THE NATURE OF THE GECKO VISUAL PIGMENT*

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The scotopic visual pigments of terrestrial animals are, like the corresponding pigment of the human retina, generally regarded as members of the rhodopsin system; *i.e.*, a group of chromoproteins possessing a characteristic spectrum with an absorption maximum at about 500 mμ and containing retinene$_1$ as a chromophore. It is expected, therefore, that geckos would possess the typical terrestrial type of visual pigment. Recently the spectral sensitivity function of one of these lizards was determined by Denton (1). He reported this curve to be displaced, by about 20 mμ, toward the red end from the position of the human aphakic sensitivity curve. This result means, either that the gecko visual pigment is a rhodopsin and that the preretinal media selectively modify the transmitted light or, alternatively, that the visual pigment is not a rhodopsin (or not a rhodopsin alone). This investigation was initiated as an attempt to ascertain the reason for the unusual sensitivity function of this lizard.

It has been commonly assumed in most of the literature dealing with the gecko visual system that rhodopsin is the photopigment of the retinal rods. Detwiler (2) implied this in his paper on the retina of *Gekko swinhonis* Guenther. Walls (3, 4) employed the word rhodopsin in writing about the pigment of the gecko rods but it is abundantly clear that he did not view this protein as a definite chemical entity but rather visualized a variety of rhodopsin pigments slightly different in different vertebrates. Underwood (5) considered the presence of rhodopsin in the rods as a feature of gecko ophthalmology. In spite of all this, there is nothing in the recent literature on the biochemical nature of this chromoprotein for the case of the geckos. Two older papers cited by A. C. Krause (6) which this writer has not seen, are apparently concerned with the gecko visual pigment. The first is by W. Krause, published in 1893 and the second is by Köttgen and Abelsdorff, published in 1895. A. C. Krause (6) listed 500 mμ as the spectral maximum for the gecko pigment examined by Köttgen and Abelsdorff but in view of the methods that were available in the year 1895 such a figure requires confirmation.

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The present investigation involved the extraction of the photosensitive pigments from the retinae of several species of geckos, the determination of the absorption spectra of these pigments, and the comparison of the absorption curves with Denton's sensitivity function. For the study, seven species of geckos, all nocturnal, were employed.[1] As yet it has not been possible to obtain *Gekko gekko*, the species used by Denton in his work. In this report the specific details will be given for the case of only one of the seven species; *i.e.*, *Phyllurus milii* (White) from the Warumbungle Mountains, New South Wales, Australia. This course was adopted in the interest of economy and is justified by the following considerations: (*a*) *Phyllurus milii* (White) is a member of the same family—the Gekkonidae—as is *Gekko gekko*; (*b*) retinal extracts from *Phyllurus* were purer than the others; and (*c*) these extracts contained the greatest concentration of photosensitive pigment. These last two characteristics made it possible to obtain the most precise data for the case of *Phyllurus*. In any case, the results obtained with this gecko are in no way strikingly different or unique. As a tentative generalization, and within the framework of ideas to be presented, the present investigation describes the main scotopic pigment of nocturnal geckos.

Analytical Procedure

The apparatus and methods were identical with those previously employed for the analysis of the lamprey pigment (7). After dark-adapting the animals for at least an hour, the heads were removed and the eyes were excised and placed in 4 per cent potassium alum for 30 to 60 minutes. The retinae were extruded through an opening in the cornea and were then placed in the alum solution for 16 to 20 hours. The hardened tissue was then washed twice with distilled water and once with borate–KCl buffer (pH = 8.3). The photosensitive pigment was then extracted from the retinae with 2 per cent digitonin made up in the alkaline borate–KCl buffer. The volume of digitonin solution varied in separate experiments from 0.5 to 1.5 ml. depending on the amount of retinal tissue which was available. The complete extraction was accomplished in two successive steps, employing two-thirds of the total volume of digitonin solution in the initial step. All these procedures were carried out in a dark room, using for illumination a deep red photographic safe light.

Since such extracts are likely to be somewhat unstable for a few days after preparation, the usual procedure was to store them in a refrigerator at about 10°C. for 1 to 3 weeks before analysis. At convenient intervals 0.5 ml. of the extract was transferred to a microcell and optical density measurements were made from 700 to 340

[1] The author is greatly indebted to the following for providing some of the animals: Mr. S. Kellner and Mr. H. G. Cogger, Sydney, Australia; Dr. P. Tardent and Dr. P. Dohrn, Stazione Zoologica, Naples, Italy; Dr. R. B. Cowles, Mr. B. Brattstrom, Mr. J. Cunningham, and Mr. D. Belkin all of Los Angeles. The author is especially grateful to Dr. G. L. Walls and to Mr. G. Underwood for a stimulating correspondence on the subject of this investigation.

