Cone and rod responses in nutritionally induced retinal degeneration in the cat

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All cats fed a specific semipurified diet developed a retinal degeneration. In the early stages electroretinograms (ERG's) from both the cone and rod systems were reduced in amplitude; those from the cone system were delayed in implicit time while those from the rod system were normal in implicit time. These ERG changes, as well as the findings on ophthalmoscopic and light microscopic examination, were consistent with the idea that initially widespread areas of cones and localized areas of rods were abnormal. In moderately advanced stages rod-implicit times were also delayed, and the slope of the function describing log stimulus intensity vs. rod ERG amplitude was abnormal; these recordings indicated that rod involvement had also become extensive. No signs of vitamin A deficiency were observed. Similarities between this retinal degeneration in the cat and retinal degenerations in man are discussed.

Key words: rod, cone, diet, vitamin A, macula, retina, electroretinogram, pigment epithelium, retinitis pigmentosa, cat eye, retinal degeneration.

Previous studies indicated that cats fed a specific semipurified diet develop retinal degenerations. Other investigations demonstrated that retinal degenerations occur sporadically in cats and raised the possibility that these degenerations were inherited. In these studies the photoreceptors of the area centralis and/or the peripheral retina were involved, but the separate effects on the cone and rod photoreceptor systems were not delineated.

This investigation was designed to resolve whether retinal degenerations previously described in the cat could be produced by feeding normal cats a specific semipurified diet. Electroretinographic testing was used to monitor the effects of this diet on the cone and rod photoreceptor systems. These effects were also studied with the light microscope and correlated with ophthalmoscopic findings. Electroretinograms (ERG's) from cats fed this semipurified diet were compared with those...
previously reported in humans with the early stages of retinal degenerations.

Materials and methods

Twelve domestic cats, approximately eight weeks old, representing eight randomly selected litters, were fed either a semipurified diet (Table I; 7 cats) or a commercial formula (Purina Cat Chow, Ralston Purina Company, St. Louis, Mo.; 5 cats). Three cats, chosen as control animals and fed the commercial formula, had litter mates fed the semipurified diet. Cats were fed water and either the semipurified diet or the commercial formula ad libitum and caged individually so that intake could be monitored. Body weight was recorded weekly. Cyclic light (12 hours on/12 hours off) was provided by overhead fluorescent (Sylvania Lifeline 40-watt warm white) lamps and average cage illumination was eight foot-candles. Room temperature was maintained at 25°C.

Cats were followed from two to twelve months, and representative stages of retinal degeneration were documented with fundus photographs.

During the course of these studies electoretinographic testing was performed in a full-field (ganzfeld) dome similar to that previously described for human testing. 2, 10 Testing was done under conditions of dark adaptation (one hour) or in the presence of a steady full-field white background light (30 foot-lamberts). Cats were anesthetized with sodium pentobarbital administered intraperitoneally (30 mg per kilogram), and pupils were maximally dilated with 1 per cent phenylephrine hydrochloride and 1 per cent cyclopentolate hydrochloride. Recordings were obtained with a double electrode (Burian-Allen) contact lens, amplified by a Tektronix RM122 preamplifier (bandpass 0.8 to 250 Hz.), displayed on a Tektronix 502A oscilloscope, and photographed on Polaroid film. Cone responses were averaged on a computer of average transients (Fabritek 1072).

This full-field system provided relatively homogeneous stimulation to the entire retina and therefore allowed careful study of the temporal aspects of the ERG. 9, 10 The time intervals between onset of the stimulus flash and either the major cornea-negative (a-wave) or cornea-positive (b-wave) response were used for the measurement of implicit times in this study. Since ERG implicit times and amplitudes are a function of stimulus intensity, implicit times were compared in normal cats and cats with retinal degeneration under stimulus conditions that elicited equal amplitude responses from both groups. Wavelength of the stimulus flash was modified by interposing filters between the light source (Grass PS2) and the inner surface of the dome. The stimulus flash was 10 μs in duration and had a maximum luminance of 30 foot-lamberts on the inner surface of the dome.

