Spectral Relations of Cone Pigments in Goldfish

FERENC I. HAROSI
From the Laboratory of Neurophysiology, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20014

ABSTRACT Dark-adapted retinal cones of goldfish were measured microspectrophotometrically. The three types of spectra so obtained were subjected to a new method of data analysis. In order of types blue (B), green (G), and red (R), the best estimates for \( \lambda_{\text{max}} \) were 453, 533, and 620 nm; for main band half width, 6,700, 4,700, and 3,900 cm\(^{-1}\). The extinction spectra of 11-cis 5,4-dehydroretinal and those of the three goldfish pigments were progressively fitted with Gaussian curves starting at the low-energy end of their spectra. The sum of the oscillator strengths of the first three Gaussian components throughout the four spectra were found to have nearly equal magnitudes. Functional relationships that connect the Gaussian parameters were obtained by curve-fitting, enabling partial absorption spectra to be generated for any \( \lambda_{\text{max}} \). The generated curves predicted the half width and peak extinction of porphyropsin-type absorption spectra more accurately than previously existing nomograms or hypothesis. The \( \epsilon_{\text{max}} \) values thus obtained were 28,500, 32,000, and 35,700 liter/mole cm for the B-, G-, and R-type goldfish pigments; these were found to be consistent with the experimental determinations of ±10% estimated accuracy.

INTRODUCTION

Dartnall (1953) observed that relative extinction (density) spectra of many rhodopsins exhibit approximately the same shape when plotted as function of wavenumber instead of wavelength. Based on this shape conformity he constructed a "nomogram" which facilitates translations in wavelength of a rhodopsin spectrum for the generation of rhodopsin-type spectra of any \( \lambda_{\text{max}} \). The principle involved here has proven to be so useful that a second template was devised for the porphyropsins, the other of the two large vertebrate visual pigment families, which were found to have slightly broader light-absorbing characteristics (Bridges, 1967a; Munz and Schwanzara, 1967).

As the accuracy of spectral measurements improved, however, evidence began to accumulate concerning spectral shape anomalies. For example, the red-absorbing cones of goldfish yielded narrower absorption spectra (Marks, 1965), and of the carp, narrower spectral sensitivity responses (Tomita et al., 1967), than either of the two templates. The same is true about the absorption spectrum of cyanopsin (Wald et al., 1953), a synthetic pigment resulting from the condensation of 11-cis 3-dehydroretinaldehyde with chicken cone opsin, as
pointed out by Bridges (1967a). The microspectrophotometric measurements of frog and tadpole rods and cones by Liebman and Entine (1968) led to the conclusion that the "green" rods have broader, the red-absorbing cones narrower, and the "red" rods about as wide spectra as the appropriate rhodopsin and porphyropsin standards. Similar results were obtained by Liebman and Granda (1971) from the rods and cones of two species of turtle, one of which was also studied by Baylor and Hodgkin (1973) with electrophysiological methods. The latter investigators confirmed the existence of systematic differences between spectral sensitivity curves and nomogram.

In an earlier study of goldfish retinal receptors Hárosi and MacNichol (1974a) confirmed the narrowness of the red-absorbing cone pigment spectrum and interpreted it assuming invariance of the main band oscillator strength. A subsequent study of amphibian photoreceptors (Hárosi, 1975) revealed that this hypothesis was qualitatively consistent with measurements, moreover, that green rod spectra are broader than the templates in the larval and adult tiger salamanders as well as in a species of tropical toad.

The former work on goldfish cones (Hárosi and MacNichol, 1974a), however, did not reveal a spectrum broader than the porphyropsin standard in the blue-absorbing cones, and did not subject the proposed hypothesis to tests that might have correlated the narrow spectrum of the red-absorbing cones with a concomitant increase in specific optical density of their outer segment.

In addition to these reasons for selecting goldfish for a repeated spectroscopic study, it is noteworthy that (a) its retina provides a tripartite cone pigment system that spans the entire visible spectrum, (b) that one of these is a visual pigment with the highest \( \lambda_{\text{max}} \) known, and (c) that all three are, almost certainly, pure porphyropsin-type pigments. The utilization of 11-cis 3-dehydroretinal as chromophore in goldfish has been previously inferred (see discussion by Hárosi and MacNichol, 1974a); another indirect evidence is the recent finding of Bridges (1973) that the goldfish pigment epithelium maintains a stockpile of 3-dehydroretinol(ester) in a more than 40-fold molar excess to the entire visual pigment content of the retina.

