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3. The diet prepared in the laboratory was described by O. A. Roels, A. Guha, M. Trout, U. Vakil, K. Yoseph, *J. Nutr.* **84**, 161 (1964); the commercial diet was purchased from General Biochemical and supplemented with a "vitamin fortification" mixture without the vitamin A. The weekly dose for A+ of *trans*-retinol (Eastman) in corn oil was 0.2 to 0.4 mg per 100 g of body weight. The *trans*-retinoic acid (Eastman) dose was 0.04 mg per 100 g of body weight, administered once or twice a week. For relief of the deficiency 0.4 to 2 mg of *trans*-retinol in 1 ml of corn oil was administered intraperitoneally on two or three consecutive days. The average amount of liver retinol after 8 weeks on the diet was 150 μg/g in the A+ animals. Liver retinol of A— animals on the diet longer than 6 weeks was either undetectable or measured as less than 0.4 μg/g; 8 to 10 days after relief it was around 40 μg/g.
4. The ERG was measured under general anesthesia (Nembutal, 3 mg per 100 g of body weight); 1 percent xylocaine was applied to the cornea and subcutaneous tissue. Light flashes were supplied by the Grass Photostimulator, with intensity No. 16 at 38 cm from the eye ("strong xenon flash," see text) attenuated by neutral density filters for testing weak stimuli. For threshold measurements, 50-msec flashes from a tungsten source were used. The light source for eliciting the ERP was a Honeywell flash unit, model 65-C, connected to the eye by 0.9-m light wire (Bausch & Lomb 3202).
5. Rhodopsin preparation: The dissected retina was fixed with alum and extracted with 2 percent digitonin or 1 percent Triton-X, 100, in 0.3 ml; the procedure was repeated thrice. The amount of rhodopsin remaining within the eye after the removal of the retina was less than 10 percent in dark-adapted A+ animals. Retinol was determined by fluorometry of xylene or cyclohexane extracts (1 ml) of the dissected retina and of the remaining eye tissue, all retinol of the remaining eye tissue being considered to reside in the pigment epithelium; exciting and analyzing wavelengths were 340 and 480 nm, respectively. After the first reading, the retinol was destroyed by exposing the samples to ultraviolet light (GE F15T8) and a second reading was made [P. K. Nakane and D. Glick, *J. Histochem. Cytochem.* **13**, 640 (1965)]. Standards were fresh solutions of retinyl acetate, prepared with spectrophotometric control. Thin-layer chromatography [B. D. Drujan, R. Castillon, E. Guerrero, *Anal. Biochem.* **23**, 44 (1968)] was performed with Eastman chromogram 6060 and a cyclohexane, ethyl ether system (4 : 1 by volume); measurement was by fluorometry of the samples taken. See also F. Crescitelli, *Vision Res.* **7**, 685 (1967).
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7. Dowling also found that the decline of rhodopsin in vitamin A deficiency can be almost wholly prevented by keeping the animals in the dark [J. E. Dowling and R. L. Sidman, *J. Cell Biol.* **14**, 77 (1962)].
8. The amplitude of the a-wave in response to the same (strong) flash of light was related to rhodopsin content by a continuous, monotonic (logarithmic) function and was a more reliable measurement than ERG threshold for estimating development and progress of the changes in the retina.
9. These experiments were conducted under "standard laboratory lighting conditions of approximately 12 hours light and 12 hours dark" (J. E. Dowling, personal communication).
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12. Our model for experiment 77 (Fig. 3) fits the data of experiment 84 with the assumption that daily retinal illumination was one-fourth that of the animals of experiment 77. However, if the variations are related to the leakage of retinol from PE, then k_5 for experiment 84 would be 0.01 instead of 0.03 per hour.
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14. The disappearance of retinol may be a threshold phenomenon (a very low concentration must be exceeded before "spilling" into the blood occurs). The computed steady-state level of retinol in PE for the condition of synthesis is about ten times lower than for the condition of cyclic light.