Table I. Semipurified cat diet

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Grams per 100 Gm.</th>
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<tbody>
<tr>
<td>Casein (vitamin free)*</td>
<td>32.1</td>
</tr>
<tr>
<td>Sucrose</td>
<td>37.4</td>
</tr>
<tr>
<td>Hydrogenated vegetable oil‡</td>
<td>12.5</td>
</tr>
<tr>
<td>Corn oil</td>
<td>12.5</td>
</tr>
<tr>
<td>Cod liver oil†</td>
<td>1.0</td>
</tr>
<tr>
<td>Salt mix§</td>
<td>4.0</td>
</tr>
<tr>
<td>Vitamin mix¶</td>
<td>0.2</td>
</tr>
</tbody>
</table>
dl-alpha Tocopheryl acetate | 0.01 |
|Choline chloride | 0.3 |

*General Biochemicals, Chagrin Falls, Ohio.
†Spry, Lever Brothers Co., New York, N. Y.
‡Cod liver oil contained 1,800 USP units vitamin A and 180 USP units vitamin D per gram.
¶The vitamins added to 385 grams of product were (in grams): niacin, 8; calcium pantothenate, 4; Pyridoxine HC1, 0.8; folic acid, 0.2; thiamin hydrochloride, 0.8; biotin, 0.04; menadione, 0.2; riboflavin, 1.0; and cyanocobalamin, 0.01.

Scotopically balanced lights were used to isolate the rod components of the ERG. Scotopic balance was achieved with a long-wave stimulus (Wratten 28, λ > 600 nm.) and a short-wave stimulus (Wratten 47, Wratten 47A, Wratten 47B, and 0.3 neutral density filter, λ < 470 nm.) that, respectively, elicited equal ERG b-wave responses near threshold (20 μV criterion) from normal dark-adapted cats. These long- and short-wave light flashes, matched near threshold in the normal cat, were presented well above normal threshold to dark-adapted cats on the semipurified diet in order to separate and monitor rod function. The responses were compared with suprathreshold responses to the same stimuli recorded from control cats fed the commercial formula.

Photopically balanced lights were used as an aid to separate the cone components of the ERG. Previous studies 11, 12 have shown that cone function in the cat can be isolated with stimuli presented at 40 cycles per second. Photopic balance was achieved with a long-wave stimulus (Cinnemoid 5 and 0.3 neutral density filter, λ > 550) and short-wave stimulus (Cinnemoid 16, λ < 550) that, respectively, elicited equal ERG b-wave amplitudes at 40 cycles per second. Single flashes of these lights, respectively, elicited identical ERG responses in the presence of a full-field white background light of at least 30 foot-lamberts.

Prior to necropsy, cats were anesthetized with sodium pentobarbital and a heparinized blood sample was drawn from the vena cava for vitamin A assay by conventional fluorometry. 13 The thorax was then opened, and a 1 per cent formaldehyde solution (1.25 per cent glutaraldehyde fixative (buffered to pH 7.4 with 0.1 M phosphate buffer) was perfused through the left heart for 5 to 10 minutes. The eyes were removed and sectioned through the
ora serrata, and the posterior globe was immersed in the same fixative for an additional 90 minutes, postfixed in 1 per cent osmium tetroxide for 120 minutes, and embedded in Epon 812. The major organs were removed and weighed and representative samples fixed in 10 per cent buffered formalin for routine light microscopy. Plastic embedded retinas were cut at 1 μ and stained with toluidine blue. In two cats perfusion was not used; the eyes were fixed for 12 hours in Bouin’s solution and embedded in paraffin. Serial sections were cut at 10 μ and stained with hematoxylin and eosin.

