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26. Although compounds **2** and **3** still possess an α-methyl-γ-lactonic moiety which might be regarded as a reactive functional group, our previous work has shown that compounds such as 2,3,11,13-tetrahydrohelenalin (*5*), which contains this moiety, lack significant cytotoxicity in vitro.
27. This investigation was supported by grants from the National Cancer Institute (CA-12360) and the American Cancer Society (CH-19) to K.-H.L. and (IN 15-P) to I.H.H. This report is part 20 in the series "Antitumor Agents"; for part 19 see K.-H. Lee, Y. Imakura, D. Sims, A. T. McPhail, K. D. Onan, *J. Chem. Soc. Commun.* (1976), p. 341.

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Photoreceptor Outer Segments: Accelerated Membrane Renewal in Rods After Exposure to Light

Abstract. *The rate of rod outer segment renewal in* Rana pipiens *tadpoles under constant light and under diurnal conditions of 12 or 2 hours light per day is significantly increased compared to that in animals in darkness. Furthermore, during 24 hours in light after 6 days in darkness the rate of renewal is three to four times that in darkness. In* Xenopus laevis *tadpoles the rate of renewal is more than five times greater during the first 8 hours of a normal diurnal cycle than during the following 16 hours. These observations demonstrate that bursts of renewal activity occur as a response to light, and suggest that a normal pattern of light alternating with darkness plays a fundamental role in the regulation of rod outer segment turnover.*

The rod outer segment (ROS) turnover process involves production and assembly of new membrane disks at the base coupled with shedding of groups of older disks at the distal tip where they are phagocytized and degraded by the pigment epithelium (*1–3*). Evidence for this concept comes from autoradiographic studies of eyes labeled with tritiated amino acids. In such studies a discrete radioactive band is formed at the base of the ROS and is gradually displaced toward the tip; eventually the label appears within phagosomes of the pigment epithelium. Shedding occurs intermittently, and light plays a major role in initiating the process (*4*). However, at a given temperature the addition of new disks is believed to proceed at a nearly constant rate that is modified only slightly by light (*2, 3*).

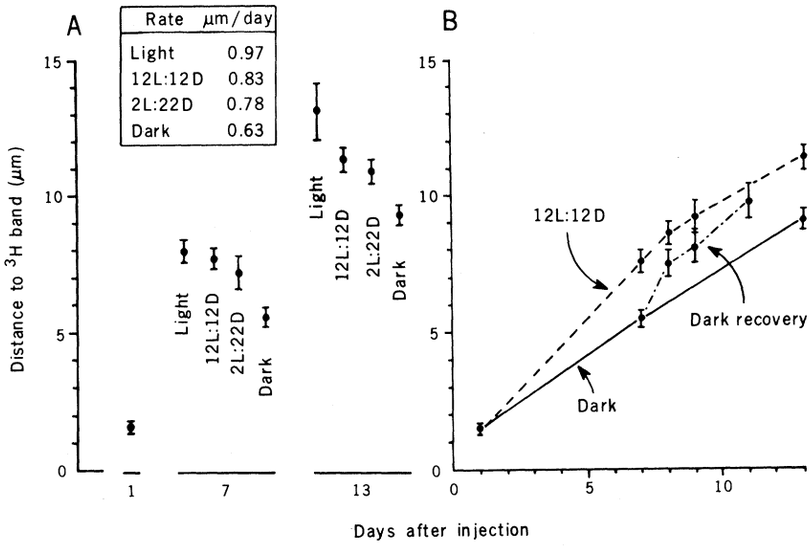
We obtained evidence that rods exposed to light after long-term dark adaptation responded with massive shedding followed by rapid restoration of ROS length (*4, 5*). Tadpoles exposed to light for 24 hours after 7 days of dark adaptation showed a rapid decline (within 2 hours) of visual pigment concentration and ROS length which was paralleled by a great increase in the number of phagosomes within the pigment epithelium. During the following 22 hours, however, visual pigment concentration and ROS length were restored to control levels. We estimated that restoration of ROS length in this experiment would have required a three- to fourfold increase in the rate of renewal. The present study was initiated to examine the effects of light on ROS renewal by autoradiography.