mμ. For these measurements, made with the extract at 20 ± 0.5°C., a Beckman DU spectrophotometer with a photomultiplier attachment was employed. Data for the absorption spectrum of each unbleached extract were first collected following the schedule given previously (7). Following this, the microcell with the extract was then transferred to a bleaching chamber, also at 20°C., in which the total volume of extract was illuminated with colored light provided by a B and L grating monochromator. To eliminate any possibility of contamination by the second order spectrum, interference filters were also used. These were placed between the exit slit of the monochromator and the entrance slit of the bleaching chamber. Colored light was employed as routine in these analyses in order to determine whether or not each extract was homogeneous with respect to photosensitive components. For a complete analysis the typical schedule of bleaching was as follows: (*a*) an initial exposure to red light (660 mμ or longer) followed by a second series of density measurements, (*b*) a second exposure to light of slightly shorter wave length (640 mμ) again followed by density measurements, (*c*) a repetition of the exposure measurement sequence using light of still shorter wave length (606 mμ). The final bleach was of sufficiently long duration to remove all remnants of the photolabile pigment. Variations from the above schedule were occasionally required in order to meet the conditions of particular extracts. Applying this procedure, and using the present equipment, it has been possible to resolve prepared mixtures of rhodopsin and porphyropsin and, in addition, to identify both of these photopigments in extracts of the same retina, in several species (data to be published).

Following the final step in the schedule listed above, the extract was usually exposed to white light or to colored light of wave length much shorter than 606 mμ. Selective density losses as the result of such an exposure do not necessarily indicate the presence of an additional photosensitive pigment in the original unbleached extract. Exposure of a bleached visual pigment solution to light containing shorter wave lengths may result in isomerization of the products of the previous bleaching (8) leading to selective density changes. One way to reduce or to eliminate isomerization is to employ a carbonyl-trapping reagent such as hydroxylamine (NH_2OH) which of itself does not destroy the visual pigment (9). For this, and for other reasons given later, each analysis of an extract contained at least one experiment with added NH_2OH as a component.

RESULTS

A. Absorption Spectrum of the Unbleached Phyllurus Extract.—A relatively pure retinal extract from this gecko yielded, before bleaching, an absorption spectrum which is typical of solutions of visual pigments (curve 1, Fig. 1). This curve is characterized by an absorption maximum at 522 mμ, an absorption minimum at 432 mμ, and a ratio, density minimum/density maximum of about 0.50. Assuming, as will later be shown to be true, that this extract contained only one photosensitive component, the position of the maximum at 522 mμ is very close to, but not identical with, the true peak of the pure pigment. On the basis of the relationship between the density

minimum/density maximum values of retinal extracts and the displacement of their absorption maxima from the true peaks of the visual pigments (10), the true peak of the *Phyllurus* pigment should be close to 524 mμ. This

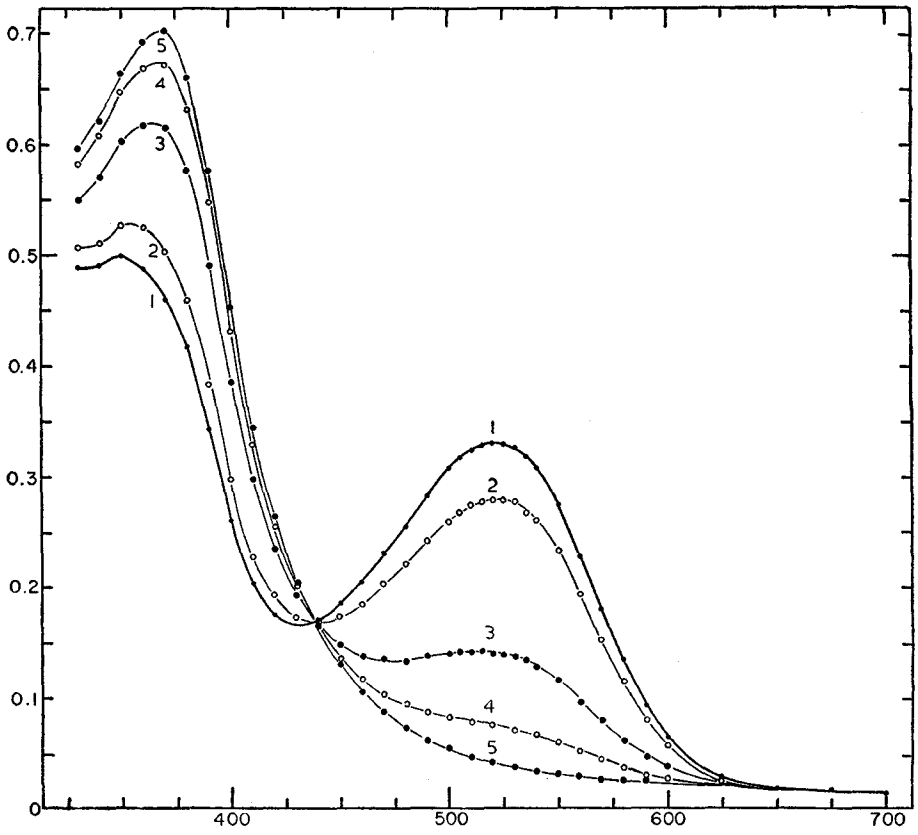

FIG. 1. Absorption spectrum of an unbleached *Phyllurus* extract (curve 1) and the results of bleaching with light at (*a*) 660 mμ for 135 minutes (curve 2); (*b*) 640 mμ for 85 minutes (curve 3); (*c*) 640 mμ for 105 minutes (curve 4); and (*d*) 606 mμ for 25 minutes (curve 5). Optical density is plotted as a function of wave length (mμ). Temperature was 20°C. The pH of the extract was 8.4.

