Alteration of Disc Formation in Photoreceptors of Rat Retina

Abstract. *Autoradiographic experiments demonstrate that the renewal process of the rod photoreceptors in the rat retina is altered during essential fatty acid deficiency. After the administration of tritiated amino acids, animals raised on a fat-free ration show no evidence of disc formation, while those raised on a normal ration show disc formation and renewal. The latter process is apparently dependent upon the availability of linoleic or linolenic (or both) fatty acids.*

Rod outer and inner segments are two components of the photoreceptor cells of the rat retina. The rod outer segments contain a series of closely packed discs that are enclosed in an outer limiting membrane and terminate to apical projections making contact with the pigment epithelium. The proximal portion of the outer segments constrict to a narrow cilium connecting to the inner segment of the visual cell. In the inner segments are located all of the organelles usually associated with anabolic functions. Rhodopsin and other proteins are synthesized by the ribosomes in the inner segments and are transported through the cilium to the outer segments, where they become part of the photoreceptor disc membranes (*1*, *2*). As new discs are formed by this process, older discs are displaced toward the apical ends of the outer segments, where they are phagocytized by the pigment epithelium (*3*). Complete renewal of photoreceptor membranes takes 10 days in rats (*1*).

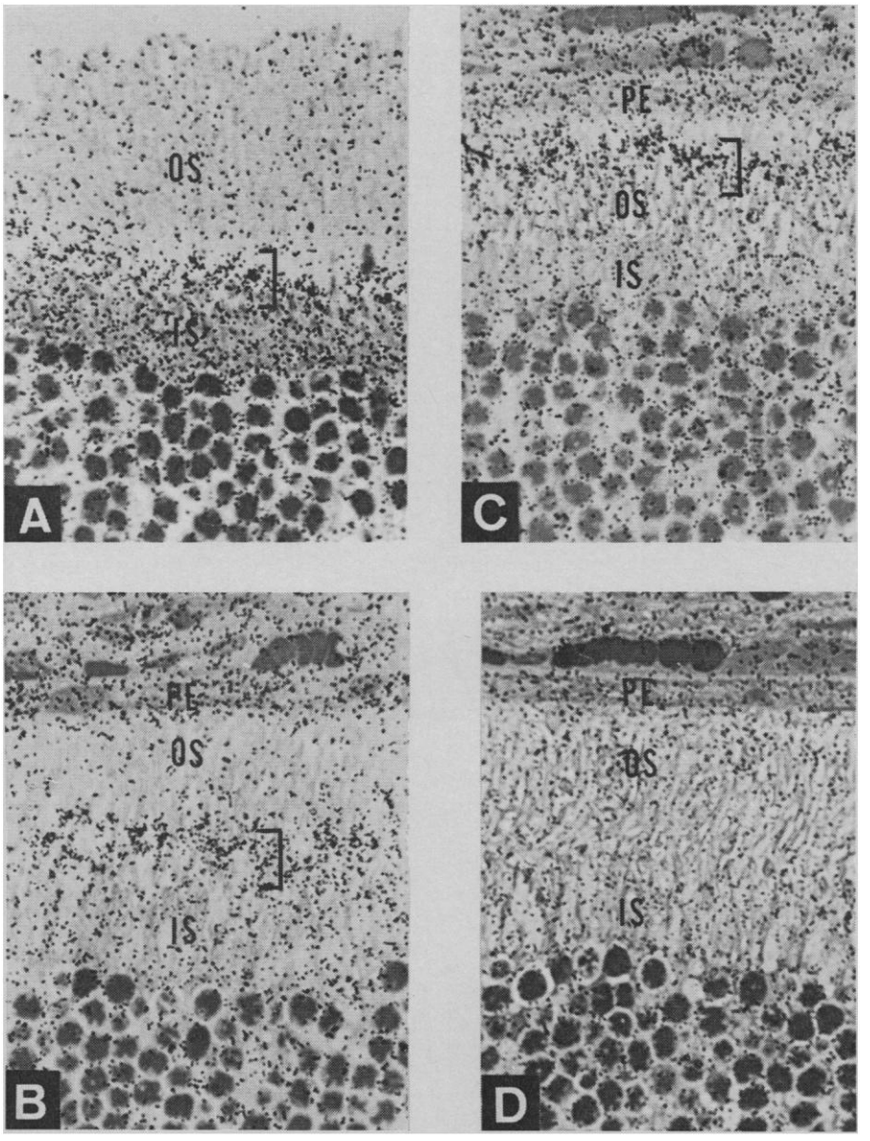

Fig. 1. (A to D) Autoradiograms of photoreceptors of control rats at 1, 3, 8, and 10 days, respectively, after administration of the tritiated amino acid mixture. A discrete band of labeled protein is seen moving from the base of the rod outer segments (*OS*) toward the pigment epithelium (*PE*). Diffuse labeling of protein is also seen; *IS*, inner segment (× 1050).

Phospholipids are a major chemical constituent of visual cell membranes and contain large percentages of long-chain polyunsaturated fatty acids (*4*, *5*). The precursors of these fatty acids are linoleic (18 : 2ω6) and linolenic (18 : 3ω3) [nomenclature explained in (*6*)], both of which cannot be synthesized de novo and must be obtained from the diet. In earlier studies in which rats were fed a fat-free diet to reduce the levels of ω3 and ω6 polyunsaturates in the visual cell, the polyunsaturated fatty acid levels remained constant even after 80 days (*5*) or 240 days (*7*) on the deficient diet. The question was: How does the retina maintain its supply of polyunsaturated fatty acids? One possibility was that rod outer segment turnover is inhibited or stopped in the absence of dietary ω3 or ω6 fatty acids. The results of autoradiographic experiments to test this possibility are reported here.

