The Electroretinogram of a Diurnal Gecko

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ABSTRACT Using the electroretinogram as the criterion of retinal activity the flicker fusion frequency, course of dark adaptation, and spectral sensitivity of the pure cone retina of the diurnal gecko, *Phelsuma inunguis*, were investigated. Both the curve relating flicker fusion frequency to stimulus intensity and that relating the amplitude of the flicker response to stimulus intensity showed a break as the intensity was increased. The dark adaptation curve was that typical of cone retinae; there was no break, adaptation was relatively rapid, and there was a total increase of sensitivity of only about 3 log units. The spectral sensitivity curve showed two maxima, a major one at about 560 mμ and another at about 460 mμ. Chromatic adaptation with red and blue lights demonstrated the presence of two independent mechanisms. Although red adaptation could not have had a direct effect on the pigment responsible for the "blue" mechanism the sensitivity of this mechanism was depressed by red adaptation. The possible relationships of the two mechanisms are discussed.

INTRODUCTION

The geckos are a group of considerable interest to the visual physiologist. Most geckos are nocturnal in habit and are usually considered to possess a pure rod retina, but there are some diurnal species. Walls (1934) believes that the rods of nocturnal geckos have been transmuted from the cones of diurnal lizard ancestors. Confirmatory evidence for this theory has since been provided by Underwood (1951, 1954) who has made a detailed study of many gecko species. Walls has further suggested that some of those gecko species which are now diurnal in habit and which possess cone-like visual cells, have reverted from nocturnal gecko ancestors and that their visual cells have undergone a second transmutation from rods back into cones. He mentioned the diurnal *Phelsuma* species as having undergone this second transmutation and made certain predictions about their ocular morphology which have since been shown to be correct (Tansley, 1961).

In view of their evolutionary history the visual pigments of the gecko retina are of especial interest. Crescitelli (1958) extracted the retinae of eleven species of nocturnal geckos and found a set of unusual visual pigments which, al-

though possessing a retinene$_1$ chromophore, had absorption maxima ranging from 518 to 528 mμ. He speculated that these might be intermediate forms between rhodopsin and iodopsin. He was unable to extract any pigment from the retina of one diurnal species, *Sphaerodactylus argus*, a common result with pure cone reptilian retinae. The absorption characteristics of the diurnal visual pigment should, however, be reflected in the spectral sensitivity of the eye and we, therefore, decided to investigate the spectral sensitivity of *Phelsuma* using the electroretinogram as an index of activity.

An additional objective was to compare the electrical activity of the *Phelsuma* eye with that of other pure cone animals (since only a few such investigations have so far been made) and with that of two species of nocturnal geckos which have recently been examined in some detail by Dodt and his collaborators (Dodt and Walther, 1959; Dodt and Jessen, 1961).

METHODS

Specimens of *Phelsuma inunguis* were flown from Mauritius where this is the only diurnal species. The animals were kept in heated glass tanks and fed on *Calliphora* and *Drosophila* specially hatched in the laboratory. Half oranges and water were also provided. With this regime the animals remained in good condition for 4 to 6 months. Some animals were killed by decapitation and used for histology.

The gecko was narcotized with ether until its movements grew sluggish. A drop of 2 per cent amethocaine was applied to the spectacle which was then removed. Anesthesia was discontinued and movements of the limbs, trunk, and tail prevented by placing the animal on a piece of sticky tape. The gecko was transferred to a cork board and its jaws opened and held apart with pins stuck in the cork. These helped to keep the head still and did not seem to inconvenience the animal. The temperature was maintained between 28 and 31°C with a small electric heater. This method of preparation was adopted after it had been found in early experiments that anesthesia or cooling resulted in small abnormal electroretinograms being recorded. If the initial administration of ether was continued until the animal was completely anesthetized a normal electroretinogram might not be recorded for some hours. Two hours or more of dark adaptation preceded the experiments. Even after the longest experiments the animals, once released, were normally active.

Silver-silver chloride wick electrodes were used. They were carefully light-screened. One wick was placed on the cornea, the other on the roof of the mouth just inferior to the floor of the orbit. The earth was placed under the tongue. The differential preamplifier, if AC-coupled, had a time constant of 2 seconds and an upper frequency response of 200 CPS. It fed a tektronix 502 cathode ray oscilloscope. The final deflection on the tube face was 40 μv/cm or, if DC recordings were made, 200 μv/cm. All traces were photographed onto 60 mm paper and measured to 0.5 mm.

