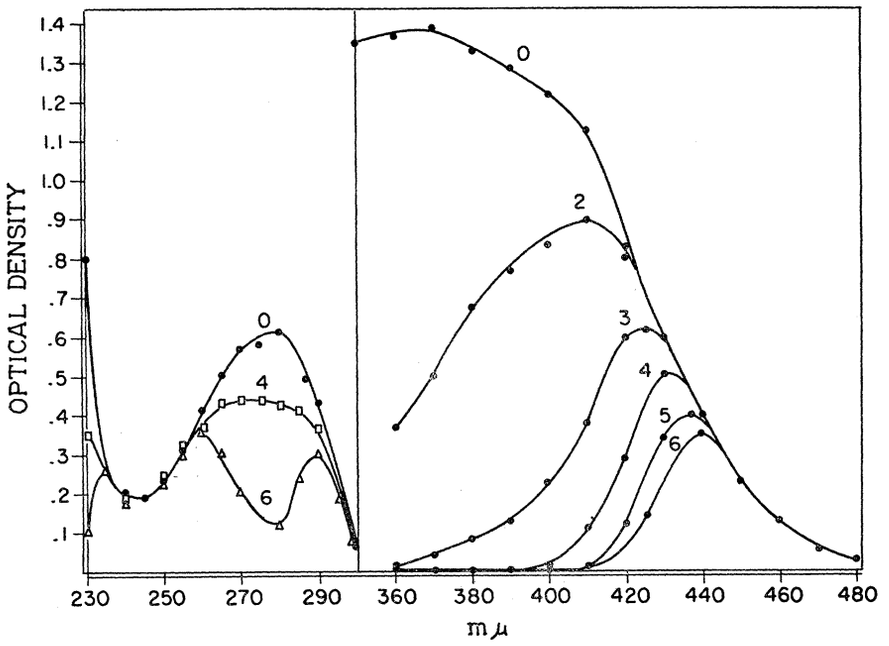

Fig. 1. Difference absorption spectra of tryptophan and 2,4-dinitrophenol, obtained with Beckman DU spectrophotometer and photomultiplier. (Left) Tryptophan. Each curve represents the difference spectrum due to $1 \times 10^{-4}M$ tryptophan in $0.05M$ phosphate, *p*H 7.3, and 0.005 percent Versene obtained as the difference between 0 and $1 \times 10^{-4}M$ (●), 4 and $5 \times 10^{-4}M$ (□), and 5 and $6 \times 10^{-4}M$ (△) respectively. (Right) 2,4-Dinitrophenol. Each curve represents the difference spectrum due to $1 \times 10^{-4}M$ dinitrophenol in $0.05M$ phosphate, *p*H 7.8, obtained as the difference between 0 and $1 \times 10^{-4}M$, 1 and $2 \times 10^{-4}M$, 2 and $3 \times 10^{-4}M$, and so forth. The indicated figure represents the lower concentration.

observed that, with solutions of only moderately high absorbance, such difference spectra may be seriously misleading.

Buffered solutions of 2,4-dinitrophenol and of tryptophan were prepared whose concentrations were $1 \times 10^{-4}M$, $2 \times 10^{-4}M$, $3 \times 10^{-4}M$, and so forth. Difference spectra were determined between succeeding pairs in the series so that in each case the absorption spectrum due to a $1 \times 10^{-4}M$ solution was obtained. The results are shown in Fig. 1.

It is clear that the nature of the *apparent* difference absorption spectrum changes as the absolute concentration of the absorbing material increases. The deviations from the true absorption spectra of these compounds are due entirely to the stray polychromatic light within the monochromator, which causes the apparent difference in optical density due to a given level of absorbing compound to decrease progressively as the absolute concentration increases. The use of a photomultiplier detector that permits determination of difference spectra between solutions of high absolute absorbance increases the likelihood of this instrumental artifact. Since this effect is most pronounced at the absorption maxima and is of least significance at the absorption minima, the entirely misleading data of Fig. 1 result. If these were difference spectra that had been obtained with a view toward identifying an unknown compound, they would be unrecognizable. In consequence, only in double-beam optical systems where the stray light is negligible may difference spectra obtained at high absolute absorbances be determined with confidence (*2*).

IRWIN FRIDOVICH, WALTER FARKAS, GEORGE W. SCHWERT, JR., PHILIP HANDLER

Department of Biochemistry, Duke University School of Medicine, Durham, North Carolina

References and Notes

1. A. H. Mehler, *Science* 120, 1043 (1954).
2. These observations were made in the course of studies variously supported by the National Institutes of Health, the U.S. Atomic Energy Commission, and the National Science Foundation.

12 March 1957

Photosensitive Pigments from Retinas of Deep-Sea Fishes

In November 1956, a new group of visual pigments was described briefly by Denton and Warren (*1*) from several species of deep-sea fishes. The new pigments were named "chrysopsins" or visual golds and characterized as having maximum sensitivity to light at a wavelength about 20 mμ less than the $\lambda_{max.}$ of rhodopsin. The chrysopsins were not extracted from the retina, but were demonstrated by determining the density changes when fresh retinas were bleached with white light.

