Table 1. Electron microprobe analysis * of krinovite, determined on specimens from the Canyon Diablo meteorite.

Oxide	Percentage (by weight)
SiO_2	48.1 ± 0.7
TiO_2	0.5 ± 0.1
Al_2O_3	0.6 ± 0.1
Cr_2O_3	19.1 ± 0.5
FeO	1.8 ± 0.04
MnO	0.1 ± 0.007
MgO	19.7 ± 0.4
CaO	0.1 ± 0.01
Na_2O	9.1 ± 0.5
K_2O	0.0
Total	99.1

* Analysis by E. Olsen and I. S. McCallum.

County meteorites are graphite, roedderite, high albite, and the amphibole richterite. This occurrence of richterite in Canyon Diablo marks the second known occurrence of amphibole in a meteorite (*6*). In addition, our specimen of Canyon Diablo contains ureyite and chromite (*7*). Wichita County has forsterite (Fo 99) but no ureyite or chromite. The associated minerals in Youndegin have not yet been thoroughly examined.

It is clear from the empirical formula that the simplest mineral reaction that forms krinovite is (volumes in cubic centimeters per mole):

$$\underset{\text{ureyite}}{NaCrSi_2O_6} + \underset{\text{forsterite}}{Mg_2SiO_4} = \underset{\text{krinovite}}{NaMg_2CrSi_3O_{10}}$$

$$63.02 \qquad 43.78 \qquad 110.30$$

$$\Delta V = +3.50 \text{ cm}^3\text{/mole (at } T = 298°\text{K)}$$

If one assumes that the sign of ΔV (change in volume) remains the same at higher temperatures, krinovite appears to be a low-pressure phase relative to ureyite plus forsterite (*8*). In this regard it is interesting that in two of the assemblages the association is krinovite plus ureyite (Canyon Diablo), or krinovite plus forsterite (Wichita County). If krinovite results from this reaction, differences in bulk composition would be expected to deplete one of the reactants, but not necessarily both. In the occurrences of ureyite (*5*, *7*) no forsterite was reported, but in one case pyroxenes and quartz were found, which suggests that the bulk composition was too siliceous for krinovite to form. With free silica, krinovite might be unstable relative to pyroxenes:

$$\underset{\text{krinovite}}{NaMg_2CrSi_3O_{10}} + \underset{\text{silica}}{SiO_2} =$$

$$\underset{\text{ureyite}}{NaCrSi_2O_6} + \underset{\text{pyroxene}}{2MgSiO_3}$$

In the case of the Wichita County meteorite, sufficient excavation into the main graphite nodule has taken place to enable us to make some very rough visual estimates of the relative volumes occupied by each silicate phase. From these some bulk elemental abundances can be computed. Magnesium is about the same as in ordinary chondrite meteorites and in the solar atmosphere, but is about 4 times higher than terrestrial basalt. Sodium, chromium, and potassium are enriched by 4, 5, and 20 times, respectively, over the chondrites and the sun's atmosphere, and by 2, 20, and 2 times, respectively, over terrestrial basalt. On the other hand, calcium and aluminum are depleted by 2 and 5 times, respectively, relative to chondrites and the sun's atmosphere and are depleted by 28 and 12 times below terrestrial basalt. Even allowing for the large errors in these estimates, it is clear that these small isolated silicate systems within these iron meteorites represent an unusual fractionation of elements which is different from both ordinary chondrite meteorites and the lower crust of the earth.

Krinovite was named by us for E. L. Krinov, noted Russian investigator of meteorites and Scientific Secretary of the Committee on Meteorites of the Academy of Sciences of the U.S.S.R. (*9*, *10*).

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Table 2. X-ray powder diffraction pattern of krinovite.* Camera diameter, 114.59 mm; nickel filtered; copper radiation. b, Broad.

d (Å)	Intensity (visual)	d (Å)	Intensity (visual)
7.92	60	2.281	20
7.27	50	2.186	20
6.25	40	2.165	10
4.75	30	2.080	70
4.62	10	2.049	60
4.130	50	1.969	50
3.949	10	1.927	10
3.713	10	1.920	10
3.639	60	1.886	20
3.412	40	1.873	20
3.104	60	1.779	10
2.934	10	1.702	40 b
2.893	80	1.657	30
2.804	30	1.642	10
2.754	30	1.598	50
2.713	30	1.570	20
2.655	90	1.530	30
2.528	20	1.484	40
2.501	100	1.470	50
2.429	30	1.456	50
2.379	40	1.448	50
2.300	20		

* There were 12 additional spacings to 0.8337.