Two similar light systems were employed, one for adapting the eye and the other for evoking electroretinograms. The optical system focused the image of a tungsten filament projection lamp upon the gecko's cornea. The light intensity and wavelength

were altered by filters, the lamp current being maintained constant. Ilford neutral gelatin filters were used for altering the stimulus intensity. The colour filters used for altering the stimulus wavelength were ten Schott "depal" double interference filters with maximum transmissions at 428, 447, 469, 487, 505, 526, 549, 569, 586, and 608 mμ respectively. The beam in which the filters were interposed was a slightly convergent one but with these filters this amount of convergence does not affect the transmitted wavelength. For the colour adaptation experiments two filters, Ilford bright spectrum violet and Ilford 610, were used in the adapting beam. The violet filter had a cut-off at about 510 mμ. Single stimuli were provided by a self-cocking photographic shutter (ibsor) the usual flash duration being 1.1 seconds. Repeated stimuli were produced by a rotating drum shutter giving a light: dark ratio of 1:3. The intensity provided by this optical system was one-tenth that of the single flashes. The photocell monitor of the stimulus was displayed on the cathode ray tube.

Removal of the gecko's spectacle caused the iris to become fixed and the cornea opalescent. For this reason the stimulating and adapting sources illuminated a very large fraction of the retina so that the fact that they were merely placed side by side and did not share the same optical system was of no importance. The intensities of the stimulating and adapting systems were compared visually and found to differ by less than 0.5 log unit. The stimulating intensity was determined by luminance matching (human eye, photopic conditions). The diameter of the gecko pupil under the experimental conditions was about 1 mm, but the posterior nodal distance, measured in sections through the whole eye, is only about 1.5 mm, allowing for shrinkage during preparation. The equivalent retinal illumination for the gecko is, therefore, about 19,500,000 trolands.

All filters were calibrated on a spectrophotometer. The relative intensities of the monochromatic stimuli were measured by putting a calibrated photomultiplier in the light path. The energies were then expressed (for the calculation of the sensitivity curves) in terms of the relative number of quanta they contained.

The spectral sensitivity curves were calculated as follows: electroretinograms for each wavelength were evoked by stimuli of different intensities and amplitude/intensity curves constructed, usually for the *b*-wave but in some cases for the off-effect as well. The neutral density filter necessary to reduce the response to a constant amplitude was read off from these graphs and the sensitivity curves calculated accordingly using the energy corrections obtained for each colour filter. In order to compensate for apparent changes in the sensitivity of the eye during the experiments (these were for the most part due to slight movements of the electrodes) records were obtained for the stimulus at 608 mμ directly before or after each of the other wavelengths, and all sensitivities were calculated as logarithmic fractions of that at 608 mμ. The means and standard errors were calculated from these results. Where sensitivities are expressed linearly the values were obtained by taking the antilogs of these results and appropriate scaling. The average *b*-wave amplitude taken as a criterion was 90 μv, but in the light-adapted experiments the electroretinogram was so much reduced in amplitude that lower criterion values had to be used in order to include the short wavelengths.

For the dark-adapted curves an interval of 0.5 to 1 minute was allowed between

stimuli, the actual interval depending on the stimulus intensity used. For the light-adapted curves the eye was continuously exposed to the adapting light and the stimulus interval was reduced to 15 seconds.

Dark adaptation curves were obtained by evoking a series of electroretinograms at different intensities at measured intervals after the end of a period of light adaptation, and plotting a series of curves showing the increasing response with time in the dark for each intensity. From these a series of amplitude/intensity curves could be constructed for any given moment of dark adaptation. The method has been described in detail by Dodt and Jessen.

RESULTS

The microscopic structure of this retina is like that of *Phelsuma madagascariensis* and has already been described (Tansley, 1961). This is a pure cone retina.

Useful records were obtained from twenty-six animals.

The actual shape of the electroretinogram varied slightly from animal to animal but *a*- and *b*-waves and off-effects were all produced under suitable conditions; *c*-waves were always absent. The voltage recorded for the *b*-wave was usually less than 200 μv so that a high gain AC-coupled system was employed. In dark adaptation and with low intensity stimuli the *b*-wave was smooth and rounded with a long culmination time and an *a*-wave and off-effect did not develop. As the stimulus intensity was increased the *b*-wave became less rounded and the culmination time decreased. A small *a*-wave was present if the stimulus intensity was not too low, but an off-effect was only produced at the highest stimulus intensities (Fig. 1). There was no significant alteration in the dark-adapted wave form when the wavelength of the stimulus was altered through the spectrum (Fig. 2). The slight distortion of the later phase of the electroretinogram of Fig. 2 *B* is caused by movement of the animal. Such artefacts ruled out any except crude comparisons of waveform. When the eye was light-adapted a well marked off-effect was present at all but the lowest stimulus intensities.

One component of the electroretinogram, a maintained DC potential, was seriously distorted by the time constant of the amplifier. With DC amplification and a high stimulus intensity the dark-adapted electroretinogram, after an initial *a*-wave, presented the unusual appearance of two positive steps, one at the beginning and one at the end of the flash (Fig. 3 *A*). In light adaptation, however, the wave form altered, the *a*-wave and off-effect becoming larger and the potential being no longer maintained during the stimulus, but falling instead nearly to the baseline (Fig. 3 *B*). When longer stimuli were used the potential actually fell below the baseline. It is this alteration in the maintained potential that causes the light- and dark-adapted electroretinograms to appear so different when recorded with a condenser-coupled system.