Results

A retinal degeneration was produced in all seven cats fed the semipurified diet and in none of the five cats fed the commercial formula. In all cats the degeneration was first visible with the ophthalmoscope after administration of the semipurified diet for three to seven months. Fig. 1 shows a normal fundus from a cat fed the commercial formula (A) and representative stages of the retinal degeneration (Figs. 1 B, C, D, E, and F) from five cats fed the semipurified diet. The earliest visible abnormality was a zone of granularity in the area centralis (B) usually associated with a central, white, highly reflexive spot. In moderately advanced stages (Figs. 1 C, D, and E), the white lesion occupied an oval zone in the area centralis bordered by a narrow margin of hyperpigmentation, and the surrounding fundus was diffusely granular. In some cats a second focal lesion was seen just above and nasal to the disc. These two lesions became confluent. At the same time the entire fundus appeared granular and pale, and some arterioles were narrowed. In a very advanced stage (Fig. 1 F), the original focal zone of discoloration could no longer be clearly defined, and the fundus had a blue-green appearance. Changes were symmetrical in both eyes of each cat, and the typical animal proceeded from the initial stage (Fig. 1 B) through one or more moderately advanced stages (Fig. 1 C, D, and/or E) to the very advanced stage F within nine months. When ophthalmoscopic changes were barely visible, ERG amplitudes were diminished. In the subsequent figures, ERG’s labeled I were recorded from cats fed the semipurified diet with the early stage of retinal degeneration (i.e., fundus photograph, Fig. 1 B) and ERG’s labeled II were recorded from cats fed the semipurified diet with moderately advanced stages of retinal degeneration (i.e., fundus photographs, Figs. 1 C, 1 D, or 1 E).

Fig. 2 demonstrates dark-adapted ERG responses to scotopically matched short-wave (left) and long-wave (right) light stimuli for a normal control cat fed the commercial formula (N), and for two cats fed the semipurified diet with retinal degeneration (Stages I and II). The stimuli, matched to elicit equal amplitude responses from the rod system of a normal dark-adapted cat near threshold, elicited responses from the normal cat (N) to suprathreshold stimuli that were virtually identical (top pair) except for a small early cornea-positive oscillation (presumably from the cone system) seen only in the response to the long-wave flash (top right). In the cats with retinal degeneration these same suprathreshold stimuli elicited identical responses (lower pairs) indicating that these responses were generated by the rod system. These rod ERG responses were reduced in amplitude compared with the normal responses. The rod b-wave implicit times for the cat with the early stage of retinal degeneration (I) were normal, while the rod b-wave implicit times were slightly delayed in the cat with moderately advanced retinal degeneration (II).

Fig. 3 shows rod-system responses to short-wave light stimuli and compares the rod b-wave implicit time of cats with the early (I) and moderately advanced (II) stages of retinal degeneration with the rod b-wave implicit times from a normal control cat (N). A vertical line (left column) has been extended from the peak of the normal rod b-wave (N) through the responses of cats with retinal degeneration (I and II) to the same intensity flash (0.0). Neutral density filters (0.3 to 1.5 log units) were placed in front of the normal cat eye (right column) to obtain amplitudes com-
Fig. 1. Fundus photographs of the left eye of a control cat fed a normal commercial diet (A) and cats fed the semipurified diet (B through F). All cats fed the semipurified diet developed retinal degeneration: early stage, photograph B; moderately advanced stages, photographs C, D, and E; and advanced stage, photograph F. In each cat the findings were symmetrical in both eyes.
Fig. 2. ERC recordings (top to bottom) under dark-adapted conditions for a normal cat (N), a cat with early retinal degeneration (I), and a cat with moderately advanced retinal degeneration (II); responses were obtained to suprathreshold, scotopically matched, short-wave (left) and long-wave (right) light stimuli. Calibration symbol (lower right corner) signifies 40 msec. horizontally, 40 μV vertically. Two or three successive responses are illustrated; stimulus onset is vertical hatched line; cornea-positivity is an upward deflection. Normal rod implicit times under these test conditions were 67 msec. ± 3 msec. and normal rod amplitude was 100 μV ± 20 μV. Rod b-wave implicit times were normal for cat I and slightly delayed for cat II (see text).

