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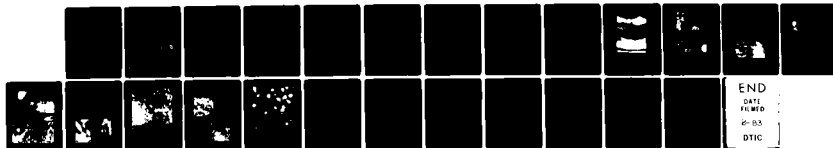
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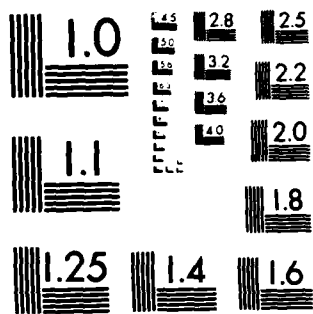
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first subject received 24 cumulative hours of exposure, the second, 40 hours and the third, 42 hours. The radiances of the hemisphere were 6, 12 and 440 uw/cm²/sr, respectively. The eyes were enucleated 4, 11, and 7 days after the last exposure. Observations were made on 1.3 mm long sections through the foveola (temporal to nasal). The number of basal bodies (4.9 ± 1.3 BB/mm) and striated rootlets (7.6 ± 1.3 SR/mm) in the apex of the RPE cells of the exposed eyes was 2.5 the number observed in the occluded eye (2.0 ± 1.2 BB/mm and 3.0 ± 1.6 SR/mm) for the first two animals. In these exposed maculas, the outer segments were separated from the RPE and the space was filled with proteinaceous fluid. No separation was observed in the maculas of the occluded eyes or eyes of the third animal which showed a smaller increase in the number of BBs and SRs. The increase in BBs and SRs was correlated with exposure to DALR and may represent the lower end of a continuum of morphologic change that is initiated by light in the RPE. These results suggest changes in the metabolic milieu of the RPE - photoreceptor relationship which may be related to the observation of the speckle pattern produced by coherent sources.

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**BASAL BODY AND STRIATED ROOTLET CHANGES
IN PRIMATE MACULAR RETINAL PIGMENTED EPITHELIUM
AFTER LOW LEVEL DIFFUSE ARGON LASER RADIATION**

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and
DIVISION OF OCULAR HAZARDS**

SEPTEMBER 1982

**LETTERMAN ARMY INSTITUTE OF RESEARCH
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-- Schuschereba, Zwick, Stuck, and Beatrice**

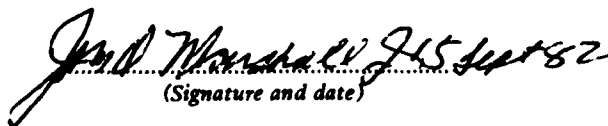
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**BASAL BODY AND STRIATED ROOTLET CHANGES IN PRIMATE MACULAR RETINAL
PIGMENTED EPITHELIUM AFTER LOW LEVEL DIFFUSE ARGON LASER RADIATION**

-Schuschereba, Zwick, Stuck, and Beatrice

ABSTRACT

Basal bodies or centrioles (BB - microtubule organizing centers) and striated rootlets (SR - bundles of 60 A actin-like filaments) have a close association in primate retinal pigmented epithelial (RPE) cells. The frequency of occurrence of these structures was evaluated in the macular RPE after repeated exposure to low level diffuse argon laser radiation (DALR). The awake chaired animal's head was restrained and positioned near the center of a 0.75 m hemisphere which was diffusely irradiated with 514.5 nm laser radiation. The right eye of each subject was occluded during the two hour exposure session. The first subject received 24 cumulative hours of exposure, the second, 40 hours and the third, 42 hours. The radiances of the hemisphere were 6, 12 and 440 uw/cm²/sr respectively. The eyes were enucleated 4, 11 and 7 days after the last exposure. Observations were made on 1.3 mm long sections through the foveola (temporal to nasal). The number of basal bodies (4.9 + 1.3 BB/mm) and striated rootlets (7.6 + 1.3 SR/mm) in the apex of the RPE cells of the exposed eyes was 2.5 times the number observed in the occluded eye (2.0 + 1.2 BB/mm and 3.0 + 1.6 SR/mm) for the first two animals. In these exposed maculas, the outer segments were separated from the RPE and the space was filled with proteinaceous fluid. No separation was observed in the maculas of the occluded eyes or eyes of the third animal which showed a smaller increase in the number of BBs and SRs. The increase in BBs and SRs was correlated with exposure to DALR and may represent the lower end of a continuum of morphologic change that is initiated by light in the RPE. These results suggest changes in the metabolic milieu of the RPE - photoreceptor relationship which may be related to the observation of the speckle pattern produced by diffuse coherent sources.

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**BASAL BODY AND STRIATED ROOTLET CHANGES IN PRIMATE MACULAR RETINAL
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--Schuschereba, Zwick, Stuck, Beatrice

In recent years, much has been done in the area of low-level light effects on the retinal; this has been summarized by Lianum (1). Such effects result from nonthermal rather than thermal damage mechanisms. Numerous explanations have been proposed. Most evidence suggests that nonthermal retinal effects (photochemical) are restrictive to the short and intermediate visible and near visible spectrum. Photochemical damage processes may reside in either the retinal pigment epithelium (RPE), the photoreceptor, or between the photoreceptor and the RPE and may represent a breakdown of complex metabolic processes that normally occur between the photoreceptors and the RPE. We have examined a novel morphologic aspect of this problem, the distribution of basal bodies and striated rootlets in the retinas of monkeys exposed to chronic diffuse visible laser light.

