Reaction Patterns of Dark- and Light-Adapted *Hydra* to Light Stimuli[1]

That fresh water polyps of the genus *Hydra* can perceive light and react to it has been known since the work of TREMBLEY[2] and has been confirmed subsequently by various other authors[3–7]. The response to light stimuli manifests itself in a positive or negative phototropism determining the locomotory activities of the semi-sessile animals; or it can consist of a fast stepwise contraction of the entire animal including the tentacles (see Figure 1). This phobic reaction is performed by polyps which are exposed to light of high intensities.

In *Hydra* nothing has been established so far about the existence and localization of photoreceptors. Electronmicrographs[8] of the ectodermis have revealed particular nerve elements featuring rudimentary ciliary structures and these may act as primitive photoreceptors.

H. pirardi[7] is known to be particularly sensitive to blue light ranging between 350 and 500 nm (see also[5]). In this spectral segment lie the absorption peaks of most of the carotinoids that are incorporated in the entodermal cells of *Hydra*[9]. Thus, according to FELDMAN and LENHOFF[10] the carotinoids may be involved somehow in the process of photoreception.

This note is concerned with the reactions of *H. attenuata* Pall.[11] to various light conditions (white light only) with special reference to the question whether there is adaptation to light or not. Consequently all experiments were carried out with 2 groups of animals: 'light adapted' specimens which prior to the experiment were exposed for at least 10 days to a constant illumination (3000 Lux) and 'dark adapted' animals which were kept in complete darkness for as long as 6 months. Both 'dark- and light-adapted' polyps were then exposed to repeated light stimuli (2 min exposure) alternating with dark intervals of 2 min each (Figure 3). This 'on and off'-sequence was kept constant throughout all experiments, the intensities within the light periods were varied. Each experimental run comprised 14 animals which were exposed collectively to the same sequence of alternating dark and light periods. Each polyp was placed on the tip of an upright glass capillary fixed to the bottom of a waterfilled glass-

[1] These investigations were generously supported by a grant (No. 3991) from the 'Schweizerische Nationalfonds zur Förderung der wissenschaftlichen Forschung'.

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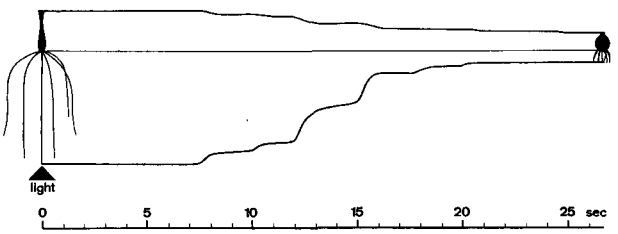

Fig. 1. Protocoll of contraction performed by a single *Hydra attenuata* after exposure (arrow) to 90,000 Lux (reconstruction of a time lapse film at 12 frames/sec).

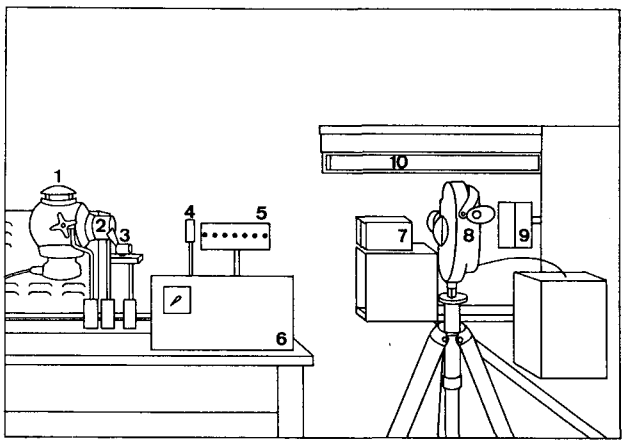

Fig. 2. Experimental set up: (1) Light source[13]; (2) water filter; (3) shutter; (4) focusing lens (biconvex 22 cm); (6) timer; (7) aquarium with experimental animals; (8) recording movie camera[12]; (9) electronic flash; (10) tube lights.

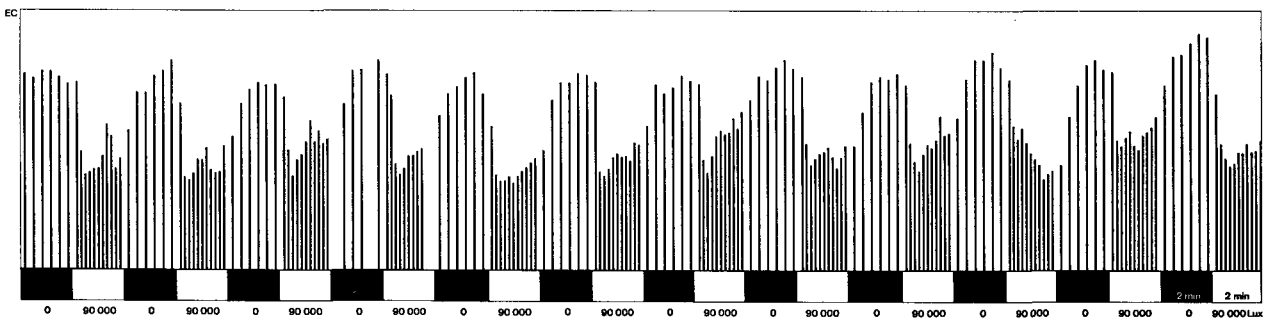

Fig. 3. Reactions of 'light adapted' hydras (14 specimens) to alternating dark and light (90,000 Lux) intervals of 2 min each (ordinate EC, extent of contraction; abszissa, time).