To obtain baseline data on the effects of different lighting conditions on renewal, *Rana pipiens* tadpoles (*6*) each received an intraperitoneal injection of 25 μc of [4, 5-^{3}H] L-leucine (New England Nuclear, specific activity 5.0 c/mmole). During the week before and the first 24 hours after injection, animals were kept at room temperature (22° to 24°C) and were exposed to light for approximately 12 hours daily. Twenty-four hours after injection some of the eyes were fixed for autoradiography (*7*). The remaining tadpoles were maintained in incubators for 12 additional days at 23° ± 0.5°C in constant light (*8*), constant darkness, or in cyclic conditions of 12 or 2 hours of light per day. Samples of eyes from each group were fixed 7 and 13 days after injection. From autoradiographs, measurements were made from the base of the outer segment to the scleral edge of the radioactive band (*9*).

Displacement of the radioactive band was consistently greater in light than in darkness both 7 and 13 days after injection (Fig. 1A), which indicates that cyclic light of low intensity and short daily duration significantly stimulates ROS renewal. The average rate of displacement was increased by 54 percent from a low of 0.63 μm/day in darkness to 0.97 μm/day in constant light. At these rates band displacement was respectively 9.1 ± 0.12 μm (mean ± standard error) and 13.1 ± 0.47 μm by the end of the experiment (Fig. 1A). However, the magnitude of the effect of light on renewal was not proportional to the duration of light exposure each day. Thus, 2 hours of light per day was sufficient to produce 44 percent of the effect observed in constant light after 12 days as opposed to the 8 percent expected if the light effect were precisely additive. In addition, the rate of band displacement was higher in all treatments during the first half of the experiment than during the second half. For example, among animals receiving 12 hours light per day the rate of band displacement was 1 μm per day between days 1 and 7 and 0.63 μm/day thereafter (see Fig. 1B) yielding an overall average renewal rate of 0.83 μm/day (*10*).

To measure the effects of light on band displacement in animals previously dark-adapted for 6 days, some of the tadpoles in the previous experiment were exposed to light on day 7 and kept for one to four additional days after fixation. The results confirmed our previous suspicion that light exposure after long-term dark adaptation results in a great increase in ROS renewal. During 24 hours in constant light after 6 days in darkness the radioactive band advanced 2.6 μm, which corresponds to a rate four times that in darkness and more than two and a half times that in constant light. Likewise, during the first 24 hours on a diurnal cycle of 12 hours of light and 12 hours of darkness after 6 days in darkness, the radioactive band advanced 2.0 μm (Fig. 1B), which corresponds to a rate three times that in darkness and two times that in constant light. The high rate of renewal measured during the first 24 hours in this group was not sustained, however. Instead, it declined during the second through fourth days after light exposure to a level comparable to that of control animals receiving 12 hours of light per day (Fig. 1B).

These results indicate that a burst of ROS renewal occurs on exposure to light, and suggest that under normal diurnal conditions there is a daily fluctuation in the rate of renewal. Because of the relatively low renewal rate in *R. pipiens* tadpoles, however, it is difficult to measure