15. We thank Drs. B. S. Kang, E. Nagel, and R. Schlosser for assistance during the early part of this project; and Dr. J. E. Dowling for review of the manuscript. Supported by grant EY-00297-05 from the National Institutes of Health and by the Buffalo Eye Bank and Research Foundation.

8 September 1970; revised 6 November 1970 ■

Irreversible Effects of Visible Light on the Retina: Role of Vitamin A

Abstract. *Diffuse retinal irradiation by visible light produces in the rat the death of visual cells and pigment epithelium. Typically, cage illumination of 1500 lux from fluorescent light through a green filter leads to severe damage when continued for 40 hours. Vitamin A deficiency protects against this damage but experiments show that retinol released by light from rhodopsin is probably not the toxic agent. Protection against light damage depends on a long-range state of cell adaptation to light itself. The normal diurnal cycle of light and dark seems to be the essential factor in controlling visual cell viability and susceptibility.*

Several years ago the surprising finding was made that visible light at intensities which are ordinarily encountered is damaging to the retina of rats (*1*). The most striking manifestation of this damage was the widespread death of the visual cells in association with the degeneration of the pigment epithelium (PE) and an irreversibly low or extinct electroretinogram (ERG). The effect was most easily produced and studied in albino rats, but pigmented (hooded) rats showed about the same damage for the same retina irradiation. The deleterious effect has also been observed in the wild-strain kangaroo rat as well as in hamsters of different strains, in the Swiss mouse, and in the nocturnal Galago monkey. The rat, however, seems to be the most sensitive animal, a finding which does not favor the indiscriminate use of rats in vision and retina studies.

The damage is a function of irradiation and exposure time. Minimum effects are produced with the equivalent of about 1 μw per square centimeter of 500-nm radiation applied diffusely over the retina (*1*). Typically, an environment illuminated by incandescent light to about 110 lux is damaging if it is maintained continuously for 7 to 10 days. Irradiation ten times stronger has a deleterious effect within 24 hours, but, when body and eye temperature are raised, damage results from exposure for 1 or 2 hours only (*1*).

The damage is graded mainly by the size of the irreversibly affected area of the retina. The weakest histological manifestation in the albino rat, exposed freely moving in a cage, is a small lesion less than ½ mm in diameter in the upper nasal region. As damage increases, this area becomes larger and may extend over the whole globe, resulting in a retina composed only of the inner layers (*1*).

The effect seems important not only because it illustrates neglected aspects of vertebrate photoreceptor biology but also because it provides new insights for the study of degenerative visual cell diseases. Ordinary daylight or artificial light has never been considered a possible hazard, except for focusing the sun or equivalent sources upon the retina, quite in contrast to exposing the skin and, in analogy, to the known relation between cochlear hair cell damage and sound pressure. Indeed, the eye seems protected against overexposure by a heavy pigment coat that limits light entry to the pupil, by pupillary reflex constriction, squinting, and others.

Four different mechanisms were considered as possible causes of the deleterious effect of visible light: (i) thermal injury, (ii) photodynamic injury

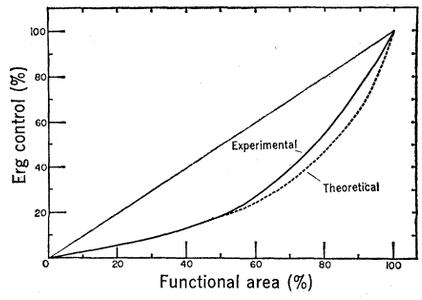


Fig. 1. Relationship between functional retinal area and the size of the ERG (a-wave or b-wave). The theoretical curve was derived from a model resistance network (*9*) based on retinal depth profile measurements of resistance and ERG potentials performed in rabbits. The nonfunctional area is assumed to include loss of the visual cells as well as of PE, which is the site of a high-resistance barrier. The loss of this barrier leads to an increase in shunt conductance and is responsible for the nonlinear relation between ERG and functional area. The experimental curve is from 54 rat eyes that had been exposed to L_{150} for 20 to 40 hours. The ERG was measured ten or more days after exposure. The eyes were serially sectioned and the spatial extent of the lesion was reconstructed (*10*). Small lesions showed variable PE destruction which probably is the main reason for the deviation between theoretical and experimental data.

caused by photosensitized oxidation of essential cell constituents, (iii) fatal metabolic or electrolyte changes resulting from excessive light "stimulation," and (iv) injury by a toxic photoproduct produced as a consequence of the absorption of light by the visual pigment.