chamber (12 × 6 × 8 cm). The behaviour of these polyps in terms of their stage of expansion or contraction was recorded by means of a motion picture camera[12] operated at a speed of 1 frame per 10 or 20 sec. The shutter of the camera was synchronized with an electronic flash so that pictures could be taken during the dark intervals also (the polyps did not react to the electronic flash). Each picture was then projected onto plotting paper. A magnification of 2 × was kept constant. The lengths of the body columns from hypostome to pedal disc of all 14 animals were summed to give a column representing the stage of contraction or expansion of all 14 animals at a given instant of the experiment (Figure 3). This procedure not only permits us to ascertain whether an animal has contracted or not, but it also provides a measure of the extent of contraction or expansion.

In order to reduce vibrations, the light source[13] and the aquarium containing the experimental animals were placed on separate tables (Figure 2). The light source was switched on and off by a programmed automatic timer which also controlled the operation of the film camera and that of the electronic flash. The light intensity was set by means of grey-filters placed in between the light source and the target. The following intensities which were recorded with a photometer[14] were used: 250, 900, 3200, 14,500, 50,000 and 90,000 ± 20% Lux.

Figure 3 shows the behaviour of 14 light adapted *Hydra* exposed to a sequence of dark and light periods. The light intensity corresponds to 90,000 Lux, comparable to weak sunlight. Approximately 10 sec after being hit by the light beam the animals start to contract as shown in Figure 1. The pattern and extent of contraction remain constant at each subsequent light stimulus. During the dark periods the animals extend to their normal length. The regularity of the reaction to light stimuli repeated at intervals of 2 min for as long as 3 h indicates that there is no habituation. This finding confirms the observations made by RUSHFORT et al.[6]. The responses of dark adapted animals to identical alternating conditions is in principle the same, but the extent of contraction in the light and that of extension in the dark are less pronounced than in 'light adapted' animals. We have no satisfactory explanation as yet for this difference.

Fig. 4. Contraction pattern of 14 dark (solid lines) and 14 light (dotted lines) adapted animals to alternating dark and light (14,500 Lux) intervals of 2 min each. The curves represent the average of 10 subsequent periods (ordinate EC, extent of contraction; abszissa, time).

[12] Bolex H16 reflex.
[13] Universal microscope lamp 'Wild' (Heerbrugg, Switzerland) with a Xenon burner XBO 162 (Osram).
[14] Tavolux 2, Metrawatt, Nürnberg (Germany).

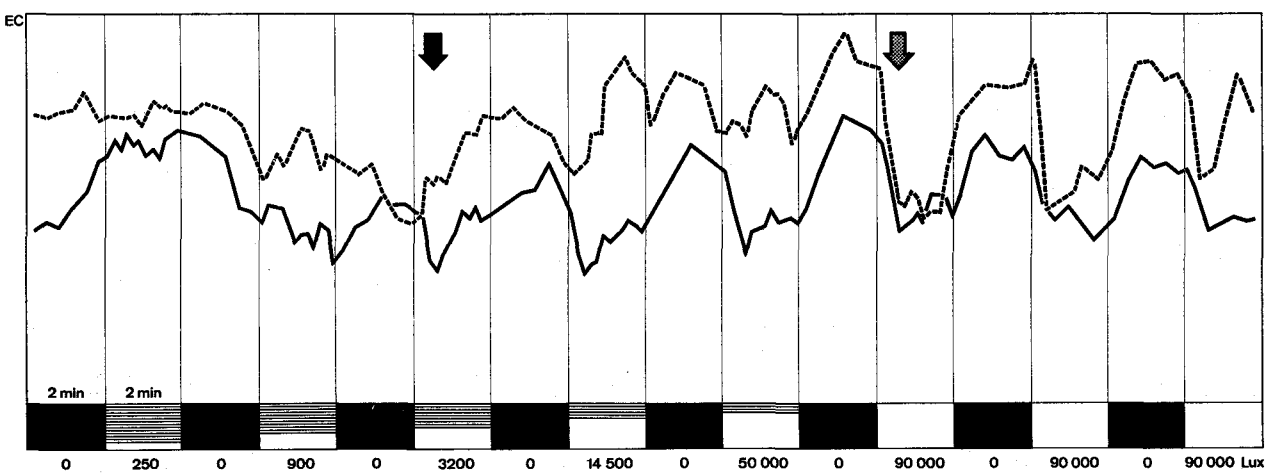

Fig. 5. Reactions of 14 light (dotted line) and 14 dark (solid line) adapted polyps to alternating dark and light intervals of 2 min each. During the sequence of light intervals, the light intensity was increased from 250–90,000 Lux. The arrows indicate at which intensities light (dotted arrow) or dark (solid arrow) adapted animals start to respond to the light stimuli (abszissa, EC, extent of contraction; ordinate, time).

