PHOTOLYTIC LIPIDS FROM VISUAL PIGMENTS*

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Kühne reported in 1878 that visual purple, the light-sensitive protein of the vertebrate retinal rods, bleached in the light with the formation of a pigment which he named “visual yellow.” Wald (1935) showed that the yellow pigment of bleached frog retinas could be extracted into chloroform and possessed a maximum spectral absorption at about 385 mµ. When treated with the Carr-Price reagent for carotenoids, SbCl₅, a blue color due to an absorption band at 664 mµ appeared. Wald has named this previously unknown carotenoid “retinene.” Hunter and Hawkins (1944) and Morton and Goodwin (1944) have shown that the oxidation of vitamin A leads to a similar chromogen, and the latter authors therefore suggest that retinene is vitamin A aldehyde.

Comparable advances have not been made in the study of daylight vision, the chemistry of which may be regarded as originating in 1937 when Wald reported the existence of a red-sensitive pigment, iodopsin, in the cone-rich retinas of chickens. Its difference absorption spectrum shows a maximum at about 570 mµ in digitonin solution, near the maximum sensitivity of the light-adapted chicken. This suggests that iodopsin is the basic light-sensitive substance of daylight vision.

The view that rhodopsin (visual purple) is a carotenoid-protein receives support from the decreased sensitivity of the eye to dim light in cases of vitamin A deficiency. Since the thresholds of both night and color vision are raised in experimental vitamin A deficiency (Hecht and Mandelbaum, 1938) it is of interest to determine whether iodopsin likewise releases retinene on exposure to light.

Preparation of Retinas

Iodopsin possesses to an even greater degree the protein-like lability of rhodopsin (Bliss, 1946). It was therefore helpful to find that iodopsin could be preserved indefinitely by freeze-drying a mass of retinas in a vacuum desiccator. This consisted of a two-section bent 1 inch diameter glass tube, one end of which contained a thin layer of retinas, while the other end was immersed in a dry ice freezing bath and thus acted as a condenser. Alcohol at dry ice temperature was poured over the vaporizer

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while pumping with a hyvac pump. The stopcock to the hyvac was then closed and
the retinas allowed to desiccate in the frozen state. A charge of about 50 retinas of
1 week old cull chicks could be dried in about 4 hours, yielding 0.4 gm. of friable pow-
der. After extraction of the retinal oils with petroleum ether the powder was stored
over drierite in the dark at 5°C.

Experimental Procedures

Since iodopsin is so much more sensitive to red light than rhodopsin the effect of
red light on the free lipid content of the retinal powder could be taken as a measure
of the lipid released by iodopsin. 100 mg. samples of powder were mixed with 5 ml.
of water in a Syracuse dish mounted in a light-tight box. A Wratten No. 70 (red)
filter was placed over the dish. A shallow glass dish holding a 1 cm. layer of water
was placed over the filter to absorb the heat of the bleaching light, a 100 watt frosted
bulb about 3 inches from the powder.

Before each bleach the suspension was bathed for ½ hour in flowing CO₂. After
illumination either with 10 minutes of red or 1 to 5 minutes of yellow light (Corning
No. 3389), the following operations were performed as rapidly as possible: the retinal
suspension was washed into a 1 inch diameter test tube, evacuated a moment to
remove trapped gas, and centrifuged 1 minute at a low speed. The supernatant
was discarded and replaced with 10 ml. of 2 per cent alcoholic petroleum ether.
A plunger operated by a windshield wiper then ground the retinas at a speed of about
75 strokes per minute for 15 minutes. The extract was next decanted into a large
test tube and pumped dry by a water aspirator with the aid of an ebuluator tube to
eliminate bumping. The dry extract was finally redissolved in 1.5 ml. of chloroform
for spectrophotometric examination by means of a potentiometric photoelectric
spectrophotometer (Bliss, 1946).

In order to distinguish the lipids released by red light from inert impurities,
control extractions were carried out without illumination. The absorption
spectrum of such extracts differed little from that of the pure solvent. An
additional problem was the presence in the chick retina of even more rhodopsin
than iodopsin. This required demonstration that rhodopsin did not bleach
significantly in the red light used to bleach iodopsin. Digitonin extracts of
powder bleached as described above showed that red light was without effect
on its rhodopsin content.

