Formation, Conversion, and Utilization of Isorhodopsin, Rhodopsin, and Porphyropsin by Rod Photoreceptors in the *Xenopus* Retina

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**A B S T R A C T** The visual pigment content of rod photoreceptors in *Xenopus* larvae was reduced > 90% through a combination of vitamin A-deficient diet and constant light. Thereafter, a dose of either all-trans-retinol or 9-cis-retinal was injected intramuscularly, leading to the formation of a rhodopsin ($\lambda_{max} 504$ nm) or isorhodopsin ($\lambda_{max} 487-493$ nm) pigment, respectively. Electrophysiological measurements were made of the threshold and spectral sensitivity of the aspartate-isolated PIII (photoreceptoral) component of the electroretinogram. These measures established that either rhodopsin or isorhodopsin subserved visual transduction with the same efficiency as the 519 nm porphyropsin pigment encountered normally. When animals with rhodopsin or isorhodopsin were kept in darkness or placed on a cyclical lighting regimen for 8 days, retinal densitometry showed that either pigment was being converted to porphyropsin; significantly more porphyropsin was formed as a result of cyclical lighting than after complete darkness.

**I N T R O D U C T I O N**

Vitamin A (retinol) cannot be synthesized by animals. It is obtained from the diet either as the preformed vitamin (all-trans-retinol) or by metabolism of the provitamin, β-carotene (Huang and Goodman, 1965). In either case, absorption is via the intestine, and delivery through the bloodstream to the eye utilizes a retinol-binding protein (RBP) to which the vitamin is complexed (Kanai et al., 1968). Inasmuch as all known visual pigments consist of the 11-cis-isomer of retinal (the aldehyde of vitamin A) joined to a protein, opsin (Wald, 1959), incorporation of the chromophore into visual pigment requires the oxidation and isomerization of the all-trans-vitamin to its 11-cis-aldehyde. In this connection it is noteworthy that, whereas significant quantities of a RBP have been found in a variety of tissues (Ong and Chytíl, 1975 a, b; Smith et al., 1975), including the neural retina and pigment epithelium (Wiggert and Chader, 1975;
Heller and Bok, 1976; Wiggert et al., 1976, 1977; Futterman and Saari, 1977), an intraocular-binding protein that exhibited a marked affinity for the 11-cis isomer of retinal was identified by Futterman et al. (1977).

Other isomeric forms of retinal can be linked to opsin, but the 9-cis-isomer is of particular interest because it combines spontaneously with opsin in vitro to yield a light-sensitive “iso” pigment (Hubbard et al., 1971). Nevertheless, attempts to produce isorhodopsin in vitamin A-starved rats by dietary or subcutaneous administration of 9-cis-retinal have not been successful, presumably because the animals converted the isomer to its 11-cis configuration before the visual pigment was formed (Crescitelli and Pearlman, 1973). There is no information available as to where this conversion took place, nor is there evidence that in its absence a mechanism exists for transporting the 9-cis-isomer to the photoreceptors. It is interesting to note, however, that isorhodopsin has been produced in situ by topical application of 9-cis-retinal to the isolated skate retina (Pepperberg et al., 1976) and by flash irradiation of photoproducts in the rat retina (Huddleston and Williams, 1977). Huddleston and Williams concluded that 9-cis-retinal can subserve visual transduction; whether it does so with the same efficacy as the 11-cis-isomer has not yet been resolved fully, and is one subject of the present investigation.

In addition to steric specificity, vitamin A occurs in two molecular forms, retinol (vitamin A$_1$) and 3-dehydroretinol (vitamin A$_2$) whose aldehydes go to form rhodopsin and porphyropsin, respectively. In some amphibians, and in some fish, the form that is utilized in visual pigments varies with the stage of development, environmental conditions, and hormonal influences (Wilt, 1959; Allen and McFarland, 1973; Bridges, 1974, 1975); occasionally both forms exist in the eye at the same time (Reuter et al., 1971). In the rods of *Xenopus* tadpoles, a vitamin A$_2$-based porphyropsin with maximal absorption ($\lambda_{max}$) at 519 nm is the only detectable photopigment (Bridges et al., 1977), and after the retina is depleted almost entirely of its content of porphyropsin, it is possible to monitor visual pigment formation after intramuscular injection of various vitamin A compounds (Engbretson and Witkovsky, 1978). The results reported here show that depending on the frequency of administration, the incubation time, and the environmental lighting conditions, *Xenopus* rods are capable of forming three different visual pigments, any one of which can subserve visual transduction. These findings suggest further that the binding proteins required at each stage in the transport of vitamin A are capable of complexing with more than one isomeric form of the vitamin or its aldehyde.