Fig. 4 shows the *b*-wave spectral sensitivity curves, plotted on a logarithmic basis, in dark adaptation (top curve) and when the eye was adapted to red (crosses) and blue light (circles), together with the standard errors of the

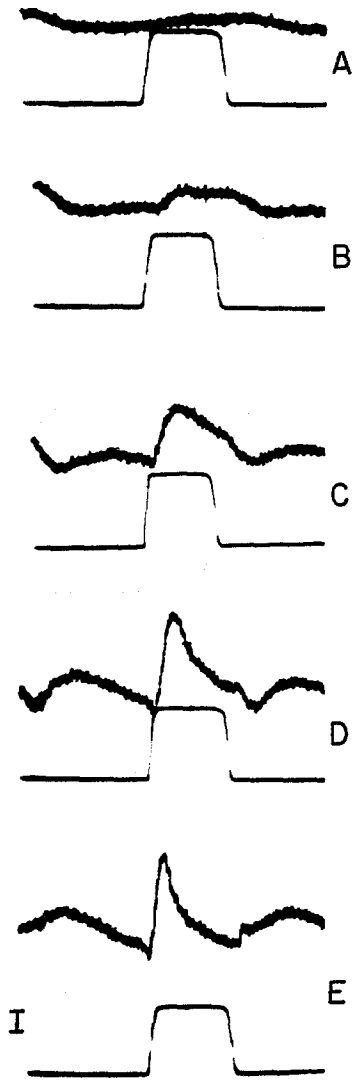

FIGURE 1. The effect of stimulus intensity on the electroretinogram. Stimulus white light with neutral tint filters of density *A*, 4.5; *B*, 3.0; *C*, 2.0; *D*, 1.0; *E*, full intensity. Dark adaptation. Calibration 80 μv. Stimulus duration $\frac{1}{6}$ sec. Time constant 2 sec.

points. No standard error can be shown for the points at 608 mμ since this was the check wavelength. In dark adaptation the maximum sensitivity lies between 550 and 570 mμ. The shape of the curve is less well defined in the short-wave part of the spectrum; there may be a subsidiary maximum around 490 mμ and there is an indication of an inflexion at about 460 mμ but the errors are too great and the number of wavelengths investigated too few for

certainty. Fig. 4 is plotted on an absolute basis and shows how far both the red and blue adapting lights depressed the sensitivity of the eye to 608 mμ. The positions of these points were calculated in two different ways. First by comparing the amplitude/intensity curves obtained in the dark and after switching on the adapting light and second by direct measurement of the average sensitivities at 608 mμ in terms of the neutral density filters which gave the criterion voltage. The results of the two methods of calculation agreed very well. It can be seen that the sensitivity was depressed by our

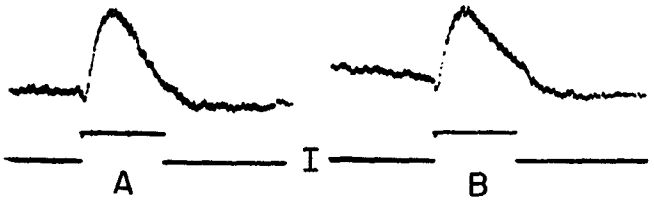

FIGURE 2. The effect of wavelength on the electroretinogram. *A*, stimulus 447 mμ + 0.75 neutral filter. *B*, stimulus 608 mμ + 2.25 neutral filter. Dark adaptation. Calibration 40 μv. Stimulus duration 1.1 sec. Time constant 2 sec.

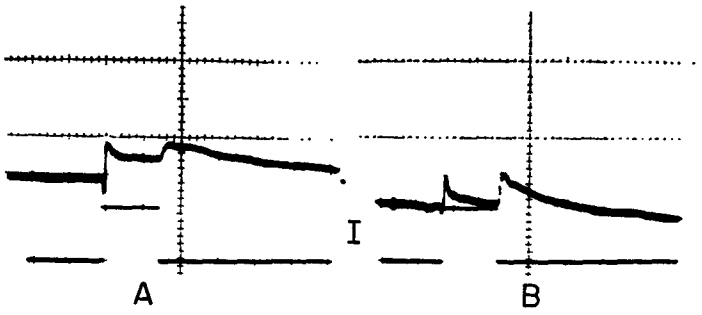

FIGURE 3. The effect of adaptation on the electroretinogram. Stimulus white light full intensity. *A*, dark-adapted; *B*, light-adapted. DC recording. Calibration 200 μv. Stimulus duration 1.1 sec.

red and blue adapting lights by about the same amount, about 1.4 log units. The reduction of sensitivity by light adaptation was not very great but this was found to be characteristic of this gecko eye as it is of other pure cone retinae (Tansley, 1957). Blue light adaptation seems to have produced little change in the shape of the spectral sensitivity curve although the possible subsidiary maximum at 490 mμ was abolished. This was not the case for red light adaptation. Here the long-wave sensitivity was more depressed than the short-wave sensitivity so that, while the 560 mμ maximum was still present, it was surpassed by another maximum at about 460 mμ which is only indicated in the dark-adapted curve.

