Exp. Eye Res. (1980) **30**, 559–574

Rod and Cone Disc Shedding in the Rhesus Monkey Retina: A Quantitative Study

DON H. ANDERSON, STEVEN K. FISHER, PAGE A. ERICKSON AND GARETH A. TABOR

Department of Biological Sciences, University of California, Santa Barbara, Santa Barbara, Ca 93106, U.S.A.

(Received 31 July 1979, New York)

Four Rhesus monkeys were maintained for several months on a daily lighting schedule of 12 hr light and 12 hr darkness (12L:12D). The animals were then fixed by intracardiac perfusion at 1 and 5 hr following the onset of light, and at 1 and 5 hr after the offset of light. The number of phagosomes/mm of retinal pigment epithelium (RPE) was counted in the light microscope for both foveal and extrafoveal locations. At extrafoveal sites, phagosomes within the RPE cell bodies and those within the ensheathing processes bordering cone outer segments were counted separately. Counts at foveal and extrafoveal locations suggest that the rate of disc shedding, and therefore the rate of membrane replacement, is probably less for foveal cones than it is for rods. The number of phagosomes above the rod tips 1 hr following light onset and their diminution several hours later suggests that an increase in rod shedding, similar to the "burst" of rod shedding reported for other species, also exists in the monkey retina. The number and location of phagosomes 5 hr after the offset of light indicates that there could be another phase of increased rod shedding somewhere around the middle of the dark period. Phagosome counts in the foveal RPE were highest 5 hr after light offset, however the counts at 1 and 5 hr following light onset were nearly as high. The number of RPE phagosomes at both foveal and extrafoveal locations was lowest 1 hr after light offset. Counts of phagosomes within the sheaths of extrafoveal cones showed that about 20% contained one or more phagosomes within their sheaths. Of those sheaths that did contain phagosomes, approximately 25% had multiple phagosomes. There appears to be a considerable period of time during which extrafoveal cone phagosomes remain within the ensheathing processes, moving from the cone tips toward the apical RPE surface. Rod and cone disc shedding in the monkey retina are probably cyclic processes—although the temporal pattern of shedding may be different than that reported for other species.

Key words: rods; cones; pigment epithelium; phagocytosis; fovea; retina.

1. Introduction

The light-sensitive portion of vertebrate rods and cones, the outer segment, is composed of a highly-ordered array of membranous discs. The organization of the disc stack is somewhat different for rods and cones (Cohen, 1961, 1968; Anderson and Fisher, 1976; Anderson, Fisher and Steinberg, 1978), although both types are thought to assemble new membrane at the outer segment base and to shed membrane-bound disc packets from the tip. The packets are engulfed by processes of the retinal pigment epithelium (RPE) after which the packets are called phagosomes (Young and Bok, 1969). In this way, the outer segments undergo a process of continual replacement (Young, 1976; Anderson et al., 1978).

Disc shedding from both rod and cone outer segments is now known to be a cyclic event in several different vertebrate species (LaVail, 1976; Basinger et al., 1976; Besharse, Hollyfield and Rayborn, 1977; Young, 1977, 1978a; O'Day and Young, 1978; Tabor, Fisher and Anderson, 1979). Recent evidence suggests that in duplex retinas most rod disc shedding is separated in time from cone shedding when animals are maintained under conditions of cyclic illumination (Young, 1978a; O'Day and Young, 1978; Tabor et al., 1979). A peak of rod shedding takes place soon after light

0014–4835/80/050559+16 $01.00/0

onset, while cone shedding appears to be largely confined to the first part of the dark period. In some species, e.g. the albino rat (LaVail, 1976; Tamai, Teirstein, Goldman, O'Brien and Chader, 1978; Goldman, Teirstein and O'Brien, 1979) and grey squirrel (Tabor et al., 1979), the increase in rod shedding is known to be an endogenous rhythm that occurs in the absence of lighting cues. It is not yet known whether the same holds true for cone shedding.

In this investigation we were interested in determining whether monkeys fixed at widely different times in the lighting cycle have different phagosome levels within the RPE. In addition, we were interested in comparing the shedding rate at foveal and extrafoveal locations. Since disc shedding from rods and cones had been documented previously in the rhesus monkey (*Macaca mulatta*) retina (Young, 1971a; Anderson et al., 1978), we maintained four animals on a schedule of 12 hr light and 12 hr of darkness (12L:12D) and then counted the phagosomes present within the RPE at four key times during the 24 hr cycle.

2. Materials and Methods

Animals

Four adult female Rhesus monkeys (*Macaca mulatta*) were maintained for several months on a lighting schedule of 12 hr light and 12 hr darkness (12L: 12D). Illumination levels ranged from 90 lx inside the cages to 170 lx directly in front of the cages. Animals ranged in weight from 4·2 to 7·8 kg, and all were judged to be in good physical condition. All of the animals were killed within one 48 hr period. An animal was fixed by intracardiac perfusion at each of the following times (measured from the time of lights-on at 0000; lights-off 1200): 0100, 1 hr after light onset; 0500, 5 hr following light onset; 1300, 1 hr following the offset of light; and 1700, 5 hr following light offset.

Fixation

Animals were anesthetized initially with an intramuscular injection of ketamine hydrochloride (Bristol). For both animals killed during the dark period, this injection was given in the darkened room while the animals remained in squeeze cages. The only room illumination came from a small flashlight fitted with a red-transmitting filter. After the initial injection, a deep level of anesthesia was induced by intravenous injection of sodium pentobarbital (Nembutal). Animals were fixed by intracardiac perfusion of an aldehyde fixative consisting of 1% paraformaldehyde, 1% glutaraldehyde in phosphate buffer (pH 7·1). After perfusion of approximately 800–1000 ml of fixative at a pressure of 90 Torr, the eyes were enucleated and the anterior structures were cut away using a double-edged razor blade and tenotomy scissors. As much of the vitreous as possible was removed from the eyecup at this time. Then, each eyecup was immersed in fixative for the next 12–24 hr. After aldehyde fixation, the eyes were transferred to buffer solution (plus 40 mg/ml sucrose), and each eye was then cut into several wedge-shaped pieces. The optic nerve head and the fovea formed the apex of one of the wedges. The specimen that included the foveal region was separated from the rest of the tissue and processed similarly. After several washes in buffer solution, the specimens were post-fixed in veronal acetate buffered osmium tetroxide (2%), washed in distilled water, dehydrated in a graded ethanol–water series, rinsed in several changes of propylene oxide, and embedded in Araldite (Cargille 6005).

Microscopy

Thick (1 μM) sections were taken from foveal, parafoveal, and near peripheral locations. The outer segments were aligned along their longitudinal axes (within the fovea it was

possible to align only the outer one-third of the outer segments), and the sections were stained with a mixture of methylene blue and azure II in 1% sodium borate. The fovea was located by sectioning the tip of the wedge-shaped foveal specimens at 100 μM intervals on an LKB Ultramicrotome III, and by examining 1 μM sections periodically for evidence of a slight thinning of the ganglion cell layer. The boundary of the foveal depression could be distinguished because fibers from Henle's layer tended to course away from the foveal region on both sides (see Anderson and Fisher, 1979). The sections were examined using a Zeiss Universal Research Microscope. Thin sections for electron microscopy (600–700 Ångstroms) were placed on 75×300 slotted mesh grids, or on formvar-coated slot grids, and then stained with 1% uranyl acetate and lead citrate.