Through the kindness of Carl L. Hubbs of the Scripps Institution of Oceanography, bathypelagic deep-sea fishes were collected on an expedition made by the Scripps vessel *Paolina-T* in February 1957. The fishes were caught at night with a midwater trawl at depths of 280 to 380 fathoms near Guadalupe Island, Baja California, Mexico. Hubbs generously sorted the trawl collections and made field identifications by dim red light (*2*). The retinas were removed, and digitonin extracts were prepared by standard methods (*3*). Spectrophotometric examination and bleaching with narrow-band colored light allowed analysis of the retinal pigments present in these extracts (*4*).

In general, the results of Denton and Warren were confirmed, $\lambda_{max.}$ of the photosensitive pigment of *Argyropelecus affinis* Garman being 478 mμ (Fig. 1). The pigment was demonstrated to be homogeneous by partial bleaching experiments (*3*). The absorption spectrum and the hydroxylamine difference spectrum of this retinal extract were in good agreement with the theoretical curve constructed from Dartnall's nomogram (*5*) for a visual pigment with $\lambda_{max.}$ of 478 mμ. The greater absorption of the extract (curve 1) in the short-wavelength portion was caused by the presence of light-absorbing impurities. The difference spectrum (curve 3) falls below the theoretical curve in this region because of the appearance of the violet-absorbing product of bleaching. The absorption of the product of bleaching was maximal at 375 to 380 mμ (*p*H 8.3) and at 365 to 370 mμ when hydroxylamine was added, indicating that retinene$_1$ was the chromophore of the light-sensitive pigment (*6*). This pigment is therefore provisionally called pigment 478_1 (the subscript means that retinene$_1$ is the chromophore).

A retinal extract of *Sternoptyx obscura* Garman of the same family, Sternoptychidae, had a different photosensitive pigment with $\lambda_{max.}$ at 485 mμ, but with the same product of bleaching. In the unrelated *Bathylagus wesethi* Bolin (family Argentinidae), however, a 478_1 pigment like that of *Argyropelecus* was mixed in the extract with a rhodopsin ($\lambda_{max.} = 500$ mμ) in the ratio of about 4/1. This was determined by selectively bleaching the red-sensitive rhodopsin with red light and then bleaching the 478_1 pigment with orange and yellow light. The products of bleaching of the two pigments were spectrally identical, suggesting that retinene$_1$ was the chromophore of each.

Further indications of diversity were found in extracts of other deep-sea fishes, each of which had only a single

photosensitive pigment with the same product of bleaching as *Argyropelecus* and *Bathylagus*. *Stomias atriventer* Garman (family Stomiatidae) and the unrelated *Lampanyctus mexicanus* Gilbert (family Myctophidae) both had 490_1 pigments. Slightly different was an extract of *Melamphaes bispinosus* Gilbert (family Melamphaidae) with a light-sensitive 488_1 pigment.

All the extracts showed surprisingly high concentrations of the photosensitive pigments. (In Fig. 1, curve 1, the optical density at 475 mμ was 0.562 in an extract made from two small eyes.) The fishes examined probably all have pure-rod retinas. With histological methods, Brauer (*7*) studied species of each genus reported here and found only rods in each of them. There is no direct evidence that the light-sensitive retinal pigments of these deep-sea fishes are visual pigments. This seems probable, however, in view of their characteristic shape, which agrees with Dartnall's nomogram, and their products of bleaching, which are indicative of retinene$_1$. Although the tendency of the retinal pigments to be shifted toward the blue end of the spectrum was common to all the deep-sea fishes studied, there is no systematic trend apparent from this preliminary survey. The extracted pigments differ from rhodopsins in their $\lambda_{max.}$ but not in their retinene. Their wavelengths of maximum absorption approach the photosensitive pigments of certain mammals which also have $\lambda_{max.}$ less than 500 mμ (*8*). Because of this continuous spread of retinene$_1$ pigments, the use of the term *chrysopsin* is confusing and therefore is not followed. The presence of a dual, light-sensitive pigment system in a pure-rod eye (*Bathylagus*), based on the single carotenoid retinene$_1$, is particularly interesting (*9*).

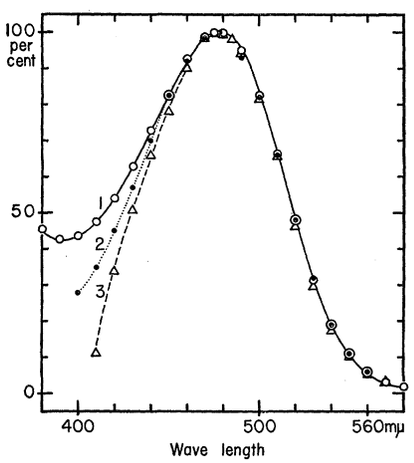

Fig. 1. Photosensitive pigment of *Argyropelecus affinis*. Curve 1, absorption spectrum of unbleached retinal extract (no significant absorption between 580 and 700 mμ); curve 2, constructed from Dartnall's nomogram, assuming a maximum at 478 mμ; curve 3, hydroxylamine experiment, difference spectrum (maximum change, 0.071 optical density units) after exposure of extract to yellow light (580 mμ).

