Hereditary Retinal Degeneration
in Drosophila melanogaster

A Mutant Defect Associated with
the Phototransduction Process

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ABSTRACT Two genes in Drosophila, rdgA and rdgB, which when defective cause retinal degeneration, were discovered by Hotta and Benzer (Hotta, Y., and S. Benzer. 1970. Proc. Natl. Acad. Sci. U. S. A. 67:1156-1163). These mutants have photoreceptor cells that are histologically normal upon eclosion but subsequently degenerate. The defects in the rdgA and rdgB mutants were localized by the study of genetic mosaics to the photoreceptor cells. In rdgB mutants retinal degeneration is light induced. It can be prevented by rearing the flies in the dark or by blocking the receptor potential with a no-receptor-potential mutation, norpA. Vitamin A deprivation and genetic elimination of the lysosomal enzyme acid phosphatase also protect the photoreceptors of rdgB flies against light-induced damage. The photopigment kinetics of dark-reared rdgB flies appear normal in vitro by spectrophotometric measurements, and in vivo by measurements of the M potential. In normal Drosophila, a 1-s exposure to intense 470-nm light produces a prolonged depolarizing afterpotential (PDA) which can last for several hours. In dark-reared rdgB mutants the PDA lasts less than 2 min; it appears to initiate the degeneration process, since the photoreceptors become permanently unresponsive after a single such exposure. Another mutant was isolated which prevents degeneration in rdgB flies but which has a normal receptor potential. This suppressor of degeneration is an allele of norpA. It is proposed that the normal norpA gene codes for a product which, when activated, leads to the receptor potential, and which is inactivated by the product of the normal rdgB gene.

INTRODUCTION

By screening chemically mutagenized Drosophila melanogaster for deficits in visual behavior, Hotta and Benzer (1970), Pak et al. (1969), Pak et al. (1970), and Heisenberg (1971) isolated many X-chromosomal mutants with altered electroretinograms (ERGs). Histological examination revealed that some of these mutants suffer from severe retinal degeneration (Hotta and Benzer, 1970; Heisenberg, 1971). All these retinal degeneration mutants fall into two complementation
groups, rdgA and rdgB. Hotta and Benzer (1970) showed that in mosaic flies with some parts genetically normal and some genetically mutant, only the eye tissue is relevant for the expression of retinal degeneration, i.e. the rdgA and rdgB defects are autonomous to the eye. In this paper, we have extended Hotta and Benzer's (1970) mosaic analysis to show that the photoreceptor cells themselves are primarily responsible for these mutant defects.

Conditions that accelerate, decelerate, or prevent hereditary retinal degeneration can offer clues to the mechanism. Dowling and Sidman (1962) found that if pink-eyed retinal degeneration mutant rats were reared in the dark, the time course of hereditary retinal degeneration was slowed. Yates et al. (1974) and LaVail and Battelle (1975) found that black eye pigmentation mimicked the dark-rearing effect. The action of light on the disease suggests that rhodopsin metabolism may be involved. Vitamin A deprivation causes retinal degeneration in mammals (Dowling and Wald, 1960) and this effect is prevented in rats by raising them in the dark (Noell et al., 1971). Furthermore, vitamin A deprivation protects against the rat retinal degeneration that is caused by strong light (Noell and Albrecht, 1971).

In this paper we examine conditions, including dark rearing and vitamin A deprivation, which are protective in Drosophila retinal degeneration mutants. This enables one to control precisely the onset of the degeneration process so that early physiological defects can be studied. Secondary mutations are also described which prevent retinal degeneration in rdgB flies.

A brief introduction to some of the anatomy, physiology, and photochemistry of the Drosophila retina should aid in the interpretation of the experiments and results presented here. The Drosophila compound eye consists of approximately 800 ommatidia each of which contains eight photoreceptor cells of three morphologically and physiologically distinct classes. The six peripheral photoreceptors, R1–6, in each ommatidium are blue and UV sensitive, (see Fig. 7), and contain a rhodopsin which absorbs maximally at about 470 nm with a secondary maximum in the UV, and which interconverts with a metarhodopsin absorbing maximally at about 570 nm (Pak and Liddington, 1974; Ostroy et al., 1974; Stark, 1975; Harris et al., 1976). The rhabdomeres of the central two photoreceptors, R7 and R8, are stacked on top of one another, and are, respectively, UV sensitive and blue sensitive (see Fig. 7); (Harris et al., 1976). R7, the distal central photoreceptor, contains a rhodopsin which absorbs maximally at about 370 nm and which interconverts with a metarhodopsin absorbing maximally at about 470 nm. R8, the proximal central photoreceptor, has a third photopigment (Harris et al., 1976). Maximal rhodopsin to metarhodopsin conversion (caused in R1–6 for example by bright 470 nm adaptation, and in R7 by 370 nm adaptation) produces a long-lived depolarization and inactivation in these cells which continues even after the termination of the stimulus (Minke et al., 1975a; Stark, 1975; Harris et al., 1976; Stark et al., 1976). The long-lived depolarization in invertebrate photoreceptors, first discovered in Limulus median ocellus (Nolte et al., 1968) and well characterized in the barnacle (Hochstein et al., 1973) has been called the prolonged depolarizing afterpotential (PDA) (Minke et al., 1973). In the dark, metarhodopsin reconverts slowly to rhodopsin in flies (Stavenga et al., 1973; Pak and Liddington, 1974), allowing PDA decay and resensitization (Minke
et al., 1975a); dark reconversion may not occur in all invertebrates (Minke et al., 1973). A much more rapid termination of the PDA and resensitization is accomplished by photoconversion of metarhodopsin to rhodopsin (caused in R1-6, for example, by 570 nm adaptation) (Hochstein, et al., 1973; Pak and Liddington, 1974; Minke et al., 1975a). In Drosophila R1-6 cells, synchronous photoconversion of substantial amounts of metarhodopsin to rhodopsin is accompanied by some fast electrical potentials, collectively called the M potential, which can be recorded in the ERG (Pak and Liddington, 1974).

The basic mechanism of excitation in photoreceptor cells is incompletely understood. Between photon capture by rhodopsin and generation of the receptor potential there may be many intermediate steps (Fuortes and Hodgkin, 1964; Baylor et al., 1974). Drosophila mutants in which the receptor potential is blocked or altered may have defects in these intermediate steps (Minke et al., 1975b; Pak, 1975).

For instance, mutants of the no-receptor-potential A (norpA) gene are deficient in an excitation step subsequent to quantum catch (Alawi et al., 1972; Pak and Liddington, 1974; Ostroy et al., 1974). The experiments presented here indicate that the rdgB defect is associated with a step in the phototransduction process subsequent to photopigment action and yet not consequent to the receptor potential. From these studies with mutants, we suggest how the normal rdgB and norpA gene products may be involved as intermediates in the phototransduction process.

MATERIALS AND METHODS

Stocks

Normal flies were from the wild-type Canton-S strain. The rdgA, rdgB, and norpA<sup>Es</sup> mutants, the multiply marked <i>y</i> <i>cho</i> <i>ev</i> <i>m<sup>3</sup></i> X chromosome, and the unstable ring-X <i>In(1)w<sup>uc</sup></i> were from the collection of Seymour Benzer at the California Institute of Technology. The <i>ora<sup>A84</sup></i> and <i>JK910</i> mutants were from John Merriam at the University of California, Los Angeles. The acid phosphatase null mutant, <i>Acp-1<sup>11</sup></i>, was from Ross MacIntyre at Cornell University. <i>s<sup>d</sup></i> was from P. T. Ives at Amherst College. <i>w</i>, <i>cn bw</i>, and <i>Df(1)g</i> were from Ed Lewis at the California Institute of Technology. <i>Df(1)KA14</i> and <i>Df(1)RA2</i> were from George Leefvre, California State University at Northridge.