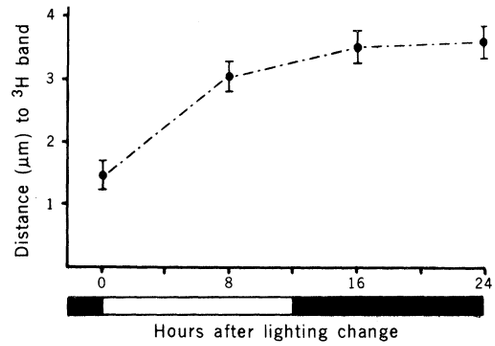


Fig. 1 (left). Effects of constant light, constant darkness, and cyclic light and darkness on displacement of the radioactive band in red rod outer segments of *Rana pipiens* tadpoles at 23° ± 0.5°C. (A) Distance to the radioactive band at 1, 7, and 13 days after injection in animals kept under specified conditions beginning on day 1. The inset gives the average rate of displacement in each group between days 1 and 13. Points are means for 50 to 90 measurements (ten per tadpole) from near the retinal center except that points for constant light are means for 20 or 30 measurements. Vertical bars extend 2 standard errors on each side of the means. (B) Band displacement as a function of days after injection in animals kept on a cycle of 12 hours of light and 12 hours of darkness (12L:12D) or in darkness compared to animals exposed to the same cycle after 6 days in darkness (dark recovery). Band displacement of 2 μm during the first 24 hours of recovery corresponds to a renewal rate more than three times that in darkness. During days 2 through 4 the rate declined to one comparable to that of controls kept on a cycle of 12 hours of light and 12 hours of darkness. Fig. 2 (right). Diurnal variations in band displacement in *Xenopus laevis* tadpoles kept on a cycle of 12 hours of light and 12 hours of darkness at 28° ± 0.5°C. Points are means for 40 measurements (ten per tadpole) and vertical bars extend 2 standard errors on each side of the means. Total band displacement was 2.1 μm for the entire day, and 80 percent of this was during the first 8 hours.

radioactive band displacement over periods of less than 24 hours. *Xenopus laevis* tadpoles, however, under comparable conditions of lighting and temperature, add disks at a daily rate more than twice that of *R. pipiens* (*11*). Consequently, we measured radioactive band displacement during a 24-hour period in *X. laevis* tadpoles (*12*) kept at 28° ± 0.5°C on a diurnal cycle of 12 hours of light and 12 hours of darkness for 7 days. On day 7 each tadpole received an intraperitoneal injection of 10 μC of tritiated leucine. A sample of eyes was fixed 24 hours later at the beginning of the next light period and at 8-hour intervals over the subsequent 24 hours. We reasoned that if the rate of disk addition were enhanced in association with the onset of light, displacement of the radioactive band would occur to a greater extent during the first 8 hours than it would later in the day. The results (Fig. 2) indicate that most of the renewal activity occurred during the first 8 hours of illumination and had declined to a low level by the end of the day. Thus, of the total 2.1 μm of band displacement, 80 percent occurred during the first 8 hours, which corresponded to a rate of 4.5 μm/day during this interval.

Adult frogs maintained in constant light of about 6400 lu/m² for 12 days showed a slightly greater radioactive band displacement than did those kept in darkness (*2, 13*); it was suggested that this "light effect" may have been related to a rise in temperature in the microenvironment of photoreceptors. It is unlikely that local heating played a direct role in our results since the magnitude of light stimulation of ROS renewal was quite high and would have required a considerable rise in temperature (*14*). Furthermore, the increase in the rate of renewal in animals exposed to light for 24 hours after 6 days of dark adaptation far exceeded that in animals maintained in constant light for 12 days. Thus, the magnitude of the increase in ROS renewal in this case was greatly increased by the preceding period of dark adaptation.

It is well established that the disks of rod outer segments are renewed throughout life, and that renewal is balanced by a disposal mechanism involving intermittent shedding of ROS tips and their phagocytosis by the pigment epithelium. The associated ideas that light has only a slight and indirect effect on renewal and that disk addition occurs continuously must now be viewed more critically. We have shown that wide variations in the rate of disk addition occur under different lighting conditions, and that light exposure after long-term dark adaptation increases the rate well above that normally observed in light. Our experiment with *X. laevis* kept under normal diurnal conditions further indicates that ROS renewal does not occur at a steady rate, but in a series of bursts of activity corresponding to the onset of illumination. Our failure to find band displacement during the last 8 hours of darkness indicates that renewal either ceases or declines to an unmeasurable level. This observation raises the possibility that disk addition may actually occur discontinuously.