In this article new results are presented which were obtained after the introduction of several technical improvements in single-cell absorption measurement and the development of a new method of spectral analysis. These results confirmed the existence of systematic deviations from the nomograms. Moreover, they yielded a phenomenological description of the three goldfish cone spectra, as well as a modified formulation of the aforementioned hypothesis. Most significantly, the chosen mathematical modeling revealed the existence of a spectroscopic continuum shared by not only the three goldfish cone pigment spectra but also by the absorption spectrum of their chromophore, 11-cis 3,4-dehydroretinaldehyde.

**MATERIALS AND METHODS**

Comet goldfish (*Carassius auratus*) of 13–14-cm length were used in these experiments. They were obtained from Ozark Fisheries (Stoutland, Mo.) and kept in an aerated aquarium at room temperature. Each fish was dark adapted, usually overnight, and
anesthetized before use in a bath containing Tricaine (Crescent Research Chemicals, Inc., Scottsdale, Ariz.) and water in 1:4,000 ratio. Although enucleation and hemisection of eyes were performed under dim red light, as before (Hárosi and MacNichol, 1974a), the retinas were removed with the aid of infrared illumination (>800 nm) and a low-power dissecting microscope equipped with an infrared image converter (Mini-Metascope, model 9969, Varo Inc., Garland, Tex.).

Isolated cone cell specimens were prepared and measured as described previously (Hárosi and MacNichol, 1974a, b) but with the following modifications: (a) The suspending saline solution was made according to the formula for fresh water fish of Forster and Taggart (1950), without the addition of phenol red. (b) The search for cells in the instrument microscope was done under infrared illumination at 320 x or 1,000 x magnification with the image converter designated above temporarily mounted on the eyepiece. The image converter was removed before each measurement, and the final focusing adjustments were made under dim visible lights of green or red color. When a cell under test was expected to have the red-absorbing pigment, the auxiliary source was filtered with a green interference filter (507-nm peak transmission, 20-nm bandwidth); for the green- and blue-absorbing cones, the filter was red (650-nm peak transmission, 20-nm bandwidth).

Additional Modifications

DIGITAL THRESHOLD DISCRIMINATION A new software program was written for the digital computer controlling the microspectrophotometer to discriminate against signal values exceeding a "window," or two preset numbers, on both analog channels of the instrument. Thus, the digital values corresponding to large "noisy" excursions could be ignored and averages computed (in floating-point arithmetic) for each datum point on both channels from the subthreshold values of 108 samples taken during each 5-nm spectral segment (swept in 10 ms) of every scan.

LACK OF BASE-LINE CORRECTION No base-line correction of any kind was used. The average dark current of the photomultiplier (about 4 nA at 22°C) was neutralized by an equal and opposite current derived from a stable bucking circuit, which was left unchanged for all recordings. Therefore, possible variation of the system (due to temperature changes or lamp aging) was taken into account only by the fact that reference and sample recordings followed each other closely in time.

