LIGHT-INDUCED CHANGES OF VACUOLAR CONTRACTION OF SENSITIZED CILIATA

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It is known that some light absorbing materials, dyes photosensitize the various organism, or rather their protoplasm (BLUM, 1941). Many enzymes, plasma proteins of the sensitized organisms may become denaturalized, and what is especially frequent with viruses, bacteria and protozoa, they may quickly die. The phenomenon called photodynam (Tappeiner and Jodlbauer, 1904) reveals important plasmic changes and is therefore a useful means in getting acquainted with the biology, the physicalchemical behaviour of the plasma. This is why it semed important to examine the photodynamic influence of known dyes on the frequency of contractile vacuoles in the *Platyophrya lata.*

Material and method

The ciliate *Platyophrya lata* KAHL breeding in root extract were made to fast after centrifugation in a solution of a determined ion composition (BICZÓK, 1961), then they were dyed with a partly purified solution of 1:100 000 dilution of fluorescein, eosin, rhodamine B, rose bengale, methylene blue, toluidine blue, trypaflavin, neutral red, Janus green B and auramin O controlled with measurements of absorption-spectrum. The isolated specimen was lit with the 500 or 25 000 lux strong light of a 15 W and 6 V lamp. We tried to eliminate the heat effect of the light by placing a thin $CuSO_4$ solution between the light source and the hanging drop. On the average we examined 25 specimens each time. After reading off 10 frequencies in each case we inserted a pausa of 5 minutes.

Result, discussion

We expected a major effect from the xanthene dyes among the sensitizing agents because they are well inducible, their photodynamic effect, their photooxidation are known to be great (HERTEL, 1906; JODLBAUER and HAFFNER, 1921; GILBERT, 1942; LÁBOS, 1966a, b). Besides this HYMAN and HOWLAND (1940) have demonstrated of two members of these dyes, that injected into an *Amoeba,* they caused over-activity of the vacuoles. Two dyes, eosin and rose bengale have since then been in the highlight of interest.

Our results are shown in the table. The measured results, the averages refer to the function of vacuoles contracting in intervals shorter than 20 sec. We have expressed the vacuolar frequency increase and decrease not only in % but also by the quotient of the average sums of the vacuolar frequency of the animals before and after dying. Evaluation was difficult because part of the sensitized animals encysted (Table). Encystment changes the normal rhytm of vacuolar pulsation before the rotating movement accompanying encystment. When the *Platyophrya* died before rotation we could not explain the fluctuation in the contraction of the vacuole. Furthermore certain dyes make their toxic influence felt even in a 1:100 000 dilution which is not identical with the damaging effect resulting from photooxidation. We think here of methylene blue, toluidin blue and Janus green B.

Taking all this into consideration the following may be stated:

1. In the majority of cases vacuolar pulsation becomes accelerated in the photosensitized animals as compared to the undyed animals. In respect of this effect the following order may be established in the xanthene dyes:

eosin > rose bengale > rhodamine B > fluorescein.

The Janus green B belonging to the mono-azo group has proved to be a very effective sensitizer; it increases vacuolar frequency by a large percentage and reacts most intensively to a stronger light. The difference between the vacuolar frequency of colourless and dyed animals functioning with decreasing tendency under the influence of light.

The photodynamic effect of neutral red an auramin O is moderate, although at 25 000 lux the quickening of the vacuolar frequency is considerable with these too.

2. The increase of light intensity caused in its total average an increase in the number of contractions with all sensitizers. As it was to be expected, the reaction of the xanthene dyes is most conspicuous in this respect also. Photoreaction at 25 000 lux took place in almost the same order of the dyes as at 500 lux.

3. The smaller part of the sensitized *Platyophryae* (also the undyed ones) responded to light with the slowing down of the vacuolar contractions. Differently from the other indexes this opposed tendency is expressed by values under 1. In my opinion these indexes have rather a precarious value in appreciating the phenomenon of photodynam since they express the frequency of decreasingly, weakly functioning vacuoles. In spite of this fact it is conspicuous, that the indexes referring to xanthene dyes do not exceed an average of 0,8, while the thyaziner are all below their value, i. e. the intervals of contraction frequency in the undyed state and those occuring at 500 lux after dying are considerably greater here.

