

THE BLEACHING AND REGENERATION OF RHODOPSIN IN THE CAT

By A. B. BONDS AND DONALD I. A. MACLEOD

*From the Biomedical Engineering Center,
Technological Institute, Northwestern University,
Evanston, Illinois 60201, and the Institute of
Molecular Biophysics, The Florida State University,
Tallahassee, Florida 32306, U.S.A.*

(Received 27 March 1974)

SUMMARY

1. The processes of bleaching and regeneration were monitored by retinal densitometry in living cats.
2. Neither bleaching nor regeneration of rhodopsin can be described by the simple kinetic equation (Alpern, 1971) found valid for man.
3. After a strong 1 min bleach, the retina contains more unbleached rhodopsin than expected on the basis of the initial bleaching rate.
4. During the first 9 min after a 1 min bleach, cats regenerate rhodopsin only slowly; density changes during this period are dominated by formation and decay of metarhodopsin III. Subsequently, rhodopsin regeneration accelerates to a rate of 50 %/11 min.
5. No such delay precedes recovery from a prolonged (20 min) bleach.

INTRODUCTION

Knowledge of the time course of the photochemical reactions in the retina of the living cat is valuable for the interpretation of many electrophysiological experiments, as well as providing clues to the processes underlying pigment regeneration. Zewi (1939) measured the time course of the regeneration of bleached rhodopsin in the cat by extraction of the pigment at various intervals after cessation of bleaching, but his technique suffered from poor temporal resolution and variation between individuals. Weale (1953*b*) first used Rushton's (1952) fundal densitometry on the living cat to yield both the difference spectrum and the time course of regeneration after bleaching the rhodopsin.

Since the cat's eye has a large pupil and high fundal reflectance (due to the tapetum) it presents an ideal subject for modern densitometric methods. Because of improvements in technique, measurements have

become virtually noise-free, removing the advantage held by *in vitro* preparations and allowing accurate recording of photochemical processes as they occur in the intact animal.

METHODS

Preparation. The regeneration of rhodopsin was measured in the left eyes of seven adult cats (2.5–5 kg). Initial anaesthetic induction was by means of ketamine (Vetalar, 25 mg/kg). Narcosis was continued with sodium thiamylal (Surital 2.5%, i.v.) until the maintenance anaesthesia, urethane (20%, 320 mg/kg), could take effect. A cannula in the femoral artery allowed continuous monitoring of the mean arterial blood pressure, which was maintained above 100 mmHg (if necessary by infusion of methoxamine HCl (Vasoxyl)) except where noted. Paralysis was introduced by i.v. infusion of gallamine triethiodide (Flaxedil, 20 mg/kg.hr) during which time the animal was artificially respired through a tracheal cannula. Body temperature was maintained with a heating pad.

During all stages of the preparation especial care was taken to prevent injury to the eyes. Atropine sulphate (1%) and neosynephrine (10%) were dropped into the conjunctival sac to dilate the pupil and retract the nictitating membrane. The eyes were then held closed with wound clips until the animal was installed in the densitometer, when completely clear plastic contact lenses were applied with wetting solution (D. N. Laboratories, York, Pa.). The results from the first cat made it obvious that even very large doses of Flaxedil (up to 50 mg/kg.hr) could not eliminate disruptive eye movements. A slit was therefore cut in the temporal margin of the eyelid and the conjunctiva and episclera were gently dissected from the surface of the globe over about one-fourth its circumference. A ring segment, attached to a rod held in a micromanipulator, was then cemented onto the cleaned limbus with Eastman 910 cement. This reduced eye movements to a minimum (Enroth-Cugell & Shapley, 1973).

Instrumentation. The densitometer used was Rushton's Florida densitometer (Hood & Rushton, 1971), slightly modified to improve the signal-to-noise ratio. A test light of a wave-length strongly absorbed by rhodopsin is projected onto the retinal region under observation. Some of this light is reflected at the fundus and emerges through the pupil. This returning beam of light has passed twice through the retina, and during each passage has been attenuated by absorption losses in rhodopsin. Changes in rhodopsin density during bleaching and regeneration increase or decrease the amount of reflected light. Dividing the amount reflected by the amount reflected in the fully bleached state we obtain a quantity whose decimal logarithm is the inferred *double density* of the rhodopsin bleached. Results are expressed in terms of the change in double density from the dark-adapted state.

To ensure that changes in reflectivity not due to rhodopsin do not spoil the measurements, the test light is rapidly alternated with a comparison light that originates from the same source but is of a different spectral composition, containing only deep red (greater than 690 nm) and infra-red radiation that is not appreciably absorbed by rhodopsin. The index of rhodopsin concentration is the ratio of the amplitude of the two reflected beams.

In the original instrument the alternation of test and comparison lights was accomplished by means of a rotating piece of polaroid. For our experiments this was replaced by a Pockels cell (Baird Atomic Electro-Optic Light Modulator JV2-UV) behind a stationary piece of polaroid. The Pockels cell acts as a retardation plate, and the retardation along its slow axis is proportional to the magnitude of an

applied electric field. When sinusoidally driven to one-half wave of retardation, the Pockels cell sinusoidally modulated the test and reference beams in opposite phase. The advantages of the Pockels cell were threefold: (1) it eliminated the mechanical vibration caused by the polaroid rotating in its bearing; (2) the optical reference channel needed for the phase-sensitive rectifier could be eliminated since the internal reference oscillator of the phase-sensitive rectifier could drive the Pockels cell directly; and (3) the beam modulation frequency could be increased (275 Hz was used) which yielded a more uniform output from the phase-sensitive rectifier. The higher reliability of the measuring system, when coupled with the stability, high fundal reflectance and large pupil (≈ 13.5 mm) of the cat eye enabled us to abandon the nulling technique normally used with this instrument and rely instead upon the amplitude output of the phase-sensitive rectifier (produced by the alternation of test and comparison lights) which, when attached to a chart recorder, allowed a continuous graphical record of the pigment density in the retina. Using a 0.1 sec smoothing network the signal read from the chart had a standard deviation corresponding to an absolute density change of ± 0.004 , or about $\pm 1.6\%$ of the total pigment density. Visual averaging of the data over a 10 sec measuring period could reduce this error to $\pm 0.5\%$ of the total pigment. The low noise level permitted reliable measurements using relatively dim test lights. The brightest test spot was 3.1 log scotopic td (at 500 nm), a value sufficient to bleach only about 6% of the pigment at equilibrium.