We report experiments to ascertain the role of vitamin A (retinol) in the mechanism of this damage. Retinol is released from rhodopsin as a consequence of light absorption; if all the rhodopsin of the dark-adapted rat retina were instantaneously bleached, the concentration of retinol in the outer segment could be $10^{-2}M$, and virtually all retinol would be in the lipid phase. The relatively slow rate of rhodopsin bleaching in vivo, the continuous regeneration of rhodopsin, and the transfer of retinol from the outer segment PE (*2*) reduces this concentration to a transient maximum one-half as large.

The possibility that the retinol from excessive rhodopsin bleaching might be the toxic agent was suggested by the known membranolytic effects of free retinol on intact cells (such as erythrocytes) or isolated cell organelles (such as lysosomes) (*3*). This possibility was considered attractive for the following reasons.

1) Monochromatic lights between 400 and 600 nm adjusted to evoke the same ERG response have the same efficiency in producing retina damage; hence, damage seems to be mediated by rhodopsin as in the physiological action of light (*1*).

2) Pathology within PE is a typical sign of severe light damage in the rat, and it is well established that retinol released from rhodopsin moves from the photoreceptors into PE (*2*).

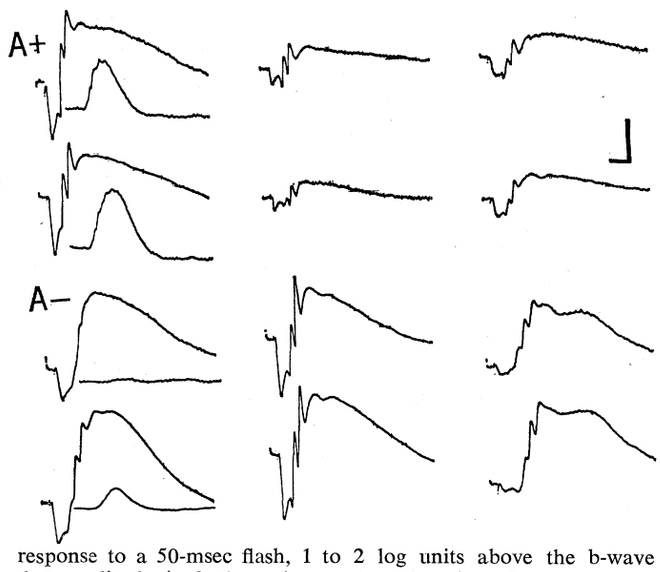
3) The early pathology of the damage involves all parts of the visual cell, including the nucleus (*1*); this favors the idea that a toxic agent diffuses from the site of light absorption to the other parts of the cell.

4) The effectiveness of light in producing the irreversible damage is very dependent on temperature (*1*), as is the lytic effect of retinol on erythrocytes and lysosomes (*3*).

5) Electron microscopic findings of early vacuolization within the visual cell as a result of damaging light (*1*) is consistent with the action of retinol on lipoprotein membranes (*3*).

To evaluate the role of retinol as the toxic agent, we compared the action of light in male albino rats deficient in vitamin A (A−) and not deficient (A+). Diet and methods have been described (*2*). The procedure for eliciting damage was a 40-hour exposure to light from circular, cool white fluorescent light (GE Cool White, 12 inches in outer diameter) delivered through a (green) 0.32-cm plastic filter transmitting from 490 to 580 nm. Illumination was measured at about 1500 lux; we refer to this light as L_{150}.

