The Photodynamic Action of Light on Hydra[1]

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Hydra were known to react to light as early as 1744 when Trembley first described their movement towards a light source. This migration of the animal was then further elucidated by Wilson in 1891, who found that *Hydra viridis* and *fusca* are maximally sensitive to blue light. Haug ('33) found that Hydra will react to a light stimulus by contracting first and then orienting; and Rushforth, Burnett and Maynard ('63) studied the contraction response in *Hydra pirardi*, the large Belgian species. Feldman and Lenhoff ('60) studied phototaxis in the animal and suggested the presence of photoreceptors.

The present work is an analysis of the influence of light intensity and wavelength on the contractility of *Hydra pirardi* employing in part the methods of study of Hecht ('20–'22) on the clam, *Mya*. It provides some evidence on the nature of light reception in Hydra and discusses the results in terms of known physiological functions.

MATERIALS AND METHODS

Hydra pirardi, cultured by the methods of Loomis and Lenhoff ('56) were used throughout the study. Animals were starved for 24 hours at 21°C prior to experimentation.

Intensity measurements

The light source was an American Optical Co. Illuminator Model 353 having a tungsten bulb (2.75 a., 6.5 v.). The intensity of the light was controlled by means of a variable transformer connected in series with the lamp. The beam was directed onto a petri dish containing 15 ml of culture water. The intensities were recorded by means of a Clairex 602 photoconductive cell and milliammeter connected to a 200–235 volt DC regulated power supply. No adjustment was made for changes in the spectral composition of the lamp at the six voltage settings used. The ambient light had an illumination of approximately 20 foot candles at the surface of the water.

The animal was transferred to the petri dish from a stock culture and after it had fully expanded, the light was turned on. The reaction time, defined as the time in seconds between onset of irradiation and completion of a single contraction of the animal, was recorded.

Wavelength measurements

Light of specific wavelengths was controlled using a Bausch and Lomb 250 mm grating monochromator with a tungsten lamp as the light source. The bulb used was a 150 watt Sylvania Tru-Flector DFA bulb connected in series with a variable transformer set at 80 volts. The light emitted through a 1.0 mm slit of the monochromator was reflected by means of a mirror through the bottom of a petri dish containing 15 ml of culture water. The beam irradiated an area of 6.25 sq cm of the bottom of the dish. The relative intensity of the light was measured by means of the photoconductive cell as in the previous experiment. The intensity measurements were adjusted for differences in sensitivity of the photocell to the differing wavelengths.

Two animals were placed in the rectangular area and separated to avoid contact. As soon as they both expanded, the animals were exposed to light of a selected wavelength and the reaction times recorded.

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RESULTS

The effect of light intensity

The results of the measurement of reaction time as a function of intensity are given in table 1. The mean and standard deviation of the reaction time are tabulated for each light intensity. The light intensity is expressed as a percentage of the intensity of the light at the highest voltage used. In figure 1 the mean reaction times together with their corresponding 95% confidence limits are plotted against the relative light intensities.

TABLE 1

Reaction time of Hydra pirardi and relative light intensity
(15 different animals at each intensity)

Relative intensity – I	Reaction time — t (Seconds)		Product of relative intensity and reaction time – It
	Mean	S.D.	
%			
6	200	128	1200
18	130	100	2340
34	66	25	2244
58	42	17	2436
70	41	14	2870
100	34	13	3400

The results show that the reaction time is inversely related to the light intensity. Except at the smallest and largest intensities, the relationship may be approximated by the Bunsen-Roscoe photochemical reciprocity law (1862) which states that the product of intensity and reaction time is constant. Measurement of spontaneous contractions of a control group of 15 animals in ambient light for ten successive contractions gives values of the mean and standard deviation of the reaction time as 199 and 63 seconds. The mean reaction time of animals tested at the lowest intensity is not significantly different from that of animals in ambient light. The variability of the reaction times of different animals tested at the same intensity, expressed as the standard deviation, is greatest at the low intensities and decreases with increasing intensity.