In conclusion, we have found that both cyclic and constant light at intensities normally encountered in nature significantly stimulate ROS renewal in *R. pipiens* tadpoles. We have also found that in *X. laevis* tadpoles the rate of ROS renewal varies significantly during a normal diurnal cycle from a high rate during the light period to near zero in darkness. These data, along with previous observations that light stimulates shedding of ROS tips, indicate that a normal pattern of light alternating with darkness plays a fundamental role in the regulation of ROS turnover (*15*).

JOSEPH C. BESHARSE
JOE G. HOLLYFIELD
MARY E. RAYBORN
Departments of Anatomy and
Ophthalmology, College of Physicians and Surgeons, Columbia University, New York 10032

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that shedding occurs as a free-running circadian rhythm in the rat [M. M. LaVail, *Science* **194**, 1071 (1976)]. Light exposure for 2 hours after dark adaptation of 1 to 7 days results in shedding of a greater number of larger phagosomes in larval frogs than does exposure to cyclic light (*16*).

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7. Eyes of *R. pipiens* tadpoles were fixed by the same procedure used previously (*16*). Eyes of *X. laevis* tadpoles were fixed with 1.5 percent glutaraldehyde in 0.067*M* cacodylate buffer at *p*H 7.4 containing 0.5 percent $CaCl_2$. All eyes were postfixed in 1 percent OsO_4 and processed for sectioning. Procedures for preparation of autoradiographs have been reported [J. G. Hollyfield, L. S. Mottow, A. Ward, *Exp. Eye Res.* **20**, 383 (1973)] (*17*).
8. The light source was a 25-watt tungsten bulb which yielded illumination of about 200 lu/m^2 at the level of the animal containers. In previous studies of the effect of light on ROS renewal, the illumination was much higher (about 6400 lu/m^2), sufficient to quickly damage albino rat photoreceptors (*2, 3*).
9. Throughout this report the rate of radioactive band displacement is assumed to bear a direct relationship to the rate of ROS disc addition (renewal) (*10*).
10. In two separate experiments the rate of radioactive band displacement declined during the second 6-day period. The meaning of this observation is not immediately obvious, and is not directly pertinent to the major points of this report. However, it does not necessarily mean that the rate of ROS disk addition changed with increasing postinjection time. For example, if ROS discs were packed together to a greater extent as they were displaced, this would produce an apparent slowing of band displacement.
11. M. S. Kinney and S. K. Fisher, paper presented at the annual meeting of the Association for Research in Vision and Ophthalmology, Sarasota, Fla., April 1976. We thank Kinney for providing us with her fixation technique for eyes of *X. laevis* (*7*).
12. Tadpoles were at stages 54 to 55 at the time of injection [P. D. Nieuwkoop and J. Faber, Eds. *Normal Table of Xenopus laevis (Daudin)* (North-Holland, Amsterdam, 1967), pp. 181–182].
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14. With knowledge of the Q_{10} of ROS renewal it is possible to predict the temperature increase due to light absorption necessary to account for a given increase in the renewal rate. Thus, if a Q_{10} of 2 is assumed (*13*), the 54 percent increase in the rate in constant light would require a 5.4°C rise in local temperature whereas the 24 percent increase in animals receiving 2 hours of light per day would require a 29°C rise in temperature during the time of light exposure. This would correspond to an average increase of 2.4°C over the entire 24-hour period. Likewise, the 300 percent increase in constant light after 6 days of dark adaptation would require a rise in temperature in excess of 20°C. The latter two values would place the temperature in the photoreceptor microenvironment outside the range compatible with cell viability.
15. Since this paper was submitted for publication, we have found that light influences renewal in adult *R. pipiens* as well. Over a period of 5 days, the renewal rate was 0.74 μm/day in darkness, 0.84 μm/day in cyclic light, and 1.14 μm/day in constant light. During 24 hours in light after 5 days in darkness, the radioactive band was displaced 1.5 μm.
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Antileukemia Activity in the Oscillatoriaceae: Isolation of Debromoaplysiatoxin from *Lyngbya*