Phagosome counts

All phagosome counts were made in the light microscope by one individual (DHA). Phagosomes were identified on the basis of their size, shape, staining characteristics, and by correlating light and electron microscopic observations. Inclusions with a diameter of less than one-half outer segment diameter were not counted. All phagosomes larger than one-half outer segment diameter were counted as a single population, since no systematic variation in size distribution was apparent. Distance measurements of RPE were made without correction for retinal curvature with the aid of a calibrated ocular micrometer. A mean number of phagosomes/mm of RPE was obtained for both foveal and extrafoveal locations for each of the four animals. A total of 50 mm of RPE, 10 mm within the foveal pit and 40 mm extrafoveally, was examined for each animal and the number of phagosomes present was recorded. The mean retinal thickness and the average width of Henle's layer (if present) was also recorded to provide a rough estimate of retinal location. Foveal measurements were made in multiples of 0·5 mm lengths of RPE opposite to the central part of the foveal depression. Each 1 μM section used for counting purposes was separated from its nearest neighbour by 3–4 μM in order to minimize the likelihood of overlapping counts. Phagosomes that appeared within the RPE processes ensheathing extrafoveal cones, i.e. the cone sheaths, were counted separately from phagosomes appearing within the RPE cell bodies. Only those cones and their ensheathing processes that were well-aligned along their longitudinal axes were included in the sampling. Extrafoveal cones were examined systematically in each animal until a total of 100 cones containing phagosomes within the ensheathing processes was accumulated, The number and location of phagosomes within the ensheathing processes was recorded for each cone included in the counts.

3. Results

The rhesus monkey pigment epithelium

The main ultrastructural features of the rhesus monkey RPE have been described previously; no detailed description need be provided here. However, there are several types of organelles that can be mistaken for phagosomes, especially in the light microscope, without careful inspection. Melanin granules are present in large numbers within the RPE, principally along the apical borders of the RPE cells. They are in the same size range as some phagosomes, but their uniform circular or elliptical profiles distinguish them from most phagosomes. In the light microscope they appear as dark brown granules, unlike phagosomes that stain an intense blue with methylene blue-azure II. There is also a large population of heterogeneous granules present that are usually termed residual bodies. These structures tend to be somewhat smaller than most phagosomes. In many cases they represent the end products of autophagic or phagocytic processes. They are circular or slightly oblong in profile, are compartmentalized within individual RPE cells (Figs 7–8), appear electron-opaque, and

typically contain one or more dense particles as part of their internal structure. In the light microscope, residual bodies stain somewhat lighter than do phagosomes.

Phagosomes within the monkey RPE can assume different appearances. In the electron microscope, they often appear as electron dense structures, staining more deeply than the outer segments. They range in size from about one-half to a little over a full outer segment diameter (1–2 μM). Their internal lamellar structure is nearly always identifiable, although the discs may be more compressed than in intact outer segments. In the light microscope, phagosomes also appear more darkly stained than do the outer segments. Discs within phagosomes may be curled, or they may retain their normal configuration. Those phagosomes that do not contain curled discs show an almost rectangular shape, while those having curled discs are almost circular.

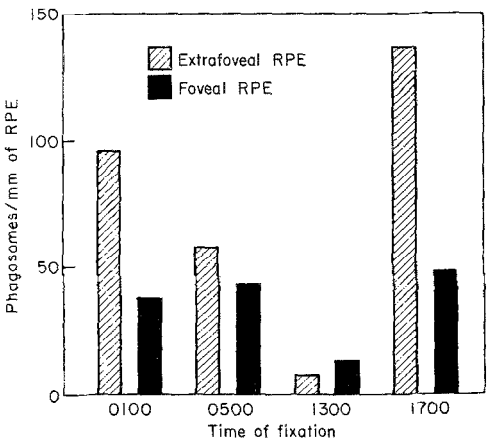


FIG. 1. Histogram showing the pattern of disc shedding at both foveal and extrafoveal sites at four different fixation times in the lighting cycle. 0000 signifies light onset and 1200 denotes light offset. Note the similar patterns of shedding at both foveal and extrafoveal sites despite differences in the phagosome levels (see Table I).

Phagosomes contain anywhere from one-half dozen to several dozen discs. Disc packets derived from extrafoveal cones could be identified easily within the RPE ensheathing processes, provided that the outer segments are properly aligned. Their size and staining characteristics, in both the light and electron microscopes, are not different from those of rod phagosomes. Multiple phagosomes are sometimes present within the cone's ensheathing processes (Figs 12–13).

Ultrastructurally, the foveal and extrafoveal RPE are indistinguishable although the relationship of foveal cone outer segments to the apical RPE is more rod-like than elsewhere (see Anderson and Fisher, 1979). All of the types of organelles found in extrafoveal RPE can also be found within foveal RPE cells. However, in the animals we studied the quantity of dense granules, i.e. residual bodies, lipofuscin and melanolysosomes appeared to be less plentiful within the foveal RPE (Fig. 4).

Phagosome counts at extrafoveal locations

Initially, phagosomes were counted separately for RPE locations within the parafovea and the near periphery of the posterior retina. The counts at these different locations showed little variability and, therefore, the data were pooled as "extrafoveal phagosomes". Phagosomes located within the RPE ensheathing processes bordering extrafoveal cone outer segments were excluded from these counts, but were included

in a separate category (see below). In this way we were able to categorize most phagosomes counted within RPE cell bodies outside of the fovea as rod phagosomes. This is almost certainly the case since the number of rods relative to cones outside of the fovea ranges from 5:1 at the parafovea to over 20:1 in the periphery (Young, 1971b). Nevertheless, it is probable that a few cone phagosomes are also included in these counts.

The number of extrafoveal phagosomes showed wide variation between different animals. As shown in Fig. 1 (and in Table I), the concentration of phagosomes was highest in the monkey fixed at 1700, 5 hr following the onset of the dark period. A substantial portion of these phagosomes were situated within the apical and mid-zones of the RPE cells (Fig. 2). In the electron microscope, such phagosomes contained several dozen well-preserved discs, and their diameter was usually close to the rod outer segment diameter. Most phagosomes appeared to be packets of recently shed discs in that they showed few signs of degradation.

TABLE I

RPE phagosomes versus time of fixation

Fixation time	Mean number of phagosomes/mm of RPE Extrafovea (±1 S.D.)	Fovea (±1 S.D.)
0100 (1 hr post-light onset)	94·2±32	38·9±11
0500 (5 hr post-light onset)	58·0±33	47·7±14
1300 (1 hr post-light offset)	8·0±4	13·5±7
1700 (5 hr post-light offset)	135·7±57	48·4±17

The number of extrafoveal phagosomes was lowest in the animal fixed at 1300, 1 hr following dark onset. In this animal, the mean value is 8·0 phagosomes/mm of RPE, approximately 6% of the 1700 maximum (Fig. 1). Phagosomes within the RPE of this monkey were so scarce (Fig. 8) that it was not possible to assign them to any predominant location within the RPE cells.

