Electroretinograms in English setters with neuronal ceroid lipofuscinosis. ELLIOT L. BERSON AND GAIL WATSON.

Full-field electroretinograms (ERGs) were recorded from three adult English setters with advanced neuronal ceroid lipofuscinosis and three normal controls. Affected setters showed 30% to 40% reductions in a-wave and b-wave amplitudes, normal cone and rod b-wave implicit times, and slightly elevated a-wave and b-wave thresholds. The ERGs of these affected setters differed from those that have been recorded from humans with neuronal ceroid lipofuscinosis (Batten's disease), humans with hereditary retinitis pigmentosa, Irish setters with rod-cone dysplasia, and miniature French poodles with progressive rod-cone degeneration.

The English setter with neuronal ceroid lipofuscinosis has been considered as an animal model for Batten's disease in humans. Both affected setters and humans with Batten's disease have brain dysfunction and ataxia, and both have shortened life-spans. Both have prominent lipopigment-containing cytoplasmic inclusion bodies in the brain and the ganglion cells of the retina; these inclusion bodies have membranes arranged in a characteristic curvilinear or fingerprint pattern. Some authors report that both affected setters and humans have a deficiency of white blood cell peroxidase activity with p-phenylenediamine as substrate; in affected setters this enzyme has been reported to be deficient in the retina and pigment epithelium. Humans with Batten's disease show macular degeneration, optic disc pallor, retinal vessel attenuation, and pigmentary abnormalities in the periphery; in contrast, no fundus abnormalities have been observed on ophthalmoscopic examination of affected dogs. Humans with Batten's disease have been reported to have very reduced or nondetectable electroretinograms (ERGs) even at an early stage; in these studies reduced responses were not subdivided into rod and cone components, and b-wave implicit times were not measured. The present study was done to evaluate ERGs of adult English setters with an advanced stage of neuronal ceroid lipofuscinosis.

Methods. Full-field ERGs were recorded from three adult English setters, ages 20 to 22 months, with neuronal ceroid lipofuscinosis. English setters affected with this autosomal recessive disease have a usual life expectancy of 23 to 26 months. Diagnosis in each affected animal was established in early life from a biopsy specimen that showed characteristic curvilinear and fingerprint bodies in brain neurons. These affected setters had ataxia at the time of this ERG testing. ERGs were also recorded from three normal English setters, ages 5, 21, and 22 months; these dogs had had no abnormalities in brain biopsy specimens. Both affected and normal dogs had clear media and no evidence of retinal degeneration on fundus examination and were active and mobile despite transport from Norway 3 to 5 days prior to testing.

Normal and affected animals were anesthetized with either sodium pentobarbital (20 to 35 mg/kg) administered intravenously or sodium pentobarbital (10 mg/kg) preceded by an intramuscular injection of acepromazine (5 mg). Pupils were maximally dilated with 10% phenylephrine hydrochloride and 1% cyclopentolate hydrochloride prior to testing. Each dog was dark-adapted for a minimum of 1 hr, and a Burian-Allen double-electrode contact lens was placed on the topically anesthetized cornea. Attention was given to the position of the eyes during testing, and recording was not done if the eyes were rolled in the extreme down position. The ERG lens was placed so that the nictitating membrane did not cross the cornea during testing. These precautions helped to ensure that affected and normal animals were tested under comparable conditions. Every effort was made to ensure adequate contact between the lens and cornea, however, the maximum ERG that could be elicited with white light from the normal dogs was 160 μV (peak-to-peak amplitude).

ERGs were recorded in a ganzfeld test system to 10 μsec single flashes of dim blue light (λ < 470 nm, Wratten 47, 47A, and 47B before 30 × 10−2 ft-Lambert white light source), single flashes of white light of gradually increasing intensity up to 80 × 10−2 ft-Lamberts, and 30 × 10−2 ft-Lambert white flickering light presented at 30 cycles/sec (cps). Stimulus intensities were measured with a United Detector Technology photometer, model 40X, and calculated taking into account the Talbot-Plateau law. Responses to blue light and white 30 cps flickering light were quantitated with respect to peak-to-peak amplitudes, and responses to single flashes of white light were quantitated with respect to a-wave (baseline to cornea-negative peak) and b-wave (baseline to cornea-positive peak) amplitudes. Responses were also quantitated with respect to rod and cone b-wave implicit times.
Fig. 1. Representative full-field ERGs from a normal adult English setter and an adult English setter with neuronal ceroid lipofuscinosis. Two or three consecutive responses for each stimulus condition are illustrated. Calibration symbol, lower right corner, designates 20 µV vertically for columns 1 and 2, and 4.5 µV for column 3, and horizontally 20 msec for all tracings. Stimulus onset is vertical hatched lines for columns 1 and 2 and vertical shock artifacts for column 3. Arrows (column 3) designate cone b-wave implicit times.

Table I. ERG response amplitudes (µV) of normal and affected English setters*

<table>
<thead>
<tr>
<th></th>
<th>Blue</th>
<th>White†</th>
<th>White 30 cps</th>
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<tr>
<td>Normal</td>
<td>29 (26-33)</td>
<td>22 (14-30)</td>
<td>97 (83-118)</td>
</tr>
<tr>
<td>Affected</td>
<td>17 (16-19)</td>
<td>14 (7-18)</td>
<td>71 (52-78)</td>
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*Average and range for 3 normal setters and 3 setters with neuronal ceroid lipofuscinosis.
†30 X 10^-3 ft-Lambert white light stimulus.
cifically, humans with early retinitis pigmentosa, Irish setters with rod-cone dysplasia, and miniature French poodles with progressive rod-cone degeneration have shown prolonged cone and/or rod b-wave implicit times. Humans with hereditary retinitis pigmentosa, tested under stimulus conditions similar to those used for testing these English setters, have shown cone and/or rod b-wave implicit times that are often delayed by as much as 10 to 15 msec. These delays seen in species with known photoreceptor cell degeneration contrast with the normal cone and rod b-wave implicit times and intact photoreceptor cells found in affected English setters with advanced systemic disease.

