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Articles

Structural and biochemical changes in vitamin A–deficient rat retinas

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The levels of rhodopsin and opsin were investigated in relation to the maintenance of retinal structure in retinas of vitamin A–deficient rats reared in low levels of cyclic illumination (1.5 to 2 foot-candles). Rhodopsin levels decreased in the deficient retinas to approximately 20% of control at 9 weeks, and this level was retained through 39 weeks on the deficient diet. Opsin levels decreased at a slower rate but reached about 20% of control levels at 32 weeks. Despite the decrease in rhodopsin levels, obvious deterioration of disc structure was not observed until 16 weeks of deficiency, when opsin levels had already decreased to 60% to 70% of control. The structural disruption of photoreceptor outer segments was localized initially in discs of the distal third. Rod cell degeneration preceded cone cell degeneration in vitamin A–deficient retinas. Most of the rods and cones persisted in the posterior retina at 23 weeks on the deficient diet; however, by 40 weeks, only 11% of the rod nuclei remained. In contrast, about 63% of the cone nuclei were present at 40 weeks of deficiency. The photoreceptor cells were affected by the deficiency to a greater extent in the inferior hemisphere than in the superior hemisphere of the eye.

Key words: vitamin A deficiency, photoreceptor degeneration, rat, rhodopsin, opsin, rods, cones

Vitamin A deficiency has been shown to produce biochemical, morphological, and physiological changes in the retina. In rat retinas, the first sign of the deficiency, night blindness, was determined by the rise in threshold for eliciting the electroretinogram (ERG). This rise in threshold was directly related to a decrease in the level of rhodopsin detected by 4 to 5 weeks on the dietary regimen.[1–4] By 10 months, no response could be elicited from retinas of the deficient rats.[1–2] Structural deterioration of outer and inner segments occurred[1, 5–8] as well as degeneration of photoreceptor nuclei.[1–3, 7, 9]

Several studies have shown the presence of cone photoreceptors in the rat retina.[10–13] Cone photoreceptor cells are affected to a

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lesser extent in some experimental lesions[13, 14] and some genetic disorders in several species.[15–18] The present study was designed to compare the rate of rod and cone degeneration and the ultrastructural deterioration of outer segments associated with decreasing levels of rhodopsin and opsin in vitamin A–deficient rat retinas.

Materials and methods

Animals. In order to accelerate the depletion of retinol from the experimental animals, pregnant female Sprague-Dawley rats were fed a vitamin A–deficient diet (basal diet*) 7 days prior to delivery and throughout lactation. This procedure results in offspring with normal blood levels of retinol, but with reduced liver stores and thus an earlier onset of tissue depletion (Bieri, unpublished observations). The male offspring were weaned at 21 days of age and divided into three groups: (1) basal diet, (2) basal diet plus retinyl palmitate (4 mg/kg diet) at 21 days of age, and (3) basal diet plus retinoic acid at 35 days of age. The supplement in group 3 was delayed for 2 weeks after weaning to prevent the known sparing of tissue retinol by retinoic acid.[21] All rats were maintained at a temperature of about 24° C with 12 hr cyclic illumination of 1.5 to 2 foot-candles.

Rhodopsin and opsin measurements. Rats were dark-adapted overnight, and the eyes were enucleated under ether anesthesia in dim red light. After rinsing in physiological saline, the lens and vitreous were removed through a slit in the cornea. To prevent loss of retinal tissue in dissection, the whole eye cup from a given rat was homogenized in 1 ml of phosphate buffer (66 mM, pH 7.1).

For rhodopsin measurement, 10 μl of the nonionic detergent Emulphogene BC 720 (General Aniline and Film) was added to each sample and allowed to stand for 1 hr at room temperature. However, for opsin measurements, both control and deficient retina homogenates were bleached for 10 min with 60 foot-candles of white light, and 0.1 μmol of 9-*cis*-retinal was added to each homogenate in the dark.[22] Each sample with retinal was incubated for 5 hr at room temperature before another 0.1 μmol 9-*cis*-retinal was added. These mixtures were incubated at 4° C overnight. On the following day, the samples were warmed to room temperature for 30 min and incubated with 10 μl of Emulphogene for 1 hr. All samples were centrifuged at 25,000 × g for 15 min to clarify the rhodopsin or isorhodopsin extracts. To each sample, 0.1 ml of hydroxylamine (0.1M, pH 7.2) was added. A Beckman Acta II spectrophotometer was used to scan the absorption spectra of the extracts before and after exposure to white light of about 60 foot-candles for 10 min. With the procedure described above for extraction of rat rhodopsin, the absorption peak was 493 nm as previously described.[23] The changes in absorbance at 493 or 485 nm after bleaching was taken as a measure of rhodopsin or isorhodopsin (i.e., opsin) content, respectively. Results were expressed as percentage of control values. Opsin was determined by the above bleaching and regeneration procedure to provide a control, to ensure that regeneration actually could occur and to compensate for variation in the degree of regeneration from one experiment to the other.

