J. Physiol. (1978), **278**, *pp.* 161–175
With 7 text-figures
Printed in Great Britain

DARK-ADAPTATION IN ABNORMAL (RCS) RATS STUDIED ELECTRORETINOGRAPHICALLY

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(*Received 19 April 1977*)

SUMMARY

1. Electroretinogram (e.r.g.) responses recorded from dark-reared rats with inherited retinal dystrophy (RCS) showed progressive decline in *b*-wave amplitude and prolongation of the time to the peak of the *b*-wave with age when compared with records obtained from dark-reared normal albino rats.

2. Dark-adaptation was followed in RCS and normal rats by recording the light intensity needed to evoke a criterion e.r.g. response at different time intervals after bleaching about 90% of the rhodopsin.

3. In normal rats, dark-adaptation was governed by two mechanisms. The first 25–35 min of recovery was determined by cones. The second branch, determined by the recovery of rods, lasted for about 3 hr and proceeded along an exponential time course with time constant of $41{\cdot}4 \pm 2{\cdot}4$ min (S.E. of mean).

4. In RCS rats, the time course of the dark-adaptation after a 90% bleach depended on age. In 25–30 day old rats the recovery curve had at least three breaks separating three different mechanisms. Rats, 35–40 days old, exhibited double exponential recovery curves, while 45–70 day old rats recovered along a single exponential curve similar in time course to the cone branch of dark-adaptation found in normal rats.

5. Action spectra obtained from RCS rats at different time intervals of the recovery curve showed that in young rats, 25–30 days old, small e.r.g. responses recorded before bleaching and at the end of the recovery period were determined by rhodopsin while those recorded during the first part of the recovery from 90% bleach were determined by a combination of rods and cones. In RCS rats of advanced age (45–70 days old), rhodopsin was the major contributor to the e.r.g. responses recorded either before bleaching or at the end of the recovery period.

6. The gradual deterioration with age of the e.r.g. in RCS rats cannot be explained by either the decrease in quantum catch due to the decrease in rhodopsin content or by the linear relationship between log e.r.g. threshold and pigment concentration.

7. Using estimates of rhodopsin density within surviving rods obtained from retinal densitometry, it was shown that in RCS rats where more than 30% of normal levels of rhodopsin was located within the functioning rods, the log intensity needed

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for a criterion e.r.g. response measured at the end of the recovery period from a 90% bleach was linearly related to the fraction of 'functional' rhodopsin.

8. No simple relationship between log e.r.g. threshold and rhodopsin concentration could be found during the course of recovery in the dark from a strong bleaching exposure in RCS rats of all ages.

INTRODUCTION

Dowling (1960), measuring the threshold electroretinographically in the rat, and Rushton (1961), measuring psychophysical threshold in a human subject, found a linear relationship between log threshold and the fraction of unregenerated rhodopsin.

$$\log \frac{I(t)}{I(\infty)} = \alpha[1-p(t)], \tag{1}$$

where $I(t)$ and $I(\infty)$ are the light intensities required for a constant effect at time t after the bleach and at the fully dark-adapted state respectively, $p(t)$ is the fraction of pigment present and α is an empirical constant. Though eqn. (1) has been confirmed in a number of other preparations (Alpern, 1971; Dowling & Ripps, 1970; Hollins & Alpern, 1973; Perlman, 1978), it has been shown to have a quite limited applicability (Pugh, 1972).

In a previous work (Perlman, 1978) the kinetics of rhodopsin *in vivo* in normal and abnormal (RCS) rats was described. It was found that only a fraction of the initial rhodopsin could regenerate in the RCS rats. The fraction of the 'regenerative' rhodopsin declined with age of the dystrophic rats in parallel to the previously described morphological changes and e.r.g. deterioration (Dowling & Sidman, 1962; Noell, 1963). However, the rate of rhodopsin regeneration was faster and its photosensitivity was higher in the RCS rats than in the normal ones.

In this study e.r.g.s were recorded from normal and RCS rats before and after exposure to light that significantly bleached rhodopsin. It was found that in RCS rats the e.r.g. dark-adaptation curves when compared to rhodopsin regeneration curves did not satisfy eqn. (1). However, the log light intensity needed to evoke a constant e.r.g. response measured at the end of the recovery period depended linearly on the estimated fraction of rhodopsin that was assumed to be present inside functioning rods in the dystrophic retina. It was further concluded that the cones were more resistant than rods to the degeneration process and that the cones were probably mediating vision in advanced stages of the dystrophic eye.

METHODS

All the animals used in this study were raised in darkness. A normal or RCS rat was anaesthetized with sodium pentobarbitone and mounted on its side on a heating pad. The eyeball was maximally exposed by drawing back the eyelids with sutures in a way that prevented increases in intraocular pressure. After aligning the rat under the image of the test light it was left for at least one half hour in the dark to recover from any light adaptation and to let rectal temperature recover.

The electroretinogram was recorded between a cotton wick electrode (with saline solution leading to a chlorided silver wire) placed on the cornea and an indifferent electrode made of chlorided silver wire and inserted in a little cut in the cheek. The signal was amplified by a preamplifier with time constant of 1 sec whose output was displayed and photographed on a DC coupled oscilloscope.