is quite obviously not a rhodopsin. Human visual purple is located at about 497 mμ (11); frog rhodopsin at about 502 mμ (12). A pigment with a peak at 524 mμ and present in the retina of a terrestrial animal is sufficiently unusual to merit further inquiry.

B. Bleaching with Non-Isomerizing Light.—The *Phyllurus* pigment is bleached by light in a manner characteristic of the visual pigments. The spectral changes which resulted from a series of successive exposures to light

of different wave lengths (Fig. 1) reveal the following features: a constant cross-over point at about 438 mμ above which the density decreased and below which it increased. The results suggest the disappearance of a photosensitive pigment and the appearance of a yellow product. This is in accord with the usual behavior of visual pigments in response to illumination (12–14).

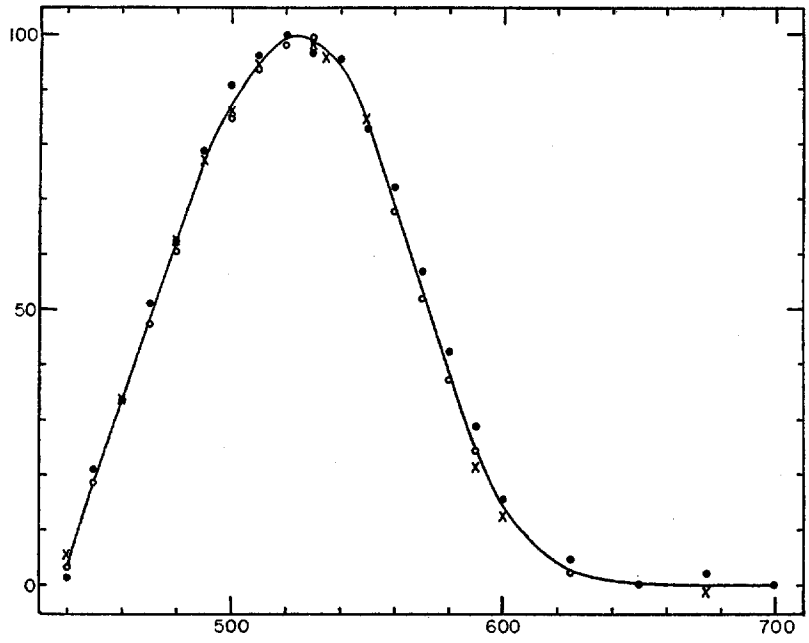

FIG. 2. Difference spectra obtained after bleaching with light at (*a*) 660 mμ (filled circles); (*b*) 640 mμ (open circles); and (*c*) 606 mμ (×'s). Each set of data represents the mean of two separate experiments. The line is the mean curve for all six experiments. The loss in density, as the per cent of the maximum, is plotted as a function of wave length.

The bleaching experiments also permit the conclusion that only one photosensitive component was present in the *Phyllurus* extracts. This statement is based on a comparison of the alkaline difference spectra obtained as a result of the several bleachings (Fig. 2). These spectral density changes indicate a striking uniformity of response, one curve, in fact, representing the density losses in response to each of the wave lengths. This curve is characterized by a maximum loss of density at about 524 mμ. The conclusion that the *Phyllurus* pigment is not a rhodopsin rests, therefore, not only on the spectral position of the absorption curve of the unbleached extract (curve 1,

Fig. 1) but also on the position of the alkaline difference spectrum (Fig. 2). The two methods of analysis yielded identical results.

C. Bleaching with Isomerizing Light.—A 10 minute exposure to white light following the final bleach with non-isomerizing colored light caused a small and selective loss in density, maximal at about 423 mμ (curve 2, Fig. 3). It is doubtful, as the results of the hydroxylamine experiment will indicate, whether this change can be interpreted as evidence of a violet-absorbing pigment. Instead this spectral change was probably the result of an isomerizing action on the products of the preceding bleaches by the short wave length components of the white light.