A mixture of L-[^{3}H]phenylalanine and L-[^{3}H]leucine (equal activities of each) in isotonic saline was injected into the jugular vein of two groups of anesthetized albino rats (60 μc per gram of body weight) that had been raised from weanlings for 12 weeks on either a fat-free or a lab chow ration. Both groups of animals appeared to be in excellent health. Rats on the fat-free diet (experimentals) were raised under normal vivarium conditions with overhead fluorescent lighting cycled on 12 hours and off 12 hours. Rats fed lab chow (controls) were maintained under these lighting conditions for 2 weeks before injection. At various times after injection, the animals were killed, and their eyes were enucleated and fixed in a buffered mixture of 2 percent glutaraldehyde and 2 percent formaldehyde (*p*H 7.35), with one exception. Eyes removed on day 1 were fixed in buffered 4 percent formaldehyde solution (*p*H 7.35) so that labeled free amino acids could be washed away during histological procedures (*8*). The pigment epithelium was detached from the retina when only formalde-

hyde was used as fixative (Figs. 1A and 2A). After fixation and dissection, the tissue was postfixed in buffered 1 percent osmium tetroxide, dehydrated in a graded series of ethanol washes, and enbedded in Araldite 502. Tissue sections were oriented so that the longitudinal view of the visual cells could be obtained and were cut at 0.5-μm thickness and transferred onto glass slides. Embedding plastic was removed with NaOH in ethanol. The slides were dipped in a Kodak NTB2 emulsion solution, stored in the dark for 37 days, and then developed and stained in a basic solution of Paragon multiple stain.

In sections of eyes removed 1 day after injection of the labeled amino acid mixture into the control animals, a discrete band of radioactive grains can be seen at the base of the rod outer segments (Fig. 1A). These grains represent the labeled proteins that have been incorporated into new discs. At 3 days, the band is located at approximately one-third the length of the rod outer segment (Fig. 1B). By 8 days, the radioactivity is near the apical tips of the rods (Fig. 1C), and after 10 days, this band has disappeared into the pigment epithelium (Fig. 1D). This demonstrates that the newly synthesized labeled proteins incorporated into the lipoprotein discs are gradually displaced toward the apical portion of the rod outer segments by unlabeled new discs, and are eventually phagocytized by the pigment epithelium. These data for control animals agree with the earlier data of Young (*1*).

Unlike the control animals, the experimental group raised on a fat-free ration shows no discrete radioactive banding in the 10-day period. This is demonstrated by comparing sections for days 1, 3, 8, and 10 for the experimental and control animals (Figs. 1 and 2). The absence of a discrete band of radioactive grains suggests that disc renewal did not take place in the experimental group during this period.

