# "Rapid Regeneration" in the Cat Retina

## A Case for Spreading Depression

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ABSTRACT    Fundus reflectometry of the cat retina showed that under certain circumstances a rapid increase in density may follow intense bleaching exposures. The spectral characteristics of the density changes indicated that neither rhodopsin nor its bleach products could be responsible for this effect. The poor condition of the animals in which the phenomenon was observed and its conspicuous absence in the majority of the experimental runs suggested that the effect was associated with a process other than the resynthesis of rhodopsin. It was shown that an extrareceptoral event, spreading depression (SD) of the retina, is the most likely source of the rapid spectral change. The well-known tissue alterations associated with SD were induced in the retina independently of pigment density change. The resultant difference spectra resembled those produced when the rapid density increase occurred spontaneously. It seems likely that the abnormal physiological condition of those cats in which the phenomenon is more frequently observed primes the retina for the light-induced generation of spreading depression.

## INTRODUCTION

Soon after he had introduced a fundus reflectometric method with which to measure photochemical changes in the living eye, Weale (1953 a) reported the results of a study on rhodopsin kinetics in the cat retina. He described a slow process of rhodopsin regeneration (seen in most cats), as well as an extremely rapid process that was observed in only a few of the cats studied. In these animals, photopigment seemed to regenerate completely within 2–3 min ($t_{1/2}$ ≈ 20 s) after a full bleach. Some years later, Bonds and MacLeod (1974), employing a modified version of the Florida Densitometer (Hood and Rushton, 1971), detected a similar phenomenon. They occasionally found density changes with half-times of 20–30 s, and noted that this "rapid recovery" occurred only in animals in poor physiological condition, i.e., with blood pressures <75 mm Hg.

Neither Weale (1953 b) nor Bonds and MacLeod (1974) were able to identify the mechanism underlying the rapid postbleach density increases, although the latter authors suggested that the phenomenon may represent a pathological process. Such fast kinetics would certainly be unusual for rho-

dopsin regeneration in a mammalian retina; even the resynthesis of cone pigments in man proceeds at a slower rate (cf. Hollins and Alpern [1973]). In addition, we (Ripps et al., 1981 b) found that rhodopsin regeneration in cat after a full bleach normally requires >1 h for completion. Nevertheless, if rapid regeneration can occur, it introduces a problem that must be considered in any study on the adaptive properties of the cat visual system. Since the recovery of visual sensitivity may be associated with the resynthesis of bleached photopigment (cf. Dowling [1960 and 1963] and Rushton [1961]), it is essential to know the circumstances under which rapid regeneration occurs and the variability in the rates of regeneration that may be encountered after a bleaching exposure.

But before we address these issues two related questions should be considered. First, is it likely that rhodopsin can regenerate thermally in the cat retina at 50–100 times the normal rate? And second, were this to occur, is it likely to do so in the retina of a sick or dying animal? We think that neither question is likely to evoke an affirmative response, and we believe that the results reported in this paper may help to resolve the enigma of "rapid regeneration."

### METHODS

Absorbance changes ($\Delta D_\lambda$ ) in the living eye were determined with the fundus reflectometric apparatus described in the first paper of this series (Ripps et al. 1981 a). In addition, spectral reflection data were collected from the cat eyecup preparation using the fundus reflectometer modified for incident light microscopy (Dowling and Ripps, 1970). The eye was excised and hemisected, and the posterior half was drained of vitreous and mounted on saline-moistened cotton pads in a silicone mold formed to the shape of the eye. The spectral test beams were directed to the preparation by a Leitz Ultropak system (E. Leitz, Inc., Rockleigh, N. J.) that produced a 2-mm diameter test area on the surface of the eyecup; light reflected from the tapetum was collected by a X 3.8 objective, and, after it passed through masking apertures, reached the photomultiplier of the reflectometer. The analytical procedures have been described (Ripps and Snapper, 1974; Ripps et al., 1978).