Intermediate between these two extremes were the animals fixed at 0100 and 0500, 1 and 5 hr respectively, after light onset (Fig. 1). At 0100 a mean value of 94·2 phagosomes/mm of RPE was obtained (about 80% of the 1700 maximum). As in the 1700 animal, there was a tendency for many phagosomes to occupy positions within the apical RPE cytoplasm just above the rod tips. However, another group of smaller phagosomes was lined up along the basal border of the RPE, adjacent to Bruch's membrane (Figs 4–5). At 0500, there was an average of 58·0 phagosomes/mm of RPE (about 43% of maximum). At this time, phagosomes were mostly located within the mid-zone or basal RPE cytoplasm.

Phagosome counts within the fovea

Variation between animals in the number of phagosomes within the foveal RPE was not as striking as it was outside of the fovea (Fig. 1). Foveal phagosomes seemed to be a little smaller than those observed extrafoveally, but the number of discs/phagosome did not differ substantially. This size difference is likely due to the slightly smaller diameter of foveal outer segments compared to rod and cone outer segments outside the fovea. As was the case extrafoveally, there were some indications that the

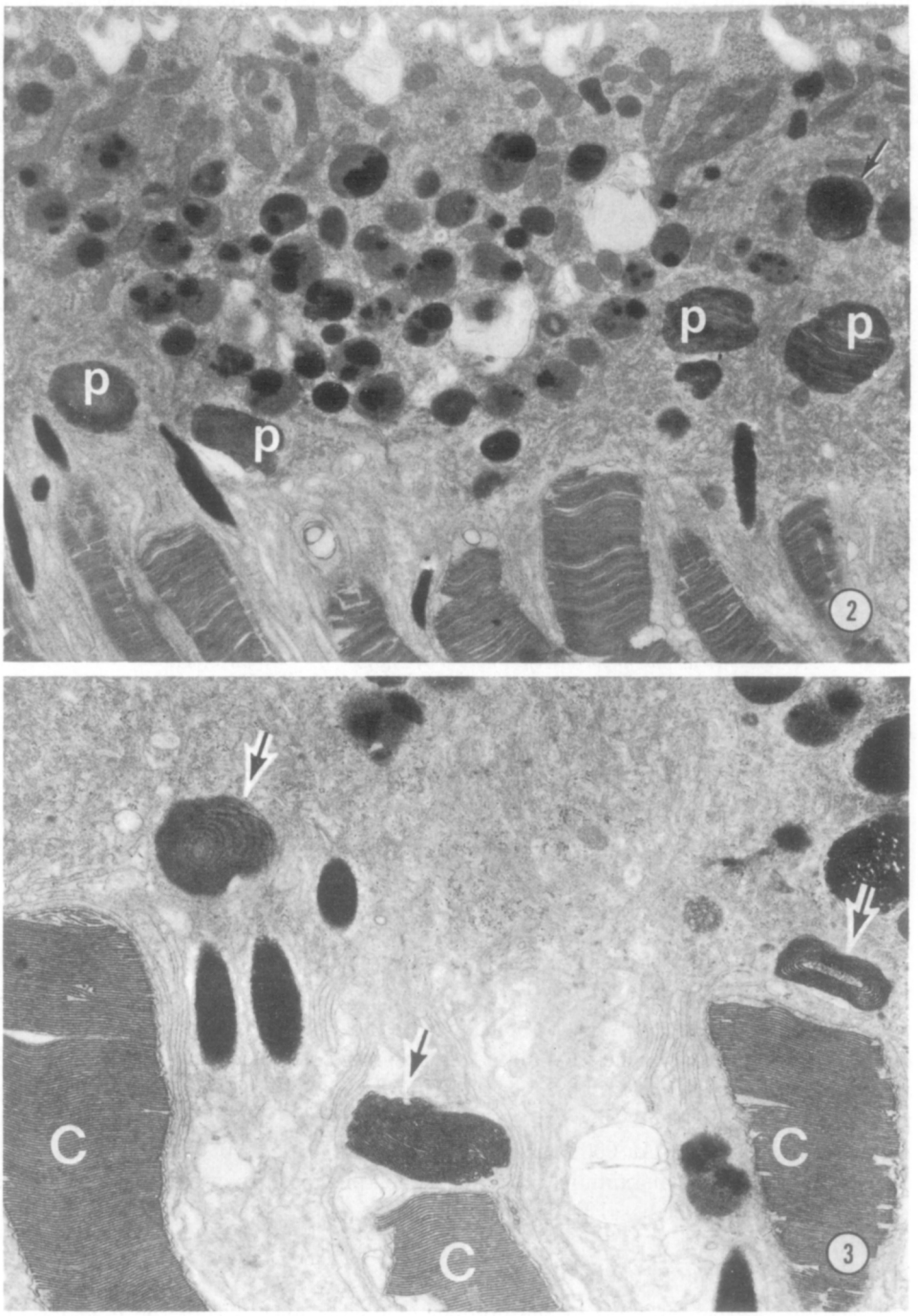


FIG. 2. Electron micrograph of the extrafoveal RPE at 1700, 5 hr following light offset. At this time, phagosomes (P) are often located above the rod outer segment tips. A partially degraded phagosome (arrow) is shown within the RPE mid-zone (×6750).

FIG. 3. Electron micrograph of the foveal RPE at 1700. Within the fovea at this time, phagosomes (arrows) also are positioned above the outer segment tips, although it is unusual to find three phagosomes above three adjacent cones (C) as shown here (×13 500).