Fig. 5 makes the point clearer. In this figure the sensitivity curves are plotted on linear coordinates and arbitrarily scaled together at 608 mμ. The mean dark-adapted sensitivity curve (points) is compared with a mean curve from experiments in which the red adapting light had only a small effect (circles) and with another mean curve from experiments in which the light was more intense and the effect was great (triangles). In the former

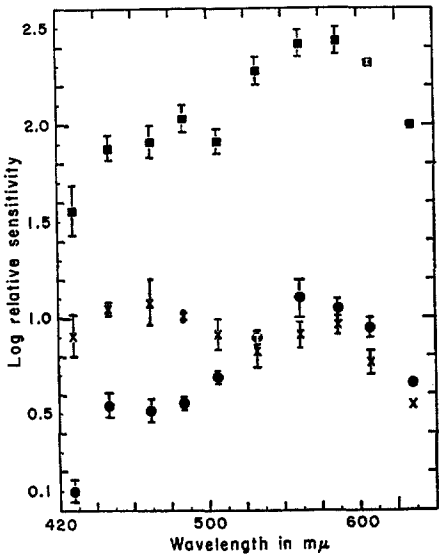

FIGURE 4. Mean spectral sensitivity curves. ■, dark adaptation; ×, red adaptation; ●, blue adaptation. Vertical lines represent twice the standard error of the points. Ordinate, log sensitivity. Abscissa, wavelength in mμ.

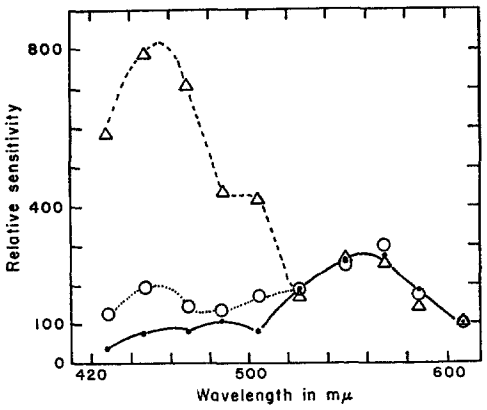

FIGURE 5. The effect of red adaptation on the spectral sensitivity curve. ●, dark adaptation; ○, red adaptation (two experiments); △, more intense red adaptation (two experiments). Ordinate, relative sensitivity. Abscissa, wavelength in mμ.

case red light adaptation only depressed the sensitivity to 608 mμ by 0.6 log unit, in the latter by 2.1 log units. Where the red light adaptation was less effective the shape of the spectral sensitivity curve was not much altered from that obtained during dark adaptation. Where the adaptation was more effective the maximum in the blue wavelengths became overwhelmingly preponderant. In this curve there is a shoulder at 490 mμ which may correspond to the high point on the dark-adapted curve at this wavelength. In connection with Fig. 5 it must be emphasized that the more effective the red adaptation was in depressing sensitivity to 608 mμ the more effective it

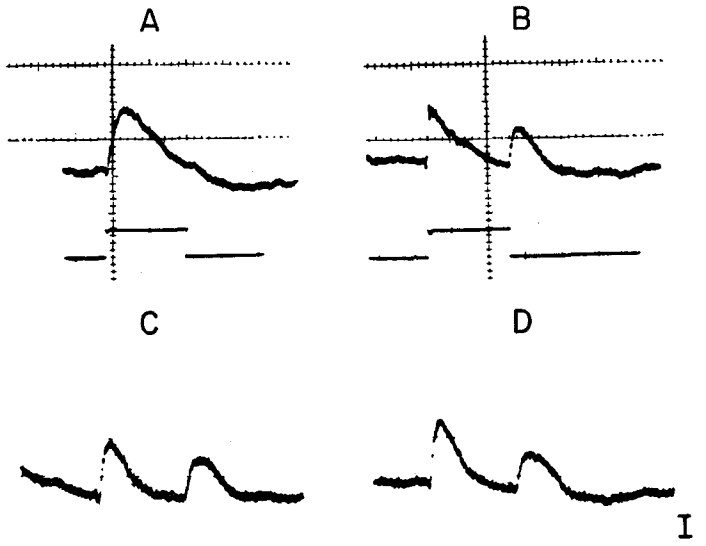

FIGURE 6. The effect of red and blue adaptation on the electroretinogram. Top row, red adaptation. *A*, stimulus 447 mμ + 0.25 neutral filter; *B*, stimulus 608 mμ + 0.25 neutral filter. Bottom row, blue adaptation. *C*, 447 mμ full intensity; *D*, 608 mμ + 1.25 neutral filter. Calibration 40 μv. Stimulus duration 1.1 sec. Time constant 2 sec.

was at all wavelengths. The dotted part of the lower red light–adapted curve in Fig. 5, if plotted on the logarithmic scale of Fig. 4, would lie between the dark-adapted and the two light-adapted curves while the dashed part of the upper curve in Fig. 5 replotted on Fig. 4 would lie below the light-adapted curves even in the short-wave part of the spectrum. The red light adaptation caused a marked depression of blue sensitivity.