In addition to the radioactive banding, we observe a diffuse labeling of protein in the rods of both control and experimental animals, which Bok and Young (*9*) attribute to newly synthesized proteins distributed among preexisting membranous discs. Further, we isolated labeled rhodopsin from the rods of both groups of animals after administration of the labeled amino acids (*10*); this demonstrates that the visual cells of rats on the fat-free diet can synthesize proteins. However, this synthesis seems to occur without new disc formation.

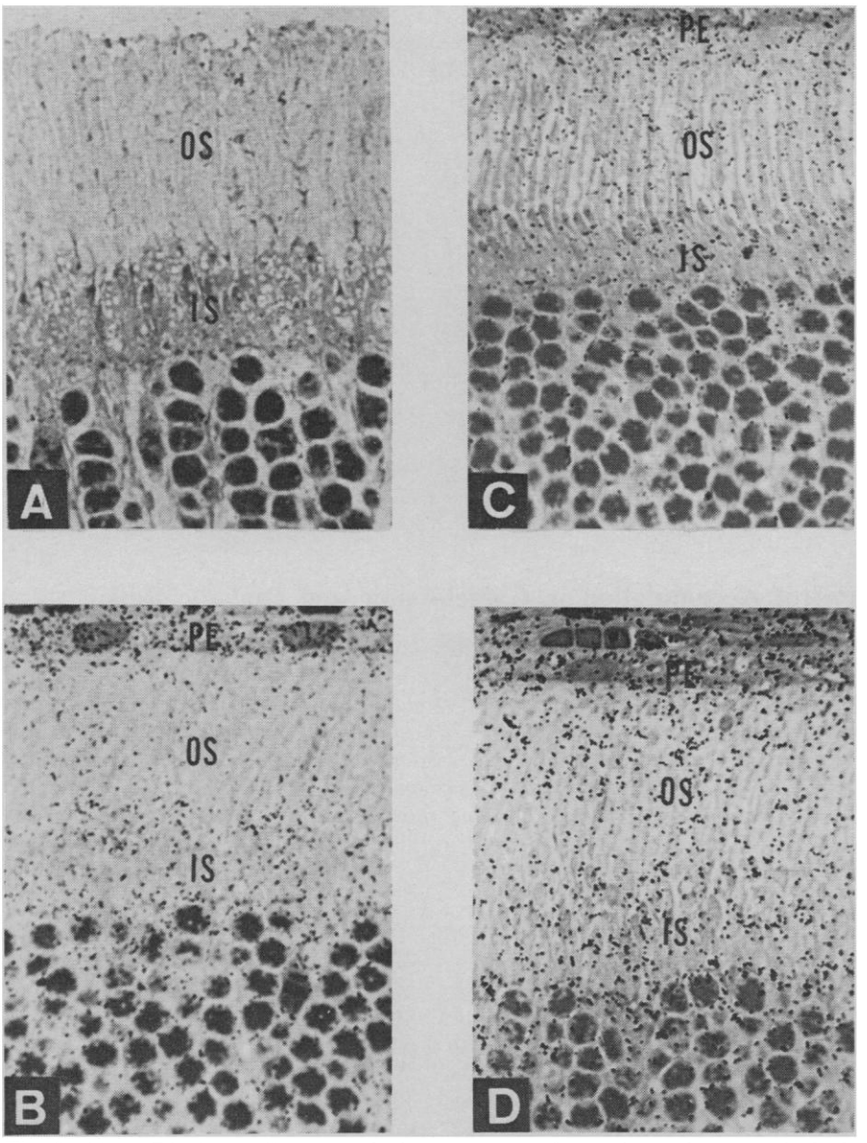

Fig. 2. (A to D) Autoradiograms of photoreceptors of experimental rats at 1, 3, 8, and 10 days, respectively, after injection of the tritiated amino acid mixture. Rats had been raised from weanlings for 12 weeks on a fat-free ration. No discrete band of labeled protein is seen between the inner segments (*IS*) and the pigment epithelium (*PE*). Diffuse labeling is seen, as in Fig. 1; *OS*, outer segments.