FREDERICK W. MUNZ*
Department of Zoology,
University of California, Los Angeles

References and Notes

1. E. J. Denton and F. J. Warren, *Nature* 178, 1059 (1956).
2. I am indebted to Alfred W. Ebeling, also of Scripps Institution of Oceanography, who determined the six species of fishes studied.
3. F. W. Munz, *J. Gen. Physiol.* 40, 233 (1956).
4. Much of the equipment required for this study was made available by Frederick Crescitelli's grant from the Division of Research Grants and Fellowships, U.S. Public Health Service.
5. H. J. A. Dartnall, *Brit. Med. Bull.* 9, 24 (1953).
6. F. Crescitelli, *J. Gen. Physiol.* 40, 217 (1956).
7. A. Brauer, *Wiss. Ergebn. deut. Tiefsee-Exped. Valdivia 1898–99. Die Tiefseefische* (Fischer, Jena, 1908), vol. 2, pp. 164 ff.
8. For example, $\lambda_{max.}$ of the retinene$_1$ photosensitive pigment of the opossum is about 493 mμ (unpublished data of F. Crescitelli).
9. A detailed account of this investigation is in preparation.

* Predoctoral fellow, National Institute of Neurological Diseases and Blindness, U.S. Public Health Service.

21 March 1957

Factors Affecting the Appearance of Picture Varnish

Among the factors that affect the appearance of a picture varnish of the spirit type, one might consider that the refractive index of the resin would play a major role. Refractive index has entered the literature regarding picture varnishes, yet I do not recall that its role has ever been demonstrated. There has certainly not been sufficient discussion of the subject to reach agreement on whether the refractive index should be low (*1*) or similar to that of aged linseed oil (linoxyn) (*2*). In seeking to develop new protective coatings for the artist and conservator, our laboratory gave consideration to this problem. This report presents reasons for believing that variations in the refractive index of the resin, within the range 1.43 to 1.54, play a relatively minor, if not negligible, role in determining the appearance of spirit varnishes on the surface of paintings. Particular attention is drawn to variations in appearance that are attributable to differences in the viscosity grades of the resin.

Fresnel's law relates the intensity of reflected light in terms of the angle of incidence and the refractive index. Under the restricted condition of perpendicular incidence, the Fresnel equation may be simplified to

$$I = \left(\frac{n-1}{n+1}\right)^2$$

where I is the intensity of light of unit amplitude which is reflected and n is the refractive index. With this equation, values as follows may be calculated: $n = 1.1$, 0.23 percent reflection; 1.3, 1.7 percent; 1.5, 4.0 percent; 1.7, 6.7 percent. These values are essentially those attained at angles of incidence up to about 40 deg.

The range of refractive indices in organic coatings applied in the conservation of art objects is limited and is perhaps no more than 0.17. Limitation is understandable if only atoms of carbon, hydrogen, and oxygen are to be used in building the molecules of durable thermoplastics. By calculations from the simplified Fresnel equation, it is estimated that a linoxyn film of refractive index 1.57 (near the upper limit) would reflect 4.9 percent of the incident light. A film of refractive index 1.467 (near the lower limit) would reflect 3.6 percent. If reflection at the varnish-oil interface is considered, application of a varnish of refractive index 1.57 would result in no reflection and application of one of refractive index 1.467 would result in less than 1-percent reflection.

Frequently, then, in picture varnish, we are concerned with absolute differences in reflection of no more than 1 or 2 percent. This is close to the limit of the sensitivity of the eye. Over a wide range of intensities, the relative threshold of just-perceptible-brightness is about 1 percent of the intensity level to which the eye is adjusted (*3*).

Regardless of the proportion of incident light which is reflected, one's impression of a surface is strongly influenced by the relative sharpness or diffuseness of the reflected light. Methods of expressing the distribution of the reflected light are aspects of "gloss" or "glossiness." Judd (*4*) lists five types, five ways of expressing gloss: specular gloss, contrast gloss, distinctness-of-image gloss, sheen, and bloom.

If one observes only the reflected light, the relative change from 3.6 to 4.9 percent is considerable. However, in viewing a painting, the eye is perhaps adjusted to the general level of illumination in the room. An absolute difference of 1 to 2 percent is frequently negligible in comparison with variations in diffuse reflection—that is, gloss. In their investigation of ceramic glazes, Dinsdale and Malkin (*5*) found that the measured and observed gloss did not follow in the order of increasing refractive index from 1.51 to 1.66.

Even with porous paint, experiment has demonstrated that the fluidity of a varnish at a given concentration of resin can play a greater role than refractive index in determining appearance. A lean paint of Bakelite polyvinyl acetate AYAT and ultramarine was prepared. The