The light stimulation apparatus consisted of two channels. The light source for the bleaching channel was a tungsten microscope lamp (6V, 18A) in a light-tight housing. The test source was a xenon flash tube (General Radio 1532-B strobolume). The spectral distribution of the test flash was calculated from the output of a photodiode with known spectral responsivity in response to a test flash stimulus attenuated by one of twenty different narrowband Baird Atomic interference filters transmitting maximally at wave-lengths spanning the entire visible spectrum (400–700 nm). The results of these measurements were in agreement with those reported by Pugh (1975). The two beams were focused by the optical system to form a uniform image 3 cm in diameter, covering a section of a ping-pong ball placed on the rat's eye. The two channels could not be used simultaneously but were interchangeable with the use of a silvered mirror placed on a revolving holder.

Throughout the experiments the criterion of a threshold response was 30 μV, just large enough to be easily distinguished from the noise. Dark-adaptation curves were constructed from the light intensity needed to evoke the criterion e.r.g. response at different time intervals following significant photopigment bleaching. The curves thus obtained were fitted, when possible, to a single exponential time course.

The bleaching strength of the bleaching light was estimated according to Cone (1963). E.r.g. responses to 20 msec flashes of different intensities (obtained by attenuating the light with neutral filters) were recorded from normal rats. The relationship obtained between the *b*-wave amplitudes and the log relative flash intensity was compared with a similar one relating *b*-wave amplitude to log absolute intensity (Cone, 1963, Fig. 1). The intensity of the unattenuated bleaching light in equivalent quanta of 502 nm absorbed per rod per flash was obtained by displacing the two curves along the abscissa until best fit was achieved at small responses (first 2 log units above threshold). The fraction of rhodopsin bleached by 5 min exposure of the normal rat to the bleaching light was then estimated from a curve describing the bleaching of rhodopsin by 5 min exposure to light, the intensity of which was given in quanta of 502 nm absorbed per rod per second (Cone, 1963, Fig. 2).

RESULTS

I. *Dark-adaptation curves*

Fig. 1 shows representative e.r.g. responses to test flashes of increasing intensities obtained from normal rat (30 days old) and RCS rats (25, 45, 60 and 90 days old). The vertical bar to the left of each response marks the onset of the flash and is of 100 μV height. The horizontal bars at the bottom of the figure are of 50 msec length. The deterioration of the dystrophic retina is evident; as age increases so does the light intensity needed for a threshold response while the maximal amplitude obtained with the brightest flash declined. The temporal characteristics of the e.r.g. also changed with age. The *b*-wave latency (time to peak) was prolonged at all intensities and the multiple splitting of the *b*-wave became pronounced especially at bright stimuli.

In all the rats studied (RCS and normal) except in RCS rats with advanced retinal degeneration (older than 70 days) a 30 μV response was well within the range where the *b*-wave amplitude was linearly dependent on the log intensity of the light stimulus. Therefore, the form of any particular relationship of the log intensity needed to evoke a 30 μV response was not dependent upon the response criterion chosen. However, the temporal characteristics of small e.r.g. responses (30 μV) differed under different conditions. In dark-adapted RCS rats the time to peak was longer than in their normal counterparts. After exposure to the bleaching light the small e.r.g. responses in both RCS and normal rats had very short time to peak which lengthened as dark-adaptation proceeded to approach the dark-adapted level.

Despite these differences in the wave form of the threshold e.r.g. it was chosen, as has been commonly done before (Dowling, 1960; Cone, 1963), to characterize the e.r.g. 'sensitivity' by the log intensity needed to evoke a 30 μV response amplitude (log I_{30}). In Fig. 2 the dependence of the prebleach dark-adapted e.r.g. sensitivity (log I_{30}) on age is shown for normal (squares) and RCS (circles) rats. In the normal rats there was a slight increase in sensitivity up to age 25 days. Thereafter the log I_{30} remained relatively constant (at least up to age 70 days). In RCS rats, on the other hand, sensitivity gradually declined with age in agreement with previous studies (Dowling & Sidman, 1962; Noell, 1963). The rats used in this study were raised in darkness from birth, therefore, the log I_{30} measured for 70 days old RCS rats was