CORRECTION FOR BLEACHING DUE TO MEASUREMENT A theoretical relationship was derived for estimating the initial (dark-adapted) cellular optical density from two sequential absorptance measurements of equal flux and duration, each causing an appreciable density loss due to bleaching. Since visual pigment concentration diminishes in bleaching according to a single-time-constant exponential law (Hecht, 1920), one may assume that the peak density at $\lambda_{\text{max}}$ will also diminish exponentially in the course of the measurement. Thus, at the end of the first $n$ spectral scans, the peak density may be expressed as $D_1 = D_o \exp(-n/\tau)$ and, similarly, at the end of the second $n$ scans, as $D_2 = D_o \exp(-2n/\tau)$. Furthermore, if $d_1$ is the average peak density of the first $n$ scans, expressible as $d_1 = (D_o + D_1)/2$ and, similarly, $d_2$ is the average peak density of the second $n$ scans, expressible as $d_2 = (D_1 + D_2)/2$, then the initial peak density $D_o$ may be found (as a result of simple algebraic manipulations) by the relation $D_o = 2(d_1)^2/(d_1 + d_2)$. When the relative loss from $D_o$ to $d_1$ is defined as $L = (D_o - d_1)/d_1$, the above relation yields $L = (d_1 - d_2)/(d_1 + d_2)$. After $L$ was obtained for each cone type from sequential recordings, each initial peak density $D_o$ was calculated by adding the loss to the first $n$-scan average; i.e., $D_o = (1 + L)d_1$. 
DATA ANALYSIS  In addition to the usual data processing and averaging of experimental runs in the dichroic microspectrophotometer (DMSP), the absorbance values corresponding to individual measurement (16-scan averages) were transferred to a PDP-10 digital computer (Digital Equipment Corporation, Maynard, Mass.). All subsequent operations on data, such as conversion from absorbance to optical density, wavelength to wavenumber or vice versa, smoothing, curve-fitting, graphics, and computations were done with the aid of the MLAB software program (Knott and Reece, 1972). For example, smoothing in MLAB is accomplished by a five-point variable-interval hyperbolic fitting process. Curve-fitting operates in an iterative manner in which the parameters of the user-specified functions are adjusted, to minimize the simultaneous sum of squares between the functions and the data points (i.e., seeks least-square errors). Whenever the data consisted of more than one observation, the variance was determined for each datum and the average observation value was weighted by the reciprocal of the corresponding variance for the purpose of curve-fitting by MLAB.

Progressive fitting of diffuse absorption spectra with a linear combination of Gaussian functions is defined for the present as a procedure in which a limited range of the spectrum, beginning at the low wavenumber end, is first fitted with a Gaussian-type function of the form

$$G_i(\nu) = E_i \exp\left[-K(\nu - M_i)^2/W_i^2\right]$$

where $K = 4(\ln 2)$ and $E_i, M_i$ and $W_i$ (for $i = 1$) are, respectively, the peak extinction, mean wavenumber, and half width of the first component. Then a second function of the same form $G_2(\nu)$ is included so that their sum, $\Sigma G_i(\nu) = G_1(\nu) + G_2(\nu)$, fitted to a somewhat extended spectral range of data. The third and subsequent Gaussian functions are gradually added as needed to fit the progressively extended range of data. It was found in the course of this work that such procedure yields a more reproducible set of final parameters than if fitting is attempted throughout the entire range of the curve at once.

The constant in the exponent of $G_i(\nu)$, $K = 4(\ln 2) = 2.772589$, is obtained as a result of $W_i$ being defined as the width at half the peak height of the $i$th component. The definite integral of $G_i(\nu)$ for all values of $\nu$ is $E_i W_i (\pi/K)^{1/2}$. Thus, the oscillator strength corresponding to the first component is obtainable as $f_1 = 4.32 \times 10^{-9} (\pi/K)^{1/2} E_i W_1$ (cf. Sandorfy, 1964).

The parameters $(E_i, M_i, W_i)$ of the Gaussian components were found to vary between the pigment spectra. For convenience, these variations were expressed in terms of a common parameter $\nu_m$, which is the main band peak wavenumber $(\nu_m = 10^4/\lambda_{\text{max}})$. Accordingly, each and every parameter was described using a power function of $\nu_m$, formally identical with

$$P_j(\nu_m) = a_j + b_j \nu_m^{c_j}.$$  

The $a_j$, $b_j$, and $c_j$ constants were then determined by fitting $P_j(\nu_m)$ to the corresponding parameter values that were previously derived by progressive curve-fitting of $\Sigma G_i(\nu)$ to a principal set of empirical absorption spectra. The "principal set" for the porphyropsin series was chosen to consist of the in situ absorption spectra of B-, G-, and R-type goldfish cone pigments plus that of 11-cis 3,4-dehydroretinal dissolved in ethanol and measured at room temperature.