Fig. 3. Rod ERG responses under dark-adapted conditions for a normal cat (N), a cat with early degeneration (I), and a cat with moderately advanced retinal degeneration (II), to a suprathreshold short-wave light (left column) without neutral density filter interposed between the light source and these cats (0.0). ERG responses in the right column were obtained from a normal cat to the same short wavelength light without a neutral density filter (0.0) and with neutral density filters (0.3 to 1.5 log units) interposed between the light source and the normal cat. Rod b-wave implicit times are designated by horizontal arrows for the normal cat (top left and right). Cats with retinal degeneration (left column, I and II) and normal cats (right column) with comparable ERG amplitudes were compared with respect to implicit time (solid vertical lines, see text). Normal rod ERG implicit time was 67 msec. ± 3 msec. Rod b-wave implicit time was normal for cat I and slightly delayed for cat II. Two or three successive responses are illustrated, and stimulus onset is indicated by a vertical hatched line. The short-wave stimulus (λ < 470 nm.) at maximum intensity (0.0) was the same stimulus used to obtained the responses in Fig. 2, left column. Calibration symbol (lower right) signifies 40 msec. horizontally, 40 μV vertically. Corneal positivity is an upward deflection.

Fig. 4 illustrates cone system responses from a normal control cat (N), a cat with early retinal degeneration (I), and a cat with moderately advanced retinal degeneration (II); all were tested in the presence of a steady 30 foot-lambert white background light sufficient to eliminate the rod contribution to the ERG (see Methods). These responses were obtained with white-light stimuli (left) or long-wave (middle) and short-wave (right) stimuli matched in
Fig. 4. Cone ERG's, successively, from top to bottom for a normal cat (N), a cat with early retinal degeneration (I) and a cat with moderately advanced retinal degeneration (II). Left column illustrates the computer summation of 128 responses to single flashes of white light in the presence of a white adapting light of 30 foot-lamberts. Center and right columns represent, respectively, the computer summation of 128 responses to photopically matched long-wave and short-wave light in the presence of the same background light. Cone b-wave implicit times are designated by arrows (see text). Normal cone b-wave implicit time to white light was 17 msec. ± 1 msec. Normal cone a-wave implicit time to white light was 6.5 msec. ± 1 msec. Normal cone amplitude (peak of the a-wave to peak of the b-wave) to this white light stimulus was 18 μV ± 3 μV. Corneal positivity is an upward deflection. Stimulus onset is a vertical hatched line. Calibration symbol (lower right) signifies 2 μV vertically for the left column and 1 μV for the center and right columns and 10.5 msec horizontally for all responses.

brightness for the cone system. The cats with both stages of retinal degeneration have b-wave responses compared with normal that are not only reduced in amplitude but also delayed in implicit time (see arrows). These reductions in amplitude and delays in implicit time were seen not only in response to white-light stimuli (left), but also in response to the long-wave light (middle) and short-wave light (right). For each stage comparable reductions in amplitude and delays in implicit time could be seen in response to stimuli from both ends of the spectrum matched for the cone system. For the cat with early retinal degeneration (I) the a-wave was also reduced in amplitude and delayed in implicit time. For the cats with moderately advanced degeneration, the a-waves were so small that implicit times could not be clearly defined.

These delays in cone b-wave implicit time in the cats with both stages of retinal degeneration could also be seen in response to white-light flashes at 40 c.p.s. Fig. 5 (left column) compares the cone b-wave implicit times of these cats (I and II) with the cone b-wave implicit times from a normal cat (N). Neutral density filters (0.3 and 0.6) were placed in front of the normal cat eye (right column) to obtain amplitudes comparable to those from the cats with retinal degeneration (left column, I and II). When cone responses from the normal cat (N) were matched in amplitude with responses from the cats with retinal degeneration (I and II), the implicit times in the cats fed the semipurified diet were longer than the implicit times in the cats fed the commercial formula.

Fig. 6 illustrates measurements of dark-adapted responses to suprathreshold white light (predominantly rod responses)* for a normal control cat (N), a cat with early retinal degeneration (I), and a cat with moderately advanced retinal degeneration (II). These measurements of log-stimulus intensity (abscissa) vs. b-wave amplitude (left ordinate) and a-wave amplitude (right ordinate) show that both a- and b-wave amplitudes are reduced below normal in the cats fed the semipurified diet (I and II). The slope of the function (linear portion) describing b-wave amplitude vs. log-stimulus intensity (solid line) is the same as the normal (N) for cat I, but is marked-