To better understand some of the unusual features of the data to be presented here, it is necessary to appreciate the essential differences between the fundus reflectometer employed in this study and the instruments used both by Weale (1953 a) and by Bonds and MacLeod (1974). In our rapid scan device,[1] 29 wavelengths (at 10-nm intervals) of the visible spectrum are sampled sequentially in ~0.3 s. The spectral form of the measured changes in absorbance ($\Delta D_\lambda$), i.e., the difference spectrum, assists in identifying the processes responsible for these changes. Thus, the bleaching and regeneration of rhodopsin give rise to difference spectra characteristic of the chemical events underlying these processes (cf. Figs. 2 and 5 of Ripps et al. [1981 a]).

On the other hand devices of the type employed in the earlier densitometric studies of cat photopigments generally make use of two wavelengths: one (usually deep red) is relatively unaffected by changes in rhodopsin concentration, and thus serves as a reference signal; the other (the test λ) lies within the absorption spectrum of the visual pigment, and monitors the photochemical changes. Kinetic data are derived from changes in reflectivity that take place at the test wavelength relative to those that occur

[1] The technique of fundus reflectometry used in our study was developed by Prof. Weale (1959).

in the reference beam. Extraneous, but spectrally neutral, changes in light output (e.g., those due to fluctuations in pupil size) affect the signals from both the test and reference beams to the same extent and no density change is recorded; when the two wavelengths are affected differentially, it is presumed to be due to the rise or fall in photopigment concentration.

### RESULTS

In the course of making measurements on the regeneration of rhodopsin, we observed absorbance changes that could not be attributed to changes in the concentration of rhodopsin. That is to say, there were abrupt, seemingly spontaneous, shifts in density whose spectral form was unlike any that had been reported in connection with photopigment kinetics. The fact that these aberrant data were obtained under experimental conditions that moments before had given quite conventional results further reinforced the view that the spectral changes were not associated with the regeneration of rhodopsin. Furthermore, we recorded these unusual density changes most often in animals exhibiting respiratory distress, irregular heart rates, lowered blood pressure, and other signs of functional deterioration (cf. Bonds and MacLeod [1974]).

Consider first the density difference spectra obtained under normal circumstances (Fig. 1). Each was recorded at the time indicated after prolonged exposure to an intense light that bleached >98% of the rhodopsin content of the test area. The gains in absorbance seen in each successive spectrum are due mainly to the slow buildup of rhodopsin, although the formation and decay of photoproducts influence the position of the $\lambda_{max}$ in the early recordings (cf. Ripps et al., 1981 $a$ and 1981 $b$). Nevertheless, the kinetics of rhodopsin regeneration can be illustrated by selecting a specific wavelength at which to display the temporal changes in $\Delta D$. In the Bonds and MacLeod (1974) study, for example, the test wavelength was 546 nm, referenced to a deep red color, i.e., $\lambda > 690$ nm. In Fig. 2, the test wavelength is 540 nm, and the density change relative to that measured at 720 nm is plotted as a function of time in darkness after the bleaching exposure. The increase in absorbance continues for at least 50 min and probably longer, although no further recordings were made. But even if the maximum density change were 0.17 (the level reached at 50 min), the half-time of regeneration ($t_{1/2}$) would be 24 min.

Fig. 3 shows the spectral shapes of the "rapid" density changes described earlier. Note that the reflectivity measurements that gave rise to these data were obtained from the *same retinal locus of the same cat* shown in Figs. 1 and 2. The point to be made, however, is that the results of Fig. 3 cannot be due to the regeneration of rhodopsin because none was bleached before the measurements. After the spectra shown in Fig. 1 had been collected and after an additional 20 min of dark adaptation had been allowed, the animal was given a lethal dose of Surital (Parke-Davis, Div. of Warner-Lambert Co., Morris Plains, N. J.). Thus, Fig. 3 shows the actual difference spectra obtained from reflectivity measurements during the 12-min period after the injection. Using the temporal changes in density for the preselected wavelengths (arrows), we produced Fig. 4. Here the gain in absorbance begins at the 3.5-min mark and continues only for ~5 min more. When the plateau is reached, the maximum

