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Abstract
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Keywords
dog, electroretinography, photoreceptor dysplasia, progressive retinal atrophy

Comments
Progress in Veterinary & Comparative Ophthalmology is now *Veterinary Ophthalmology*.

ORIGINAL ARTICLE

Photoreceptor Dysplasia: An Inherited Progressive Retinal Atrophy of Miniature Schnauzer Dogs

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A progressive retinal atrophy (PRA) affecting Miniature Schnauzer dogs is reported. Of the 287 individuals (148 female, 139 male) comprising the study population, 66 (23 percent) were affected (33 female, 33 male) and 221 animals (115 female, 106 male) were phenotypically normal. There was no sex predilection for the disease. Results of histologic and electroretinographic studies indicate that the disease is a new and different type of PRA, characterized by unique morphologic and functional deficits during rod and cone development. Accordingly, the disease has been termed photoreceptor dysplasia. Clinically, and particularly ophthalmoscopically, diagnosis is only practicable in very late stages of the disease. Electroretinography, however, can provide evidence of the disease in dogs at least as young as 8 weeks of age. Pedigree analysis and test-mating studies conclusively establish that inheritance is autosomal recessive. The gene symbol *pd* (for photoreceptor dysplasia) is assigned. (*Progress in Veterinary & Comparative Ophthalmology*, Vol. 1, No. 3, 1991, pp. 187-203; Key words: dog, electroretinography, photoreceptor dysplasia, progressive retinal atrophy.)

Generalized progressive retinal atrophy (PRA) is a clinical diagnostic category that groups together a variety of hereditary degenerative retinal diseases in domestic animals (primarily in the dog). Although an increasing number of specific diseases within this category have been defined in several canine breeds, the diverse forms of PRA in the dog share certain clinical features.[1-9] Foremost among these similarities are a consistent ophthalmoscopic appearance of the retinal disease process, and an inexorable deterioration of retinal structure and function, leading to loss of vision. Furthermore, in all canine forms of PRA studied to date, the inheritance pattern has been autosomal recessive.[1,2,4,6,7-10] Dissimilarities in manifestation of the disease, particularly among breeds, indicate that separate entities are grouped under the PRA rubric. These dissimilarities include differences in the age of emergence of clinical signs; in pathophysiology (dysplasia vs. degeneration); in the relative degree to which rod or cone cells are affected; and in biochemical abnormalities, such as deficiency of cyclic

cGMP-PDE, cyclic guanosine monophosphate phosphodiesterase; ***erd***, early retinal degeneration; **ERG**, electroretinography; **ONL**, outer nuclear layer; ***pd***, photoreceptor dysplasia; **PRA**, progressive retinal atrophy; ***prcd***, progressive rod-cone degeneration; ***rcd1***, rod-cone dysplasia type 1; ***rcd2***, rod-cone dysplasia type 2; ***rd***, rod dysplasia

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Table 1. Numbers of dogs studied, classified by age group (in years) at *first* examination, sex and diagnostic status. On each occasion, dogs were examined both by ophthalmoscopy and by electroretinography. Age group also represents the age of diagnosis for each dog because it is possible to discriminate between *pd*-affected and non-affected dogs at *all* ages using electroretinography.

	Age group (years)						
	≤0.25	>0.25 - 0.33	>0.33 - 0.5	>0.5 - 1.0	>1.0 - 2.0	>2.0	Total
Normal							
male	17	47	12	7	9	14	106
female	29	51	9	5	7	14	115
Sub-total	46	98	21	12	16	28	221
pd-Affected							
male	9	18	2	1	1	2	33
female	7	14	8	4	0	0	33
Sub-total	16	32	10	5	1	2	66
Total	62	130	31	17	17	30	287

guanosine monophosphate phosphodiesterase (cGMP-PDE) activity.[7,9,11,12]

In miniature Poodles, PRA is a late onset progressive rod-cone degeneration (*prcd*). This disease becomes evident by ophthalmoscopic examination at 3 to 4 years of age or later,[7,13] but may be detected by electroretinography (ERG) as early as 6 to 9 months of age. PRA in American and English Cocker Spaniels also is caused by a mutation at the *prcd* gene locus, as in miniature Poodles, although there are significant differences in disease manifestation among these three breeds. For example, the rate of disease progression in the English Cocker, as determined by ERG or histopathologic evaluation, is approximately half as fast as in the miniature Poodle.[7] The cause for this slower degeneration rate is unknown, but could result from different mutations at the same gene locus, and/or differences in genetic background between the breeds.

In contrast to the situation in miniature Poodles and Cocker Spaniels, different genetic loci are responsible for the several distinct forms of early onset PRA found in the Irish Setter (rod-cone dysplasia type 1, *rcd1*), Collie (rod-cone dysplasia type 2, *rcd2*); and Norwegian Elkhounds (rod dysplasia, *rd*; early retinal degeneration, *erd*). This conclusion is based on extensive breeding studies, as well as on detailed functional and structural analysis of the diseased retina.[6,9] In two breeds (Irish Setter and Collie) the same biochemical abnormality is present; elevated retinal cGMP results from deficient retinal cGMP-PDE activity.[11,12,14,15] Nevertheless, the diseases are caused by genes at different loci (i.e., are non-allelic) because all pups test-bred to have both an *rcd1* and an *rcd2* gene are phenotypically normal.[9] Therefore, the *rcd1* and *rcd2* gene loci must code for different proteins. Candidate proteins would include those involved in PDE activity (such as the different [α,β,γ] PDE subunits) or activation (such as the α,β,γ subunits of transducin), to name just two possibilities.

Although PRA has been recognized in Miniature Schnauzers — primarily in animals 4 to 5 years old[16-18] — it has not been extensively studied previously. In the present study, carried out between 1982 and 1985, the clinical, genetic, ERG and histopathologic characteristics of a progressive retinal degeneration unique to this breed are described. The disease is unusual in that the slow progression of clinical disease, assessed primarily by ophthalmoscopic observation, misleadingly suggests it is a late onset form of PRA. However, when judged by histopathologic and electroretinographic criteria, it clearly is an early onset disorder. The disease has been termed photoreceptor dysplasia and, accordingly, the symbol *pd* has been assigned to the gene.

Materials and methods

Study animals

The protocol for animal use and experimentation adhered to the Association for Research in Vision and Ophthalmology (ARVO) resolution on the use of animals in research.

The primary study population was composed of 287 Miniature Schnauzers (148 females, 2 months to 5.3 years

old; 139 males, 2 months to 12 years old) presented by their owners for examination between 1982 and 1985. Categorization of the animals studied by sex, status and age is presented in Table 1. Each of these dogs was examined both ophthalmoscopically and by ERG on one or more occasion (13 were tested twice). Selective matings between affected parental animals from the primary study population were used to produce additional dogs for correlative studies of electroretinographic and histopathologic aspects of the disease. Thirteen such dogs were utilized, as detailed below under histopathology.

Clinical examinations

All animals were examined by indirect ophthalmoscopy and biomicroscopy after appropriate mydriasis. The fundus was considered free of retinal disease in the absence of detectable vascular attenuation, tapetal hyper-reflectivity, and pigmentary margination or clumping.

Animals were assessed for behavioral evidence of visual impairment, under testing conditions designed to compare affected animals' behavior (under both dim red and standard room lighting, with obstacles placed randomly in the area) with that of animals which have no apparent visual difficulty. The animals were worked through such a system both individually and in groups. Initially, the subjects were allowed to move about in a well-lit examining room. After sufficient time had elapsed to determine how the subjects behaved in this environment, room illumination was gradually reduced until the only source of light was a red darkroom safe-light. Changes, if any, in the subject's reaction and movement were noted.

Electroretinography protocol

ERG procedures were performed on 287 animals (148 females and 139 males). In selected cases, primarily those designed to correlate electroretinographic and histopathologic studies, the ERGs were carried out at the School of Veterinary Medicine, University of Pennsylvania; the methods used to stimulate the retina and record the ERG for such studies have been previously detailed.[6-9,19] Most ERGs, however, were carried out by one of the investigators (CJP), using the methods detailed below.

All animals were fasted for approximately 12 to 16 hours; topical tropicamide 1.0 percent was used for mydriasis. Anesthesia was induced by intravenous thiamylal sodium[a] and maintained with halothane[b] or Fluothane[c] and nitrous oxide/oxygen. Sterile physiologic saline was injected ventral to the test eye to rotate the eye upward. Three silver/silver chloride electrodes were positioned as follows: the ground lead was placed subdermally near the occipital tuberosity; the corneal electrode was placed within a clear plastic corneo-scleral contact lens, and electrically coupled to the cornea with a sterile saline wetting agent; and the reference electrode was placed subdermally near the lateral canthus (it was separated from the corneal electrode by a distance approximately equal to the distance between the cornea and the posterior pole of the globe).

The light source was a 500-watt tungsten halogen bulb, which had its light path split into two pathways, one to provide background illumination and one to provide light stimuli. The two pathways were controlled independently, using suitable shutters, beam splitters, lenses and mirrors to focus the two light beams on the target end of a ¼-inch fiberoptic light guide; in turn, the guide delivered the light to the eye. The emitting end of the light guide was placed in the optical axis within 1 to 2 mm of the surface of the clear plastic corneal contact lens. Celluloid/gelatin filters, as specified in the test protocol, were used to control the spectral and/or intensity characteristics of the light. A signal detected when the target was exposed to light was recorded as part of the ERG display. Flickering light stimuli were generated by a rotating wheel, with an opening to permit equal on/off light stimulation at different test frequencies (5, 12 and 30 Hz).