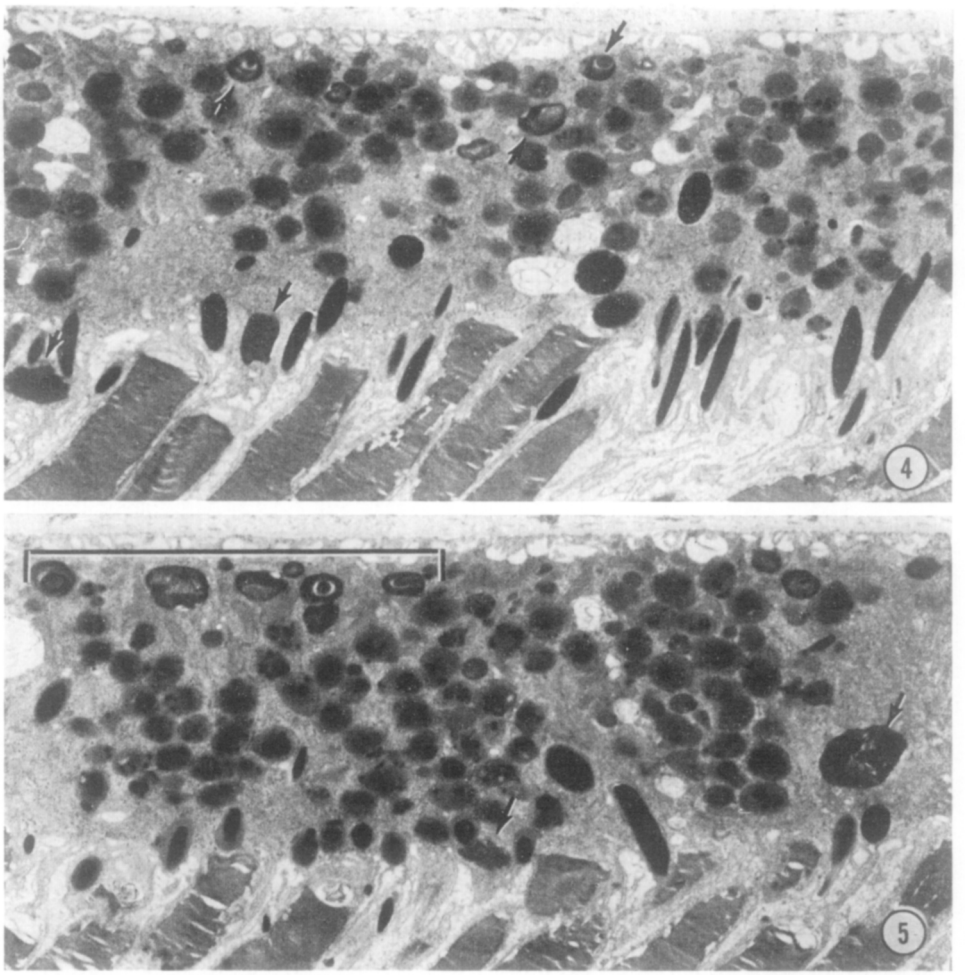


FIG. 4. Low power electron micrograph of the extrafoveal RPE at 0100, 1 hr after light onset. Phagosomes (arrows) tend to be congregated above the outer segment tips and also along the basal RPE border adjacent to Bruch's membrane (×3600).

FIG. 5. Electron micrograph from an area of extrafoveal RPE adjacent to the RPE in Fig. 4. The brackets enclose phagosomes aligned along the basal RPE border. Several other phagosomes (arrows) are situated in the apical RPE cytoplasm (×3600).

predominant location of foveal phagosomes was different in different animals. For example, there was a strong tendency for phagosomes at 1700 to be positioned within the apical cytoplasm of foveal RPE cells and, often, immediately above the cone tips (Fig. 3). At 0100 and at 0500 the phagosome concentrations were nearly equal to the 1700 value (Fig. 1), however the phagosomes were slightly smaller and more basally located within the RPE cytoplasm. We found the lowest quantity of phagosomes within the foveal RPE at 1300 (13·5 phagosomes/mm of RPE). We were unable to find more than a few examples of these phagosomes in the electron microscope (Fig. 7). In three out of four monkeys, extrafoveal phagosome counts exceeded the counts made within the foveal RPE (Fig. 1). The ratio of foveal to extrafoveal phagosomes/mm of RPE was 0·35 at 1700, 0·41 and 0100, 0·82 at 0500, and 1·7 at 1300. In the fourth animal, the 1300 monkey, foveal phagosomes outnumbered extrafoveal phagosomes, but both counts were very low in relation to the counts in other monkeys.

Counts of extrafoveal cone phagosomes

In the rhesus monkey and human retinas, extrafoveal cone outer segments do not reach the apical RPE surface. Rather, a highly-organized array of RPE processes extends down from the apical surface (up to 20 μM) and ensheaths the distal part of the outer segment (see Steinberg, Hogan and Wood, 1977; Anderson et al., 1978). Disc packets shed from the cone outer segment tips can be observed at various levels within the cone sheaths.

In order to obtain an estimate of the shedding rate for extrafoveal cones, we examined a large sample of well-aligned cone outer segments (over 400 for each animal). The sampling continued in each animal until a total of 100 cones bearing one or more phagosomes within the sheaths was counted. The proportion of cone sheaths examined that had one or more phagosomes is given in Fig. 9. These values do not show much variation in that they all fall within the range of 0·17–0·24. In other

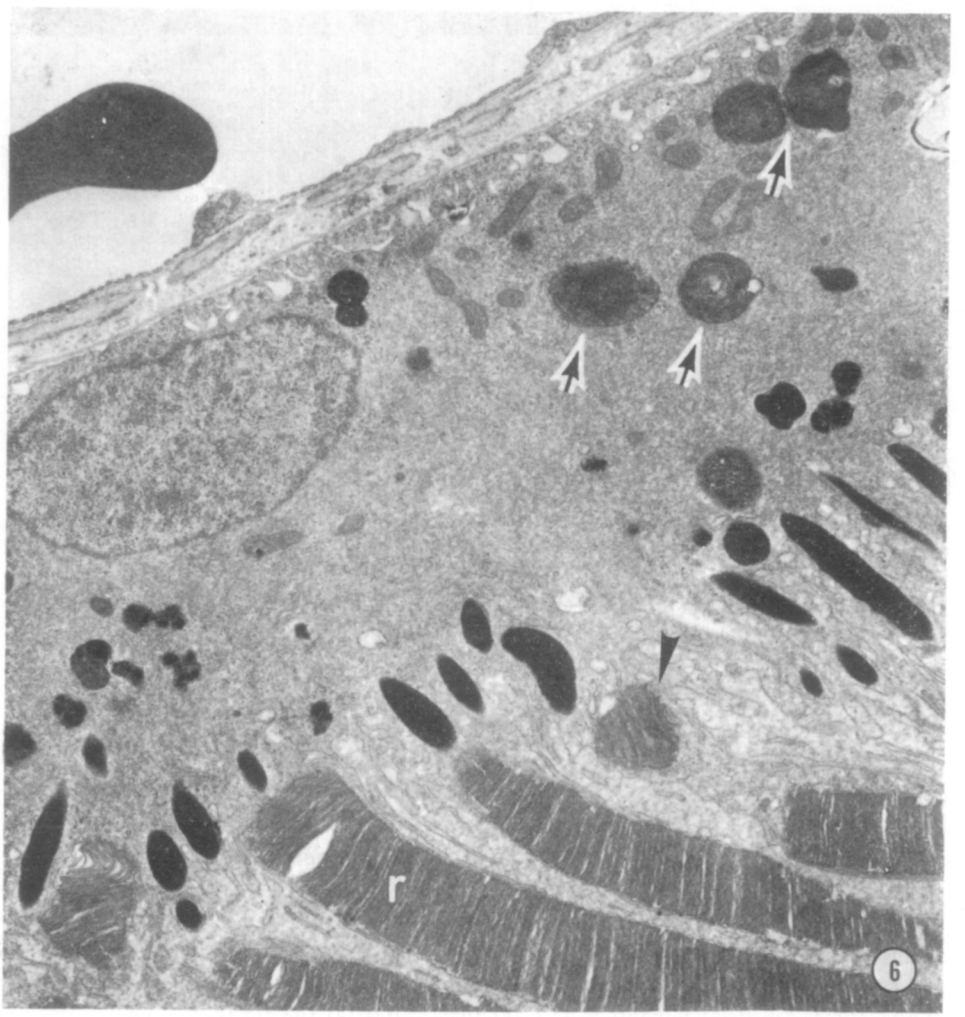
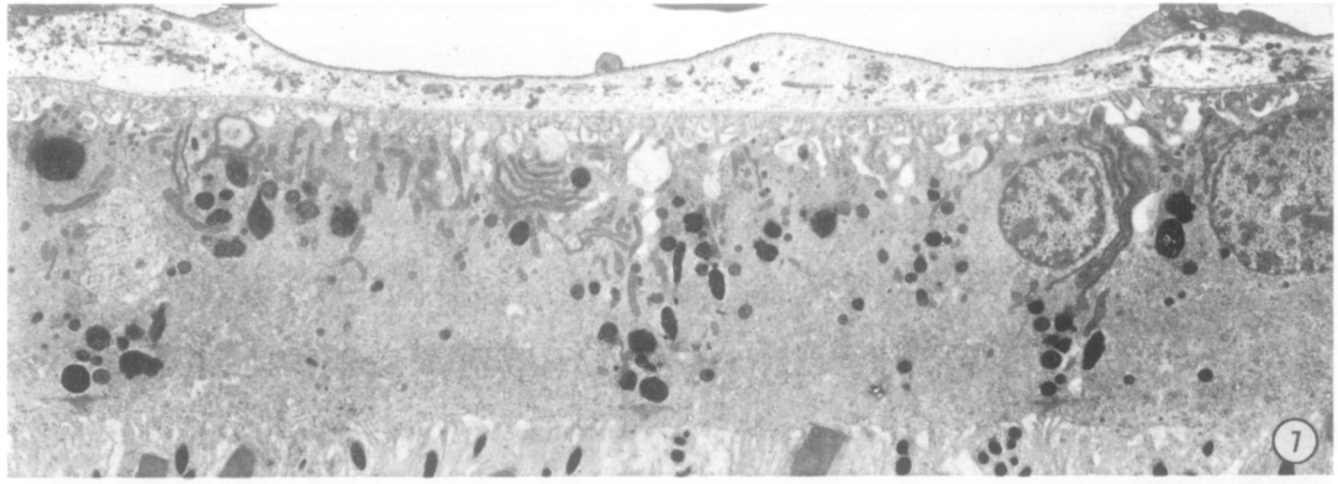


