THE EFFECT OF VISIBLE LIGHT ON THE REGENERATION OF RHODOPSIN

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SUMMARY: Both in vitro and in vivo, increased exposure to visible light decreases the regenerability of the visual pigment. Isolated opsin irradiated with increasing periods of white light decreased in pigment formation yields on combination with 9- or 11-cis retinal. The yield of regeneration of the visual pigment extracted from albino rats depended on the amount of light to which the animal had been exposed. Animals exposed to normal room light demonstrated lower regeneration yields than dark-reared animals, but these yields increased on dark adaption. Opsin from animals exposed to sunlamps did not regenerate any pigment. On dark adaption, the pigment yields increased but the opsin level remained below that for the control group.

Several investigators have now established that damage to the retina occurs from visible light at intensity levels below thresholds for thermal burns (1,2). This damage is found to be reversible in the early stages provided the pigment epithelium is intact (3), and vitamin A deficiency provides a protection (4). The mechanism of this damage is not understood. The purpose of this study was to investigate in vitro and in vivo the effect of visible light on rhodopsin regeneration in order to ascertain if the temporary breakdown in the visual cycle could be due to an alteration in the visual pigment.

MATERIAL AND METHODS: In vitro: Bovine rhodopsin was isolated from frozen dissected retinal (Hormel Co., Austin, Minn.) by sucrose floatation (5). Standard pigment regeneration systems (6) were used with 9-cis retinal generally being employed because of availability. As the rates of pigment formation in vitro for the 9-cis and 11-cis retinal have been shown to be similar (7), the choice of isomer should not be a factor. The temperature was controlled by using a constant temperature bath held at 15°C. The light source was a GE R-40 sunlamp with a ultraviolet filter (Ealing #26-4276) which omitted wavelengths below 420 nm. Opsin was prepared by exposure to sunlight for one minute in the presence of hydroxylamine followed by washing three times with buffer. The opsin was then diluted to one OD (based on A_{500} of the orginial rhodopsin) per ml phosphate buffer and irradiated with vigorous stirring. Aliquots were removed at various time intervals. Pigment regeneration was carried out on each aliquot. The yield of regenerated pigment was determined by difference spectra of the bleached and unbleached samples.

In Vivo: A control group of ten Sprague Dawley rats was dark reared from

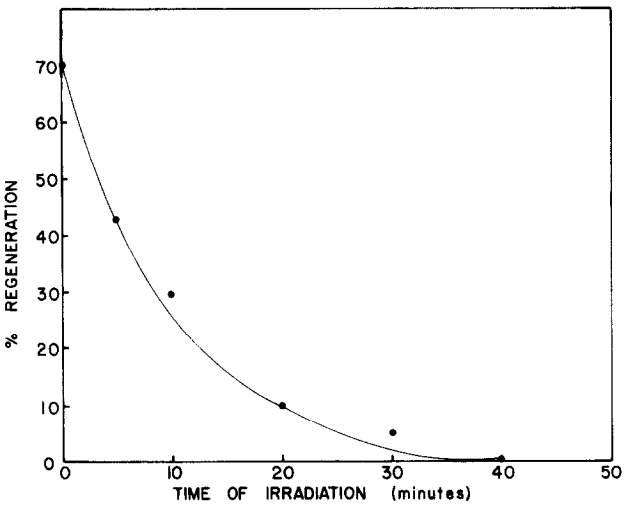


Figure 1: Percent regeneration of isorhodopsin versus time of irradiation of opsin. Bovine irradiated at 15°C with GE R-40 lamp with aliquots removed at various time intervals followed by regeneration with 9-cis retinal. Pigment quantities determined by A_{500}.

birth for 60 days. Four groups of ten each 60 day male Sprague Dawley rats (Charles Rivers Company) were maintained under cyclic room light for two weeks and then subjected respectively to the following:

1. Total darkness for 10 days.
2. Continued cyclic room light (200 foot candles) for 10 days.
3. Exposure for ninety minutes to a GE R-40 sunlamp (5000 foot candles).
4. Sunlamp (GE R-40; 5000 foot candles) exposure for ninety minutes followed by total darkness for 10 days.

The animals were allowed to move unrestained in clear plastic cages. The lighting was overhead. Care was exercised to prevent the animals from overheating during the sunlamp exposure by constant circulation of air. The decapitations and the pigment extractions were performed under dim red light. The retinae were dissected and the rhodopsin extracted by sucrose flotation (8). The quantity of rhodopsin was determined by the absorption at 500 nm. The rhodopsin samples were then bleached for one minute with sunlight in the presence of hydroxylamine. Opsin quantities were estimated from the 280 nm absorption bands. Regeneration was carried out with 9- or 11-cis retinal with incubations of 18 hours. The pigment yields were determined by difference spectra.