The bleaching beam formed a Maxwellian image that almost filled the temporal half of the pupil; the measuring beams entered at the pupil centre. On the retina, each beam produced a sharply focused image of a circular field subtending 3° at the eye, but the light beam reflected from the eye was stopped down to 2° , before it reached the photocell, by a diaphragm in the plane conjugate with the fundus.

Photometry. Illuminances were measured in photopic td (human eye) using an SEI meter in the way described by Rushton (1956a). The relative spectral distribution of the energy from the bleaching light was measured using a silicon photodiode, allowing scotopic td values to be derived from the photopic td values.

In several experiments an additional beam was provided through a monochromator illuminated by a 150 W xenon arc lamp which had been tested carefully for drift in luminous intensity. This arrangement provided the flexibility of a test (or reference) beam of continuously variable wave-length in addition to the two beams supplied by the tungsten lamp.

Technique. The animal was secured to a headholder fastened to the optical bench of the densitometer. The measuring beam was carefully aligned so that it entered the eye nearly axially, producing a good image on the retina without occlusion by the iris. The cat's eye could be moved slightly by means of the rod on which it was fastened, and we tried to place the measuring light spot near a prominent blood vessel which could later be used for identification of the test locus. Most of the measurements were found to have been made between 5 and 10° from the area centralis, a region which contains rod densities of $4.3 \times 10^5/\text{mm}^2$ and rod/cone ratios of 50:1 (Steinberg, Reid & Lacy, 1973); the cone pigment contribution to the measurements is therefore probably negligible.

The dark-adapted base line was determined by readings taken after the animal had been in total darkness for at least 30 min and before any extensive bleaching had been done. Except where noted, all bleaching runs took place after the pigment density had returned to this original base line and remained there for at least 5 min.

RESULTS

Spectral reflectance and difference spectrum

Difference spectra for the bleaching of rhodopsin in the cat eye were obtained by measuring, at several wave-lengths, the difference in fundal reflectance between the completely dark-adapted condition and the light-adapted state caused by the prolonged application of the bleaching light (10–25 min at 6.0 log td). Ealing-TFP interference filters of 10 nm bandwidth defined the spectrum of the measuring beam. The average double densities from three cats are shown as filled circles in Fig. 1*a*. When compared with a Dartnall nomogram pigment (Wyzecki & Stiles, 1967) absorbing maximally at 500 nm, the match of the curve at longer wave-lengths is good. The data gave no indication of a contribution from cone pigments, which would manifest itself as a density increase near 560 nm. The only deviations from the nomogram occur at wave-lengths shorter than 500 nm; the density is much lower at 436 nm, and actually negative (i.e. increases during bleaching) at 405 nm. This can be tentatively attributed to the formation, as a result of bleaching, of substances which absorb these short wave-lengths (Rushton, 1956*a*). The peak double density, using a white bleaching light (6 log td) and measuring at 500 nm, ranged from 0.20 to 0.255, with a mean of 0.223 in six cats. This value is slightly higher than twice the single density of 0.09–0.1 reported by Weale (1953*b*).

An artificial eye with physical characteristics chosen to resemble the cat eye was constructed using a lens of 13 mm diameter and 15 mm focal length carefully focused on a magnesium oxide diffusing screen. By comparing the luminance of the aerial image emanating from the cat's fundus with that from the magnesium oxide screen of the artificial eye, the relative spectral reflectance of the cat's eye (including the double attenuation by the eye media) was determined. The results, shown in Fig. 1*b* in relation to the reflectance for the infra-red comparison beam, indicate a much higher reflectance for visible wave-lengths than for either the infra-red or ultra-violet. This enhancement is no doubt due to the tapetum, which has been shown to have much higher reflectance for visible light (Weale, 1953*a*).

Estimates of absolute reflectance were obtained for one cat. Expressed as a percentage of the reflectance of magnesium oxide, the reflectances at 500 nm were 14% in the regenerated state and 24% in the bleached state. These values are not much lower than the 32% found by Weale (1953*a*) for the tapetum alone.