Lipids from Chick Retinas

As may be seen in Fig. 1 the lipids from both iodopsin and rhodopsin showed
the peak at about 390 mμ characteristic of retinene. The Carr-Price test was
attempted but was unsuccessful due to the formation of turbidity on addition
of the antimony trichloride reagent. Drying at 50°C. under vacuum for 5
minutes provided no improvement.

While the position of the peak at 390 mμ agreed with the published spectra
of retinene (Krause and Sidwell, 1938), the absorption at longer wavelengths
was far too high. In particular there appeared to be a hump about 470 mμ, a region where previously published spectra of retinene show practically no absorption. The trend of successive curves was strongly indicative of a labile substance with a maximum density about 470 mμ.

**FIG. 1.** Spectra of lipids from chick retinas, dissolved in 1.5 ml. of chloroform and measured in a 1 cm. cell. The curves are drawn through the average of duplicate experiments. Upper curve, effect of white light. Lower curve, effect of red light.

**Lipids from Frog Retinas**

In order to determine whether these anomalies represented a species difference or were due to the methods used, *Rana pipiens* powder was prepared and used in the same manner as the chick powder. It was found that 2 per cent alcoholic petroleum ether released retinene even in the dark. On reducing the alcohol to ½ per cent retinene was released only by bleaching by light and showed even more strikingly the peculiar lability mentioned above. The results of this experiment are shown in Fig. 2. The extrapolated curve for zero time of solution in chloroform is strikingly suggestive of the “transient orange” stage of rhodopsin solutions bleached in the cold (Lythgoe and Quilliam, 1938) and the “second dark component” noted by Wald (1938) in rapid, automatically recorded spectra obtained after bleaching at room temperature.

The height of the absorption spectra in the vicinity of 470 mμ varied widely,
with Fig. 2 presenting in one experiment the different types of retinene spectra found in the course of many extractions. The usual spectra of frog retinene resembled those of chick retinene, as shown in Fig. 1. In all cases the curves passed through the sequence of Fig. 2, ending with the relatively stable spectrum of typical retinene.

![Figure 2](image)

Fig. 2. Spectra of lipids from 14 frog retinas, dissolved in 1.5 ml. of chloroform and measured in a 1 cm. cell. The curves are drawn through experimental points at 20 μm intervals at the following times: 0 minutes in chloroform-extrapolation, indicated by heavy line; 2 to 14 minutes; 15 to 28 minutes; 52 to 65 minutes; 192 to 207 minutes. Chloroform was added prior to the third and last series to replace loss due to evaporation. All curves were determined in order of increasing wavelength.

Because of the lability of the long wavelength component of the spectra, an attempt was made to minimize its breakdown by decreasing the temperature and time of bleaching and extraction. However, 10 minute extractions at 5°C, with a bleaching time of 1 minute did not differ significantly from 30 minute extractions at 27°C, with bleaching for 10 minutes.

**DISCUSSION**

It has become increasingly clear that the bleaching of rhodopsin is a complex chain of "light" and "dark" reactions. Some of the reactants in this chain have already been identified by Wald and incorporated into his well known cycle involving rhodopsin, retinene, and vitamin A. This cycle is certainly incomplete since it does not include Lythgoe's transient orange and indicator yellow.
Krause (1946) describes a complex lipid, provisual red, which he considers equivalent to transient orange. Provisual red shows absorption peaks at 330 and 440 μm in absolute alcohol.

Indicator yellow is differentiated by Krause (1941) from visual yellow, which he believes to correspond to retinene, and which does not show the pH dependence of indicator yellow (Chase, 1936; Lythgoe, 1937). The lability of the retinene spectra found in the present experiments may well be due to the involvement of these poorly understood intermediates.

In any case the similarity of the bleaching products of both rhodopsin and iodopsin, extending even to their complex lability, testifies to the parallelism of rod and cone processes emphasized by Hecht (1937).

SUMMARY

A method is described for the preservation of iodopsin, the labile photopigment of daylight vision, by freeze drying in vacuo.

The lipids released by the action of light on rhodopsin and iodopsin are found to be similar and to possess a labile absorption spectrum in chloroform, with a rising peak at about 390 μm and a declining peak in the region of 470 μm. After the change is complete the absorption spectrum resembles closely that of retinene.

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BIBLIOGRAPHY

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