value of $\Delta D_{540}$ ($\approx 0.22$) is even greater than the value attained 50 min after the bleaching exposure (cf. Fig. 2). Although the circumstances under which these data were collected were quite different from those that prevail when "rapid regeneration" occurs spontaneously, the spectral changes are similar to those
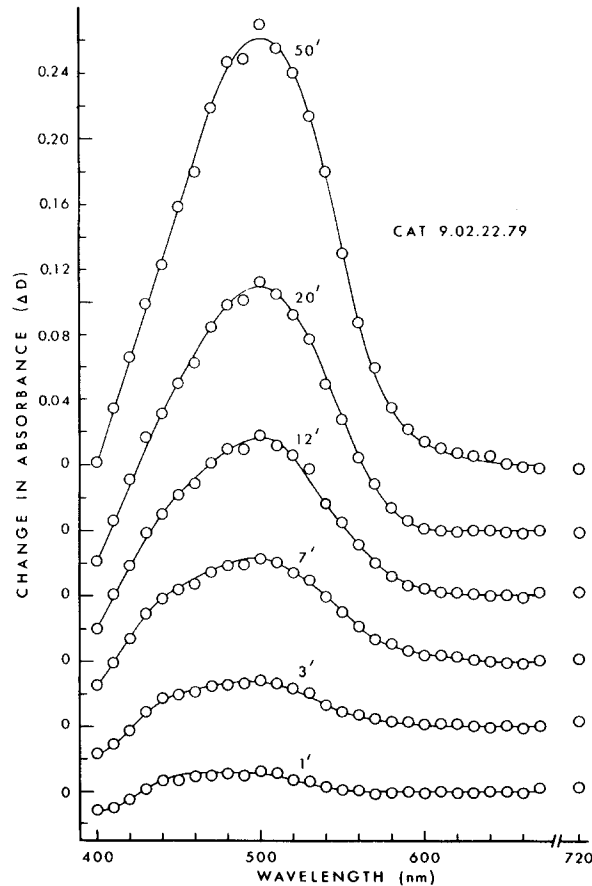


FIGURE 1.    Difference spectra obtained during normal recovery from a 98% bleach in cat. The values of $\Delta D$ at each wavelength represent the absorbance difference between the measurements at the indicated time in the dark and that recorded immediately after the bleach. Bleaching conditions: 8.27 log scotopic td-s (cf. Ripps et al., 1981 a). Data other than those obtained at 50 min have been displaced downward for clarity.

observed during such events. More important, the results illustrate one of the procedures followed in our attempt to identify the source of the phenomenon.

*Spreading Depression*

The experimental conditions under which the results shown in Figs. 3 and 4 were obtained and the fact that the absorbance changes of Fig. 3 are not

spectrally neutral led first to a consideration of the possibility that vascular changes underly these density difference spectra. However, neither the absorbance differences between oxygenated and deoxygenated blood (Dacie and Lewis, 1968; Sidwell et al., 1938) nor those between normal and exsanguinated tissue (Flower et al., 1978) account for the spectral form of any of the data representative of "rapid regeneration." On the other hand, the fact that such density changes could be induced by intense lights in the retinae of animals in poor physiological condition led to the suggestion that the phenomenon may be due to spectral changes in retinal transmissivity associated with spreading depression.[2]
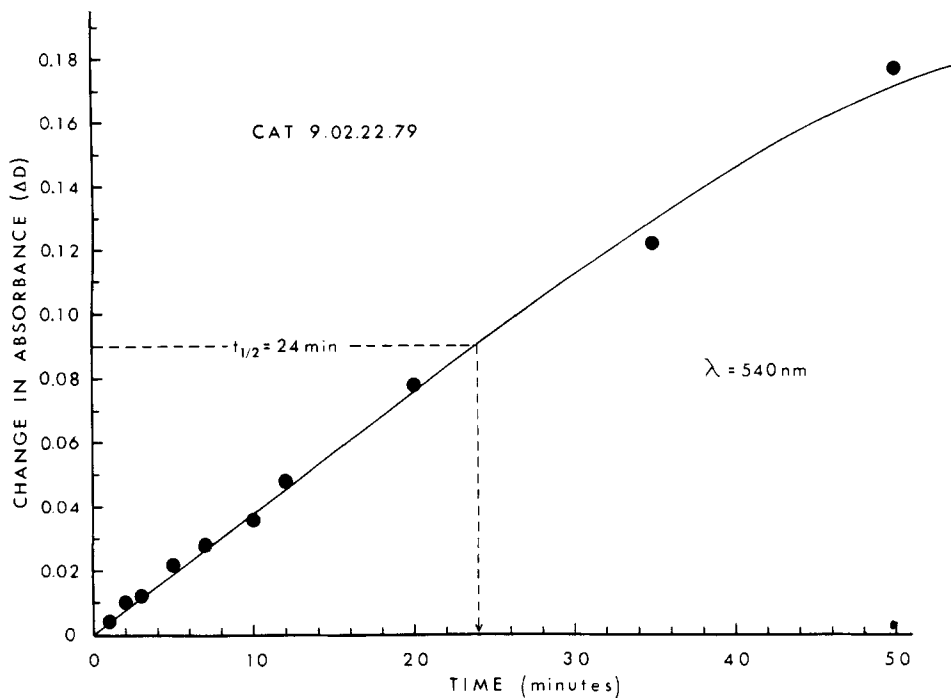


