PURPOSE. To describe and classify the morphologic changes in a naturally occurring dog model of early-onset cone–rod dystrophy (CRD) and to correlate these with earlier described clinical characteristics of the disease in dogs.

METHODS. Purpose-bred Standard Wire-Haired Dachshunds (SWHDs) derived from a large pedigree of dogs with early-onset CRD were euthanatized at defined ages to characterize morphologic changes in the disease process. Specimens were examined by light microscopy, including morphometric studies, electron microscopy, and immunohistochemistry. Peanut agglutinin (PNA), protein kinase C (PKC), synaptophysin (Syn), rhodopsin (Rh)-63, glial fibrillary acidic protein (GFAP), and short-wavelength cone opsins (OS) were used for immunohistochemical characterization.

RESULTS. The photopic cone-system–derived ERG amplitudes were already significantly reduced or nonrecordable in CRD-affected dogs at 5 weeks, the earliest age studied. The outer retina was morphologically most severely affected initially, with a subsequent degeneration of the inner retina. Cone degeneration was more pronounced than rod degeneration in young CRD-affected dogs. There was a marked phenotypic variation based on morphologic findings in the affected dogs. At the earliest time point studied (5–8 weeks) cone photoreceptor and glial cell abnormalities were observed, in accordance with earlier studies based on electrophysiological and clinical findings in which day blindness and abnormal cone ERGs were observed in young affected SWHD puppies. Preliminary genetic studies have indicated an autosomal recessive mode of inheritance for the defect.

CONCLUSIONS. Through functional and structural characterization, early-onset cone abnormalities were found, consistent with a cone dysplasia at an age when rod structure was normal. Further studies are in progress to identify the gene(s) involved in this retinal disease process. The presently described natural animal model of primary cone dysplasia followed by rod degeneration may provide further insight into the human counterpart. Further studies are needed to ascertain an autosomal recessive mode of inheritance for CRD in the SWHD. (Invest Ophthalmol Vis Sci. 2008;49:1106–1115) DOI:10.1167/iovs.07-0848

Cone-rod dystrophies (CRD) comprise a heterogeneous group of naturally occurring inherited retinal disease entities described in humans and dogs. The group of disorders is characterized by a predominant loss of cone function, with relative preservation of the rod function.1,2 CRD has recently been described in the literature in three different dog breeds: the Standard Wire-Haired Dachshund (SWHD), the Miniature Long-haired Dachshund (MLHD), and the Pit Bull Terrier (PBT). The few existing reports on mode of inheritance of CRD in dogs indicate an autosomal recessive mode of inheritance.3–6

In humans, autosomal recessive, X-linked, and autosomal dominant modes of inheritance have been described for this group of diseases, the latter being by far the most common.2 A colony of CRD-affected SWHD-derived dogs was established through back-crossing the founder, a male affected with CRD, with his daughters. The colony comprised 82 dogs (Fig. 1). Results of clinical and electrophysiological studies in affected dogs of the colony have recently been described.3,4 In short, there was a great variation in age of onset and development of clinical signs in the CRD-affected SWHDs. One of the most characteristic clinical findings was pin-point–sized pupils, observed in 60% of the 8-week-old CRD-affected puppies. Older CRD-affected dogs displayed dilated pupils and delayed pupillary light reflexes (PLRs) when stimulated with a focal light source (for details, see Ropstad et al.4), in contrast to that reported from studies of other CRD-affected dog breeds, in which pin-point–sized pupils was never observed.3,4 Age at onset of funduscopic changes in the SWHDs varied from 10 months to 3 years of age. Initial changes were a subtle cellophane-like sheen in the tapetal fundus and a characteristic motting of the pigment in the nontapetal area, followed by a marked increase in tapetal sheen and attenuation of the retinal vessels with subsequent hyper reflectivity in the tapetal fundus and marked pigment migration in the nontapetal fundus (see Fig. 2). A bilateral generalized retinal atrophy was evident within the age of 6 years.5 Electoretinographic (ERG) examinations of CRD-affected SWHDs showed that the photopic cone-derived amplitudes were significantly lower and never reached the levels recorded in control dogs. In affected dogs there was no increase with age in amplitudes recorded using 30.1- and 50.1-Hz flicker stimuli, in contrast to the control groups where the photopic b-wave amplitude recorded at 50.1-Hz increased significantly with age. In affected animals, scotopic rod–derived amplitudes were significantly lower for most recordings than those of control dogs. Both a- and b-wave implicit times were significantly longer in the youngest affected group when compared to the age-matched control group at 0.6 log cd · s/m2 and 5.1-Hz single-flash light stimuli. In the control dogs, however, there was a significant shortening in a-wave implicit times from age 5 to 8 weeks, and in a-and...
b-wave implicit times recorded at 5.1-Hz single flash stimuli from age 5 to 52 weeks.