Several of these mutants were combined to study their interaction or to eliminate screening pigments from the eye. The following stocks were constructed by standard genetic techniques: (a) <i>y w rdgA<sup>PC17</sup></i>; (b) <i>w sn<sup>6</sup> rdgA<sup>B212</sup></i>; (c) <i>w rdgB<sup>ES22</sup></i>; (d) <i>y cho rdgB<sup>ES22</sup></i>, <i>Acp-1<sup>11</sup></i>; (f) <i>rdgB<sup>ES22</sup></i>, <i>ora<sup>JK910</sup></i>; (g) <i>w norpA<sup>Es</sup></i>; (h) <i>norpA<sup>Es</sup> rdgB<sup>ES22</sup></i>, <i>cn bw</i>; (i) <i>norpA<sup>Es</sup> rdgB<sup>ES22</sup></i>; (j) <i>rdgB<sup>ES22</sup></i>, <i>JK910</i>.

The single mutation <i>w</i> (white) and the double mutation <i>cn bw</i> (cinnabar brown) are equally effective at eliminating screening pigments from the eye while not interfering with the functioning of the photoreceptor cells (Alawi et al., 1972). Since <i>cn</i> and <i>bw</i> are located on the second chromosome while <i>w</i> and most of the visual mutants are on the first, it was often easier to use <i>cn bw</i> than <i>w</i> in the construction of white-eyed multiple mutants. Flies were raised at 25°C on standard yellow cornmeal medium (Lewis, 1960) in a 12 h:12 h light-dark cycle unless otherwise stated.

Isolation of Suppressor Mutations

To find X-linked suppressors of degeneration <i>rdgB<sup>ES22</sup></i> and <i>rdgA<sup>KA14</sup></i> males were muta-
genized with ethyl methane sulfonate according to the protocol of Lewis and Bacher (1968) and mated to virgin females having attached X chromosomes marked with yellow and forked (XX, yf). 5-day old male progeny were checked for retinal degeneration by the pseudopupil technique (see below). Those that showed no degeneration were pair mated to XX, yf virgin females, and the male progeny tested by the same method. Suppressors were kept as stocks. One of these, found to be allelic to norpA and designated norpA<sup>null</sup>, was combined with various other mutations to produce the following stocks: (a) norpA<sup>null</sup> rdgB<sup>8222</sup>; (b) norpA<sup>null</sup>, cn bw; (c) norpA<sup>null</sup> rdgB<sup>8222</sup>, cn bw; (d) norpA<sup>null</sup> rdgB<sup>8045</sup>.

**Examination of the Eye in Living Animals**

A technique devised by Kirschfeld and Franceschini (1968) allows analysis of the photoreceptor optics in living flies. The pseudopupil is formed by the superposition of the images of the rhabdomere tips from several neighboring ommatidia. It was observed by placing the fly on a glass slide and illuminating the head from below with a narrow beam of intense light, while focusing just below the surface of the eye with about ×20 magnification in a compound microscope. Alternatively, individual rhabdomeres were examined directly, without sectioning the eye, by the technique of optical neutralization of the cornea (Franceschini and Kirschfeld, 1971). In this case, the head of the fly to be examined was cut off at the neck with a razor blade, mounted on a glass slide with clear nail polish, and examined under oil at about ×400 magnification.

**Histology**

For light and electron microscopy, heads of flies were cut off, sliced midsagittally, and fixed immediately by the techniques of Poodry and Schneiderman (1970). They were then embedded in Epon-Araldite mixture. 1.5-μm sections for light microscopy were collected on a glass slide and stained with toluidine blue. Thin sections of about 1,200 Å were picked up on copper grids, and stained with lead citrate (Reynolds, 1963).

**Production of Mosaics**

The first method was to use males carrying the retinal degeneration mutation of interest linked to recessive eye and body color mutations (y, yellow body color, and che, chocolate eye color). These were mated to females heterozygous for the unstable ring-X chromosome In(I)w<sup>nc</sup> which contains dominant normal alleles of the genes for retinal degeneration, body color, and eye color. Approximately 7% of the progeny of such crosses were haplo-X diplo-X gynandromorphs in which the mutations were expressed in the hemizygous male tissue but not in the heterozygous female tissue (see Hotta and Benzer, 1970).

The second method was to X-ray female first and second instar larvae heterozygous for the white eye color and retinal degeneration mutations to induce somatic crossing over (Stern, 1936). The dose used was 1,200 rad, 325 rad/min, 50 kV, 20 mA, 13 cm from two 1-mm Al filters to target. In this way, small patches of homozygous mutant tissue were produced in a background of heterozygous normal tissue.

**Stimulation and Recording**

These methods were similar to Stark's (1975). Monochromatic stimuli were from a 150 W xenon arc (Hanovia 901C) with a Bausch & Lomb 500-mm monochromator (Bausch & Lomb, Inc., Rochester, N.Y.). Achromatic optics were used to focus the light onto the specimen, and the intensity was adjusted with Inconnel-on-glass neutral density filters (Bausch & Lomb 31-34-38 series). Energy calibrations at the locus of the preparation were made with a calibrated United Detector Technology PIN-10 photodiode (United Detector Technology Inc., Santa Monica, Calif.). Electroretinograms were recorded DC by use of
a Medistor (A-35) or ELSA-4 electrometer with saturated NaCl-filled microelectrodes inserted through the cornea. Responses were displayed on a Tektronix (5100 series) oscilloscope (Tektronix, Inc., Beaverton, Ore.) and a Physiograph DMP-4B recorder and photographed on a Grass C4R camera (Grass Instrument Co., Quincy, Mass.). Spectral sensitivities were determined as in Harris et al. (1976). Intense flashes of white light for generating the M potential were from a Vivitar (152) camera flash attachment. Intense 10-s adaptation conditioning flashes of $10^{17}$–$10^{18}$ quanta/cm², unless otherwise stated, were followed by approximately 1 min of dark before data were collected.

**Spectrophotometry**

Samples were obtained from dark-reared w and w rdgB·X522 flies by placing approximately 100 flies in a small glass bottle which was then dipped in liquid nitrogen for 1 min. Vigorous shaking of the bottle decapitated the frozen flies. Nylon mesh filters were used to separate the heads from the bodies. The heads were then homogenized in ~0.6 ml of 0.1 M phosphate buffer, pH 7.2, and the homogenate was then placed in a cuvette for spectrophotometry at room temperature. A dual wavelength spectrophotometer constructed by Dr. Edward Lipson at the California Institute of Technology and described in Harris et al. (1976) was used to measure light-induced absorption changes of the Drosophila photopigments.

**Vitamin A Deprivation**

Drosophila were vitamin A deprived by raising sterilized eggs aseptically on Sang's synthetic diet, medium C (Doane, 1967). For vitamin A-enriched medium, β-carotene (Nutritional Biochemicals Corp. 101287) was added to a final concentration of 125 mg/100 ml. See Stark and Zitzmann (1976) for details.

**ATPase Assay**

100 retinas each were dissected from cold-anesthetized w and w rdgB·X522 dark-reared flies, kept overnight at 4°C, then homogenized in 200 μl of reaction buffer. For total or ouabain-sensitive ATPase determination, 25 μl of homogenates were added to 75 μl of buffer at 30°C, and the reaction was started with 10 μl of 25 mM 32P-γ-ATP (New England Nuclear, Boston, Mass.). The reaction was terminated after 30 min by addition of 50 μl of ice-cold 20% TCA. When ouabain was present, its final concentration was $2 \times 10^{-4}$ M. Determination of inorganic 32P was by the method of Fahn et al. (1968). Specific activity was determined after assaying for protein (Lowry et al., 1951).

**RESULTS**

**Mutants**

**Mapping** Genetic mapping of the rdgA gene places it at position 26.3 ± 1.2 on the X chromosome; the recessive rdgA is uncovered by the small deletion Df(1)KA14, which spans salivary chromosome region 7F1·2-8C6, but is not uncovered by Df(1)RA2, which spans 7D10-8A4·5. Therefore rdgA is within the 8A4·5-8C6 region. Mapping of rdgB by recombination placed it at 42.7 ± 0.7 on the X chromosome. rdgB was uncovered by the deletion Df(1)g4 and is therefore in salivary region 12A-12E.