Exclusion of ω3 or ω6 polyunsaturated fatty acids from the diet of albino rats results in an alteration of amino acid uptake. Specifically, the discrete banding of protein observed in controls does not occur in animals maintained on a fat-free ration. Two possible explanations are that (i) the renewal of rod outer segments is halted or (ii) new discs are formed without protein. We reject the latter because we found that the eyes of experimental and control animals contain equivalent amounts of rhodopsin. Thus, the most reasonable interpretation of our data is that the normal orderly turnover of discs ceases in rats deficient in ω3 or ω6 (or both) polyunsaturated fatty acids. We suggest that a deficiency of these fatty acids prevents the packaging of a lipoprotein complex required for formation of visual membranes.

DENNIS J. LANDIS
PETER A. DUDLEY
ROBERT E. ANDERSON
Department of Ophthalmology, Baylor College of Medicine, Houston, Texas 77025

References and Notes

1. R. W. Young, *J. Cell Biol.* **33**, 61 (1967).
2. — and B. Droz, *ibid.* **39**, 169 (1968); M. O. Hall, D Bok, A. D. Bacharach, *J. Mol. Biol.* **45**, 397 (1969).
3. R. W. Young and D. Bok, *J. Cell Biol.* **42**, 392 (1969).
4. R. E. Anderson and M. B. Maude, *Biochemistry* **9**, 3624 (1970); R. E. Anderson and L. Sperling, *Arch. Biochem. Biophys.* **144**, 673 (1971).
5. R. E. Anderson and M. B. Maude, *Arch. Biochem. Biophys.* **151**, 270 (1972).
6. The fatty acid nomenclature is as follows. The number before the colon is the number of carbon atoms in the fatty acid chain, and the one after the colon is the number of double bonds. (This system is used for methylene-interrupted *cis* double bonds.) Omega 3 means that the first double bond is three carbons from the methyl end of the molecule. We refer to $\omega 3$ and $\omega 6$ fatty acids, two families of polyunsaturates derived from linolenic ($18:3\omega 3$) and linoleic ($18:2\omega 6$) acids, respectively.
7. S. Futterman, J. L. Downer, A. Hendrickson, *Invest. Ophthalmol.* **10**, 151 (1971).
8. T. Peters, Jr., and C. A. Ashley, *J. Cell Biol.* **33**, 53 (1967).
9. D. Bok and R. W. Young, *Vision Res.* **12**, 161 (1972).
10. Rats raised for 10 to 12 weeks on either lab chow or fat-free diets were injected with the tritiated amino acid mixture (3 μc per gram of body weight). Rhodopsin was obtained by agarose column chromatography. Plots of specific activity of rhodopsin against time for control animals showed a complete turnover of rhodopsin in 9 to 10 days, with a peak of radioactivity at 4 to 5 days. In contrast, specific activity of rhodopsin in experimental animals was much lower than for controls and did not show any peak of radioactivity or apparent turnover.

11. Supported in part by NIH grants EY00871 and EY00244; the Institute of Ophthalmology, Texas Medical Center; Research to Prevent Blindness, Inc.; and a Fight for Sight student fellowship SF-367 (to P.A.D.) financed by a grant from the Abraham Silver Memorial Fund of Fight for Sight, Inc., New York. We gratefully acknowledge the technical assistance of V. Blanchard and the secretarial assistance of T. Marston.

11 July 1973

Polyol Accumulation in Galactosemic and Diabetic Rats: Control by an Aldose Reductase Inhibitor

Abstract. *An orally active inhibitor of aldose reductase, 1,3-dioxo-1H-benz[de]-isoquinoline-2(3H)acetic acid (AY-22,284), prevented cataractous changes in cultured lenses exposed to high concentrations of galactose. When given orally, AY-22,284 markedly decreased the accumulation of polyols in the lenses and sciatic nerves of galactosemic rats and rats with streptozotocin-induced diabetes. In addition, treatment of galactosemic rats with AY-22,284 effectively suppressed the formation of cataracts.*

Insulin therapy has greatly reduced death from acute complications of diabetes. However, the resulting longevity of diabetic patients has led to complications such as neuropathy, nephropathy, retinopathy, and cataracts. The sorbitol pathway appears to be involved in metabolism of the excess glucose in diabetic tissues; this may lead to development of some of these complications (*1*). In this report we describe the effects of the compound AY-22,284 in galactosemic and diabetic rats. The changes in treated animals are consistent with the capacity of AY-22,284 to inhibit aldose reductase, one of the enzymes of the sorbitol pathway.