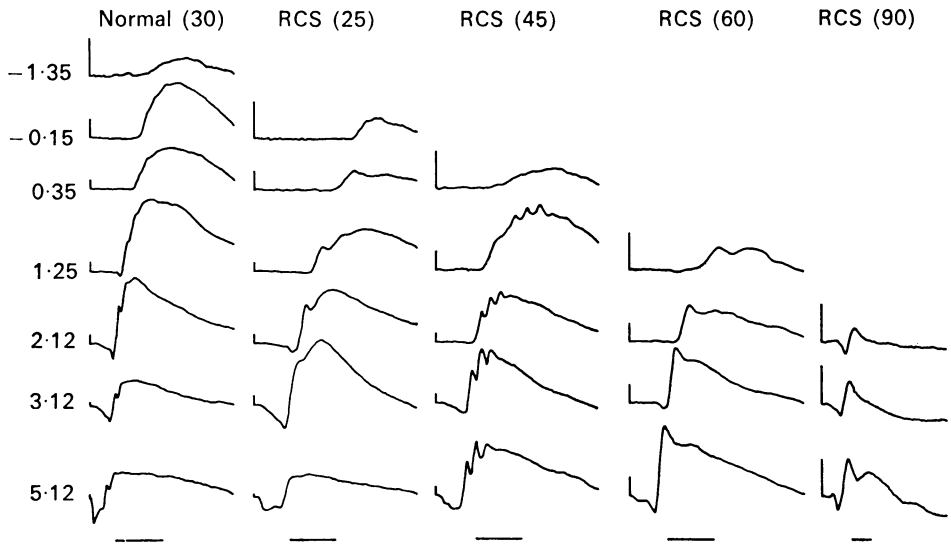

Fig. 1. Representative electroretinogram responses obtained from 30 day old dark-reared normal rat and dark-reared RCS rats 25, 45, 60 and 90 days old. The intensities of the flashes used are given in log quanta (502 mm) absorbed per rod per flash in the normal eye on the left of the responses. (Absolute intensity determination is described in the Methods section.) The vertical bars mark the flash artifact and are of 100 μV height. The time calibration is given by the horizontal bars at the bottom of the figure and are of 50 msec length.

elevated by only 3 log units above normal. No e.r.g. response could be recorded from RCS rats of the same age that were raised in cyclic light–dark environment (Dowling & Sidman, 1962).

After e.r.g. responses had been recorded from the dark-adapted rat, the eye was exposed for 5 min to the bleaching beam. This exposure was estimated to bleach about 90 % of rhodopsin in the normal rat. The rat was then left in the dark and the recovery of the e.r.g. sensitivity was followed. Representative dark-adaptation curves are shown in Fig. 3. The big symbols at the extreme right of this figure show the prebleached dark-adapted log I_{30} for each rat. Normal adult rats (older than 25 days) exhibited recovery curves similar to the one in Fig. 3 (open squares). The curve is composed of two branches. The first branch was relatively fast, lasting for the

first 25–40 min in the dark and covering about 0·5 log units of recovery. The second branch followed an exponential time course with mean time constant of 41·4 ± 2·4 min (S.E. of mean) obtained from nine rats. Within 3–4 hr after the bleach, recovery was complete and log I_{30} reached its dark-adapted level.

The time course of dark-adaptation after the same bleaching exposure in RCS rats depended on the age of the animal. In rats, 25–30 days old, three branches could be detected in the dark-adaptation curve (Fig. 3, filled circles). The first branch reflected

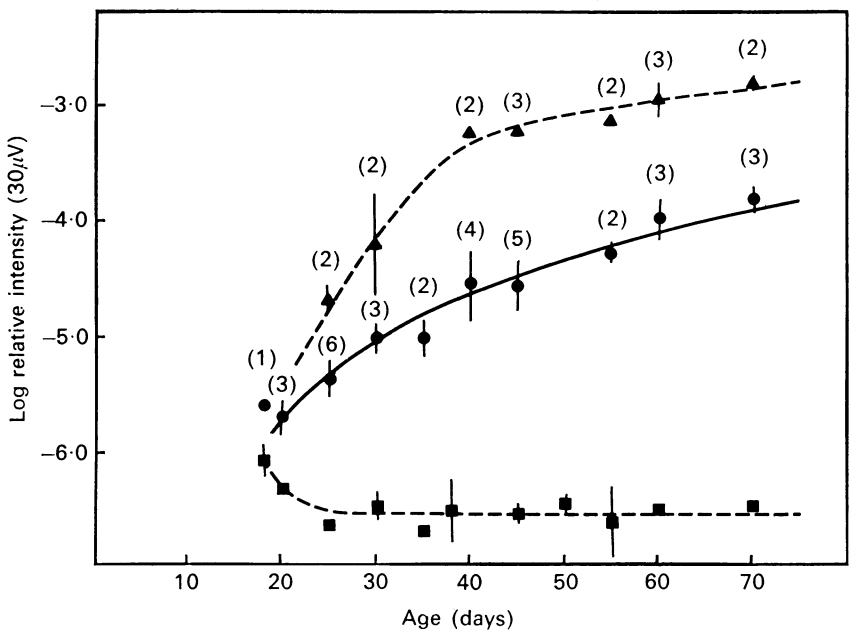

Fig. 2. Dependence of log light intensity needed to evoke a 30 μV response on age of dark-reared normal and RCS rats. Squares and circles describe the relative flash intensity needed to evoke a *b*-wave criterion from dark-adapted normal and RCS rats respectively before bleaching. The triangles represent e.r.g. sensitivity measurements in RCS rats after 5 hr of dark-adaptation following significant bleaching exposure. The dashed and continuous curves were drawn by eye through the data points. Number of rats used for each point are indicated in parentheses.

fast recovery similar to the first branch of the normal curve. The second branch could be described by an exponential time course with time constant of about 32 min while the third branch was very slow with time constant of about 65 min. This slowest phase of recovery was absent when dark-adaptation was followed in RCS rats 35–40 days old (Fig. 3, filled squares). RCS rats, 45 days and older, exhibited a fast single exponential dark-adaptation curve (Fig. 3, open circles). The mean time constant in ten rats was 8·9 ± 1·9 min (S.E. of mean).

The dark-adaptation curves obtained from RCS rats of all ages showed an initial fast recovery that lasted for about 25–40 min. The log intensity needed for a 30 μV e.r.g. measured at the 'end' of the fast recovery in RCS rats 25–70 days old (change in slope or plateau in the recovery curves) was elevated above normal by less than 0·5 log units while log I_{30} measured 5 hr after termination of the bleaching light could

be elevated above normal by as much as 3·8 log units (in RCS rat 70 days old). It is therefore concluded that the mechanism responsible for the fast phase of dark-adaptation is considerably less affected by the degeneration process in the dystrophic retina than the mechanism responsible for the slow phase of the recovery curve.