Spectral sensitivity

The previous experiment demonstrates an inverse relation between reaction time and the intensity of the light in the contraction response. Thus in order to study reaction time as a function of the wavelength of the light stimulus, it was necessary to correct for differences in intensities of the various wavelengths. The corrections applied to the observed reaction

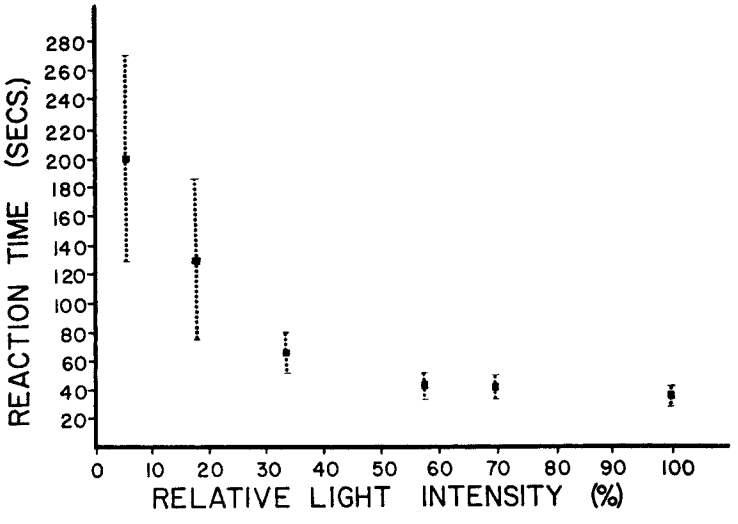

Fig. 1 Reaction time of *Hydra pirardi* and relative light intensity. The solid squares represent the mean reaction times of groups of 15 hydra. The dotted lines represent the 95% confidence limits for the mean reaction times.

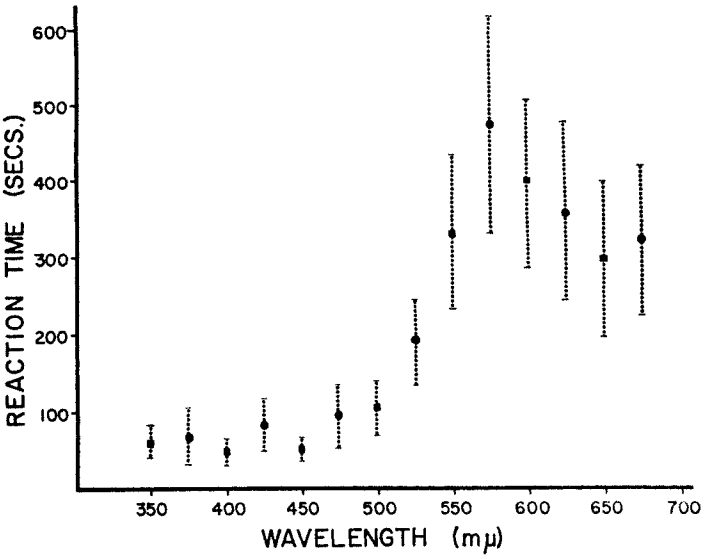

Fig. 2 Reaction time of *Hydra pirardi* and wavelength of the light stimulus. The solid squares represent the mean reaction times of groups of 20 hydra in the first experiment; the solid circles represent the mean reaction times of different groups of 20 hydra in a second experiment performed two weeks later. Squares and circles alternate. The dotted lines represent the 95% confidence limits for the mean reaction times.

times were obtained using the photocell measurements of the intensities at the various wavelengths R_λ, together with the wavelength sensitivities of the photocell S_λ. The corrected measurements, expressed as percentages, were read from a graph of the spectral sensitivity of the cell furnished by the manufacturer. The photocell reading R_λ for a wavelength was divided by the sensitivity S_λ to give the relative intensity of the light at the wavelength I_λ. Since the contraction time was previously shown to be inversely related to the intensity of the light, the corrected reaction times $t_{\lambda,c}$ were obtained by multiplying the observed reaction times $t_{\lambda,o}$ by the respective relative intensity. Thus $t_{\lambda,c} = t_{\lambda,o} \times I_\lambda = t_\lambda \times (R_\lambda/S_\lambda)$. This correction assumes the reciprocity law to hold for the reaction of the animal to the light stimulus.