White light adaptation depressed the sensitivity at all wavelengths but did not alter the shape of the spectral sensitivity curve. In other words there was no Purkinje shift.

It was found that the two types of chromatic adaptation did not have identical effects on the electroretinogram wave form (Fig. 6). During blue light adaptation stimuli of all wavelengths evoked typically light-adapted

responses with marked off-effects. With red light adaptation normal light-adapted records were produced by stimuli of wavelength 487 mμ or longer while stimuli of shorter wavelength resulted in electroretinograms with more rounded *b*-waves and no off-effect, like those produced in the dark-adapted eye. Spectral sensitivity curves for the off-effect were plotted for three experiments with red light adaptation. The curve for one of these is shown in Fig. 7 together with the corresponding part of the curve for the *b*-wave. The two curves are plotted on a linear scale and arbitrarily scaled together at the 549 mμ maximum. It will be seen that there is good agreement between these two

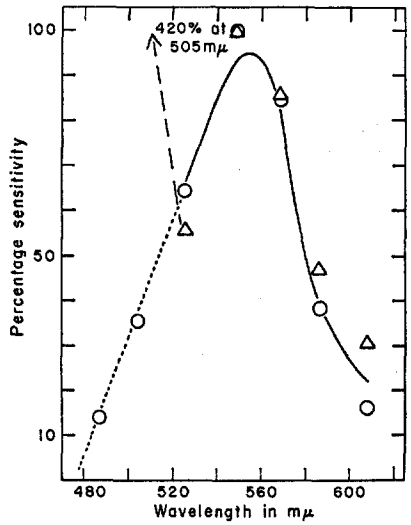

FIGURE 7. Spectral sensitivity curves for the "on" and "off" responses in red adaptation. ○, off response; △, on response (*b*-wave). Ordinate, percentage sensitivity. Abscissa, wavelength in mμ.

curves for the "on" and "off" responses at the longer wavelengths but that at the shorter wavelengths the *b*-wave curve rises to a second maximum which is absent in the curve for the off-effect.

The lens of *Phelsuma inunguis* is a very pale yellow. Owing to its extremely small size we have not been able to measure its spectral transmission and the values given for the spectral sensitivity curves have not therefore been corrected for the lens tranmission. This omission should not affect the long wavelength part of the curves but the values for the blue wavelengths as given will be a little too low.

The wavelets produced by intermittent stimulation of this eye are very small and it was easier to get measurable traces with a light:dark ratio of 1:3. With a slow flicker of approximately 2 stimuli per second the responses were similar to those obtained with single flashes. Recording with DC coupling

demonstrated that the slow positive potentials produced by each stimulus were summated so that the general potential level rose (Fig. 8). The response to each stimulus superimposed on this general potential was a large *a*-wave followed by a *b*-wave which became more and more nearly submerged in the general DC shift as the flicker frequency was increased (Fig. 8 *B* and *C*). At the higher frequencies the *a*- and *b*-waves came together to produce a simple saw-tooth record (Fig. 8 *D*). With AC coupling it could be seen that the

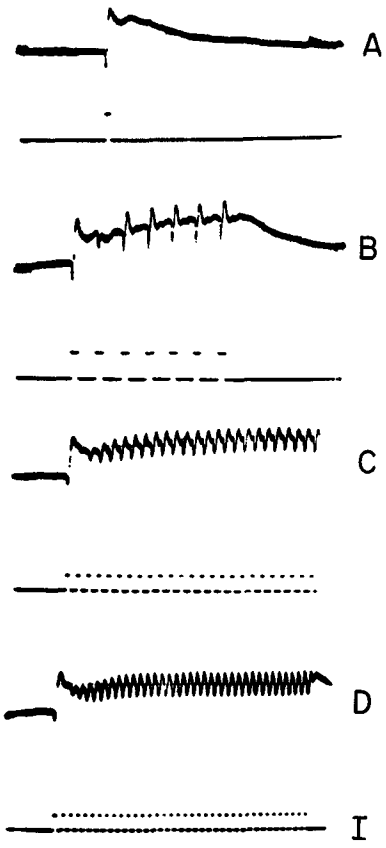

FIGURE 8. The development of flicker. Stimulus white light full intensity. *A*, single flash 1/10 sec.; *B*, 1.5 flashes per sec.; *C*, 3 flashes per sec.; *D*, 6 flashes per sec. DC recording. Calibration 200 μv.

interacting *b*-wave broke up into a series of wavelets (Fig. 9) similar to those produced by other pure cone eyes (Tansley, Copenhaver, and Gunkel, 1961 *a*) and by the human eye under photopic conditions (Rendahl, 1958). This is unlike the single stimulus *b*-wave which was always simple in form under our experimental conditions. This pattern of development of flicker responses was followed in all cases in which the stimulus intensity was high enough to produce an *a*-wave. The flicker records taken at lower intensities where there was no *a*-wave were so small that it was impossible to be sure how the flicker responses developed.