A common feature found in the dark-adaptation curves of RCS rats of all ages was that even after 5 hr in the dark e.r.g. sensitivity was below the prebleach level. The e.r.g. sensitivity (log I_{30}) at the 'end' of dark-adaptation in RCS rats was derived by fitting an exponential time course to the last branch of the measured recovery. The mean ± 2 S.E. of mean of these values are shown in Fig. 2 (triangles).

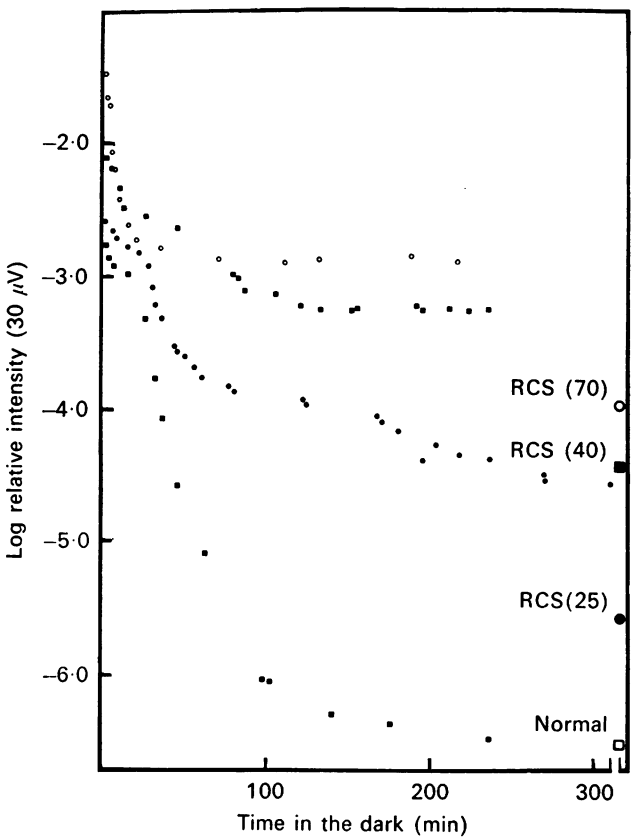

Fig. 3. E.r.g. dark-adaptation after 90% rhodopsin bleach in adult normal rat (open squares) and RCS rats 25 days old (filled circles), 40 days old (filled squares) and 70 days old (open circles). The big symbols on the extreme right indicate the level of log I_{30} measured before each rat was exposed to the bleaching light.

In normal rats two mechanisms are responsible for the recovery of e.r.g. sensitivity (log I_{30}) after a strong bleach (Fig. 3, open squares). Dark-adaptation after a full bleach was followed with two coloured test flashes (blue and red). The dark-adaptation curves revealed that the mechanism responsible for the first fast recovery was more sensitive at long wave-length than expected from the rod action spectrum (personal experience; Dodt & Echte, 1961). It has already been shown that under

photopic conditions, the action spectrum measured electroretinographically (Green, 1971, 1973) and the behavioural spectral sensitivity curves (Birch & Jacobs, 1974) could not be fitted by a rhodopsin absorption spectrum due to higher than expected sensitivity in the long wave-length region of the spectrum. Therefore a cone mechanism has been postulated to contribute to the e.r.g. threshold responses under photopic conditions and during the first 25–40 min of recovery from strong bleaching exposures.

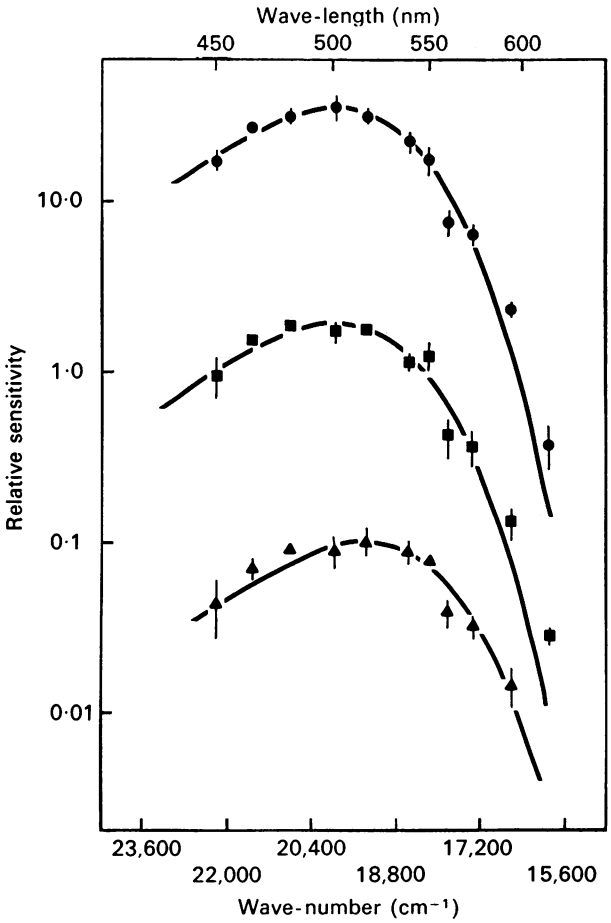

Fig. 4. Averaged action spectra for five RCS rats, 25–30 days old, obtained before bleaching the eyes (circles), during the first 10–20 min of recovery from the bleach (triangles) and after staying for 5 hr in the dark (squares). The three sets of data points, shifted vertically for clarity, were fitted by absorption spectra calculated from a Dartnall nomogram (continuous curves). The circles and squares (obtained before bleaching and at the end of recovery) fit a 501 nm pigment while the triangles fit best a pigment with $\lambda_{max} = 515$ nm.

