Unlike the results from mucopolysaccharide staining, no change is detected in staining for IRBP prior to photoreceptor degeneration. The intensity of anti-IRBP staining decreases in the RCS rat after P18, paralleling temporally the loss of photoreceptors. These findings suggest that IRBP may be synthesized in the photoreceptors but is not abnormal in amount or distribution prior to the disorganization of outer segments found in the RCS model of inherited retinal degeneration.

Key words: retinoid-binding protein, development, retina, RCS rat retina, intercellular retinoid-binding protein

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References

Structural Periodicities Observed in Mammalian Rod Outer Segments with Nomarski Optics

Lory D. Andrews

Structural periodicities have been observed in isolated rod outer segments of several mammalian species using differential interference contrast (Nomarski) optics. The spacing among the observed structural inhomogeneities is somewhat variable. If these measured spacings are corrected for shrinkage, estimated by comparing ROS widths in unfixed cells to those in light microscopic autoradiographs, the mean values correlate well with published rates of ROS renewal in rats and dogs. Invest Ophthalmol Vis Sci 26:778-782, 1985

The retinal rod outer segments (ROS) of a variety of vertebrate species have been studied intensively for many years. The relevance of this work to human ocular disorders has been diminished by the frequent use of ROS from nonmammalian species, which have the advantage of being several times larger in diameter than are mammalian ROS. Among the many consequences of the smaller diameter of mammalian outer segments is that they are very dim when viewed in the polarizing microscope. This makes it more difficult to detect any potentially interesting variations in their structure such as those reported in amphibians. Kaplan has reported seeing the type of periodicities of birefringence in monkey ROS, but no one has yet reported seeing the type of periodicities of birefringence shown to correlate with ROS renewal rate in amphibians. In this article, we report the existence of distinctive periodicities in mammalian ROS when viewed with Nomarski (differential interference contrast) optics. While these periodicities are considerably less regular than those seen in amphibian ROS, there is a correlation between their spacing and published data on the rate at which mammalian ROS are...
renewed. A preliminary report of this work has appeared in abstract form.

**Materials and Methods.** Species used in this study included the rat, guinea pig, rabbit, and dog. Studies using these animals were performed in conformation with the ARVO Resolution on the Use of Animal in Research. All were chronically (greater than 1 month) maintained under cycling light (12 hr light, 12 hr dark—12L:12D). Animals were killed by decapitation (rat), or pentobarbital overdose (guinea pig, rabbit, and dog). After death, eyes were enucleated and hemisected (immediately except for the dogs, for which there was a delay of about 1 hr during which the eyes were wrapped in wet paper towels, sealed in glass jars, and kept on ice). The retina was then dissected away from the pigment epithelium, and a portion (except rat retinas, in which the entire retina was used) was rapidly agitated in one to two drops of either vitreous humor from the same eye or mammalian Ringer's solution (0.1 M NaCl, 0.03 M KCl, 0.014 M CaCl₂, 4 mM NaHCO₃, 2 mM glucose, 2 mM MgCl₂; pH 7.4, 290–300 mOsml).

For microscopy a small drop (about 10 μl) of the suspension obtained was placed within a boundary drawn on a freshly cleaned microscope slide with vacuum grease. A coverslip was then placed over the drop and gently pressed down into the grease to form a seal around the flattened drop. The slide was then examined in a Nikon Optiphot-Pol microscope (Nikon Instruments, Inc; New York, NY) equipped with rectified Nomarski optics, including 20X, 40X, and 100X oil immersion objectives and using a 100W Mercury light source. Cells were photographed with a Nikon UFX system with 35-mm camera back. Ilford XPI-400 film (ASA 400, fine-grained, Ilford, Ltd; Basildon, Essex, England) was used, and exposures typically were about 2 sec.

For morphometry, all measurements were made on prints using a digital image analysis device (MOP-30, Zeiss; New York, NY). All dimensions were calibrated using a certified graticule with 10-μm calibration marks (ser. no. A156, Graticules, Ltd; Tonbridge, Kent, England), which was photographed at the end of each roll of film; these pictures were printed in tandem with the experimental pictures and were used to internally calibrate all the dimensions listed below.