Flicker fusion frequency was investigated by photographing superimposed traces on a time base which was triggered by the photocell monitor at increasing repetition rates. The eye was exposed to the flickering light for about 1 minute at each frequency and therefore the state of adaptation was wholly dependent on the stimulus intensity used. The criterion of fusion was that no wavelets were visible on the superimposed traces and this point

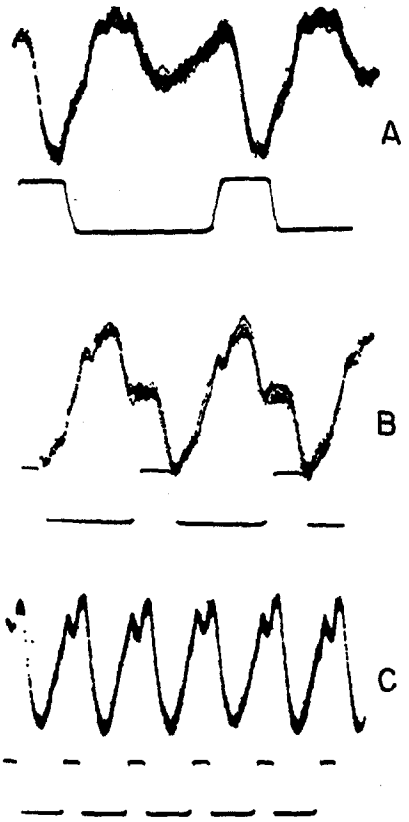

FIGURE 9. The development of flicker. Stimulus white light full intensity. *A*, 9.3 flashes per sec.; *B*, 13.2 flashes per sec.; *C*, 31.3 flashes per sec. Calibration 40 μv.

depended to some extent on the gain employed and on the noise level. The sweep speeds were adjusted so that the traces all had much the same appearance. In other experiments continuous records were made on moving paper while the flicker rate was increased but, while both techniques gave similar results, the former was the more accurate. Fig. 10 shows (for one experiment) fusion frequency/intensity curve (circles), the total amplitude/intensity curve at a constant frequency of 2 per second (triangles), and the *a*-wave amplitude/intensity curve at the same frequency (lowest curve). It

is clear that the first two curves have two branches and that the break in the first is at a frequency of about 10 per second. The intensity at which the break appears is near that at which the *a*-wave first becomes apparent.

Before beginning an experiment for the determination of the dark adaptation curve the sensitivity of the dark-adapted eye was measured and the criterion of success was that the sensitivity should finally return to the same level. A typical dark adaptation curve is shown in Fig. 11. In this experiment the maximum intensity of the adapting light was used but this only depressed

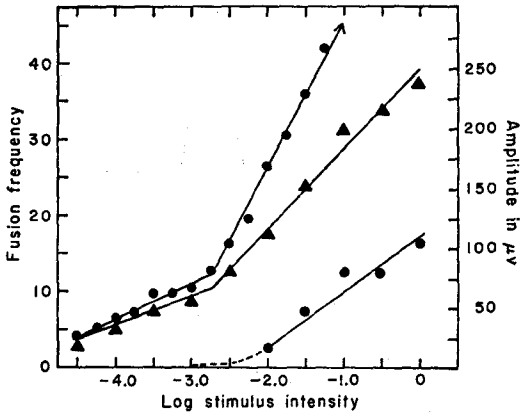

FIGURE 10. The effect of stimulus intensity on flicker responses. ●, curve relating flicker fusion frequency to stimulus intensity; ▲, curve relating amplitude of response to stimulus intensity at a constant frequency of 3 per sec.; ● (lower curve), curve relating *a*-wave amplitude to stimulus intensity at a constant frequency of 3 per sec. Ordinate, (left hand), fusion frequency; (right hand), amplitude in microvolts; abscissa, log stimulus intensity. AC recording.

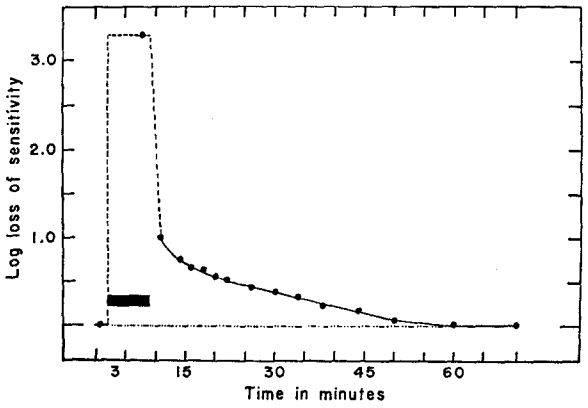

FIGURE 11. Dark adaptation curve. Ordinate, log loss of sensitivity; abscissa, time in minutes. The black bar represents the period of light adaptation. Stimuli of 1.1 sec. duration.

the sensitivity by 3.2 log units. Less than 2 minutes after the adapting light was turned off the sensitivity loss had decreased to 1 log unit and complete recovery took place over 40 to 50 minutes. The dark adaptation curve is unusual in that the change of sensitivity is small and the curve has no break (*cf.* Dodt and Jessen). Adaptation to red and blue lights was tried using coloured test lights. The time course of dark adaptation appeared to be similar in all these cases but the experiments were not very satisfactory owing to the small sensitivity loss caused by the weaker coloured adapting lights.

