPE, must be of cone origin since the sheath contacts no rods. As with the RPE phagosomes, there were definite differences in the number of cone phagosomes during the 24-hr cycle, but these changes are not easily shown photomicro-

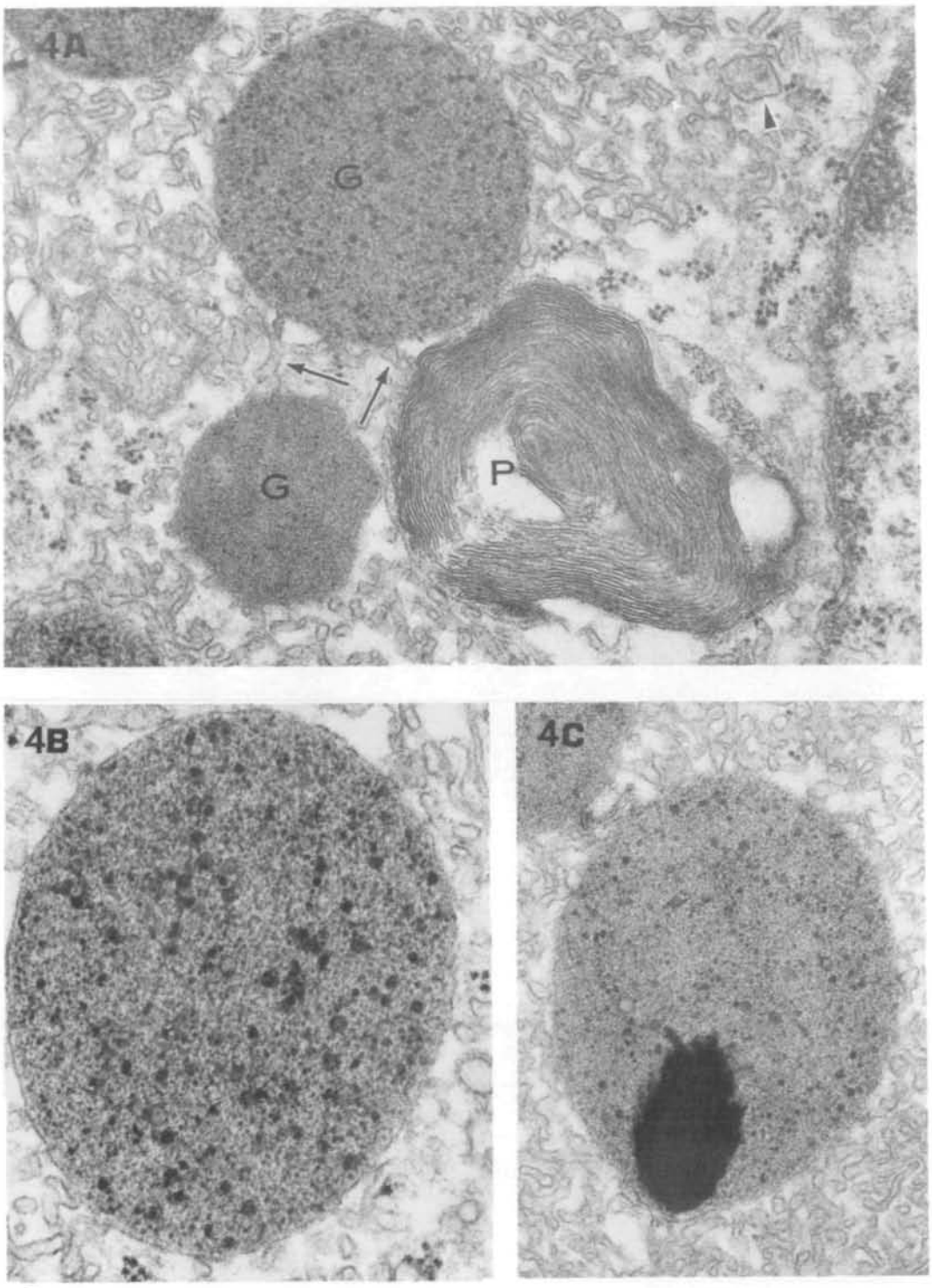

Fig. 4. A, possible interaction between the dense granules (G) and between them and a phagosome by way of the SER (arrows). A microperoxisome is indicated by the arrowhead (×37,000). B, a common form of the granules in which larger particles are distributed in the matrix (×58,000). C, a portion of a melanin granule within one of the dense granules (×45,000).