The present study was performed to characterize the morphologic changes in the CRD-affected SWHDs and to correlate these with the clinical findings of the disorder.

**MATERIALS AND METHODS**

**Animals**

The colony, including the founder, comprised 82 SWHD-derived dogs of which 27 were found to be affected with CRD based on electoretinographic examinations (ERG; Fig. 1). Dogs that died without undergoing ERG examinations were not included in the study. Unrelated age-matched dogs without any history of eye disease served as controls. Purebred Dachshunds were used as controls for ERG interpretations. In the present study, 22 eyes of 22 dogs were used for morphologic evaluations (Table 1). Two affected littermates and a normal control dog were used for correlations between clinical findings, including ERGs and results of the morphologic studies (Fig. 3).

All procedures were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the Norwegian Animal Research Authority (Forskolethyttanet).

**Clinical Examinations**

The dogs were subjected to complete ophthalmic examinations from age 5 to 8 weeks and then at regular intervals during the first year of life. Visual behavior and studies of the pupillary light reflexes (PLRs), slit lamp biomicroscopy, and indirect ophthalmoscopy, were performed, the latter two examination procedures using a short acting mydriatic (tropicamide 5 mg/mL; Mydrian; CIBA Vision AG, Hettingen, Switzerland). All dogs included in the study were subjected to bilateral full-field ERG examinations on several occasions, starting with 5 to 7 weeks as the earliest time point studied in most dogs (Table 1).

**Electroretinography**

ERG was performed as previously described. In short, dogs were dark adapted for at least 1 hour, after which general anesthesia was induced in the dark under red dim light. The dogs were premedicated with xylazine hydrochloride (Narcoxyl vet, 20 mg/mL; Intervet int. BV, Boxmeer, The Netherlands). All dogs were intubated at the induction of anesthesia, which was induced and maintained with propofol 10 mg/mL (4 mg/kg followed by 20 mg/kg/h IV; Fresenius Kabi AB, Uppsala, Sweden) administered by an infusion pump. Pancuronium 2 mg/mL (Pavulon, 0.01 mg/kg IV; NV Organon, Oss, The Netherlands), was used to prevent downward rotation of the eye. Inhaled and exhaled O2 and CO2, SpO2, pulse, and respiration frequency were continuously monitored. Oxygenation and artificial ventilation were instantly initiated at SpO2 levels below 92%. Pupils were dilated by the use of tropicamide (Mydrian 5 mg/mL; Ciba Vision AG). Each ERG session was recorded from −6.0 to 0.6 log cd·s/m2 by use of a selected sequence of white light stimuli. Brightness was increased at intervals of 0.5 log from −6.0 to −4.0 log cd·s/m2, intervals of 0.3–1.0 log from −4.0 to 0.6 log cd·s/m2. The average of eight flashes at a frequency of 0.5 Hz were used for the least bright recordings, an average of four flashes at the same frequency at −4.0 log cd·s/m2 and a single flash with an interval of 1 minute between recordings from −3.0 to 0.6 log cd·s/m2. The animals were then light adapted (37 cd/m2) for 5 minutes. Photopic ERGs were elicited by use of 0.0 log cd·s/m2 of light stimuli performed at 5.1-Hz and 0.3 log cd·s/m2 at 30.1- and 50.1-Hz flicker.