FIGURE 2.   The temporal course of absorbance changes at 540 nm with respect to changes at the red end of the spectrum (720 nm). Data were obtained directly from the difference spectra of Fig. 1.

Spreading depression can be induced in a variety of ways (cf. Brinley et al. [1960] and van Harreveld [1978]), and Fig. 5 shows results obtained from reflection measurements on the cat eyecup at various times after application of a drop of 1 M KCl at the edge of the retina ~6–10 mm below the recording site (Sugaya et al., 1975). The increases in absorbance were similar in some respects to those obtained after the Surital injection (Fig. 3), and were accompanied by a semitransparent milky wave that moved across the retina as originally described by Gouras (1958). The observation by Weale (1957) that the density increase associated with rapid regeneration is followed by a

[2] We are indebted to Dr. Geoffrey Arden of the University of London for this suggestion.

decrease in density after 2–3 min is consistent with the notion of a transient wave. After the wave had passed and the color change had fully dissipated (~10 min), the procedure could be repeated with similar results.

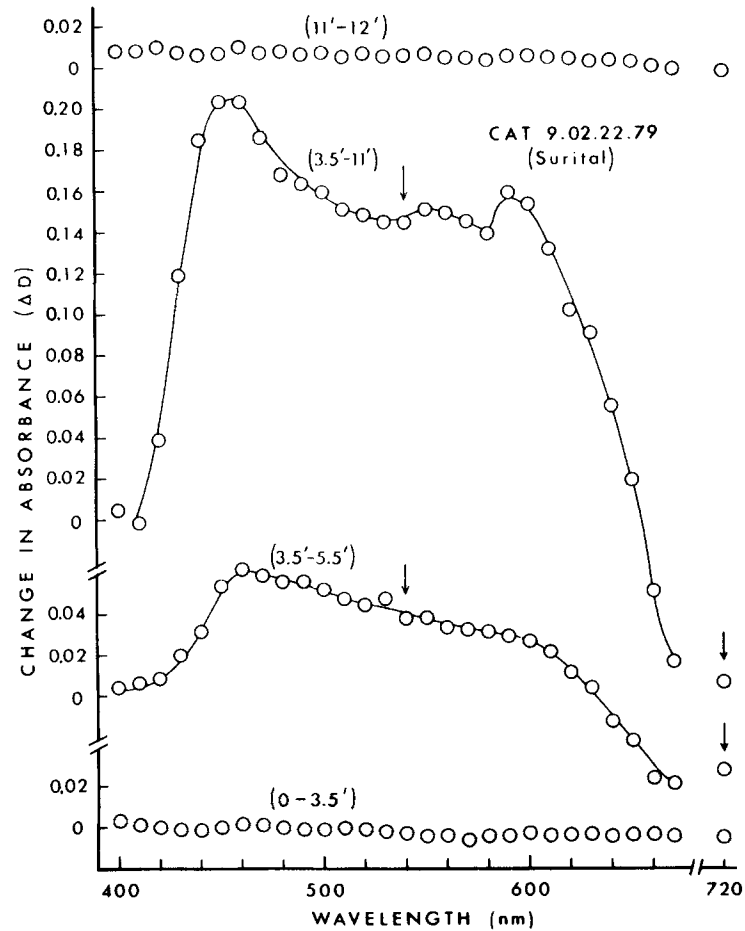The irregular curves of Fig. 5 are typical of the results usually obtained