The unanesthetized and unrestrained rats, mostly two at a time, were in circular wire mesh cages that were surrounded by lamps and a filter and ventilated by cooled air of variable



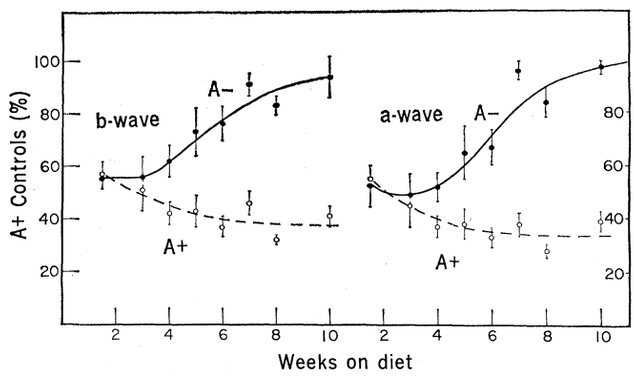


Fig. 2 (left). The ERG's of A+ and A− animals of same experiment exposed for 40 hours to damaging light (L_{150}) during the 10th week after weaning. The A− animals are relieved of vitamin A deficiency 24 hours after the exposure. The degree of damage is indicated by the reduction in ERG 10 days after exposure. Each row shows ERG's of same animal. Before (1st column): Tests 24 hours prior to exposure, the inserts are the response to a 50-msec flash, 1 to 2 log units above the b-wave "threshold"; the time scale of inserts is 2½ times slower, and the amplitude is 2 times larger than for the other responses. 2nd column: All animals were given a high dose of retinol 1 day after exposure, and ERG tests were made 9 days later (10 days after exposure). 3rd column: ERG 1 day after exposure prior to relief. Calibration: 20 msec, 500 μv. Fig. 3 (right). Average a- and b-wave amplitude (± S.E.M.) 10 days after exposure to damaging light (L_{150}). After the test on day 10, the ERG did not change significantly.

Table 1. The irreversible effect of light on ERG in relation to different conditions before and during exposure to L_{150}. Numbers in parentheses indicate the numbers of animals. S.E.M., standard error of the mean.

Prior conditions	Exposure (hr)	10 to 30 days after L_{150} exposure and subsequent relief			
		a-Wave (μv + S.E.M.)		b-Wave (μv + S.E.M.)	
		A−	A+	A−	A+
		Experiment a			
Dark	2*	540 ± 35 (10)	280 ± 60 (10)	1100 ± 46 (10)	523 ± 127 (10)
		Experiment b			
Cyclic	40	610 ± 22 (4)	250 ± 30 (4)	1300 ± 130 (4)	560 ± 60 (4)
	80	340 ± 57 (4)	80 ± 17 (4)	840 ± 60 (4)	290 ± 50 (4)
		Experiment c			
Cyclic†	40	240 ± 39 (4)	90 ± 40 (4)	570 ± 61 (4)	265 ± 89 (4)
		Experiment d			
Dark	40	43 ± 23 (4)	20 ± 9 (4)	120 ± 49 (4)	85 ± 44 (4)
Cyclic	40	590 ± 18 (7)		1225 ± 54 (7)	

* Body temperature during exposure was 39°C in response to hot and humid air environment (*2*). Normal body temperature in experiments b to d. † Relieved 6 days before exposure.

flow so that inside the cage the temperature was maintained at 24° ± 1°C. The rhodopsin content of the retina was about 0.2 nmole at the end of 2 hours of exposure compared to more than 2.0 nmole for A + rats maintained continuously in darkness (*2*). Both A− and A+ animals were exposed simultaneously under identical conditions. Vitamin A deficiency was relieved 24 hours after exposure by giving a high dose of retinol (*2*) to both groups of animals or to A− only.

For quantitative assessment of the damage by light, the ERG of each eye of every animal was tested with a strong xenon flash (*2*) 24 hours (just prior to relief) after exposure and at 10 to 12 days. The amplitude of the a-wave and the ERG threshold provided the indication in vivo of the degree of rhodopsin depletion in the deficient animals. The a- or b-wave of the strong-flash ERG 10 to 12 days after exposure indicated the degree (spatial extent) of irreversible damage (Fig. 1).