Histological procedures. The eyes were enucleated from some rats of each group between 4 and 40 weeks. All eyes were fixed by immersion in 4% glutaraldehyde in 0.15M Na-K phosphate buffer at room temperature for about 30 min. Portions of the posterior eye cup, including the retina, were excised from the inferior hemisphere within 2 mm of the optic nerve head. In the peripheral region of the same hemisphere, segments approximately 2 mm in length were excised. These segments were postfixed with 1% osmium in the Na-K phosphate buffer for 30 min at room temperature and 30 to 45 min at 4° C. The tissue was processed for embedding in Epon. For light microscopy, sections were cut at 1 to 1.5 μm and stained with a solution of 1% toluidine blue in 1% tetraborate buffer. Ultrathin sections, stained with uranyl acetate and lead citrate, were examined with an electron microscope.

Counts of rod and cone nuclei were made in the posterior and peripheral retina of the inferior hemisphere. Rod and cone nuclei were distinguished by their nuclear morphology. Cone nuclei contain multiple small clumps of heterochromatin, and rod nuclei contain a large central clump.[10, 11, 14] In the posterior and peripheral regions, seven consecutive segments 90 μm in length were examined in each of four sections. Counts were made within 2 mm of the optic nerve head in the posterior retina and beginning 180 μm from the ora serrata moving posteriorly in the peripheral

*Percentage composition: vitamin-free casein, 20; DL-methionine, 0.3; cellulose, 5.0; corn oil, 5.0; AIN-76 mineral mix,[19] 3.5; vitamin mix-3 without retinol,[20] 2.0; sucrose, 64.2. DL-α-Tocopheryl acetate was added at 20 mg/kg.

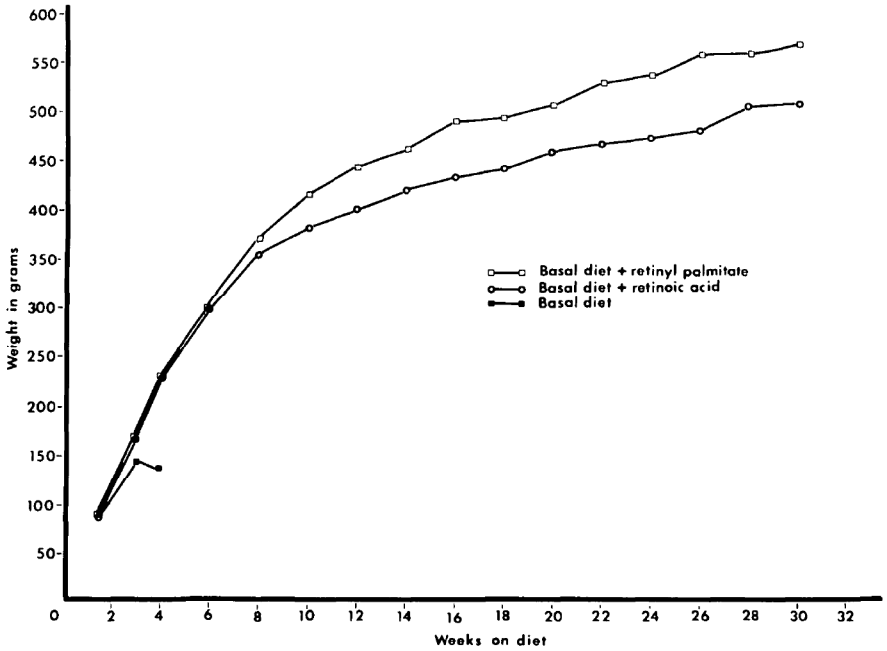

Fig. 1. Weight of rats on basal diet, basal diet + retinoic acid, and basal diet + retinyl palmitate. Each point represents the mean weight of 4 to 8 animals.

retina. The numbers of rod and cone nuclei were recorded in each region, and the mean, standard error of the mean, and percentage of remaining rod and cone nuclei were calculated.