graphically because of the wide spacing between adjacent cones. Fig. 8 shows the diurnal variation in numbers of cone phagosomes. There is a large increase in cone disc shedding shortly after light onset, with a peak reached in two animals about 2 hr after light onset. As in the case of the total RPE phagosomes, cone phagosomes also show an increase in number beginning about half-way through the dark

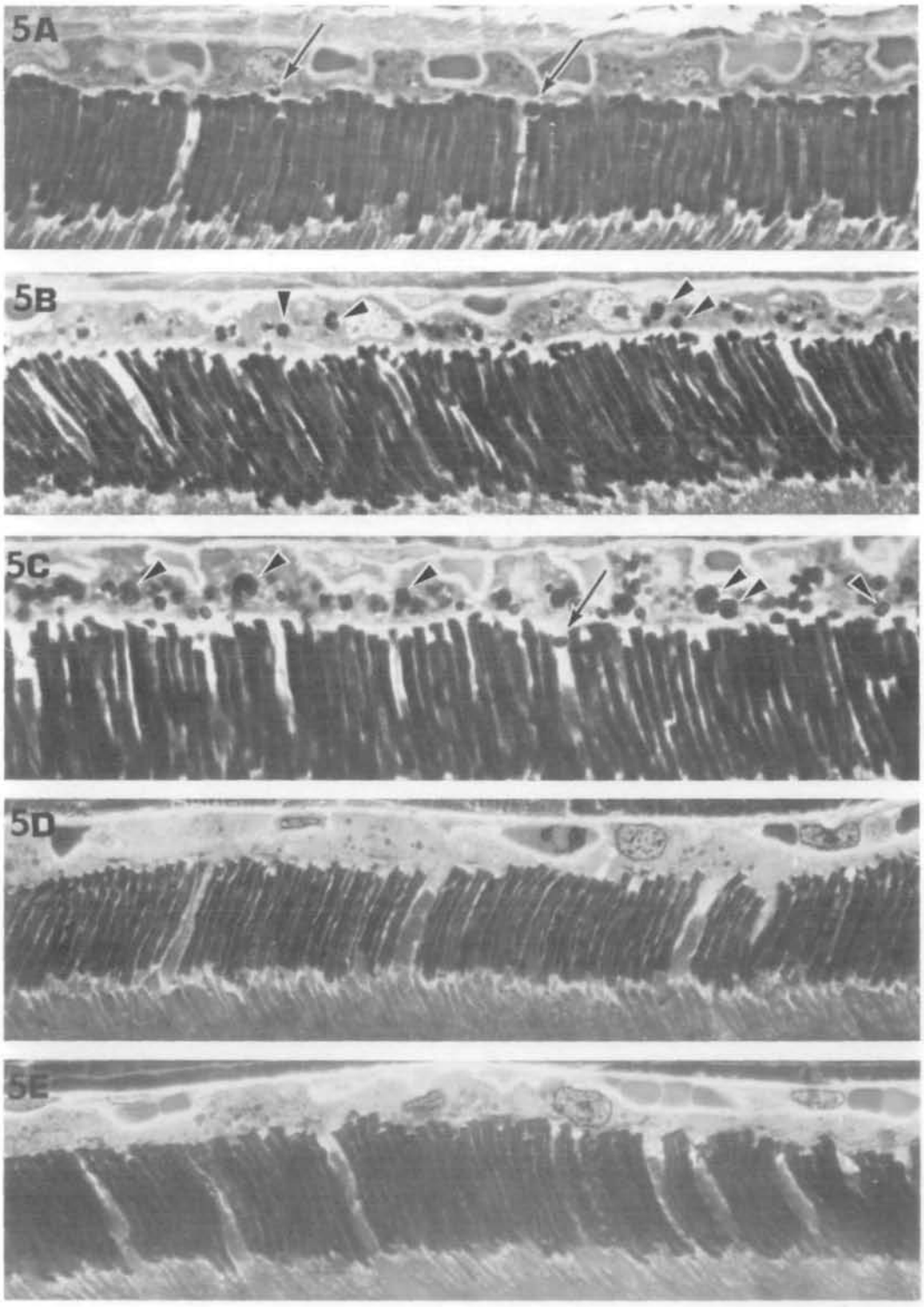

Fig. 5. A series of light micrographs of the photoreceptor outer segments and RPE of cats killed at light onset (**A**), 1 hr (**B**), 2 hr (**C**), 3 hr (**D**) after light onset, and 1 hr after light offset (**E**). Two freshly-shed phagosomes are indicated by arrows in A. There are large numbers of phagosomes in the RPE only in **B** and **C** (arrowheads). A cone phagosome in the supracone space is shown at the arrow in C (×1200).

portion of the daily cycle. By 3 hr after light onset, the number of cone phagosomes drops to nearly 20% of the value in the two animals at 2 hr; and at 13 and 14 hr (1 and 2 hr after the beginning of darkness), there are about 20 times fewer phagosomes than during the peak of shedding. Although the individual

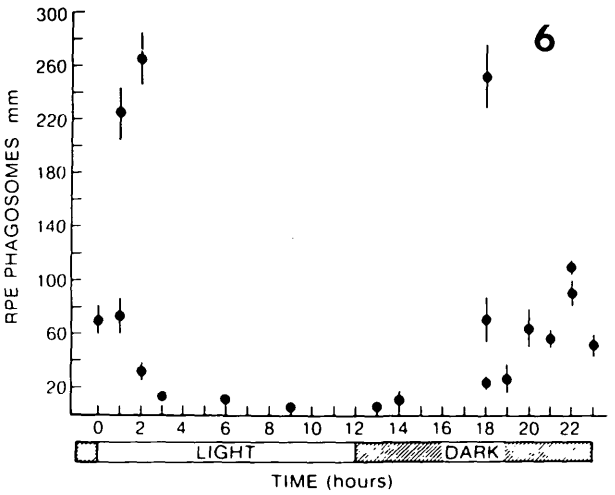

Fig. 6. The pattern of RPE phagosome counts in light-entrained cats over a 24-hr light-dark period. Each data point is the mean number of phagosomes in a single animal. Error bars = 1 standard deviation (at 3, 6, 9, and 13 hr, the error bars lie within the black circles).