D. Bleaching in the Presence of Hydroxylamine.—There are two reasons for using NH_2OH in experiments designed to analyze retinal extracts. One—to test for isomerization—has already been discussed. The second reason involves the nature of difference spectra. Since such spectra are determined by the spectral properties both of the products of bleaching and of the original pigment, it is necessary, if the difference spectrum is to be informative, to reduce absorption by the products to a minimum. This, in fact, is the contribution of the NH_2OH technique. Curve 1 (Fig. 3) is an example of an hydroxylamine difference spectrum obtained as a result of a total bleach of a *Phyllurus* extract with light of 606 mμ. This curve is characterized by a distinct break between the positive (loss in density) and negative (gain in density) segments. The positive and negative segments have maxima at 524 and 369 mμ, respectively. The 524 mμ peak is in accord with the peak of the absorption curve (Fig. 1) of the unbleached extract (when corrected, as explained, for the presence of impurities) and with the peak of the alkaline difference spectrum (Fig. 2). The fact that the maxima of the two difference spectra agree, is excellent confirmation of the view that the products of bleaching did not significantly determine the long wave length portion of the difference spectra and that 524 mμ is the true maximum of the visual pigment. This follows logically from the fact that the 524 mμ peak was also obtained when NH_2OH was employed, in which case the oxime which was formed has an absorption peak shifted toward shorter wave lengths. This shift was readily revealed by a comparison of the peaks of the negative segments of the difference spectra with and without NH_2OH. These peaks were at 369 mμ (with NH_2OH) and at 377 mμ (without NH_2OH). Moreover, the cross-over points for the two experiments were at 416 mμ (with NH_2OH) and at 438 mμ (without NH_2OH). Clearly, the NH_2OH resulted in a distinct spectral separation of the product and the original photolabile pigment. This effect is clearly understandable in terms of a reaction of NH_2OH with a retinene to form the corresponding oxime.

The NH_2OH experiment also yielded evidence that the selective spectral change following the terminal exposure to white light in the experiment cited

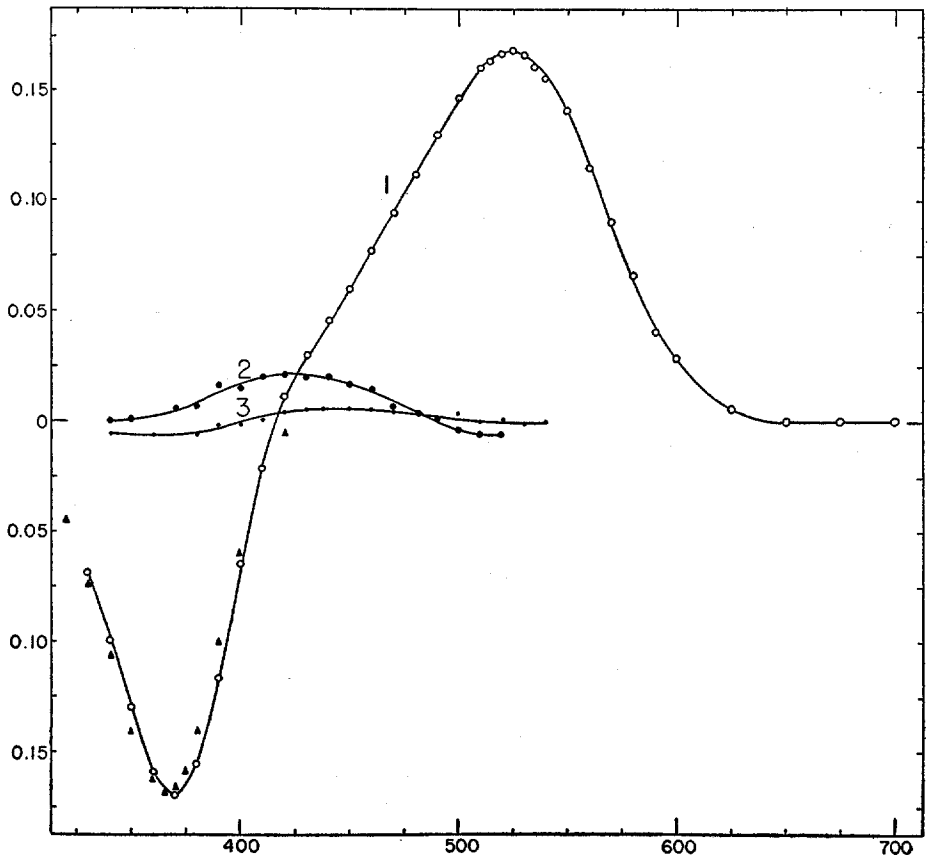

FIG. 3. The NH_2OH difference spectrum following a total bleach with light at 606 mμ (curve 1). Curve 2 is the alkaline difference spectrum (without NH_2OH) obtained after bleaching with white light following total bleach of the extract with red light. Curve 3 is another experiment similar to that represented by curve 2 except for the presence of NH_2OH in the extract. Ordinate values above the zero indicate loss of density; value below zero indicate gain of density. The filled triangles are the spectral densities for a digitonin solution (pH = 8.3) of crystalline all-trans retinene$_1$ to which was added NH_2OH to yield the oxime. These points were scaled for plotting with the difference spectrum.

previously (curve 2, Fig. 3) was probably the result of isomerization. The presence of NH_2OH in the extract almost entirely abolished the white light effect (curve 3, Fig. 3). In view of this no serious defense can be made of the idea that a violet-absorbing pigment was present in the *Phyllurus* extract.