It was found that vitamin A deficiency protects against damage by visible light (L_{150}). The ERG's of Fig. 2 are from animals which had been kept since weaning (10 weeks) on a 12-hour light (40 watt), 12-hour darkness cycle (*4*). The ERG of the two A− animals prior to exposure L_{150} (first column) indicates a moderate degree of deficiency (*2*); the a-wave is reduced (425 μv compared to 635 μv in the A+), the b-wave is high and smooth and the ERG threshold (inserts) is increased (Fig. 2). Ten days after the exposure, the ERG of the A+ animals is markedly reduced; a- and b-waves are 12 to 15 percent their normal size. In contrast, the ERG of the A− animals is as high as the control ERG of A+. Because retinol had been administered 1 day after exposure, the A− animals have recovered from the deficiency but, in addition, they recovered from whatever effect the 40-hour exposure might have had. The "damaging" light did not result in irreversible change.

The data for the 10-day test from A+ animals (142) and A− animals (79) exposed after 2 to 10 weeks on the diet are plotted in Fig. 3. The difference between A− and A+ became progressively more evident after 3 weeks on the diet and was very pronounced at 10 weeks.

During the 10-week period after weaning, the rhodopsin of the A+ animals increased from 1.5 to 1.9 nmole; it decreased in A− to an average of 1.3 nmole. After exposure and 24 hours recovery in the dark, the measured rhodopsin of A− was 0.7 nmole, while retinol in PE and retina combined was the same as before exposure. The ERG of A− at this time (24 hours after exposure and just prior to the relief of the vitamin A deficiency) has a much smaller a-wave than before exposure and two prominent b-wave humps as illustrated in the third column of Fig. 2. These abnormalities are typical for advanced vitamin A deficiency, that is, for advanced retinol depletion of the retina (*2*). Indeed, they vanish within the next 9 days (second column of Fig. 2) after the administration of a high dose of vitamin A. In contrast, the ERG reduction of the A+ animals persists, manifesting the irreversible damage by the exposure to L_{150}. The effect of L_{150} on A−, hence, was merely to increase the rate of loss of retinol from the retina.

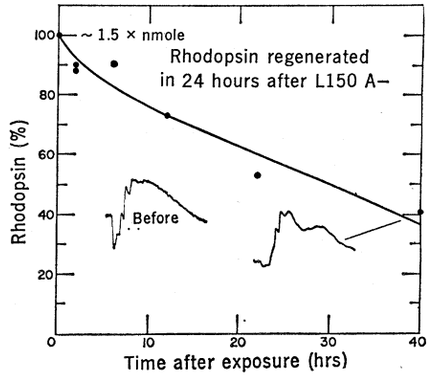


Fig. 4. The disappearance of retinol in A− animals as measured by determining rhodopsin recovery after 2 to 40 hours exposure to L_{150} light. Rhodopsin is expressed as percentage of the value before exposure. It was measured 24 hours after the end of exposure together with the retinol of PE, immediately after ERG testing (see insert from 40-hour light exposure). At this time all available retinol had been used for rhodopsin regeneration. The animals were on the deficient diet for 8 to 12 weeks and had been maintained in darkness or weak cyclic light. The drawn curve is theoretical, obtained by application of a model of the reaction sequence for the loss of retinol in vitamin A deficiency (*2*).

Figure 4 provides additional data on the loss of retinol during exposure of A− animals to L_{150}. This loss was 10 percent for 2 hours exposure, 28 percent for 12 hours, and 60 percent for 40 hours. The simple model for the disappearance of retinol from the retina in vitamin A deficiency (*2*) was applied to these data, and a reasonable fit was obtained on the assumption that the measured loss in the strong light occurred by the same process as in the weak cyclic light ($k_5 = 0.03$ per hour) (*5*). Thus, two quite different actions of the strong light are revealed: (i) in A+ (or normal) animals L_{150} damages the outer retinal layers, and (ii) in A− animals it accelerates the loss of retinol from the retina. Moreover, in condition (ii), condition (i) does not occur (or is greatly diminished); that is, the A− animals are protected against the damage.