References and Notes

1. Microprobe data corrected by the methods of J. V. Smith [*J. Geol.* **73**, 830 (1965)] with a combination of natural and synthetic standards: natural albite, olivine, chromite, rutile, microcline, and synthetic anorthite and tephroite. The standards are all from the microprobe laboratory in the Dept. of Geophysical Sciences, Univ. of Chicago. The samples of krinovite analyzed were all separated grains mounted in carbon-coated epoxy within brass mounts. Normal operating conditions were 15 kv; sample current, 0.01 μa; counting times, 10 seconds; and 1 to 2 μ beam.
2. G. Donnay and R. Allmann, *Carnegie Inst. Wash. Year Book* **66**, 485 (1968).
3. P. Moore and J. Bennett, *Science* **159**, 524 (1968).
4. Cell dimensions were obtained by rotation on all three axes in order to obtain consistency. Twinning was quite prominent; however, examination of several crystals led to identical cell dimensions.
5. C. Frondel and C. Klein, Jr., *Science* **149**, 742 (1965).
6. E. Olsen, *ibid.* **156**, 61 (1967).
7. Ureyite in Canyon Diablo also has been noted by U. Marvin [*Trans. Amer. Geophys. Union* **48**, 167 (1967)].
8. Mole volume data for ureyite and forsterite were taken from R. Robie, P. Bethke, K. Beardsley [*U.S. Geol. Surv. Bull. No. 1248* (1967)]. Mole volume of krinovite was calculated.
9. Name approved by the Commission on New Minerals and Mineral Names of the International Mineralogical Association.
10. The meteorites used are from the collection of the Field Museum: Canyon Diablo (catalog No. Me 1249); Wichita County (No. Me 885); Youndegin (No. Me 877).
11. Work performed, in part, under the auspices of AEC. We thank S. Siegel, J. Whitaker, and B. Tani for the single-crystal x-ray work, and F. Gallagher for performing the high-pressure experiments (all are from Argonne National Laboratory). E.O. was supported by NSF grant GA-307, and this support is gratefully acknowledged.

1 July 1968

Visual Pigment Renewal in the Mature Frog Retina

Abstract. *It has been demonstrated by autoradiography that radioactive amino acids serve as precursors for proteins which are subsequently incorporated into retinal rod outer segment discs in mature animals. By the isolation and purification of visual pigment from retinas of adult frogs after injection of tritiated leucine and phenylalanine, it has been shown that at least part of this labeled protein consists of visual pigment (rhodopsin).*

From autoradiographic studies, Young (*1*, *2*) has suggested that the outer segments of retinal rods are constantly being renewed. In rod photoreceptors of adult rats, mice, and frogs injected with radioactive amino acids, protein synthesis predominates in the ergastoplasm of the inner segment. A major portion of the newly formed, radioactive protein then migrates to the outer segment, where it is incorporated into the basal discs of that structure.