**Results.** Figures 1–4 show freshly isolated ROS from the retinas of a rat, guinea pig, rabbit, and dog, respectively. All display alternating light and dark “bands” oriented perpendicular to the long axis of the cell. It is important to note that the cell must be properly oriented in the microscope for the bands to be observed since the axis of the underlying structural variations must be parallel to the shear axis of the Nomarski optics. Thus there is only one suitable axis. This differs from the case with polarized light optics, in which birefringence bands are observed in cells oriented in either of two perpendicular axes.

Measurements were made of band separations in selected cells of the rat and dog. These measurements were made from the visually estimated centers of either bright or dark bands. These cells were selected for sharpness of focus (which is variable, since the cells were free to float around under the coverslip) and for the presence of bands of high contrast, i.e., that were easily and reliably measurable. It should be emphasized that while every cell observed had bands, they were not always of the contrast illustrated in Figures 1–4. Thus, there may be a selection bias in the data presented, but it is a bias that is difficult to avoid.

Band spacing in the rat was 3.1 μm (95% confidence interval: 2.7–3.4), and in the miniature poodle was 4.1 μm (95% confidence interval: 3.5–4.6). Variability...
Fig. 2. "Bands" in isolated rod outer segment of a dog observed with Nomarski optics. Note that adjacent ROS with different orientations do not display these bands. Bar = 10 μm.

Fig. 3. Bands in an isolated outer segment of a guinea pig revealed with Nomarski optics. Again note the importance of the correct orientation for observation of the bands. Bar = 5 μm.

was encountered both within cells and among different cells. In the rat, LaVail estimates that a total of 9 days was required to renew the entire ROS in his control animals. Measurements of the figures in this paper indicate an average ROS length of about 28 μm, giving a daily renewal rate of 3.1 μm/day, which agrees with the average band spacing observed with Nomarski optics. Aguirre et al. however, report an average renewal of 2.4-2.6 μm/day in control dogs.

To facilitate comparison of the band separation data of current study with data on the outer segment renewal rate determined autoradiographically, an estimate was obtained of ROS width. Width was chosen rather than length because in isolated cells it is difficult to determine with assurance if a particular ROS is intact or broken. ROS width in the current study was 1.75 μm, while the width of outer segments in Aguirre et al. (Fig. 2b) was approximately 1.0 μm. These data indicate that a shrinkage of approximately 40% occurred in ROS width in this autoradiographic study. Thus, in order to compare results, a correction factor must be applied to the renewal rate data. If it is assumed that axial and radial shrinkage were nearly equal, then the "corrected" renewal rate becomes 4.2 μm/day, again in agreement with the band spacing reported above.

In the cells depicted in Figures 1-4 one end of each ROS appears especially dark and one end light. This probably arises from the abrupt change in refractive index occurring at the edge of the ROS, and is not related to the phenomena discussed in this paper.

Discussion. The basic question raised by this study is whether the apparent coincidence between the structural inhomogeneities of the ROS described above and published renewal rates in the rat and dog (the latter with a correction for shrinkage) represents a real relationship in these species. This question is more difficult in the material examined in the current study than in previous work with the birefringence
bands of frog ROS, since the bands observed with Nomarski optics in mammalian ROS have greater variability in their spacing.

It must be decided if the occurrence in a given ROS of some bands which are separated by a distance quite different from the accepted rate of ROS renewal for that species constitutes grounds to reject the idea that these bands represent the production of new disks by that cell on some previous day. Such a rejection does not seem justified, given that ROS renewal rates represent mean values around which there is considerable variation, ie, the bands of radioactivity that have been reported in autoradiographic studies of ROS renewal rate in mammals are not sharp. This represents evidence that, within a population of photoreceptors, there is some variation in the amount of new ROS disks produced during the interval between the radioisotope injection and death. There is no data on the occurrence of variability in the amount of new ROS material assembled from day to day within a single cell. This paper presents observations which suggest that this variability may be quite large.