variability in the number of cone phagosomes is much less than in the RPE phagosomes, there is some scatter in the data during the last half of the dark part of the cycle. During this period, no cone phagosomes were found in two animals (20 and 23 hr) while as many as 4.5/100 cones were found in 1 animal killed at 22 hr.

By electron microscopy, freshly shed cone phagosomes look identical to the freshly shed RPE phagosomes. The cone phagosome in Fig. 7 sits just above the cone tip, within the cytoplasm of one of the RPE apical processes. Although the phagosome is considerably more electron dense than the cone outer segment from which it was shed, its lamellar structure is still evident. We measured 22 cone phagosomes along their scleral-vitreal axes in the animal with 14.7 phagosomes/100 cones (1 hr). Their average size is 1.7 ± 0.4 μm and they contain an average of 52 discs.

Cone phagosomes are not usually vacuolated or otherwise disrupted while within the sheath. In fact, most of the cone phagosomes examined in this study are located in the sheath near the apical border of the RPE (Fig. 5C), not near the cone tip, and have essentially the same appearance as the phagosome shown in Fig. 7.

The total number of RPE and cone phagosomes shed during one light-dark cycle was estimated by connecting the maxima on Figures 6 & 8 for each hour sampled and then by measuring the area under the two curves (the very high value at 18 hr for RPE phagosomes was excluded for this purpose). These measurements yielded 1141 RPE phagosomes/mm and 77 cone phagosomes/100 cones.