FIG. 6. Electron micrograph of extrafoveal RPE at 0500, 5 hr after light onset. Phagosomes tend to be located within the basal RPE cytoplasm (white-bordered arrows). A cone phagosome (black arrow) appears within the ensheathing processes. Rod outer segment (r) (×8100).



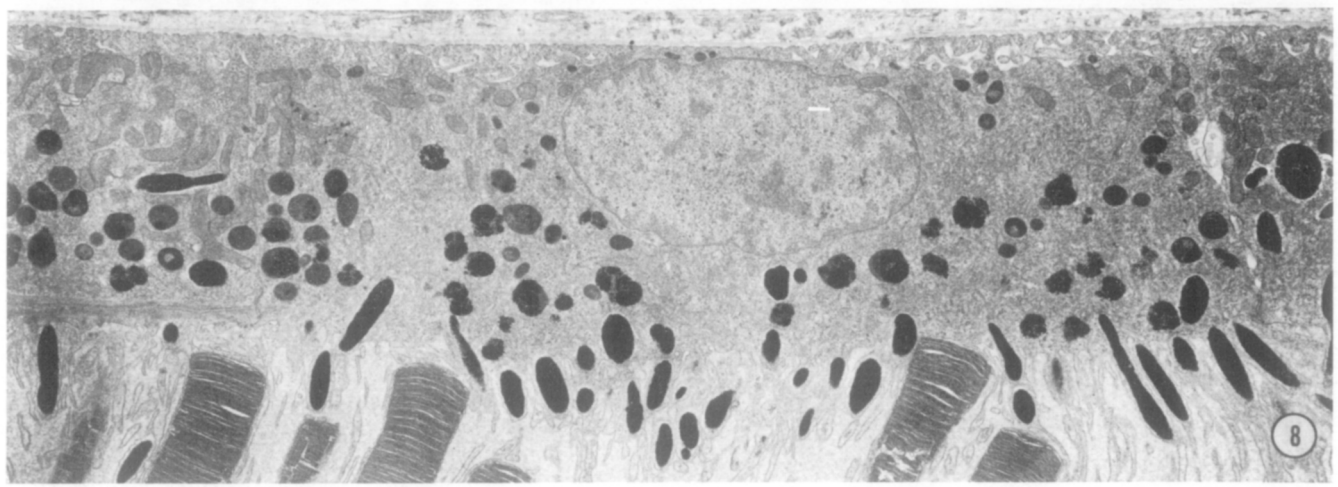


FIG. 7. Low power electron micrograph of the foveal RPE at 1300, 1 hr after light offset. Very few phagosomes are present (none are shown), and the number of residual bodies and other dark granules is low relative to their numbers in some of the other animals (see Figs 4–5) (×3510).

FIG. 8. Low power electron micrograph of the extrafoveal RPE at 1300, 1 hr after light offset. As in the fovea, phagosomes are scarce at this time in the lighting cycle (×4940).

words, for each monkey, approximately one out of five cones contained at least one phagosome within the ensheathing processes. The only noteworthy difference occurs in the animal fixed at 1300, when a somewhat smaller proportion of the cone sheaths contained phagosomes than at any other time. The difference between the proportions at 1300 and 1700 is statistically significant ($P < 0{\cdot}05$).* As shown in Fig. 10, about one out of four cone sheaths that did contain phagosomes had multiple phagosomes. The average proportion for all four animals was 0·26. Once again, the variability between animals was low. But there was a detectable, although not statistically significant, difference between the 1300 and 1700 monkeys.

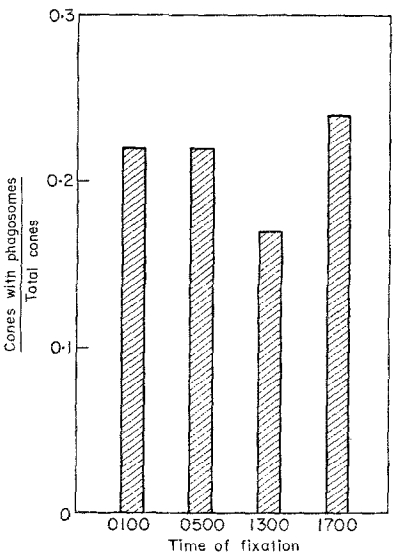


FIG. 9. Histogram showing the proportion of cones bearing one or more phagosomes within the ensheathing processes at each of the four times in the lighting cycle (total sample of over 400 cones/time point). Overall, about one out of five cone sheaths examined contained at least one phagosome. The shedding pattern in extrafoveal cones is similar to the shedding patterns shown in Fig. 1. This implies that a degree of synchrony exists between rod and cone disc shedding.