Dark-adaptation curves of RCS rats exhibited abnormal time courses. The curve can be composed of either three, two or one branches depending on the age of the animal. In order to characterize the mechanisms responsible for the e.r.g. dark-adaptation, action spectra were measured at different times during the recovery process by inserting various narrow band interference filters in the test beam and

recording the intensity needed for a 30 μV response. Fig. 4 shows the averaged action spectra for five RCS rats, 25–30 days old, obtained before bleaching (circles), at the beginning of recovery (10–20 min in the dark; triangles) and at the 'end' of dark-adaptation (squares). The three action spectra, shifted vertically for clarity, were fitted with spectra calculated from the Dartnall nomogram (Wyszecki & Stiles, 1967). The spectra obtained from the dark-adapted rat and 5–6 hr after termination of the bleaching exposure (circles and squares, respectively) fit a pigment with λ_{max} = 501 nm, indicating a rod mechanism. However, the data points representing the e.r.g. sensitivity during the first 10–20 min after termination of the bleaching light (triangles) fit best a nomogram with λ_{max} = 515 nm. This action spectrum is presumably the result of the contributions of cones to the e.r.g.; these results confirm previous findings (Green 1971, 1973) on the existence of a photopic mechanism in the normal rat.

In RCS 35–70 days old action spectra implied rod dominance in both the prebleach state and after 5–6 hr in the dark following a significant bleaching exposure. The time course of dark-adaptation in RCS rats 45–70 days old (Fig. 3, open circles) is very fast, similar to the cone branch of normal rats. This suggests that in RCS rats of advanced age dark-adaptation after significant bleaching may be determined by either cone-like photoreceptors that contain a rhodopsin-like photopigment or by rod-like photoreceptors that contain a cone-like photopigment. These two possibilities depend on whether the fast cone dark-adaptation is due to the features of the cone photoreceptor or to the characteristics of the cone pigment.

II. *The relation between rhodopsin concentration and e.r.g. sensitivity in RCS rats*

It is evident from Fig. 2 that sensitivity to light, as expressed by the e.r.g., is declining with age in RCS rats, confirming previous studies (Dowling & Sidman, 1962; Noell, 1963). Does the decrease in sensitivity reflect the decline in rhodopsin content of functioning and surviving rods in the dystrophic eye? The assumptions that (*a*) total rhodopsin content of the eye is the same in RCS and normal rats (Perlman, 1976, 1978), (*b*) all rhodopsin located within functioning rods in the RCS retina can regenerate after a full bleach (Delmelle, Noell & Organisciak, 1975) and (*c*) RCS and normal rods contain the same amount of rhodopsin and respond similarly to each quantum caught, lead to the prediction that the elevation of log intensity needed for a 30 μV response of dark-adapted RCS rats above the normal level will be linearly related to the fraction of total rhodopsin that can regenerate after a full bleach. The relationship between the elevation of log I_{30} measured in dark-adapted RCS rats above that in normal rats (from Fig. 2) and the fraction of the 'regenerative' rhodopsin (Perlman, 1978, Fig. 9) is shown in Fig. 5. The dashed curve describes the expected relationship if the only effect of the decrease in rhodopsin is to decrease the probability of catching quanta. The solid line describes the linear relationship between e.r.g. sensitivity defined by log I_{30} and pigment concentration (eqn. 1) found in normal rats (Perlman, 1978, Fig. 3).

If the retinal degeneration in the dystrophic eye involves loss of pigment from relatively intact rods as in bleaching experiments or vitamin A deficiency then the data points should follow the continuous line (Dowling, 1960). On the other hand if the degeneration process is expressed in the shortening of the rods, due to loss of

pieces of outer segments, with no change in the concentration of rhodopsin within the rods then I_{30} might depend linearly on rhodopsin levels (dashed curve) as was found in *Xenopus* tadpoles during development by Witkovsky, Gallin, Holleyfield, Ripps & Bridges (1976). The data points do not fit either the dashed or the continuous curves. It is possible that the degeneration process involves both loss of pieces of outer segments as well as some decrease in the concentration of rhodopsin in the remaining intact rod.