FIGURE 3.   Examples of the difference spectra recorded after a lethal dose of Surital administered after 70 min of dark adaptation. Arrows indicate the two wavelengths used to determine the temporal changes in absorbance shown in Fig. 4. 720 nm provides the reference point against which absorbance changes at 540 nm are computed. Data are from the cat used to obtain the data in Figs 1 and 2.

after spreading depression had been induced in the eyecup preparation, but not all of the difference spectra resulting from this maneuver were as far removed in appearance from the absorbance spectrum of rhodopsin. Fig. 6 shows the results of an experiment on the fellow eye of the same cat. In this case, the difference spectra resulting from the KCl-induced opacification

resembles the bell-shaped curves of photopigment regeneration. However, the $\lambda_{max}$ is at a longer wavelength than is characteristic of rhodopsin, and both the long- and the short-wavelength regions show peculiar fluctuations in absorbance. But most significant is that the *visual pigments were not bleached before or during the measurements*.

### DISCUSSION

The results of extensive experimentation on the in vivo regeneration of rhodopsin in cat, tested by spectral-scan fundus reflectometry, have failed to reveal a single instance of the extremely fast regeneration process said to
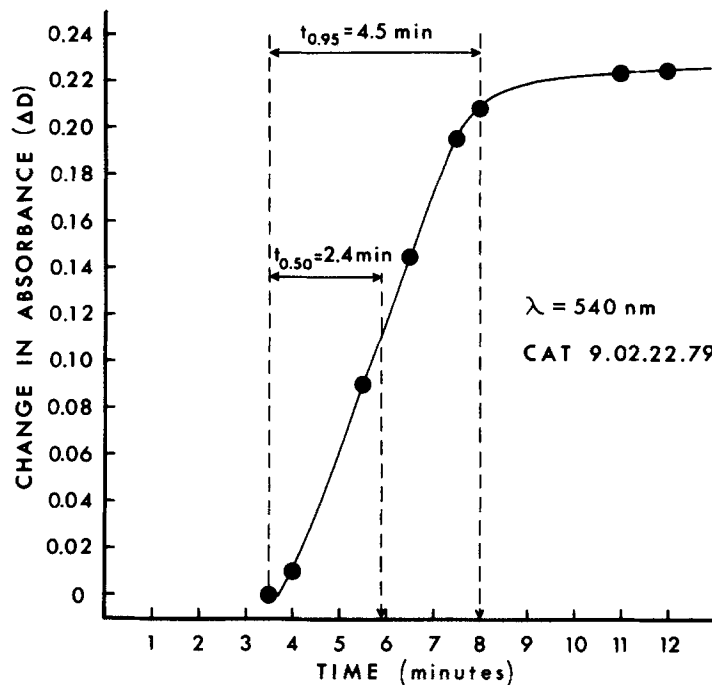


FIGURE 4. Time-course of absorbance changes at 540 nm with respect to changes at 720 nm. The data were taken from difference spectra spanning the full range of test wavelengths (see Fig. 3).

occur, although infrequently, in the cat retina (Weale, 1953 *a* and 1953 *b*; Bonds and MacLeod, 1974). On several occasions, however, the absorbance changes recorded after an intense bleaching exposure revealed a progressive increase in optical density in the midregion of the visible spectrum relative to that measured at the long-wavelength end. The changes ranged from about 0.2 to 0.3 density units and occurred over 5 min or less, in agreement with the magnitude and time-course of the changes reported by Weale (1959) and Bonds and MacLeod (1974). The shapes of the difference spectra, however, left little doubt that these changes could not be due to a buildup of rhodopsin or any other photopigment (cf. Fig. 3). Nevertheless, it is apparent that when

such spectral changes are manifest, attempts to assess the kinetic process by taking measurments at only one or two test wavelengths (cf. Fig. 4) yield data that may be interpreted erroneously as demonstrating a rapid regenerative process.

