direct interaction such as charge-transfer or redistribution of electronic charge within the thyronine molecule.

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Rod-Cone Dysplasia in Irish Setters: A Defect in Cyclic GMP Metabolism in Visual Cells

Abstract. *An abnormality in retinal guanosine 3',5'-monophosphate (cyclic GMP) metabolism is demonstrated in the inherited rod-cone dysplasia of Irish Setter dogs. Affected visual cells are deficient in cyclic GMP phosphodiesterase activity and have elevated levels of cyclic GMP. The biochemical abnormalities observed in affected retinas of Irish Setters are similar to those in the retinas of mice with inherited retinal degeneration before visual cell degeneration begins. A defect in cyclic GMP metabolism may be characteristic of early-onset degenerative diseases of the retina, possibly including those that affect humans.*

Retinal degenerations that cause blindness are known to occur in several species of animals, including humans (*1*). In certain of these diseases, photoreceptor cells of the retina degenerate selectively before reaching maturity. These early-onset disorders (dysplasias) are inherited, and some may share a common biochemical etiology.

Detailed studies on mice with inherited retinal degeneration (*rd* mice) have shown that an abnormality in the metabolism of guanosine 3',5'-monophosphate (cyclic GMP) occurs before visual cells begin to degenerate (*2*). The metabolic abnormality is related to a deficiency in cyclic GMP–phosphodiesterase activity that results in the accumulation of cyclic GMP within affected photoreceptor cells (*3*). A causal relationship between the elevation of cyclic GMP and photoreceptor cell degeneration has been confirmed in vitro in normal eye rudiments of *Xenopus laevis* cultured in the presence of phosphodiesterase inhibitors (*4*).

Now we report that a derangement in cyclic GMP metabolism is present in the dystrophic retina of Irish Setters, and that the defect is biochemically similar to that of *rd* mice. This raises the possibility that a defect in cyclic GMP metabolism can exist in retinal degenerative diseases in other species including man.

Irish Setters carry an autosomal recessive mutation (*5*) that causes retinal degeneration (rod-cone dysplasia) in homozygous animals (*6*). Electroretinographic measurements have proved useful for establishing the degree of rod or cone degeneration in living animals and, therefore, the progress of the disease. In vivo, the physiological response of affected rods to light is greatly diminished during early postnatal life, while that of cones is altered to a lesser extent. Morphologic studies have demonstrated failure of postnatal differentiation of visual cells in affected retinas. Following their initial development, affected visual cells soon appear pathological and form only small and disorganized outer segments. By 18 to 20 weeks, all rod cells have degenerated, leaving the retina with only a small number of cone cells in the photoreceptor layer.

Affected Irish Setters (8 and 12 weeks of age) were studied physiologically to assess visual cell function, morphologically to determine visual cell pathology, and biochemically to assess whether cyclic nucleotide metabolism was normal. Unaffected heterozygote Irish Setters of similar age were used as control animals. These animals were considered the best available control subjects; their morphology and retinal cyclic GMP content were comparable to those of two other normal dog breeds (beagles and foxhounds). Electroretinograms were recorded from halothane-anesthetized animals; in affected dogs, rod-mediated responses to light were completely absent. Cone responses were present but abnormal; the response amplitude was reduced and the peak latencies were increased. All responses were normal in the control animals.

Morphologic studies revealed that the visual cells from the retinas of affected dogs were abnormal at both 8 and 12 weeks of age (Fig. 1). The photoreceptor layer contained a minimum of rod outer segment material, short inner segments, and fewer visual cells than that of a control retina. Cones were prominent, their inner segments broad and club-shaped, the outer segments short and abnormal. The inner retinal layers remained unchanged and normal in appearance. In the Irish Setter disease, the early onset of rod degeneration and the maintenance of cone integrity is thus similar to the pattern of visual cell degeneration observed in retinas of *rd* mice and comparable to that suggested from electrophysiological studies of the human disease, retinitis pigmentosa (*7*).

Cyclic nucleotide metabolism in control dog retinas appears to be similar to that of other species (Table 1) in that retinal homogenates show two apparent values of the Michaelis constant (K_m) for phosphodiesterase with cyclic GMP as substrate. The phosphodiesterase with the high value (K_m-A) has been localized within the photoreceptor layer of the retina of several animals, and that with the lower value (K_m-B) is associated with the inner layers (*8*). In affected Irish Setters, the activity of K_m-A is below the level of detection, and that of the inner retina is comparable to the control. Therefore, a deficiency in cyclic GMP-phosphodiesterase activity can be demonstrated in the affected retinas at the time of photoreceptor cell degeneration.

The deficiency in cyclic GMP–phosphodiesterase activity of affected visual cells impairs their ability to hydrolyze cyclic GMP. The concentration of cyclic GMP in the retinas of affected dogs is about ten times greater than that in control retinas (Table 1). In affected retinas, however, the deficiency in phosphodiesterase activity does not alter the concentration of adenosine 3',5'-mono-

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phosphate (cyclic AMP). Furthermore, we assessed whether cyclic GMP concentrations were generally elevated throughout the tissues of affected dogs (for example, liver, brain, and the pigment epithelium–choroid unit of the eye). The negative findings suggest that the abnormality in cyclic GMP metabolism is restricted to cells of the neural retina and, most likely, to visual cell outer segments.