Two factors could clearly be responsible for the protection: (i) a decreased concentration of rhodopsin for light to act upon, and (ii) a decreased concentration of light-produced retinol, as a consequence of (i) and of the transfer of retinol from PE into the blood in depleted animals (*2*).

The following experimental facts, for which examples are given in Table 1,

suggest, first of all, that the loss of retinol during exposure of A— is not a factor of primary importance in the mechanism of protection.

1) When body temperature is raised to about 39°C, L_{150} produces irreversible damage with exposure for 2 hours only. As shown in experiment a (Table 1), A— animals were significantly protected in this case despite the fact that the loss of retinol during short exposure is probably small (Fig. 4).

2) Whereas A— animals were little affected by 40 hours of L_{150} in the standard experiment (Fig. 3), extension of the exposure to 80 hours resulted in significant change as in experiment b (Table 1). This damage developed despite the fact that a considerable fraction of the light-produced retinol disappears during the 40-hour exposure and that, at the start of the extension, less than 8 percent of rhodopsin was unbleached.

3) A— animals relieved of the deficiency prior to exposure as in experiment c (Table 1) were less affected than A+ by an exposure 5 to 10 days after relief. Recovery to normal ERG threshold had occurred at least 1 to 2 days before exposure.

4) Protection was surprisingly weak when A— animals kept continuously in darkness were exposed as illustrated by experiment d (Table 1). After the animals were on the diet for 7 weeks, rhodopsin of the A— maintained in darkness was within 15 percent the same as in the corresponding A+. Damage from simultaneous L_{150} exposure was also about the same in these two groups, in contrast to its absence in the A— animals which had been kept in cyclic light (initial rhodopsin 30 percent lower than in the others).

The important factor, therefore, would seem to be the concentration of rhodopsin either as (i) the source of retinol or (ii) the mediator of the damaging reaction through a mechanism that does not involve retinol directly or decisively. The facts given above actually may be extended in their interpretation to indicate that retinol is probably not the toxic agent responsible for the damage by light.

Great difficulties, however, were encountered in the attempt to establish a consistent relation between rhodopsin and the degree of damage or protection. Especially for near-normal concentrations of rhodopsin, results were too variable and inconsistent for postulating a reaction starting with, and dependent mainly upon, the initial rhodopsin. Protection or damage seemed to be determined by another variable, in addition to rhodopsin.

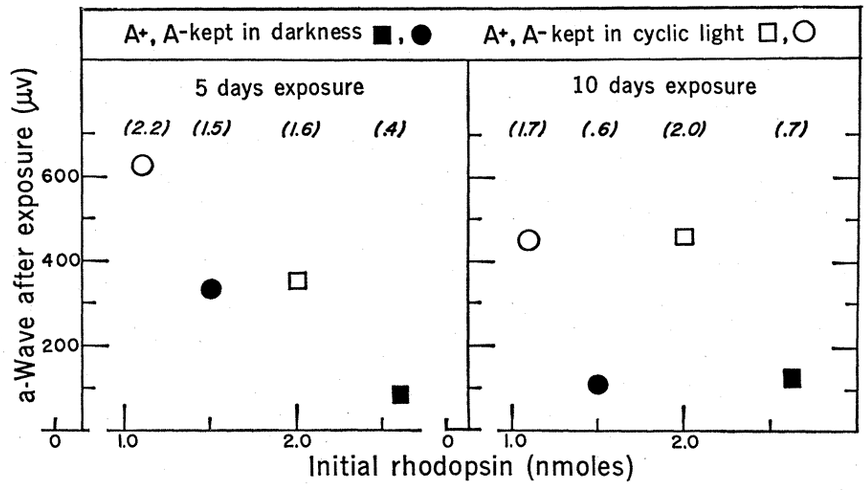


Fig. 5. Average a-wave, 3 weeks after exposure, is related to the average initial rhodopsin level obtained in other animals of the same groups. All animals were of the same age and were reared together in darkness or cyclic light (experiment 84); four animals were used for each point. The A— animals were relieved of the deficiency 14 hours after exposure. The numbers in parentheses at the top are values for rhodopsin measured after the 3-week ERG test of the same eyes. These values provide an additional criterion for the extent of the damage.