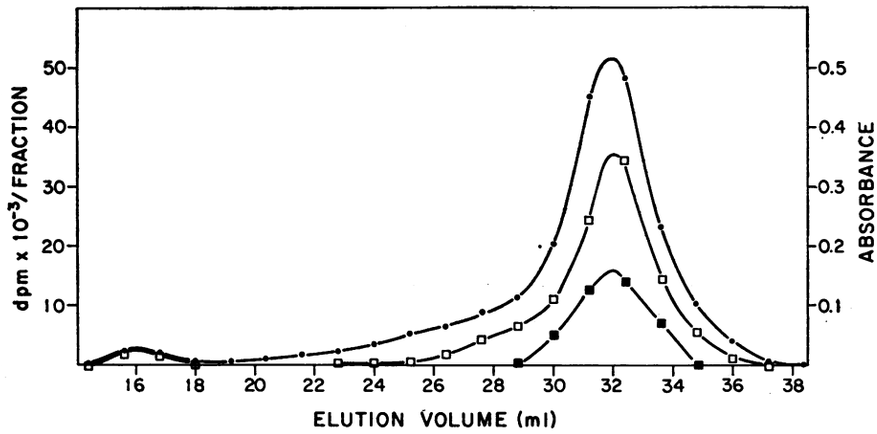

Fig. 1. Purification of frog visual pigment on an agarose column (0.7 by 98 cm). The column was developed with 0.04*M* hexadecyltrimethylammonium bromide, *p*H 7.1 (*3*). Fractions of 1.2 ml were collected in the dark. Symbols: ●, disintegrations per minute; □, A_{280}; ■, A_{500}.

These labeled discs are gradually displaced in the direction of the sclera, along the outer segment. Upon reaching the extremity of the rod, the radioactivity suddenly disappears from the cell. Apparently, the outer segment of the rod is renewed by repeated assembly of new discs at its base, coupled with a balanced removal of older outer segment material at its apical tip.

These findings raise the question of whether visual pigment (rhodopsin)—allegedly an integral part of the rod outer segment discs—is also renewed in the mature retina. The results of an experiment designed to investigate this problem are reported here.

A mixture of tritium-labeled L-leucine (29.1 c/mmole) and L-phenylalanine (5 c/mmole) containing equal amounts of radioactivity due to each amino acid, was administered intravenously to four groups of five adult frogs (*Rana pipiens*) at doses of 1, 2, 5, and 10 μc per gram of body weight (average body weight, 25 g). The animals were killed 7 days after administration of the labeled amino acids when, on the basis of autoradiographic evidence (*1*), the labeled protein had been displaced about 15 percent of the distance along the outer segment of the rod. The results from all four groups of frogs were qualitatively identical. Those from the group given 1 μc per gram of body weight are discussed below.

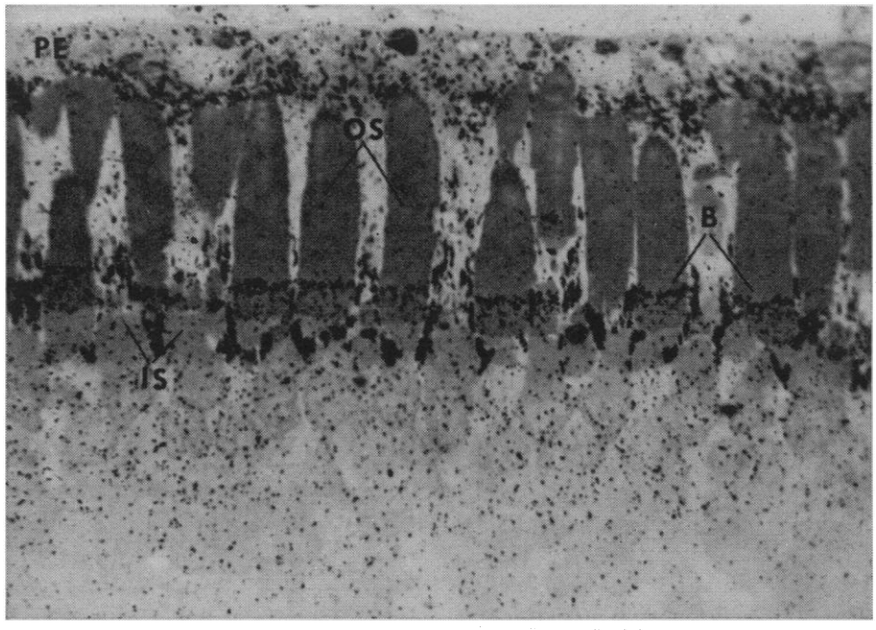

Fig. 2. Autoradiogram of frog photoreceptors 7 days after an injection of tritium-labeled amino acids. The outer segments of the rod (*OS*) extend from the pigment epithelium (*PE*) to the inner segments (*IS*) of the rod. A band of silver grains (*B*) extends across the width of each outer segment of the rod, revealing the presence of radioactive protein molecules in a disc-shaped component within the outer segment (× 700).