*Fig. 4 illustrates that the maximum cone contribution to suprathreshold white light for a normal cat was 20 μV in this test system. The same white-light flash elicited at least a 450 μV response from the normal dark-adapted cat. This demonstrated that the rod contribution to the dark-adapted ERG in response to white light is approximately 95 per cent of the total ERG response amplitude, assuming linear summation of cone and rod system components.
Fig. 5. Cone ERG responses to suprathreshold white flickering light (40 c.p.s.) for a normal cat (N), a cat with early (I), and a cat with moderately advanced (II), retinal degeneration (left column) to maximum intensity stimulus flash (0.0). The ERG's in the right column are responses from a normal cat (N) to the same white flickering light (top) and to successively dimmer stimuli (0.3 and 0.6 log units). Vertical shock artifacts designate stimulus onset in all traces. Cone b-wave implicit times are shown by arrows (see text). Each trace represents the computer summation of 256 sweeps. Corneal positivity is an upward deflection. Calibration symbol (lower right) signifies 2 μV vertically and 10.5 msec. horizontally.

ly reduced for cat II. Similarly, the slope of the function describing a-wave amplitude vs. log stimulus intensity (dashed line) is the same as the normal (N) for cat I but is markedly reduced for cat II.

Histologic examination of the retinas revealed that cats fed the semipurified diet had degenerative changes of the photoreceptors in the area centralis in the early stages; in the advanced stages areas of photoreceptors in the peripheral retina were also involved. The most extensive loss of photoreceptors occurred where the concentration of ganglion cells was greatest. In the zone surrounding the area centralis, some preservation of photoreceptor nuclei could be seen in the same animal. Photoreceptor nuclei appeared intact in many areas of the peripheral retina. In areas of minimal disruption, the outer segments appeared shortened and fragmented with a few pyknotic photoreceptor nuclei (Fig. 7 B) in contrast to the normal (Fig. 7 A). In areas where loss of the inner and outer segments was complete, loss of the outer nuclear layer was also evident (Figs. 7 B, 7 C, and 7 D). In areas where the outer nuclear layer was absent, the inner nuclear layer appeared to have a widened profile (Fig. 7 D) possibly related to proliferation of glial cells in this area. The pigment epithelial cell layer appeared unaltered, but these light microscopic studies were inconclusive with regard to possible alterations in the fine structure of these cells, particularly during the early stages.

Plasma vitamin A levels were 71 ± 2 μg per 100 ml. for the cats fed the semipurified diet and were 66 ± 10 μg per 100 ml. for the control cats fed the commercial formula. Food intake was reduced in the cats fed the semipurified diet when compared to those fed the commercial formula, and this was associated with a reduced
Fig. 6. Log-stimulus intensity (abscissa) vs. ERG amplitude (b-wave left ordinate; a-wave right ordinate) for a normal cat (N), a cat with early retinal degeneration (I), and a cat with a moderately advanced retinal degeneration (II). Solid symbols (•, ■, ▲) and open symbols (○, □, △) represent measurements of b-wave and a-wave amplitudes, respectively, for each cat. Solid and dashed lines (see text) were drawn, respectively, through the linear portion of the b-wave and a-wave intensity-amplitude functions. Each symbol represents the average measurement of 3 or more responses.

mean body weight at nine months of age (2,630 ± 261 grams vs. 3,998 ± 640 grams). Histologic sections of cornea, lung, and the parotid duct system showed no squamous cell metaplasia.

Discussion

This study confirms that cats fed a specific semipurified diet (Table I) develop a retinal degeneration. Furthermore, the “feline central retinal degeneration” described by Bellhorn and Fischer as a possible hereditary disease and the “feline degenerative retinopathy” described by Morris as a dietary-induced diffuse retinal degeneration appear to be early and advanced stages of the degeneration produced in this study. Scott, Greaves, and Scott studied cats fed a similar diet and reported widespread retinal degeneration associated with systemic manifestations of vitamin A deficiency and concluded that a defect in utilization and storage of vitamin A was present. Signs of vitamin A deficiency (xerosis of the conjunctiva, corneal vascularization, and squamous metaplasia of the corneal epithelium and parotid duct system), reported by Scott, Greaves, and Scott, were not present in these cats. In addition, plasma vitamin A levels in the cats fed the semipurified diet were comparable to levels measured in control cats raised on the commercial formula. The specific deficiency or factor in this semipurified diet responsible for this degeneration is not known, although casein as the principal source of protein is a dietary component common to all studies in which nutritionally induced retinal degeneration in the cat has been reported.