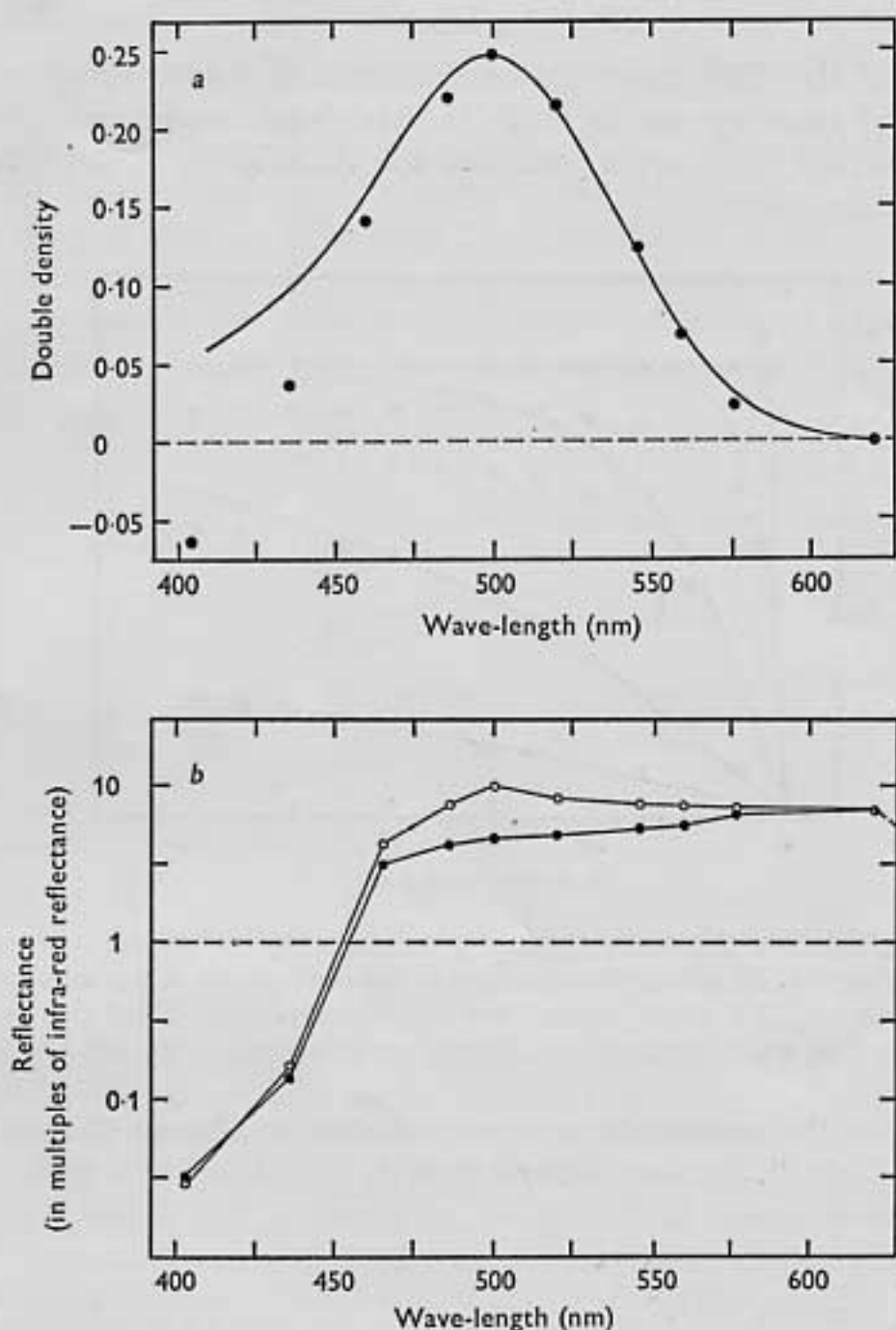


Fig. 1. *a*, the difference spectrum (double density), from measurements before and after bleaching the previously dark-adapted eye. White light bleach, 6 log td for at least 5 min. The curve is the standard shape for visual pigments, with maximum absorption at 500 nm. Points are averages from three cats. *b*, spectral reflectance of intact living cat eye relative to the infra-red (greater than 690 nm). Filled circles, dark-adapted eye; open circles, bleached eye. Four runs in three cats.

Bleaching kinetics

Because of the differences between individual cats, averaging the bleaching and recovery curves tends to mask phenomena more clearly seen in single runs. The curves presented will therefore be of single representative performances.

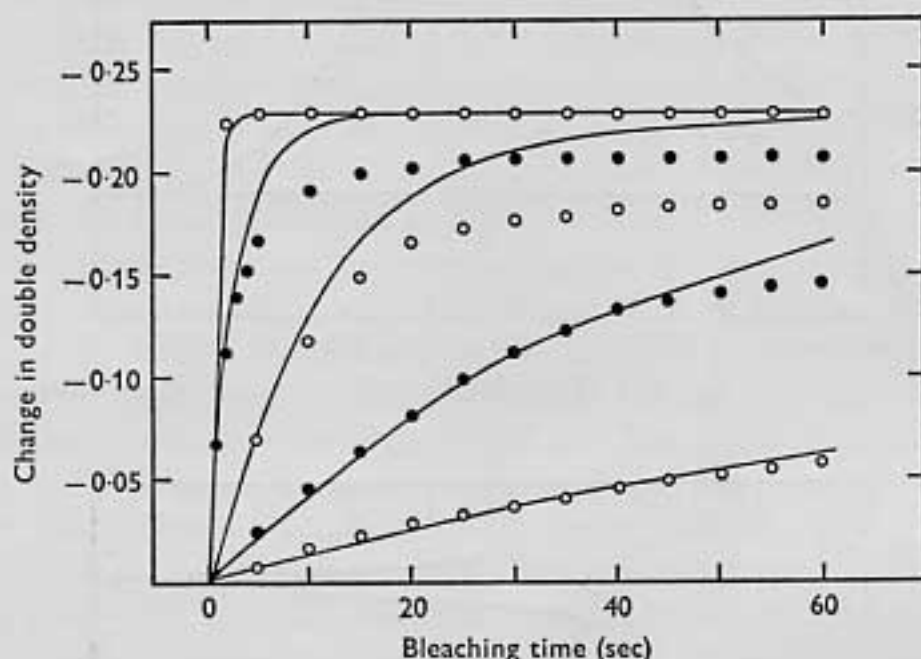


Fig. 2. Points show the change in double density (500 nm) during a 60 sec bleaching period. Bleaching illuminances in log scotopic td: 4.8 (lowest set of open circles), 5.4 (filled circles), 6.0 (open circles), 6.6 (filled circles), 7.2 (uppermost set of open circles). Curves are solutions of eqn. (1). Cat 7.

Fig. 2 shows the results of five 60 sec bleaches at different illuminances on one cat. Each illuminance differed from its neighbours by a factor of 4. The continuous curves in Fig. 2 are solutions of the simple equation which describes pigment kinetics in man both in cones (Rushton, 1958) and in rods (Alpern, 1971):

$$-\frac{dp}{dt} = \frac{Ip}{Q_e} - \frac{1-p}{t_0}, \quad (1)$$

where p is the amount of pigment present, t_0 is the exponential time constant of regeneration, I is the illuminance of the bleaching light, and Q_e is the bleaching energy (illuminance multiplied by bleaching duration) that would bleach all but $1/e$ of the pigment in the absence of regeneration. In calculating the curves, t_0 was set at 900 sec and Q_e was set at 7.04 log td.sec, a value selected to obtain a good fit to the initial rates of bleaching. The density changes from equation (1) have been scaled so as to obtain a good fit to the data for the brightest bleaching illuminance. Fig. 2 shows

that the time course of bleaching differs from the predictions of equation (1): the stronger bleaches leave the retina less transparent than equation (1) would predict. Bleaching curves from three other cats confirmed this behaviour.

A possible explanation of this departure from equation (1) is contamination of the rhodopsin measurement by the formation of a photoproduct absorbing at 500 nm. The most likely candidate is metarhodopsin III which has a λ_{\max} around 465 nm. Let us assume that it was produced in sufficient quantity to cause the deviations seen in Fig. 2, where measurements were made at 500 nm. If we then measured at 486 nm, the deviation