**MATERIALS AND METHODS**

Most of the procedures are described in detail in the preceding paper (Engbretson and Witkovsky, 1978). *Xenopus* larvae obtained from breeding pairs injected with gonadotrophin extracts were raised on a vitamin A-free diet in constant light. Intramuscular injections of vitamin A ($2.1 \times 10^{4}$ molecules in 0.1 $\mu$l ethanol) were made under microscopic control. 9-cis-retinal was obtained from Hoffman-La Roche Inc., Nutley, N. J., all-trans-retinol from Sigma Chemical Co., St. Louis, Mo., and 11-cis-retinal from Prof. P. K. Brown, Biological Laboratories, Harvard University. Vitamin A in crystalline form was stored under nitrogen in light-proof containers in a freezer before use.
Solutions were freshly prepared just before injection, after which the animals were kept at room temperature either in the dark or in an illuminated chamber.

Measurements of photopigment spectra were made upon retinas isolated under dim red illumination and placed in a chamber under a drop of Ringer solution containing 10 nM hydroxylamine. The chamber was located on the stage of a compound microscope that formed part of a computer-assisted transmission densitometer (Ripps and Snapper, 1974). Density difference spectra ($\Delta D_\lambda$ for $\lambda = 360-720$ nm) were calculated from transmissivity data obtained through a 0.126 mm² area of the central retina under the following conditions: (a) dark adaptation in excess of 24 h; (b) after a 30-s exposure to a deep red light produced by passing the bleaching beam through a Wratten 29 filter (Eastman Kodak Co., Rochester, N. Y.) that transmitted $\lambda > 610$ nm; and (c) after a 20-s exposure to an intense "white" light that bleached completely all of the available visual pigments. Irradiation with deep red light tends to bleach selectively any long wavelength-sensitive pigments, whereas the subsequent exposure denudes the retina of its remaining photopigments (Dartnall, 1962). Thus, shifts in the $\lambda_{\text{max}}$ of the density spectra obtained under the various conditions were useful in detecting the presence of an admixture of photopigments.

We also measured the density spectra of digitonin extracts. Eyes were removed with the aid of an infrared viewing system, then ground in Mcllvaine's citrate buffer, pH 7.4 in a Potter-Elvehjem glass-Teflon tissue grinder (Markson Science Inc., Del Mar, Calif.). The suspension was centrifuged at 11,000 rpm for 20 min at 4°C and the supernate was discarded. The pellet was resuspended in 0.2 ml of 2% digitonin solution (Bridges, 1977) and stored overnight at 4°C. After centrifugation as above, the supernate was placed into a cuvette, diluted 1:1 with pH 8.5 borate buffer containing 10 mM hydroxylamine (final concentration), and examined spectrophotometrically in a Zeiss PMQ II spectrophotometer (Carl Zeiss, Inc., New York).