The earliest lesions visible with the ophthalmoscope, oval in shape and located in the area centralis, followed a pattern consistent with high cone density. Histologic examination revealed that the degree of photoreceptor damage was greatest in the area of high ganglion cell density and therefore cone density as well. Furthermore, the oval pattern observed during the early stages of this degeneration closely resembled the oval pattern of cone distribution determined by Steinberg, Reid, and Lacey through direct photoreceptor cell counts in the normal cat retina. The wide-
Fig. 7. Photomicrographs of retina from control cat fed commercial formula (A) and three cats fed the semipurified diet (B, C, and D). Anatomic layers are identified for the control only ganglion cell layer Gcl; inner plexiform layer, IPS; inner nuclear layer, INL; outer plexiform layer, OPL; outer nuclear layer, ONL; inner segment, IS; outer segment, OS; pigment epithelium, PE; and tapetum, TAP. Early (B), moderately advanced (C), and advanced stages (D) of the degeneration are represented (see text). Toluidine blue ×400.

spread involvement of the cone system even during the early stages of this degeneration could be seen in the cone ERG's which showed not only reductions in amplitudes but also delays in a- and b-wave implicit times.16 The mechanism underlying delays in cone system implicit time in cats with retinal degeneration remains to be defined.

In contrast to the delays in cone system ERG b-wave implicit times, rod system ERG b-wave implicit times in the early stage were normal. Previous studies in man have shown that focal or patchy destruction of rods and cones is associated with reductions in ERG b-wave amplitudes with preservation of normal b-wave implicit times.16 The present study has demonstrated that focal or patchy destruction of rods in the area centralis is associated with reductions in rod ERG b-wave amplitude and normal rod b-wave implicit time. The idea that only a localized area of rods in the central retina is destroyed during the early stages is consistent with the normal histologic appearance of large areas of photoreceptors in the rod-dense peripheral retina. This is also consistent with the observation that the slope of the function describing the rod-dominated dark-adapted ERG amplitude vs. log-stimulus intensity was normal during the early stage. In moderately advanced stages large areas of rod photoreceptors were abnormal. The slope of the ERG amplitude vs. log-stimulus intensity function also became markedly abnormal, and the implicit times of the rod responses were delayed; both changes have been described in widespread degenerative retinopathies that occur in man.9,17-19

Nutritionally induced retinal degeneration in the cat may prove to be an important model for the study of retinal degenerations in man. The delays in cone ERG b-wave implicit times seen in the early stages of practically all types of retinitis pigmentosa17-19 have been stimulated for the first time in an animal model. When stimulus flash intensity was reduced so that cone system b-wave amplitudes from a normal subject could be matched with the reduced cone b-wave amplitudes seen in patients with retinitis pigmentosa, the implicit times of the normal subject were faster than the implicit times observed in these

*The ratio of rods to cones in the area of highest cone density (area centralis) of the cat retina has been shown to be approximately eleven rods to one cone.16
patients.\textsuperscript{10} This same phenomenon was observed in cats fed a semipurified diet when their cone system ERG b-wave amplitudes and implicit times were compared with responses matched in amplitude from normal cats. Furthermore, this condition closely resembles one specific degeneration\textsuperscript{20} described in man, “progressive cone-rod degeneration.” During the early stages of “progressive cone-rod degeneration,” bilateral macular involvement is clearly visible with the ophthalmoscope and ERG responses indicate widespread involvement of the cone system and involvement of localized or patchy areas of the rod system. Investigations are in progress in the cat to define the specific nutritional factor(s) responsible for this degeneration, to study the relationship between the photoreceptor outer segments and pigment epithelium in greater detail, and to evaluate the effects of light\textsuperscript{21-23} on the pathogenesis of this degeneration.

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REFERENCES