It is noteworthy that both in the study by Bonds and MacLeod (1974) and in the present work, the unusually fast density changes referred to above were
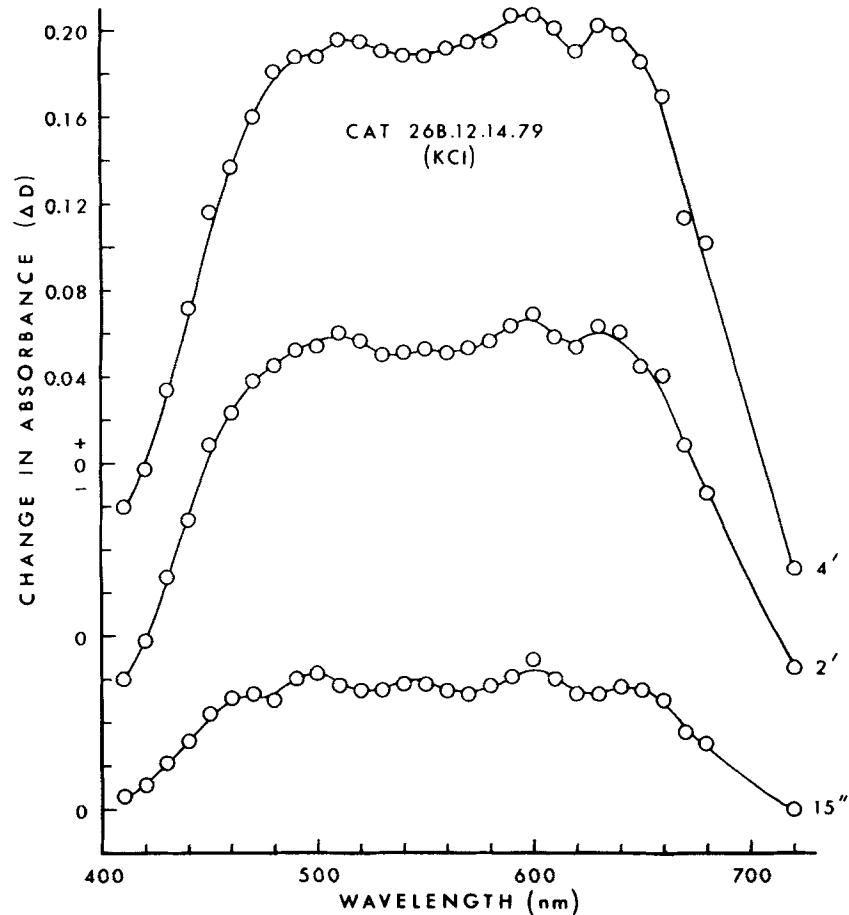


FIGURE 5.  Difference spectra obtained during passage of a wave of spreading depression induced in an eyecup preparation by the application of one drop of 1 M KCl to the edge of the retina. The values of $\Delta D_\lambda$ at each time are with reference to the absorbance before application of the KCl.

detected in animals in poor condition. Indeed, analysis of the absorbance-difference spectra associated with morbid events (Fig. 3) led us to explore the notion that a light-evoked spreading depression (SD) was responsible for these unique spectral changes. It is well known that SD, a transient pathological condition discovered by Leão (1944) in cerebral cortex, is accompanied by an efflux of potassium into the extracellular space (Grafstein, 1956), and the

magnitude of the $K^+$ efflux seen post-mortem approximates that which occurs during SD (Brinley et al., 1960).

Spreading depression was first observed in the vertebrate retina by Gouras (1958), who showed that the retinal changes presented the same basic features as the cortical phenomenon. In addition to describing the profound loss of sensitivity that marks this condition, Gouras's account of the visible changes
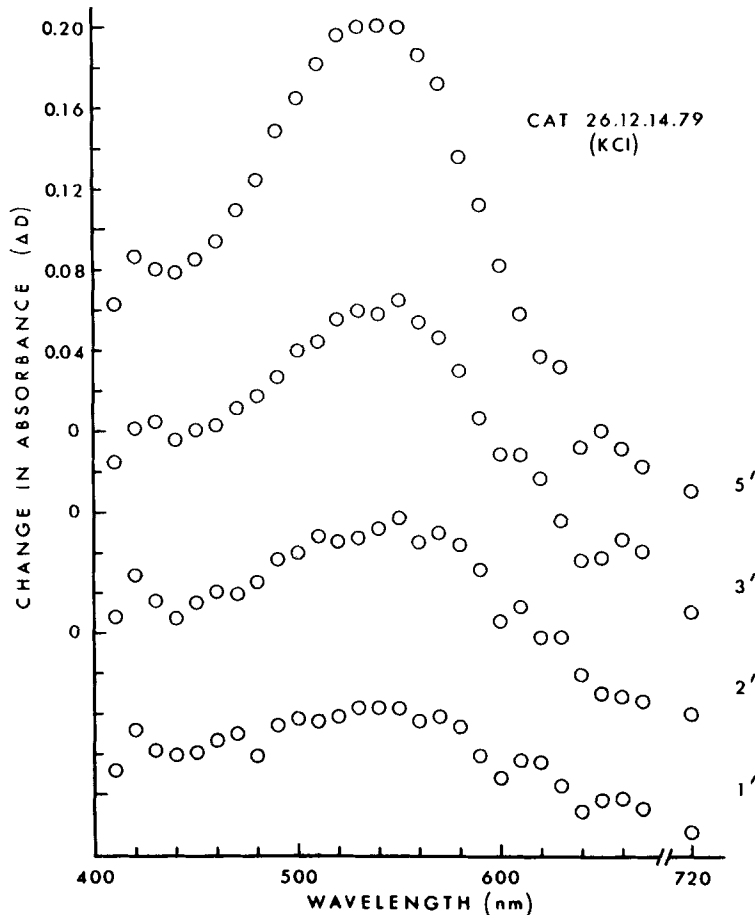