To record the ERG, a standard preamplifier[d] (high and low frequency cutoffs were 1 kHz and 0.1 Hz, respectively) and dual trace oscilloscope[e] were used. With this equipment, it was possible to identify responses greater than 5 μV in amplitude. Permanent records of the ERG responses were made by photographing the monitor screen with Polaroid film. The ERG protocol used throughout this study was designed for identification and separation of rod and cone responses.[6-9,19]

ERG records were evaluated by parameters designed to examine the effect of light adaptation; photopic to scotopic shift during dark adaptation; responses to white and scotopically balanced red and blue stimuli; initial and average b-wave amplitude and implicit time for rod flicker (5 and 12 Hz); initial and average b-wave amplitude and implicit time for cone flicker (5, 12 and 30 Hz); and overall character of the ERG. The extracted data were then recorded and evaluated utilizing a computer spreadsheet program. Because ERG response amplitudes are known to change with age[8] as a function of retinal maturation it is necessary to compare data from affected and non-affected dogs at similar ages. In this study, it was elected to make such comparisons using six age groups (≤0.25 years; >0.25 to 0.33 years; >0.33 to 0.5 years; >0.5 to 1.0 year; >1.0 to

Table 2. Electroretinographic response parameters evaluated.

	Response	Parameter
1	light adapted, white light	a-wave amplitude
2	light adapted, white light	b-wave amplitude
3	20 minute dark adapted, dim red	b-wave amplitude
4	dark adapted scotopic red	b-wave amplitude
5	dark adapted white light	a-wave amplitude
6	dark adapted white light	b-wave amplitude
7	dark adapted white light	b_1-wave amplitude
8	rod flicker – 5 Hz	initial b-wave amplitude
9	rod flicker – 12 Hz	initial b-wave amplitude
10	cone flicker – 5 Hz	initial b-wave amplitude
11	cone flicker – 12 Hz	initial b-wave amplitude
12	cone flicker – 30 Hz	initial b-wave amplitude

2.0 years; and >2.0 years) for the specific parameters detailed in Table 2. The appropriate data were, therefore, sorted by age group, by the ERG parameters listed in Table 2, and by assigned disease/diagnostic status (that is, either PRA-affected or non-affected).

In the initial phase of this study, assessment of disease/diagnostic status in Miniature Schnauzers was dependent on comparison of a given dog's ERG response characteristics with those of normal and PRA-affected dogs of other breeds. As this study progressed and the data base presented in this report developed, it was possible to establish tables of normal and *pd*-affected values for the defined set of ERG parameters in Table 2. In general terms, the following ERG criteria were used for classifying animals as PRA affected: failure to achieve normal amplitudes and implicit times to a dim red light stimulus after 20 minutes of dark adaptation; failure to have equal amplitudes and similar waveform responses to scotopically balanced red and blue light stimuli; failure to generate a high amplitude b-wave dominated dark-adapted response to a high-intensity, white light stimulus; failure to produce oscillatory potentials; failure to achieve normal amplitudes, implicit times and waveforms to flicker stimuli that isolate rod and cone responses; and failure of any ERG parameter evaluated to reach amplitudes within one standard deviation of the established normal mean values.

Histopathology

Eyes from selected animals that had undergone ERG testing were used for morphologic characterization of retinal disease. In most cases, the eyes were collected from dogs deeply anesthetized with intravenous pentobarbital; following enucleation, the dogs were euthanized with a barbiturate overdose. In three cases, however, the dogs were anesthetized with halothane following induction with a thiobarbiturate, and unilateral enucleations were performed. These three dogs were allowed to recover and, subsequently, another ERG and/or a second terminal enucleation performed. Except where noted, eyes from individual PRA-affected dogs were examined at the following ages: 24 days, 8 and 19 weeks, 4.4, 5, 6 (n=2), and 7.5 (n=2) months, 2.3, 3, 4.2 and 5 years. Eyes from normal, non-Miniature Schnauzer dogs of different ages served as control.

All eyes were processed for light and electron microscopic examination of the retina using one of two fixatives: (a) a double fixative protocol using 2.5 percent cacodylate buffered-glutaraldehyde, followed by 1 percent osmium tetroxide; or (b) a triple-fixative protocol using 3 percent glutaraldehyde/2 percent formaldehyde, 2 percent glutaraldehyde/1 percent osmium tetroxide, and 2 percent osmium tetroxide. After dehydration, the specimens were embedded in an epoxy resin[f], sectioned at one micron and stained with azureII/methylene blue, with or without a paraphenylenediamine (PPDA) counterstain. In general, the one micron sections extended from the optic disc to the ora serrata in the superior and inferior meridians; in some specimens, sections also were cut of the temporal and nasal meridians. Details of the fixation and sectioning methods have been described previously.[8,13] To further characterize the photoreceptor abnormalities at different stages of the disease, retinas were examined by electron microscopy. For this, areas were selected from specific regions of the 1-micron-thick light microscopy sections and 60-nm sections were cut and stained with uranyl acetate-lead citrate and examined using electron microscopes[g].

Test mating

To obtain information regarding the inheritance of this retinal degenerative disorder, the results of 28 informative matings were analyzed. The following breedings (detailed in Table 3) were performed: affected to affected (8

Table 3. Summary of breeding results by informative mating types.

Mating type[1]		Numbers of pups					
Sire	Dam	Number of litters	*pd*-Affected males	*pd*-Affected females	Non-affected males	Non-affected females	Status unknown[2]
A	A	8	9	12	0	0	2
C	A	6	8	4	4	7	5
A	C	9	2	10	13	5	9
A	N[3]	5	0	0	14	7	2

1. C=Carrier (*pd*- heterozygous); A = affected with photoreceptor dysplasia (*pd*-homozygous); N = homozygous normal at the *pd*-locus.
2. Status unknown = dogs either not surviving to diagnostic age or lost to follow-up.
3. The 5 AxN litters were bred from two non-affected female Miniature Schnauzers, test-proven (bitch 1, $p<0.00025$; bitch 2, $p<0.002$) homozygous normal at the *pd*-locus. See text for details.

litters), carrier male to affected female (6 litters), affected male to carrier female (9 litters), and affected male to homozygous normal female (5 litters).

Results

Ophthalmoscopy

Ophthalmoscopic diagnosis of early PRA in these Miniature Schnauzers was complicated by the unusual and variable appearance of the tapetal fundus in normal dogs of this breed. The visible extent of the tapetum varied widely, and frequently covered significantly less than the usual superior ⅓ to ½ of the fundus. Its color varied among dogs, ranging from yellow through yellow-green, yellow-blue to green-blue. Irregularity and discoloration of the tapetal reflectivity (commonly referred to clinically as "salt and pepper spotting" and "bronzing") were observed both between litters and among members of the same litter, particularly when examined with reduced light intensity. Using brighter illumination, a metallic sheen to the tapetum often was apparent, which emulated the "green reflex" of tapetal hyper-reflectivity that is recognized as an indicator of retinal thinning in early PRA.

Of 66 study dogs determined to have PRA based on ERG testing (77 ERGs), 21(20 less than 2 years old) had ophthalmoscopic abnormalities detected that were considered indicative of early PRA; the remaining 45 were ophthalmoscopically normal (see Table 1 for specific categorization of these dogs by disease/diagnostic status, sex and age at diagnosis). Abnormalities noted in the 21 "ophthalmoscopically affected dogs" included one or more of the following signs, usually considered indicative of early PRA: a radial pattern of varied reflectivity, suggesting choroidal vascular ridging of the tapetum; irregular intensity of tapetal reflectivity; and a brownish discoloration ("bronzing") of the tapetal reflectivity when dim, oblique illumination was used. However, the lack of reliability of these indicators in the Miniature Schnauzer was evident in the results of ophthalmoscopic examination of 221 dogs classified as normal on the basis of ERG testing; of these, 13 dogs had similar ophthalmoscopic "abnormalities" noted. Conclusive ophthalmoscopic evidence sufficient to permit reliable and accurate diagnosis (diffuse tapetal hyper-reflectivity, margination of pigment in the non-tapetal zone, marked vascular attenuation) was not apparent until the very late stages of the disease. Such lesions usually were not found in affected animals until 2 to 5 years of age, and did not always develop in littermates at the same time. In several animals, diagnostic fundus lesions did not develop until 5 years of age or later. Figures 1 (A-H) and 2(A-C) illustrate the pertinent ophthalmoscopic features of the disease in the Miniature Schnauzer. Of the 66 dogs found to have PRA, based on the results of ERG testing, only one dog had cataracts.

Vision testing

Success or lack of success at retrieving, response to menace stimuli, and avoidance of fixed or moving objects in the animal's visual space were factors used as crude estimators of visual function. These observations were made in various lighting conditions, but objective scoring of visual performance was very difficult and often futile.