To compare the effects of the deficiency on the four quadrants of retina, eyes from two rats supplemented with retinoic acid were examined at 36 weeks on the diet. The left eyes were bisected through the optic nerve along the vertical meridian and the right eyes along the horizontal meridian after fixation in glutaraldehyde. Portions of the cornea were removed to indicate inferior and nasal hemispheres. The half hemispheres were post-fixed and embedded in Epon as described above. Sections were cut to include the cornea; thus, in a given section, temporal and nasal regions or inferior and superior regions were present. Seven consecutive segments, 90 μm in length, were examined in each of four sections beginning 180 μm from the ora serrata moving posteriorly. The number of photoreceptor cells was recorded for each region, and the mean and standard error of the mean were calculated.

Results

Animals. Animals in all three groups gained weight at about the same rate for the first 2 weeks of the diet. Around the third week, rats in the group receiving only the basal diet weighed approximately 20 gm less than those supplemented with retinoic acid or retinyl palmitate. Rats fed only the basal diet also showed porphyrin around their eyes. By 4 weeks, many animals lost weight (Fig. 1), became lethargic, and showed hind limb weakness. All rats in this group were sacrificed by the fifth week. Rats in the other two groups continued to gain weight. Those supplemented with retinoic acid did not gain as much as the ones supplemented with retinyl palmitate (control group) beginning at 8 weeks on the diet through the period studied. These differences in weight were not statistically significant (mean ± S.D.: plus retinoic acid 481 ± 47 gm; plus retinyl palmitate, 521 ± 53; $p > 0.2$). Rats maintained with retinoic acid appeared as healthy as those supplemented with retinyl palmitate.

Rhodopsin and opsin levels. The amount of rhodopsin in the retinas of vitamin A–deficient rats (plus retinoic acid) was below control levels as early as 2 weeks on the diet

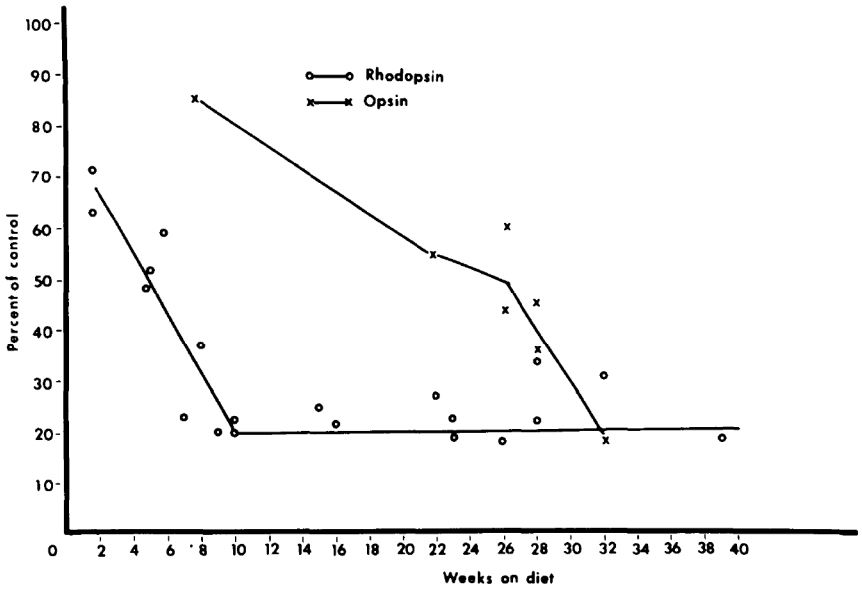

Fig. 2. Rhodopsin and opsin levels in vitamin A–deficient retinas expressed as percent of control. The curves are drawn to show the general trend of rhodopsin and opsin levels.

(Fig. 2). The level of rhodopsin was about 70% of the normal value at 2 weeks. Over the following 8 weeks, the percentage of rhodopsin decreased to approximately 20% of control values. (Analyses were made only on eyes from rats supplemented with retinoic acid, since those on the basal diet were sacrificed by 5 weeks. Hereafter, rats referred to as deficient were fed the basal diet plus retinoic acid.) The amount of rhodopsin in the retinas of the deficient rats remained relatively constant between 10 and 39 weeks on the diet. Opsin levels remained high in the retina during the early stages of vitamin A deficiency. After 8 weeks on the deficient diet, the retinas contained 85% of the control value of opsin (Fig. 2). By 28 weeks, the level of opsin was around 40% of control. The percentage of opsin dropped to 19% of control levels by 32 weeks of deficiency.

Histology. Retinas of rats in all groups appeared normal through 5 weeks on their respective diets. By 7 weeks, the distal two thirds of the outer segments in the posterior retina of rats supplemented with retinoic acid stained less intensely with toluidine blue, but no other morphological changes were seen. However, around 16 weeks on this diet, the outer segments began to show morphological abnormalities. Some discs were distended and disrupted into small vesicles ranging from 0.19 to 0.35 μm in diameter. This abnormal structure was confined largely to the distal third of the outer segment. The length of these outer segments in the posterior retina of rats receiving retinoic acid was comparable to controls (mean ± S.E.M.: plus retinoic acid, 21 ± 0.4 μm; control, 20 ± 0.3; n = 10 outer segments).