Basal bodies (BB) or centrioles are widely known as microtubule organizing centers of the cell. They contain a ninefold symmetry of microtubule doublets or triplets and represent the preferred site within the cell for microtubule polymerization. They usually occur in pairs, at right angles to each other, and seem to divide autonomously. Striated rootlets (SR), bundles of 60 Å actin-like filaments cross-linked with a longitudinal periodicity of about 700 Å are often associated with BBs. Little is actually known about the functional significance of the striated rootlets (2). Both of these structures have a close association in the apical portion of primate retinal pigment epithelial cells. While the relationship of these structures to other organelles remains unclear for now, their significance to visual function may be of consequence. Recent morphological investigations of mammalian photoreceptors indicate that the striated rootlet courses the entire length of both Rhesus monkey rods and cones (3). Furthermore, recent psychophysical evidence suggests that the photoreceptors may have the ability to make small alignment changes (4,5) which could involve the striated rootlet system. The present experiment was initiated to examine the subtleties of morphologic change possibly correlated with alterations in spectral sensitivities produced in Rhesus monkeys exposed to low levels of diffuse argon laser radiation (DALR) (6). In earlier work, we were unable to distinguish morphological alterations between exposed and nonexposed eyes (7), although outer segment lamellae changes have been a common characteristic found in mammalian retinas exposed to low-level light (1). This work attempts to correlate the more subtle aspects of

retinal alteration with FAHR. Preliminary observations have been reported elsewhere (8).

METHODS

Three untrained Rhesus monkeys (*Macaca mulatta*, approximately 7 years old weighing about 3.5 kg) with normal appearing fundi were exposed to diffuse argon laser radiation at 514.5 nm. The chaired animal's head was restrained and positioned near the center of a 0.75 m hemisphere located inside a standard primate cubicle. The hemisphere was irradiated nearly uniformly by reflecting the laser beam into the cubicle and diffusing the radiation onto its surface (6,7). The right eye of each subject was occluded during the two hour exposure sessions. The measured pupil diameters of the three subjects after adapting to the stimulus ranged from 4.0 to 4.4 mm. Animal 1 received 24 cumulative hours of exposure (average hemisphere radiance = $6.1 \mu\text{W}/\text{cm}^2/\text{sr}$) in 12 sessions over an 18-day period. Enucleation occurred 5 days following the last exposure session. Animal 2 received 40 cumulative hours of exposure (average hemisphere radiance = $12 \mu\text{W}/\text{cm}^2/\text{sr}$) in 20 sessions over a 37-day period. Enucleation occurred 11 days after the last exposure session. Animal 3 received 42 cumulative hours of exposure (average hemisphere radiance = $440 \mu\text{W}/\text{cm}^2/\text{sr}$) in 21 sessions over a 42-day period. Enucleation occurred 7 days after the last exposure session.

Two nonexposed maculas from two other Rhesus monkeys (animals A and B) were evaluated as controls (Table I). All enucleations were performed approximately at 10:00 a.m. on the day of sacrifice (tranquilized with 50 mg/kg ketamine intramuscularly and anesthetized with 10 mg/kg of sodium pentobarbital intravenously).

The enucleated eyes were fixed in 3% glutaraldehyde-2% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) at room temperature. The maculas were isolated in nasotemporal blocks (1.5 mm long x 0.5 mm wide). After a brief rinse, blocks were en bloc stained in aqueous 2% uranyl acetate then processed by routine transmission electron microscopy (TEM) techniques. Plastic one micron serial sections were cut until the foveola was reached. Blocks with the foveola in the center were thin sectioned, and non-serial sections were collected on formvar coated slot grids and stained with ethanolic 2% uranyl acetate and lead citrate. In defect-free sections the apex of the RPE was scanned at 7000x (TEM). The number of isolated striated rootlet bundles and basal bodies was counted for each macula and standardized for 1 mm length of RPE (Table 1).

TABLE 1

Rootlets and Centrioles in Macular Retinal Pigment Epithelium*

SUBJECTS	EXPOSED EYES		OCCLUDED EYES	
	ROOTLETS/mm	CENTRIOLES/mm	ROOTLETS/mm	CENTRIOLES/mm
1	8.3 $\pm$ 1.2	5.5 $\pm$ 1.5	3.0 $\pm$ 1.5	2.2 $\pm$ 1.1
2	6.9 $\pm$ 1.1	4.2 $\pm$ 0.71	2.6 $\pm$ 1.7	2.1 $\pm$ 1.4
3	4.9 $\pm$ 3.2	4.3 $\pm$ 2.5	4.4 $\pm$ 1.5	2.7 $\pm$ 0.92
CONTROL EYES				
	2.6 $\pm$ 1.2	0.9 $\pm$ 0.40		
	3.5 $\pm$ 1.0	2.0 $\pm$ 0.60		

*The mean number of striated rootlet filament bundles and basal bodies (centrioles) per unit length of retinal pigment epithelium through the foveola. Striated rootlets and basal bodies were counted in 8-12 nasotemporal sections at the TEM level. The total length of each section evaluated was approximately 1.3 mm and the foveola was situated approximately in the center.