In addition to the in situ measurements, spectral analyses were obtained on visual pigments synthesized in vitro. *Xenopus* opsin was isolated in a sucrose gradient according to a variation on the method described by Papermaster and Dreyer (1974). 25–30 retinas, each ~5 mm² were pooled in 34% sucrose solution containing 66 mM NaCl, 2 mM MgCl₂, and 5 mM Tris-acetate buffer, pH 7.4, and sonicated. This solution was overlain by a similar one containing 24% sucrose, 1 mM MgCl₂, and 10 mM Tris-acetate, pH 7.4. The suspension was centrifuged at 4°C for 20 min at 25,000 rpm in a SW50-1 Sorvall rotor (DuPont Co., Instrument Products Div., Wilmington, Del.) The resulting concentration of rod outer segments at the interface was pipetted off, diluted twofold with 10 mM Tris-acetate buffer, pH 7.4, and spun at 12,000 rpm for 20 min in a Sorvall HB-4 rotor (DuPont Co., Instrument Products, Div.) The supernate was decanted and the pellet was resuspended, without washing, in Mcllvaine's citrate buffer at pH 6.1; to this a ~1-5 M equivalent of vitamin A was added in a small (<1% total) volume of ethanol. NADP (Sigma Chemical Co.) at a final concentration of 1–2 × 10⁻⁷ M was added and the mixture was incubated overnight in the dark at 4°C. It then was centrifuged at 12,000 rpm for 20 min, the supernate was decanted, and 0.2 ml of 2% digitonin (prepared according to Bridges, 1977) was added to the pellet and stirred with a glass probe. After a 24-h incubation in the dark at 4°C, the mixture was centrifuged at 12,000 rpm for 20 min; the supernate was placed in a cuvette and then diluted with pH 8.5 borate buffer containing 10 mM hydroxylamine. Absorbance spectra were obtained in a Zeiss PMQII manual spectrophotometer.

Electrophysiological measures of photoreceptor responsiveness were obtained as described in the preceding paper. After 24 h dark-adaptation, one eye of a vitamin A-deficient larva was excised, its anterior portion was dissected away, and a drop of 100 mM sodium aspartate was added to the vitreous body to eliminate the b-wave of the
Electroretinogram (ERG) (Sillman et al., 1969) and permit measurement of the fast PIII (receptoral) component of the transretinal voltage. An additional 20-60-min dark-adaptation was allowed in the experimental chamber to ensure recovery from exposure to the red dissecting light. Responses were elicited by 1-s test flashes, varying in intensity from below threshold to above rod saturation. The animal then received an intramuscular injection of \(2.1 \times 10^{16}\) molecules of 9-cis-retinal in 0.1 \(\mu l\) of ethanol, and was maintained in the dark for 48 h before testing the remaining eye.

**Figure 1.** Difference spectra of vitamin A-deprived Xenopus tadpoles, obtained 48 h after a single injection of \(2.1 \times 10^{16}\) molecules of 9-cis-retinal. (○) Results \(\lambda_{\text{max}} = 522\) nm obtained after partial bleaching with a deep red light. (●) Absorbance change \(\lambda_{\text{max}} = 496\) nm resulting from a subsequent exposure to an intense white light. (◯) The full difference spectrum gives the change from the dark-adapted to the completely-bleached state, i.e., the sum of the two partial bleaches, \(\lambda_{\text{max}} = 498\) nm.

**RESULTS**

9-cis-Retinal as a Source of Visual Pigment

Transmissivity measurements taken 48 h after a single injection of \(2.1 \times 10^{16}\) molecules of 9-cis-retinal showed that retinae, previously depleted of visual pigment, now contained a large quantity of light-sensitive substance. The difference spectra of Fig. 1 indicate that at least two photopigments were present after the injection. The difference spectrum produced by the partial bleach with deep red light is maximal at \(~520\) nm, but a second bleach with white light shows the overwhelming preponderance of an iso pigment with \(\lambda_{\text{max}} = 496\) nm. Because spectral measurements on the retinae of vitamin A-starved animals sometimes gave small density differences with \(\lambda_{\text{max}}\) in the region of 520-525 nm, the density changes resulting from the red bleach in Fig. 1 may have
been due to the remnants of the naturally occurring porphyropsin. It is appropriate to note here that the wavelengths of peak absorption reported in this paper are calculated as the average of the two half-maximal points on the spectral curves. With regard to the location of the $\lambda_{\text{max}}$, it will be recalled that these are difference spectra, and the photoproduct formed attenuates the short wavelength side of the curve. This results in a small ($\approx 4 \text{ nm}$) displacement of peak to longer wavelengths. In addition, small amounts of cone pigments might contribute to a shift to longer wavelengths.

**Threshold Changes Induced by the Injection of 9-cis-Retinal**

Fig. 2 shows the averaged voltage-intensity data of animals obtained before and 48 h after the injection of 9-cis-retinal; for each animal, the recorded voltages were elicited by 1-s flashes of nearly monochromatic light and are expressed as percentages of the maximum potential elicited by a supersaturating test flash; errors bars are $\pm 1 \text{ SD}$. The quantal flux needed to elicit a half-maximal response (dashed line) fell by 2.21 log units (vertical arrows) after 9-cis-retinal administration.