To summarize the preceding sections, it appears that only one photosensitive pigment was present as a significant component of the *Phyllurus* ex-

tract. The absorption spectrum of the relatively pure extract as well as the difference spectra with and without NH_2OH agree in showing that this pigment is characterized by an absorption maximum at 524 mμ and is, therefore, not a rhodopsin.

E. The Nature of the Chromophore.—The evidence of the preceding sections leads to the conclusion that the *Phyllurus* chromoprotein is a typical visual pigment with retinene as a prosthetic group. The position at 524 mμ raises the obvious question whether this pigment may be a porphyropsin and therefore, a remarkable exception to the generalization that porphyropsin is a retinal constituent of animals with a fresh water habitat (15). The original definition of porphyropsin (16) requires that retinene$_2$ be a part of the chromoprotein. It is doubtful whether the data on the *Phyllurus* pigment would conform with this definition. A good indication as to the nature of the chromophore is often obtained from the position of the product peaks (indicator yellow and retinene oxime) under conditions of constant pH. The product peaks as determined from the negative difference spectrum occur approximately at 377 mμ without NH_2OH (curve 2, Fig. 4) and at 369 mμ with NH_2OH (curve 1, Fig. 3). These figures are to be compared with the corresponding average product peaks—377 and 369 mμ—of the rhodopsin system of a number of Amphibia which have been examined in this laboratory. In contrast, the product peaks of the porphyropsin system in Amphibia (unpublished data from this laboratory) occur at 397 and 383 mμ. These comparisons suggest that the *Phyllurus* pigment, in spite of its position at 524 mμ, contains as a carotenoid moiety, not retinene$_2$ but retinene$_1$. This conclusion is reinforced by two further comparisons. The first of these involves a comparison, under similar conditions, of the negative difference spectrum in the NH_2OH experiment with the absorption spectrum of a retinene$_1$ oxime (Fig. 3). The retinene$_1$ oxime was prepared by adding to a solution of the crystalline all-trans retinene$_1$ in 2 per cent digitonin (pH = 8.3) a small volume of freshly neutralized NH_2OH.[2] The absorption curve of this oxime (filled-in triangles), when corrected to scale with the difference spectrum, strongly suggests that retinene$_1$ oxime was in fact the product of bleaching of the *Phyllurus* pigment in the NH_2OH experiment. In the second comparison (Fig. 4) the alkaline difference spectra obtained after bleaching of a rhodopsin solution (curve 1), a porphyropsin solution (curve 3), and the *Phyllurus* extract (curve 2) are shown plotted together on a comparable scale. It is clear that, whereas most of the positive section of the *Phyllurus* difference spectrum is similar to the corresponding portion of the porphyropsin, the negative section, which is related to the products of bleaching, resembles the corresponding section of the rhodopsin. The *Phyllurus* chromo-

[2] The crystalline retinene was kindly provided by Dr. Grove Baxter of Distillation Products Industries.

protein is clearly not a porphyropsin but a member of the retinene$_1$ class of photopigments. The presence of retinene$_1$ as a chromophore of the *Phyllurus* pigment accounts for one result which is usually not obtained in work on visual pigments. This is the agreement in position of the peaks of the differ-