RESULTS

Light and electron micrographic evidence of the nonexposed and exposed retinas of Rhesus monkeys appears in Figures 1 through 7.

While a separation of photoreceptor outer segments from the RPE was observed in sections through the foveola of the exposed eyes of animals 1 and 2 (Figures 1b and 2b) no separation was observed in any of the nonexposed eyes (Figures 1a and 2a) nor in the exposed eye of animal 3. The measured area of the largest observed separation (animal 2) was 0.2 mm² centered on the foveola and was associated with the longest time between the last exposure and enucleation (11 days). The subretinal space produced by the separation was filled with proteinaceous fluid.



Figure 1. a. Light micrograph of nonexposed rhesus macular (nasal-temporal section). b. Light micrograph of animal 2 (Table 1) after repeated low level exposure to diffuse argon laser radiation (DALR). A diminished outersegment-retinal pigment epithelial adhesion is present (small arrows). Peripheral zone shows more normal outersegment adhesion to RPE (large arrows).

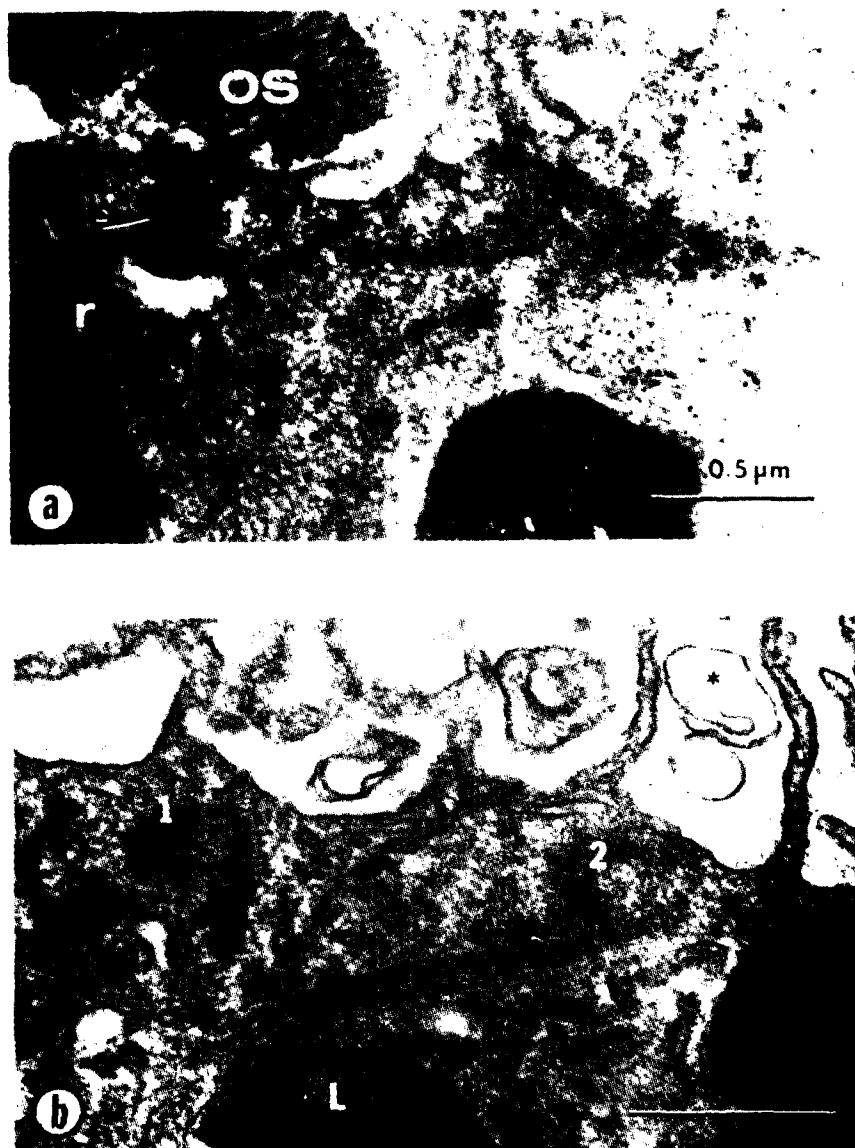


Figure 2. **a.** Electron micrograph on nonexposed rhesus macular RPE. The basal body (arrow) and rootlet (r) in the cell apex are closely associated with a photoreceptor outersegment (OS). **b.** Electron micrograph of rhesus macula exposed to diffuse argon laser radiation (DAIRR). Two basal bodies (1 and 2) appear to have interconnecting rootlets. Asterisk (*) represents zone of outersegment separation from RPE. Lysosome = L.