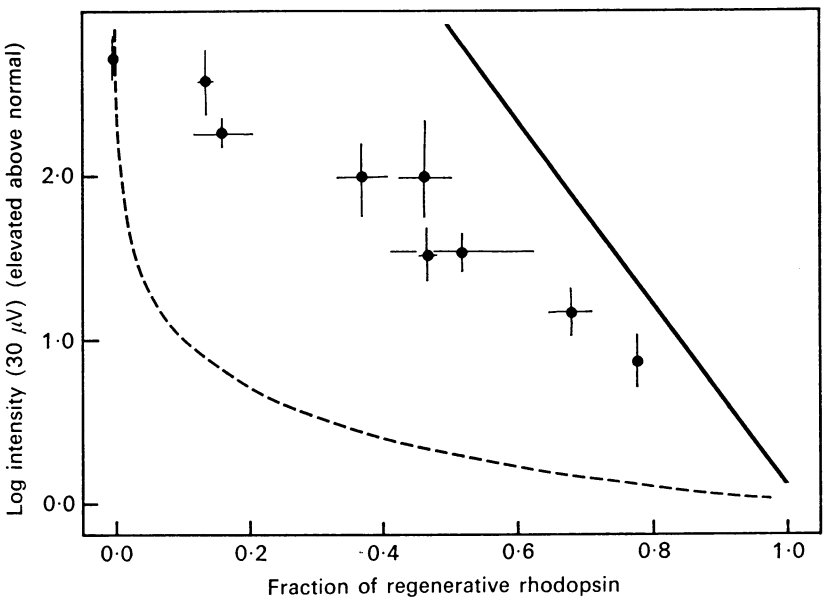

Fig. 5. The elevation of prebleach dark-adapted threshold in RCS rats above normal adult level as a function of the fraction of total rhodopsin that regenerates in RCS rats after 5 hr of recovery from a full bleach. The dashed curve describes the expected threshold elevation due to the reduction in quantum catch. The continuous line describes the log-linear relationship between e.r.g. threshold and rhodopsin content found for normal rats (Perlman, 1978, Fig. 3).

Delmelle *et al.* (1975) concluded that all the rhodopsin in the surviving rods can regenerate after a full bleach, implying that visual threshold should also completely recover to the dark-adapted level. Figs. 2 and 3 show that this is not the case. E.r.g. sensitivity did not recover completely in RCS rats of any age even after 5–6 hr in the dark following a significant bleach. In order to investigate this point further log I_{30} elevation of RCS rats above dark-adapted normal rats is plotted against the fraction of 'functional' rhodopsin present in the dystrophic retina after recovery from a full bleach as shown in Fig. 6. The fraction of 'functional' rhodopsin is a measure of the relative amount of rhodopsin present in rods in the RCS eye compared to rhodopsin content in the normal eye. The density β_λ of rhodopsin at wave-length λ was estimated from retinal densitometry using the equation

$$\frac{T_o - T_d}{T_o} = (1-S)\,[1-\exp(-2\beta_\lambda)], \qquad (2)$$

where T_0 and T_d are the wedge transmittances needed to balance the 'red' and 'λ' beams in full-bleached and dark-adapted states respectively, S is the fraction of the light reaching the photomultiplier tube which is stray light. Assuming (*a*) that the fraction of stray light is very small and constant for all rats studied and (*b*) the density, β_{550}, of rhodopsin is small enough to allow expansion of eqn. (2) into a Taylor series and neglecting the high order terms then the fraction of 'functional' rhodopsin in RCS rats is given by

$$((T_0-T_p)/T_0)^{RCS}_{550}/((T_0-T_d)/T_0)^{N}_{550},$$

where $((T_0-T_p)/T_0)^{RCS}_{550}$ is an estimate of rhodopsin density at 550 nm in RCS rods and $((T_0-T_d)/T_0)^{N}_{550}$ is an estimate of rhodopsin density at 550 nm in the normal

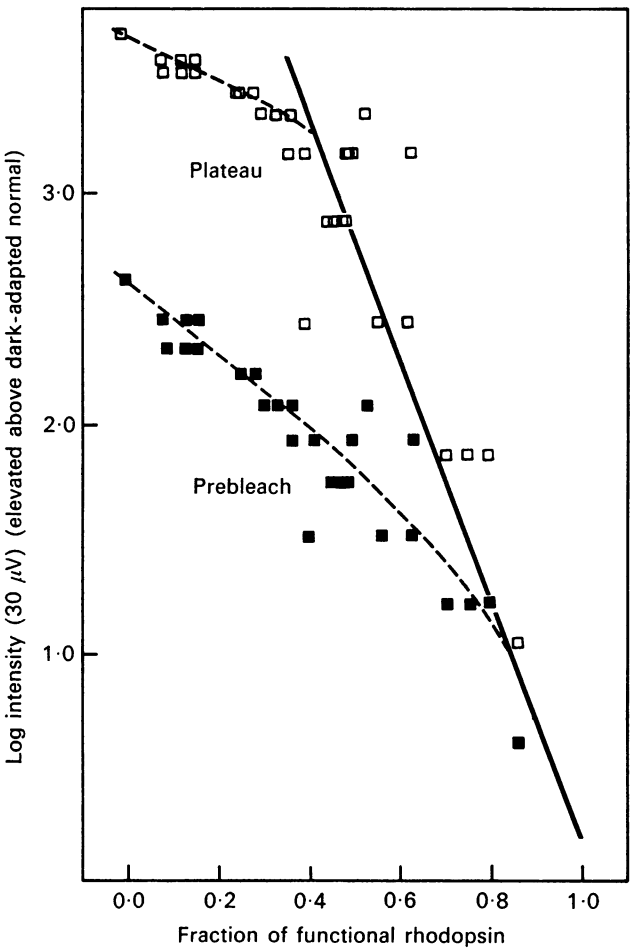

Fig. 6. The relation between elevation of log intensity needed for 30 μV response in RCS rats above dark-adapted normal level and the fraction of normal rat's rhodopsin that regenerates in RCS rats ('functional' rhodopsin). The filled and open squares describe respectively log I_{30} measured in RCS rats before being bleached and after 5 hr of dark-adaptation following a significant bleach. The continuous line describes the linear relationship between the log intensity needed for a 30 μV response and the rhodopsin concentration in normal rats (Perlman, 1978, Fig. 3). The dashed curves were drawn by eye through the data points.