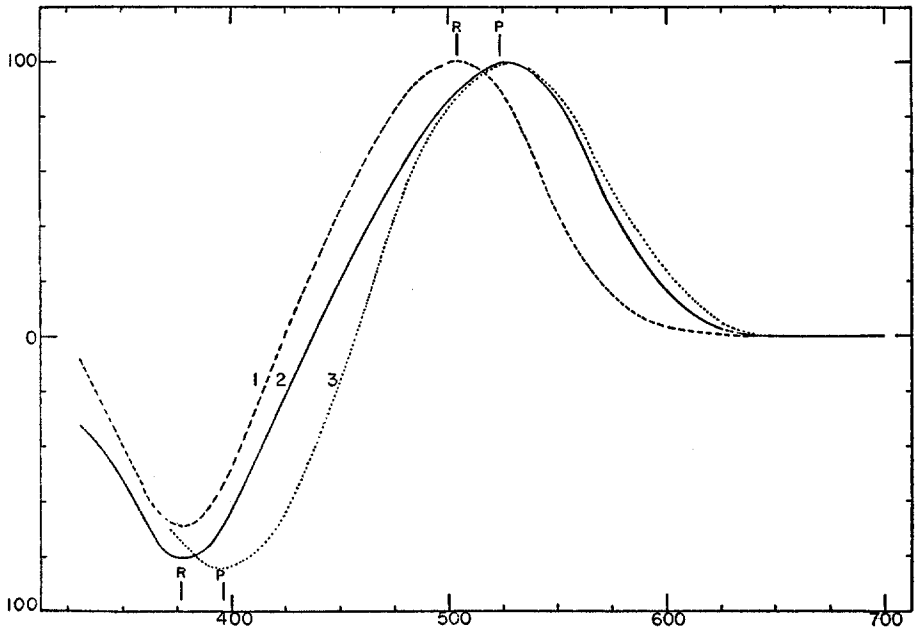

FIG. 4. A comparison of the alkaline difference spectra obtained with a rhodopsin extract (curve 1); a porphyropsin extract (curve 3); and the *Phyllurus* pigment (curve 2). The rhodopsin and porphyropsin extracts were prepared from the retinae of the alligator and carp, respectively. Density change as per cent of the maximum loss is plotted as a function of wave length. Values above the zero ordinate indicate loss of density; values below zero indicate gain of density. The conditions were identical for all three extracts. The short vertical lines represent the mean positions of maxima for density loss and for density gain for amphibian rhodopsins (*R*) and amphibian porphyropsins (*P*).

ence spectra with and without NH_2OH. This agreement is undoubtedly due to the large spectral separation which already exists between the gecko pigment (at 524 mμ) and retinene$_1$ indicator yellow (at 377 mμ).

As an additional reinforcement of this general argument, it should be pointed out that biochemical agreement exists between the retina and the liver. This point has been carefully tested, employing the liver and retinal systems of the banded gecko, *Coleonyx variegatus*. Using the same methods and arguments which were applied to the analysis of the *Phyllurus* pigment,

it was found that *Coleonyx* possesses a retinal photosensitive pigment with a maximum at about 520 mμ and that the product of bleaching of this pigment is retinene$_1$. The liver of this lizard was found to contain a quantity of vitamin A_1 but no vitamin A_2. This fact is demonstrated by the antimony-trichloride reaction (Fig. 5 A) performed on a chloroform extract of the *Coleonyx* liver and by the ultraviolet absorption spectrum of this extract compared with the spectrum of a chloroform solution of crystalline vitamin A_1 (Fig. 5 B).

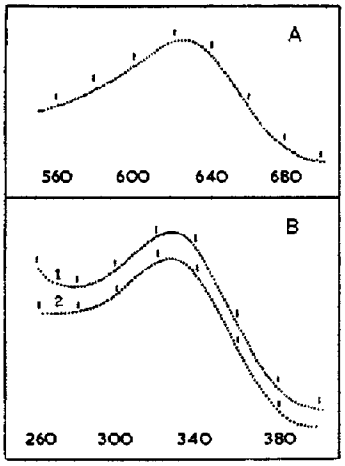

FIG. 5 A. Antimony trichloride reaction performed on a chloroform extract of the liver of *Coleonyx*. The peak at about 618 mμ is characteristic of vitamin A_1.

FIG. 5 B. The ultraviolet absorption spectrum of (*a*) a chloroform extract of the liver of *Coleonyx* (curve 1) and (*b*) a chloroform solution of crystalline vitamin A_1 (curve 2). All three curves of Fig. 5 were traced from original records made with a recording spectrophotometer.

DISCUSSION

A. The Absorption Spectrum of the Phyllurus Pigment.—Neither the absorption spectrum of the unbleached retinal extract (Fig. 1) nor the difference spectrum (Fig. 2) represents the true absorption spectrum of the visual pigment of this gecko. The former is distorted, especially in the region of shorter wave lengths, by the presence, in the extract, of yellow impurities. The latter is distorted also at shorter wave lengths by the occurrence of yellow products of beaching, although this effect is minimized in the NH_2OH experiment (Fig. 3). In spite of these distortions the absorption spectrum of a relatively pure extract and the NH_2OH difference spectrum, when plotted together on a comparable scale (Fig. 6), agree reasonably well down to about 520 mμ. Since these curves represent only one photosensitive component, it may be concluded that the

absorption curve of the pure pigment down to about 520 mμ is directly and accurately pictured by these results. Another useful procedure in the analysis of visual pigments is to apply the Dartnall nomogram (17). Using 524 mμ as the peak of the absorption spectrum the application of this nomogram yielded curve 3 (Fig. 6). This reconstructed curve agrees well with the experimental data down to about 500 mμ. Below this wave length the recon-