Discussion

Tapetal RPE Cells

There have been very few published descriptions of RPE cells in the cat.[16] Ultrastructurally, the tapetal RPE cells in this species differ slightly from the general descriptions given elsewhere for mammalian RPE cells.[17,18,19,20] In other species, the small amounts of RER and free ribosomes that are present in the RPE are concentrated in the apical zone of the cells.[18,19] In the cat, however, there are fairly extensive zones of these organelles along the apical and basal borders and around the nucleus. Except for this perinuclear ring, these structures are nearly absent from the midzone of the cell that is filled with extensive tubules of SER.

The small homogeneous granules observed by light microscopy are heterogeneous in structure when studied by electron microscopy. The presence of an electron lucent halo between the granular matrix and the limiting membrane of these organelles, along with the presence of various inclusions such as membrane fragments and melanin, indicates that they are probably lysosomes.[21,22] There is also a possibility that these granules are peroxisomes[23] or that they form a mixed population of the two; this identification only can be made cytochemically. Lysosomes clearly must be an important component of these cells, considering the large quantity of membrane degraded by them on a daily basis.[24] It appears that the tapetal RPE cells in the cat have an efficient lysosomal system for removing degradative products, since we did not observe the presence of any structures that fit the description of "lipofuscin."[25] Lipofuscin is known to accumulate with age.[25] Although we do not know the age of the cats used in this study, all were mature animals and generally presumed to be young adults (less than 5-years-old). Perhaps lipofuscin occurs in the RPE of much older cats, although the accumulation of large amounts of lipofuscin (usually described as a yellow to brown pigment[25]), as occurs in the RPE of some species,[25] would almost certainly interfere with the tapetal reflectance.

Cyclic Disc Shedding

Diurnal variations in the number of RPE phagosomes have now been reported in the retinae of albino[1] and pigmented[26] rats, mouse,[27] Rana, Xenopus, lizards,[8] chicks,[5] goldfish,[4] gray squirrels,[6] monkeys,[28] and opossums.[9] Thus, there is increasingly strong evidence that disc shedding from all photoreceptors occurs in a diurnal pattern, although the exact timing of the peak of shedding varies, depending upon the species and type of receptor.[6,9,28] In albino rats,[1,29,30]

gray squirrels,[31] and Xenopus,[3] there is evidence that the cycle is circadian.

Disc Shedding from Rods

A diurnal pattern in RPE phagosome counts is apparent in the cat retina. Because the cones comprise a relatively small proportion of the total receptor population outside the area centralis,[10] we conclude that this pattern almost certainly represents a diurnal cycle of rod disc shedding. The increase in rod phagosome counts 1 to 2 hr after the onset of light is fully consistent with results in other vertebrates that are maintained under similar experimental conditions (see references above).

It is possible that a second increase in rod disc shedding occurs mid-way through the dark period in the cat. One of three cats killed at that time (18 hr) had a very large number of RPE phagosomes. Herman and Steinberg[7] reported similar results in the retina of the opossum; two of four animals killed 8 hr into the dark period in their study had an increase in the number of phagosomes. (A large number of RPE phagosomes was also reported in a rhesus monkey retina fixed 5 hr after the offset of light.[28]) We have no evidence that the cat with a large phagosome count was abnormal or unhealthy; indeed, the number of cone phagosomes was in agreement with other animals killed at the same time. Our results suggest that a rather high degree of individual variability occurs in cats killed in the later part of the dark cycle. This variability cannot be attributed to a lack of entrainment to the lighting cycle, because the three animals killed at 18 hr were all entrained for several months. Nor can it be attributed to the lighting conditions during dissection, because in the animal with a large number of RPE phagosomes and in one of the animals with a low number, the tissue was only exposed to dim red light until it was in fixative. The other animal with a low number of phagosomes was killed in red light, but the dissection was done under dim room illumination. Both rod and cone phagosome counts tend to rise in the latter part of the dark cycle, as if anticipating the morning peak. Similar results also were reported for albino rats.[1] Thus, on any given day, some animals may enter their period of maximal shedding prior to the main burst exhibited by the population—a conclusion tempered by the relatively small numbers of animals used in this study.