RESULTS

In the course of a recent study of cone morphology (Stell and Hárosi, 1976), the absorption of 124 isolated goldfish cone outer segments was measured micro-
spectrophotometrically. 55 cones were identified as red absorbing (R), 50 as green absorbing (G), and 19 as blue absorbing (B). The records were carefully screened for artifacts and the best three spectra were chosen in each group. The averages of these spectra after having been processed by type in the measuring instrument (DMSP) are shown in Fig. 1.

Numerical values of absorptance-wavelength pairs of individual recordings (consisting of 16 bidirectional scan averages) were converted to optical density-wavenumber pairs; then for each point of each spectral type the average density and corresponding variance were computed. To arrive at approximate figures for \( \lambda_{\text{max}} \) and \( \Delta \nu \), the average density spectra were smoothed. The values obtained by this method for the B, G, and R types were, respectively, 450.0 nm, 6,891/cm; 530.0 nm, 5,035/cm; and 620.0 nm, 4,011/cm. The peak transverse specific densities were also determined from the smoothed curves; they are listed for purpose of reference in Table I.

The most accurate representation of cone pigment spectra was considered to be that which obtains when the measured, averaged spectral points were progressively fitted with a sum of three Gaussian functions such that each point was weighted in inverse proportion to the corresponding variance. This indeed yields a statistically correct representation of data, provided the individual measurements are independent of one another and the values are normally distributed about the averages. The fitted results are shown in Fig. 2.

![Absorption spectra of dark-adapted goldfish cone outer segments](image)

**Figure 1.** Absorption spectra of dark-adapted goldfish cone outer segments, measured sideways with linearly polarized light in the DMSP. Each recording consisted of the average response to the first 16 bidirectional spectral scans. The average of three single-cell recordings obtained from blue-absorbing (a), green-absorbing (b), and red-absorbing (c) types.
analytic functions were differentiated; the first derivatives were found to approach zero at wavenumbers corresponding to λ_max of 453.4, 533.8, and 616.7 nm for the B, G, and R types. The densities at these peaks were found to be 0.0602631, 0.0461324, and 0.0537078, whereas the widths at half of these values were 6.821/cm, 4.503/cm, and 3.896/cm (see Table I).

Based on photomicrographs that were taken subsequent to spectral recordings, the outer segment diameters were determined for each type. The average diameters (±1 SD) were found to be 4.44 ± 0.35, 3.49 ± 0.23, and 3.48 ± 0.21 μm for the B-, G-, and R-type cones, respectively, yielding transverse specific densities (before correction) of 0.0136/μm, 0.0132/μm, and 0.0154/μm.

The loss of pigment caused by the measuring light during a recording was estimated from sequentially obtained records. Several cells of each type were measured twice in sequence by recording identical 16-scan averages from them. The first and second spectra were then averaged for each cone type, and from the densities at λ_max, the losses were calculated (see Methods). It was found that the peak transverse densities obtained as the first 16-scan averages could be brought to their dark-adapted level by increasing their values by 2.3, 2.9, and 2.6% for the B, G, and R types, respectively. The corrected specific densities, therefore, were 0.01391/μm, 0.01358/μm, and 0.01589/μm.

In order to estimate pigment properties in situ, the cellular dichroic ratios were first obtained as the ratios of densities at λ_max of the two polarized components, both of which were represented by progressively fitted sums of three Gaussian curves. The ratios found for the B, G, and R types were, respectively, 2.01, 2.02, and 1.66. It was then possible to calculate the product of in situ pigment concentration and molar extinction coefficient (εε_max) in each

| Table I | GOLDFISH CONE PIGMENTS: SPECTRAL AND CELLULAR DATA |
|---------|--------------------------------------------------|
| Method of determination | Cell type | λ_max | Δw | D₁ | ε* | D₂/ε₁ | R | (ε_max) | ε_max |
| Previous estimates | B | 455±3 | 4,822±100 | 0.0590078 | 50,000 |
| | G | 550±3 | 5,085 | 0.0452512 | 50,000 |
| | R | 625±5 | 5,085±100 | 0.0527444 | 50,000 |
| Smoothed averages | B | 450.0 | 5,891 | 0.0390078 | 40,000 |
| | G | 550.0 | 5,085 | 0.0452512 | 40,000 |
| | R | 620.0 | 6,011 | 0.0527444 | 40,000 |
| Progressively fitted | B | 453.4 | 6,821 | 0.0590078 | 159.1 | 2.01 | 115.3 | 50,000 |
| (weighted averages) | G | 538.8 | 5,505 | 0.0461324 | 155.8 | 2.02 | 112.5 | 50,000 |
| | R | 616.7 | 3,896 | 0.0537078 | 150.0 | 1.66 | 156.6 | 50,000 |
| Current best estimates | B | 455 | 6,700 | 28,500 |
| (generated curves) | G | 555 | 4,700 | 32,000 |
| | R | 620 | 3,900 | 35,700 |

