Key words: proliferative retinopathy, diabetic retinopathy, vitreous-soluble proteins, electroretinography, intravitreal neovascularization, retina, vitreous.

REFERENCES


Essential fatty acid deficiency and renewal of rod outer segments in the albino rat.

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Biochemical studies in albino rats fed a lab chow diet (control) showed a 9 to 10 day turnover time for rhodopsin in rod photoreceptor membranes, whereas the turnover time in animals raised on a fat-free diet (experimental) was not easily measurable. The number of phagosomes in the pigment epithelium of the control group was three times that found in the experimental. These studies support earlier autoradiographic data which suggested that the renewal of new photoreceptor discs in the rat retina is controlled by the availability of polyunsaturated fatty acids.

†Dennis Landis, our friend and colleague died March 14, 1975.
collected at 4°C in the dark and scanned on a Cary 118C spectrophotometer. Rhodopsin concentration was determined directly or as the retinal oxime. Column fractions were assayed for radioactivity in a Beckman LS-230 Liquid Scintillation counter equipped with an external standard. DISintegrations per minute (D.P.M.), determined by the channels ratio method, were related to millimicromoles of rhodopsin to determine specific radioactivity.

Groups of six animals each of experimental and control rats, raised for 12 weeks under the same conditions and treated identically except for diet, were anesthetized with Nembutal and perfused with an intracardiac catheter using a mixture of 2 per cent glutaraldehyde and 2 per cent formaldehyde buffered in 75 mM sodium cacodylate (pH 7.35). Eyes were enucleated, immersed in the perfusion fluid for 24 hours, and prepared for electron microscopy by previously described procedures. Ultrathin tangential sections of pigment epithelium were cut and examined on an AEI-801A transmission electron microscope. Tangential sections (rather than meridional) were cut to provide a greater area for counting phagosomes. Only those tangential sections clearly passing through the middle to apical portions of the cells were used. The same region from six retinas of each group were examined and the number of phagosomes per pigment epithelium cell per ultrathin section was counted in over 100 cells from each group. The criteria used to characterize a phagosome was that it was clearly membrane bound and contained recognizable membraneous lamellae resembling photoreceptor discs.

Fig. 1 is an elution profile of rhodopsin from experimental and control animals four days after injection of the radioactive amino acid mixture. Counts per minute and absorbance at 498 nm. are plotted against fraction number. Some radioactive material eluted ahead of rhodopsin; however, ratios of 278/498 nm. absorbance and rhodopsin specific radioactivity were constant in the peak tubes, indicating that extraneous protein was not making a selective contribution to the rhodopsin radioactivity in either group.

The data in Fig. 2 are plots of the rhodopsin specific radioactivity (disintegrations per minute per millimicromole) versus time after injection. It is evident that the specific activity from control animals rises sharply during the first 24 hours, remains high until the eighth day, and then precipitously falls. This is consistent with the incorporation of a pulse of radioactive protein into the base of the outer segments which, because of continued synthesis of new unlabeled discs, is displaced toward the pigment epithelium. After 10 days, the labeled discs reach the tips of the rod outer segments and are shed and phagocytized by the pigment epithelium. The specific activity curve for the experimental animals does not show the clear turnover seen in the controls. While the kinetic incorporation of label into visual pigment is similar for the first 24 hours, the specific activity in experimental animals is less than half of that for controls by the fourth day. Further, the specific activity curve does not undergo a rapid change in slope indicative of the removal of radioactive discs. Rather there is a slow change in slope suggesting random insertion and removal of rhodopsin.

Figs. 3 and 4 are electron micrographs of tan-
Fig. 3. Electron micrograph of pigment epithelium from a control animal. Arrows point to typical phagosomes. ×10,000.

In other studies employing meridional sections, the number of rod outer segments making intimate contact with the pigment epithelium was approximately the same for the two groups of animals. However, there is a noticeable decrease in the...

gential sections of pigment epithelium of control and experimental animals, respectively. The morphology of the pigment epithelial cells of both groups of animals is quite similar and cells from both groups are approximately the same size.
number of phagosomes in the pigment epithelial cells in the animals raised on the essential fatty acid-deficient diet. The phagosome density counts for the control group is $4.4 \pm 0.34$ (S.E.M., $n = 101$) phagosomes per pigment epithelial cell per thin section, whereas the experimental group contain $1.4 \pm 0.14$ (S.E.M., $n = 102$) phagosomes per pigment epithelial cell per thin section. The probability that these means are from the same population is $< 0.001$. 

Fig. 4. Electron micrograph of pigment epithelium from an experimental animal. Arrow points to a single phagosome of the size and morphology counted in this study. ×10,000.
The two pieces of data presented in this paper, along with our previously published autoradiographic study,\textsuperscript{5,6} support our contention that renewal of photoreceptor membranes by new disc formation is a function of the polyunsaturated fatty acid availability. We cannot state unequivocally that outer segment disc renewal in the experimental animal has ceased completely. Indeed, the phagosome density studies presented here would argue against this. However, our biochemical and autoradiographic procedures indicate that turnover has been significantly altered in the experimental animals.

Why are polyunsaturates so important in photoreceptor membranes? The answer probably lies in a role for polyunsaturates in maintaining a functional membrane by providing either the proper viscosity for events associated with visual excitation or a unique hydrophobic environment in which these events take place. Recent studies have shown that a minimal change in polyunsaturated content of photoreceptor membranes selectively decreases the amplitude of the a-wave of the ERG relative to the b-wave, the latter serving as an internal control.\textsuperscript{8}

How do polyunsaturates control photoreceptor membrane renewal? The answer to this exciting question is unknown. However, since rhodopsin, the visual pigment of the rod outer segments, is synthesized in the experimental animals, it seems reasonable to suggest that the control of membrane biogenesis is not at the level of protein synthesis. Perhaps phospholipids containing certain polyunsaturated fatty acids are necessary to form a lipoprotein complex with rhodopsin that can be incorporated into new photoreceptor discs.

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REFERENCES