Disc Shedding from Cones

Previous studies of cyclic disc shedding in cones have not produced such consistent results as those in rods. Our study strongly suggests that in the cat retina, there is a diurnal pattern of cone disc shedding that closely matches the cycle in rods. Cones in the cat

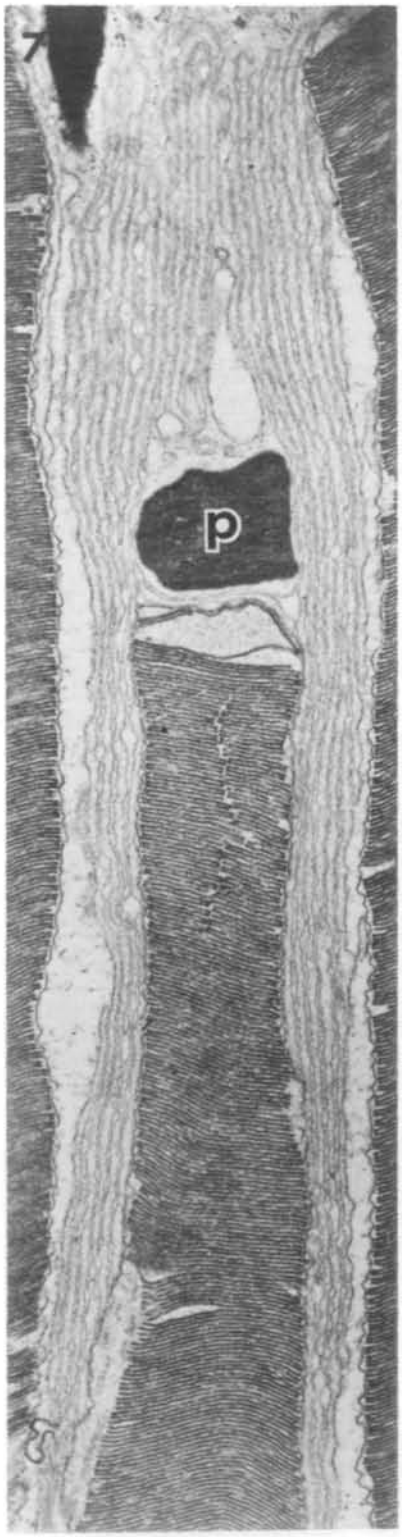

Fig. 7. A phagosome (P) located just above the tip of a cone outer segment and within one of the RPE apical processes forming the cone sheath. (×18,600).

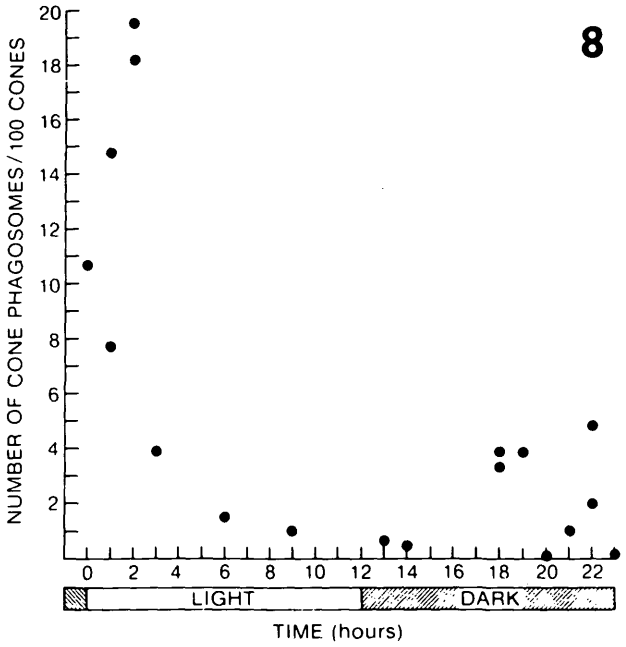

Fig. 8. The pattern of cone phagosome counts in light-entrained cats over a 24-hr light-dark period. Each data point represents the number of cone phagosomes counted per 100 well-aligned cone outer segments.