An increase in the number of macular basal bodies and striated rootlets per millimeter of RPE section was observed in the exposed eyes when compared with values obtained in nonexposed eyes (Table 1). Both the basal bodies and rootlets were primarily observed in the cell apex, near the cell border. In some sections, two or more basal bodies were observed in the same cell with associated rootlets which suggests interconnection (Figures 2b, 2c and 3a). Both rootlets and microtubules of the cilium appeared more prominent in exposed eyes (Figures 3b, 5b and 7a) than in nonexposed eyes (Figures 2a and 5a). The basal body of the cilium in exposed eyes appeared normal and was anchored in the cellular cytoplasm (Figures 3b and 3b inset 1) while the ciliary microtubules were present in an intracellular space (Figures 3b and inset 2).

The rootlets in the exposed maculas were observed in the base of microvilli of the fovea (Figure 4a) and in the base of lamellapodia that contact cone outer segments in the paramacula (Figure 4b). In addition, exposed eyes showed an increased association of rootlets with many organelles (lysosomes - Figures 2b and 7a, mitochondria - Figure 4b, melanin granules - Figure 5b, golgi - Figure 6c, smooth endoplasmic reticulum - Figures 6a, b and 7c, and nucleus - Figure 7b). Rootlet periodicities (640 to 760 Å per striation), the number of cells/mm of the RPE, and the thickness of the RPE in exposed eyes were comparable to values obtained in nonexposed eyes.

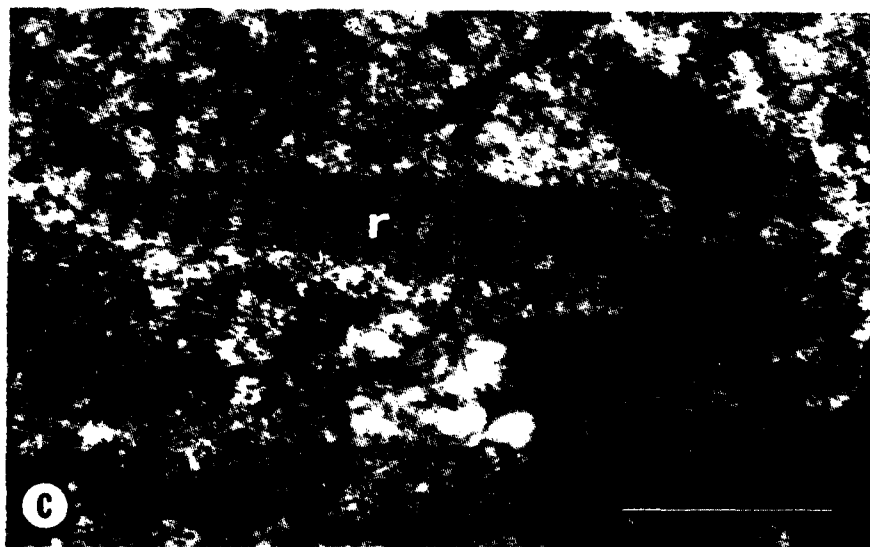


Figure 2, concluded. C. Electron micrograph of cross-section of RPE apex of exposed rhesus macula shows two basal bodies connected by single rootlet (r). Cell border is at the lower right. (Bars on all electron micrographs = 0.5 μ m.)



Figure 3. Electron micrographs of rhesus macaque exposed to DAFR. **a.** Three dense regions (arrows) suggestive of basal bodies appear adjacent to two bundles of rootlets. **b.** An inwardly directed cilium with well-developed microtubules (arrow) is present in an intracellular space (*). Rootlet bundle (R) courses horizontally to the cell apex. **Inset 1.** Cross section of a basal body in plane of section 1. Basal body is surrounded by cytoplasm and comprised of 9x0 triplets (microtubule granule (MD)). **Inset 2.** Cross section of the cilium microtubules in plane of section 2. Cilium is surrounded by an intracellular space (*) and is comprised of 9x0 doublets. (Bars = 0.5 μ m).