* Pathlength, assumed to equal average outer segment diameter.
† Corrected for bleaching by increases of D₁ values by 2.3% for B, 2.9% for G, and 2.6% for R.
‡ Computed by a previously developed method (Eq. 3, Häröni, 1975).
§ Assuming ε_max = 30,000 liter/mole cm for G, and that ε is identical in B, G, and R.
¶ Häröni and MacNichol (1974 a).
class with the aid of a previously developed method (cf. Eq. 5, Hárosi, 1975). These products were 0.01155/μm (B), 0.01125/μm (G), and 0.01366/μm (R). By assuming $e_{\text{max}} = 30,000$ liter/mole cm for G, $c = 3.75$ mM is obtained; and if the same pigment concentration prevails in B and R types as well, then their molar extinction coefficients should be 30,747 and 36,427 liter/mole cm, respectively (see Table I).

The absorption spectrum of 11-cis 3-dehydroretinal has been available in the literature (Bridges, 1967b). Based on the determination of Planta et al. (1962), this substance has the main peak extinction of $E_1^{1\text{cm}} = 882$ at $\lambda_{\text{max}} = 398$ nm when dissolved in ethanol (at room temperature). The peak molar extinction was calculated to be 24,900 which results from the multiplication of the above number with 28.24 (mol wt/10); the molar extinction spectrum was then constructed by scaling the published absorption data (Fig. 2d). It was found that the experimental points may be approximated to a fair degree of accuracy with the sum of five Gaussian functions. Furthermore, it was also found that the sum of the oscillator strengths of the progressively fitted first three (low-energy) Gaussians approximately equals the sum of the oscillator strengths of the first three components in the goldfish cone pigment spectra. This property was considered encouraging in the effort of finding a manageable description of the spectral transformations.

In order to arrive at a phenomenological description of the changes that take place in the absorption spectra among the visual pigments of the same prosthetic group but differing $\lambda_{\text{max}}$, the progressively fitted Gaussian parameters were plotted as function of $\nu_m$, the main band peak frequency. The parameters, component by component, are depicted in Fig. 3. The variations in their values observable in the plots prompted the supposition that they are continuous and monotonic functions of $\nu_m$. This was assumed to be operative and then simple power functions of identical form were fitted to the four values of each parameter. The results are drawn in Fig. 3; the numerical values of functions are summarized in Table II.

If one assumes that the progressively fitted three Gaussians provide reasonably good approximation to physiologically relevant regions of visual pigment absorption spectra and that the fitted power functions faithfully describe the variations of the Gaussian parameters, then, with the aid of Table II, spectra for all porphyropsin-type pigments for any $\lambda_{\text{max}}$ may be generated. Such generated functions are shown in Fig. 4. The $\lambda_{\text{max}}$ values for the four curves were chosen to correspond to the original spectra of the principal set. The analysis of these generated curves indicate that they reproduce all the spectroscopic properties of the corresponding pigments according to expectations (cf. Table I).