**Anatomical Defects** Upon eclosions all rdgA and rdgB mutants raised and kept as adults in standard conditions (12 hr light: 12 h dark at 25°C) have
normal-looking photoreceptors, as judged by electron microscopy and by pseudo-
pupil examination. 7 days later, however, all mutants showed degeneration of the outer six receptor cells, R1-6, of every ommatidium. The central two photoreceptors, R7 and R8 (see Figs. 1 and 2) were preserved in almost every

![Figure 1](image)

**Figure 1.** Normal eye. (a) Pseudopupil (bar = 100 μm); (b) rhabdomeres viewed by optical neutralization of the cornea (bar = 10 μm); (c) light microscopy of retina (bar = 10 μm); (d) electron micrograph of ommatidium (bar = 2 μm).

ommatidium in \( \text{rdgB}^{K5222} \) and \( \text{rdgB}^{K045} \), in about 60% of the ommatidia in \( \text{rdgB}^{K5100} \) and \( \text{rdgA}^{K3199} \), and in fewer than 10% of the ommatidia in \( \text{rdgB}^{BE170} \), \( \text{rdgA}^{K014} \), and \( \text{rdgA}^{BS12} \). These results suggest that R1-6 are more sensitive to the effects of the \( \text{rdgA} \) and \( \text{rdgB} \) mutations, and that the alleles of each retinal
degeneration gene can be ordered with respect to how much R7 and R8 are affected in each mutant. Thus, for \textit{rdgB} the order is: \textit{rdgB}^{E519} > \textit{rdgB}^{K5100} > \textit{rdgB}^{K516} = \textit{rdgB}^{K5200} > \textit{rdgB}^{K045} = \textit{rdgB}^{K5222}. For \textit{rdgA}: \textit{rdgA}^{R512} = \textit{rdgA}^{K014} > \textit{rdgA}^{K519} > \textit{rdgA}^{PC47}.

\textbf{FIGURE 2. Degenerate \textit{rdgB}^{K5222} eye.} (a, b, c, and d) as in Fig. 1.

\textbf{PHYSIOLOGICAL AND BEHAVIORAL DEFECTS} ERGs of 7-day old adult retinal degeneration mutants, raised in normal conditions, showed reduced receptor potentials and the absence of on-transients (Benzer, 1971; also see Figs. 7, 10, 14 and 17). The alleles in which R7 and R8 were most affected gave the smallest receptor potentials. In \textit{rdgB}^{K5222} and \textit{rdgB}^{K045} in which R7 and R8 are least affected, a receptor potential of up to 5 mV was common. This is of the same
order as the maximal response of R7 and R8 (Minke et al., 1975a). In rdgA\textsuperscript{K014} and rdgB\textsuperscript{EE170} in which R7 and R8 are most affected, no receptor potential greater than 0.5 mV was found. These results suggest that the residual receptor potential in these mutants originates from R7 and R8. Indeed, Harris et al. (1976) have shown that the receptor potential in \textit{rdgB\textsuperscript{Ks222}} has a spectral sensitivity corresponding to that of R7 and R8 in normal eyes (Fig. 8). The on-transient is absent in all of the mutants in which R1–6 have degenerated. This is consistent with the idea that this transient arises in the lamina (see Goldsmith and Bernard, 1974), since only the axons of R1–6 have synapses in the lamina; the axons of R7 and R8 pass through the lamina and have their first synapses in the medulla (Trujillo-Cenoz and Melamed, 1966).

Phototaxis, measured by counter-current distribution (Benzer, 1967), was strongest in those retinal degeneration mutants in which R7 and R8 were most preserved. Spectral analysis of this behavior (Harris et al., 1976; Stark et al., 1976) showed that R7 and R8 mediate the residual phototactic response in these mutants.

\textbf{TIME COURSE OF DEGENERATION} The time course of degeneration was measured for \textit{rdgB\textsuperscript{Ks222}}. 10 groups of about 20 \textit{rdgB\textsuperscript{Ks222}} flies raised in normal conditions were collected within 1 h of eclosion, kept in constant room light at 25°C, and examined at various intervals by the pseudopupil technique. Fig. 3 shows as a function of time the percentage of pseudopupils in which no defect was evident. Since the sharpness of a normal pseudopupil is dependent upon the precise optical alignment of the photoreceptors in about 20 ommatidia (Franceschini 1972), this is a sensitive assay for anatomical signs of photoreceptor degeneration. By 24 h degeneration was beginning in some flies, at 72 h degeneration was well underway in almost all \textit{rdgB\textsuperscript{Ks222}} flies. The steepness of the decline in Fig. 3 does not necessarily indicate an abrupt change from a nondegenerate to a degenerate state, but is more likely to reflect the threshold of the technique used for revealing anatomical changes. A pseudopupil was judged to be normal whenever the trapezoidal pattern of seven dots (Fig. 1a) was visible. Genetically normal flies in the same conditions showed no degeneration whatsover.

Although anatomical signs of degeneration do not occur until after emergence of the adult, it is evident from the ERG that R1–6 are already functionally defective at eclosion in all \textit{rdgA} and \textit{rdgB} mutants. Since the photoreceptors of \textit{Drosophila} are fully developed in late pupal life (Waddington and Perry, 1960), the initial degenerative process (i.e. the irreversible physiological malfunction of the photoreceptors, as distinguished from their subsequent structural degeneration) probably begins before emergence.

\textbf{Localization of Defect}

\textbf{TISSUE LOCALIZATION} By mosaic analysis of ERG deficits Hotta and Benzer (1970) found that the eye was the focus of both the \textit{rdgA} and \textit{rdgB} defects. Pseudopupil examination of 100 \textit{y cho rdgA\textsuperscript{K014}} and 100 \textit{y cho rdgB\textsuperscript{Ks222}} mosaics produced by ring loss (see Materials and Methods) confirmed their results. Even in mosaics in which all external landmarks were genetically normal except for one
eye, that eye showed retinal degeneration. Furthermore, a mosaic dividing line often (in about 20% of the mosaics) passed through an eye. In these cases the genetically mutant part of the eye showed degeneration while the genetically normal part did not. This was true for both \textit{rdgA^{K014}} and \textit{rdgB^{K5222}}. The most closely related internal tissue, in terms of fate map position, is the first optic ganglion. The latter is very rarely (<2%) split by mosaic dividing lines and is, in 10% of these mosaics, of genotype different from the retina (Kankel and Hall, 1976). Therefore, the degeneration defects must be autonomous to the retina.

**CELLULAR LOCALIZATION OF THE DEFECT** Ready et al. (1976) have shown that the cells of a \textit{Drosophila} ommatidium are not clonally related; a single ommatidium at a mosaic borderline may be composed of both normal and mutant cells. Examination by light and electron microscopy of borderlines in the eyes of

\begin{figure}[h]
  \centering
  \includegraphics[width=\textwidth]{figure3}
  \caption{Time course of degeneration in \textit{rdgB^{K5222}}. The percentage of flies with no retinal degeneration (as judged by observation of the pseudopupil) as a function of age after eclosion (see text for details).}
\end{figure}