DISCUSSION

Histological investigation has shown that although the receptors of the *Phelsuma* retina are of different types they are all more like cones than rods in appearance. Further, the comparatively thick inner nuclear layer and the number of ganglion cells suggest a cone retina (Tansley, 1961). However, the electroretinogram of this animal shows some differences from those of the other pure cone retinae examined. These tend to produce relatively bigger *a*-waves and off-effects even in dark adaptation, and although it is true that in the squirrel both *a*-waves and off-effects disappeared in the dark-adapted eye as the stimulus intensity was decreased (Tansley, 1957), such changes occurred at much higher intensities in the gecko where it was the exception to see an off-effect in the dark-adapted eye. The electroretinogram of *Phelsuma* is, in this respect, much more like those of the two nocturnal geckos investigated by Dodt and Jessen, animals which have been shown to have rod-like visual cells (Tansley, 1959). These findings suggest that some caution should be used before making too rigid an identification between a particular type of electroretinogram and a particular form of visual cell.

The absence of a Purkinje shift in *Phelsuma* suggests that the visual cells are all cones, but the break in the curve relating flicker fusion frequency to stimulus intensity might be though to argue against such a belief particularly since in mixed retinae such a break is considered to mark the transition from rod to cone responses. However, a similar break was found by Dodt and Jessen in the curve for a nocturnal gecko which has a pure rod retina and also no Purkinje shift. In discussing this finding together with similar results from the guinea pig (Granit, 1944) and rabbit (Dodt and Walther, 1958) these workers point out that the presence of anatomically different sense cells is not necessary for the production of photopic and scotopic responses in the electroretinogram. They suggest that in speaking of the duplicity of the retina the emphasis should be shifted from the types of visual cell involved (rods or cones) to "such subsequent retinal structures as the bipolars and other constituents of the inner nuclear layer which may contribute to the electroretinogram."

The dark adaptation curve, on the other hand, is typical of those traditionally associated with pure cone function. The maximum light intensity available to us, nearly 19,500,000 trolands, only depressed the sensitivity by about 3 log units and all but 1 log unit of the subsequent recovery was complete in less than 2 minutes. There was no break in the curve, which is rather similar both in its time relations and in its excursion to the first part of the bifid curve obtained by Dodt and Jessen from nocturnal geckos. Dark adaptation in *Phelsuma* is, of course, much slower than that of the human fovea obtained by psychophysical methods but this is not so surprising when one considers the very slow rates usually found in experimental animals when the electroretinogram is used as the criterion of sensitivity, for instance 4 to 6 hours to reach completion in the rat (Dodt and Echte, 1961).

One object of this investigation was to obtain information about the visual pigments of *Phelsuma*. It is, however, impossible to argue from electroretinogram spectral sensitivity curves to pigments unless one adopts rigid criteria. The electroretinogram cannot be used to measure thresholds, for estimation of a minimal response depends upon baseline noise or involves the assumption, which is certainly wrong, that the amplitude/intensity curve is linear. Consequently the sensitivity curves are equal response functions and can only be related to the absorption spectra of visual pigments if the responses are not only equal but identical. It is not sufficient merely to measure the size of the *b*-wave: the wave form and amplitude/intensity relationship should also be considered.

The shapes of the sensitivity curves suggest that there are two distinct mechanisms in the *Phelsuma* retina, one with a sensitivity maximum at about 460 mμ (the "blue" mechanism), the other with its maximum in the region of 560 mμ (the "yellow" mechanism). It seems possible that detailed comparisons with visual pigments might be made for the dark–adapted and blue light–adapted curves because in these the wave form of the response did not change throughout the spectrum (Figs. 2 and 6 *C* and *D*). However, caution must be employed because in the red light–adapted experiments the wave form did change, and in such a way as to suggest that red light did not light adapt the blue mechanism. This is easily understandable if the mechanisms reflect the absorption characteristics of typical visual pigments. While these all absorb light at wavelengths considerably shorter than that of their maximum they have relatively sharp cut-offs at longer wavelengths (Dartnall, 1953). The filter used for red light adaptation came from a pair of dark adaptation goggles designed to cut out light which would affect human rhodopsin with its maximum absorption at 502 mμ. It should, therefore, not excite receptors containing a pigment absorbing maximally at 460 mμ.