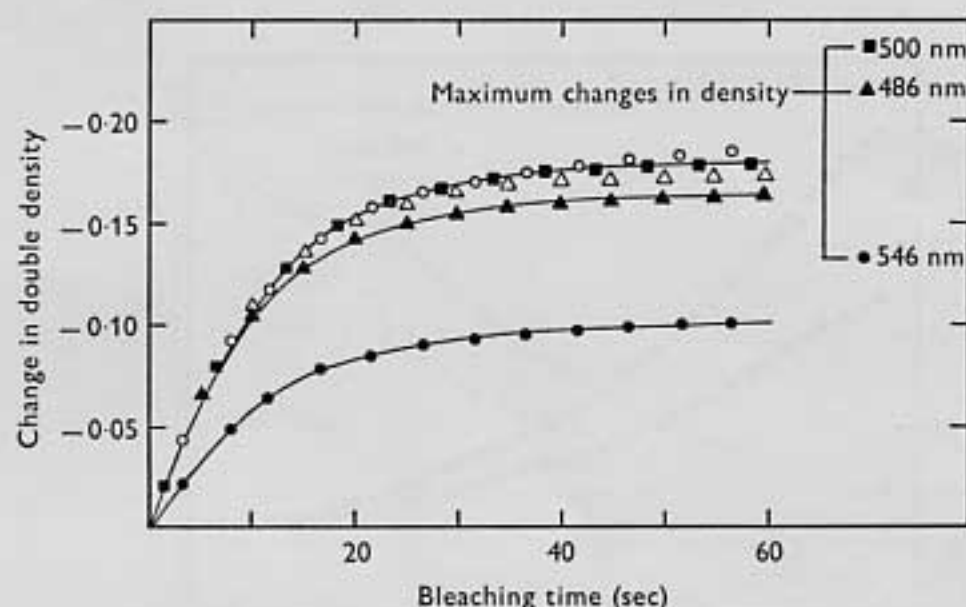


Fig. 3. Filled points show the changes in double density seen at various measuring wave-lengths during a 60 sec exposure to a white bleaching light of 6 log scotopic td. Circles, measuring wave-lengths 546 nm; squares, 500 nm; triangles, 486 nm. Also shown are the greatest changes in double density seen at each wave-length after exposure to a prolonged bright bleach. Cat 5, one run at each wave-length.

Open circles and triangles show the density changes at 546 and 486 nm respectively, normalized for rhodopsin (normalization factors: 1.06 for 486 nm, 1.85 for 546 nm). If all density changes were due to removal of a rhodopsin with maximum absorption at 501 nm and an absorption spectrum of the standard shape, these normalized points and the filled squares (500 nm data) would lie on the same curve.

from the expected value would be greater than at 500 nm due to the greater density of metarhodopsin III nearer its λ_{\max} ; and measurement at 546 nm would produce little or no deviation. Fig. 3 shows the results of this experiment (filled symbols; one run at each wave-length). Also shown are the density changes seen after prolonged exposure to 7.2 log td. It is clear that even at 546 nm (filled circles, Fig. 3) the retina is by no

means fully transparent after a 1 min exposure to 6 log td. To assist interpretation, the changes in density have been normalized by dividing the changes seen at 486 nm (filled triangles) and 546 nm (filled circles) by the extinction coefficient of a standard rhodopsin of λ_{\max} 501 nm, a manoeuvre which superimposes the rising phases of the three curves. The normalized points (open symbols) deviate by relatively small amounts from the 500 nm data. This suggests that the large deviations from equation (1) probably do reflect changes in the density of rhodopsin (or the spectrally similar isorhodopsin) without much contamination by metarhodopsin III. One minute exposures fail to bleach as much rhodopsin as would be expected from the initial bleaching rates.

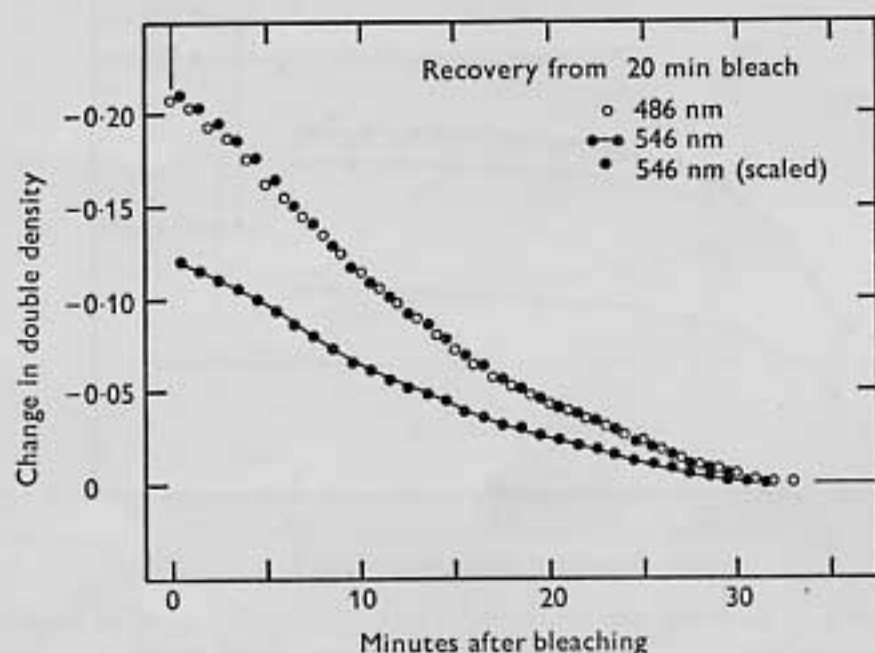


Fig. 4. Recovery from a prolonged (20 min) bleach at 6 log td. Linked filled circles, density measured at 546 nm; open circles, 486 nm. To obtain the upper (unlinked) set of filled circles, the 546 nm data were multiplied by a scaling constant (1.74) equal to the ratio of the densities of a 501 nm rhodopsin for the 486 and 546 nm measuring beams. Where only rhodopsin regeneration is being measured, the normalized 546 nm points should lie on the same curve as the open circles.

Regeneration

After prolonged bleach. After bleaching the retina by exposing it for 20 min to a white light of 6.0 log scotopic td, recovery was tracked at two different wave-lengths, 486 nm and 546 nm, by changing the filter in the measuring beam at 30 sec intervals. In Fig. 4 filled circles show the results obtained at 546 nm and open circles show results at 486 nm. The density at each wave-length rises monotonically to its previous dark-adapted value, but the time course does not follow the exponential that would be

expected if the regeneration rate were proportional to bleached rhodopsin (equation (1)). Rather, the density increase during the first 15 min appears to be linear in time; half of the return to the dark-adapted base line is achieved in 630 sec. There follows a gradual decrease in the rate of rhodopsin formation, and complete regeneration is attained in about 35 min. Density changes at 486 nm and 546 nm are closely proportional. To illustrate this, the 546 nm density changes have been divided by the ratio of the extinction coefficients of a 501 nm rhodopsin for the two measuring lights. The normalized 546 nm data (upper set of filled circles, Fig. 4) are nearly superimposed on the 486 nm data, demonstrating that there is little interference here by photoproducts spectrally distinguishable from rhodopsin.

