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Light-induced Rhabdomeric Degeneration in the Norway Lobster, Nephrops norvegicus (L)

by

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Summary

The visual pigment of Nephrops norvegicus (L) was measured microspectrophotometrically in isolated rhabdoms and found to have maximum absorbance at 498 nm. This pigment bleached to a long-lived photoproduct, λ-max 484 nm, which broke down slowly in the dark. The bleaching of the visual pigment affected the structural stability of the rhabdoms resulting in deteriorative changes and eventual degeneration of the rhabdoms. These in vitro effects were found to occur in vivo after exposure of Nephrops caught and maintained in the dark to low-level illumination from a single fluorescent tube. Exposure of 3 hours duration was enough to cause complete degeneration of the rhabdoms within 2 days even though the animals were kept in the dark following exposure. It is suggested that in those studies using Nephrops brought to the surface during daylight, rhabdomeric degeneration is a probable consequence and results obtained using these animals must be interpreted with this in mind.

Introduction

There have been several reports of photoreceptor degeneration in living rats and pigeons after exposure to low-level illumination (Gorn and Kuwabara, 1967; Grignolo et al., 1969; Kuwabara and Gorn, 1968; Noell et al., 1966; O'Steen, 1970). That such effects of light on visual photoreceptors are uncommon is obvious from the large number of animals which retain perfect visual function even after long exposures to relatively high light levels such as might be encountered by animals active at mid-day. It is generally accepted that mechanisms such as migrating shielding pigments, photomechanical movement, and variable apertures (i.e. pupils) keep the light incident on the photoreceptors at a tolerable level and allow even nocturnal animals to tolerate bright light without damage to the visual cells. The surprising finding with rats is that light intensities similar to those found in well lit animal rooms can cause degeneration of the photoreceptors. I report here an apparently related phenomenon in the Norway lobster, Nephrops norvegicus (L).

Methods

The initial observations were made during a microspectrophotometric (MSP) study of isolated rhabdoms to determine the

visual pigment. The Nephrops used had been caught in outer Loch Torridon in traps set 60 metres down during the day and left in place until after nightfall. After raising to the surface, the animals were thereafter kept in light-proof containers of sea water with all manipulations carried out under dim red light. Nephrops so obtained and kept had a deep "white spot" in the compound eyes indicating that the shielding pigments were in the "dark adapted" position (Eguchi and Waterman, 1967). For MSP examination the eyes were excised under dim red light and the rhabdoms isolated by grinding the compound eye with a glass rod in 2 ml of sea water (pH 7.6). A drop of the resulting suspension was placed on a cover slip, diluted with a drop of fresh sea water, and sealed using another cover slip rimmed with silicone grease. A description of the MSP and associated techniques is given elsewhere (Liebman, 1972). All positioning and alignment of the rhabdoms was done in infra-red light using an appropriate image converter for visualization. Microscopic examination was generally made in infra-red light; however, in the case of compound eye "squash" preparations white light was used after staining with methylene blue.

Results

Absorption spectra of isolated rhabdoms yielded a single rhodopsin-like pigment with a λ_{max} at 498 nm. This bleached on exposure to light to a long-lived photoproduct (i.e. metarhodopsin) of λ_{max} 484 nm (Fig. 1). This product broke down in the dark with a half-time at room temperature (18°C) of 180 - 210 minutes. Attempts to photo-regenerate the 498 nm pigment from the 484 nm product were unsuccessful. These properties are similar to those of other crustacean visual pigments (Goldsmith, 1972). In measuring the breakdown of the metarhodopsin, I noticed an increase in absorbance above 700 nm during the first 60 minutes after the bleach, which I interpreted as a sign of rhabdomeric deterioration. This was confirmed by visual inspection of the same rhabdoms, which showed definite signs of swelling and vesiculation. Examination of unbleached rhabdoms at the same time, however, showed no evidence of similar structural changes and it was only after an hour and a half that any signs of deterioration could be detected in them. To test this effect of bleaching further, small areas of single rhabdoms were bleached with white light. Examination during the first hour after the bleach showed structural changes only in the bleached areas.