Figure 11 shows the predominant location of phagosomes identified within the cone sheaths for each fixation time. Phagosomes were arbitrarily placed into one of three categories: Those at or close to the cone tips were placed into the vitreal category; those about midway between the cone tips and apical RPE surface were placed into the midzone category; and those adjacent to the apical RPE surface were put into the scleral category. Thus, the total number of phagosomes was tabulated as a function of their location within the processes of the *supracone* space. This measure yielded some interesting differences between the animals. At 0100, there was a strong tendency for phagosomes to be situated next to the cone tips or at mid-level within the sheaths (Fig. 13). Less than one out of 10 phagosomes bordered the apical RPE surface. This same trend was clearly evident at 1700, although it was not quite so pronounced (Fig. 12). In contrast, the trend at 1300 was reversed. Only 23 out of 139 phagosomes were placed close to the cone tips. At 0500, most of the phagosomes were

* This is a Chi-square statistic used to test the difference between two proportions of two independent samples, where p_1 = proportion in sample 1; p_2 = proportion in sample 2; $p = (p_1+p_2)/2$ and $q = 1-p$; N_1 = observations in sample 1; N_2 = observations in sample 2.

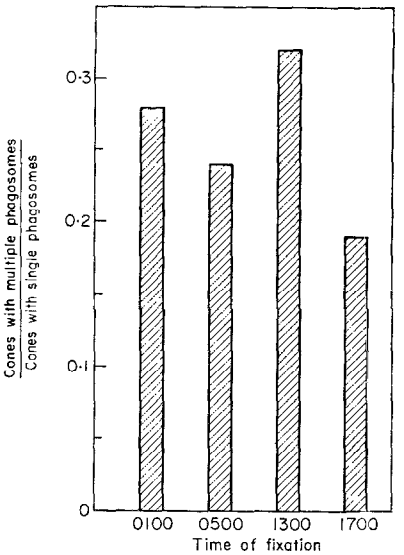


FIG. 10. This histogram shows the proportion of cone sheaths that contained multiple phagosomes at each of the four fixation times. The proportion was derived by forming the ratio of sheaths bearing multiple phagosomes to those bearing a single phagosome. Overall, about one out of four sheath contained more than one phagosome. The difference between the 1300 and 1700 proportions is statistically significant. (See note on p. 568).

localized at mid-level within the sheaths, with the remainder being nearly equally divided between those found at the cone tips and adjacent to the RPE surface.

The number of phagosomes within the vitreal part of the cone sheaths tends to decline during the light period and just after light offset, but to increase 5 hr following light offset. In contrast, phagosome counts within the scleral portion of the sheaths increase during the light period and immediately after light offset, and then fall 5 hr

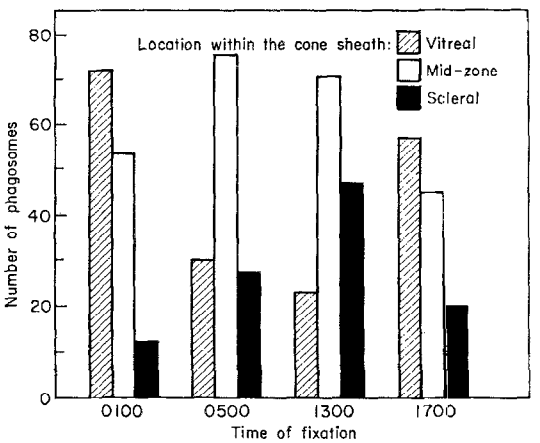


FIG. 11. Histogram showing the predominant location of phagosomes identified within the cone sheaths for each fixation time. Phagosomes were arbitrarily assigned to one of three categories: those near the cones were put into the vitreal category; those about midway between the outer segment tips and the apical RPE surface were placed into the mid-zone category; and those close to the apical RPE surface, but not within the fovea, were assigned to the scleral category. In this way, the number of phagosomes was tabulated as a function of their location within the *supracone space*.

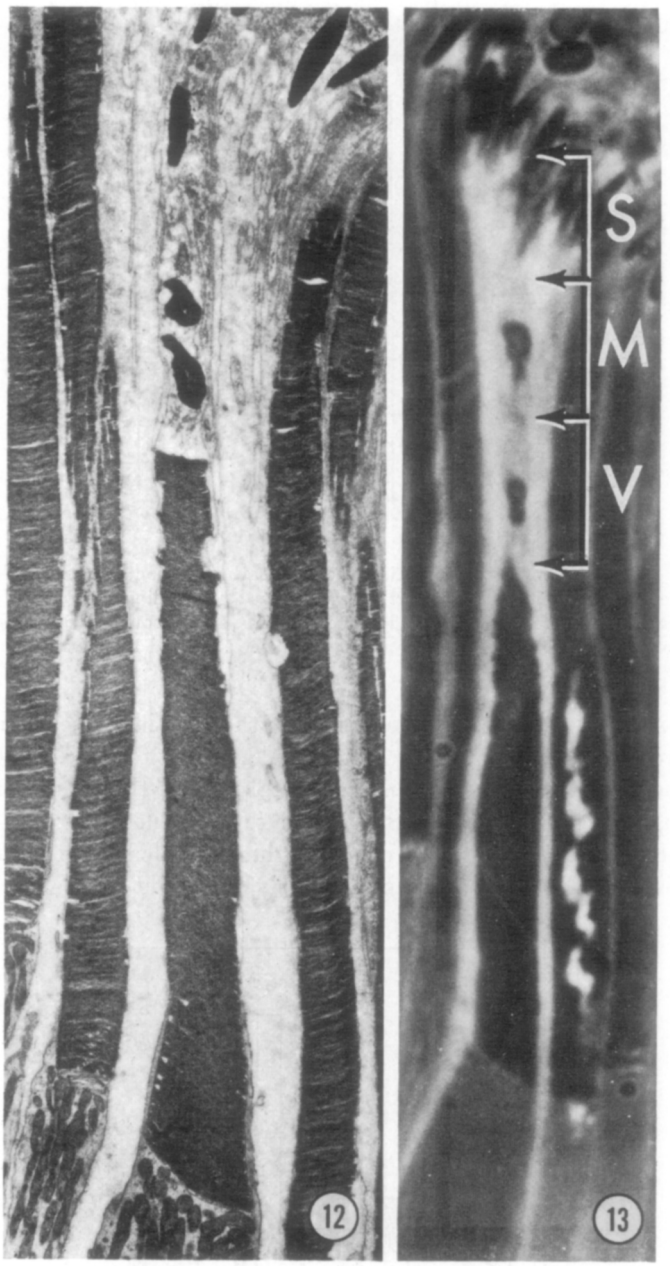


FIG. 12. Electron micrograph showing an extrafoveal cone outer segment from the rhesus monkey fixed at 1700, 5 hr after light offset. Two cone phagosomes are located close to the outer segment tips within the processes of the cone sheath (×9000).