**Morphologic Studies**

In close proximity to ERG examinations, specific dogs were euthanized. As sedation, a mixture of tiletamin-zolazepam (Zoletil Vet; Virbac SA, Carros, France) xylazine (Narcoxyl vet, 20 mg/mL; Intervet Int. BV), and butorphanol tartrate 10 mg/mL (Torbugesic vet, 0.15 mL/kg; ScanVet Animal Health A/S, Fredensberg, Denmark) was used, followed by an intravenous overdose of barbiturates. The eyes were enucleated within 2 minutes and transected at the ora ciliaris retina. The anterior segment, lens, and vitreous body were discarded. The eye cups were sectioned into two halves by a vertical incision from the superior to the inferior retinal margins through the optic disc. The nasal part of the right eye and the temporal part of the left eye were used for lectin and immunohistochemical examinations. For the two latter, the specimens were fixed in 4% phosphate-buffered formaldehyde (generated from paraformaldehyde in 0.1 M Sorensen’s buffer, adjusted to pH 7.4) at 4°C for 4 hours. The tissue was rinsed and cryoprotected by stepwise transference through solutions with increasing concentrations of sucrose (10%, 15%, and 20%) in Sorensens’s buffer. The specimens were embedded in Yawalla media (30% egg albumen and 3% gelatin in water) and mounted for sectioning. Approximately 50 sections, each 12 μm thick, were cut in the vertical plane from the central part toward the periphery by using a cryostat, comprising a total width of approximately 2 mm horizontally. The sections were collected on chrome alum-coated slides, air dried, and stored at −20°C until used. Several sections from this batch were stained with hematoxylin and eosin for routine light microscopy. Sections for lectin and immunohistochemical examination were thawed rapidly at room tem-

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**Table 1. Morphologic and Clinical Diagnostics Used in the Study**

<table>
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N, normal; A, affected; M, male; F, female; ERG, electoretinography; IHC, immunohistochemistry; LM, light microscopy on toluidine blue-stained sections; EM, electron microscopy.
ture and washed in phosphate-buffered saline (PBS) at pH 7.2 with 0.25% Triton X-100 (PBST; Merck). Bovine serum albumin 1% (Sigma-Aldrich, St. Louis, MO) was added to the PBST for diluting the primary and secondary antibodies. The sections were then incubated in the appropriate dilution of the primary antibodies (see Table 2) for 24 hours at 4°C. After 1 hour at room temperature, the slides were rinsed in PBST and incubated for 45 minutes in darkness with the appropriate fluorescein isothiocyanate (FITC) or Texas red–conjugated antibodies. The remaining halves of the eye cups were immediately placed in an electron microscopy (EM) fixative consisting of 1.25% glutaraldehyde, 2% paraformaldehyde, 0.13M Na-cacodylate, and 0.13 mM CaCl2, and washed in 0.17 M sodium cacodylate (pH 7.4), postfixed with osmium tetroxide, 2% paraformaldehyde, 0.13M Na-cacodylate, and 0.13 mM CaCl2. The specimens were examined with an electron microscope (model 1200EX; JEOL, Tokyo, Japan).

For morphometric studies, the number of photoreceptor nuclei within each vertical row in light microscopic sections was collected on 200-mesh, copper, thin-bar grids with a diamond knife. The remaining halves of the eye cups were immediately placed in an electron microscopy (EM) fixative consisting of 1.25% glutaraldehyde, 2% paraformaldehyde, 0.13M Na-cacodylate, and 0.13 mM CaCl2, and washed in 0.17 M sodium cacodylate (pH 7.4), postfixed with osmium tetroxide, 2% paraformaldehyde, 0.13M Na-cacodylate, and 0.13 mM CaCl2. The specimens were examined with an electron microscope (model 1200EX; JEOL, Tokyo, Japan).