In previous work on the correspondence between birefringence bands and ROS renewal in the frog it was considered important to test their apparent relationship experimentally. This was done by exposing animals to cold, thus reducing ROS renewal rate, and observing that the birefringence band spacing changed accordingly.\(^2\) Analogous work is underway in the dog, for which there exists an inherited retinal degeneration featuring a reduced rate of ROS renewal.\(^7\) It is obviously difficult to lower a mammal's body temperature for a period of days in order to study ROS renewal at reduced temperatures, and ROS renewal apparently stops during hibernation, at least in the squirrel.\(^8\) ROS renewal rate in mammals has not been shown to be significantly modulated by environmental light.\(^9\)

An unresolved question is the physical basis for these observations in terms of ROS structure. Nomarski optics generate contrast on the basis of gradients in bulk refractive index. The observed bands could represent gradients of disk membrane composition (eg, differing protein:l lipid ratio), disk spacing, disk diameter, or combinations of all of these.

Results have been presented that structural periodicities are discernable in the ROS of mammalian species beyond the rat and dog, namely, the guinea pig and rabbit. Quantification of the structural periodicities in the latter species and extrapolation to yet other species are the goals of ongoing research.

Key words: Nomarski optics, rod outer segment, renewal, structural periodicities, mammals

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References
Barium Removes the Ouabain-Induced Increase in the Rod Response to Light

A. E. Walter* and A. J. Sillman

The mass receptor potential of the excised, superfused retina of the bullfrog was isolated with aspartate. Rods were selectively stimulated by using very dim flashes of light. In the presence of 0.1 mM ouabain, the amplitude of the receptor response was found first to increase transiently and, subsequently, to decrease progressively. The ouabain-induced transient increase in receptor response was completely eliminated by 0.4 mM barium chloride. However, barium did not affect the rate at which the response decayed in the presence of ouabain. The ability of barium to remove the ouabain-induced transient increase in the amplitude of the receptor response is discussed in terms of reducing the coupling ratio of the postulated electrogenic sodium–potassium pump of rods. Invest Ophthalmol Vis Sci 26:782–785, 1985

It is commonly known that cardiac glycosides such as strophanthidin or strophanthin (ouabain) poison sodium–potassium pumps. It is not surprising, therefore, that such substances cause a decrease in the amplitude of the photoreceptor potential, since suppression of the pump causes the collapse of the sodium and potassium gradients that are necessary to generate the response. What is surprising, however, is that when rod photoreceptors are exposed to strophanthidin and stimulated with dim flashes of light, the amplitude of the receptor potential first increases and, then, decreases. This transient increase in the receptor potential amplitude may be explicable in terms of the postulated electrogenicity of the sodium–potassium pump of rods. If so, then altering the electrogenicity should also alter the transient. Interestingly, barium ions have been shown to make the sodium–potassium pump in muscle less electrogenic. Thus, the present study was undertaken to determine whether barium removes the transient increase in rod receptor response amplitude that is induced by cardiac glycosides.

Materials and Methods. The methods employed in this study have been described before and will not be repeated in detail here. Experimental animals were treated in conformity with the ARVO Resolution on the Use of Animals in Research. Prior to each experiment a bullfrog, Rana catesbeiana, was dark-adapted overnight. Under dim red illumination the animal was decapitated and double-pithed, after which an eye was enucleated and hemisected. The eyecup was then immersed in control Ringer solution where the retina and pigment epithelium were dissected out. The retina, minus the pigment epithelium, was then mounted in the superfusion chamber which, in turn, was placed in a metal block. Control and experimental solutions were held in reservoirs above the perfusion chamber and before entering the chamber passed through separate water-jacketed condensers. The temperature of water circulating through the jacketed condensers and the metal block was controlled such that the temperature of the preparation was maintained at 18 ± 0.2°C. The base of each condenser fit into a manifold that conducted the solutions to a common outlet that was connected, in turn, to the inlet of the chamber. To decrease turnover time, the valves that controlled the flow from each of the condensers were exposed to strophanthidin and stimulated with dim flashes of light, the amplitude of the receptor potential first increases and, then, decreases. This transient increase in the receptor potential amplitude may be explicable in terms of the postulated electrogenicity of the sodium–potassium pump of rods. If so, then altering the electrogenicity should also alter the transient. Interestingly, barium ions have been shown to make the sodium–potassium pump in muscle less electrogenic. Thus, the present study was undertaken to determine whether barium removes the transient increase in rod receptor response amplitude that is induced by cardiac glycosides.

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