It thus appears that bleaching of the visual pigment and/or the production and subsequent breakdown of metarhodopsin decreases the structural stability of Nephrops rhabdoms *in vitro*. Experiments were then carried out to see if this effect could be demonstrated *in vivo*. An opaque tank having an 8 watt, daylight fluorescent tube fitted into the lid was partially filled with sea water to a depth of 20 cm. The tube was 20 cm above the water line and the average luminance under these conditions was 200 cd $\cdot$ m^{-2}. Two Nephrops were placed into the tank and the light turned on. Almost immediately the "white spot" in the eyes disappeared indicating that the shielding pigment had migrated between the rhabdoms (Eguchi and Waterman, 1967). After

18 hours the light was turned off and an eye was removed from one of the animals. Rhabdoms isolated from this eye showed signs of extensive structural damage with loss of the typical rhabdomeric banding and veriform appearance. MSP measurements showed no signs of visual pigment, and only a trace of metarhodopsin. After 2 hours in darkness the other eye was removed from the same animal and it, too, yielded structurally damaged rhabdoms. Again, no visual pigment was found indicating a lack of visual pigment regeneration within the 2 hour period. No photoproduct was present. The second Nephrops was kept in darkness an additional 18 days before the compound eyes were removed and whole squash preparations made. In neither of the eyes were any identifiable rhabdoms found. Moreover, the "white spot", indicative of the dark adapted condition, failed to reform during the 18 day dark period. In a second experiment, a Nephrops was exposed to the above light intensity for only 3 hours and then kept in the dark for 2 days. At the end of this period there were few identifiable rhabdoms and those that were present showed signs of severe degeneration. No visual pigment or metarhodopsin was found in these rhabdoms and the "white spot" had failed to reform. Controls maintained in the dark were periodically examined during the course of the above experiments and were found to have well-formed "white spots", normal rhabdoms and visual pigment.

Discussion

The mechanism underlying the deterioration and eventual loss of rhabdoms *in vivo* after light exposure is unknown, but it might have resulted from the rapid accumulation of the less thermally-stable metarhodopsin within the photoreceptor membrane. The slow thermal breakdown of this product in the dark would not only release the visual pigment chromophore, presumably retinal$_1$, but would also alter the conformation of the opsin moiety and perhaps affect the structural integrity of the membrane. It is not yet known if there is a time-intensity relationship governing rhabdomeric breakdown or if there is a critical visual pigment/photoproduct ratio above which there is no degeneration. Increasing the body temperature of rats exposed to light accelerates degeneration of the outer segments. It might be expected, therefore, that reducing the temperature would have a stabilizing effect. This suggests that Nephrops living at a depth of 60 metres, where the temperature is only about 8°C, might not suffer the degeneration observed at 15° - 18°C in the laboratory.

The observations reported here were not completely unexpected and may serve as a guide in the study of any animal from a dim light environment. Some time ago an attempt was made in this laboratory by Professor Dartnall to extract the visual pigment of Nephrops. A photostable pigment with a λ_{max} at 480 nm was found which broke down slowly in the dark. These characteristics suggest that it was the long-lived photoproduct of visual pigment bleaching. The history of the Nephrops used in this unsuccessful attempt is significant. Although they were caught at the same depth as those used in the present study, they had been raised to the surface during the day. This means that on the way up they were exposed to increasing light levels and once on the surface had been exposed to direct sunlight. They were removed from the traps

and placed in the dark for several hours to dark adapt before the eyes were removed and stored in the dark on ice. The assumption was implicit that once placed in the dark the Nephrops would recover from light exposure and any bleached pigment would be regenerated. It is now evident that this was not the case. Not only would the visual pigment of Nephrops exposed to direct sunlight fail to regenerate in the dark, but such animals would experience rhabdomeric degeneration rendering them effectively blind and useless for experiments requiring intact primary photoreceptors.

Chapman and Rice (1971) have found that light intensity is an important factor in determining the diurnal activity pattern of Nephrops living at various depths. The animals observed in their study were caught and tagged in daylight before being returned to the sea bed, so rhabdomeric degeneration probably occurred. Since the tagged animals continued to show an apparent light-dependent activity pattern in the absence of visual cells, the entrainment of the circadian rhythm is probably mediated by a dermal light sensitivity or direct stimulation of nervous tissue. However, the possibility still exists that some environmental factor not controlled in the laboratory experiments can prevent the deterioration of the rhabdoms even after exposure to direct sunlight.