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RESULTS

Breeding Studies

A total of 81 offspring were obtained from the founder of the colony (Fig. 1). Eight puppies were stillborn or died before determination of disease status. Thus, 74 dogs, including the founder of the colony, were available for examination. No sex bias was observed in disease transmission. Evaluation of the breeding pedigree supported an autosomal recessive mode of inheritance for CRD in these SWHDs.

Clinical Studies

Ophthalmoscopy. Age of onset of ophthalmoscopic changes in the CRD-affected SWHDs varied from 10 months to 3 years. The initial findings consisted of a characteristic decoloration and a pigment clumping in the nontapetal region and a subtle thinning of the retinal vessels and cellophane-like hypo- or hyperreflective sheen in the tapetal region (Fig. 2). These changes have formerly been described as being bilaterally symmetric, but in recent studies, they have been shown to be initially asymmetric in some individuals. With progression of disease the changes became bilaterally symmetric consisting of hyperreflectivity in the tapetal area, thinning of the retinal blood vessels, pallor of the optic nerve head, and a significant decoloration with hyperpigmented ridges in the nontapetal area. A marked individual variation in the rate of progression of the ophthalmoscopic changes was observed; however, a complete bilateral retinal atrophy was evident by the age of 5 to 6 years.

Electroretinography. The photopic cone-system derived ERG amplitudes were already significantly reduced or nonrecordable in CRD-affected dogs at the earliest age studied (5 weeks). In contrast to the control dogs, there was no significant increase in the respective amplitudes with age in the affected dogs, except for an increase in the 5.1-Hz single-flash b-wave from the age of 5 to 8 weeks. The scotopic rod- and mixed rod-cone-system–derived amplitudes were significantly lower in most recordings in the CRD-affected SWHDs than in the control dogs. There was an increase in the amplitudes...
recorded in the affected dogs with age, although they never reached the same level as those in the age-matched control animals (for details, see Ropstad et al.4). There was a marked heterogeneity in ERG recordings between CRD-affected age-matched dogs, even within litters (Fig. 3).

**Morphology and Morphometry**

**Light Microscopy.** Although, there was a pronounced variation among individuals, affected dogs showed a successive thinning of the photoreceptor inner and outer segment layers with progression of the disease.

Pigment granules were observed in all retinal layers in affected dogs, most markedly in the inferior periphery (IP) of the most severely affected dogs.

CRD-affected SWHDs displayed a reduced number of nuclei in the outer nuclear layer (ONL) compared with the control dogs, although there was a great variation between affected individuals (Table 3). In two affected dogs, the ONL was reduced to a single row of nuclei interrupted by areas devoid of photoreceptor nuclei. In affected dogs the mean photoreceptor cell nuclei number was highest in the AC (4.99), intermediate in the SP (4.79), and lowest number in the IP (4.01). In control dogs, these numbers were 10.08, 8.38, and 7.98, respectively. Age-matched affected dogs showed a marked difference in thickness of the outer retinal layers and in the number of photoreceptor cell nuclei in the ONL in particular (Fig. 4). Even at more advanced stages of the disease, the cell layers of the inner retina appeared fairly normal, except for the
nerve fiber layer (NFL), which appeared thicker and more vacuolated in the affected dogs than in the age-matched control subjects.

**Electron Microscopy.** A marked clumping of pigment in the retinal pigment epithelium (RPE) and an abundance of pigment in the apical microvilli was observed in the IP of affected dogs as early as the age of 48 weeks. In some cases of advanced disease, these changes were also found in the SP and AC. There appeared to be an increased number of phagosomes in the RPE in affected animals. In advanced cases, the photoreceptor atrophy resulted in the inner nuclear layer (INL), adjacent to the RPE layer, with little or no remnants of photoreceptors in the subretinal space. The structures found in the subretinal space were highly disorganized and constituted phagosomes, pigment-filled macrophages, pyknotic photoreceptor nuclei, and photoreceptor inner- and outer segment remnants.