shed maximally 1 to 2 hr after light onset, then the phagosome numbers decline rapidly to a low level and begin to rise again during the last part of the dark period. Since there is no suggestion of a nighttime peak of cone shedding in the cat, our results are very different from those published on some other species. In the retinae of the Western fence lizard[8] and chick,[5] cone shedding reaches a maximum about 2 hr after light offset. In the goldfish,[4] the peak is about 4 hr after light offset and in the gray squirrel,[6] it is delayed to 7 hr into the dark period. However, in our earlier study of disc shedding in rhesus monkeys, the highest count of cone phagosomes located at the cone tips occurred 1 hr after light onset.[28] In a recent study of the cone dominated retina of the tree shrew, we also have found evidence that cone shedding reaches a maximum shortly after light onset.[33]

Cone Outer Segment Renewal

In rods, the rate of disc replacement or outer segment turnover time usually is obtained by measuring the displacement of the radioactive band that forms after labeling of the cells with ^{3}H-amino acids.[34] In cones, no band forms; instead, the outer segment is diffusely labeled, preventing the direct measure of renewal time.[35] To date, the only method used to estimate renewal time in cones relies on estimating the amount of outer segment shed and the frequency of shedding (phagosome counts).[8,31] The amount of membrane shed can be estimated fairly accurately by measurements of phagosome size or disc number. The estimate of frequency is more problematic. Young[8] used the maximum phagosome count as an estimate of shedding frequency, while Tabor et al[31] relied on the assumption that cones shed two packets of discs every other day.

We obtain values for the renewal time of cat cone outer segments ranging from 5.4 to 20.8 days, depending on the method used to estimate the frequency of shedding. An average cone phagosome in our study measures 1.7 μm in diameter and cone outer segments in the portion of the retina used for the counts average 6.2 μm in length. Thus, a phagosome represents 27% of the outer segment length. The maximum shedding frequency, taken from Fig. 8, at 2 hr after the onset of light is about 20% or, in other words, each cone sheds a disc packet once every 5 days. This yields a turnover time of 18.5 days $[(0.27/5)^{-1}]$. Similarly, we found that an average cone phagosome has 52 discs and the cone outer segments are composed of about 215 discs. In this case, a phagosome represents 24% of the outer segment length and this yields a turnover time of 20.8 days $[(0.24/5)^{-1}]$.

For this method to be useful in calculating renewal time, the maximum phagosome count must be a reasonable estimate of the true shedding rate. An alternative value for the latter was obtained from the area under a curve constructed from the graph in Fig. 8. This method is valid if the degradation time is less than 1 hr but not so rapid that many phagosomes are missed by sampling at hourly intervals. This yields a shedding value of 77 phagosomes per 100 cones, which gives a renewal time of 5.4 days $[(0.24 \times 0.77)^{-1}]$.

Since these two estimates of cone renewal time differ so greatly, we need some other means of deciding which method is more accurate. We can obtain similar values for the rod data, shown in Fig. 6, and, in this case, we know that the renewal time calculated from autoradiographic data is 6.5 days (S. A. Bernstein and S. K. Fisher, unpublished data). The maximum RPE phagosome count is 265/mm (2 hr after light onset) and the rod linear density in these same tissue sections is 884 rods/mm. A shedding frequency of 33% is estimated from this data. The "fresh" phagosomes average 1.5 μm in diameter in this cat and the rod outer segments average 15 μm long. This gives a renewal time of 33 days $[(0.1 \times 0.33)^{-1}]$. On the other hand we estimate that the total RPE phagosomes shed per day is 1141/mm or 129% of the rod density. This gives a renewal time of 7.75 days $[(0.1 \times 1.29)^{-1}]$, a value much closer to the renewal time obtained from autoradiographic data.