![Figure 2](image-url)

**Figure 2.** $V$-$\log I$ data for vitamin A-deprived *Xenopus* tadpoles (○, $n = 23$) before and (●, $n = 8$) 48 h after an intramuscular injection of $2.1 \times 10^{16}$ molecules of 9-cis-retinal. Voltages are expressed as percentages of the maximum potential elicited by a supersaturating test flash; errors bars are $\pm 1 \text{ SD}$. The quantal flux needed to elicit a half-maximal response (dashed line) fell by 2.21 log units (vertical arrows) after 9-cis-retinal administration.

Any comparison of absolute dark threshold for *Xenopus* rods containing rhodopsin, isorhodopsin, or porphyropsin must take into account the differences in molar extinction and quantum efficiencies of absorption for these three
photopigments. The values are listed in Table I. If it is assumed that the photopigment subserving the receptive response of Fig. 2 is a 9-cis pigment, then allowance for the differences in molar extinction and quantum efficiency between porphyropsin and isorhodopsin equates the above sensitivity difference with 1.93 log units of sensitivity change due to porphyropsin. It is interesting to note that the irradiance required to elicit a half-maximal response (0.5 \( V_{\text{max}} \)) after administration of 9-cis-retinal, namely 9.19 log incident quanta cm\(^{-2}\)s\(^{-1}\), does not differ significantly from that obtained after injection of the same quantity of all-trans-retinol (9.48 log quanta; Engbretson and Witkovsky, 1978); moreover, both values are in good agreement with the quantal flux (9.65 log quanta) that elicits a 0.5 \( V_{\text{max}} \) response in the normal, porphyropsin-containing retinæ of *Xenopus* larvae having rods of comparable length (stage 54-56, Witkovsky et al., 1976).

Further evidence that visual function was being mediated by an isopigment was provided by the spectral sensitivity of the PIII (photoreceptoral) response. Spectral sensitivity curves were calculated from voltage-intensity functions generated by a series of monochromatic test flashes; from these the reciprocal of the quantal flux required to elicit a criterion response (0.25-0.50 \( V_{\text{max}} \)) was determined. The filled squares of Fig. 3 show the mean ± 1 SD of four such runs; the curve represents the absorbance spectrum of a 487 nm pigment, corrected for an axial density of 0.5. The axial absorbance value of 0.5 is a function of the specific absorbance of the rod (0.010-0.016/μm) (Liebman, 1972) times its mean length (35.1 μm); see Engbretson and Witkovsky, 1978. The rationale for selecting a \( \lambda_{\text{max}} \) of 487 nm will be made clear shortly. Note that the mean sensitivity departs significantly from the curve for wavelengths longer than 490 nm; the data of Fig.1 and the results of a chromatic adaptation experiment described below suggest that this was due to the presence of a second photopigment, probably the naturally occurring prophyropsin (\( \lambda_{\text{max}} = 519 \) nm) found in this animal (Bridges et al., 1977).

Spectral sensitivity was tested in the presence of a yellow (Wratten 15, Eastman Kodak Co.) background field, adjusted in intensity to depress the peak sensitivity by ~0.5 log unit. The results (Fig. 3, ○) show that in the presence of the yellow adapting field there is a greater degree of correspondence between experimental points and the absorption spectrum than was found when stimuli fell on a dark background.
It is also worthwhile to compare the foregoing results with the spectral sensitivity function obtained on vitamin A-deprived larvae before injecting 9-cis-retinal. The data (●) are fit well by the absorption spectrum of *Xenopus* porphyropsin (Bridges et al., 1977), although they are displaced downward by $>2$ log units on the sensitivity scale as compared with the sensitivity data of animals after injection.