eye. These values were obtained from a retinal densitometry study of rhodopsin in normal and RCS rats (Perlman, 1978, Fig. 10). In Fig. 6 the elevation of log I_{30} measured in RCS rats before bleaching (filled squares) and at the 'end' of dark-adaptation (open squares) above that in dark-adapted normal rats are plotted against the fraction of 'functional' rhodopsin. The continuous line in Fig. 6 describes the linear relationship between log threshold and rhodopsin content in normal rats while the dashed curves were drawn by eye.

The reasonable agreement between the open squares and the solid line suggest that in RCS rats younger than 45 days, where the rhodopsin content of the functioning rods at the completion of recovery from a full bleach is more than 30% of the

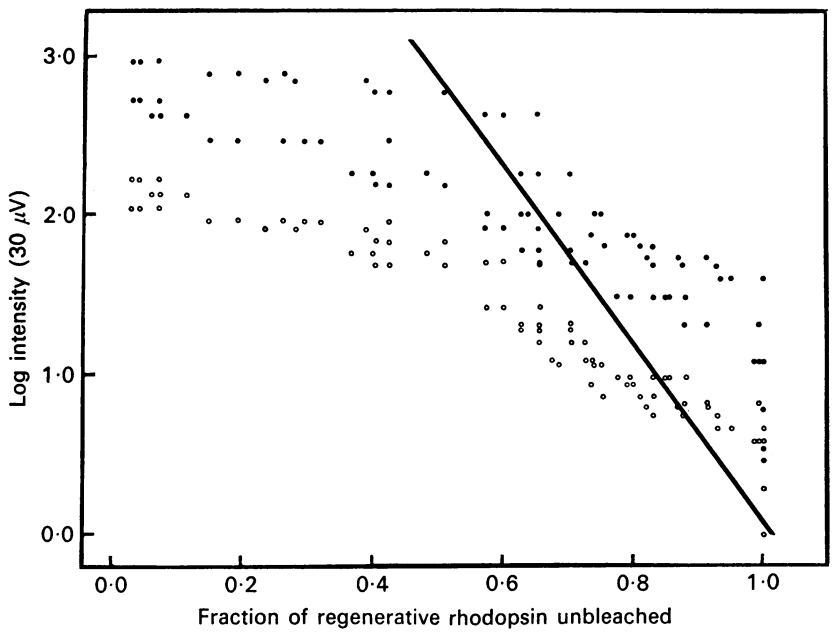

Fig. 7. The relation between log intensity needed for 30 μV response and rhodopsin regeneration in 25-day-old RCS rat during recovery from a strong bleach. The filled and open circles represent respectively the elevation of log I_{30} above the prebleach dark-adapted level and above the value measured after staying in the dark for 5–6 hr following the bleach. The values along the abscissa were obtained from retinal densitometry by normalizing the regeneration curve assuming that the plateau level of the regeneration curve represented the total rhodopsin located within surviving rods. The continuous line describes the relation between log I_{30} and rhodopsin level during recovery of normal rats from a strong bleach.

normal rhodopsin level, the light intensity needed for a criterion e.r.g. response measured at the end of dark-adaptation is related to rhodopsin according to eqn. (1). In older RCS rats, where the fraction of 'functional' rhodopsin is smaller than 0·3, the measured I_{30} is lower than expected from eqn. (1), that is e.r.g. sensitivity at the 'end' of dark-adaptation is not determined by rods. This is not an unexpected finding since in normal rats I_{30} is also related to rhodopsin according to eqn. (1) only when rhodopsin level is at least 0·3 of that in the fully dark-adapted eye. When the rods

contain less than 30% of the initial rhodopsin the cones are the main contributors to the e.r.g. at threshold (Perlman, 1978, Fig. 3).

In normal rats eqn. (1) describes the relation between log I_{30} and rhodopsin concentration throughout the rod branch of the dark-adaptation process (Dowling, 1960; Perlman, 1978). In RCS rats, on the other hand, no such simple relationship could be found, as can be shown by comparing the time courses of rhodopsin regeneration (Perlman, 1978, Fig. 7) and e.r.g. dark-adaptation (Fig. 3) obtained from rats of the same age. In Fig. 7 the relation between log I_{30} and rhodopsin regeneration in 25 day old RCS rat during recovery from a strong bleach is plotted. The values along the abscissa were obtained from retinal densitometry assuming that the plateau level of the regeneration curve represented the total rhodopsin in surviving rods. The elevation of log I_{30} above the dark-adapted level is shown by filled circles, and above the level measured after staying for 5–6 hr in the dark by open circles. Neither set of points fall along the continuous line as expected if eqn. (1) held. Rhodopsin regeneration is faster in 25 day old RCS rat than dark-adaptation. Therefore there must exist a slow mechanism that keeps the dystrophic retina desensitized even after regeneration is completed. Such a mechanism, e.g. removal of desensitizing photoproducts, allows the eye slowly to resensitize to a level determined by the amount of rhodopsin within the rods according to eqn. (1).