Abstract. *Chloroform extracts of several seaweeds, of the family Oscillatoriaceae, from Enewetak Atoll, Marshall Islands, display activity against P-388 lymphocytic mouse leukemia. A P-388 active compound, debromoaplysiatoxin, has been isolated from* Lyngbya gracilis *and characterized. This compound also has dermonecrotic activity and may be the dermatitis-producing substance in* L. majuscula, *the causative agent of "swimmers' itch" outbreaks in Hawaiian waters.*

Over the past two decades a few reports of biological and pharmacological activities of extracts of marine blue-green algae have appeared in the literature, but little progress has been made on the isolation and identification of the active principles. Lipid extracts of *Lyngbya majuscula* Gomont, the causative organism in sporadic outbreaks of a contact dermatitis (swimmers' itch) among swimmers in Hawaiian waters, show dermonecrotic activity (*1*). *Schizothrix calcicola* (Ag.) Gomont, an alga suspected of being associated with the appearance of toxic fish on the atoll of Marakei in the Gilbert Islands (*2*), contains two lipid-soluble toxins (*3*). Extracts of several species of *Hydrocoleum* (*4*) and *L. majuscula* (*5*) have demonstrated antibiotic properties. Antiviral activity has also been reported for extracts of *L. majuscula* (*5*).

We have found that marine blue-green algae are potential sources of anticancer compounds. In the fall of 1975, specimens of several blue-green algae were collected at Enewetak Atoll in the Marshall Islands. Chloroform extracts of these algae were tested for activity against P-388 lymphocytic leukemia in mice. Extracts of seaweeds belonging to the family Oscillatoriaceae consistently displayed activity in the P-388 assay, three extracts, those of *Lyngbya*, the *Oscillatoria-Schizothrix* mixture, and *Symploca*, being particularly active (Table 1).

The availability of a large amount of a *Lyngbya* from a single location prompted us to select this alga for initial study. Frozen *L. gracilis* Gomont (*6*) (3 kg wet weight) collected from Reefer 8 Pinnacle, Enewetak lagoon, was homogenized and extracted with a mixture of chloroform and methanol (1 : 2 by volume). Water was added to the filtrate and

Table 1. Activity of chloroform extracts of blue-green algae collected at Enewetak Atoll against P-388 lymphocytic leukemia in mice. The activity is expressed as ratio of the mean survival time of the diseased treated (T) mice to the mean survival time of the diseased control (C) mice ×100. The dose indicates the amount of extract injected intraperitoneally twice a day for 10 days commencing 24 hours after injection of the cancer cells. Dosages were not optimized.

Alga	Collection site	Activity (T/C × 100)	Dose (mg)
	Family Oscillatoriaceae		
Lyngbya gracilis	Reefer 8 pinnacle	144	0.011
Lyngbya gracilis	South Elmer pinnacle	137	0.013
Oscillatoria nigroviridis and *Schizothrix calcicola* (1:1)*	Enewetak, seaward side	140	0.0047
Oscillatoria nigroviridis and *Schizothrix calcicola* (1:1)*	Enewetak, lagoon side	122	0.74
Symploca muscorum	Enewetak, lagoon side	142	0.15
Microcoleus tenerrimus	Ananij, seaward side	120	0.27
Schizothrix calcicola and *Calothrix crustacea* (10:1)*	Enewetak, seaward side	125	0.44
	Family Rivulariaceae		
Calothrix crustacea	Enewetak, seaward side	100	0.98
Calothrix crustacea	Reef flat near Mike and Koa Craters	117	0.40
	Family Nostocaceae		
Nostoc muscorum	Enewetak, terrestrial	104	0.31

*Inseparable mixture. Relative amounts of algae as indicated.