**Spectral Maxima of Rod Pigments in Solution**

Another method of demonstrating that isorhodopsin can be formed in vivo was through analyses of visual pigment extracts. *Xenopus* larvae were injected with a
single dose of either all-trans-retinol or 9-cis-retinal, and then maintained in the dark for 12-50 h. The animals were decapitated, the eyes were removed with the aid of an infrared image converter, and a digitonin extract of the visual pigment was prepared. The difference spectra of the extracts are shown in Fig. 4. Injection of all-trans-retinol led to the formation of a pigment with a $\lambda_{\text{max}}$ of 503 nm, whereas administration of 9-cis-retinal resulted in a pigment peaking at 493 nm. These data strongly suggest that the 9-cis-isomer is incorporated into isorhodopsin whereas the all-trans-retinol is converted to the 11-cis-isomer to form rhodopsin. Because of the tendency to convert these pigments to the 519 vitamin A$_2$ pigment normally found in Xenopus rods (see below) the curves of Fig. 4 may reflect an admixture of a second pigment. To explore this possibility and to provide further evidence of the separate identity of the isopigment, we attempted to synthesize isorhodopsin and rhodopsin in vitro.
The Synthesis of 9-cis and 11-cis Pigments

Visual pigments were formed in vitro by incubation of 9-cis- or 11-cis-isomers of vitamin A₁ with *Xenopus* opsin, in the presence of NADP (see Methods). Difference spectra obtained from these mixtures are graphed in Fig. 5. They show that the isorhodopsin formed had a peak absorption at 487 nm, whereas the rhodopsin peaked at 504 nm. The experimental points correspond reasonably well to curves of corresponding \( \lambda_{\text{max}} \) calculated from the Dartnall nomogram (Dartnall, 1953). The scatter in the data, visible primarily on the short wavelength side of peak, may be attributable to the small yields \((D \leq 0.02)\).

Photoproduct formation is not shown, because its true spectrum was obscured by the strong absorbance at \( \lambda \leq 380 \) nm due to free chromophore.

Taken together then, the data strongly support the contention that after its injection, the 9-cis-isomer is delivered to *Xenopus* rods and incorporated as an isorhodopsin. Furthermore, the isorhodopsin can subserve visual transduction with the same efficacy as either the rhodopsin induced by an injection of all-trans-retinol or the naturally occurring photopigment, porphyropsin (Witkovsky et al., 1976).

Interconversion of Visual Pigments in *Xenopus* Rods

Although normal *Xenopus* rods, like those of many fish, contain a vitamin A₂-based pigment, the animal’s diet provides only vitamin A₁. Thus, larvae must possess a suitable enzyme for interconverting these two forms of the vitamin. It
seemed appropriate, therefore, to determine whether the artificially induced isorhodopsin or rhodopsin pigments would be converted in the course of time to porphyropsin. To answer this question, groups of animals were placed on one of four different regimens. Some were given a single injection of \(2.1 \times 10^{16}\) molecules of 9-cis-retinal or all-trans-retinol, and then were allowed 8 days, either in complete darkness or in 12 h/12 h cyclical lighting, before testing. Another group of animals received four injections totaling \(2 \times 10^{16}\) molecules of vitamin A on test days 1, 3, 5, and 7. These also were maintained either in the dark or on the cyclical lighting protocol. After the various procedures, the retinae were isolated under dim red illumination for transmission densitometry. Difference spectra were obtained by the methods described earlier, measurements being taken in the dark, after a 30-s exposure to a deep red (Wratten 29) bleaching light, and after a full bleach with white light. Spectra obtained 1-20 min after the final bleach established that no measurable regeneration of pigment occurred.

Figs. 6 and 7 show representative difference spectra from singly-injected animals maintained in darkness (Fig. 6) or in cyclic light (Fig. 7). In each figure, the results of the partial bleaching exposure to red light (○) were similar, and indicate the presence of a light-sensitive substance absorbing at long wavelengths (\(\lambda_{\text{max}} = 523-526\) nm). However, a subsequent bleach with white light gave different results in these two cases. For the animals maintained in darkness, the second bleaching exposure to white light effected a shift to 505 nm, indicating the presence of rhodopsin (Fig. 6); it is not possible in this instance to distinguish between the 9-cis-and 11-cis-isomers. On the other hand, animals maintained in cyclical light gave a nearly symmetrical change in absorbance (\(\lambda_{\text{max}} = 521\) nm) after the second bleach (Fig. 7). The fact that both bleaches gave rise to difference spectra with \(\lambda_{\text{max}}\) in the same spectral region is evidence that the only pigment detectable was porphyropsin.