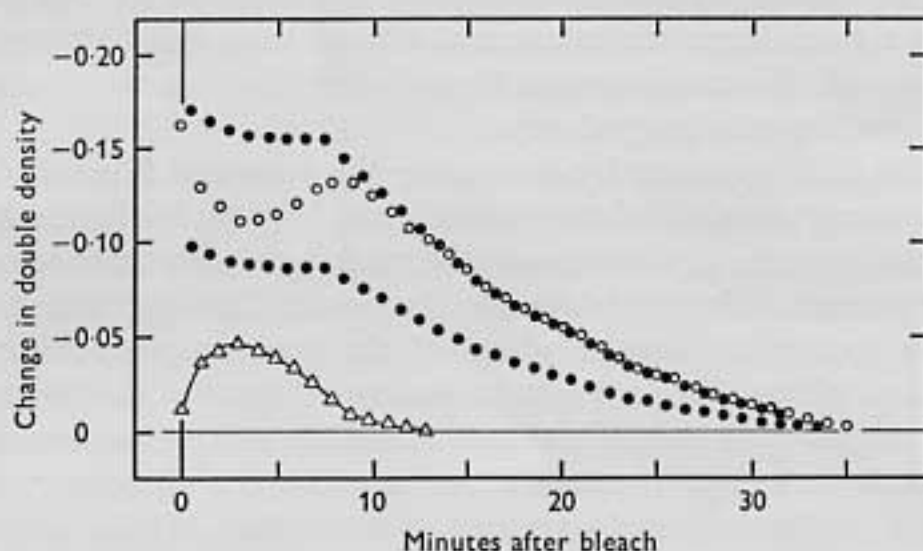


Fig. 5. Recovery from a shorter bleaching exposure than in Fig. 4 (60 sec, 6 log td). Linked filled circles, density at 546 nm; open circles, density at 486 nm. Upper set of filled circles, normalized 546 nm data (densities at 546 nm multiplied by 1.74). Triangles, excess density at 486 nm.

One minute bleach. The time course of recovery from a short (60 sec) intense bleach is remarkably different (Fig. 5). In Fig. 5 recovery was again tracked simultaneously at 486 and 546 nm. At 546 nm (filled circles, Fig. 5) the density is seen to level off to a plateau within a few minutes, after a recovery of about 15%. The duration of this initial phase of recovery varied from 8 min 30 sec to 9 min 50 sec (eight runs in three cats). At this point, a break in the curve marks the start of a second phase of recovery, similar in time course to the recovery observed after a long bleach; the rate of increase of density is constant (50%/660 sec) until about 15 min after extinction of the bleaching light, and then decreases gradually. Recovery is again virtually complete in 35 min. At 486 nm (open circles, Fig. 5) the rapid initial density increase is followed

by a reversal and a subsequent period of decreasing density; then, shortly after the break in the 546 nm curve, another reversal occurs and the density begins to increase once again, at the same rate as after a prolonged bleach.

All phases of recovery at 546 nm are probably due mainly to rhodopsin. The initial drop to the plateau cannot be attributed to cone pigments because the difference spectra show no cone pigment contribution sufficient to cause this effect. As to the plateau itself, the simplest interpretation would be that the density changes introduced by a continual regeneration of rhodopsin at a constant rate, are cancelled by the fading of metarhodopsin III. But the results at the shorter wave-lengths contradict that analysis: if metarhodopsin III were present in quantities sufficient to account for the departure from a linear time course at 546 nm, it would create a much larger disturbance at 486 nm than the slight reversal actually observed. We must assume that during the plateau, rhodopsin itself is regenerating relatively slowly.

Nevertheless, it is apparent by comparing the open and filled circles of Fig. 5 that a strong transient photoproduct is masking the initial rhodopsin regeneration at 486 nm. The nature of this masking at short wave-lengths is clarified by normalizing the data for rhodopsin. The normalized data run together during the second phase of rhodopsin regeneration; but during the first phase there appears an excess of density at 486 nm. In Fig. 5 the triangles show the difference between the 486 nm curve and the normalized 546 nm curve. It is clear that a substance absorbing at the shorter wave-lengths is already present at extinction of the bleaching light (see also the bleaching curves of Fig. 3), rises to a peak concentration 3 min after the bleach, and decays almost totally in 10 min. The time variation of its concentration is approximately described by a model having exponential production and decay with time constants 130 and 225 sec respectively.

To determine more closely the spectral properties of this substance, the arc lamp and monochromator (set at 470 nm) were substituted for the densitometer reference beam, and the former reference beam and test beam were equipped with 460 and 486 nm interference filters; the densitometer then measured the density difference between 470 and either 486 or 460 nm. Between 1 and 6 min after bleaching, the density first increased and then decreased, just as in Fig. 5*b*. The changes in density were greatest at 470 nm, and least at 486 nm. The photoproduct responsible therefore absorbed most strongly between 460 and 470 nm, and so must be metarhodopsin III (see Discussion).

Another transient photoproduct was seen in the ultra-violet in one cat. Measuring at 405 nm, the density increased very quickly with the onset

of the bleaching light and at extinction decreased exponentially with a time constant of 90–120 sec (three runs). This product is presumably metarhodopsin II ($\lambda_{\max} = 380$ nm) the decay of which is reflected in the formation of metarhodopsin III. Further attempts to define the density and time course of the presumed metarhodopsin II in other cats were frustrated; density changes were small and inconsistent.

Rapid regeneration

Weale (1953*b*) reported a 'rapid' mode of recovery requiring only 2 min or less for almost complete recovery. In one such case the density measured at 540 nm increased 0.2 log units in less than 1 min. While we have never found a density change as large as that (under any conditions) at 546 nm, we have noticed instances of this 'rapid recovery'. In one cat the density increased 0.138 in 20 sec (out of a measured total increase of 0.24) at 500 nm; in another, the density increase at 546 nm was found to be 0.055 (out of 0.11 total) in 30 sec. Similar changes were seen in two other cats. In all cases, the 'rapid recovery' was seen only when the animal was in poor physiological condition, indicated by a blood pressure of less than 75 mmHg. The phenomenon is therefore probably a pathological one. The effect was repeatable with the application of brief 'topping up' bleaches of about 40 sec between recoveries, allowing a very coarse determination of the difference spectrum in one case. 'Rapid regeneration' is conspicuous at 546 nm, and is still noticeable (albeit much weaker) at 620 nm, so it is unlikely that a rapid-forming photoproduct of shorter wave-length than rhodopsin (such as metarhodopsin III) is responsible. Weale (1965) suggests this effect may be caused by prelumirhodopsin, which has a λ_{\max} of 543 nm. Confirmation of the mechanism behind 'rapid regeneration' will be problematic because of the transience of both the phenomenon and the animal when the conditions are ripe for its appearance.