If, instead of 90,000 Lux, lower light intensities are used in the same sequence of 2 min dark-light intervals, 'dark- and light adapted' animals behave totally differently (Figure 4). 'Light adapted' polyps do not show any reaction when exposed to 14,500 Lux while the dark adapted group clearly responds by contracting.

This marked difference between the 2 experimental groups also manifests itself when the sequence of dark and light periods is modified in the following manner: instead of using the same light intensity throughout a series we increased gradually the intensity in each subsequent light period starting from 250 Lux and ending up with the highest possible intensity of 90,000 Lux.

The comparison (Figure 5) between the behaviour of 'light- and dark adapted' animals under these conditions clearly shows that in dark adapted polyps the contractions already become evident when a light intensity of 3200 Lux is reached, whereas light adapted animals do not start reacting until hit by 90,000 Lux. Thus, the threshold for the response to white light is considerably lower in 'dark adapted' than in 'light adapted' animals.

This differential behaviour of polyps that had been kept in complete darkness and those subjected to continuous illumination can be interpreted as being a manifestation of an adaptation to particular light conditions. We have no information so far about the mechanism of adaptation and the level at which this adaptation takes place. Investigations about the possible role of the carotinoids are in progress.

Zusammenfassung. Die Kontraktionsintensität bleibt auch dann unverändert, wenn die Polypen von *Hydra attenuata* dem Dunkel-Hell-Wechsel (je 2 min) während 3 h ausgesetzt werden. Dunkeladaptierte Hydren reagieren auf Lichtreize auffallend empfindlicher als bei 3000 Lux helladaptierte Tiere.

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Changes in Ascorbic Acid Content in Denervated Frog Gastrocnemius Muscle

It is shown that there is continuous local accumulation of ascorbic acid in the gastrocnemius muscle of rat after denervation[1]. In the present study, an attempt was made to examine certain causative factors for the increase of ascorbic acid content in the denervation atrophy of the muscle.

Unilateral denervation of the hind limb of the common Indian frog, *Rana hexadactyla*, was carried out according to KRISHNAMOORTHY and DAS[2]. The gastrocnemius muscles were excised, weighed and homogenized in ice-cold 5% metaphosphoric acid containing 1% $SnCl_2$[3]. The homogenates were centrifuged at 2000 rpm for about 20 min and the acid-soluble fraction was used for the assay of ascorbic acid. L-ascorbic acid (AsA), dehydro-L-ascorbic acid (DHA) and diketo-gulonic acid (DKA) were determined by the 2,4-dinitro-phenyl hydrazine method of ROE and KUETHER[3]. The same procedure was followed for the liver, kidney and adrenals after norit treatment[3] to remove interfering pigments. The blood was collected into a hypodermic syringe through the inferior vena-cava and pooled from 4–5 specimens for assay. The data were statistically analysed[4].

AsA, DHA and DKA concentrations were not changed in the kidney and adrenal tissues of frog after denervation (Table I), instead of an increase as in the gastrocnemius muscle. DHA and DKA were singularly absent in the

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Table I. The levels of catabolic products of ascorbic acid in the tissues of normal and denervated frog (period of denervation, 60 days)

Tissue	Catabolic product	Normal frog (mg %)	Denervated frog (mg %)	*t*-test value	Incidence of change on denervation
Serum	AsA	0.12 ± 0.007	0.11 ± 0.15	0.17	no change
	DHA	nil	nil	–	–
	DKA	nil	nil	–	–
Liver	AsA	80 ± 1.14	95 ± 3.00	7.80[a]	increase, $p = < 0.01$
	DHA	25 ± 1.94	24.5 ± 3.50	0.09	no change
	DKA	6 ± 1.23	7 ± 1.58	0.21	no change
Kidney	AsA	149 ± 8.35	157 ± 12.75	0.99	no change
	DHA	33 ± 3.24	35 ± 1.0	1.02	no change
	DKA	6 ± 1.58	7 ± 1.32	1.05	no change
Adrenal gland	AsA	98 ± 5.49	100 ± 5.83	0.25	no change
	DHA	24 ± 2.59	27 ± 4.66	0.97	no change
	DKA	7 ± 1.32	6 ± 1.93	0.74	no change

[a] Mean of 4 samples ± standard deviation.