Several factors referable to the patient's attitude and demeanor made it difficult to demonstrate a vision deficit in many affected dogs. The normally friendly, outgoing, playful behavior of the dogs make them seek attention. The slightest hint, therefore, of the presence of a "friend" (animal or human) could make objective evaluation of

visual performance difficult. Therefore, animals often needed to be tested both in groups and as individuals in a strange environment, with obstacles placed randomly in the working areas. Sometimes, animals had to be tested in the presence of a strange group of animals. Occasionally, a dog would perform very well as a "follower," only to act greatly confused when the companion was removed from the area. Animals that had been trained to use vision in a working situation, or demonstrated a desire and ability to retrieve objects, were much easier to evaluate. Affected

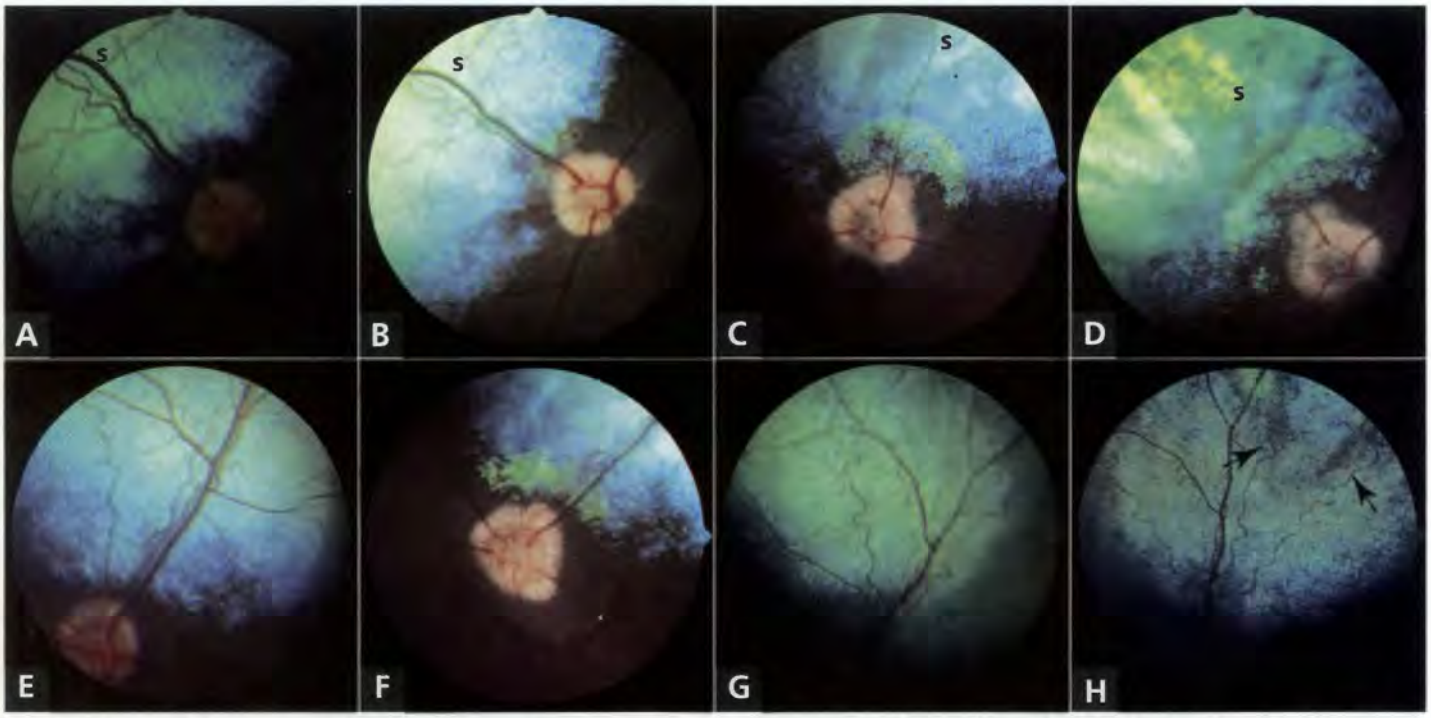

Figure 1. Fundus photographs of *pd*-affected Miniature Schnauzers at different ages. A-D: Right eye of female at 24 weeks (A), and at 1.7 (B), 3 (C) and 4.2 (D) years of age. The retina appears normal at 24 weeks (A), shows early disease at 1.7 years (B) and advanced disease at 3 (C) and 4.2 (D) years of age. In the late stages, there is diffuse hyper-reflectivity, vascular attenuation and loss of vessels. E,F: Right eye of female at 1.3 (E; fundus is normal) and 2.9 (F; fundus shows mid/late stage disease) years of age. A distinct peripapillary atrophic halo is apparent when the animal is older and has more advanced disease. G,H: Right eye of male at 2.6 (G) and 3.9 (H) years of age. Note the progressive attenuation and/or loss of retinal vessels, and the prominence of dark radial ridges (arrows) in the tapetal zone. Refer to Figure 11 legend for disease stages. Figures B-D and F-H have been taken with reduced flash intensity (1 log unit neutral density) to overcome the increased reflectivity from the tapetum lucidum. In A-D, "s" indicates the same position in the superior fundus at the 12-o'clock meridian.

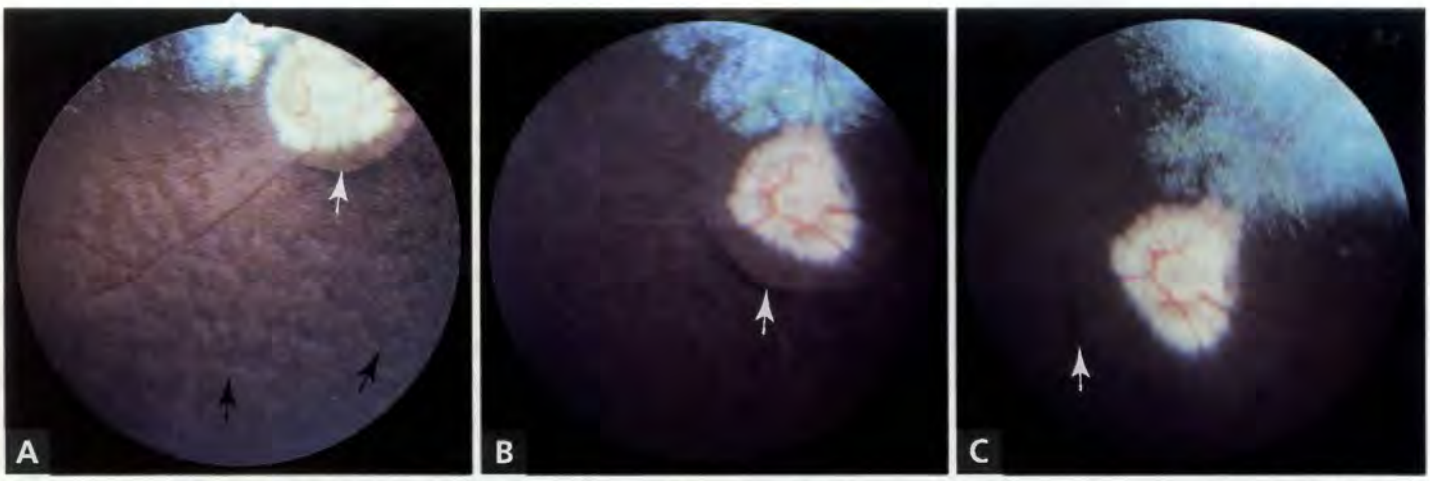

Figure 2. Fundus photographs showing the characteristic changes in the right optic disc and non-tapetal region of a *pd*-affected male dog at 3.2 (A), 3.8 (B) and 4.9 (C) years of age. Focal atrophic changes result in irregular pigmented lines (black arrowheads), which give the non-tapetal zone a scalloped appearance at 3.2 years (A). With disease progression, there is expansion of the peripapillary atrophic ring (white arrows) and thinning of the retinal vasculature.

animals often compensated very well behaviorally for their loss of vision, a phenomenon clinicians frequently recognize with slowly progressive blindness that develops in young animals. Handlers often reported, however, that affected animals displayed reduced social status in their interactions with other animals.

The most successful method for assessing visual performance involved observation of the animal under standard room lighting conditions, followed by observation as the light intensity was reduced and replaced by low-intensity red illumination. The system could be taught to owners, and was found to be reasonably reliable in evaluating visual