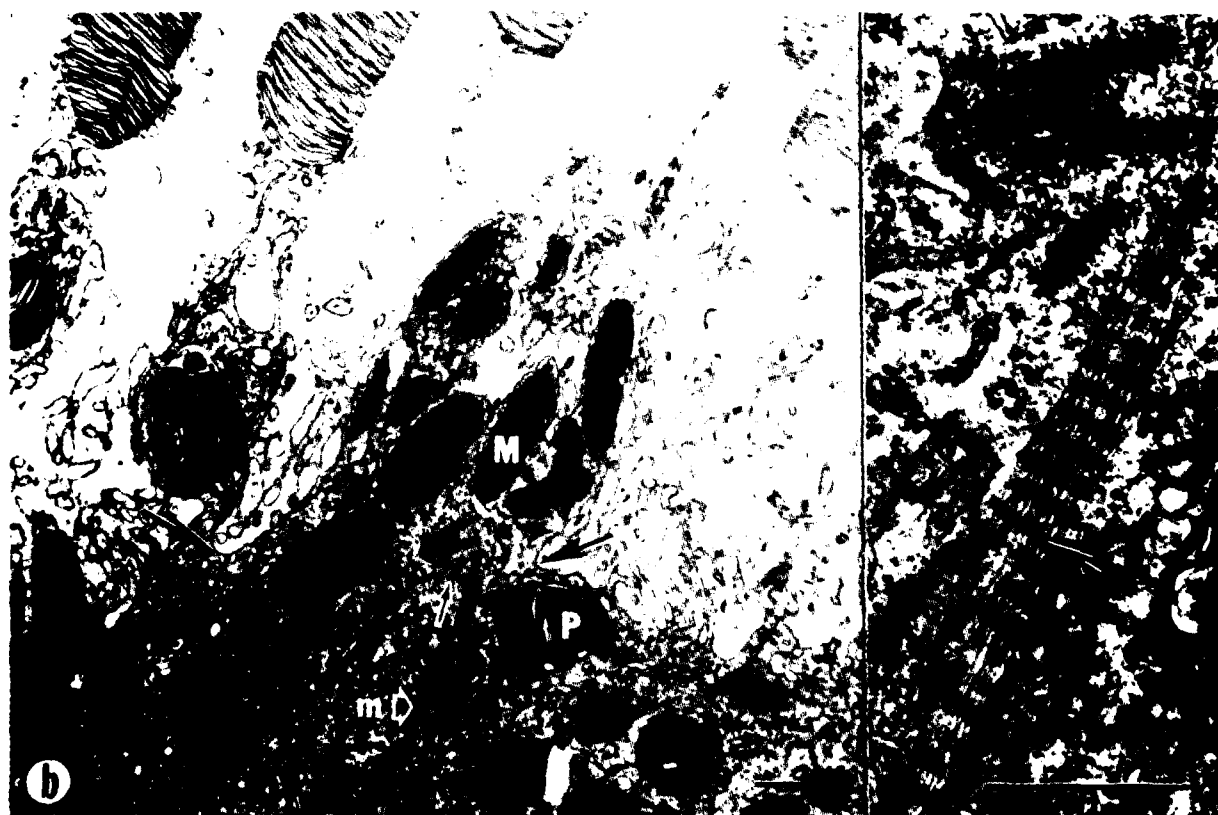


Figure 4 (opposite page). **a.** Electron micrograph of rhesus macula exposed to DALR. Rootlet filaments (**r**) in the RPE apex are oriented in register with the long axis of the foveal RPE microvilli (**MV**). **b.** Electron micrograph of paramacular region exposed to DALR. A striated rootlet-basal body complex is located at the base of an RPE lamellapodium that extends to and contacts the short cone outer segments. The large cytoplasmic process is delimited by large arrows in longitudinal section. A dense body between the rootlet and basal body (*small arrow*) is suggestive of a secondary basal body. The striated rootlet and basal body complex plus the cytoplasmic extension represents a uniquely specialized region of the RPE that is associated with cones. (**M**) = melanin granule, (**m**) = mitochondria, and (**p**) = phagosome. *Inset*, electron micrograph of rhesus paramacula exposed to DALR. High magnification of rootlet pictured in *Figure 4b*. The dark striations are formed by increased thickness and cross-bridging of rootlet filaments (*arrow*). Bars = 0.5 μ m.

Figure 5 (below). **a.** Electron micrograph of nonexposed rhesus macula. A basal body is directed nearly horizontal with the RPE apex at the base of a microvillus. No microtubular cilium development is evident (*). (**M**) = melanin granule. **b.** Electron micrograph of rhesus macula exposed to DALR. A basal body is directed inwardly and is situated below a melanin granule (**M**) of a microvillus. An asterisk (*) denotes the microtubular development of the cilium within an intracellular space. The difference can be seen by comparing the sites on **a** and **b**. A rootlet (**r**) appears to insert into a dense granule (*arrow*) located between the basal body and melanin granule.