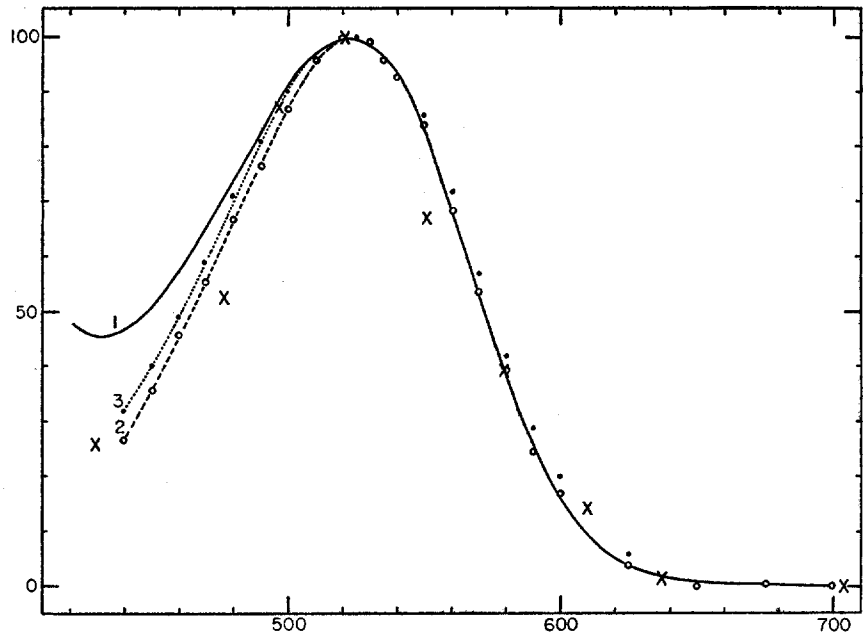

FIG. 6. Summary graphs which show the absorption spectrum of a relatively pure unbleached *Phyllurus* extract (curve 1), the NH_2OH difference spectrum (curve 2), and the reconstruction based on Dartnall's nomogram (curve 3). The points indicated as ×'s are from Denton's data on the visual sensitivity curve of *Gekko gekko* expressed on a quantum basis. All curves were scaled so that each maximum was at 100 per cent.

structed curve falls in between the other two curves but is closer to the difference spectrum. In the light of all this, two statements may be made about the *Phyllurus* pigment: (*a*) for all practical purposes the absorption spectrum is reasonably well established down to about 460 mμ by the reconstructed curve and (*b*) this chromoprotein is a typical broad banded visual pigment with an absorption spectrum which has the same form as the other visual chromoproteins fitted by the Dartnall nomogram (17). The only distinctive feature about it is its location at about 524 mμ which is unique for pigments in terrestrial animals.

B. Physiological Correlations.—It is now possible to correlate the justified portions of the absorption curves (Fig. 6) with Denton's spectral sensitivity data. Through the kindness of Dr. Denton, who made his data available to the writer, this correlation is shown in Fig. 6. The points marked with X's represent the sensitivity data. There is no reasonable doubt that the physiological and biochemical data are in accord. This agreement leads to two mutually related conclusions: (*a*) the visual sensitivity function of the gecko is satisfactorily explained in terms of the retinal pigment and (*b*) the *Phyllurus* chromoprotein is undoubtedly a visual pigment and is, therefore, of physiological interest.

C. The Visual Pigment of Other Geckos.—The general description of the *Phyllurus* pigment as given here is not unique for this species alone. The retinal extracts of six other species of nocturnal geckos were examined by the methods applied to the *Phyllurus* preparations. Though differences were noted in the spectral positions of the pigments from the different species, the two notable characteristics of the *Phyllurus* visual protein—location in the region, 518 to 528 mμ, and the presence of retinene$_1$ as a chromophore —also apply to the pigments from the other geckos. It is likely that these two characteristics also apply to other nocturnal geckos and to *Gekko gekko*, the species employed by Denton. It should be emphasized, however, that when applied to geckos as a whole, generalizations cannot yet be made. The geckos are a diverse group of animals and this is especially true from an ophthalmological point of view. Thus far only an insignificant number of genera have been examined and one important family, the Sphaerodactylidae, is missing from the results.

D. The Visual Pigment of Other Reptiles.—A few attempts have been made already to extract, and to identify, the visual pigments of reptiles (18, 19). These attempts have led to little success probably because of the presence of these proteins in such low concentrations in these cone-rich retinae. By a direct visual method Walls (20) noted the occurrence of a photolabile pigment in the retinae of several reptiles. He referred to it as visual purple. The finding of an unusual pigment in geckos raises the question whether a unique pigment, and not rhodopsin, is also a retinal constituent of other reptilian groups. Using the present methods, the successful extraction and identification of visual pigments have been accomplished in the case of two reptiles: the alligator (*Alligator mississippiensis*) and the Pacific rattlesnake (*Crotalus viridis helleri*).[3] In both cases rhodopsins were found. The difference spectrum of the alligator pigment is reproduced in Fig. 4 (curve 1). A similar difference spectrum was obtained as the result of bleaching the retinal extract of the rattlesnake. The gecko results need not be extrapolated to apply to other

[3] Dr. R. B. Cowles kindly provided the rattlesnakes and was most helpful in the risky experiment of handling these animals in the dark room.

reptiles. In the light of present information geckos appear to be unique among terrestrial animals in the possession of an atypical pigment system.