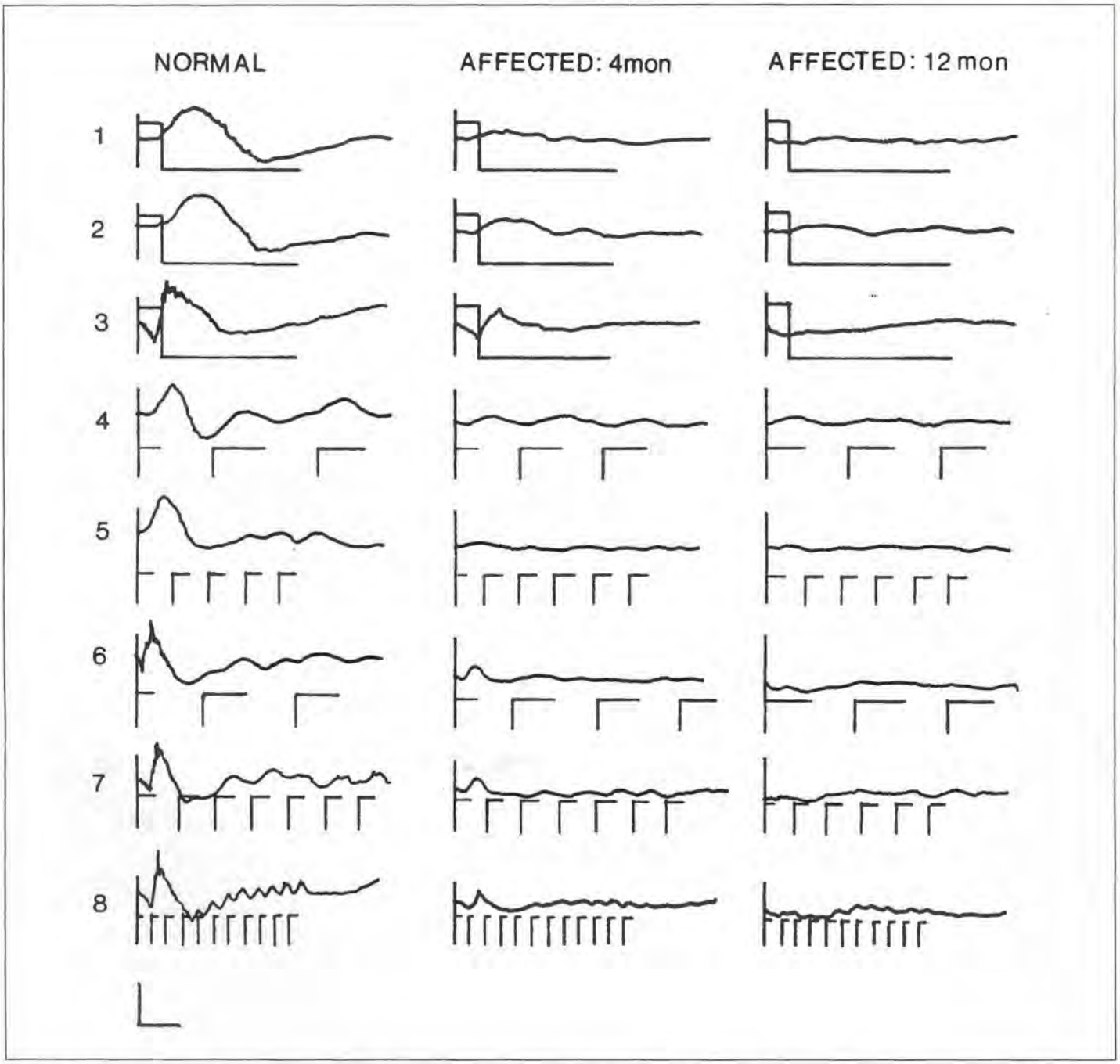

Figure 3. Dark-adapted ERG responses recorded from normal and affected (4 and 12 months) Miniature Schnauzers in response to various stimuli presented sequentially: 1) scotopic red; 2) scotopic blue; 3) white light; 4) rod flicker, 5 Hz; 5) rod flicker, 12 Hz; 6) cone flicker, 5 Hz; 7) cone flicker, 12 Hz; 8) cone flicker, 30 Hz. In the young affected dog, response amplitudes are low and waveforms are abnormal compared to those of the normal control; both rod and cone system responses are affected. By 12 months, the ERG responses are very low in amplitude. Vertical line preceding each response is the stimulus onset; square wave under each tracing indicates stimulus duration. Calibration mark in lower left: vertical=100 μV, horizontal = 50 msec for single stimuli and 100 msec for flicker stimuli.

performance. PRA-affected dogs with early disease showed whimpering; refusal or hesitancy to move about; searching movements; immediate tendency to lower the head and "smell" for familiar ground; blundering into fixed objects; lack of curiosity; and congregation, with apparent fear to break away from familiar groupings of animals. When these signs became apparent as lighting intensity was reduced, the animal was considered to have a dim-light vision deficit indicative, primarily, of rod functional deficits. Reversal of these signs usually occurred when illumination was returned to pre-test levels. These deficits were subtle and not apparent in all dogs. Animals with advanced PRA

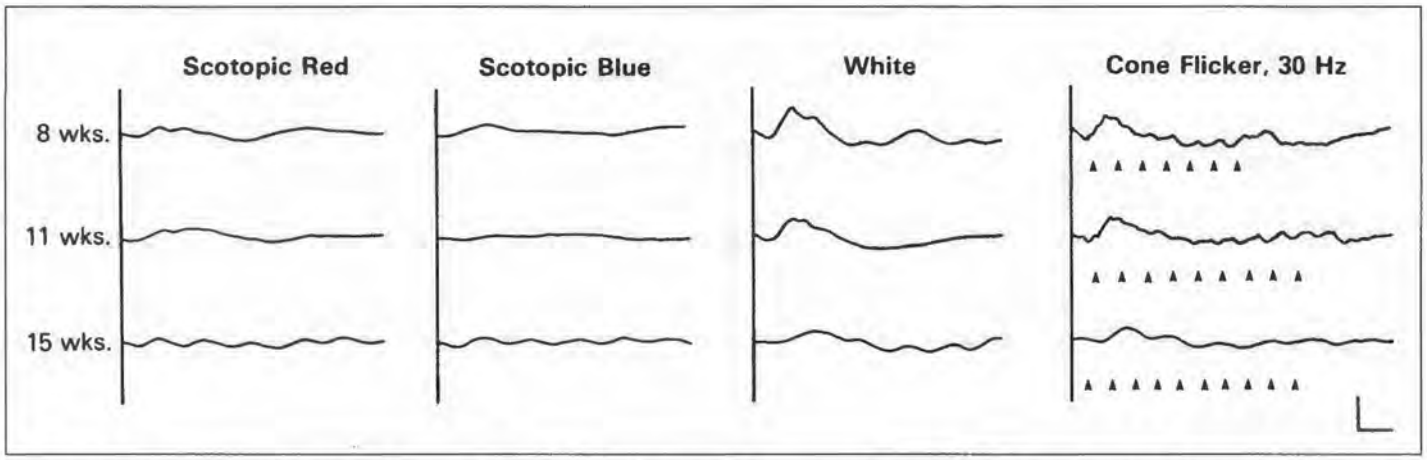

Figure 4. ERG responses recorded from the same female *pd*-affected Miniature Schnauzer at different ages (left eye = 8 weeks; right eye= 11 and 15 weeks). Severe abnormalities in ERG rod and cone function are detectable by 8 weeks and progress over a seven-week period. Calibration mark in lower right: vertical=100 µV, horizontal = 50 msec. (Refer to Figures 1E/F, 6C, 8 and 9 for the fundus photographs and retinal pathology of this case.)

Table 4. Discriminatory values for selected ERG parameters. Parameters are identified by their numbers as listed in Table 2. Because ERG amplitudes vary with age, both for normal and affected dogs, parameter values are presented for both normal[1] (phenotypically non-affected) and *pd*-affected dogs in a series of age groups ranging from 0.25 to greater than 2 years of age.

Parameter No:	1	2	3	4	5	6	7	8	9	10	11	12
>0.25 - 0.33												
Normal	7	28	36	63	24	91	54	65	58	83	83	92
pd	8	37	22	33	15	67	24	44	36	69	65	72
>0.33 - 0.50												
Normal	8	24	39	62	24	88	55	64	60	81	80	87
pd	12	21	26	38	24	66	20	40	35	66	64	69
>0.50 -1.0												
Normal	6	25	38	73	27	100	57	77	68	97	85	94
pd	10	26	19	19	30	43	0	31	21	44	44	45
>1.0 - 2.0												
Normal	5	26	40	59	28	89	66	71	62	83	89	93
pd	7	18	19	31	20	53	18	27	26	65	62	65
>2.0												
Normal	6	15	33	54	25	77	50	61	51	75	77	75
pd	9	20	19	17	15	29	4	24	22	32	31	32

1. The normal value for each parameter represents the mean amplitude for that parameter for non-affected Miniature Schnauzers (in microvolts) *minus* 1 standard deviation. The *pd* value for each parameter represents the mean amplitude for that parameter for *pd*-affected Miniature Schnauzers (in microvolts) *plus* 1 standard deviation.

and vision deficits (marked rod and cone deficiency) could readily be detected by failure to retrieve, absent menace response, and failure to negotiate an obstacle maze in good lighting conditions.

Indications of decreased day vision coincided with advanced ERG signs of disease. However, correlation between the degree of night vision loss with ERG evidence of rod dysfunction was more difficult to demonstrate, without a considerable amount of experience with vision testing in dim red lighting conditions. Despite these difficulties, however, the disease in this breed was consistently found clinically to show initial night blindness followed by progressive day vision loss.

Electroretinography

Diagnosis of PRA status by electroretinography was possible at all ages studied. Table 1 summarizes, by age and sex, the 287 Miniature Schnauzers that had ERG testing for diagnosis. There were approximately equal numbers of males and females in both the normal and PRA-affected groups.

Figure 3 illustrates representative electroretinographic responses recorded from normal and PRA-affected Miniature Schnauzers at 4 and 12 months of age. Affected animals had a dramatic depression of the dark-adapted b-wave response amplitudes to white and scotopically balanced red and blue stimuli. Initial and trailing flicker responses also were markedly depressed. These abnormalities in ERG function were present at an age when retinal function in the normal dog is becoming adult in character.[8] At later ages, the response amplitudes were even more decreased and the waveforms became abnormal.

The severity of the early ERG abnormality and the rapidity of decay in ERG function was somewhat variable among affected dogs. Regardless of this variability, however, distinct ERG functional abnormalities were present in *all* affected animals tested early; these abnormalities were worse in older animals, and showed distinct progression in animals having sequential ERGs. Figure 4 illustrates the responses of one affected dog, which showed severe functional abnormalities of both rods and cones at 8 weeks of age (top tracings). During the next seven weeks, this dog's retinal function progressively deteriorated (middle and bottom tracings). At this time, the dog's fundus appearance was normal (Figure 1E), and conventional vision testing showed "normal" visual function under bright and dim light conditions; however, visual performance was very poor when the dim background light was removed. Repeat examinations showed no change in ophthalmoscopic appearance at 14 months of age but, by 3 years of age, there was diffuse hyper-reflectivity of the tapetal zone, moderate attenuation of the major arterioles and venules, and a peripapillary ring of retinal and pigment epithelial atrophy (Figure 1F). By this time, the dog was night blind and had very compromised day vision. Blindness did not develop until after 4 to 5 years of age. This dog illustrates the great temporal differences between the presence and severity of ERG functional abnormalities, and the development of clinical signs characteristic of PRA.

For two reasons, not all ERG parameters measured were equally effective as discriminators between affected and non-affected groups of dogs. The first is because of unequal differences for the various parameters, between groups, and the second is the variability of these parameters within groups. Table 4 is designed to demonstrate the relative utility of several ERG parameters in discriminating between affected and non-affected dogs. Note that values for each parameter for normal animals represent the mean amplitude in microvolts *minus* one standard deviation (that is, the low side of the range for the normals) and, for PRA-affected animals, the mean *plus* one standard deviation (that is, the high side of the range for affecteds). This method of presenting the data apparently minimizes differences between the two groups, but assures that when differences in the values are noted between the two groups they are more reliable.