FIG. 13. Light micrograph showing an extrafoveal cone from the monkey fixed at 0100, 1 hr after light onset. This is an example of the way extrafoveal cone phagosomes were identified and categorized using the light microscope. Two phagosomes are present within the cone sheath (brackets). One phagosome lies just above the outer segment tip and is categorized as being in the vitreal (V) part of the sheath. A second phagosome lies within the mid-zone (M) of the sheath. The scleral (S) portion of the sheath contains no phagosomes (×3750).

following the offset of light. It is 1 hr after light offset, at 1300, when vitreal phagosomes are lowest, scleral phagosomes are highest, and when the proportion of multiple phagosomes is greatest (Fig. 10).

4. Discussion

The rate of rod versus cone disc shedding in the monkey retina

Until quite recently, information concerning the rate of membrane replacement in the rods and cones of a single species was unavailable. The rate of replacement in cones could not be directly determined by autoradiographic techniques because the displacement rate of radioactive molecules within the outer segment could be traced only in rods (Young, 1969). Estimates of the disc shedding rates in several vertebrate retinas have now led to some rough comparisons between rods and cones in the rate of membrane turnover within the outer segments (Young and O'Day, 1978). These estimates suggest that the replacement rate for cones is less than it is for rods.

Insofar as the shedding rate can be used as a rough gauge of the membrane replacement rate, the phagosome counts at foveal versus extrafoveal locations in the monkey retina point toward the same conclusion (Fig. 1). Despite the fact that cone density within the foveal region greatly exceeds the maximum rod density (at the perifovea) (Young, 1971b), the maximum number of foveal phagosomes (cone-derived) that we found is only about one-third the maximum number of extrafoveal phagosomes (mainly rod-derived). Although there is a distinct possibility that cone shedding could be much greater at other times in the lighting cycle, we tentatively conclude that the rate of membrane replacement for monkey foveal cones is less than it is for rods.

Evidence for cyclic disc shedding in the monkey retina

Some substantial differences in phagosome levels are present in the monkeys fixed at different times in the lighting cycle, and the location of these phagosomes within the RPE appears to vary systematically between different animals. These observations can be summarized as follows: 1 hr following light onset there is a population of phagosomes situated immediately above the rod outer segment tips. A second population of smaller, partially degraded phagosomes lie adjacent to Bruch's membrane. Phagosomes derived from extrafoveal cones are found close to the cone tips. Within the fovea, the phagosomes are scattered throughout the RPE cytoplasm. Five hours after light onset rod phagosomes are not as numerous and they tend to be more basally positioned. Extrafoveal cone phagosomes are often found about midway within the ensheathing processes. Within the fovea, phagosomes are slightly more numerous than at 0100. One hour following light offset, phagosomes at both foveal and extrafoveal sites are scarce. Extra-foveal cone phagosomes, when present, tend to occupy the scleral part of the sheaths next to the apical RPE surface. The proportion of sheaths bearing phagosomes is lowest at this time; however, of those sheaths that do contain phagosomes, a higher proportion contain multiple phagosomes. Phagosome levels in both the foveal and extrafoveal RPE are highest 5 hr after light offset. Most of these phagosomes are found within the apical RPE, close to the outer segment tips, and they appear to be recently shed in that there are few morphological signs of degradation. Phagosomes derived from extrafoveal cones also appear close to the outer segment tips. More cone sheaths contain phagosomes at this time than at any of the other times.

On the basis of these findings from four animals, we cannot characterize a complete cycle of disc shedding activity in light-entrained monkeys. In addition to small sample size there are other potential influences, such as individual variability and age-related changes, that limit our ability to offer any definitive conclusions regarding the shedding cycle. As such, our findings must be regarded as provisional until an investigation utilizing many more animals is completed. Until then, these results should be interpreted as a first step in defining the temporal pattern of disc shedding in the primate retina. Despite these limitations, these results do suggest that many of the observed differences between animals, in both the number and location of phagosomes, probably reflect an underlying rhythm of disc shedding and phagocytosis. For example, all of the reports of rod shedding emphasize that a significant increase takes place within a couple of hours following light onset in animals entrained to cyclic illumination (LaVail, 1976; Basinger et al., 1977; Young, 1978a; O'Day and Young, 1978; Tamai et al., 1978; Tabor et al., 1979). The number and location of phagosomes observed above the rod tips 1 hr after light onset, and their diminution several hours later (at 0500), strongly suggests that the so-called "morning burst" also occurs in the monkey retina. The similarities between the rhesus monkey and human retinas implies that it occurs in human rods as well.

Thus far, there have been no indications that an increase in rod shedding takes place at any time other than after light onset. However, it is noteworthy that some rods do shed during the phase of maximal cone shedding in the tree squirrel retina—5–8 hr after light offset (Tabor et al., 1979; Tabor, Fisher and Anderson, 1980). In the 1700 monkey, the large number of phagosomes and their apical location within the extrafoveal RPE suggest that there may be another phase of increased rod shedding that occurs somewhere around the middle of the dark period. A "midnight" phase of increased rod shedding in the monkey retina could account for the phagosome population aligned along the basal RPE border in the 0100 monkey, Figs 4–5). These phagosomes could be the remnants of the shedding that occurred 8 hr previously. In any case, it seems probable that further work will show that both the timing and number of shedding peaks may vary from species to species.

Disc shedding by cones

An analogue of the shedding increase by rods following light onset is reported to occur in the cones of several vertebrates—in a species of diurnal lizard (Young, 1977), in the chick (Young, 1978a), in the goldfish (O'Day and Young, 1978), and the tree squirrel (Tabor et al., 1979, 1980). These experiments indicate that the majority of cone shedding takes place during the dark period. In the lizard and chick retinas, the increase follows shortly after the onset of darkness, while in the goldfish and squirrel retinas it may peak several hours later into the dark period.

Our results in the monkey retina are in only partial agreement with those obtained in other species. Unlike the situation in both the lizard and chick retinas (Young, 1977, 1978a), we find little evidence of cone shedding in the interval immediately following light offset. On the other hand, there are some indications from the counts and the location of phagosomes in the foveal RPE that shedding is underway several hours later at 1700.

Balanced against these observations are the counts from both the 0100 and 0500 monkeys which show foveal phagosome counts that are approximately equal to the 1700 value (Table I). Although many of these phagosomes tend to occupy more basal

locations within the foveal RPE, it seems unlikely that they are *only* the residuum from the phase of night-time shedding. The numbers (Fig. 9–10) and location (Fig. 11) of extrafoveal cone phagosomes at 0100 and 0500 also makes this interpretation appear unlikely. If most cone shedding in the monkey retina was restricted to the dark period, we would not have predicted such results.

Observations of primate extrafoveal cones in retinas fixed during the daytime hours also show multiple phagosomes within the RPE ensheathing processes (Steinberg et al., 1977; Anderson et al., 1978). We have seen as many as 6–7 phagosomes contained within a single cone sheath in some monkey retinas (Anderson and Fisher, unpublished observations). These observations imply that there is a considerable period of time during which the phagosomes remain within the processes, moving from the cone tips to the scleral portion of the sheaths. Thus, the number and location of phagosomes within the ensheathing processes reflects the shedding history of a particular cone for many hours or even days prior to the actual time of fixation (Steinberg et al., 1977). The results from the present study allow some speculation concerning the minimum time phagosomes require to transit the sheath.