DISCUSSION

Regeneration. The data for regeneration in the dark show plainly that the rate of regeneration of cat rhodopsin is not simply proportional to the amount of still unregenerated pigment in the way that Rushton (1958), Ripps & Weale (1969) and Alpern (1971) have found true for cone and rod pigments in man. After a prolonged bleach (Fig. 4) the pigment is restored at an almost constant rate until most of it has been regenerated, instead of following the exponential curve observed in man. It is therefore likely that in the cat, 11-*cis* retinal is not present in excess but is made available at an approximately constant slow rate for the reaction with free opsin. Enzymes are thought to be involved in the chain of reactions

leading to the production of 11-cis retinal (Baumann, 1972*a*); whatever the rate-limiting step in regeneration might be, initial formation of rhodopsin at a constant rate following a bleach is to be expected if the enzymes do not suffer from a lack of substrate.

Regeneration curves beginning with a linear portion have previously been observed by Weale (1953*b*) in the cat; by Zewi (1939), Peskin (1942) and Reuter (1966) in the frog; by Dowling (1960) in the rat; by Dowling & Ripps (1970) in the skate; and by Hagins & Rushton (1953; Rushton, Campbell, Hagins & Brindley, 1955) in the rabbit. The cat restores half of its rhodopsin in about 11 min (Figs. 4 and 5; Weale, 1953*b*). Regeneration in the cat is thus less rapid than in man (half regeneration in about 4.75 min; Rushton *et al.* 1955; Alpern, 1971) but more rapid than in frog (25–30 min at normal temperatures; Zewi, 1939; Peskin, 1942; Reuter, 1966), rabbit (24 min; Rushton *et al.* 1955), rat (40 min; Tansley, 1931; Dowling, 1963), skate (60 min; Dowling & Ripps, 1970), or guinea-pig (Weale, 1955).

Metarhodopsin III. The curves of recovery from a 1 min bleach (Fig. 5) show, superimposed on the expected regeneration of rhodopsin during the 10 min following the bleach, the formation and subsequently disappearance of another substance that absorbs strongly in the visible spectrum but is spectrally distinguishable from rhodopsin. This is suggested also by the observations of Weale (1957) who found a difference spectrum peaking near 470 nm, when a clearing bleach was applied to a retina already partially bleached. By a mathematical analysis of regeneration curves in man, Weale (1967) and Ripps & Weale (1969) have shown that a visible absorbing photoproduct occurs in man also, following bleaching by xenon flashes; Alpern (1971), however, reports that when bleaching was effected with an incandescent lamp, no intermediate photoproduct contaminates the rhodopsin regeneration curve measured by retinal densitometry in man.

In the cat the spectral variation of the measurements during recovery from a 1 min bleach shows that changes in photoproduct concentration are not only significant but actually dominate the density changes observed during the first 10 min of recovery. If for simplicity we suppose that the photoproduct is completely transparent to 546 nm, the maximum photoproduct density is 0.045 at 486 nm (Fig. 5, triangles) and 0.048 at 460 nm (absolute densities may vary between animals however). This implies maximum absorption between 465 and 470 nm. Among the products of rhodopsin bleaching known to occur under physiological conditions, only metarhodopsin I ($\lambda_{\text{max}} = 478$ nm) and metarhodopsin III ($\lambda_{\text{max}} = 465$ nm; Ostroy, Erhardt & Abrahamson, 1966) have even approximately appropriate absorption spectra. Since metarho-

dopsin I is thermally unstable, with a half-life of only about 0.1 msec in the living mammal (Hagins, 1956; Ebrey, 1968; Cone & Cobbs, 1969) the spectral properties of the stable photoproduct dealt with here allow it to be firmly identified as metarhodopsin III. The time course of the formation and disappearance of metarhodopsin III (Fig. 5, difference curve) appears to follow two first-order reactions (Baumann, 1972*b*) and agrees well with the results of Dowling & Ripps (1970) in the skate and of Ripps & Weale (1969) and Alpern (1971) in man. At $\frac{1}{130} \text{ sec}^{-1}$, the rate of formation is close to the rate of metarhodopsin II decay obtained by Cone & Cobbs (1969) in living rats and by Ebrey (1968) in excised rat eyes; the rate of decay ($\frac{1}{225} \text{ sec}^{-1}$) is, however, much faster than the rates reported for rats.

The agreement between the rates of formation of metarhodopsin III and disappearance of metarhodopsin II supports the view that metarhodopsin III is formed in the living eye by thermal decay of metarhodopsin II. A simple model, in which metarhodopsin III is formed by a first-order reaction from the metarhodopsin II which exists when the bleaching light is extinguished, and is removed by a slower first-order reaction, provides a reasonable description of the time course of metarhodopsin III concentration during recovery. However, calculations assuming nomogram spectral sensitivities (Wyzecki & Stiles, 1967) and equal absorbances for metarhodopsin III and rhodopsin show that the amount of metarhodopsin III formed is never more than about 0.7 times that predicted (and in some cats as little as 0.4 times). Baumann (1972*b*) and Weale (1973) have observed this phenomenon in frog receptors and have concluded that some metarhodopsin II is hydrolysed into retinal and opsin and does not form metarhodopsin III.

Delayed regeneration following short bleaches. Retarded regeneration immediately after short bleaches has been previously reported by Zewi (1939) for the frog and by Dowling & Hubbard (1963) for the rat. Dowling & Hubbard point out that immediately after a brief bleaching exposure all-trans retinal remains attached to the chromophoric site for some time. A molecule cannot be regenerated until the all-trans retinal has left the original binding site on opsin, to allow its replacement by 11-cis retinal. The very pronounced delay reported here for the cat (Fig. 5) cannot therefore be accounted for on this basis. Computer calculations show that if 0.3 or more of metarhodopsin II is hydrolysed directly to retinal and free opsin and only the remainder follows the route through metarhodopsin III, a large surplus of free opsin is available throughout most of the plateau and until regeneration nears completion. The delay following a short bleach cannot be attributed to any lack of free opsin, but it may result from a lack of the 11-cis retinal that must combine with the opsin.