For example, the amplitude of the a-wave (Table 2, parameter 1) — in the response to the light-adapted white light stimulus — usually was of very similar magnitude for PRA-affected and normal dogs. In contrast, the dark-adapted b-wave response amplitude to red (parameters 3 and 4) or white light (parameter 6) stimuli was usually lower in the affected than in the control dogs. In affected dogs, we found that the amplitude of the wavelet, which immediately followed the peak of the dark-adapted b-wave response to white light, was consistently of lower amplitude in PRA-affected than in normal dogs. We have referred to this wavelet as b_1 (parameter 7). These observations emphasize the need for a very thorough ERG evaluation of retinal function in dogs suspected of being PRA-affected. Moreover, the evaluation must rely not only on assessment of the ERG waveform, but also on precise measurement of responses that represent rod, cone or mixed contributions.

Histopathology

Normal development of the canine retina has previously been described, and compared to abnormal photoreceptor differentiation, in two different hereditary retinal

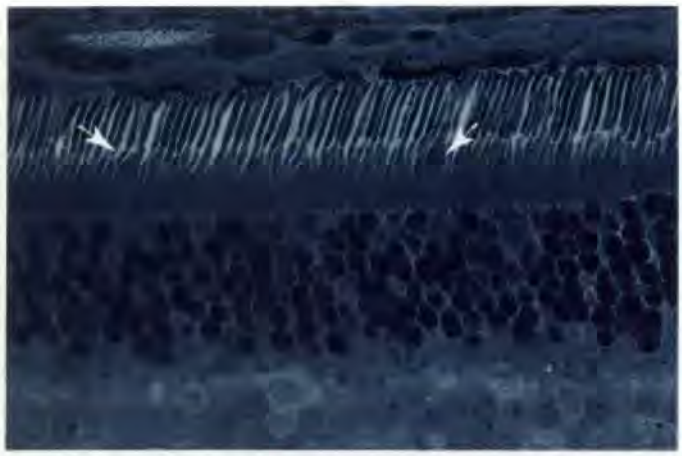

Figure 5. Photomicrograph of normal dog retina fixed in cacodylate buffered-glutaraldehyde/osmium tetroxide and embedded in an epoxy resin. This procedure assures excellent retinal preservation and orientation and permits the identification of individual rod and cone (white arrows) photoreceptors. Azure II/methylene blue stain, X630.

degenerations (*rcd1* in Irish Setters[2,14,20] and *erd* in Norwegian Elkhounds[8]). For that reason, detailed descriptions of normal development are not presented here. Figure 5 illustrates the structure of normal dog retina when fixed and processed as in this study.

Retinal development was abnormal at 24 days of age, the earliest time point evaluated morphologically (Figure 6A,B). These abnormalities were limited to the photoreceptors, and consisted of a marked retardation in development. The visual cell layer contained large, prominent, structurally normal cone inner segments that spanned the ventricular space. In appropriately oriented sections, short outer segments were associated with these broad inner segments; in most, distinct cone outer segments were not readily apparent. Rod inner segments, on the other hand, formed two distinct populations of cells. One population was elongate and slender, and extended beyond the cone inner segment apex. These inner segments had prominent connecting cilia and abnormal outer segments. A second population of rods consisted of cells having diminutive inner segments and prominent connecting cilia, but no outer segments. At this age, the outer segment layer consisted of short profiles of disorganized and disoriented disc membranes; these formed a distinct layer in the interphotoreceptor space between the tips of the inner segment and the apical surface of the pigment epithelium (Figure 7). Although there was some slight variation in disease severity, these changes were consistent throughout the retina. [In contrast to diseased retina, normal retina at this stage of postnatal development has inner segments that have elongated and terminally differentiated into distinct rod and cone inner segments. Similarly, normal outer segments, although shorter than in mature retina, have become aligned and display the regular disc membrane stacking characteristic of this structure.[2,14,20]]

By 8 weeks of age, disease severity was greatest in the photoreceptor outer segment layer (OSL). Most outer segments had disappeared, and only a few short, well-organized stacks of disc membranes were present (compare Figure 5 and 6C). What remained was a zone of detritus, consisting of disoriented, disorganized and degenerate clusters of disc profiles, as well as membranous vesicular profiles (Figures 8 and 9). These profiles were different from those reported for *prcd*-affected Poodles[7] in that they had a greater variability in size, shape and content; the latter varied from granular to electron dense. These vesicular profiles looked like membrane debris formed from degenerate outer segment structures. There were minimal changes in inner segments at this age. Some rod inner segments had early degenerate changes, and their cytoplasm was markedly electron dense. In appropriate sections, these inner segments were found to have pyknotic nuclei and degenerating synaptic terminals. Pyknosis of visual cell nuclei was prominent at all levels of the outer nuclear layer (ONL), which was reduced in width from 10 to 11 nuclei, as found in normals, to 7 to 9 nuclei (Figure 6C).

Degenerative changes were more severe at 19 weeks and at 7.5 months. The seven dogs examined in this time period showed advancing rod degeneration: the density of rods in the photoreceptor layer was decreased by more than 50 percent (Figure 6D). The remaining rod inner segments were diminutive. They also appeared broader, presumably because of loss of lateral support, resulting from visual cell loss. Cones, on the other hand, appeared to have been selectively spared; the prominent, club-shaped cone inner segments were the predominant cell type remaining in the visual cell layer. Although a small number of cone inner segments (and to a lesser extent rod inner segments) had short but abnormal outer segments, neither receptor type contained any significant amount of outer segment material (Figure 10). In the outer segment layer, distinct macrophages were present adjacent to the retinal pigment epithelium (RPE) (Figure 6D). The apical surface of the RPE, in many areas, had prominent irregular cytoplasmic processes that extended into the interphotoreceptor space. These pathologic changes affecting the photoreceptors and RPE were present throughout the retina, but their appearance was influenced, to some degree, by the normal heterogeneous distribution of photoreceptor classes in different regions of the eye; that is, loss of rods was more apparent

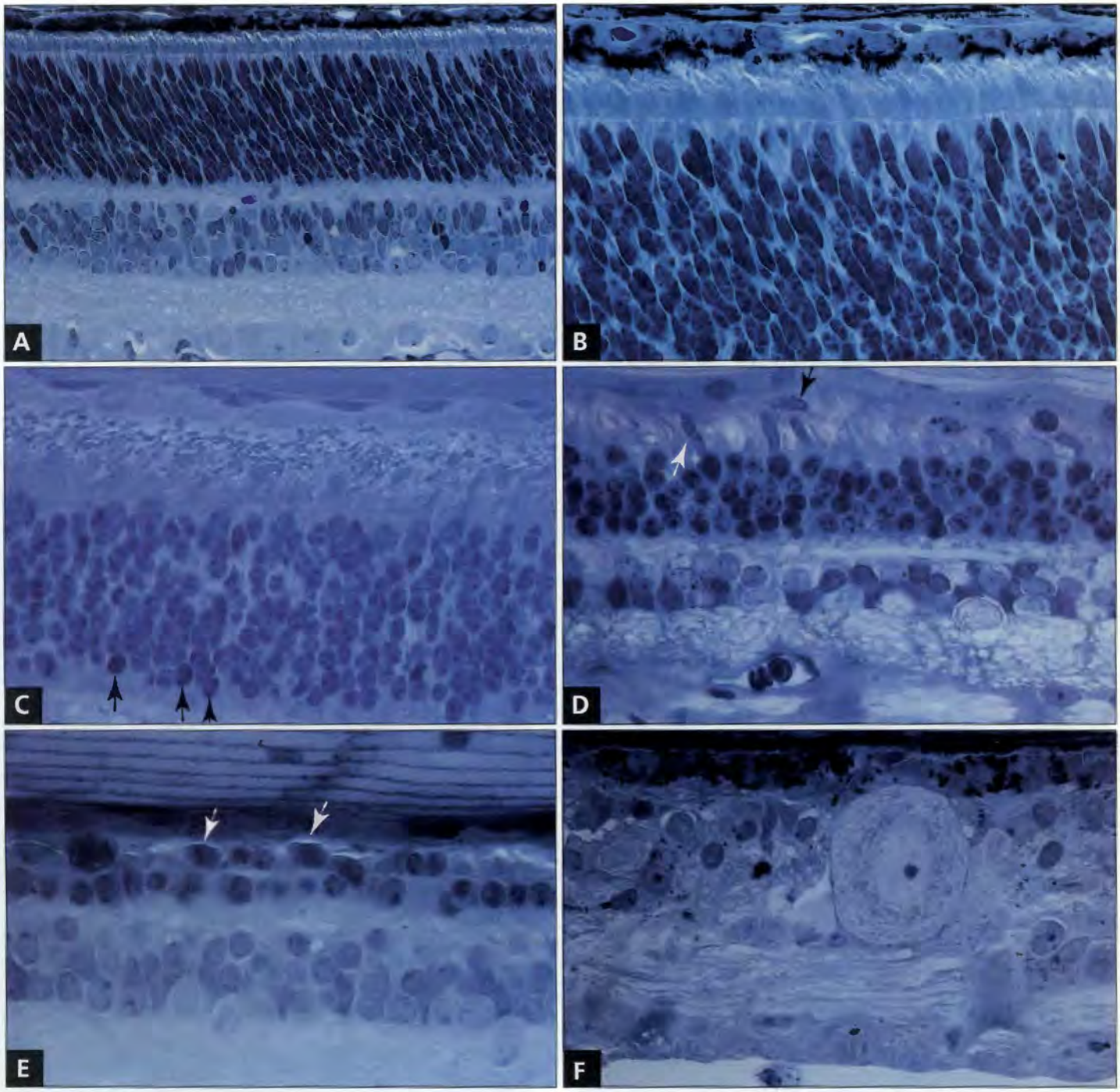

Figure 6. Photomicrographs of *pd*-affected retinas at different ages and stages of disease. A and B: 24 days of age. At this age, the retinal layers are organized normally (A), but the photoreceptor layer shows a retardation of development; there is minimal outer segment material and that which is present is in disarray. C: 8 weeks of age. There is extensive disorganization and disorientation of the remaining outer segment material. Note pyknotic nuclei (black arrowheads) in the ONL. D: 6 months of age. There is a marked thinning of the photoreceptor layer; the cone inner segments (white arrow) are prominent and macrophages (black arrow) are located in the interphotoreceptor space. E: 3 years of age. The photoreceptor layer is severely narrowed and several ectopic nuclei are located within the photoreceptor inner segments (arrows). The ONL is only one-nucleus wide, but the inner retinal layers are normal. F: 5 years of age. There is complete loss of retinal layer organization, and a prominent ganglion cell is now adjacent to the pigment epithelial layer. Azure II/methylene blue stain; A = X315; B-F = X630.