Figure 5 illustrates that this variable is the environmental light condition which precedes the acute exposure to strong light. The a-wave 3 weeks after exposure is plotted against the initial rhodopsin concentration; a decrease of the a-wave indicates irreversible damage. Exposure was in a special chamber to fluorescent light through the green plastic filter, giving a cage illumination of only 180 lux. A+ and A— animals had been on the diet for 12 weeks, but each class had been divided into two groups: one was maintained continuously in darkness and the other, from the 6th week, was maintained in cyclic light.

With continuous exposure of 5 days (preceded by 1 day in darkness for all animals, and followed after 14 hours by relief), the irreversible effect was strongest for the A+ animals kept in darkness and weakest (or absent) for A— kept in cyclic light. Correspondingly, the initial rhodopsin concentration was high in A+ and low in A—. There was no difference in damage, however, between A+ kept in cyclic light and A— kept in darkness, although their initial rhodopsin level differed, rhodopsin in A— being lower than in A+. The damage was of moderate degree, but considerably less for the "light-adapted" than for the "dark-adapted" A+, and in a similar manner for A—. Exposure was extended (in other animals of the same experiment) to 10 days with the result that the irreversible effect segregated exclusively in accordance with the light condition preceding the continuous exposure to the damaging 180 lux.

We conclude from this experiment and others of similar results that the major factor determining the protection against damage by light is a state of "cell adaptation" to light itself. Retinol deficiency provides this protection so effectively because it develops as a function of light (*2*), producing a steady state of adaptation (*6*) which can be reversed by the administration of retinol but not by darkness. Because protection should closely relate to the mechanisms of damage, it follows that the damage by visible light probably is the result of a reaction to the sudden and prolonged step of cell activity from a level of functioning in the dark or weak light to one in strong light (*7*). There is much evidence to indicate that the mammalian visual cell is a metastable, highly differentiated cell and that its cellular activities are delicately balanced for survival (*8*). The main result of our analysis, therefore, is the recognition that this balance may intricately depend upon the diurnal cycle of light and darkness.

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4. In the preceding paper (*2*), illumination during the cyclic light was obtained by 100-watt incandescent light instead of 40-watt. Rhodopsin levels of A— after 10 weeks on the diet (Figs. 2 and 3, this paper), therefore, are not as low as in figure 3 of (*2*).
5. The A— animals used for measuring the loss of retinol in strong light were selected for minimum light damage on the basis of their ERG's 24 hours after exposure. Measurements of retinol at the end of exposure were also made but gave less reliable information than rhodopsin regeneration because criteria for light damage could not be applied. Damage itself (as in A+) was associated with the rapid loss of retinol because of PE deterioration. Hence, measurements of retinol as well as of rhodopsin during and shortly after exposure were inconclusive if used for comparing A+ and A—. Experimental analysis had to be restricted to relating different initial conditions and different types of exposure to the magnitude of the damage.
6. The postulated "cell adaptation" is clearly different from the "dark" or "light-adaptation" measured in visual physiology. As used here, adaptation relates to cellular activities in a general way including metabolism, catabolism, electrolyte balance, acid-base balance, membrane permeability, and the like.
7. It must be understood that this reaction to light is only one of several mechanisms by which strong light can damage the visual cells. Another one, for instance, is by thermal injury as in eclipse and laser burns [B. S. Fine and W. J. Geeraetz, *Acta Ophthalmol.* **43**, 684 (1965)]. Vitamin A deficiency also may produce visual cell death but only after many months (6 to 8) as most recently shown [H. A. Hansson (*Virchow's Arch. Abt. B Zellpathol.* **4**, 368 (1970)]. Light exposure is essential to render the deficiency effective upon the retina (*2*). Hence, while one type of damage by light can be prevented by "cell adaptation" to light or by the retinol depletion occurring in vitamin A deficiency from daily exposure to weak light, weak light itself initiates changes that make vitamin A deficiency detrimental to the visual cells if the condition is maintained for a considerable length of time. In Hansson's experiments the animals were exposed to 50 lux for 10 hours daily. Concerning the "cell adaptation" to light, it is an intriguing fact that light "stimulation" of vertebrate photoreceptors produces hyperpolarization, not depolarization, as in axon and nerve cell. Photoreceptor membrane conductance is highest in darkness; light reduces it in a graded manner [B. Baylor and M. G. F. Fuortes, *J. Physiol.* **207**, 77 (1970)]. Similarly, oxygen consumption of retina decreases with illumination [I. Hanawa and W. K. Noell, *Physiologist* **5**, 153 (1962); W. Sickel, in *Clinical Electroetinography*, H. M. Burian and J. H. Jacobson, Eds. (Pergamon, New York, 1966), p. 115].
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Retrograde Amnesia: Electroconvulsive Shock Effects after Termination of Rapid Eye Movement Sleep Deprivation