E. The Biological Significance of the Gecko Pigment.—A discussion of the significance of this unusual visual chromoprotein involves phylogenetic considerations. The basis for this statement is the transmutation theory of Walls (3, 4) which has received support from the studies of Underwood (5). According to this concept the ancestral gecko was a diurnal lizard with retinal cones. In the course of evolution a transmutation of cones to rods occurred in association with the development of the secretive, nocturnal habit. At some time during this evolution the high concentration of visual pigment in the outer segments is assumed to have developed. Underwood's interesting study of retinal cytology has brought to light a number of transition forms among living genera of geckos which support the transmutation theory in a convincing manner. Perhaps the most unexpected support for Walls' theory, in so far as geckos are concerned, is the finding by Crozier and Wolf (21) that the critical fusion frequency contour of the gecko, *Sphaerodactylus inaquae* Noble and Klingel, is similar to that obtained from *Pseudemys*, a turtle with a predominantly pure cone retina. Except for these few studies the transmutation theory has stood almost alone while overwhelming support has been marshalled in favor of the duplicity theory and no one, except for Walls' qualification about nomenclature, already referred to, seems to have questioned the view that the gecko visual pigment is anything but a typical rhodopsin. The present results, demonstrating, as they do, that this view is untenable, may be interpreted as supporting the transmutation theory. The so called rods of nocturnal geckos are characterized biochemically by a pigment of the retinene$_1$ class but one which is roughly intermediate in spectral position between the other two retinene$_1$ chromoproteins: the typical rod constituent, rhodopsin and the cone pigment, iodopsin. Is it possible that this arrangement is of evolutionary significance possibly representing substances which had their origin from a common ancestral retinene$_1$ pigment?

The data for the seven species of geckos from which retinal extracts were prepared suggest that the visual pigments are not spectroscopically identical in all these lizards. Minor, but significant, variations appear to exist in the position of the pigment from each of the seven species. It remains to be discovered whether a systematic, taxonomically significant variation occurs in the pigment system of geckos as a whole. Underwood (5) has recently proposed a classification of geckos into three families: (*a*) Eublepharidae, (*b*) Sphaerodactylidae, and (*c*) Gekkonidae. Transmutation is supposed to have occurred independently in these three gecko stocks. It will be of interest in future work to inquire whether the evolution of the visual pigment also occurred independently in these three families or whether the biochemical pattern was fixed in the common ancestral form. It would not be too surprising

to discover in future work, gecko pigments in several other spectral positions and all members of the retinene$_1$ system. Even typical rhodopsin may turn out to be present in some species. It will be of special interest to determine which, if any, visual pigment can be extracted from the retinae of those diurnal geckos whose retinal cells consist of cones presumably derived tertiarily from rods. The geckos appear to offer fruitful prospects for future physiological and biological queries.

F. The Matter of Nomenclature.—The discovery of a visual pigment at the position of porphyropsin yet containing retinene$_1$, raises the question of a proper nomenclature for this group of pigments. The classical system of describing them according to their color (rhodopsin, porphyropsin, iodopsin, or visual purple, visual violet) is obviously insufficiently informative in the case of the *Phyllurus* pigment which has the same color as the porphyropsins but which differs from them in the nature of its chromophore. The same objection can be made to Dartnall's system (22) of naming each pigment numerically according to the spectral location of the absorption maximum. There are several alternative methods which might be adopted including modification of the present systems by the addition of the subscript 1 or 2 according to whether retinene$_1$ or retinene$_2$ is obtainable from the products of bleaching. In view of the possibility of further discoveries in this field the present author is not yet prepared to offer suggestions for a definitive system of nomenclature even though it is clear that present systems are unsatisfactory in a number of respects.

SUMMARY

Retinal extracts of the Australian gecko, *Phyllurus milii* (White), have revealed the presence of a photosensitive pigment, unusual for terrestrial animals, because of its absorption maximum at 524 mμ. This pigment has an absorption spectrum which is identical in form with that of other visual chromoproteins. It is not a porphyropsin, for bleaching revealed the presence, not of retinene$_2$, but of retinene$_1$ as a chromophore. Photolabile pigments with characteristics similar to those of the *Phyllurus* visual pigment were also detected in retinal extracts of six other species of nocturnal geckos.

The presence of this retinal chromoprotein adequately accounts for the unusual visual sensitivity curve described by Denton for the nocturnal gecko. This pigment may have special biological significance in terms of the unique phylogenetic position of geckos as living representatives of nocturnal animals which retain some of the characteristics of their diurnal ancestors. The occurrence of this retinene$_1$ pigment, intermediate in spectral position between rhodopsin and iodopsin, is interpreted in support of the transmutation theory of Walls. The results and interpretation of this investigation point up the fact that, from a phylogenetic point of view, too great an emphasis on the

duplicity theory may serve to detract attention from the evolutionary history of the retina and the essential unitarianism of the visual cells.

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