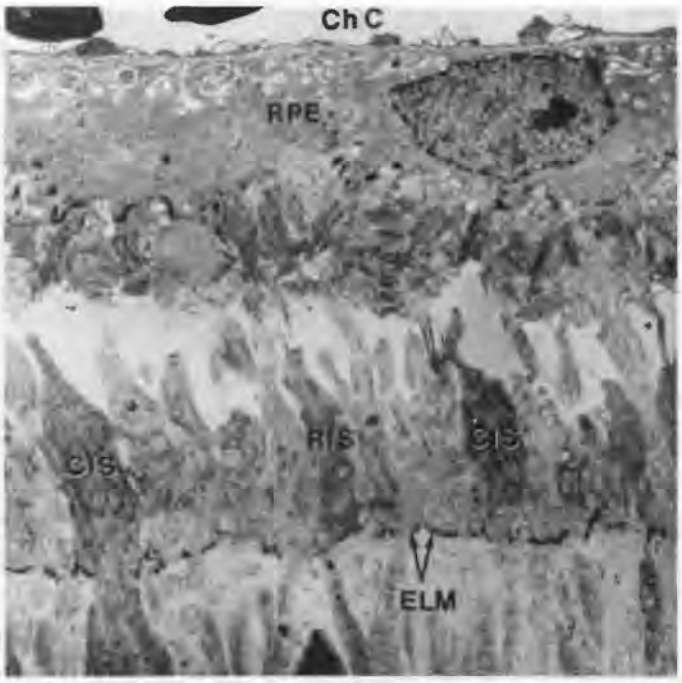

Figure 7. Electron photomicrograph of 24-day-old *pd*-affected retina. The receptor layer has prominent cone inner segments (CIS), and rod inner segments that are elongate (RIS) or short (arrow). Outer segment material is limited in amount, disoriented and clustered adjacent to the normal retinal pigment epithelial (RPE) apex. ELM = External limiting membrane, ChC = Choriocapillaris. X3,630.

in the posterior pole and equator because the remaining cones were prominent and created a striking contrast to the surviving diminutive rod inner segments. In the periphery, however, cone density is lower and the loss of receptors in this region was represented by gradual thinning of the visual cell layer.

By 2.3 and 3 years of age, the ONL in the posterior polar and equatorial regions showed a double row of surviving photoreceptor nuclei (Figure 6E). One row of rod or cone nuclei was located in the normal position, below the external limiting membrane. The second "row" consisted of rod and cone nuclei that had been displaced into the photoreceptor layer. Detailed light microscopic examination of these nuclei indicated that some were displaced into the inner segments of the few surviving photoreceptor cells. In other instances, however, the entire photoreceptor appeared to be rounded up and extruded directly into the interphotoreceptor space. Beyond the equator, there was loss of all receptor elements; the ONL disappeared and only disorganized remnants of inner retina remained. In the oldest animals examined (4.3 and 5 years) a one-nucleus-wide ONL was inconsistently present in the posterior pole; more peripherally, advanced gliosis and disruption was present in all retinal layers (Figure 6F).

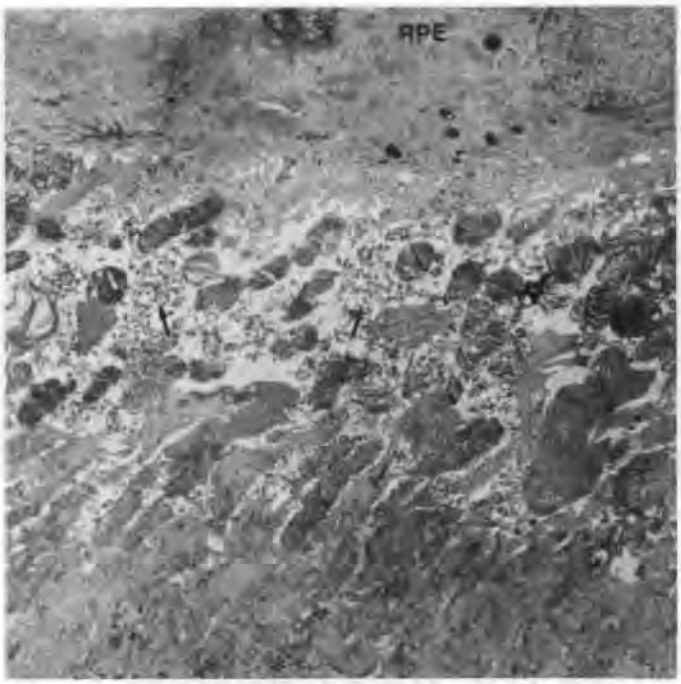

Figure 8. Electron photomicrograph of 8-week-old *pd*-affected retina. Outer segment material is markedly disorganized and disoriented and membranous detritus (arrows) is present in the interphotoreceptor space. Inner segments are short and irregular in contour. The RPE is normal. * = External limiting membrane. X3,683.

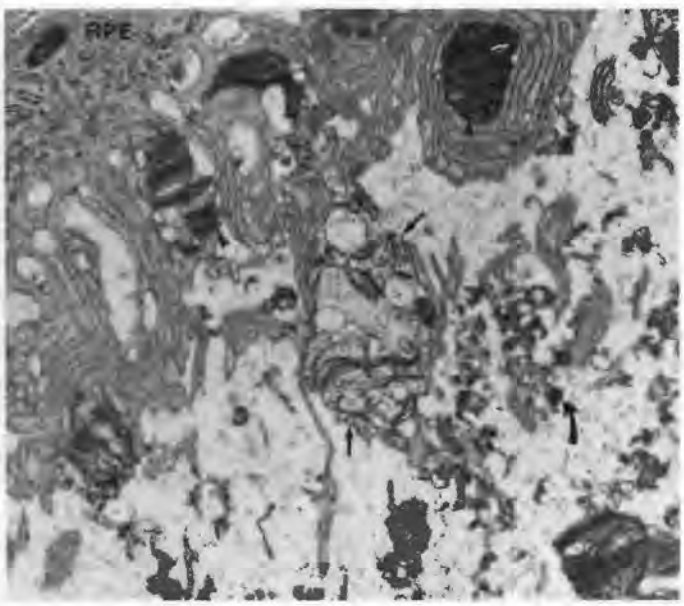

Figure 9. Higher magnification of the RPE-outer segment interface from an 8-week-old *pd*-affected dog. Fine, elongate RPE apical microvilli extend into the interphotoreceptor space. They surround a few rod outer segment profiles (arrowheads) that are structurally better preserved than the remaining outer segments which are severely disrupted (arrow) or form vesicular profiles (curved arrow). The amount of outer segment material is significantly reduced from normal. X9,275.

The time course of loss of ONL nuclei had two phases. Between 24 days and 7.5 months, the number of nuclei decreased rapidly, almost linearly; but, thereafter, the rate of nuclear loss was much more gradual (Figure 11). During the early, rapid phase of nuclear loss, pyknosis in the ONL was prominent; in the later stages of the disease pyknotic nuclei were rarely found in this layer. This two-phase loss of ONL nuclei appeared to be significant in relationship to the discrepancy between the ages when ERG and morphologic abnormalities are present and when ophthalmoscopic diagnosis is possible.

To examine this issue, the clinical staging of the disease by age was compared with structural characteristics of the outer retinal layers in 12 affected dogs. This comparison is illustrated in Figure 11. It is apparent from this figure that early clinical disease was not evident until the ONL thickness was reduced to 3 nuclei. Thereafter, nuclear loss was more gradual and midstage clinical disease was not seen until the ONL thickness was reduced below 2 nuclei (at 2.3 to 3 years of age). The two-phase rate of decay in ONL thickness, therefore, accounts for the temporal separation between the presence of ERG and/or morphologic disease, and the diagnosis of disease by ophthalmoscopy; the former are evaluated directly at the photoreceptor level, while the latter requires an appreciable reduction in outer retinal thickness for recognition.