\textit{y cho rdgA^{K014}} and \textit{y cho rdgB^{K5222}} mosaics reveals that within a single ommatidium some receptors may degenerate while others may not. This was also shown for another allele of \textit{rdgB} by Benzer (1971). The degeneration is not dependent on the genotype of neighboring pigment cells since degenerate and nondegenerate photoreceptors can be found next to the same pigment cells. By X-ray-induced somatic crossing over, small patches of \textit{w rdgB^{K5222}} mutant tissue may be made. In these the pigment cells and the photoreceptors can be scored individually for the absence of screening pigments (caused by the \textit{w} mutation). These results also indicate that \textit{rdgB^{K5222}} is autonomous to the photoreceptors themselves. In the diagrammatic reconstruction of part of such a patch (Fig. 4) normal photoreceptors are next to mutant pigment cells and vice versa. Furthermore, the only photoreceptors which survive in spite of being mutant are the central ones, R7 and R8, as expected in \textit{rdgB^{K5222}}. This means that the act of photoreceptor degeneration is consequent only on the genotype of the individual photoreceptor cell.
SUBCELLULAR LOCALIZATION OF THE DEFECT In the mutant ora^{JR84} isolated by Koenig and Merriam (1975), the rhabdomeres of the outer photoreceptors R1-6 fail to develop. By combining this mutant with the retinal degeneration mutant rdgB^{K5222}, doubly mutant flies were obtained. These had R1-6 photoreceptor cells, without rhabdomeres, carrying the rdgB^{K5222} mutation. When these double mutants were raised and kept as adults at 18°C their photoreceptor cells did not degenerate even after 20 days in constant light. That no degeneration occurs at 18°C in rdgB^{K5222} ora^{JR84} double mutants while considerable degeneration occurs in rdgB^{K5222} single mutants raised in identical conditions is explained by the light-deprivation effect (see below) because these photoreceptors with no rhabdomeres have no light response (Harris et al., 1976). However, when these double mutant flies were kept as adults at higher temperature (25°C) they did degenerate after about 10 days independent of light condition (Fig. 5). Thus, the cells are defective even in the absence of rhabdomeres. While the ora^{JR84} single mutants raised in given conditions do not degenerate, these double mutant
photoreceptors do, even though no rhabdomeres are present (Fig. 5). This indicates that the \textit{rdgB}^{K8222} mutant defect is not localized to the rhabdomere itself; some other part of the cell must be defective, (of course, the rhabdomere may be also).

\textit{Altering the Time Course of Degeneration}

\textbf{TEMPERATURE} Temperature has an accelerating effect on retinal degeneration. Mutant \textit{rdgB}^{K8222} flies were raised and kept as adults at 18°C, 25°C, or 30°C either in constant light (i.e. in glass food bottles 0.5 m in front of a GE 15 W cool white fluorescent lamp General Electric Co., Cleveland, Ohio) or in darkness. In constant light at 18°C, pseudopupil examination showed that degeneration became evident in about 3 days postemergence and approached completion in about 12 days. In constant light at 25°C, degeneration was evident 1 day postemergence and became complete in about 7 days. In constant light at 30°C, newly emerged flies already showed some degeneration, which reached completion in about 3 days.

In the dark, temperature also had a large effect. As will be discussed below,
flies raised and kept in darkness at 18°C showed little or no degeneration even up to 30 days postemergence. At 25°C, degeneration became evident by about 7 days. At 30°C, degeneration was evident within 2 days postemergence. Normal flies showed no retinal degeneration under any of the above conditions.

**ACID PHOSPHATASE DEPRIVATION** Lysosomal enzymes, including acid phosphatases, are involved in digesting cellular debris and degenerating tissue. Acid phosphatase activity changes markedly during retinal degeneration in the mouse **rd** mutant (Sanyall, 1970). Therefore, it was of interest to combine the Drosophila mutant **Acph-1**

flies and about 60 y cho **rdgB**

flies were raised at 25°C in constant light, and adults were examined by the pseudopupil technique when 24, 48, and 72 h old.

At 24 h, degeneration was evident in 49% of the flies carrying the **Acph-1**

gene, while only 12% of the flies carrying the null **Acph-1**

gene showed degeneration. By 48 h, 98% of **Acph-1**

flies showed degeneration as compared to 55% for **Acph-1**

flies. By 72 h, degeneration neared completion in the **Acph-1**

flies. Thus the absence of acid phosphatase activity does not prevent hereditary retinal degeneration in Drosophila but does seem to delay it by about 24 h.

**Prevention of Degeneration by Light Deprivation**

**BASIC EFFECT** Flies of each of the **rdgA** alleles were raised at 18°C in the dark from the egg until about 5 days postemergence. Controls were raised in constant light at the same temperature. All showed the same amount of degeneration in light or dark.

A different result was obtained with **rdgB**. In this case, flies of all the **rdgB** alleles showed considerably more degeneration when raised in the light. The effect was most pronounced in **rdgB**

flies and **rdgB**

flies, which showed no signs of degeneration in the dark, as judged by the pseudopupil method or in histological sections. Fig. 6 shows an example of 10-day old adult **rdgB**

flies from the same parents, which had been separated as larvae into two groups. The dark-raised group showed very little degeneration after 10 days compared to the light-raised group. Even after 30 days in the dark at 18°C, most **rdgB**

flies showed little or no retinal degeneration.

The ERGs of white-eyed **rdgB**

flies raised in the dark at 18°C were recorded. If these flies were prepared for physiological examination under dim red light, the flash-elicited ERGs looked normal in all respects (Fig. 7). Spectral analysis of the ERG (Fig. 8) showed the high sensitivity two-peaked curve shown by Harris et al. (1976) to be generated by R1-6.

After exposure of these dark-raised mutants to intense stimulation (see below) or brief room light, at 20°C, the ERG waveform was of the R7-8 type (Fig. 7), and showed R7-8 spectral sensitivity (Fig. 8). 3 days later the first anatomical signs of degeneration became evident by pseudopupil examination.
Figure 6. Induction of degeneration in rdgB^{K3222} flies by exposure to light. Flies were kept for 10 days at 18°C in the dark or in the light then examined by the pseudopupil technique. 50 flies each group.

Figure 7. Typical ERG waveforms for cn bw and w rdgB^{K3222}. 470-nm flashes (traces below ERGs) were about $2 \times 10^{13}$ quanta/cm²·s (top) and $5 \times 10^{13}$ quanta/cm²·s (bottom). The obtainability of ERG on and off transients in 570 nm-adapted cn bw and dark-reared w rdgB^{K3222} but not in 470 nm-adapted cn bw and light-reared w rdgB^{K3222}, as well as the higher sensitivity in the former cases is consistent with the idea that the ERGs in the top panels are dominated by photoreceptors R1-6, and those in the bottom panels by photoreceptors R7 and R8. Such waveforms at similar intensities were obtainable after about 1 min of dark adaptation after 570 or 470 nm bright adaptation.
LIGHT-SENSITIVE PERIOD Dark-raised and light-raised (both at 18°C) rdgB<sup>K3222</sup> flies were shifted to the opposite lighting condition at various times during development and adulthood. Shifting from light to dark was effective in preventing degeneration provided it was done before the adult photoreceptors were formed in the late pupal stage. Shifting from dark to light was always effective in inducing degeneration even when it was done in adulthood. This result indicates that it is the adult photoreceptor which is sensitive to light-induced degeneration and not, for example, a precursor cell.

DARK RECOVERY Reversibility of light-induced degeneration was tested by raising rdgB<sup>K3222</sup> flies in the dark at 18°C until about 3 days post-eclosion and then exposing them to one of three light regimes shown in Fig. 9 (series I): (a) 1 day (24 h) in light (in glass food vials 0.5 m from a GE 15 W cool white fluorescent lamp) followed by 7 days in darkness; (b) 8 days in light; or (c) 8 days in darkness. The technique of optical neutralization of the cornea was used to count the number of normal R1–6 photoreceptors. Fig. 9 shows that 8 days in light caused severe degeneration. The number of R1–6 rhabdomeres remaining per ommatidium was 0.9 ± 0.2 (SEM) (n = 50 ommatidia examined—10 each from five flies). 8 days in darkness caused little if any degeneration (5.7 ± 0.1 R1–6/ommatidium). After 1 day in light followed by 7 in darkness there was mild degeneration (4.3 ± 0.1 R1–6/ommatidium). Genetically normal flies after 8 days in the light or the dark showed no degeneration.

In these flies, it is possible that the degeneration had proceeded slightly in 1

![Figure 8](image-url)
day of light and was halted by 7 days of dark, or that there was some recovery in the dark. To distinguish between these possibilities, 3-day old rdgB \textit{Ks222} adults, dark raised at 18°C, were transferred to one of the four light-dark schedules shown in Fig. 9 (series II). It is clear from these results that 4 consecutive days of light caused more retinal degeneration than 4 days of light separated by 3 days of dark. This recovery may occur only in photoreceptor cells that have not yet reached a critical stage in the degeneration process, since the gross histological retinal degeneration in those rdgB \textit{Ks222} flies kept in constant light for 8 or 10 days was not reversed by putting the flies into the dark at 18°C.

