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PHOTO-INDUCED RESPIRATION OF THE SENSITIZED TETRAHYMENA PYRIFORMIS GL, INHIBITED WITH KCN

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Unicellular animals are multifunctional organisms. Their metabolism is, in a lot of connections, identical with that of *Metazoa*. That is verified by respiratory investigations performed on various *Protozoa* (Leichsenring, 1925; Wittner, 1957; Hall, 1938, 1941, etc.) and by the inhibition of respiration with its classic inhibitor, KCN (Pace, 1945; Reich, 1955; Sarojini and Nagabhushanam, 1966). For investigations like these a suitable culture of *Tetrahymena pyriformis* (Syn. *Tetrahymena geleii)* is very good (Pace and Lyman, 1947; McCashland and Pace, 1952; McCashland, 1956; Hunter and Hunter, 1957; G. van de Vijver, 1966).

The respiration can be influenced by different endogenous and exogenous factors; like temperature (Wittner, 1957; Pace and Kimura, 1944), pH (Sarojini and Nagabhushanam, 1966), the different effects of radiation and light (Giese, 1953). The light effects proved to stimulate the respiration temporarily. This phenomenon was obvious in the presence of adequate sensitors. The light-induced O_2-consumption of *Sacharomyces cereviseae* stained with Rose bengale was, e.g., much higher than otherwise and in the dark (Freaman and Giese, 1952). It proved instructive to be established how the respiration of *Tetrahymena pyriformis* was influenced by being light-sensitized, and the whole process by KCN.

Materials and Methods

The strain GL of *Tetrahymena pyriformis* was cultivated sterilely in the dark, in room temperature (22 $\pm$ 1.5°C), in a rat-liver extract. The stagnant cultures were inoculated in every third week. For the investigations cultures of 5—10 days were used. The substance was centrifuged twice and starved for 24 hours (McCashland and Pace, 1952) in a medium of defined ion-composition set to pH 6.9 with phosphate buffer (Biczók, 1961). The O_2-consumption, resp. the inhibition of the photoinduced respiration with KCN was measured at 25°C with the conventional Warburg technique (G. van de Vijver, 1966a,b). The animals were formerly sensitized with photodynamic substances of a concentration of to 50 000, namely with Rose bengale (RB) and methylene blue (MB).

5 ml substance (in the medium of the ioncompostion and pH mentioned) was poured into small vessels of 15 ml content, with 0.3—0.5 million animals. The concentration of the KCN inhibitor was $5 \cdot 10^{-4}$, resp. $5 \cdot 10^{-3}$M. The vessels were illuminated with two F-tubes built in the water-bath of Warburg's apparat, the luminous intensity of which was measured 1105 erg $cm^{-2}sec^{-1}$ (measured with an iron-constantan thermo-column of silica valve built into a vacuum, tested by measuring the radiated energy emitted by a 100 Watt wolfram electric bulb in a

1 m long black paper tube of 10 cm diameter (I. Horváth, Botanical Institute, A. J. University, Szeged, and J. Zimonyi, Biochem. Inst., Budapest). The shake-speed of vessels was 130 oscillation a minute. As a gas phase of measurement, atmospheric air was used. The exact number of animals after being measured and fixed in alcoholic formalin (the animals depose well), was established with Bürker's chamber, resp. Petri dish, wellproved in practice and applicable fast, putting a paper of sq.mm division on its bottom The cell respiration was denoted in the O_2-consumption per hour of a million organisms given in μl.

Result and discussion

1. Oxygen uptake without sensitor

The O_2-consumptions of the *Tetrahymena pyriformis* starved and non-starved, illuminated and breathing without light effect, have been compared on the basis of 142 measurements. The centrifuged animals cultivated in liver-extract have consumed 101 μl/mil. (h. oxygen) average of the quantities measured in the light and in the dark, in the anorganic medium. The average of the one hour oxygen consumption of the starved animals, measured in the light and in the dark, is 84 μl, that means 17 p.c. decrease opposite to the O_2-consumption of those well-fed.

2. Oxygen uptake in the presence of a sensitor

It is ascertained by the analyses of our direct measurements and photos that the speed of the movement of *Tetrahymena pyriformis* unstained and, all the more, of that sensitized with RB and MB, has considerably increased and then decreased as a result of light, depending on wave-length and intensity, and finally the animals have ceased moving; those of them that were sensitized were rounded off and often cytolysed. Temporarily also the number of frequency of the contractile vacuole increased (Biczók, 1966). In consideration of the photo-oxidation connected with these phenomena, it was to be expected that the oxygen uptake of sensitized animals as compared with those unstained increases. This supposition has been supported by our measurments (Table).

The numerical data have called the attention to some remarkable phenomena:

a) The respiration of the *Tetrahymena* unstained was increased by light, if only in a lower degree. That is obviously connected with the presence of the "photoreceptoric molecules" that may be responsible (as absorbing light and having a capacity of being induced) indirectly for the increase of the speed of motion following the photo-reception.

b) The MB sensitor that, in case of the *Tetrahymena pyriformis* suspended in tap-water, has considerably increased the O_2-uptake (49 p.c. — van de Vijver, 1966a), at our in vivo investigations has though resulted but in a stimulation of minor degree (in the dark 12 p.c., as influenced by light 20 p.c.), yet this stimulation has surpassed 4 p.c. that of RB known as of a stronger photodynamic effect.