The "mechanisms" referred to above, however, include much more than just different types of receptors for they have been identified by changes in

the *b*-wave which has been shown to be produced by the neurons of the inner nuclear layer (Brown and Wiesel, 1961; Brown and Tasaki, 1961). It appears that the yellow and blue mechanisms occupy parallel and distinct pathways in the retina as far as the bipolar cells. These pathways are not completely independent for, although red light adaptation does not produce off-effects to blue stimuli, the sensitivity of the blue mechanism is, nevertheless, depressed (Fig. 4). This cannot be due to a direct effect of red light on the blue receptors for in this case the blue and red adapting lights should have identical effects on the wave form. There must, therefore, be a transretinal pathway whereby the yellow mechanism can depress the sensitivity of the blue without causing the other changes associated with light adaptation. Since the amplitude/intensity function of the *b*-wave is flattened by activity in this pathway just as it is by light adaptation it seems likely that the interaction occurs at or after the first synapse and is not interreceptoral.

Such retinal interactions have not previously been demonstrated in work with the electroretinogram and the reason is doubtless that in no other animal has it so far been possible to demonstrate two such widely separated mechanisms. The separation is necessary if one is to be sure that a coloured adapting light can only affect one mechanism. It is perfectly possible that the two *Phelsuma* mechanisms operate symmetrically, so that the blue mechanism can depress the sensitivity of the yellow by the "non-light-adapting" transretinal pathway discussed above, but it is impossible to demonstrate this, for light which can adapt the blue mechanism will, owing to the shape of the visual pigment absorption curves, also light adapt the yellow.

The problem raised by the demonstration of a second method (apart from light adaptation) of alteration of sensitivity is strictly relevant to the consideration of electroretinogram sensitivity curves, for such an interaction may well operate rapidly and affect the response to a test light. However, the fact that the shape of the yellow sensitive part of our curve is unaffected by adaptation to blue, red, or white light and is the same for the *b*-wave and off-effect does suggest that a comparison between absorption spectra and electroretinogram sensitivity curves may be valid for this region of the spectrum.

In the *Phelsuma* red-adapted sensitivity curve there is a fairly clear maximum in the neighbourhood of 460 mμ and this might possibly be related to the visual pigment with a maximum absorption in the same region found by Crescitelli in a nocturnal gecko, *Oedura monilis*. In addition there is a hint in *Phelsuma* of a mechanism maximally sensitive at 490 mμ which could correspond to another pigment which Crescitelli extracted from two nocturnal species, *Hemidactylus frenatus* and *Oedura robusta*. The most striking part of the *Phelsum* sensitivity curve is that showing a peak at about 560 mμ. This peak corresponds to that of the chicken pigment, iodopsin. But if the whole of this

part of the sensitivity curve is compared with the absorption spectrum of iodopsin the fit is not too convincing (Fig. 12). Similar difficulties of interpretation were experienced by Forbes, Fox, Milburn, and Deane (1960) in two species of diurnal lizard. The gecko curve is clearly too narrow. Because of the uncertainties of such spectral sensitivity curves this difference does not necessarily mean that iodopsin is not the pigment responsible for the 560 mμ peak in *Phelsuma*. However, the gecko result bears an interesting relationship to that obtained with other pure cone eyes. In the grey squirrel, digitonin extracts of the retina contain a rhodopsin with a maximum absorption at 502 mμ in spite of the absence of rods (Dartnall, 1960). The spectral sensitivity curve of this animal shows a narrow hump displaced about 30 mμ towards

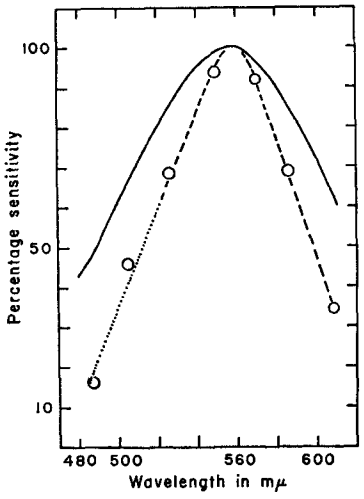

FIGURE 12. Spectral sensitivity of the "red" mechanism. O, mean spectral sensitivity curve for the long-wave part of the spectrum. Continuous line, absorption curve of iodopsin. Ordinate, per cent sensitivity; abscissa, wavelength in mμ. Dotted line, off effect only.

the longer wavelengths (Arden and Tansley, 1955; Tansley, Copenhaver and Gunkel, 1961 *b*). Nocturnal geckos have visual pigments with the usual broad absorption spectrum with a maximum at about 524 mμ (Crescitelli, 1958) and their spectral sensitivity curve calculated by means of the electroretinogram is also broad with its maximum in the same part of the spectrum (Dodt and Walther, 1958). The spectral sensitivity curve of the diurnal gecko, on the other hand, has a narrow hump again displaced about 30 mμ towards the long wavelengths. No pigment has yet been extracted from the *Phelsuma* retina. It seems possible that the spectral sensitivity of a pure cone eye bears a constant relationship to the sensitivity of the visual pigment of related nocturnal species and is due to some special transformation of the pigment *in vivo* or interaction in the receptors between the pigment and its photosensitive bleach products as suggested by Dartnall (1960).

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