\textit{Properties of Mutant Photoreceptors on First Exposure to Light}

\textbf{PHOTOPIGMENT CONVERSIONS} The question arises of whether the light-induced degeneration in rdgB \textit{Ks222} flies is an invertebrate analog of light-sensitive degeneration in mammals deprived of vitamin A. Dowling and Wald (1960) showed that in mammals, vitamin A deficiency caused an inability to regenerate rhodopsin from opsin, and also that the opsin was a structurally less stable protein than rhodopsin. Thus, rod outer segment membranes, which are normally composed mostly of rhodopsin, disintegrate under vitamin A-deprived conditions. In invertebrates, including \textit{Drosophila}, rhodopsin is converted by light of one range of wavelengths into metarhodopsin which is stable at room temperature and is converted back into rhodopsin by light of a second range of wavelengths (Hamdorf et al., 1971; Pak and Liddington, 1974; Ostroy et al., 1974; Harris et al., 1976). In the squid, metarhodopsin is structurally less stable than rhodopsin (Hubbard and St. George, 1958), suggesting that the defect in rdgB \textit{Ks222} might be in the regeneration of rhodopsin from metarhodopsin.
To test this idea, a spectrophotometric analysis was carried out on the visual pigment from white-eyed \textit{rdgB}^{KS222} flies raised at 18°C in the dark. It showed that \textit{rdgB}^{KS222} contained as much rhodopsin as do normal flies (see Harris et al., 1976) and that the photointerconversion of R1-6 rhodopsin and metarhodopsin in dark-raised \textit{rdgB}^{KS222} was normal. That the pigment regenerates properly in vitro does not mean it will do so in vivo, so a second experiment was done. Pak and Liddington (1974) and Grabowski and Pak (personal communication) have characterized two fast potentials in the \textit{Drosophila} eye that are similar in some respects to the vertebrate early receptor potentials (ERPs). In the \textit{Drosophila} case, Pak and Liddington (1974) showed that these are generated by the conversion of metarhodopsin back to rhodopsin, since they have the spectral sensitivity of \textit{Drosophila} R1-6 metarhodopsin and are proportional to the amount of metarhodopsin converted by the flash. For this reason, these potentials are collectively called the M potential (Pak and Liddington, 1974). Fig. 10 shows the M potentials in white-eyed control flies and \textit{rdgB}^{KS222} flies. In 18°C dark-raised \textit{rdgB}^{KS222} flies the M-potential properties were normal and remained so for several hours after the R1-6 receptor potential had vanished, indicating that the rhodopsin-metarhodopsin interconversion was normal in vivo. Only after several days in light, when the R1-6 photoreceptors had completely degenerated, was the M potential no longer obtainable (Fig. 10). Similar results have also been obtained by Grabowski and Pak (personal communication) in the same and another allele of \textit{rdgB}. The normal in vitro and in vivo interconversion of the photopigment does not necessarily mean that everything about the photopigment is normal;
these experiments do not rule out the possibility that some other aspect of photopigment function may be defective in these mutants.

**Prolonged Depolarizing Afterpotential.** When intense 470-nm light, which converts R1–6 rhodopsin to metarhodopsin, is presented to a normal, dark-adapted white-eyed *Drosophila*, the R1–6 cells stay depolarized for up to 6 h (Minke et al., 1975a). This has been called the prolonged depolarizing afterpotential (PDA) and is observed in the ERG as a corneal-negative afterpotential (Minke et al., 1975a). During a maximal PDA, photoreceptor cells R1–6 are not responsive to stimulus flashes of light (Minke et al., 1975a). Exposure to intense 570-nm light immediately resensitizes and repolarizes these receptors, and the R1–6-dominated ERG can once again be observed (see Fig. 7 and Fig. 11a, b).

With white-eyed *rdgB*<sup>ks22</sup> (dark-raised at 18°C) an intense 470 nm flash caused a PDA which lasted only for 30 s to 2 min (Fig. 11f). The ERG is an extracellular measure of current flow, so the final membrane potential of the receptor cells is not known. If an intense 570 nm light was presented to a *w rdgB*<sup>ks22</sup> eye before the PDA current had run down, say after 10 s (see Fig. 11d), then, after the ERG had returned to base line, R1–6 were still capable of responding (see Fig. 11c–f). If the PDA current was allowed to run down without interruption by 570-nm light, the R1–6 cells became completely unresponsive (Fig. 11g). At this point, even intense 570-nm light was incapable of reactivating them.

**Receptor Potential and Degeneration**

**Vitamin A Deprivation.** The intensity of 470 nm adaptation required to produce irreversible loss of R1–6 sensitivity in *w rdgB*<sup>ks22</sup> is the same as for reversible loss (and PDA) in normal white-eyed flies (Fig. 12). This suggested that the PDA-generating mechanism might be defective in the mutant flies. Since vitamin A deprivation has been found to block the PDA and R1–6 inactivation in normal flies (Stark and Zitzmann, 1976) while decreasing sensitivity by about 2.0 log units (Zimmerman and Goldsmith, 1971), *w rdgB*<sup>ks22</sup> flies were vitamin A deprived. These deprived flies, raised at 18°C in the dark and kept for several days as adults before testing, showed R1–6 activity which consistently survived intense stimulation including 24 h of room light (Fig. 14), conditions which eliminated R1–6 activity in vitamin A-enriched controls reared in exactly the same conditions (Fig. 14). The mutant and normal vitamin A-deprived flies showed a nearly identical sensitivity decrease induced by 470 nm adaptation without a PDA (Fig. 13), but in this case the sensitivity loss in both mutant and normal was reversible. This protection caused by vitamin A deprivation, however, did not last indefinitely as judged by ERG recordings and pseudopupil examinations.

**Receptor Potential Deprivation.** Raising *rdgB*<sup>ks22</sup> flies in the dark, eliminating the rhabdomeres (by *ora*<sup>fs64</sup>), and desensitizing the photoreceptors by vitamin A deprivation all protect against degeneration; also, the mutant defect does not appear to be in the rhodopsin-metarhodopsin photoconversions. It was therefore conjectured that the defect might be electrical, i.e. that depolarization was lethal to the mutant photoreceptors.

Mutations of the *norpA* gene can completely block the receptor potential...
FIGURE 11. Responses prolonged afterpotentials in cn bw (a, b) and w rdgB⁸⁵⁰⁷⁸ⁱ⁷⁴⁵ (c, d, e, f, g). (a) Response of cn bw to intense (5.75 × 10¹⁶ quanta/cm²·s) 1-s flash of 470 nm light followed by an equally intense 570 nm 1-s flash. (b) Response to the 470-nm flash alone causing a PDA (see text) which in this case is not terminated by 570 nm light. (c) First response of a dark-reared w rdgB⁸⁵⁰⁷⁸⁴⁵ fly to a dim 3.25 × 10¹¹ quanta/cm²·s 470 nm light. It is essentially normal (like dark-adapted cn bw; Fig. 7). (d) shows the response to a 470 nm followed by a 570-nm flash (same intensity as in a and b). The 470-nm flash elicits a large (though not as large as in cn bw) receptor potential with a slow repolarization after the 570-nm flash. This stimulation sequence does not inactivate R1-6 as assayed by the normal waveform in the subsequent response to 3.25 × 10¹¹ quanta/cm²·s of 470 nm, shown in (e). (f) First response of dark-reared w rdgB⁸⁵⁰⁷⁸⁴⁵ to a single 470 nm flash (same intensity as a, b, and d). The extracellularly recorded PDA current decays to base line in about 30 s. After this stimulus, R1-6 responsivity is lost as judged by reduced sensitivity and loss of ERG transients, shown in (g) (here stimulus intensity was 2 log units greater than in c and e). In at least 10 experiments such as these, 570-nm stimulation was never found to reactivate R1-6 if applied after about a 30-s delay, while it could after a 10-s delay. In these experiments, the PDA current decay took typically 30–100 s.
Figure 12. Sensitivity and adaptation of vitamin A-enriched cn bw and w rdgB^K222. The curves plot the sensitivity of the ERG receptor component as inverse threshold (3.0 mV criterion) in log quantum flux of 470-nm flashes as a function of adaptation at 470 nm (log intensity). Typical adaptation curves for cn bw (top) and dark-reared w rdgB^K222 (bottom) are shown. For cn bw, PDA (1 min poststimulus) is also plotted (dotted line) against the right ordinate. The threshold change and afterpotential for cn bw are reversible by long-wavelength adaptation; the threshold change for w rdgB^K222 is irreversible. The threshold data were obtained between 1 and 2 min subsequent to each bright adaptation conditioning flash.