The corrected reaction times together with their corresponding 95% confidence limits based on the results of two experiments are plotted in figure 2. This action spectrum shows that the contraction of *Hydra pirardi* is most easily induced by blue light (400–450 mμ) and less so by light of longer wavelengths. Sensitivity markedly decreases above 500 mμ and then increases slightly above 575 mμ.

DISCUSSION

These studies on the contraction of *Hydra pirardi* to light show that the reaction time is inversely proportional to the light intensity (except at the lowest and highest intensities) satisfying the Bunsen-Roscoe photochemical reciprocity law. Some initial experiments with *Hydra pseudooligactes* yielded similar results. Deviation from the law occurs at low and high intensity levels [see for example the recent discussion by Giese ('62) and Prosser and Brown ('61)]. Our results resemble those of Hecht ('20) on *Mya arena*, who showed that the light response obeys the Bunsen-Roscoe law and concluded that the initial action of the light is photochemical.

The results of the spectral sensitivity of *H. pirardi* indicate that the reaction time is shortest in blue light (400–500 mμ). The sensitivity of the animal markedly decreases above 500 mμ. Haug ('33) also reported a differential contraction of Pelmatohydra oligactis to blue light, but he did not distinguish between the relative energies transmitted at different wavelengths. Recently Passano and McCullough ('62) claimed that exposure to blue light changes both the frequency and origin of rhyth-

mical electrical potentials in *Hydra pirardi*. Such changes were not affected by wavelengths above 500 mμ.

The action spectrum of *Hydra pirardi* strongly resembles that reported for other animals and plants. The response, whether it be that of turning toward light (e.g., plants and hydroids among animals) or the movement to or away from light (seen widely among animals), is in general attributed to light in the blue end of the spectrum (see review of Wald, '45–'46). *Euglena viridis* and other related species are especially sensitive in the blue, 473–482 mμ (Mast, '17). In the marine worm *Arenicola* and the earthworm, maximal sensitivity is at 483 mμ (Mast, '17), and in the clam, *Mya*, at approximately 490 mμ (Hecht, '20–'21). In the orientation reactions of holothurians, Uexkull ('04) observed the sensitivity of spicules surround the anus to blue light and Crozier ('14–'15) of the entire body surface. The sixth abdominal ganglion of the ventral cord of the crayfish is maximally sensitive in the blue (Kennedy, '58). In addition to Wilson's early studies of phototaxis in Hydra in which he demonstrated that maximum sensitivity is in the blue, Loeb and Wasteneys ('15) studied the orientation of hydroid polyps to light and noted maximum sensitivity at 474 mμ.

Whatever the motile response, the general similarity in action spectrum of these widely ranging forms has suggested a similar underlying photochemical mechanism (Wald, '45–'46). This mechanism is based on the reception of light energy by a photosensitive pigment, almost always found to be a carotenoid. It seems unlikely, considering the action spectrum of *Hydra pirardi* characterized in figure 2 that the animal should deviate in its photochemical pigment from that common in both the animal and plant kingdoms. Indeed, the review of Fox and Panthin ('44) cites the existence of carotenoids in almost every species of coelenterates, particularly in Hydra. Furthermore, Burnett ('59) demonstrated the presence of carotenoids in the digestive cells of *Pelmatohydra oligactes* concentrated in the hypostome and basal disk.

In Hydra no special structure is known for the reception of light such as the stigmata of Euglena and the vertebrate eye, although Eakin and Westfall ('62) have indicated presence of photoreceptors in a marine coelenterate. A lack of specialization in Hydra, however, does not preclude a refined molecular arrangement whereby the energy of light is absorbed to initiate a photochemical reaction resulting in motility.

SUMMARY

Hydra pirardi contracts in response to a light stimulus. The reaction time, except at lowest and highest intensities, is inversely proportional to the light intensity, satisfying the Bunsen-Roscoe photochemical reciprocity law. The reaction times of individual animals to light is quite variable. Inspection of the spectral sensitivity of the animal indicates that the reaction time is shortest in blue light (400–450 mμ). The sensitivity markedly decreases above 500 mμ.

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