Perhaps, cats like rats (Dowling, 1960) are unable to store retinal, so that the ingredients for the formation of 11-cis retinal must be provided by the bleaching light itself. During a prolonged bleach the synthesis of 11-cis retinal can proceed (partially or to completion) during the bleaching exposure, allowing regeneration to begin at its maximum rate once the bleaching light is extinguished (Fig. 4). After a short bleach, 11-cis retinal must be synthesized *ab initio*, and regeneration must await the completion of the synthesis. Since the maximum rate of regeneration is the same following prolonged exposures and 1 min exposures (Figs. 4, 5) the rate-limiting reaction is probably not one of those which can proceed during exposure to the bleaching light.

Photoreversal. There is an obvious discrepancy between the amount of rhodopsin bleached in cat at higher luminances (Fig. 2) and that predicted by the kinetic equation which holds for man (Alpern, 1971). Deviations in a similar direction, i.e. less rhodopsin bleached than expected at higher luminances, have been seen in rabbit (Weale, 1964) and frog (Baumann, 1966; Bäck, Donner & Reuter, 1965). Weale (1964) and Bäck *et al.* (1965) found the measured density to actually *increase* when illuminances were increased from moderate to very bright (8 log td) levels. While Weale suspected this effect to be due to oedema in retinal tissues, Bäck *et al.* confirmed their findings by measuring the density of extracted rhodopsin, thereby ruling out any circulatory or cellular effects. Reuter (1966) attributed the phenomenon to photoreversal of a long-lasting intermediate, and he has subsequently established (T. Reuter, in preparation) that metarhodopsin III is readily photoisomerized to a mixture of isorhodopsin and rhodopsin, the latter predominating.

Photoreversal of metarhodopsin III is an attractive interpretation of the deviations of the bleaching curves (Fig. 2) from equation (1), since metarhodopsin III is the only known stable photoproduct that is sufficiently sensitive to the bleaching light. In computer simulations of simple models involving photoreversal of metarhodopsin III, no model has exactly reproduced the curves of Fig. 2, but all are qualitatively successful in accounting for the deviations from equation (1).

Photosensitivity. The inverse photosensitivity constant, Q_e (Alpern, 1971), measured in three cats from the initial slopes of the bleaching curves, ranged from 6.74 to 7.04 log scotopic td.sec, with a mean of 6.87 log scotopic td.sec. In estimating approximately the corresponding retinal quantum fluxes, we proceed by assuming that the cat's relative sensitivity to monochromatic (500 nm) light and to white light, at the cornea, can be equated with that of human rod vision. It follows that for cat as for man, 1 scotopic td.sec is visually equivalent to 4.46×10^5 quanta (500 nm) per square degree of visual angle, at the cornea (Wyszecki

& Stiles, 1967). To obtain the effective retinal quantum flux this must be multiplied by 0.79 to allow for losses in the cat eye media (Dodt & Walther, 1958; Weale, 1954) and again by 1.32 to allow for reflexion at the tapetum (Weale, 1953*a*). And with a posterior nodal distance of 13 mm (Bishop, Kozak & Vakkur, 1962), one square degree corresponds to $5.15 \times 10^{-4} \text{ cm}^2$ at the retina. One scotopic td.sec is therefore equivalent to 9×10^8 quanta/ cm^2 (500 nm) at the cat retina, and the measured Q_e value of 7.4×10^6 td.sec corresponds to 6.7×10^{15} quanta/ cm^2 . The photosensitivity at 500 nm is therefore $1.49 \times 10^{-16} \text{ cm}^2$. The slight difference between this and Dartnall's values (1968) of 1.01 to $1.09 \times 10^{-16} \text{ cm}^2$ at λ_{max} for A_1 rhodopsins in solution with hydroxylamine is easily accounted for by the fact that in the living eye the molecules are arranged in planes approximately orthogonal to the incident light rays. But the precision of our photometry and the accuracy of our assumptions are not sufficient to exclude the small intensity amplification by optical funnelling, postulated by Rushton (1956*a*) and Alpern & Pugh (1974) for human rods.

Quantum catching by cat and man. Of the quanta (500 nm) incident at the retina, cat rhodopsin with a single density of 0.11 would absorb a fraction 0.22 at first passage, and a further 0.055 after reflexion at the tapetum (assuming Weale's value (1953*a*) of 0.32 for tapetal reflectance at 500 nm). If attenuation by the media is allowed for, the total fraction absorbed corresponds to 24% of the quanta incident on the cornea. Similar calculations for man, assuming an ocular transmittance of 50% and a rhodopsin single density of 0.09 (Rushton, 1956*b*) show that 9% of the quanta incident on the human cornea are absorbed by rhodopsin. On this basis, therefore, the cat's quantum catch is 2.7 times that of man. In addition, the pupil area of the dark-adapted cat (160 mm^2 ; Kappauf, 1943) is about 3.2 times greater than that of man (51 mm^2 ; DeGroot & Gebhard, 1952). Hence if a human eye and a dark-adapted cat eye are to absorb quanta at the same rate, the light source must be nine times brighter for the man than for the cat. The dimmest lights detectable by man are of 6 or 7 times the lowest intensity visible to cats (Gunter, 1951). The superiority of the cat's vision in the dark is therefore entirely accounted for by the greater quantum-catching power of the cat eye: the number of bleached molecules that will suffice to produce a visual sensation is about the same in cat and man.

Experiments were carried out at Florida State University, Tallahassee, Florida, with the support of NSF grant GU-2162 and NIH grant EY00684 to W. A. H. Rushton. We thank Professor Rushton for his kind permission to use his densitometer. We are also grateful to the Davis & Geck Division of American Cyanamid for supplying Flaxedil. A.B.B. was supported by PHS training grant GM00874 and a special travel grant from the Research Committee of the Graduate School, Northwestern University.