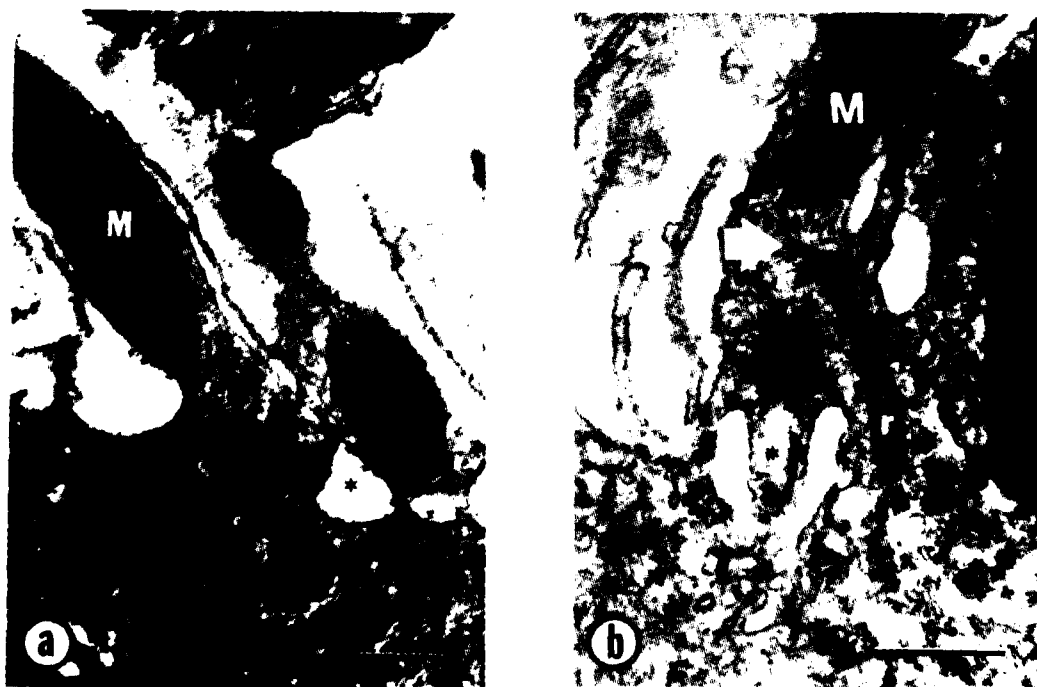


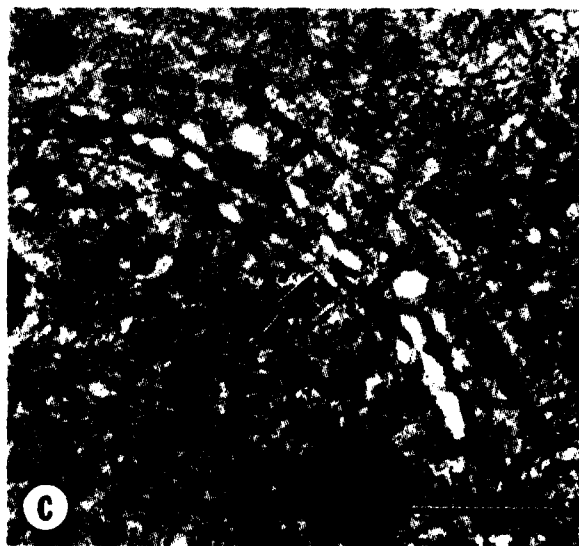
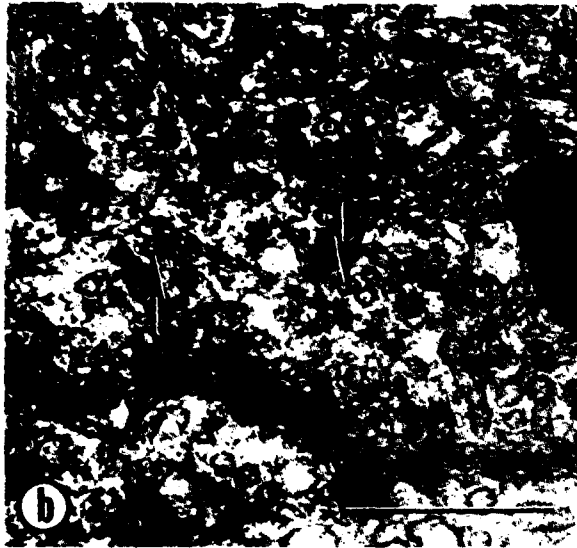


Figure 6. Electron micrograph of rhesus macula exposed to DALR. **a.** (above) Zones of dense smooth endoplasmic reticulum (small arrows) are present throughout the RPE cell. A rootlet (large arrow) appears to associate closely with the zones of dense SER --continue, opposite page, b and c.

Figure 6, concluded.

b (*below, left*). Higher magnification of rootlet pictured in *Figure 6a*. Note close association to normal SER (arrows).

c (*below, right*). A rootlet (r) closely courses around a Golgi apparatus (arrow).