**Discussion**

In comparing the present results with those of an earlier study of isolated goldfish cones (Hárosi and MacNichol, 1974a), a major discrepancy is evident; namely, the half width of the type B spectrum previously estimated to be 4,832 ± 100 cm$^{-1}$ was found near 6,800 cm$^{-1}$ in this work. The latter value is considered to be more reliable because of the following reasons: (a) Whereas earlier the
Figure 2. Progressively fitted and empirical absorption spectra. The continuous curves are sums of the dashed Gaussian components, which were fitted to the experimental values marked by circles. The experimental data in parts a, b, and c were derived from the transverse components of the B-, G-, and R-type goldfish cone spectra depicted in Fig. 1. The data presented in part d correspond to the absorption properties of 11-cis 3-dehydroretinaldehyde in ethanol as given by Bridges (1967b) and Planta et al. (1962).
width of the B-type spectrum was judged on the basis of a visual comparison of transverse absorptance (averaged from three cells) with a template of relative extinctions, the current result was obtained analytically from averaged smoothed as well as fitted spectra. (b) The base-line correction method used earlier was found to introduce "over-correction" errors from time to time. This could
Figure 3. Parameter variations corresponding to the first three Gaussian components fitted to the absorption spectra of B-, G-, and R-type goldfish cones and 11-cis 3-dehydroretinal. (a) Mean frequencies of the three components ($M_1, M_2, M_3$) for the four spectra. (b) Peak extinctions of the three components ($E_1, E_2, E_3$) and of the main bands ($EMB$). (c) Half widths of the components ($W_1, W_2, W_3$) and of the main bands ($WMB$). (d) Oscillator strengths corresponding to the first three components ($O_1, O_2, O_3$).
happen if, during the prescan sampling of dark currents, the photodetector would produce positive noise-bursts, thereby raising the apparent short-term background levels. Then, as a result of subtracting the inflated base-line values from all subsequent sample values of the scan, the resulting spectra could
become trimmed at the base. The fact that previous measurements yielded slightly lower peak densities, smaller half widths, and larger dichroic ratios is consistent with that assessment. The practice was discontinued (see Methods). (c) A re-examination of the old records revealed that one of the three spectra used in the previous average was in fact distorted, appearing excessively narrow. (d) In the present study a much greater number of records were available to select from than before, and the selection proceeded analytically rather than visually by smoothing single-cell spectra and computing their main parameters before averaging.

The half width of the main band of the four absorption spectra studied here

### Table II

**Porphyropsin-Type Pigments: Parameter Values for a Partial Synthesis of Absorption Spectra**

| Parameters          | Gaussian components |
|---------------------|---------------------|
|                     | $G_1$               | $G_2$               | $G_3$               |
| Mean frequency $a_1$| $-11,795.6$         | $-75,350.1$         | $-68,973.4$         |
| M $b_1$             | $75.2967$           | $12,051.2$          | $15,025.8$          |
| $c_1$               | $0.609767$          | $0.211814$          | $0.188107$          |
| Peak extinction $a_2$| $15,792.6$         | $15,600.4$          | $12,191.8$          |
| E $b_2$             | $0.435458 \times 10^{13}$ | $-0.137813 \times 10^{-4}$ | $0.115786 \times 10^{-3}$ |
| $(\text{liter/mole cm}) c_2$ | $-2$              | $2$                 | $2$                 |
| Half width $a_3$    | $2,812.8$           | $4,649.1$           | $8,328.9$           |
| W $b_3$             | $0.84202 \times 10^{-13}$ | $-0.230687 \times 10^{29}$ | $-0.351486 \times 10^{29}$ |
| $c_3$               | $6$                 | $-6$                | $-6$                |

$M$ - $E$ - $W$ - parameter $(\nu_m) = a_j + b_j \nu_m^2$.

$\nu_m = 10^7/\lambda_{\text{max}}$ (nm).

Spectrum $(\nu) = \sum_{i=1}^{3} G_i(\nu)$.

$G_i(\nu) = E_i \exp[-2.772589(\nu-M_i)^2/W_i^2]$.  

The hypothesis of invariance of the main band oscillator strength also needs modification. Although qualitatively correct, the numerical predictions based upon it fall short of the experimentally found values (cf. Table I). One of the difficulties appears to be the diffuse nature of visual pigment absorption spectra, whereby the main band is ill-defined, particularly for blue-absorbing pigments. The results of the present study suggest that the total oscillator strength corresponding to the first three Gaussian components remains nearly invariant (cf.
Figure 4. Generated functions in the porphyropsin series. Numbers near the peaks are $\lambda_{\text{max}}$ values in nanometers used in the computations. (a) Partially synthesized absorption spectra of the principal set: the R-, G-, and B-type goldfish cone pigments and 11-cis 3-dehydroretinal. (b) The same set of spectra when plotted as function of wavelength.
In this formulation, however, the hypothesis is no longer easily applicable.