REFERENCES

- ALPERN, M. (1971). Rhodopsin kinetics in the human eye. *J. Physiol.* **217**, 447-471.
- ALPERN, M. & PUGH, E. N. (1974). The density and photosensitivity of human rhodopsin in the living retina. *J. Physiol.* **237**, 341-370.
- BÄCK, I., DONNER, K. O. & REUTER, T. (1965). The screening effect of the pigment epithelium on the retinal rods in the frog. *Vision Res.* **5**, 101-111.
- BAUMANN, CH. (1966). Der Einfluss von Metarhodopsin auf die Sehpurpurbleichung in der isolierten Netzhaut. *Vision Res.* **6**, 5-13.
- BAUMANN, CH. (1972a). The regeneration and renewal of visual pigment in vertebrates. In *Handbook of Sensory Physiology*, vol. 7/1, ed. DARTNALL, H. J. A. Berlin: Springer-Verlag.
- BAUMANN, CH. (1972b). Kinetics of slow thermal reactions during the bleaching of rhodopsin in the perfused frog retina. *J. Physiol.* **222**, 643-663.
- BISHOP, P. O., KOZAK, W. & VAKKUR, G. J. (1962). Some quantitative aspects of the cat's eye. *J. Physiol.* **163**, 466-502.
- CONE, R. A. & COBBS, W. H., III. (1969). Rhodopsin cycle in the living eye of the rat. *Nature, Lond.* **221**, 820-822.
- DARTNALL, H. J. A. (1968). The photosensitivity of the visual pigments in the presence of hydroxylamine. *Vision Res.* **8**, 339-358.
- DEGROOT, S. G. & GEBHARD, J. W. (1952). Pupil size as determined by adapting luminance. *J. opt. Soc. Am.* **42**, 492-495.
- DODT, E. & WALTHER, J. B. (1958). Netzhautsensitivität, Linsenabsorption und physikalische Lichtstreuung. *Pflügers Arch. ges. Physiol.* **226**, 167-174.
- DOWLING, J. E. (1960). Chemistry of visual adaptation in the rat. *Nature, Lond.* **188**, 114-118.
- DOWLING, J. E. (1963). Neural and photochemical mechanisms of visual adaptation in the rat. *J. gen. Physiol.* **46**, 1287-1301.
- DOWLING, J. E. & HUBBARD, R. (1963). Effects of brilliant flashes on light and dark adaptation. *Nature, Lond.* **199**, 972-975.
- DOWLING, J. E. & RIPPES, H. (1970). Visual adaptation in the retina of the skate. *J. gen. Physiol.* **56**, 491-520.
- EBREY, T. G. (1968). The thermal decay of the intermediates of rhodopsin *in situ*. *Vision Res.* **8**, 965-981.
- ENROTH-CUGELL, C. & SHAPLEY, R. M. (1973). Adaptation and dynamics of cat retinal ganglion cells. *J. Physiol.* **233**, 271-309.
- GUNTER, R. (1951). The absolute threshold for vision in the cat. *J. Physiol.* **114**, 8-15.
- HAGINS, W. A. (1956). Flash photolysis of rhodopsin in the retina. *Nature, Lond.* **177**, 989.
- HAGINS, W. A. & RUSHTON, W. A. H. (1953). The measurement of rhodopsin in the decerebrate albino rabbit. *J. Physiol.* **120**, 61P.
- HOOD, C. & RUSHTON, W. A. H. (1971). The Florida densitometer. *J. Physiol.* **217**, 213-229.
- KAPPAUF, W. E. (1943). Variation in the size of the cat's pupil as a function of stimulus brightness. *J. comp. Psychol.* **36**, 125-131.
- OSTROY, S. E., ERHARDT, F. & ABRAHAMSON, E. W. (1966). Protein configuration changes in the photolysis of rhodopsin. II. The sequence of intermediates in thermal decay of cattle metarhodopsins *in vitro*. *Biochim. biophys. Acta* **112**, 265-277.
- PESKIN, J. C. (1942). The regeneration of visual purple in the living animal. *J. gen. Physiol.* **26**, 27-47.

- REUTER, T. (1966). The synthesis of photosensitive pigments in the rods of the frog's retina. *Vision Res.* **6**, 15-38.
- RIPPS, H. & WEALE, R. A. (1969). Rhodopsin regeneration in man. *Nature, Lond.* **222**, 775-777.
- RUSHTON, W. A. H. (1952). Apparatus for analysing the light reflected from the eye of the cat. *J. Physiol.* **117**, 47 P.
- RUSHTON, W. A. H. (1956a). The difference spectrum and the photosensitivity of rhodopsin in the living human eye. *J. Physiol.* **134**, 11-29.
- RUSHTON, W. A. H. (1956b). The rhodopsin density in the human rods. *J. Physiol.* **134**, 30-46.
- RUSHTON, W. A. H. (1958). Kinetics of cone pigments measured objectively on the living human fovea. *Ann. N.Y. Acad. Sci.* **74**, 291-304.
- RUSHTON, W. A. H., CAMPBELL, F. W., HAGINS, W. A. & BRINDLEY, G. S. (1955). The bleaching and regeneration of rhodopsin in the living eye of the albino rabbit and of man. *Optica Acta* **1**, 183-190.
- STEINBERG, R. H., REID, M. & LACY, P. (1973). The distribution of rods and cones in the retina of the cat. *J. comp. Neurol.* **148**, 229-248.
- TANSLEY, K. (1931). The regeneration of visual purple: its relation to dark adaptation and night blindness. *J. Physiol.* **71**, 442-458.
- WEALE, R. A. (1953a). The spectral reflectivity of the cat's tapetum measured *in situ*. *J. Physiol.* **119**, 30-42.
- WEALE, R. A. (1953b). Photochemical reactions in the living cat's retina. *J. Physiol.* **121**, 322-331.
- WEALE, R. A. (1954). Light absorption in the crystalline lens of the cat. *Nature, Lond.* **173**, 1049-1050.
- WEALE, R. A. (1955). Bleaching experiments on eyes of living guinea-pigs. *J. Physiol.* **127**, 572-586.
- WEALE, R. A. (1957). Observations on photochemical reactions in living eyes. *Br. J. Ophthalm.* **41**, 461-474.
- WEALE, R. A. (1964). Comparison of reactions of human and rabbit fundi to photic exposure. *J. opt. Soc. Am.* **54**, 120-126.
- WEALE, R. A. (1965). Vision and fundus reflectometry. *Documenta ophth.* **19**, 252-286.
- WEALE, R. A. (1967). On an early stage of rhodopsin regeneration in man. *Vision Res.* **7**, 819-827.
- WEALE, R. A. (1973). Metarhodopsin III. In *Biochemistry and Physiology of Visual Pigments*, ed. LANGER, H., pp. 101-104. Heidelberg: Springer-Verlag.
- WYSZECKI, G. & STILES, W. S. (1967). *Color Science*. New York: John Wiley.
- ZEWI, M. (1939). On the regeneration of visual purple. *Acta Soc. Sci. fenn.* **2**, 1-56.