DISCUSSION

Electroretinogram responses recorded from RCS rats of all ages indicate not only a decline in sensitivity with age but also abnormal temporal characteristics (Fig. 1). The time to peak of the *b*-wave is prolonged and the subwaves present prior to the *b*-wave (normally evident only at moderate light intensities) are more pronounced and appear even at intensities close to threshold. By 90 days of age the e.r.g. at threshold is composed of a fast biphasic wave and only a bright stimulus evokes a normal looking *b*-wave.

It is difficult to compare RCS rats with patients suffering from retinitis pigmentosa because the latter are divided into many different classes. However, in general, excluding the sector variety of retinitis pigmentosa, all patients studied have shown a reduction in the amplitude and a prolongation of the time to peak of both the rod and cone responses (Berson, Gouras & Gunkel, 1968; Berson, Gouras & Hoff, 1969; Berson, Gouras, Gunkel & Myrianthopoulos, 1969*a*, *b*; Berson & Kanters, 1970). Moreover, e.r.g.s from some of the patients show abnormal splitting of the *b*-wave and pronounced subwaves (Berson *et al.* 1968, Berson *et al.* 1969*b*) similar to the records obtained from RCS rats of advanced age. Retinitis pigmentosa is also referred to as a night blindness disease because the first symptoms noticed are loss of night vision due to degeneration of rods. In early stages of the disease patients may lose night vision and still exhibit quite normal day vision. In the RCS rats of all ages e.r.g. dark-adaptation was relatively fast during the first 25–40 min in the dark following bleaching exposure. The light intensity needed for a criterion e.r.g. response measured at the end of the first phase of recovery was only about 0·5 log units elevated above the normal level while if measured after 5 hr in the dark it was elevated above normal by at least 2 log units in the 25 day old RCS rat and by as much as 3·5 log units in

the 70 day old RCS rat. Thus, just as in the human condition, the cone mechanism responsible for the fast dark-adaptation appears to be more resistant to the degeneration process than the rod mechanism.

In a recent study on the damaging effects of light exposure on the retina of the albino rat it was shown that the cones were more resistant than the rods to the damaging light as revealed by a considerable elevation of the rod branch of the dark-adaptation curve with little effect on the cone branch (Cicerone, 1976). Thus light damage seems to mimic the retinal degeneration in RCS rats.

One common feature seen in the dark-adaptation studies of RCS rats of all ages was the elevation of the intensity needed to evoke a criterion e.r.g. response above the prebleach level even after 5 hr of dark-adaptation following a significant bleach (Figs. 2, 3). There are at least four different ways to explain the incomplete recovery of the e.r.g. sensitivity. (*a*) Rhodopsin in the 'debris' may contribute to the e.r.g. in the dark-adapted prebleach state but not after bleaching because it cannot regenerate. (*b*) A bleaching photoproduct of the 'debris' rhodopsin may act to desensitize functioning rods. (*c*) Only a fraction of the rhodopsin present in the functioning rods may regenerate after being bleached and (*d*) the bleaching exposure may cause some damage to sites proximal to the photoreceptors but distal to the generators of the *b*-wave, thus causing the *b*-wave to deteriorate even though functional rhodopsin fully regenerates.

Possibility *a* seems quite unlikely because rhodopsin in the 'debris' has no known way to convey information down to the neural retina where the *b*-wave is generated. Alternative *b* is based on the finding that bleached rhodopsin causes desensitization of the e.r.g. more than would be expected simply from the decrease in quantum catch (Dowling, 1960). However, it remains to be shown that extracellular bleached rhodopsin manifests a similar desensitizing role.

There is no easy way to decide between possibilities *c* and *d*. Previous studies on light damage in the retina of the albino rat (Kuwabara & Gorn, 1968; Kuwabara & Funahashi, 1976) pointed to the photoreceptors as the prime target for the damaging light. The first morphological change was seen simultaneously in the tips of the outer segments and in the synaptic terminals of the photoreceptor cells. These damaged cells might be able to synthesize rhodopsin while signal transmission down to the neural retina is deteriorating. Some support for the light damage hypothesis comes from comparing dark-adaptation of rats raised in darkness with that of rats raised in cyclic light–dark conditions. Dark-reared normal rats, after being fully bleached, completely regenerate rhodopsin along a time course similar to the one measured in normal rats raised in a cyclic light–dark environment. E.r.g. dark-adaptation, on the other hand, proceeded slower in the dark-reared rats than in the cyclic-reared animals and did not reach the prebleach level (Perlman, 1976). Therefore a milder bleach (90 %) was employed in the previously described experiments. In RCS rats of all ages e.r.g. dark-adaptation was faster than in normal rats raised either in darkness or in cyclic light–dark environments, suggesting that light damage was not solely responsible for the incomplete recovery after a significant bleaching that caused no detectable damage to normal rats raised in darkness. Moreover, the elevation of log I_{30} measured in RCS rats after 5 hr of dark-adaptation above the normal dark-adapted level is related to the fraction of 'functional' rhodopsin

(density of 'regenerative' rhodopsin in RCS/density of rhodopsin in normal) according to eqn. (1), suggesting that in the RCS rats the incomplete e.r.g. dark-adaptation was due to incomplete regeneration of the rhodopsin located within the functioning rods.

I thank Professor M. Alpern for his support and assistance. This work was supported by a grant EY-00197-18 to M. Alpern from the National Eye Institute.

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