FIGURE 6. Difference spectra obtained as for Fig. 5 but from the other eyecup of the same cat. The difference spectra resemble in some respects those produced during photopigment regeneration. Note, however, the location of the peak at ~545 nm and the irregularities in the data at the ends of the spectrum.

accompanying SD accurately describes the effects we observed after applying KCl to the retina of the cat: "The phenomenon is characterized by a spontaneous 'milky' wave that periodically marches across the surface of the retina and lasts for 2–3 minutes at any one point." The color change noted by Gouras and others has been exploited by Martins-Ferreira and DeOliveira Castro (1966), who monitored the concomitant fluctuations in light scatter by

the retinal tissue and thereby were able to time the passage of the wave. The reflectivity measurements of the present study, which provide yet another means for estimating the time-course of the SD, show that the density changes are neither spectrally neutral nor entirely consistent in spectral shape (cf. Figs. 5 and 6).

Results obtained from both light-scattering and electrophysiological studies have shown that SD originates in the inner plexiform layer (Martins-Ferreira and DeOliveira Castro, 1966 and 1971; Mori et al., 1976), although other retinal layers appear to contribute to the wave of opacification. We have not yet explored the possibility that, in the cat, changes in tapetal reflectivity are involved secondarily in affecting the transmissivity data (Weale, 1953 c). In this connection, we should mention that some preliminary observations of SD in the isolated frog retina, measured by transmission spectrophotometry, gave absorbance spectra similar to those of Figs. 3 and 5, and Weale (1957) reported that the 'rapid effect' occurs also in the frog eye.

The fact that recurrent waves of SD can be elicited by optical stimulation either with flickering light (Martins-Ferreira et al., 1974), or at the offset of intense illumination in retinae bathed in chloride-free solutions (Olsen and Miller, 1977) raises the possibility that the high rate of neural activity resulting from these stimulus conditions (cf. Miller and Dacheux [1975]) may contribute to the production of SD (Gouras, 1958; Martins-Ferreira and DeOliveira Castro, 1971). However, the wide variety of stimuli capable of initiating a wave of SD (cf. Leão and Morrison, 1945; Leão, 1947; Sugaya et al., 1975) suggests that any maneuver that significantly alters the ionic composition of the extracellular space or the integrity of neuronal membranes suffices to produce the phenomenon. In any event, it seems likely that the rapid increases in retinal absorbance seen in some cats are not due to photochemical events. Rather, the abnormal physiological condition of these animals primes the retina for the light-induced generation of SD.

The dramatic changes in retinal absorbance that occasionally follow exposure to bright light and that we attribute to SD may be relevant to the growing concern with nonthermal photic injury to the retina (Calkins and Hochheimer, 1980; Sperling, 1980). Intense light sources have been shown to be capable of producing in primates pathological changes in the retina (Tso, 1973) as well as long-term visual defects (Harwerth and Sperling, 1971). Although the mechanism of these damaging effects has yet to be identified, it is possible that SD is associated with an early stage in the pathological process. If so, retinas already compromised due to existing disease may be particularly susceptible to this form of injury.

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