Table II summarizes the data obtained under the various experimental conditions. One particularly noteworthy point is the fact that rhodopsin or isorhodopsin was formed within 24-48 h after a single injection of all-trans-retinol or 9-cis-retinal, respectively. The subsequent changes that took place when the animals were kept for 8 days in different environments parallel those described above and graphed in Figs. 6 and 7. Thus, after an 8-day incubation, all animals, whether recipients of 9-cis-retinal or all-trans-retinol gave full-bleach difference spectra (right-hand column, Table II) with \(\lambda_{\text{max}}\) in the range 505-523 nm. These spectra proved to be variable mixtures of rhodopsin and porphyropsin as shown by the results of partial bleaches (left-hand and middle columns, Table II). It is clear that in each instance, bleaching with deep red light revealed the presence of the long-wavelength-absorbing pigment (porphyropsin). Comparison of these data with difference spectra obtained after a subsequent white light exposure, showed that the fraction of porphyropsin was greater when animals were kept in a cyclical-light environment as compared with animals maintained in complete darkness. Multiple injections also favored a conversion to porphyropsin compared to a single injection of the same total amount.
A principal finding of the present study is the formation, in the rods, of an isorhodopsin after systemic administration of 9-cis retinal. Recent work has established that vitamin A is transported within the body by a series of binding proteins that exhibit different degrees of selectivity for the various compounds of vitamin A. The mammalian extraocular retinol-binding protein found in a number of tissues (Ong and Chytil, 1975 a) distinguishes between retinol, on the one hand, and either retinal or retinoic acid on the other (Ong and Chytil, 1975 b), but will bind either retinol or 3-dehydoretinol in various isomeric forms. The pigment epithelium-neural retina complex contains at least two species of binding protein (Wiggert et al., 1976, 1977). Of these, the lighter molecule shows greater specificity, in that it will not bind retinol, and moreover, preferentially binds 9-cis- and 11-cis-retinal over their all-trans counterpart. If the vitamin A transport mechanisms described in mammals pertain also to *Xenopus*, systemically administered 9-cis-retinal would have to be reduced to 9-cis-retinol before transport to the eye and then reconverted to its aldehyde form before incorporation into visual pigment.
Isorhodopsin and Photoreceptor Threshold

The question of whether isorhodopsin is capable of subserving visual transduction was also of concern in this study, although it had been probed in the two papers cited earlier (Pepperberg et al., 1976; Huddleston and Williams, 1977). Pepperberg et al. applied 9-cis-retinal to the receptoral surface of the isolated skate retina after bleaching away 40% of the available rhodopsin; uptake of the isomer and formation of isorhodopsin was shown by transmission densitometry. Partial recovery of ERG threshold was found over 30-60 min, but the spectral sensitivity of the response was not obtained, hence it was not established unequivocally that the isorhodopsin rather than the remaining rhodopsin mediated visual transduction. Huddleston and Williams (1977) utilized flash bleaches to produce mixtures of rhodopsin and isorhodopsin in intact rat eyes. Based on the location of $\lambda_{\text{max}}$ (between the 498-nm rhodopsin and 487-nm...
isorhodopsin spectra) they estimated that ~30% of the mixture was isorhodopsin. Although spectral curves obtained from ERG measures also indicated a shift to shorter wavelengths as a result of the flash bleach, the associated changes in receptor threshold were not charted. The results of the present study show that isorhodopsin can subserve visual transduction. Theoretical considerations based upon the presumed values for quantum efficiency and molar extinction of rhodopsin, porphyropsin, and isorhodopsin lead to the prediction that absolute thresholds of the rods will vary by 0.3–0.5 log unit, depending upon the nature of the photopigment. Although the variability in our data may have obscured these differences, a comparison of the present data with those of previous studies (Witkovsky et al., 1976; Engbretson and Witkovsky, 1978) showed that the dark threshold of *Xenopus* rods containing isorhodopsin were not greatly different from those rods containing either rhodopsin or porphyropsin.