Nevertheless, the present observations upon the oscillator strengths of visual pigment spectra are interesting when compared with those made upon the retinal isomers. Sperling (1973) reported that the four isomers of retinal he studied have about the same overall oscillator strength, independent of solvent and temperature.

There appear to be analogous spectral properties between the 11-cis chromophore in different solvent systems and temperatures and the different visual pigments. The various "opsins" of visual pigments might be thought of as so many "solvent systems" in which the 11-cis chromophores are dissolved. On the other hand, the increase in peak extinction and narrowing of the main band in red-absorbing pigments are like lowering the temperature for the 11-cis chromophores. Thus, with the exception of large red shifts in $\lambda_{\text{max}}$, the effects of an opsin can be mimicked by appropriate choices of solvent and temperature.

In all fairness, however, the nonuniqueness of the Gaussian spectral synthesis should be emphasized. Although progressive fitting results in nearly reproducible Gaussian parameters, the sum of the areas under the components are somewhat uncertain because the third Gaussian components are less reliable due to insufficient data. Also, despite the use of weighted averages which probably best represent the experimental values, the variances for only three observations may be biased in undetectable manners. The specific density determinations of cells are estimated to be accurate to $\pm 10\%$, and hence relative $\varepsilon_{\text{max}}$ values are uncertain to the same extent. A few percent inaccuracy must be ascribed even to the $\varepsilon_{\text{max}}$ values determined in solutions. The validity of assumptions must also be tested in the final analysis; for example, whether all the parameters are continuous or discontinuous between the spectra of visual pigments and that of the prosthetic groups, or whether ethanol or some other solvent would be most appropriate for the determination of the intrinsic $\varepsilon_{\text{max}}$ of the chromophores.

Although the absorption spectra could be synthesized with other functions or perhaps with more Gaussians, or the parameter variations might be described in more detail for better accuracy, no such modification seems productive unless more accurate spectra and $\varepsilon_{\text{max}}$ values become available. Meanwhile, as discussed in a separate communication (Hárosi, in preparation), the scheme derived from the goldfish cone pigment spectra above appears to be applicable to many other vertebrate visual pigments. For example, the 533-nm curve generated to describe the G-type spectrum compares well with the porphyropsin absorption spectrum obtained by Bridges (1967a), while the 620-nm curve describes the cyanopsin spectral shape of Wald et al. (1953) to a good approximation. Moreover, a qualitatively identical scheme is found to connect the absorption spectra of rhodopsin-type pigments with that of their prosthetic group, 11-cis retinaldehyde.

**SUMMARY**

**Main Assumptions**

(a) The chromophore of goldfish cone pigments is 11-cis 3,4-dehydroretinaldehyde whose molar extinction coefficient in ethanol is 24,900 liter/mole cm. (b)
The peak molar extinction of the green-absorbing cone pigment ($\lambda_{\text{max}} = 533$ nm) is equal to that of the yellow perch porphyropsin (with similar or possibly identical $\lambda_{\text{max}}$) at 30,000 liter/mole cm. (c) The concentration of visual pigment is the same in the outer segments of all three goldfish cone types. (d) The absorption spectrum of 11-cis 3,4-dehydroretinal dissolved in ethanol and that of the in situ visual pigments using the same molecule as prosthetic group are related to one another through transformations in which the spectral component parameters are continuous, monotonic functions of frequency (i.e., of the wavenumber $\nu_m$ of the main absorption band peak).

**Main Results**

(a) A phenomenological analysis of absorption spectra led to the observation of the apparent existence of a spectroscopic continuity between the chromophore absorption spectrum and that of the corresponding visual pigments. (b) By the use of curve-fitting to empirical data, a set of analytic functions were determined which to a good approximation describe the physiologically relevant portions of vertebrate visual pigments. (c) The generated curves reproduce the shapes (half widths) as well as the amplitudes (molar absorptivities) of the pigment spectra. (d) The spectral transformation between the pigments and their chromophore is such that the sum of the oscillator strengths of the three lowest-energy Gaussian components remain nearly invariant. (e) The phenomenological explanation for the gradual increase in peak extinction coupled with the narrowing of the main absorption band as the chromophore is bound into more and more red-shifted pigments is that the first two Gaussian components move closer and closer together while the third one gradually falls behind.