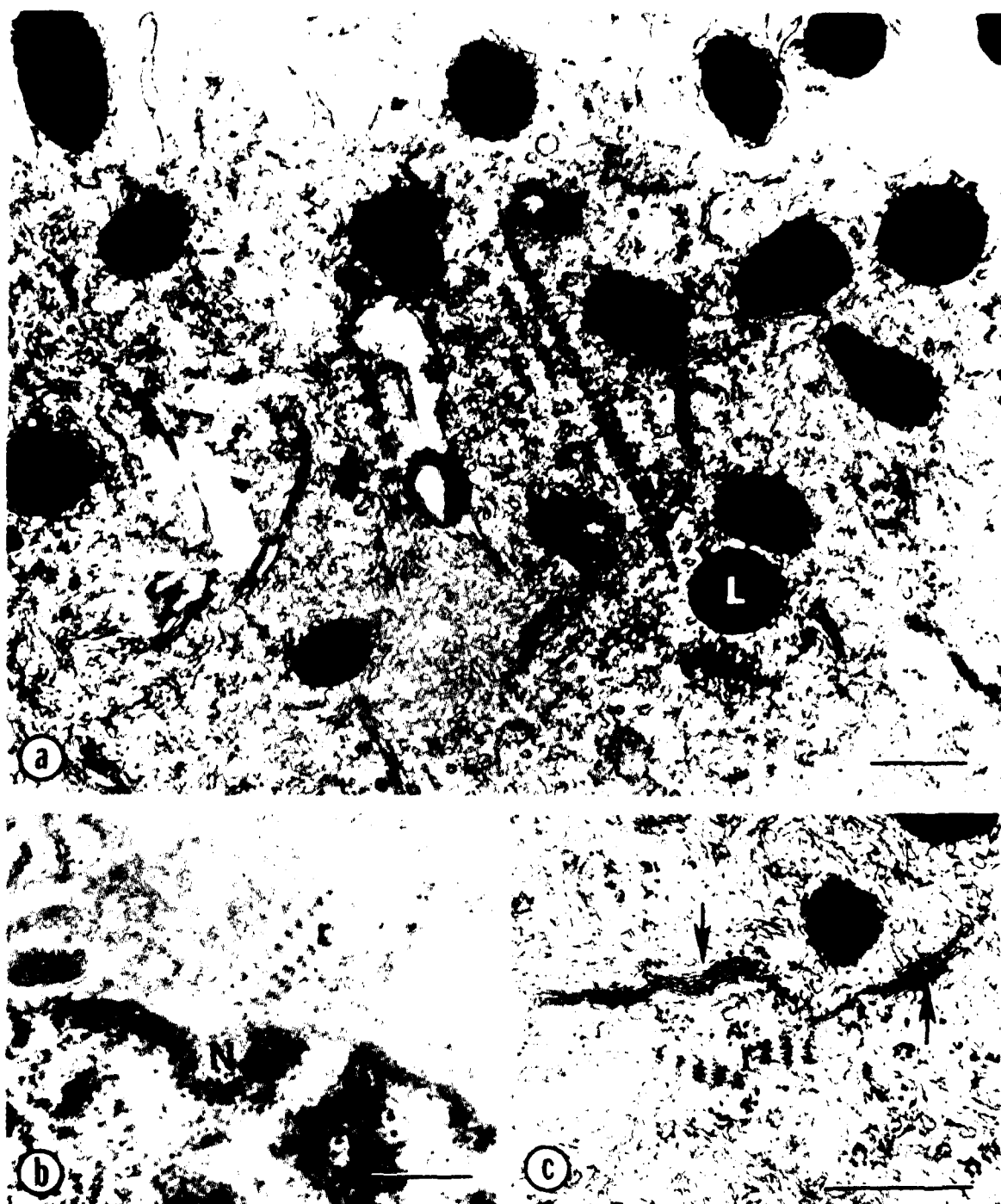


Figure 7. Electron micrographs of rhesus macula exposed to DALR. **a** (*top*) A well-developed rootlet courses from a basal body toward a lysosomal granule (L). The rootlet filaments taper at both ends suggesting an origin at the basal body and an end near the lysosomal granule. **b** (*bottom left*) Rootlet filaments pass near an RPE nucleus (N). **c** (*bottom right*) Two rootlet fragments (r) are closely associated with the dense regions of the SER (arrows).

DISCUSSION

Light effects to the macula have not been widely reported. Previous studies documenting either photoreceptor or RPE changes have utilized light sources that are greater than those encountered in this experiment. Furthermore, we are reporting on the chronic effects of low-level light exposure to the macula with the evaluation of tissue 2 hr after the last exposure.

After DALR, BB and SR complexes increase and form a more elaborate communication network in the macular RPE apex between the cellular organelles. The SR and BB network appears to be located at the base of the apical processes that communicate with cone outer segments (9). The increased occurrence of BBs and SRs in the RPE after the maculas are exposed to DALR strongly suggests that these structures have a greater involvement with some cone-specific function than previously suspected. This contention is supported, first by their increased occurrence in the base of paramacular cone sheaths and, secondly, by their increase in the cone-rich region of the fovea.

These morphologic changes to low-level coherent light have not been reported previously for similar light exposure conditions. While they may be unique in this regard, they may represent the lower end of a continuum of morphologic changes that are initiated in the RPE. Previous investigations (1) that have stressed the RPE as a mediating influence in low-level light effects implied a close interrelationship between photoreceptor metabolic requirements and the role of the RPE in such requirements.

An important consideration in the present results is that the effects were observed in the central retina. It should be noted that diffuse environmental light exposure in humans tends to affect visual functions that are more central in their retinal mediation (10), and recent investigations (11) in Rhesus monkeys where diffuse fluorescent light exposure was employed support this notion. In this latter study (11) paramacular sites were observed to be less sensitive to low level light alteration than macular sites. In parallel behavioral investigations where DALR was employed spectral sensitivities for central retinal functions were affected first and in fact indicated that the reduction of such central vision resulted in a slight increase in sensitivity for peripheral visual processes (6).

No changes between exposed and nonexposed eyes could be made based on gross photoreceptor outer segment (OS) or RPE alteration. Sykes et al. (11) report on a rod and cone differential in OS alteration (macular) as a result of fluorescent light exposure. Tso and Fine (12), however, utilizing a single 600 μ m spot size from an argon laser (488 nm) in the macula do not show a similar OS alteration trend. Since Sykes et al. (11) sacrificed their animals 15 hr after

the last exposure, the changes seen in the rod OSs may be a result of later RPE decompensation that may affect the functional integrity of the photoreceptors. As a result the morphologic characterization of light-induced lesions maybe clearly dependent on such variables as exposure time, recovery time, intensity, and spectral characteristics.

Several other investigations (13-15) involving the delineation of nonthermal retinal mechanisms have implicated important structures in the RPE without necessarily singling them out in the macula. Ham et al. (13) point to a hypopigmentation of the RPE occurring to short wavelength light and have suggested that the photochemical mechanism for this effect lies in the ability of the melanin to respond to highly energetic short wavelength light in a manner that is different from its response to less energetic long wavelength light, i.e. in a photochemical manner to short wavelength light and in a thermal manner to long wavelength light. Corroberating action spectra have been obtained by Lawwill et al. (14) but with much less specification of an actual morphological site in the retina for the mediation of these processes.