Pedigree analysis and test matings

Pedigree information was obtained on the propositus — a purebred, male, PRA-affected Miniature

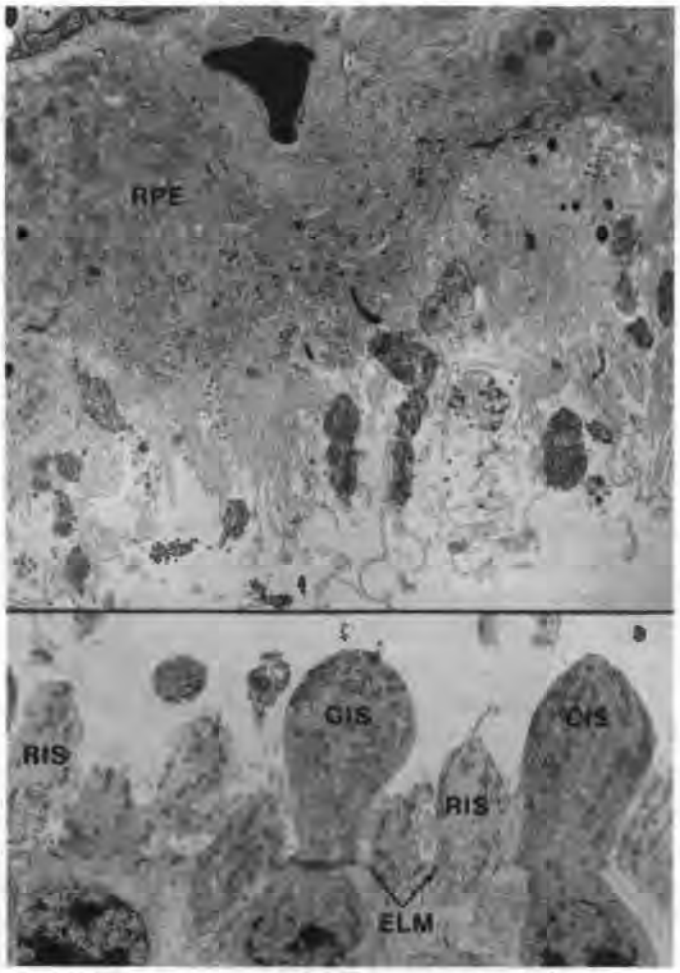

Figure 10. Electron photomicrograph composite of the RPE-photoreceptor layer from a 19-week-old *pd*-affected dog. There is a very limited amount of outer segment material, and this remains entrapped within the apical microvilli of the RPE. The photoreceptor layer has prominent cone inner segments (CIS), and diminutive rod inner segments (RIS). Loss of receptors results in the lateral expansion of the remaining visual cells. ELM = External limiting membrane. X3,520.

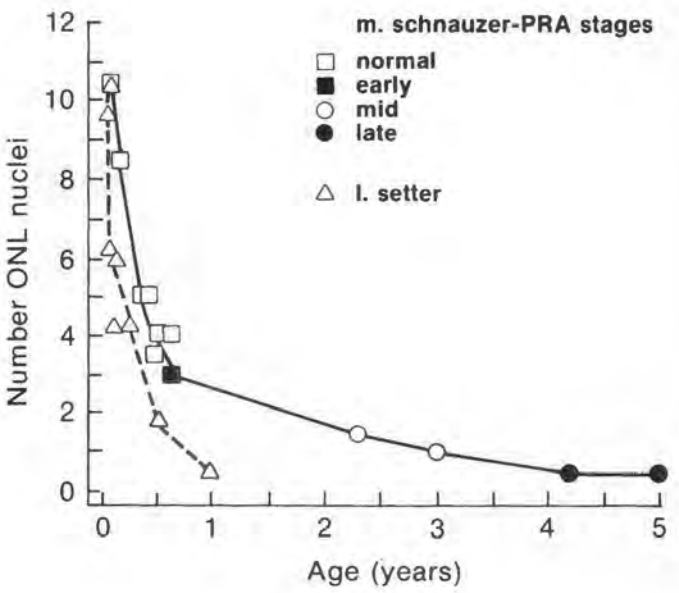

Figure 11. Comparison of changes in ONL width (in nuclei) by age, and by ophthalmoscopic staging of PRA disease for 12 *pd*-affected Miniature Schnauzers with similar data from *rcd1*-affected Irish Setters. Miniature Schnauzers with *pd* show an initially rapid, but then more gradual decay in the number of ONL nuclei. In contrast, *rcd1*-affected Setters show a more consistently rapid and early loss of ONL nuclei (ONL width values for normal Irish Setters = 11 to 14 nuclei from 13 to 34 days of age; 8 to 10 nuclei from 42 days to 2 years[21]). PRA disease staging: normal = fundus within normal limits; early = generalized but mild increase in tapetal reflectivity, vessels are normal; mid = marked increase in tapetal reflectivity, mild attenuation of retinal vessels and loss of 4th vascular branches; late = diffuse hyper-reflectivity of tapetal zone, geographic areas of more advanced atrophy, marked attenuation and loss of retinal vessels, secondary optic atrophy *(Data for rcd 1-affected Irish Setters was originally published in Schmidt SY, Aguirre GD 1985 Reductions in taurine secondary to photoreceptor loss in Irish Setters with rod-cone dysplasia.* Invest Ophth Vis Sci *26:679.)*

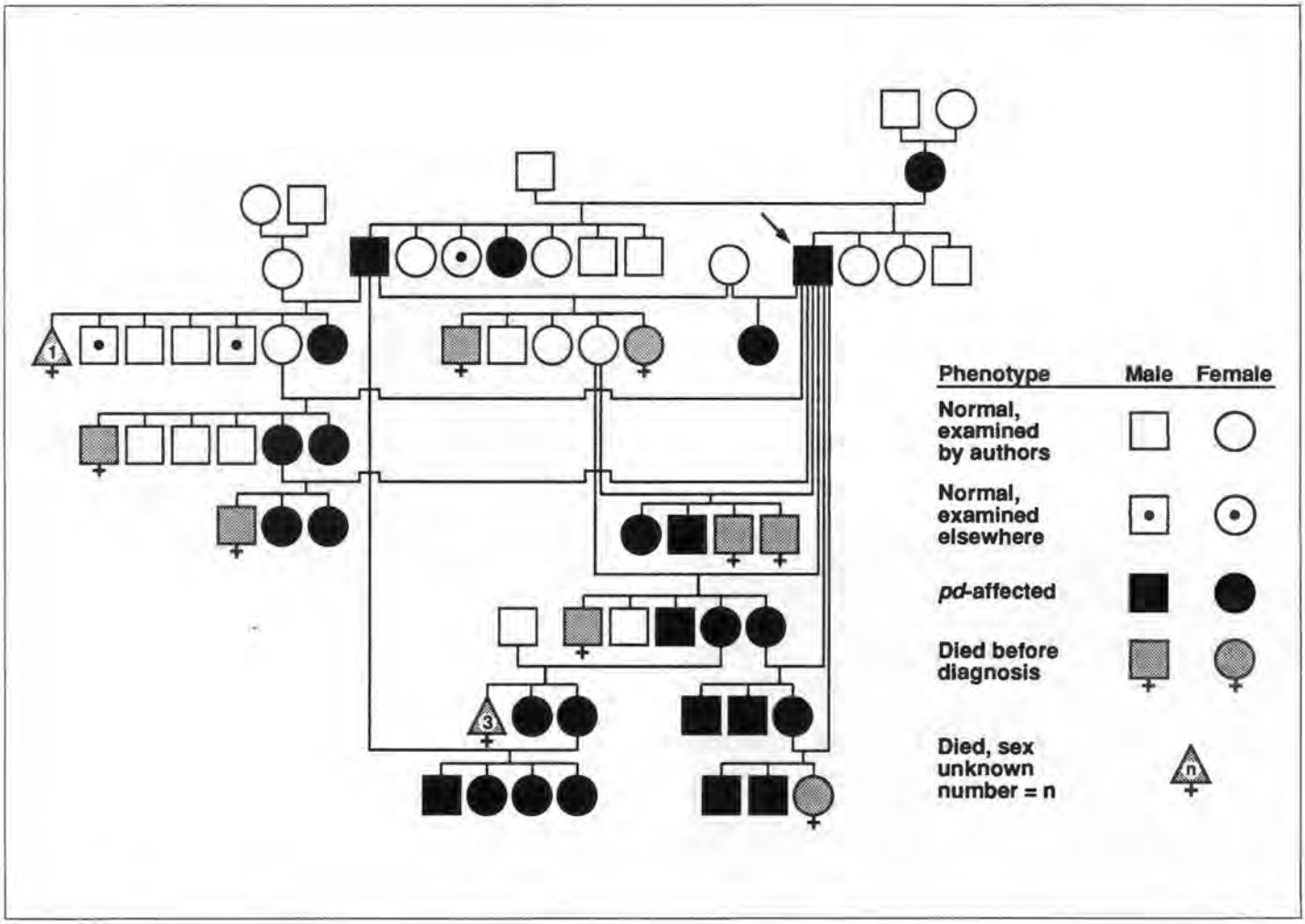

Figure 12. Abbreviated Miniature Schnauzer pedigree. For clarity, not all breedings nor all individuals utilized in this investigation have been included. Pedigree demonstrates those relationships critical to establish the mode of inheritance of photoreceptor dysplasia. The propositus (arrow) and several other dogs in this pedigree were used in our breeding studies to produce additional litters, not illustrated, but included as data in Table 3. Note all *pd*-affected dogs descend from a single common affected (female) ancestor, the daughter of non-affected parents; affected to affected breedings always produce only affected progeny; non-affected males can produce *pd*-affected daughters. These results effectively prove that photoreceptor dysplasia is transmitted as an autosomal recessive disorder.

Schnauzer — and on as many relatives as possible. All available dogs in the pedigree were examined by ophthalmoscopy and ERG. Additional elective breedings were performed utilizing the propositus and several of his close relatives and offspring. These breedings are illustrated in Figure 12, and their results are summarized in Table 3.

The dam of the propositus was affected, but his sire was phenotypically normal (as were his three littermate siblings). Note that neither parent of the propositus' affected dam were themselves affected. A second litter from the parents of the propositus contained a further seven full siblings to the propositus: these included two affected (one of which was female) and five non-affected (two male, three female) dogs.

There was no evidence for any sex predilection for the disease. Of the total number of dogs studied (see Table l) the number of affected males was exactly equal to the number of affected females (33). Similarly, from the breedings performed for informative mating analysis there were 19 affected males produced, and 26 affected females. The latter numbers are not significantly different (χ^2 = 1.089, degree of freedom [df] = 1, $0.20 < p \leq 0.30$).