From the rod data it appears that the area under the disc shedding curves may reflect the true frequency of shedding and thus might better estimate the renewal time. If this is the case, our calculations indicate that the renewal time is similar for cat rods and cones. The autoradiographic data from ^{3}H-leucine labeled photoreceptors shows a band displacement rate of 2.3 μm/day in cat rods (S. A. Bernstein & S. K. Fisher, unpublished data). If the cones average 6.2 μm in length and turnover about every 5½ days, then the rate of membrane production must be about 1.13 μm per day. Tabor et al[31] estimated a turnover time of about 5 days for gray squirrel cones; since these cones are about 7 μm long, they must produce about 1.4 μm of membrane per day. ^{3}H-band displacement in autoradiograms of squirrel rods occurs at a rate of 2.4 μm/day.[30] Thus, in both these mammals, it appears that the rate of membrane production may be significantly less in cones than in rods, but since cone outer segments are shorter than rod outer segments, their total turnover time may be nearly the same.

The concept of turnover time or renewal rate for cones, as discussed here, refers only to the replacement of a standard length of outer segment. The continuity of cone discs and the random diffusion of molecules inserted into fluid membranes means a continuous mixing of old and new membrane constituents within the outer segment. Thus, the turnover of a given volume of an outer segment would occur more rapidly than the turnover of its molecular components.[31] On the other hand, since rod outer segments are composed of discrete discs, turnover time for the outer segment and its molecular components are essentially the same.

Synchrony of Rod and Cone Shedding

Both rod and cone shedding in the cat reach a peak at approximately the same time after the onset of light, fall to a low level rapidly, and then remain low until the latter part of the dark period when the cycle begins again. However, precise synchrony between the two types of shedding does not appear in individual animals. Fig. 9 shows the results of this study plotted so that the magnitude of rod and cone shedding in each animal can be compared. For instance, when the cone shedding rate is high (■ 1 hr, ■ 2 hr) the rod shedding response in the same eye may be very low (● 1 hr, ● 2 hr). In the 18-hr animal with a very large number of RPE phagosomes, the rod shedding response is very high (●) while the cone shedding rate is low (■). Thus, the peaks of rod and cone disc shedding in an individual animal may be slightly displaced temporally. There are two possible

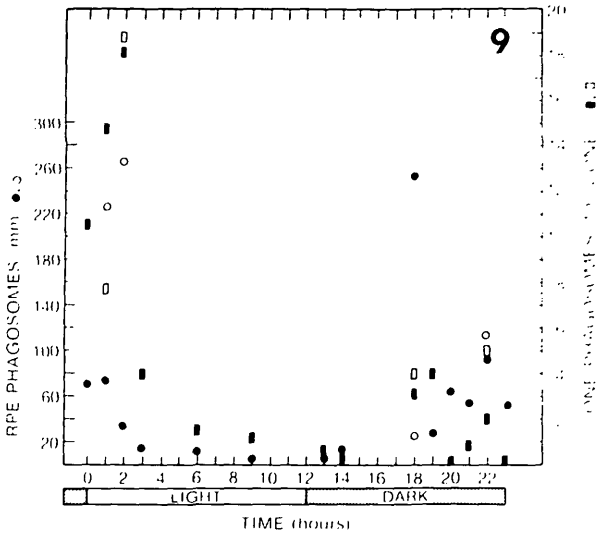

Fig. 9. A combined plot of the data in Figs. 6 and 8 to allow comparison of the shedding response from rods and cones in individuals. Circles represent rod phagosome counts, rectangles represent cone phagosome counts. When data from more than one animal is shown at a given time, the filled rectangle and filled circle are data from the same animal, while the open rectangle and open circle are from the other animal.

explanations for this asynchrony in individual animals. First, it is possible that the mechanism for initiating shedding may be different for rods and cones. Secondly, if the stimulus for rod and cone shedding originates from a single source, the timing of its effect on the two receptor types may be slightly different.