Another report (12) documenting changes to the macula from argon laser exposure argues that the RPE is functionally decompensated and that it later affects the retina. Our findings tend to support the concepts that coherent light induced changes are a result of a reaction within the RPE or are due to some interaction between the RPE and photoreceptors.

If the RPE is the first to show light-induced changes it may be a result of a gradual decompensation with failure to maintain the blood-retinal barrier, resulting in edema, which we believe is responsible for the small (0.2 mm^2) subretinal separation confined to the foveola. Depending on animal variability, the amount of adaptation, and the time of sacrifice after the last exposure, these conditions may or may not be manifested. In addition, the changes reported in this study are reported at the quantitative level. These changes may not be obvious when observation is random and unsystematic.

Tso and Fine (12) also reported accumulation of edema 4 years after argon laser exposure. In addition, the occurrence of intracytoplasmic filaments or membranes and the large collection of oriented and banded basement membranes 4 years after a single 1.0 to 1.5 W/20 min argon laser exposure have also been documented by Tso and Fine (12). These changes were related to a chronic RPE decompensation effect with resultant cytoid changes (12).

The animal that received the highest dose in our study (animal 3) showed inner retinal changes similar to what Tso and Fine (12) called cytoid changes. In this animal, however, we observed a lower SR and BB count. Higher energy retinal exposures may preclude detection

of these structures. We report these changes after 40 days with cumulative low-level exposures while Tso and Fine (12) report these changes 4 years later in higher energy lesions.

The increased development of the basal body microtubules after DALR appears to result in an inwardly directed cilium that is similar in appearance to that found within photoreceptor cells (16). Cilia have previously been reported to be a normal constituent of embryonic mammalian RPE but not of the adult (17). The increased development of RPE cilia after DALR suggests that the cell may be reverting to a more embryonic stage, perhaps directing its cytoplasmic milieu for proliferation after an insult (18).

Others (19) have suggested that rootlets orient the cilia or coordinate their beat. Indeed investigators (20,21) have reported localization of myosin antibodies and actin-like filaments in the RPE apex. Since active processes have been implicated in the RPE apex for photoreceptor alignment toward the exit pupil (22), the BB and SR may subserve this function. A similar structure has been recently reported to traverse the entire course of the rod photoreceptor in mammalian retina (23) and more recently are reported in both rods and cones of Rhesus monkey retina (3). While the response of such systems to light is unknown, their complexity and frequency of occurrence suggests activation by coherent light.

Still others (24) have suggested that cilia are concerned with regulating the ionic balance between rods, cones, and the RPE. Undoubtedly, factors influencing the RPE-photoreceptor processes, such as, phagocytosis and retinal adhesion, would influence ionic regulation. The increase in BBs and SRs and the development of basal body microtubules may be a consequence of an ionic imbalance after DALR.

The possibility that the morphologic effects observed in the present study are related to the coherency of our diffuse light source should be considered (25). While previous low-level light experiments have been done with incoherent light exposure, the present study involved coherent light at very low exposure power levels. Low-level coherent light exposure may involve a different mechanism for visual functional alteration (13). The speckle pattern in the retinal plane (independent of the ocular optics) is a random distribution of intensity produced by constructive and destructive interference. This pattern contains diffraction limited irradiated areas resulting in a stimulus with high spatial frequencies and unity contrast, that moves with small eye or head movements. Theoretical calculations (26) indicate that there is a small but finite probability that peak retinal irradiances can be greater than a factor of 100 above the average retinal irradiance. This speckle property when coupled with the narrow laser bandwidth (less than 0.05 nm) uniquely stimulates the

retinal photoreceptors and the RPE and may produce morphologic changes.

CONCLUSIONS

Our morphologic observations show that increases in the macular RPE BBs and SRs and concomitant foveal separation are associated with repeated exposure to DALR at average retinal irradiances well below those required to produce either an acute thermal injury or those levels maximally encountered in our environment that produce nonthermal effects. Although the functional significance of BB and SR increases in the RPE are not clear, these increases may reflect functional or metabolic changes in either the RPE, the macular cones, or both. We wish to stress, however, that the mechanism for retinal alteration underlying the changes observed in this experiment may be unique, differing from previous metabolic nonthermal types of mechanisms described in other low-level light studies. How much of the morphologic change represents damage remains unknown. Our findings stress the RPE as a site of alteration as opposed to the photoreceptors. Yet, the structures we have discussed are closely related to photoreceptors and perhaps their metabolic processes. It remains to be seen if similar increases in BBs and SRs occur in more peripheral retinal regions after DALR.

RECOMMENDATION

- It is recommended that the current study be continued and that correlative data on paramacular with macular sites be obtained.
- Additional studies should be conducted using more animals to support these findings.
- Other studies should also be initiated to determine the underlying physiological and functional ramifications of the increase in striated rootlets and basal bodies in the retinal pigment epithelium. The physiological and functional implications should be assessed in terms of the laser-safety objectives. Specifically, it is strongly recommended that previous interactions with Army laser system developers be continued to insure maximum optimization of all laser system parameters based on these research findings.
- A correlative study utilizing behavioral, morphological and biochemical approaches should help reveal the significance of the present findings.

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