Table 1. Inhibition of the respiration of Tetrahymena pyriformis, strain GL, unstained and sensitized, with KCN

	O_2-uptake as a result of light						O_2-uptake in the dark					
	Without inhib.	Stand. dev.	KCN 5.10^{-4}	Stand. dev.	KCN 5.10^{-3}	Stand. dev.	Without inhib.	Stand. dev.	KCN 5.10^{-4}	Stand. dev.	KCN 5.10^{-3}	Stand. dev.
Degree of inhibition			22 p.c.		28 p.c.				20 p.c.		24 p.c.	
Rose bengale (RB)	97 ul	± 6.8	76 ul	± 3.8	70 ul	± 2.1	87 ul	±6.6	70 ul	±3.1	66 ul	± 2.8
Degree of inhibition			17 p.c.		46 p.c.				15 p.c.		57 p.c.	
Methylene blue (MB)	101 ul	±10	84 ul	±14.7	55 ul	± 7.2	92 ul	±6.7	78 ul	±14	40 ul	±16.1
Degree of inhibition			10 p.c.		45 p.c.				16 p.c.		43 p.c.	
Unstained	87 ul	± 8.3	78 ul	± 8.8	48 ul	±10.4	81 ul	±9.1	68 ul	±6.4	46 ul	± 8.7

The respiration of Tetrahymena unstained for light has increased 7 p.c., of those stained with RB 16 p.c., of those stained with MB 20 p.c. (Values compared with those of dark unstained ones).

In the dark, RB has increased respiration 7 p.c., MB 12 p.c.

The respiration of those stained with RB has increased by light 11 p.c., of those stained with MB 9.8 p.c.

The intensity of respiration has considerably been influenced by the inhibitor KCN. On the basis of the Table it can be established that, depending upon the concentration of this compound, it inhibits not only the respiration of unstained animal but also that of the sensitized ones, in the light and dark equally. The inhibiting effect of KCN was not defended by the sensitors; the oxygen uptake has altered in about such a degree as it could be expected on the basis of the photodynamic character of stain.

Our results are in close connection with the statement that *Tetrahymena pyriformis* is cyanidesensitive (Hall, 1941) and that the degree of the respirative inhibition is expressed by the KCN-concentration (McCashland, 1952; 1956). The $5 \cdot 10^{-3}$M concentration of KCN, applied by us, has resulted in a significant inhibition; at the $5 \cdot 10^{-4}$M concentration there occurred here and there a stimulated respiration, as well.

KCN has inhibited not only the animals without sensitor but also the respiration of those stained with RB and MB, stimulated to the light more, to the dark less. The inhibition seemed to support the data published by Baker and Baumberger in 1941 (in Lwoff, 1951) concerning the role of cytochromes, of cytochrome-oxidase in the respiration of *Protozoa*. The resepiration is actually complicated. The investigations of van de Vijver (1966b) raised namely doubts as to the existence of cytochrome-c oxidase at *Tetrahymena pyriformis*. As the inhibition of KCN was not defended by MB the oxygen uptake has not increased at the animals inhibited as a result of MB, it seems reasonable to suppose the absence of cytochrome-c, resp. of cytochrome-c-oxidase.

References

Biczók, F. (1961): Examination of the protoplasmic changes during the process of cystment. — Progress in Protozool. I. — Internat. Congr. on Protozool. Prague 213—217.

Biczók, F. (1966): Light-induced changes of vacuolar contraction of sensitized *Ciliata*. — Acta Biol. Szeged *12*, 87—91.

Freeman, P. J. — Giese, A. C. (1952): Photodynamic effects on metabolism and reproduction in yest. — J. Cell Comp. Physiol. *39*, 301—322.

Giese, A. C. (1953): *Protozoa* in Photobiological research. — Physiol. Zool. *26*, 1—22.

Hall, R. H. (1938): The oxygen consumption of *Colpidium campylum*. — Biol. Bull. Woods Hole *75*, 395—408.

Hall, R. H. (1941): The effects of cyanid on oxygen consumption of *Colpidium campylum*. — Physiol. Zool. *14*, 193—208.

Hunter, N. W. — Hunter, R. Jr. (1957): Enzyme systems of *Colpoda cucullus*. I. Oxidation of certain Krebs cycle intermediate. — J. Cell. Comp. Physiol. *50*, 341—346.

Leichsenring, J. M. (1952): Factors influencing the rate of oxygen consumption in unicellular organisms. — Amer. J. Physiol. *75*, 84—92.

Lwoff, A. (1951): Biochemistry and Physiology of *Protozoa*. — New-York.

McCashland, B. W. — Pace, D. M. (1952): The influence of KCN on growth and respiration in *Tetrahymena gleii*. — Growth. *16*, 75—82.

McCashland, B. W. (1956): Adaptation of *Tetrahymena pyriformis* to KCN. II. Adaptation against respiratory inhibition. — J. Protozool. *3*, 131—135.

Pace, D. M. — Lyman, E. D. (1947): Oxygen consumption and carbon dioxide elimination in *Tetrahymena pyriformis*. — Biol. Bull. Woods Hole *92*, 210—216.
Pace, D. M. (1945): The effect of cyanide on respiration in *Paramecium caudatum* and *P. aurelia*. — Biol. Bull. Woods Hole. *89*, 76—83.
Reich, K. (1955): The effect of cyanide and azide on the respiration of the *Amoeba, Mayorella palestinensis*. — Physiol. Zool. *28*, 145—151.
Sarojini, R. — Nagabhushanam, R. (1966): The oxygen consumption of the ciliate, *Coleps hirtus*. — Brotéria *35*, 45—55.
van de Vijver, G. (1966a): Studies on the metabolism of *Tetrahymena pyriformis* GL. I. Influence of substrates on the respiratory rate. — Enzymol. *31*, 363—381.
van de Vijver, G. (1966b): Studies... II. Some properties of the terminal oxidation. — Enzymol. *31*, 382—391.
Wittner, M. (1957): Effect of temperature and pressure on oxygen poisoning of temperature and pressure on oxygen poisoning of *Paramecium*. — J. Protozool. *4*, 20—23

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