Abstract. *Mice that were deprived of rapid eye movement sleep for 2 days immediately after one-trial training in an inhibitory avoidance task and were given an electroconvulsive shock after deprivation displayed retrograde amnesia on a retention test given 24 hours later. Electroconvulsive shock produced no amnesia in comparable groups of animals that were not deprived of rapid eye movement sleep.*

It is well documented that susceptibility of a memory trace to disruption by an electroconvulsive shock (ECS) decreases as the interval between the learning trial and the application of the amnesic treatment is increased. Generally, little or no amnesia is obtained if the ECS is given several hours or longer after training (*1*). This evidence supports the general hypothesis that memory trace consolidation processes are time-dependent.

Evidence from a recent pilot study (*2*) suggests that, if ECS is given immediately after several days of rapid eye movement (REM) sleep deprivation (REMD), the memory trace of an event experienced just before the REMD may be disrupted. Our report examines this finding further. Separate groups of mice were subjected to continual REMD for the 48 hours after their training in a one-trial inhibitory avoidance (passive avoidance, or PA) task. They were then administered an ECS at one of several intervals after termination of the REMD. On a 24-hour retention test, the mice displayed a retrograde amnesia gradient that varied with the interval between termination of REMD and administration of ECS.

We used as subjects 226 naive Swiss Webster mice that were 50 to 60 days old (*3*). The training apparatus was an alleyway (2.6 by 19.8 cm) leading to an open field (16.0 by 16.0 cm), with a grid floor mounted 0.6 cm above the base. The grid was constructed of 2.4-mm stainless steel rods, 7 mm apart, covering the entire floor of the enclosure. The apparatus was connected to a shock scrambler. All animals were given one training trial and one retention test trial. On each trial, the animal was placed in an alleyway, and the latency of its entrance into an open field (all four legs in the field) was recorded to the nearest 1/100 second. On the first trial, a scrambled shock of 500 msec at 0.6 ma was delivered to the floor of the open field as a gate behind the mouse was closed. On the retention test trial no shock was given; latency of entering the open field was recorded as in the training trial, with a maximum latency of 30 seconds allowed. In order to control for diurnal rhythm effects, care was taken to train and test each animal at the same time of day (within a few minutes). Since small groups of animals were run each day, control for age differences was maintained by starting the animals in the experiments when they were no younger than 50 days and no older than 60 days. Control for seasonal differences was maintained by assigning animals at random to each of the groups through the course of the experiment. REMD was produced by placing each mouse on a Plexiglas pedestal 3 cm in diameter, approximately 4 cm above the floor of the cage in which the animals were maintained. The floor was covered with water 3 cm deep. Food and water were always available (Fig. 1). Fishbein (*4*) has described this technique as it is used with mice. The method of deprivation takes advantage of the fact that during REM sleep there is a generalized inhibition of spinal motor activity (*5*). Since this inhibitory process appears to take place only during the REM periods, postural tonus persists during the non-REM (NREM) synchronized, high voltage, slow wave sleep. This means that the mice can sleep while crouching or sitting on the small pedestals. However, when the mice begin to enter the REM phase and postural tonus of the whole body, particularly of the muscles of the head and neck, diminishes, they either awaken briefly or fall