Cone outer segments (OS) were sparse in affected dogs compared with control dogs, and those present were disrupted and in the process of undergoing degeneration. This phenomenon was observed at the first time point studied in a 5-week-old affected dog. Cone inner segments (IS) in affected dogs contained fewer and irregularly shaped mitochondria compared with those in control dogs, and in some instances the mitochondria were clustered in the vicinity of the external limiting membrane (ELM; Fig. 5C). Rod outer and inner segments displayed no apparent morphologic changes until the advanced stages of the disease. There were already a decreased number of cone photoreceptor nuclei in the ONL in the less severe stage of the disease. The cone nuclei present appeared

![Figure 2](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933445/)

**Figure 2.** Tapetal (A) and nontapetal (B) fundus of a 132-week-old CRD-affected dog. (A) The retinal vessels are slightly attenuated (arrow) and there is a moderately increased tapetal reflectivity (arrowhead). (B) There are characteristic depigmented areas together with hyperpigmented ridges in the nontapetal part of the fundus.

![Figure 3](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933445/)

**Figure 3.** Full-field ERG recordings in a control dog (red, dog 5) and in two affected siblings at different stages of the disease at age 11 months (turquoise and blue, dogs 17 and 18, respectively). (A) Photopic (37 cd/m²) light adapted single flash (5.1 Hz at 0.0 log cd s/m²); (B) 30.1-Hz flicker (0.3 log cd s/m²); (C) dark adapted low (−2.0 log cd s/m²); and (D) high-intensity (0.6 cd s/m²) scotopic responses. Amplitudes (ordinate) are in microvolts and implicit times (abscissa) in milliseconds.
abnormally shaped, with a more abundant perinuclear cytoplasm than in the cone nuclei of unaffected dogs (Fig. 5). In some of the dogs, the shape of the cone nuclei appeared more elongated than that observed in the normal age-matched dogs. Further, some cone nuclei were observed to be displaced into the subretinal space. In more advanced stages of disease, these cell nuclei appeared shrunken and irregularly shaped. Most of the rod photoreceptor cell nuclei, although decreased in number, appeared normal in shape, even when the outer and inner photoreceptor segments were completely atrophied. With the progression of disease, rod photoreceptor nuclei eventually underwent pyknosis. The external limiting membrane (ELM) appeared more distinct and thickened in CRD-affected dogs under went pyknosis. The external limiting membrane (ELM) appeared more distinct and thickened in CRD-affected dogs compared with that observed in unaffected dogs (Figs. 5A, 5B).

A marked thinning of the outer plexiform (OPL) was observed at more advanced stages of the disease in the CRD-affected dogs, resulting in the outer nuclear layer (ONL) and inner nuclear layer (INL) being almost adjacent to each other (Fig. 6). In contrast to the outer retinal structures, the inner parts (INL and inner plexiform layer [IPL]) did not show any morphologic changes on electron microscopy at the moderately advanced stage of disease. Only at the most advanced stage studied, when the retinas were almost completely atrophied, were there changes in these inner retinal structures (Fig. 7).

**Immunohistochemistry and Lectin Histochemistry.** A composite of lectin and immunohistochemical labeling for selected retinal proteins used in the present study is shown in Figure 8.

Immunohistochemically, the cone photoreceptors initially appeared much more severely affected than did the rod photoreceptors. The overall number of cone photoreceptors, shown by PNA staining, was severely reduced in affected SWHDs compared with unaffected control dogs already at the earliest age studied. Furthermore, selective OS staining for short-wavelength cones (S) cones showed a reduced number of the respective cones in CRD-affected dogs. Immunolabeling of rod outer segments with Rho-63 showed no obvious differences between CRD-affected and control dogs at any age studied.