Visual pigment was isolated from outer segments of the rod, and was purified (*3*), by using sucrose flotation of the outer segments, extraction with hexadecyltrimethylammonium bromide, and agarose-gel filtration (Bio-Gel A-1.5M, 100 to 200 mesh). All dissection, extraction, and purification procedures were performed under dim, red light on dark-adapted animals. After gel filtration on a column, portions from each of the collection tubes were counted by liquid-scintillation spectrometry, and the results were corrected for quenching.

Two additional frogs were injected with 20 μc per gram of body weight of the tritiated amino acid mixture. Their retinas were then processed for autoradiography to monitor the progress of displacement of radioactivity in the outer segment. These animals were also killed 7 days after injection. The retinas were fixed in formaldehyde to extract free amino acids.

Prior to agarose-gel filtration, the ratio A_{280}/A_{500} for the crude visual pigment was 3.2. After gel filtration, the absorption at 280 nm and at 500 nm was measured in all fractions collected. Portions from those tubes which contained 500-nm absorption were combined, and the ratio was determined again. The ratio of A_{280}/A_{500} was now 2.0. From Fig. 1, the main peak of 280-nm absorption corresponded exactly with the 500-nm absorption of the visual pigment. The ratio A_{280}/A_{500} in each tube over the peak varied between 1.9 and 2.3, with an average of 2.0.

The principal peak of radioactivity coincided with the 500-nm peak, and with the main peak of 280-nm absorption (Fig. 1). Sixty-five percent of the total radioactivity applied to the column was recovered in the visual pigment peak. The ratio of disintegrations per minute (dpm) to A_{280} and dpm to A_{500} were calculated for each tube, and they were essentially constant over the peak tubes. Rechromatography of the purified visual pigment on the same column of agarose gave single corresponding peaks of radioactivity, A_{280} and A_{500} with an elution volume identical to that found on the initial chromatography. Although there is slight variation in

these ratios across the peak, the values are within the limits of experimental error. These results therefore strongly indicate that the isolated protein is homogeneous, consisting only of native visual pigment.

Autoradiographic analysis revealed a discrete reaction band which extended across the width of each outer segment of the rod near its base (Fig. 2). This indicated that protein formed during the period immediately after injection was now concentrated in a disc-shaped constituent of the outer segments, presumably in membranous discs which had been displaced from the base of the outer segment by newer discs formed in the 7 days after the injection.

Thus we have isolated purified, radioactively labeled visual pigment from retinal rod outer segments after injection of labeled amino acids in adult frogs. Since visual pigment is considered an integral part of the outer segment disc membrane, this finding supports the hypothesis (*1*) that the outer segment of the rod is renewed by continual synthesis of new discs.

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1. R. W. Young, *J. Cell Biol.* **33**, 61 (1967).
2. ——— and B. Droz, *ibid.*, in press.
3. J. Heller, *Biochemistry*, in press.
4. Tritium-labeled L-leucine and L-phenylalanine from Nuclear-Chicago Corp., Des Plaines, Ill.; agarose beads from Bio-Rad Laboratories, Richmond, Calif. We thank Drs. R. W. Young and J. Heller for help and advice. Supported by PHS grants NB-03807 and NB-06592; and by a Fight for Sight grant-in-aid of the National Council to Combat Blindness, Inc., New York, N.Y.

1 July 1968

Vernolepin: A New, Reversible Plant Growth Inhibitor

Abstract. *Vernolepin (5 to 50 micrograms per milliliter), a novel sesquiterpenoid dilactone obtained from* Vernonia hymenolepis, *inhibits extension growth (from 20 to 80 percent) of wheat coleoptile sections. Inhibited tissues appear normal and their respiration is unaffected. If the inhibited sections are washed and subsequently treated with indole-3-acetic acid, the tissues respond to the auxin, but the degree of elongation is determined by the length of prior treatment with vernolepin. Administered simultaneously, increasing concentrations of auxin will significantly reduce the inhibitory effect of vernolepin, but there is no evidence for a competitive interaction between the two substances.*

A number of sesquiterpenoid plant growth inhibitors have been isolated from higher plants (*1*). The recent discovery of the widespread occurrence of (+)-abscisic acid in plants (*2*) and the strong inhibitory effects of this substance at very low concentrations suggest that it may have a regulatory function. Another sesquiterpene, heliangine (*3, 4*), has been found to inhibit extension growth of *Avena* coleoptile sections.