Phagosome Degradation

Phagosome degradation in the cat's RPE appears to occur in the same manner as in other species. The steep decline in phagosome number after the peak of shedding is presumably due to rapid enzymatic digestion of the disc packets by lysosomal enzymes.[38,39] Many, perhaps all, phagosomes proceed through a stage in which they become more electron dense and compact but in which the discs remain intact. In later stages, discs occur in larger, distorted aggregates that appear to contain remnants of more than one phagosome plus other granular material. These large aggregates tend to occur mainly in mid and basal RPE, while the fresh and electron dense phagosomes appear most frequently in apical cytoplasm. Similar results have been reported for phagosome degradation in the opossum.[37] Although a few of the round, granular inclusions found in abundance in these cells contain fragments of membrane clearly derived from phagosomes, we have no evidence they are the end product of phagosome degradation. Their number did not vary systematically with the number of phagosomes,

nor did we find them in sufficient number to account for the large number of phagosomes shed during the peak. The majority of these granules are homogeneous in structure and contain only the lightly staining granular matrix. In this respect, they differ from the residual bodies described in the lizard[8] and from lipofuscin described in a variety of RPE cells.[27] Both residual bodies and lipofuscin are thought to be breakdown products of phagosomes and other structures, and in the lizard, their number varies in a clear diurnal pattern.[8]

Cone phagosomes in the cat probably do not undergo lysosomal degradation while in the apical projections but only after they are transported into the RPE cell bodies. This is in contrast to results in the rhesus monkey, where cone phagosomes show extensive structural signs of enzymatic degradation while still in the apical cone-ensheathing processes.[28] In that species, we were able to identify lysosome-like vesicles fused with phagosomes in the apical projections. No such structures were found in cat apical projections.

Our data strongly suggest that RPE phagosomes are degraded rapidly in the cat. The peak phagosome count at 2 hr is reduced by 95% 1 hr later (Fig. 6). This conclusion is supported by the fact that both partially degraded and fresh phagosomes occur in the RPE of all three cats exhibiting maximal shedding. Likewise, cone phagosomes disappear rapidly from the cone sheaths. Within 1 hr after the morning peak, their number is reduced by 80% and within 4 hours by 93% (Fig. 8). The cat seems to be different from the rhesus monkey in this respect, since cone phagosomes may linger in the apical processes of that species.[28]

To date, studies of cyclic cone disc shedding in various species have not revealed as consistent a pattern as is found for rod shedding. As this process is studied in more species, it will be interesting to see if a pattern emerges that can be correlated with some aspect of the animal's visual physiology, biochemistry, or behavior. At this point, none is apparent. Nighttime shedding is found in two poikilotherms (lizard, goldfish)[4,8] and two homeotherms (chicks, gray squirrels),[5,6] while daytime shedding predominates in two homeotherms (cat, tree shrew).[33] Lizards, chicks, and gray squirrels all have nighttime cone shedding peaks and are diurnal,[38] but tree shrews are also strongly diurnal and have a morning peak. On the basis of similarity in retinal histology, the squirrel and tree shrew might be expected to show similar patterns of disc shedding, but they do not. The domestic cat, with a morning peak like the tree shrew, is usually described as nocturnal[18,38] (although its daytime activity is well known[39]). On the basis of earlier work it was proposed that the two classes of photoreceptors shed discs shortly after they cease to subserve vision,[9] that is, cones would shed at the beginning of the dark part of a daily cycle while rods would shed at the beginning of the light portion. On the basis of our results in the gray squirrel, monkey, tree shrew, and cat, this hypothesis now seems unlikely. On the contrary, we strongly suspect that the pattern of shedding in the human retina will prove to be similar to the pattern displayed in the duplex retina of the cat, with both rod and cone shedding occurring shortly after light onset.

Key words: cones, rods, disc shedding, photoreceptors, diurnal cycles, cat, retina

Acknowledgment

The authors wish to acknowledge the technical assistance of Kenneth Linberg.

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