Table 1. Kinetic characteristics of cyclic GMP–phosphodiesterase and cyclic nucleotide concentrations in dog tissues. Dogs were anesthetized with pentobarbital, and the eyes (10-week-old controls, $N = 8$ eyes; 8- and 12-week-old affected, $N = 10$ eyes) and other body tissues removed. Visual cortex and liver samples were obtained from 27-week-old control and 12-week-old affected animals. The neural retina was quickly dissected from the pigment epithelium–choroid unit, and all tissues were either frozen in liquid nitrogen and lyophilized or immediately placed in 10 percent perchloric acid solution and sonicated. Cyclic nucleotides then were determined by two methods. (i) Portions of the 10 percent perchloric acid extract (0.5 ml) were taken and the cyclic nucleotides separated and purified by chromatography on Dowex AG 1- × 8; following succinylation, the concentration of cyclic nucleotide (in picomoles per milligram of protein) was measured by radioimmunoassay (*9*), as previously described (*10*). Recovery of cyclic nucleotide was 70 to 80 percent. (ii) Samples of lyophilized tissue were homogenized and extracted with boiling 0.1*N* HCl, and the supernatant fractions were adjusted to *p*H 4.5 or 7.6 for the determination of cyclic AMP and cyclic GMP, respectively. Assays were then carried out according to the binding protein method (*11*). The two methods of determining the cyclic nucleotides gave comparable data and the results were combined. Values are means ± standard errors shown where six or more samples were assayed. Cyclic GMP phosphodiesterase was measured in homogenates of lyophilized neural retina by a two-step procedure (*8*). Individual K_m values were obtained by interpolating plots of 1/velocity versus 1/substrate, and the reported value is the mean of four to six determinations. Protein in all cases was measured by the method of Lowry *et al.* (*12*).

Group	Neural retina				Other tissues		
	Cyclic GMP—phosphodiesterase		Cyclic nucleotides (pmole/mg)		Cyclic GMP (pmole/mg)		
	K_m-A	K_m-B	GMP	AMP	Pigment epithelium–choroid	Visual cortex	Liver
Control	$1.2 \times 10^{-4} M$	$2.0 \times 10^{-5} M$	12 ± 0.7	12	9 ± 1.2	3 ± 0.2	2 ± 0.1
Affected	0	$1.8 \times 10^{-5} M$	102 ± 8.6	12	6 ± 0.9	5 ± 0.4	5 ± 0.3

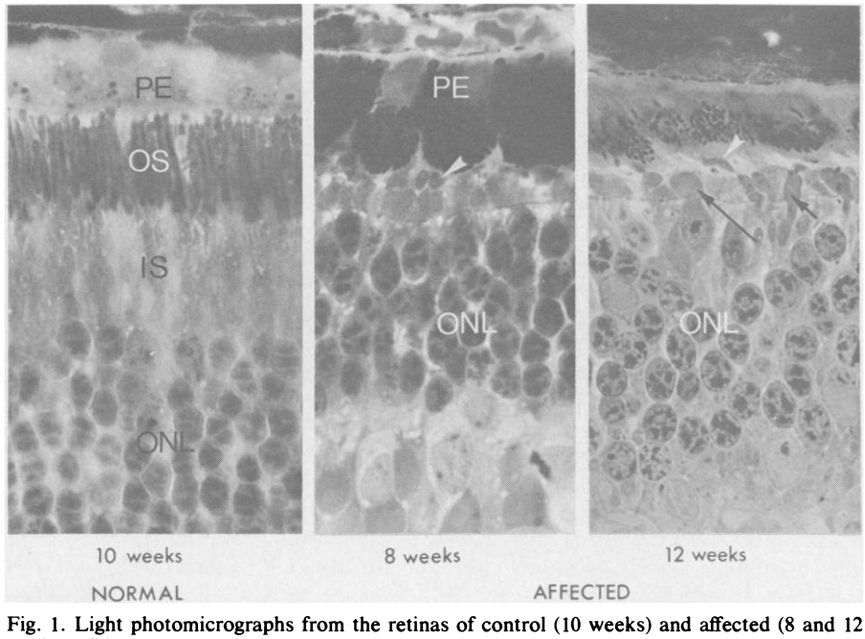

Fig. 1. Light photomicrographs from the retinas of control (10 weeks) and affected (8 and 12 weeks) Irish Setters. In the tapetal zone of the control retina, photoreceptor inner segments (*IS*) and outer segments (*OS*) are elongated and abut the nonpigmented pigment epithelium (*PE*). The sections from the affected retinas are taken from the nontapetal zone, where the pigment epithelium is variably pigmented. The photoreceptor layer of the diseased retinas is reduced in width and density; the inner segments are diminutive, and there is minimal outer segment material (white arrowheads), especially in the older dog. Rod photoreceptor loss causes increased prominence of cones (black arrows) and reduction in the width of the outer nuclear layer (*ONL*). This is more striking in the more peripheral section of the 8-week-old retina. Tissues were fixed in 2.5 percent glutaraldehyde and 2 percent osmium tetroxide, dehydrated, and embedded in Epon. Sections were stained with azure II–methylene blue (×965).

The inherited disorder of Irish Setters, therefore, can best be characterized as an abnormality in visual cell differentiation that leads in early life to photoreceptor degeneration and blindness. The abnormality in cyclic GMP metabolism is an early biochemical defect that may be related to the etiology of visual cell degeneration as assessed morphologically and electrophysiologically. A causal relationship between the biochemical defect and cell degeneration is inferred from the study of *rd* mice in which an accumulation of cyclic GMP precedes visual cell degeneration (*2*).

While studying an inherited disease there is always concern for whether a biochemical abnormality is primary to the genetic mutation or a secondary response of an affected cell. This issue has not been resolved for the disorders of *rd* mice or Irish Setters. It is significant, however, that early onset diseases of the retina in two very different animal species show identical biochemical abnormalities. Derangement in cyclic GMP metabolism may thus be a common feature of degenerative diseases of the retina that cause blindness in early life.

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