The results shown in Fig. 11 demonstrate that there is a decline in the number of "vitreal" (i.e. recently shed) phagosomes and a concomitant rise in "scleral" (i.e. older) phagosomes between 0100 and 1300. This, in combination with the data in Fig. 10 which shows the greatest proportion of multiple phagosomes at 1300, suggests that within this 12 hr period there is a movement of phagosomes away from the cone tips and an accumulation of partially-degraded phagosomes within the scleral region of the sheaths. The decline in the number of "scleral" phagosomes at 1700 makes it likely that many of the phagosomes then enter the RPE cell bodies; however, the increase in "vitreal" phagosomes is another indication that an increase in cone shedding occurs around this time.

5. Conclusion

In addition to disc shedding and phagocytosis, there are other cellular events in the photoreceptors and RPE which recently have been shown to occur cyclically. In the frog retina RNA and glycoprotein synthesis (Hollyfield et al., 1979), the assembly of new outer segment discs (Besharse et al., 1977), and changes in RPE cell organelles (Matthes, Hoffman and Basinger, 1977) all have been reported to show rhythmic variations. In the teleost retina, the cones undergo length changes according to a circadian rhythm (Burnside, 1979). In the rat retina, diurnal variations in the rate of autophagy reportedly take place (Remé and Sulser, 1977). It has been suggested that daily rhythms, similar to those described in the above species, might also occur in the human retina (Young, 1978b). Although further work is clearly required our results in the closely-related rhesus monkey retina, coupled with descriptions of rod and cone disc shedding in the human retina (Spitznas and Hogan, 1970; Steinberg et al., 1977), make this possibility a near certainty.

ACKNOWLEDGMENTS

This research was supported by Research grants EY 02082 and EY 00888 from the National Eye Institute. The authors wish to thank Dr Walter Stern, in whose laboratory all of the surgical procedures were performed; Mr Joe Immel for computer assistance; and Mr Randy Falls for surgical assistance.

REFERENCES

Anderson, D. H. and Fisher, S. K. (1976). The photoreceptors of diurnal squirrels: outer segment structure, disc shedding, and protein renewal. *J. Ultrastruct. Res.* **55,** 119–41.

Anderson, D. H., Fisher, S. K. and Steinberg, R. H. (1978). Mammalian cones: disc shedding, phagocytosis, and renewal. *Invest. Ophthalmol. Vis. Sci.* **17,** 117–33.

Anderson, D. H. and Fisher, S. K. (1979). The relationship of primate foveal cones to the pigment epithelium. *J. Ultrastruct. Res.* **67,** 23–32.

Basinger, S. F., Hoffman, R. and Matthes, M. (1976). Photoreceptor shedding is initiated by light in the frog retina. *Science* **194,** 1074–6.

Besharse, J. C., Hollyfield, J. G. and Rayborn, M. E. (1977). Turnover of rod photoreceptor outer segments. II. Membrane addition and loss in relationship to light. *J. Cell Biol.* **75,** 507–27.

Burnside, M. B. (1979). Circadian effects on teleost cone length. *Invest. Ophthalmol. Vis. Sci.* (ARVO Abstracts) **18,** p. 227.

Cohen, A. I. (1961). The fine structure of the extrafoveal receptors of the rhesus monkey. *Exp. Eye Res.* **1,** 128–36.

Cohen, A. I. (1968). New evidence supporting the linkage to extracellular space of outer segment saccules of frog cones but not rods. *J. Cell Biol.* **37,** 424–44.

Goldman, A. I., O'Brien, P. and Teirstein, P. (1979). The effect of lighting schedules on rat ROS shedding rhythms. *Invest. Ophthalmol. Vis. Sci.* (ARVO Abstracts) **18,** 226.

Hollyfield, J. G., Basinger, S. F., Medford, D. I., Moss, M. E. and Rayborn, M. E. (1979). Cyclic metabolism of the retina and pigment epithelium. *Invest. Ophthalmol. Vis. Sci.* (ARVO Abstracts) **18,** p. 225.

LaVail, M. M. (1976). Rod outer segment disc shedding in the rat retina: Relationship to cyclic lighting. *Science* **194,** 1071–4.

LaVail, M. M. and Ward, P. A. (1978). Studies on hormonal control of circadian outer segment disc shedding in rat retina. *Invest. Ophthalmol. Vis. Sci.* **17,** 1189–92.

Matthes, M., Hoffman, R. and Basinger, S. F. (1977). Diurnal changes in morphology of frog pigment epithelium. *Invest. Ophthalmol. Vis. Sci.* (ARVO Abstracts) **16,** p. 73.

O'Day, W. T. and Young, R. W. (1978). Rhythmic daily shedding of outer segment membranes by visual cells in the goldfish. *J. Cell Biol.* **76,** 593–604.

Remé, C. and Sulser, M. (1900). Diurnal variation of autophagy in rod visual cells in the rat. *Albrecht v. Graefes Arch. klin. exp. Ophthal.* **203,** 261–70.

Spitznas, M. and Hogan, M. J. (1970). Outer segments of photoreceptors and the retinal pigment epithelium. *Arch. Ophthalmol.* **84,** 810–19.

Steinberg, R. H., Wood, I., and Hogan, M. J. (1977). Pigment epithelial ensheathment and phagocytosis of extrafoveal cones in human retina. *Phil. Trans. R. Soc. Lond.* [*Biol.*] **277,** 459–74.

Tabor, G. A., Fisher, S. K. and Anderson, D. H. (1979). Evidence for a circadian rhythm of disc shedding in light-entrained gray squirrels. *Invest. Ophthalmol. Vis. Sci.* (ARVO abstracts) **18,** p. 81.

Tabor, G. A., Fisher, S. K. and Anderson, D. H. (1980). Rod and cone disc shedding in light-entrained tree squirrels. *Exp. Eye Res.* **30,** 545–57.

Tamai, M., Teirstein, P., Goldman, A., O'Brien, P. and Chader, G. (1978). The pineal gland does not control rod outer segment shedding and phagocytosis in the rat retina and pigment epithelium. *Invest. Ophthalmol. Vis. Sci.* **17,** 558–62.

Young, R. W. (1971a). Shedding of discs from rod outer segments in the rhesus monkey. *J. Ultrastruct. Res.* **34,** 190–203.

Young, R. W. (1971b). The renewal of rod and cone outer segments in the rhesus monkey. *J. Cell Biol.* **49,** 303–18.

Young, R. W. (1976). Visual cells and the concept of renewal. *Invest. Ophthalmol. Vis. Sci.* **15,** 700–725.

Young, R. W. (1977). The daily rhythm of shedding and degradation of cone outer segment membranes in the lizard retina. *J. Ultrastruct. Res.* **61,** 172–85.

Young, R. W. (1978a). The daily rhythm of shedding and degradation of rod and cone outer segment membranes in the chick retina. *Invest. Ophthalmol. Vis. Sci.* **17,** 105–16.

Young, R. W. (1978b). Visual cells, daily rhythms, and vision research. *Vision Res.* **18,** 573–8.