The presence of the sorbitol pathway has been established in several tissues, in which it is localized in certain cell types—for example, the lens (*2*), Schwann cells of peripheral nerves (*3*), kidney papilla (*4*), and the islets of Langerhans in the pancreas (*5*). The sorbitol pathway consists of two enzymes, aldose reductase (E.C. 1.1.1.21) and sorbitol dehydrogenase (E.C. 1.1.1.14):

$$\text{D-Glucose} + \text{NADPH} + \text{H}^+ \xrightarrow{\text{aldose reductase}} \text{sorbitol} + \text{NADP}^+$$

$$\text{Sorbitol} + \text{NAD}^+ \xrightarrow{\text{sorbitol dehydrogenase}} \text{fructose} + \text{NADH} + \text{H}^+$$

(NADPH, reduced nicotinamide adenine dinucleotide phosphate; $NADP^+$, nicotinamide adenine dinucleotide phosphate; NAD^+, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide.)

Aldose reductase has low substrate specificity and reduces several aldoses and other compounds containing an aldehyde group (*6*). Since the affinity of aldose reductase for glucose and galactose is low (Michaelis constants approximately 70 and 20 m*M*) (*6*), maximal rates of aldose reductase–catalyzed formation of sorbitol or galactitol can be attained only with high intracellular concentrations of glucose, as in diabetes, or of galactose, as in galactosemia. The polyols thus formed accumulate because they are neither efficiently metabolized nor readily diffused through cell membranes. Accumulation of polyols causes hypertonicity followed by osmotic swelling. This sequence of events is the common mechanism of sugar cataract formation (*2*, *7*, *8*). Sugar cataracts like those in diabetic and galactosemic patients can be produced in experimental diabetic rats and in rats fed galactose or xylose (*9*), and can be simulated (*8*) and prevented (*10*) in lens cultures.

Recognition of the role of aldose reductase in the pathogenesis of sugar cataracts led us to consider the possible involvement of the sorbitol pathway in diabetic neuropathy. Sorbitol and fructose, present in the nerves of normal rats, are increased in amount in the nerves of rats with diabetes produced by alloxan (*11*) or streptozotocin (*12*); aldose reductase is localized in the Schwann cells (*3*), which are involved in myelin formation and maintenance; and damage of Schwann cells results in

Table 1. Effect of AY-22,284 on galactitol accumulation in galactosemic rats. For lens measurements, rats had access to a diet containing 10 percent galactose for 3 days; for measurements in sciatic nerve, a diet with 30 percent galactose was given for 7 days. The diet of treated animals contained 0.7 percent AY-22,284 (approximately 1.2 g/kg per day). Eight rats were in each group. Galactitol was determined in trichloroacetic acid extracts by a modification of a method for glycerol determination (*19*). Values have been corrected by subtracting the background galactitol contents found in normal rats. In separate experiments, similar values were obtained by gas-liquid chromatographic analyses for the lens (by J.H.K.) and sciatic nerve (by K.H.G.); S.E., standard error.

Group	Galactitol (micromoles per gram of tissue)	
	Lens (mean ± S.E.)	Sciatic nerve (mean ± S.E.)
Control	14.43 ± 0.77	3.29 ± 0.82
AY-22,284	7.19 ± 0.49*	0.82 ± 0.16†
Change	− 50 percent	− 75 percent

* $P < .01$. † $P \leqq .02$.

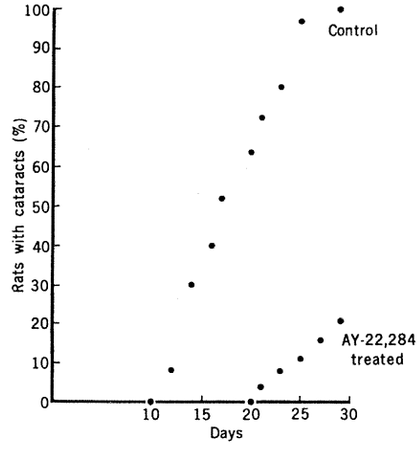

Fig. 1. Effect of AY-22,284 (0.7 percent of diet, or approximately 0.96 g/kg per day) on formation of macroscopically detectable cataracts in rats fed a diet containing 30 percent galactose. There were 23 or 24 rats per group.