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Figure Legend

1. Original MSP absorption spectra from an isolated rhabdom. Each spectrum represents one forward and one reverse wavelength scan between 400 nm and 650 nm. a) Absorption spectrum of the unbleached visual pigment; b) absorption spectrum of the same rhabdom after a 2 minute white-light bleach. The maximum absorbance is shifted to a shorter wavelength and the extinction coefficient is increased; c) absorption spectrum 20 minutes after the bleach showing the stability of the photoproduct.

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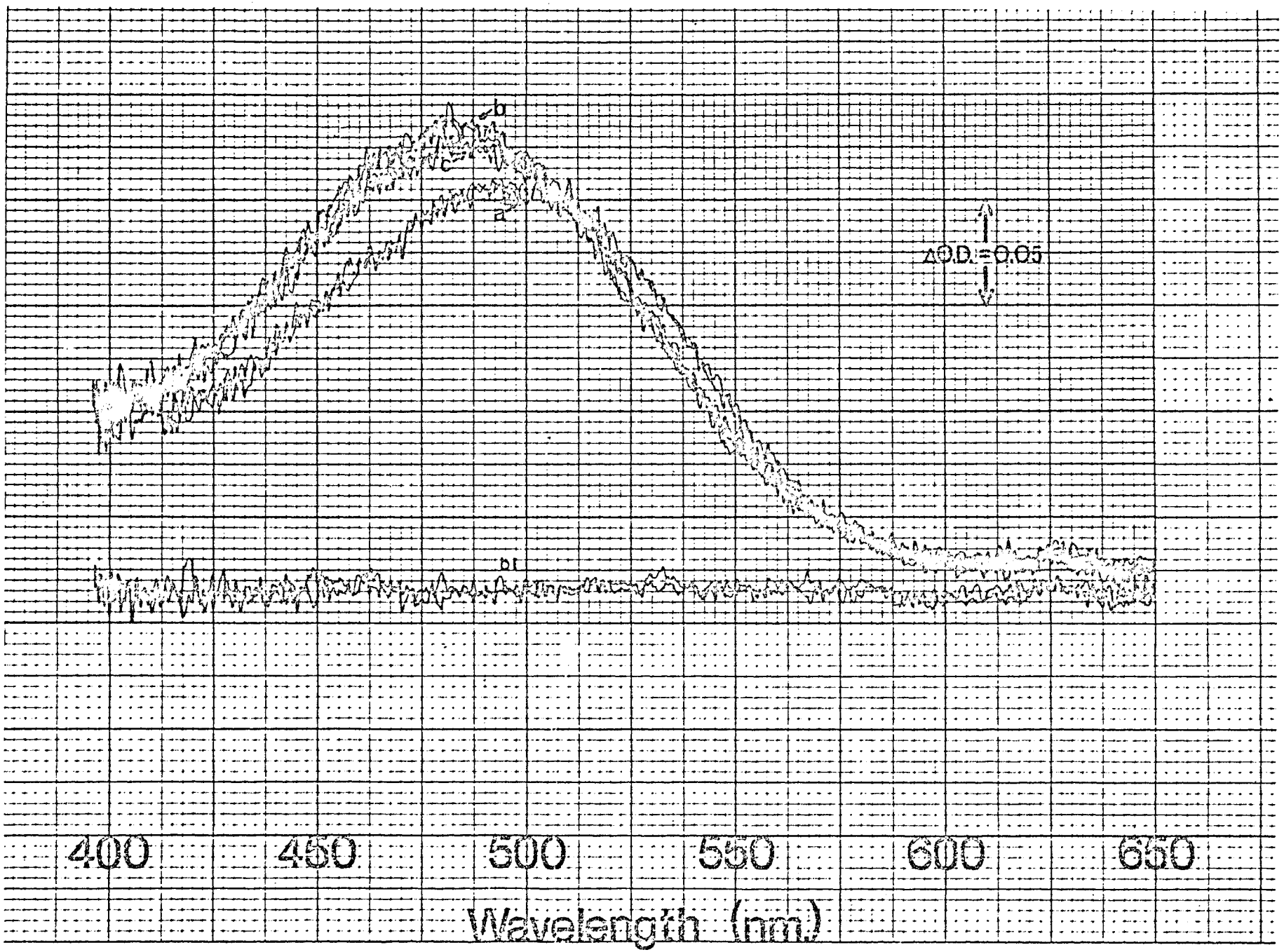

FIG.1