Figure 13. Sensitivity and adaptation of vitamin A-deprived cn bw and w rdgB^K222. Typical threshold changes as a function of adaptation for vitamin A-deprived cn bw (top) and w rdgB^K222 (bottom) are shown. These threshold changes are considerably less than those of vitamin A-enriched flies due to the fact that in neither deprived case is R1-6 inactivated as assayed by the obtainability of ERG on transients. Furthermore, in both cases the threshold changes are reversible by long-wavelength stimulation. The threshold data were obtained between 1 and 2 min subsequent to each bright adaptation conditioning flash.
(Hotta and Benzer, 1970; Alawi et al., 1972). This block occurs at a step in the transduction process after rhodopsin conversion since photopigment levels in these mutants are large fractions of normal levels (Ostroy et al., 1974) and photopigment properties appear identical to normal (Pak and Liddington, 1974). To test whether blocking the receptor potential would inhibit degeneration, the norpAEE5 mutation which completely blocks the receptor potential (Fig. 15) was genetically combined with rdgBKS222. The double mutants were checked by backcrosses to assure that both mutations were present. As in norpAEE5, the double mutants had normal M-potential properties but no receptor potential (Fig. 15), indicating photoinduced pigment conversions. Preventing the receptor

\[ \text{vit. A deprived} \]
\[ \text{vit. A enriched} \]

**Figure 14.** ERG waveforms of vitamin A-deprived and enriched rdgBKS222 flies which had been raised and aged for 7 days at 18°C in the dark and exposed for 24 h to white light at room temperature immediately before running. Responses in both cases were elicited by 470 nm flashes of \( 3.2 \times 10^{14} \) quanta/cm\(^2\)-s. The transients and larger (top) receptor potential indicate that R1-6 are still functioning in the deprived mutant but not in the enriched.

potential in this way also prevented morphological signs of retinal degeneration (Fig. 16). These norpAEE5 rdgBKS222 flies had normal-looking R1-6 photoreceptors, as judged by electron microscopy, even after 20 days in constant light at 18°C. This result suggests that a receptor potential may be necessary for photoreceptor degeneration to occur.

**DEPRIVATION OF ON AND OFF TRANSIENTS** The norpAEE5 mutation eliminates both the receptor potential and the transient components of the ERG. Therefore, rdgBKS222 was also combined with a mutation that eliminates the on and off transients of the ERG but not the receptor potential component. The mutant JK910 was used for this (Koenig and Merriam, 1975). In the double mutant rdgBKS222, JK910, retinal degeneration proceeded just as rapidly as in the single mutant rdgBKS222. Thus it appears that the receptor potential, not the transients, is important in the retinal degeneration process.
FIGURE 15. ERGs and M potentials of $w$ norpA<sup>EES</sup> and norpA<sup>EES</sup> rdgB<sup>K3222</sup>; cn bw. Stimulation and adaptation as in Fig. 10. In both cases the M potentials could be seen after 470 nm adaptation but were considerably reduced after 570 nm adaptation. The later receptor potential component of the ERG (seen in Fig. 10) is completely absent in these mutants.

FIGURE 16. Prevention of degeneration by norpA<sup>EES</sup>. These results are plotted as in Fig. 6, except in this case both groups were exposed to constant light 18°C for 10 days.

SODIUM POTASSIUM PUMP The ouabain-sensitive $\text{Na}^+-\text{K}^+$ ATPase is involved in photoreceptor repolarization at the cessation of the light stimulus (Brown and Lisman, 1972). One possible hypothesis that might account for the correlation of degeneration with the receptor potential is that this enzyme is
defective in the \textit{rdgB}^{KS222} mutant. Failure of this mechanism might also account for the slow repolarization at the 570 nm-induced termination of the PDA (Fig. 11d). The ATPase level was measured directly by a biochemical assay. It was found that about 60\% of the ATPase activity in the retina of normal white-eyed flies was sensitive to 0.2 mM ouabain. Dark-reared white-eyed \textit{rdgB}^{KS222} mutants showed essentially an identical (within 5\%) amount of total and ouabain-sensitive ATPase activity.

\textbf{Suppressors of Degeneration}

\textbf{SCREENING FOR SUPPRESSORS} To investigate further the relation between the receptor potential and retinal degeneration, mutants were sought that would prevent retinal degeneration in the presence of the \textit{rdgB}^{KS222} mutation. Since one mutant, \textit{norpA}^{EES}, which eliminates the receptor potential suppresses degeneration, one might expect other mutations which eliminated the receptor potential to be among the suppressors. If all suppressors of degeneration in \textit{rdgB}^{KS222} flies were found to have no receptor potential, that would suggest that the receptor potential is both necessary and sufficient for causing degeneration in \textit{rdgB}^{KS222} flies.

Suppressors on the X chromosome were sought by mutagenizing \textit{rdgB}^{KS222} and \textit{rdgA}^{PC47} males and mating them to attached-X females. Male progeny of this cross carry the X chromosome with the \textit{rdg} mutation and any other mutations that the mutagen might have caused. Approximately 1,000 mutagenized \textit{rdgA}^{PC47} and 1,000 \textit{rdgB}^{KS222} flies were checked by pseudopupil examination for retinal degeneration. No suppressors of the \textit{rdgA}^{PC47} were found. Three suppressors of \textit{rdgB}^{KS222} were found, all of which proved to be alleles of the \textit{norpA} gene and were named \textit{norpA}^{suI} etc.

Two of these, \textit{norpA}^{mut} and \textit{norpA}^{null}, gave very small receptor potentials. Like other \textit{norpA} mutants these were recessive. Thus, \textit{norpA}^{mut}\textit{rdgB}^{KS222}+/+ \textit{rdgB}^{KS222} did show a receptor potential and also retinal degeneration. Neither allele complemented \textit{norpA}^{EES}. Thus \textit{norpA}^{null} \textit{rdgB}^{KS222}/\textit{norpA}^{EES} \textit{rdgB}^{KS222} had almost no receptor potential and did not degenerate. The suppression of degeneration caused by these mutants can be understood as a mimicking of \textit{norpA}^{EES}, i.e. preventing the receptor potential and hence preventing degeneration.