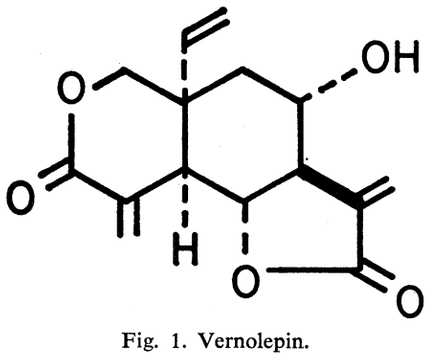

Fig. 1. Vernolepin.

When applied in combination with indole-3-acetic acid (IAA), however, activity curves ran parallel over a wide range of concentrations, which suggests a constant, independent physiological action of this inhibitor. Similar results have been reported for the so-called inhibitor-β from sycamore (*5*), now thought to consist mainly of abscisic acid (*2*).

During an evaluation of the effects on plant growth of several tumor-inhibitory compounds derived from plants, it was noted that several sesquiterpenoid dilactones, specifically elephantin (*6*), elephantopin (*6*), and vernolepin (*7*), were strong inhibitors of extension growth of wheat coleoptile sections. The biological activity of these compounds was determined by means of the straight-growth technique described by Sequeira and Kelman (*8*). Briefly, the technique consists of exposing 4-mm portions of wheat (var. Atlas 66) coleoptiles, removed 2 mm below the apex and soaked in $MnSO_4$ solution (1 μg/ml) for 2 to 3 hours, to various concentrations of the inhibitors. The inhibitors were dissolved in 2 percent sucrose and dispensed in vials, each containing three coleoptile sections. For controls, coleoptile sections were placed in vials containing 2 percent sucrose only. The vials were placed horizontally on a wheel rotating at 1 rev/min, and, after incubation in the dark (20 hours), the sections were removed and measured under a dissecting microscope. Inhibition or promotion of growth of treated coleoptiles was calculated as percentage of control growth.

Of the sesquiterpenoids tested, vernolepin (Fig. 1), isolated from *Vernonia hymenolepis* A. Rich. (*7*), was the most effective inhibitor. Extension growth was significantly inhibited by vernolepin at 5.0 μg/ml ($1.8 \times 10^{-5}M$) (Fig. 2). Above 50 μg/ml ($1.8 \times 10^{-4}M$), vernolepin almost completely inhibited extension growth.

Tests were then made to determine whether coleoptile sections that had been inhibited approximately 50 percent by vernolepin (25 μg/ml) could resume active growth after they had simply been washed with distilled water and transferred to solutions containing IAA at different concentrations. The degree of reversibility of inhibition would indicate whether the substance was directly toxic to the cells or had growth-regulatory properties. For this purpose, 4-mm wheat coleoptile sections were exposed to vernolepin at 25 μg/ml ($9.0 \times 10^{-5}M$) for 4, 12, and 18 hours. After thorough washing with distilled water, the sections were incubated with IAA for 14, 12, and 18 hours, respectively. The concentrations

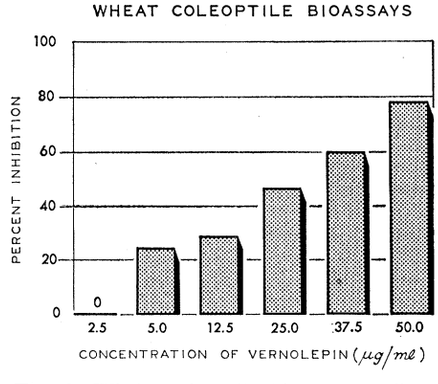

Fig. 2. Wheat coleoptile bioassays. Effect of various concentrations of vernolepin on growth of wheat coleoptile sections after 20-hour incubation in the dark. Methods as described in the text.