Received for publication 30 December 1975.

**REFERENCES**

BAYLOR, D. A., and A. L. HODGKIN. 1973. Detection and resolution of visual stimuli by turtle photoreceptors. *J. Physiol. (Lond.)* 254:163–198.

BRIDGES, C. D. B. 1967a. Spectroscopic properties of porphyropsins. *Vis. Res.* 7:349–369.

BRIDGES, C. D. B. 1967b. Biochemistry of visual processes. In Comprehensive Biochemistry. M. Florkin and E. H. Stotz, editors. Elsevier, Amsterdam. 27:31–78.

BRIDGES, C. D. B. 1973. Interrelations of visual pigments and vitamins A in fish and amphibia. In Biochemistry and Physiology of Visual Pigments. H. Langer, editor. Springer-Verlag, New York. 115–121.

DARTNALL, H. J. A. 1953. The interpretation of spectral sensitivity curves. *Br. Med. Bull.* 9:24–30.

FORSTER, R. P., and J. V. TAGGART. 1950. Use of isolated renal tubules for the examination of metabolic processes associated with active cellular transport. *J. Cell Comp. Physiol.* 36:251–270.

HÁROSI, F. I. 1975. Absorption spectra and linear dichroism of some amphibian photoreceptors. *J. Gen. Physiol.* 66:357–382.

HÁROSI, F. I., and E. F. MACNICHOL, JR. 1974a. Visual pigments of goldfish cones. Spectral properties and dichroism. *J. Gen. Physiol.* 65:279–304.

HÁROSI, F. I., and E. F. MACNICHOL, JR. 1974b. Dichroic microspectrophotometer: A
computer-assisted, rapid, wavelength-scanning photometer for measuring linear dichroism in single cells. *J. Opt. Soc. Am.* 64:903–918.

Hecht, S. 1920. Photochemistry of visual purple. I. The kinetics of the decomposition of visual purple by light. *J. Gen. Physiol.* 3:1–13.

Knott, G. D., and D. K. Reece. 1972. MLAB: A civilized curve-fitting system. Proceedings of the ONLINE '72 International Conference. Brunel University, England. 1:497–526.

Liebman, P. A., and G. Entine. 1968. Visual pigments of frog and tadpole (*Rana pipiens*). *Vis. Res.* 8:761–775.

Liebman, P. A., and A. M. Granda. 1971. Microspectrophotometric measurements of visual pigments in two species of turtle, *Pseudemys scripta* and *Chelonia mydas*. *Vis. Res.* 11:105–114.

Marks, W. B. 1965. Visual pigments of single goldfish cones. *J. Physiol. (Lond.)*. 178:14–32.

Munz, F. W., and S. A. Schwanzara. 1967. A nomogram for retinene<sub>2</sub>-based visual pigments. *Vis. Res.* 7:111–120.

Planta, C. V., U. Schwieker, L. Chopard-Dit-Jean, R. Ruegg, M. Kofler, and O. Isler. 1962. Physikalische Eigenschaften von isomeren vitamin-A- und vitamin-A<sub>2</sub>-Verbindungen. *Helv. Chim. Acta.* 45:548–561.

Sandorfy, C. 1964. Electronic Spectra and Quantum Chemistry. Prentice-Hall, Inc., Englewood Cliffs, N. J.

Sperling, W. 1973. Conformations of 11-cis retinal. In *Biochemistry and Physiology of Visual Pigments*. H. Langer, editor. Springer-Verlag, New York. 19–28.

Stell, W. K., and F. I. Harosi. 1976. Cone structure and visual pigment content in the retina of the goldfish. *Vis. Res.* 16:647–657.

Tomita, T., A. Kaneko, M. Murakami, and E. L. Paultre. 1967. Spectral response curves of single cones in the carp. *Vis. Res.* 7:519–531.

Wald, G., P. K. Brown, and P. H. Smith. 1955. Cyanopsin, a new pigment of cone vision. *Science (Wash. D. C.)*. 118:505–508.