\textbf{SUPPRESSOR II} The notion of the receptor potential's being both necessary and sufficient for degeneration was shattered by the third allele, \textit{norpA}^{null}, which suppressed degeneration yet permitted a normal receptor potential (Fig. 17). That is, \textit{norpA}^{null} \textit{rdgB}^{KS222} flies had a normal ERG yet showed no degeneration. This suppressor, like other \textit{norpA} mutants, was recessive. Thus, \textit{norpA}^{null} \textit{rdgB}^{KS222}/+ \textit{rdgB}^{KS222} degenerated. Mapping experiments done with \textit{norpA}^{null} using the suppression of degeneration as a character for scoring recombination placed it at 1-6.3 ± 0.6. Previous maps of other \textit{norpA} mutants by using ERGs placed \textit{norpA} at 1-6.5 ± 0.7 (Pak, 1975). Furthermore, \textit{norpA}^{null} did not complement with \textit{norpA}^{EES}. Thus, \textit{norpA}^{null} \textit{rdgB}^{KS222}/\textit{norpA}^{EES} \textit{rdgB}^{KS222} flies have an ERG but do not degenerate. The presence of an ERG in \textit{norpA}^{null} is dominant to its absence in \textit{norpA}^{EES}. From these results, it is clear that \textit{norpA}^{null} is an allele of \textit{norpA}. 
The norpA\textsuperscript{null} mutation does not suppress degeneration by simply lowering the sensitivity of the photoreceptors. This was shown by genetically separating the norpA\textsuperscript{null} mutant from rdgB\textsuperscript{K3222}, making it white eyed, and testing the responsivity. The intensity of 470-nm light needed to elicit a 3.0 mV response in norpA\textsuperscript{null}; cn bw (log quantum flux = 10.56 ± 0.37 SD, n = 4) was identical to that for normal white-eyed flies (10.65 ± 0.25, n = 8). Furthermore, the waveform, the maximal flash-induced ERG receptor waves (about 25 mV), the intensity-response functions, and the PDA properties were normal (Fig. 18). The suppression of degeneration was not perfect, however; 15 days' exposure to room light and temperature caused some degeneration in about 15% of 60 norpA\textsuperscript{null} rdgB\textsuperscript{K3222} adults examined, while in rdgB\textsuperscript{K3222} control flies under identical conditions there was degeneration in 100% of the flies. The existence of a suppressor of retinal degeneration with a normal receptor potential shows that the receptor potential, while perhaps necessary, is certainly not sufficient for retinal degeneration to occur.

**THE INTERACTION OF norpA AND rdgB** When a mutational change in one protein is compensated with restoration of function by a mutational alteration in a second, interaction between these two proteins can usually be inferred (e.g., Wood and Bishop, 1973). This raises the possibility that the gene product of the normal rdgB gene [call it gp(rdgB\textsuperscript{+})], interacts with the gene product of the normal norpA gene [gp(norpA\textsuperscript{+})], and that the defect in gp(rdgB\textsuperscript{K3222}) is counteracted by the defect in gp(norpA\textsuperscript{null}). In other words, gp(norpA\textsuperscript{null}) is specifically
FIGURE 18. Left side shows typical responsivity of norpA<sup>null;</sup> cn <i>bw;</i> right side of <i>cn bw</i> controls. The top figure shows intensity-response functions for the ERG negative (receptor) potential elicited by 1-s 470-nm flashes from a 570-nm then dark-adapted condition. They are calculated for 0.5, 1, 3, 6, 10, 15, and near-maximal 22 mV with standard errors between preparations shown (for norpA<sup>null;</sup> cn <i>bw;</i> <i>n</i> = 3; for <i>cn bw</i> <i>n</i> = 6). Typical 1-s flash-elicited ERGs are shown for both strains for a 1.5 mV receptor potential (elicited by almost 10<sup>10</sup> quanta/cm<sup>2</sup>-s of 470-nm light, first pair of traces with stimulus monitor below and 4 mV positive calibration) and for a 7 mV receptor potential (elicited by about 10<sup>11</sup> quanta/cm<sup>2</sup>-s, second pair of traces with 10 mV calibration). At the bottom are responses to intense (about 5.75 × 10<sup>18</sup> quanta/cm<sup>2</sup>-s<sup>2</sup>-s) 2-s stimuli in the sequence 470, 470, 570, 570 nm to show the afterpotential properties in the two strains. Within limits of experimental variability, responsivity in norpA<sup>null;</sup> cn <i>bw</i> and <i>cn bw</i> are the same.
tailored to interact with \( gp(\text{rdgB}^{\text{ES22}}) \). If this were so then one might expect the \( \text{norpa}^{\text{null}} \) mutation to be allele specific, i.e. it might not suppress the degeneration caused by other \( \text{rdgB} \) mutations. On the other hand, one would not expect \( \text{norpa}^{\text{EE5}} \) suppression to be allele specific since there is no restoration of function in \( \text{norpa}^{\text{EE5}} \text{rdgB}^{\text{ES22}} \) double mutants, i.e. since \( \text{norpa}^{\text{EE5}} \) completely prevents the receptor potential it should suppress the degeneration in all \( \text{rdgB} \) mutants. The allele \( \text{rdgB}^{\text{RO45}} \), though physiologically similar to \( \text{rdgB}^{\text{ES22}} \), was induced by a separate mutational event. To test the action of \( \text{norpa}^{\text{null}} \) and \( \text{norpa}^{\text{EE5}} \) on this allele the appropriate double mutants were constructed and checked for degeneration by pseudopupil examination. \( \text{norpa}^{\text{null}} \text{rdgB}^{\text{RO45}} \) double mutants did show retinal degeneration which proceeded at the normal rate, while \( \text{norpa}^{\text{EE5}} \text{rdgB}^{\text{RO45}} \) double mutants did not. Thus, \( \text{norpa}^{\text{null}} \) suppression is indeed allele specific while \( \text{norpa}^{\text{EE5}} \) is not.

**DISCUSSION**

**Anatomical Localization of the Defect**

In order to understand the mechanism of hereditary retinal degeneration it is important to identify the tissue primarily responsible for the defect. By making mosaic individuals, part normal and part mutant, it is possible to determine the primary focus of the defect, i.e., the tissue that must be mutant in order for the mutant property to appear. Such mosaic analysis has been used in mouse hereditary retinal degeneration caused by the \( \text{rd} \) mutation to show that the photoreceptor cells themselves are probably responsible for the defect (LaVail and Mullen, 1974). In rat retinal degeneration, however, the pigment epithelium has been implicated (Herron et al., 1969; Bok and Hall, 1969, 1971) and shown by mosaic analysis to be the primary focus of the defect (Mullen and LaVail, 1976). Genetic mosaics in humans caused by random inactivation of the X chromosomes in females heterozygous for sex-linked mutations (Lyon, 1961) revealed that some cases of hereditary retinal degeneration are autonomous to the retina (Goodman et al., 1965; Berson et al., 1969). Another type of hereditary retinal degeneration in humans is caused by a defect in absorption of vitamin A in the intestine (Gouras et al., 1971).

In Drosophila various techniques are available for making mosaics (Hall et al., 1976). Hotta and Benzer (1970) used mosaics to show that the \( \text{rdgA} \) and \( \text{rdgB} \) defects are autonomous to the eye. In this study, histological examination of mosaic retinas shows that it is the photoreceptors which are defective. Furthermore, a mutant, \( \text{ora}^{\text{R64}} \), which blocks the formation of rhabdomeres in the outer photoreceptor cells but still allows retinal degeneration (at high temperature) in \( \text{rdgB} \) flies, shows that the defect is not restricted to the rhabdomeres and must be present in the cell body.

**Physiological Localization of the Defect**

Given that the photoreceptor cells are responsible for their own degeneration, what is wrong with them? The \( \text{rdgB} \) mutants are conditional in that retinal degeneration is light sensitive. By turning light on and off at various times in the life of an \( \text{rdgB} \) mutant, it is possible to show that the photoreceptor is light
sensitive only when fully differentiated. This does not necessarily mean that
the defect first appears only in the adult. The immature photoreceptor cell
could, for instance, already be defective in the uptake of some substance that is
necessary for the adult photoreceptor's response to light.

Several studies with light deprivation and vitamin A deprivation in rodents
have suggested that defective photopigment metabolism may be important in
leading to degeneration (Dowling and Sidman, 1962; Herron et al., 1969; Bok
and Hall, 1971; Noell et al., 1971; Noell and Albrecht, 1971; LaVail et al., 1972;
Yates et al., 1974; LaVail and Battelle, 1975). Similar experiments, described
here, on the rdgB mutants of Drosophila also suggest that the photopigment
metabolism may be defective in these mutants. However, direct studies of the
photointerconversion of rhodopsin and metarhodopsin showed that, both in
vivo and in vitro, there are normal conversions of the photopigment. This
suggests that the defect is expressed at a step in the transduction process
subsequent to photopigment conversion.