At 23 weeks, the structure of outer segments in the posterior region of deficient rat retinas appeared more disorganized (Fig. 3). Many discs in the distal third of the outer segments were distended or dispersed into vesicles approximately 0.1 to 0.3 μm in diameter. Some outer segment discs were oriented parallel to the long axis. Numerous densely stained, membranous vesicles, 0.2 to 0.4 μm in diameter, presumably portions of disc membranes, were present in the pigment epithelium and between pigment epithelial cell processes. In contrast, discs in the outer segments of retinas from rats supplemented with retinyl palmitate (control

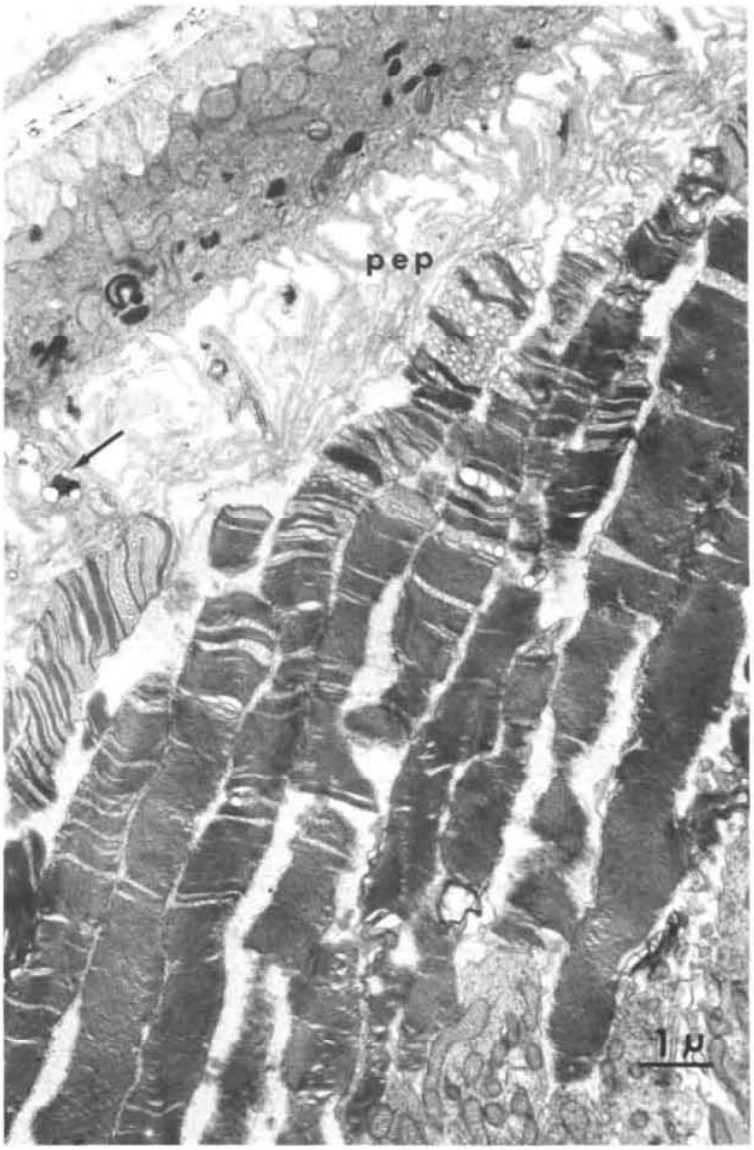

Fig. 3. Posterior retina of vitamin A–deficient rat maintained on the diet 23 weeks. The discs in the distal portion of many outer segments are disrupted into small vesicles. Some small vesicles are present between and in pigment epithelial processes (arrow). Processes of the pigment epithelium *(pep)* have lost their normal contact with outer segments.

group) were intact (Fig. 4). The outer segments were also shorter by 5 μm at 28 weeks in the posterior retina (mean ± S.E.M.: deficient, 14 ± 0.3 μm; control, 19 ± 0.1; n = 10 outer segments).

The intimate contact between the pigment epithelial cells and outer segments was lost in light-adapted deficient rat retinas (Fig. 3). Also, the lipid droplets in the pigment epithelium of the deficient retinas were quite homogeneous in density, except for an electron-dense cortical region (Fig. 5). Most

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