### Table I

**DIFFERENCE SPECTRA RESULTING FROM PARTIAL BLEACHES OF LARVAL *Xenopus* RETINAS**

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Dark → Red</th>
<th>Red → White</th>
<th>Dark → White</th>
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<tbody>
<tr>
<td><strong>A. Injection of 2.1 x 10⁴ molecules all-trans-retinol</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1 injection, 24 h in dark</td>
<td>516 ± 3</td>
<td>508 ± 1</td>
<td>504 ± 1</td>
</tr>
<tr>
<td>1 injection, 8 days in dark</td>
<td>527 ± 2</td>
<td>516 ± 2</td>
<td>520 ± 1</td>
</tr>
<tr>
<td>cyclic light</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>4 injections, 8 days in dark</td>
<td>522 ± 1</td>
<td>502 ± 1</td>
<td>505 ± 1</td>
</tr>
<tr>
<td>4 injections, 8 days in 12h/12h</td>
<td>526 ± 1</td>
<td>511 ± 1</td>
<td>516 ± 1</td>
</tr>
<tr>
<td>cyclic light</td>
<td></td>
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<tr>
<td><strong>B. Injection of 2.1 x 10⁴ molecules 9-cis-retinal</strong></td>
<td></td>
<td></td>
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<tr>
<td>1 injection, 48 h in dark</td>
<td>523 ± 1</td>
<td>497 ± 1</td>
<td>500 ± 2</td>
</tr>
<tr>
<td>1 injection, 8 days in dark</td>
<td>525 ± 1</td>
<td>505 ± 1</td>
<td>511</td>
</tr>
<tr>
<td>1 injection, 8 days in 12h/12h</td>
<td>524 ± 1</td>
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<td>cyclic light</td>
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<td>4 injections, 8 days in 12h/12h</td>
<td>524 ± 1</td>
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Maxima of the Visual Pigments

The *Xenopus* porphyropsin at λ<sub>max</sub> = 519 nm is typical of its class. Not surprisingly, therefore, the same protein, when combined in vitro with either 9-cis- or 11-cis-isomers of vitamin A<sub>a</sub> produced pigments with a λ<sub>max</sub> of 487 nm and 504 nm, respectively, which also are within the range of reported values for isorhodopsin and rhodopsin (Hubbard et al., 1971). The spectral maxima obtained from densitometric runs after a single injection of the 9-cis-isomer and only 24–48 h dark adaptation are displaced to slightly longer wavelengths than
those cited above. Some of this difference is attributable to a slight admixture of a second pigment absorbing at longer wavelengths. The other potential contributory factor is spectral interaction between parent pigment and photoproduct described earlier (see Results).

Interconversion of Visual Pigments

The ability to generate either isorhodopsin or rhodopsin in the place of the normal porphyropsin pigment in *Xenopus* probably depends on the relatively greater speed of the transport system compared to the rates of the enzymatic processes responsible for isomerization and interconversion of vitamins A₁ and A₂. This conclusion is buttressed by the observation that, when administered in less massive doses over several days, the same total amount of 9-cis-or *all-trans*-vitamin A₂ resulted in the formation of a relatively higher fraction of the A₂-based pigment (Table II). Furthermore, exposure to light during the incubation period favored formation of porphyropsin. Although the experimental situation may be considered artificial, the results are consistent with the known capability of amphibian eyes to incorporate either vitamin A₁ or A₂ into visual pigment.

Another interesting finding of this study is the demonstration of an isomerization in darkness of 9-cis-retinal or *all-trans*-retinol to the 11-cis form of 3-dehydroretinal which serves as the chromophore of porphyropsin. The 519 photopigment (Table II) resulting from an 8-day incubation of either precursor is evidence of such an isomerization. Hubbard (1956) described an enzyme, "retinene isomerase" which was capable of converting *all-trans*-retinal to 11-cis-retinal in the dark, albeit at a very slow rate. The reaction reached equilibrium when the 11-cis-isomer comprised about 5% of the mixture. However, with free opsin present to combine with the 11-cis-isomer (as was the case in our study), the reaction equilibrium would not be reached. Thus, after an 8-day incubation, sufficient *all-trans*-retinal could be converted to 11-cis and incorporated as visual pigment.

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