In affected setters the combination of 30% to 40% reductions in cone and rod ERG amplitudes, normal b-wave implicit times, slight elevations of a-wave and b-wave thresholds, and structurally intact photoreceptors remains difficult to explain. The biochemical abnormalities reported in retina and pigment epithelium of affected setters could possibly lead to photoreceptor malfunction with ERG changes. A secondary effect on the retina due to systemic disease must also be considered. Another possibility is that the heavy accumulation of lipopigment in ganglion cells, reported in the inner retina, so that the intensity of the stimulus flash reaching the photoreceptors is reduced. A screening effect of lipopigment, if present in the cornea or lens, could also be contributory. Spectrophotometric analyses of retina, cornea, and lens of normal and affected English setters are needed to help resolve whether or not lipopigment proximal to the photoreceptor cells absorbs incident light and thereby leads to the reductions in the ERG recorded at the cornea.

We thank Dr. Nils Koppang, Department of Pathology, Veterinary College of Norway, Oslo, for allowing us to study his colony of English setters, and Dr. Donald Armstrong, University of Colorado, for his cooperation so that English Setters en route from Oslo, Norway, to Denver, Colorado, for ultrastructural and biochemical studies could first have electroretinographic testing in Boston.

From the Berman-Gund Laboratory for the Study of Retinal Degenerations, Harvard Medical School, Massachusetts Eye and Ear Infirmary, Boston, Mass. This work was supported in part by National Eye Institute Research Grant EY00169 and Research Career Development Award EY70800 (E. L. B.) and in part by the National Retinitis Pigmentosa Foundation, Baltimore, Md., and The George Gund Foundation, Cleveland, Ohio. Submitted for publication June 25, 1979. Reprint requests: Dr. E. L. Berson, Berman-Gund Laboratory, 243 Charles St., Boston, Mass. 02114.

Key words: Batten’s disease, neuronal ceroid lipofuscinosis, retinal degeneration, retinitis pigmentosa, retina, dog, rod, cone, electroretinogram, hereditary

REFERENCES
7. Armstrong D, Siakotos A, Koppang N, and Connole E: Studies on the retina and the pigment epithelium in hereditary canine ceroid lipofuscinosis. I. The distribution of enzymes in the whole retina and
Collagen production by cultured retinal capillary pericytes. 

Marco P. Cohen, Robert N. Frank,* and Ahmed A. Khalifa.

The cells that proliferate from microvessels isolated from calf retinas and placed in tissue culture have been found to derive from the intramural pericytes. When these cells were cultured in medium supplemented with ascorbate and pulse labeled for 24 hr with [14C]proline, about 2% of the [14C]proline in the nondialyzable protein secreted into the culture medium by these cells was hydroxylated, and [14C]collagen polypeptides were recovered in large-molecular-weight aggregates (>300,000 daltons) which were largely converted to chains of approximately 95,000 MW = 180,000 and 270,000 when the medium was subjected to reduction and alkylation prior to chromatography. The findings indicate that cultured retinal pericytes elaborate collagen and suggest the production of type III collagen. Retinal microvessel cells in culture may facilitate study of abnormalities in retinal pericyte function and collagen synthesis that occur in retinal vascular disease.

Retinal capillaries contain endothelial cells, which line the vessel lumen, and intramural pericytes, which lie outside the basement membrane surrounding the endothelial cells and which are themselves enveloped by a basement membrane. Although the anatomy of the retinal pericytes has been described in numerous ultrastructural investigations, details of their physiology and biochemistry are largely unknown. Examination of their biosynthetic products would offer one approach toward gaining insight into the functional role of these cells. Such an opportunity is provided with tissue culture of viable retinal capillaries from adult animals and juveniles beyond the neonatal stage. Such capillary fragments produce monolayers of cells with relatively uniform morphology that derive from intramural pericytes. Since collagen production has been described in cultures derived from other vascular tissues, examination of cultured retinal pericytes in culture elaborates collagen and suggests the production of type III collagen. Retinal microvessel cells in culture may facilitate study of abnormalities in retinal pericyte function and collagen synthesis that occur in retinal vascular disease.

Table I. [14C]proline incorporation into nondialyzable peptides and [14C]hydroxyproline synthesis by cultured retinal pericytes

<table>
<thead>
<tr>
<th>Exp 1</th>
<th>Medium</th>
<th>105.0</th>
<th>1.7</th>
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<tr>
<td>Cell layer</td>
<td>82.3</td>
<td>1.4</td>
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<tr>
<td>Exp 2</td>
<td>Medium</td>
<td>105.0</td>
<td>2.1</td>
</tr>
<tr>
<td>Cell layer</td>
<td>82.3</td>
<td>1.7</td>
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After 24 hr of continuous labeling with [14C]proline, the media were collected, and the cell layers were harvested by mechanical scraping followed by homogenization in 0.5M acetic acid. Both fractions were extensively dialyzed against 0.5M acetic acid in the cold until the dialysates were free of radioactivity. [14C]proline and [14C]hydroxyproline were determined as described in the text on triplicate aliquots in each experiment. Values presented have been corrected for recovery, determined by simultaneous performance of a series of labeled proline and hydroxyproline standards.

*H/T = ratio of [14C]hydroxyproline to total [14C] label in the experimental sample, expressed as a percentage.