Conversion of a net amount of rhodopsin to metarhodopsin induces a pro-
longed depolarizing afterpotential (PDA) (Hochstein et al., 1973; Minke et al.,
1973), which lasts up to 6 h in normal Drosophila (Minke et al., 1975a). The
duration is less than 2 min in rdgB mutants on their first exposure to 470-nm
light, after which the photoreceptors become permanently inactive. Vitamin A
deprivation prevents the PDA in normal Drosophila (Stark and Zitzman, 1976)
and delays degeneration in rdgB mutants. The intensity of 470-nm light needed
to cause a PDA approximates the intensity needed to produce long-term damage
to rdgB photoreceptors. These results suggest that long-lasting depolarization
of the photoreceptors is causally related to the degeneration in these mutants. This
idea was confirmed by depriving the photoreceptors of depolarization by use of
the norpA^{re} mutation. The norpA mutants have normal photopigment metabo-
lism but are defective in the generating mechanism for the receptor potential
(Pak, 1975). The norpA^{re} mutation results in no receptor potential and prevents
rdgB photoreceptors from degenerating. Thus, the rdgB defect was shown to act
during or subsequent to the action of the norpA gene product. The finding of a
suppressor of degeneration with normal receptor potential, norpA^{+}, demon-
strated that the degeneration process is not consequent to the receptor potential.
Thus, the rdgB defect is associated with a step in the phototransduction process
of the adult photoreceptor which begins after the photopigment action, is after
or during the norpA^{+} gene product action, and is not consequent on the receptor
potential.

Model of Drosophila Photoreceptor Degeneration

We propose the following scheme for degeneration in Drosophila rdgB mutants.
Each absorbed photon converts one rhodopsin to metarhodopsin and, as a
result, one or more molecules of gp(norpA^{+}), the gene product of the normal
norpA gene, is either directly or indirectly activated. This activated gp(norpA^{+})
which may be an enzyme, an internal transmitter, a channel, etc., is somehow
involved in the eventual generation of a receptor potential. In nonmutant flies
circulating gp(rdgB^{+}), the gene product of the normal rdgB gene, terminates the
action of gp(norpA^{+}) by direct interaction with it. In rdgB mutants, however, the
defective gp(rdgB<sup>K5222</sup>) is incapable of terminating the action of gp(norpA<sup>+</sup>), and this abnormal state of affairs leads somehow to cell death.

This model explains the results of this paper. In the dark, gp(norpA<sup>+</sup>) does not become activated and thus does not have to be inactivated, so degeneration is prevented. Vitamin A deprivation in flies reduces the amount of rhodopsin (Razmjoo and Hamdorf, 1976). This would lead to a reduction in the amount of activated gp(norpA<sup>+</sup>). This should delay the onset of degeneration, as observed. In mutants such as norpA<sup>EE5</sup>, there is no receptor potential because gp(norpA<sup>EE5</sup>) is absent or nonfunctional. Therefore, it does not need to be inactivated for the cell to be protected against damage. In the norpA<sup>null</sup> mutant, which was selected for suppression of degeneration in the presence of the rdgB<sup>K5222</sup> mutation, a modified gp(norpA) molecule is produced so that it can act in the usual way to produce a receptor potential. The modified form of the molecule, however, is such that it can be inactivated by gp(rdgB<sup>K5222</sup>) so that there is no degeneration.

The genetic evidence so far obtained can be considered in light of this model. The rdgB defect is recessive; this would be expected if, in rdgB/<sup>+</sup> heterozygotes, there is half the normal level of gp(rdgB<sup>-</sup>) and that is still sufficient to inactivate all the gp(norpA<sup>+</sup>). The suppression of degeneration in norpA<sup>EE5</sup> and norpA<sup>null</sup> is recessive since in norpA<sup>EE5</sup>/<sup>+</sup> and norpA<sup>null</sup>/<sup>+</sup> heterozygotes there is still half the normal level of gp(norpA<sup>-</sup>) which cannot be properly inactivated by any amount of mutant gp(rdgB<sup>K5222</sup>). The norpA<sup>null</sup> suppression of degeneration is allele specific whereas the norpA<sup>EE5</sup> suppression is not because gp(norpA<sup>EE5</sup>) has been specifically modified to be inactivated by gp(rdgB<sup>K5222</sup>) while gp(norpA<sup>EE5</sup>) is simply inactive; it cannot produce a receptor potential and therefore does not have to be inactivated. The model predicts suppression of degeneration in norpA<sup>null</sup> rdgB<sup>K5222</sup>/norpA<sup>null</sup> rdgB<sup>K045</sup> heterozygotes since in this case while gp(rdgB<sup>K045</sup>) cannot inactivate any gp(norpA<sup>null</sup>), gp(rdgB<sup>K5222</sup>) can inactivate it all. The model also predicts no suppression in norpA<sup>EE5</sup> rdgB<sup>K045</sup>/norpA<sup>null</sup> rdgB<sup>K045</sup> heterozygotes because even though gp(norpA<sup>EE5</sup>) is inactive, gp(norpA<sup>null</sup>) can generate a receptor potential and its action cannot be terminated by gp(rdgB<sup>K045</sup>). These heterozygotic combinations were constructed and found to conform to prediction.

Two of the results presented in this paper may, at first, appear contradictory to the model proposed. The first is that the time needed to irreversibly damage the photoreceptors of the rdgB<sup>K5222</sup> mutant with a bright flash is on the order of tens of seconds (Fig. 11f, g), whereas the visual excitation process takes only a few milliseconds (see, for example, Fig. 10). Thus, one might argue that this rdgB<sup>K5222</sup> phenomenon is much too slow to be involved in the excitation mechanism. The role proposed for gp(rdgB<sup>-</sup>), however, is one of de-excitation rather than excitation. According to the model, the rdgB mutations should, therefore, have no effect on the initial rise time of the receptor potential. It may be fairer to propose, then, that gp(rdgB<sup>-</sup>) is involved in the adaptation rather than transduction phenomena in the broadly defined processes of excitation. The second apparently troublesome result is that degeneration can proceed in the absence of rhabdomeres, i.e. in the rdgB<sup>K5222</sup>; ora<sup>K045</sup> double mutant (Fig. 5). There is good evidence in flies that the rhabdomere contains the visual pigment (Langer and Thorell, 1966; Stavenga et al., 1973) and that the photoreceptor current in some
invertebrates flows through the rhabdomeric membrane or closely associated membranes (Hagins et al., 1962; Lasansky and Fuortes, 1969). Thus, it might seem unexpected that a defect in the transduction process should be expressed in the absence of so much transduction machinery. That there can be degeneration in \( \text{rdgB}^{K222} \) mutants at high temperature without rhabdomeres argues that there may be some cytoplasmically located intermediates in the phototransduction process such as proposed by Cone (1973). For instance, if \( \text{gp(norpA}^+ \) and \( \text{gp(rdgB}^+ \) are cytoplasmic, and if \( \text{gp(norpA}^+ \) can be thermally activated, one might expect to see degeneration at high temperature in the \( \text{rdgB}^{K222}; \text{ora}^{K84} \) double mutant. It is important to recall, however, that the degeneration seen in these double mutants proceeds only slowly and only at high temperature. Eliminating the rhabdomeres does have a substantial saving effect on the photoreceptors, approximately equivalent to dark-rearing. This kind of protection is just what the model predicts.

Alawi et al. (1972), Pak and Liddington (1974), and Ostroy et al. (1974) have shown that the \( \text{norpA} \) mutants are defective in a step in the transduction process between quantum catch and receptor depolarization. Minke et al. (1975b) have shown that \( \text{trp} \), another \( \text{Drosophila} \) mutant, which leads to a transient receptor potential, is also defective in an intermediate step in phototransduction. In this study we have presented evidence suggesting that the \( \text{rdgB} \) gene also codes for a step in the transduction process. The evidence for direct interaction between the products of the \( \text{norpA} \) and the \( \text{rdgB} \) genes, while based solely on genetic evidence, engenders the hope that the further study of interactions among these and other \( \text{Drosophila} \) visual mutants at genetic, physiological, and biochemical levels will yield a complete stepwise description of the phototransduction process.

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