Synaptophysin immunoreactivity was observed within both the OPL and IPL in all dogs examined, and no differences were observed between affected and control dogs. The area between the first- and second-order neurons appeared thinner in the affected dogs compared with that observed in control dogs.

With the PKC-ε antibody, no differences were found in the bipolar cells or photoreceptor terminals between CRD-affected and control dogs. The CRD-affected dogs showed markedly increased immunostaining of glial cells (GFAP) with marked thickening of the NFL and extensions from the GCL toward the outer retina, which was not observed in control dogs.

**DISCUSSION**

The cone-rod degenerations described in humans show a great diversity in time of onset and clinical signs. This difference has also been described in our previous clinical and electrophysiological studies of CRD-affected dogs. Results of morphologic investigations from the present study have corroborated these clinical findings, which show a great phenotypic variability between affected and normal age-matched SWHDs. In the present study, two CRD-affected siblings euthanatized at the same age (48 weeks, dogs 17 and 18) showed a marked difference in the number of photoreceptor nuclei in all three sections (Table 3). The mean number of photoreceptor nuclei in the most severely affected dog varied from 25.6% to 27.0% for the three sections examined, compared with her littermate. Similar findings (0%-5.7%), were found in two other CRD-affected dogs euthanatized at 26 and 28 months of age (dogs 19 and 20, respectively; Table 3). This is in accordance with earlier morphologic descriptions of hereditary photoreceptor degenerations where a spatial distribution of disease has been shown, with the lowest number of photoreceptor nuclei found in the IP. Cones were found to be affected early in the disease with diminutive or a complete lack of outer segments and abnormalities in cone cell bodies. Further, it appeared that short-wavelength cones were most severely affected, as shown by OS immunostaining. The nonuniform distribution and the relatively small number of S-cones, however, have to be taken into consideration when interpreting this result. The electrophysiologic findings in young dogs corroborated these findings, in that 30.1-Hz flicker recordings were barely recordable or nonrecordable, and single-flash cone recordings were of low amplitude or even nonrecordable.

Ultrastructural studies showed the cone nuclei to be shrunken in advanced cases. Further, several aberrant cone nuclei were observed in the subretinal space. The rod outer
segments seemed to be spared, at least morphologically, early in the disease process, as shown by EM and immunolabeling for Rho-63. Even at the latest time point studied (dog 22) there were no differences when comparing immunohistographs of Rho-63-labeled retinas from this CRD-affected dog and the normal control dog (Fig. 8). OPL was thinner, however, probably because of the degeneration of cone synaptic structures.

At an early stage of disease, in 5-week-old dogs, no evident electron microscopic changes were found in rod photoreceptors, although there were significantly reduced rod-derived ERG amplitudes, as described in previous electrophysiologic studies. This could be due to interaction between rod- and cone bipolar cells at the level of amacrine II cells or cone OFF bipolar cells. The nerve fiber layer of affected dogs was observed to be thicker and more expanded than normal, with extensions observed in the outer retina. Earlier studies have shown extensive neurite sprouting in human retinitis pigmentosa (RP) and in the rd/rd mouse. In advanced cases of CRD-affected dogs, the subretinal space appeared completely collapsed due to a lack of both cone and rod outer and inner segments, resulting in the RPE apical microvilli’s being in direct contact with the external limiting membrane. Similar findings have been described in human RP.

GFAP is the major component of glial intermediate filament found in astrocytes. In the retina, this protein is highly expressed in astrocytes and minimally in resting Müller cells. Local or generalized retinal injury caused by laser photocoagulation, light damage, and genetically determined retinal degenerations have been shown to increase GFAP expression in Müller cells in RCS rats, cats, and humans. Similar changes were found in retinas from affected SWHDs, with intense staining for GFAP, indicating Müller cell reactivity and neurite sprouting. Increased Müller cell reactivity was also confirmed on EM through a thickening and a more distinct ELM.