From the data presented in Figure 12 and Table 3 the following critical facts emerge:

- 21 affected progeny and no non-affected progeny were produced from affected to affected breedings. If dominant inheritance was postulated, most of the

parental animals in these breedings would, by pedigree analysis, have to be heterozygous for the mutant gene. Under this hypothesis, at least one non-affected pup would be expected from these breedings. Thus, dominant inheritance is effectively ruled out.

- non-affected males can produce *pd*-affected daughters. Four such daughters are included in Figure 12, effectively ruling out X-linked inheritance.
- a total of 24 affected and 29 non-affected pups were produced from affected to carrier matings, of both types. This is not significantly different from the expected segregation ratio (0.5) for autosomal recessive inheritance (segregation ratio = 0.45, $\chi^2 = 0.472$, df = 1, $0.30 < p \leq 0.50$).

These results conclusively establish that photoreceptor dysplasia is transmitted as a single gene defect, and is an autosomal recessive disorder.

As soon as this evidence was at hand, a program was instituted to identify homozygous normal animals. Two candidate female Miniature Schnauzers have been bred to affected males and produced (in five litters) a total of 21 pups that survived to diagnostic age. None of these pups were affected, establishing that both bitches had been test cleared as homozygous normal (bitch 1, $n=12$, $p < 0.00025$; bitch 2, $n=9$, $p < 0.002$). The fact that this program has succeeded conclusively disproves the possibility of autosomal dominant inheritance, and is final confirmation of the autosomal recessive inheritance of photoreceptor dysplasia.

Because the morphological and functional studies indicate that this disease is a defect of differentiation affecting both rods and cones, it was named photoreceptor dysplasia. Because it is recessively inherited it was assigned the symbol *pd* to refer to the gene for photoreceptor dysplasia.

Discussion

The form of progressive retinal degeneration reported here is unique to the Miniature Schnauzer breed. All references to PRA in Miniature Schnauzers prior to this study suggested that the disease was a late onset type.[16,17] The results of this study, however, indicate that it is an early onset disorder in which the clinical manifestations are uniquely delayed. By histology and ERG, the disease is evident (in fact well advanced) at an age when normal retina is approaching the end of postnatal differentiation. These studies reveal the presence of a defect very early in life, which causes marked abnormalities in retinal structure and function. The ophthalmoscopic hallmarks of PRA, however, are not apparent until very much later in life. Furthermore — and unlike other early onset disorders (e.g., *rcd1* in Setters, *rcd2* in Collies) — visual function is only subtly affected in young affected animals. Moreover, vision (particularly photopic function) remains relatively normal for many months to years. This is surprising because of the severity of the structural lesions, especially when most of the rod and cone outer segment material is either absent or very degenerate. In no other form of PRA is there such a discrepancy between the biologic and clinical aspects of the disease.

Because of this discrepancy, ERG is a critical tool for the identification of affected Miniature Schnauzers prior to breeding age. This step is essential if practicable control programs (e.g., test-matings for carrier detection) are to be established within the breed. ERG has contributed greatly to the study of canine retinal function and dysfunction since the early 1970s. Quantitative ERG evaluation has made possible the identification of hereditary rod and/or cone abnormalities prior to the presence of ophthalmoscopic and/or clinical signs in several discrete forms of PRA.[8,19] Since ophthalmoscopic signs often are only demonstrable after animals reach their prime breeding age (particularly so for late onset forms of PRA) the ERG has been important in the study of these diseases, both for the identification of affected animals and for characterization of the disease process. In affected Miniature Schnauzers, the ERG detects retinal dysfunction so much earlier than it does in other forms of PRA (i.e., relative to the onset of clinical disease) that it adopts a crucial importance for understanding and controlling this disease.

The ERG indicates that this disease in the Miniature Schnauzer is a developmental rather than a degenerative disorder of the visual cells. This conclusion is based on finding that a normal ERG was not recordable in affected animals soon after the completion of postnatal retinal maturation,[6,8] and that the abnormal ERG rapidly deteriorated. In contrast, in the late onset degenerative hereditary retinal diseases (*prcd* in the miniature Poodle, English and American Cocker Spaniels), a normal ERG is present early after postnatal development is completed, and the rate of amplitude decay is much slower than in affected Schnauzers.[7]

Histopathology confirms the developmental nature of this disorder. Both rods (inner segments, outer segments) and cones (outer segments) appear to develop abnormally before undergoing very rapid degeneration. These abnormalities represent not only a retardation of normal development (e.g., the degree of development in the 24-day-old

affected retina resembled that of a 10- to 12-day-old normal) but also an aberrant differentiation process. Rod and cone photoreceptors fail to become structurally normal and subsequently degenerate. Degeneration, present by 8 weeks of age, is recognized by the presence of numerous pyknotic nuclei in the ONL, and necrotic photoreceptor elements with massive disruption of cellular organelles and remaining outer segments. That degeneration has been well-established by 8 weeks of age is most clearly evident by the almost 20 percent reduction in the number of ONL nuclei at this time. Progression of the disease results in the further loss of photoreceptors and their nuclei. This degeneration primarily affects rods and, by 6 months of age, prominent cone inner segments are the predominant photoreceptor class that remains. Subsequently, photoreceptors slowly disappear, and disease progresses to affect the inner retinal layers. The changes found in the early stages of the disease are specific to the photoreceptor cells, their nuclei and synaptic terminals; the inner retinal layers remain unaffected. Following the complete loss of the visual cell layer, inner retinal degeneration begins. These later degenerative and atrophic changes result in gliosis and loss of retinal layer organization. In addition, there is pigment epithelial atrophy, focal hyperplasia and intraretinal pigment migration. These changes are not specific for photoreceptor dysplastic Miniature Schnauzers, but occur in the late stages of any progressive, inherited retinal degenerative process in dogs.

The disorder has been termed photoreceptor dysplasia to indicate that it represents a defect in postnatal differentiation of the rods and cones. However, it is distinctly different from other forms of PRA classified as developmental disorders (*rcd1* and *rcd2* in the Irish Setter and Collie breeds, respectively; and *rd* and *erd* in the Norwegian Elkhound). It differs from *rd* in that cone responses are not only abnormal, but also rapidly deteriorate.[6] It also differs from *erd* because, in addition to the functional photoreceptor abnormalities, *erd* affected retinas also have a defect in signal transmission across the synaptic terminals in the outer plexiform layer; thus the ERG is a-wave dominated, with minimal contribution from the b-wave generators.[8] The affected Miniature Schnauzer ERG is similar to that of *rcd1*- or *rcd2*-affected dogs; there are extensive functional abnormalities affecting both rod and cone systems.[4,9] It differs, however, in that Schnauzers have greater functional cone disease and, in some cases, there is a distinct rod contribution to the recorded response. Moreover, although photoreceptor dysplastic Miniature Schnauzers show an extremely rapid progression of the ERG functional abnormalities, they retain vision for a longer period of time. Future work will be needed in order to critically examine these differences.

In both *rcd1* and *rcd2*, ERG and structural abnormalities are detectable by 6 weeks of age. The defects are qualitatively similar in severity to those present in *pd*. However, disease progression is rapid in *rcd1* and *rcd2*, and ophthalmoscopic diagnosis of PRA is possible by 16 weeks of age. In *pd*, on the other hand, fundus abnormalities are not apparent in most affected dogs until relatively late in the disease. This difference probably reflects the more rapid loss of photoreceptor nuclei that occurs in the *rcd1*-affected Irish Setter (see Figure 11).

Another factor common to both *rcd1* and *rcd2* is that both result from abnormalities in retinal cyclic nucleotide metabolism.[11,15] Early retinal degeneration in Elkhounds, on the other hand, is different in that cyclic nucleotide metabolism is normal in affected visual cells.[9] Unpublished studies in the Miniature Schnauzer (G.J. Chader and R.T. Fletcher) indicate that it too is a developmental disorder of the visual cells, not associated with abnormal cyclic nucleotide metabolism. They have found that the 19-week-old photoreceptor dysplastic retina has normal levels of cGMP, and normal activity of a calmodulin independent cGMP-PDE.[18] Even though there was advanced degeneration at 19 weeks of age, the comparable disease stage in the Irish Setter would still have showed a 7- to 10-fold elevation in retinal cGMP levels. Thus, cyclic nucleotide abnormalities can be ruled out in the pathogenesis of photoreceptor dysplasia.

Histology, ERG and clinical examinations have shown that this new form of PRA in Miniature Schnauzers represents a defect in postnatal differentiation of the rods and cones of the retina. Pedigree analysis and breeding studies have conclusively established that it is inherited as an autosomal recessive disorder. Accordingly, the disease is termed photoreceptor dysplasia, and assigned the symbol *pd* to represent the gene.

At the same time that the currently reported studies were in progress, a number of Miniature Schnauzers were identified (not included in this study) that had ERG responses which were completely normal both in waveform and in the proportional contribution of rods and cones to the response, but which were low in amplitude for the dogs' ages. These animals have been identified as "low amplitude" to characterize the salient feature of the ERG response. It is the authors' view that these animals are not normal, but are affected with a disorder different from *pd*. These animals have been followed for several years by repeated ERG and histopathologic studies. It has been

found that they have an extremely slow progression of their ERG abnormality, i.e., further amplitude reduction. Morphologic assessment of their retinas indicates that the dogs have a lower number of structurally normal photoreceptors, and that the receptor number decreases with age. The "low amplitude" abnormality appears to be an unrelated heritable defect, but needs to be characterized further and its significance determined.

Acknowledgements

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Footnotes

a. thiamylal sodium, Surital, Park Davis, or Biotal, Bio-ceutics
b. Halothane, Halocarbon Laboratories, Inc.
c. Fluothane, Aveco Co., Inc.
d. Tektronix 502, Tektronix, Beaverton, OR
e. Tektronix D13, Tektronix, Beaverton, OR
f. Epon 812 or Polybed
g. Zeiss EM 9S2 or EM 109 electron microscopes

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