4. Some degree of regularity may be detected the frequencies of the unchanged, quickening and slowing contractions shown in brackets. In eahc series of experiments the number of vacuolar frequency of undyed, then sensitized specimens under the influence of ligth was as follows:

unchanged < quickening > slowing,

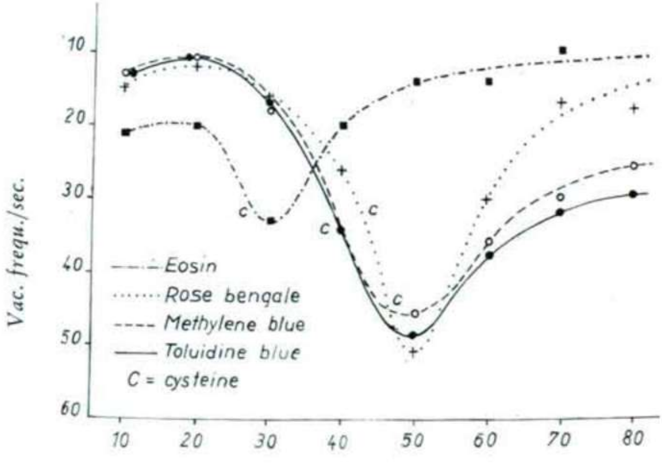
i. e. under the influence of light the vacuoles contracting at slower frequencies become quicker, those functioning quickly do not change or rather slow down.

5. Under the influence of light the vacuolar rfequency of the photosensitized animals changed, usually becoming quicker. Depending on the quality of the

Table

Dyes	Frequency of vacuole contractions: after dying at 500 lux (Average frequencies in brackets)			Frequency of vacuole contractions: index showing ratio of original state and change after dying		Frequency of vacuole contractions: index showing ratio of change after dying at 500 and 25 000 lux respectively	Encystment during examination
	unchanged	faster	slower	increase of frequency	decrease of frequency		
	%	%	%				
Eosin	5 (11)	75 $\left(\frac{14,6}{10,8}\right)$	20 $\left(\frac{10,3}{12,3}\right)$	1,352	0,837	1,408	8
Rose bengale	15,4 (12,5)	69,2 $\left(\frac{14,4}{11,2}\right)$	15,4 $\left(\frac{13}{16}\right)$	1,286	0,813	—	3
Rhodamine B	5 (15)	60 $\left(\frac{15,5}{12,8}\right)$	35 $\left(\frac{11,4}{13,6}\right)$	1,211	0,838	1,500	2
Fluorescein	21,1 (13,5)	52,6 $\left(\frac{14,2}{12,6}\right)$	26,3 $\left(\frac{15,4}{19,2}\right)$	1,119	0,802	1,416	6
Methylene blue	9,5 (8,5)	76,2 $\left(\frac{15,5}{11,6}\right)$	14,3 $\left(\frac{19,3}{32,3}\right)$	1,336	0,597	1,408	—
Toluidine blue	14,8 (11)	51,9 $\left(\frac{13,1}{10,4}\right)$	33,3 $\left(\frac{13,7}{24,3}\right)$	1,250	0,564	1,323	—
Trypaflavin	19,1 (10)	47,6 $\left(\frac{14}{11,6}\right)$	33,3 $\left(\frac{11,3}{15,9}\right)$	1,207	0,711	1,296	9
Janus green B	9,5 (12)	71,4 $\left(\frac{15,6}{11,5}\right)$	19,1 $\left(\frac{14}{25,8}\right)$	1,357	0,543	1,525	—
Neutral red	26,1 (10)	30,4 $\left(\frac{16,9}{13,9}\right)$	43,5 $\left(\frac{12,1}{16}\right)$	1,216	0,756	1,385	1
Auramin O	5,3 (10)	47,35 $\left(\frac{15,4}{12,6}\right)$	47,35 $\left(\frac{12,3}{13,9}\right)$	1,222	0,885	1,376	4
Colourless (at 500 and 25,000 lux)	29,9 (12,6)	39,3 $\left(\frac{14,1}{12,9}\right)$	30,8 $\left(\frac{13,1}{14,1}\right)$	1,093	0,929	—	3

dye and the intensity of the light the acceleration decreased after 10—15 minutes, sometimes after a longer period and ceased almost simultaneously with the loss of the ability of locomotion. With some dyes this condition occured after long hours (e. g. with fluorescein, trypaflavin, auramin O). We may have here a case of sensitized photooxidation which may inactivize DNA (SIMON and HELEN VAN VUNAKIS, 1946; SUSSENBACH and BERENDS, 1964), important enzymes (TAPPEINER and JODLBAUER, 1904; RAPOPORT and co-workers, 1965), important structureforming, SH-groups containing compounds (WITTNER, 1957; WACKER and co-workers, 1963; RENSBURG and co-workers, 1965). Many antioxidants, cystein among them, give effective protection against this damaging influence. Therefore we made an attempt to reactivate animals sensitized with the enumerated dyes and not yet noticeably damaged by light. We gave cystein (15—25 mM) to the weakly moving specimen with slow vacuolar pulsation. Those *Platyophryae* in the protoplasm of which there were already vesicles distinguishable, colescent vacuoles, they died quickly. Otherwise ciliar motion and vacuolar activity became quicker (Graph). The reaction shows, that the SH-groups which are oxidated during the effect of photodynamism play an important part in the function of the *cilia* contractile vacuoles.



Time in min.

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