RESULTS: Increased exposure of isolated rhodopsin to white light resulted in a decreased yields of regeneration (Figure 1) terminating in complete failure of pigment formation. The presence of retinal did not change yields of pigment regeneration. The use of pure, retinal-free opsin prepared by

TABLE I

The Effect of Light on Rat Visual Pigments

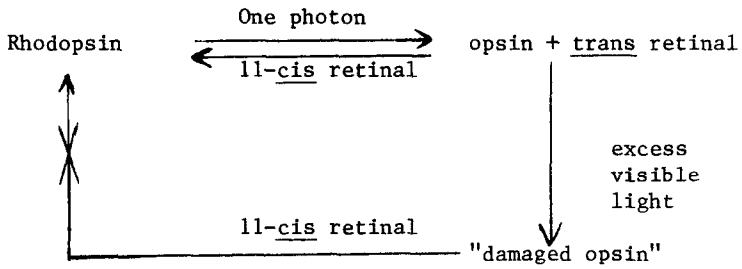
Group Control	Light Exposure	% Rhodopsin	% Opsin	% Relative Regeneration
Control	Total darkness from birth	100	100	100
1	Cyclic room light[1] followed by total darkness (10 days)	100	100	100
2	Cyclic Room light[1]	30	100	50
3	Sunlamp[2] (90 minutes)	0	100	0
4	Sunlamp[2] (90 minutes) followed by 10 days total darkness	60	70	60

1) Foot Candles = 200
2) Foot Candles = 5000

All values ± 5%; Rhodopsin based on 0.05 Extinction/Eye; opsin concentration based on 280 absorbance: the regeneration yields were the same with 9-*cis* or 11-*cis* retinal; relative regeneration based on 100% value for dark reared animals.

the method of Rothschild *et al*. (9) showed the same degree of failure in regeneration on irradiation as did opsin routinely prepared which has been demonstrated to contain some chromophore. Likewise the addition of two equivalents of *trans* retinal did not enhance the light effect. The *in vivo* experiments demonstrated the regenerability of the opsin is affected by the amount of light to which the animals have been exposed. The rhodopsin content per eye was determined to be the same as reported earlier for dark adapted animals (10). The animals under cyclic light completely recovered their rhodopsin levels on dark adaption for ten days. The sunlamp exposed animals did not have a complete recovery of the rhodopsin on ten day dark adaption. Although the rhodopsin concentration is dependent on light exposure, the opsin levels based on the absorbance at 280 nm were about the same for all the groups except the group exposed to the sunlamp and followed by dark adaption. The regeneration yields based on the dark reared group were found to be dependent on the amount of light to which the animals had been exposed (Table I).

DISCUSSION: We have demonstrated that opsin which is exposed to "excess" visible light fails to regenerate pigment on the addition of 9- or 11-cis retinal.



The chromophore retinal apparently is not envolved in the mechanism as no variation in the irradiation effect could be detected either in its absence or in the presence of a twofold excess of the all-trans isomer. Since the isolated opsins of both the rat and bovine species were found to be affected by visible irradiation, the phenomenon is not species specific.

The in vivo studies demonstrate the rhodopsin content of the eye is dependent on the amount of light to which the animal has been exposed as has been demonstrated in previous studies (10). The total opsin/rhodopsin content was not found to be dependent on light conditions. However, the ability of this opsin to regenerate pigment was found to be dependent on the light history of the animal.

The eyes of animals maintained under normal room light conditions (200 foot candles) contained the same amount of opsin on bleaching as the control dark-reared group but this opsin formed pigment on combination with 9- or 11-cis chromophore in only 50% yield. However, by dark adapting the animals for 10 days, the rhodopsin now present reformed pigment to the same degree as the control group. Noell et. al. (1) have previously shown the visual acuity of albino rats is affected by low intensity, normal room lighting. Our results demonstrate at this illumination level the ability of the animal to regenerate new rhodopsin is impaired due to some "damage" to the opsin and thus the visual process is deminished. Hall et. al. (11) have shown rhodopsin is not renewed

within the rod discs. New rhodopsin is formed in the rod inner segment and progresses to the outer segment within the disc. The rod outer segment requires ten days for complete renewal in the rat (12). The fact that the animals regain their regeneration ability on dark adapting for the time period required for the renewal of the rod outer segment thus lends support for the theory that the visual pigment itself is being affected by light of low intensity. The particular alteration to the protein or phospholipid and the mechanism by which it occurs is currently being studied.

The opsin extracted from the animals exposed to the sunlamp (5000 foot candles) failed to regenerate any pigment on combination with chromophore. On dark adapting the animal for ten days after the sunlamp exposure, the extracted opsin formed pigment but in lower yield. These results indicate some permanent damage had occurred.

In conclusion, we have demonstrated in vitro that rhodopsin decreases in pigment formation with increasing exposure to visible light. Futhermore, the yield of regeneration of the visual pigment extracted from albino rats depends on the amount of light to which the animal has been exposed. The effect was reversible at low intensity with dark adaption at sunlamp levels, permanent damage appeared to have occurred. From these results we conclude the reversible damage observed from low intensity visible light is due to some alteration of the visual pigment and once new rod discs containing new rhodopsin are synthesized, the visual process is restored.

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