In the present study the variation in progression of the disease stage and in progression of the disease among CRD-affected individuals was best visualized by the clinical differences between age-matched CRD-affected dogs. Morphologically, this great variability was most readily observed through evaluation of ONL thickness and degree of photoreceptor OS and IS changes.

Canine inherited retinal diseases affecting the photoreceptors have been grossly subdivided into photoreceptor dyspla-
sias and retinal degenerations, the former with changes occurring before the time of retinal maturation in the dog (7 weeks) and in the latter with changes observed after this time point.20,21 In the affected SWHDs of the present study, cone-derived ERG recordings never increased with age nor reached the same levels as in the control dogs. Further, cone photoreceptors were morphologically abnormal at the age of 5 weeks, whereas rod photoreceptors showed normal ultrastructure initially, but were found to degenerate at later stages in the disease process, making it possible to classify the disease in the SWHDs as a cone dysplasia with subsequent rod degeneration.

Based on the large pedigree obtained by breeding CRD-affected and carrier SWHDs, the mode of inheritance is most consistent with an autosomal recessive disorder. Molecular genetic investigations are ongoing. So far, 11 candidate genes have been excluded as causative of CRD in the SWHDs (Wiik A et al. unpublished results, 2007). One of the genes excluded is RPGRIP1, which has been identified as mutated in MLHDs with CRD,5 suggesting that the CRDs in the two Dachshund breeds are caused by mutations in different genes.

The end stage of the CRD in the SWHD is complete blindness in all affected dogs. The disease in the presently described strain of SWHD may be a valuable naturally occurring animal model for the human counterpart.

In summary, clinical findings in affected SWHDs correlated well with results of the morphologic studies. The heterogeneity observed clinically was also observed by LM, immunohistochemistry, and EM. Further, both electrophysiologic and morphologic studies showed that cones never develop normally, allowing the disease to be classified as a cone dysplasia with late-occurring rod degeneration.

**FIGURE 6.** Electron micrographs showing the inferior periphery of a 52-week-old control dog (A, dog 5) compared with the ultrastructural changes found in the same region in a 48-week-old affected dog (B, dog 17). There was thinning of the OPL in the affected dog resulting in the outer and inner nuclear layers being almost adjacent to each other. No marked differences were found between the INLs in the two dogs. The ONL was markedly thinner in dog 17 (mean thickness, 1.16 nuclei) compared with dog 5 (mean thickness, 7.25 nuclei). In dog 17 there was a marked gliosis caused by hypertrophied Müller cells (arrow) and the subretinal space (✽) was filled with debris from degenerated photoreceptors. Magnification, ×1500.

**FIGURE 7.** Electron micrograph of a 307-week-old dog (no. 22) with pigment migration into the subretinal space and the inner retina (arrows). No normal outer or inner segments were observed in the subretinal space, in which remnants of photoreceptor nuclei (Ph) and inner segments (IS) were found. Magnification, ×5000.
FIGURE 8. Immunohistochemistry of selected retinal proteins. Antibodies against OS, PNA, Rho-63, PKC, Synaptophysin, and GFAP were used on central retinal sections from 16-week-old control (A, C, E, G, I, K) and affected dogs (B, D, F, H, J, L). The dog in (F) was 312 weeks old. There was a decreased number of blue cones in the affected dog (B) as shown by OS immunostaining. PNA staining in the affected dog (D) showed a marked disorganization of the cone photoreceptors with changes in inner and outer segments. There are no differences in rod photoreceptor inner and outer segments between control and affected dogs (E, F) when labeled with Rho-63, nor are there any obvious differences in bipolar cell immunoreactivity when labeled with PKC (G, H). There was a thickening of the nerve fiber layer in the affected dog (L) as shown by GFAP immunolabeling, whereas no differences were seen when labeled with synaptophysin (I, J).
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References