MUTAGENIC ACTIVITY OF THIOPYRONINE AND METHYLENEBLUE IN COMBINATION WITH VISIBLE LIGHT

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Recently Wacker, Türck and Gerstenberger (1963) showed parralleles between the quantitative differences of photodynamic activity of the dyes pyronine, thiopyronine and methyleneblue and their individual capacity for oxydative destruction of guanine in vitro and in vivo. The destruction of guanine moieties in DNA seems to be a specific photodynamic reaction as also found by Simon and van Vunakis (1962) in case of methyleneblue. A destruction of the other DNA bases could not be found in the presence of thiopyronine and small doses of visible light, inactivating more than 99 % of bacteria (Wacker et al., 1963) and DNA-viruses (Herzberg et al., 1963). Therefore it seemed to be of interest to test the mutagenic activity of thiopyronine (TP) and methyleneblue (MB) in combination with visible light.

Experimental — Two mutant strains of Proteus mirabilis were used: VI/str-d-3 (streptomycin dependent being tested for reversion to streptomycin independence); VI/99 (met^- $phen^-$, being tested for reversion to phenylalanine prototrophy). The mutagenic treatment was done as follows: overnight broth-cultures were washed in buffer and the cell number was adjusted to app. 2.5×10^8/ml. After washing the bacteria were resuspended in TP and MB solutions, respectively (10 µg/ml in phosphate buffer pH 7.0); one part of the suspension was irradiated immediately in a petri-dish with a daylight lamp (100 W; distance 35 cm). Aliquots were taken for the estimation of survival and mutant frequency before irradiation as well as after different irradiation times and plated

immediately. Determination of inactivation was done on nutrient agar (+ 50 µg dihydrostreptomycin in case of strain str-d-3), determination of mutant frequency in strain str-d-3 on streptomycin free nutrient agar, in strain VI/99 on minimal agar (Böhme 1962) supplemented with 20 µg/ml methionine. From the second part of the suspension aliquots were taken in the same way and after identical time intervals but without irradiation for determination of inactivation and mutant frequency. With exception of irradiation all experimental procedures were done in the dark or under dim yellow light. Incubation 72 hours at 37° C.

Results — TP and MB proved to be mutagenic in combination with visible light in both strains tested. For photodynamic inactivation of a definite fraction of Proteus mirabilis cells more light energy is necessary in the case of MB than with TP (Fig. 1 a and b). The same is true for mutation induction, at least for strain VI/99 (Fig. 2 a and b). With the same amount of light energy the mutagenic effectivity in this strain was found to be higher in the presence of TP. Both dyes did not induce mutations without irradiation.

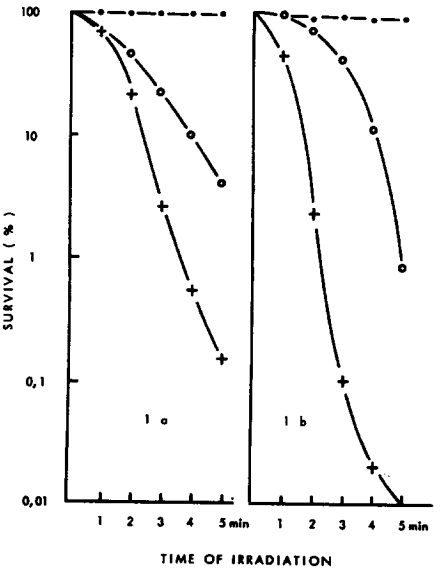

Fig. 1. Inactivation of P. mirabilis VI/str-d-3 (1a) and VI/99 (1b) by thiopyronine (+ — +) and methyleneblue (o — o) in combination with visible light. (• — •) methyleneblue without irradiation. The curve for thiopyronine without irradiation is identical with that for MB; it has therefore been omitted here and in Fig. 2.

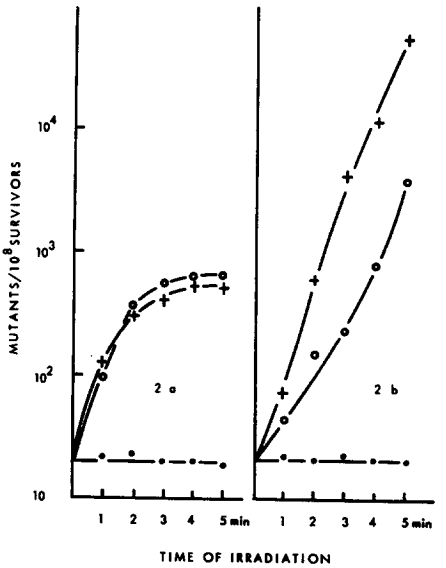

Fig. 2. Mutagenicity ot thiopyronine (+ — +) and methyleneblue (o — o) in combination with visible light in strain str-d-3 (2a) and VI/99 (2b). (• — •) methyleneblue without irradiation.

Significant differences of the mutagenic activity of these dyes on different mutational sites of the genetic material should be expected, if the mutagenicity of TP as well as MB in the presence of visible light is in fact the consequence of a selective destruction of guanine. Experiments for elucidating this question are planned. The relatively low mutant frequency induced in the streptomycin dependent strain VI/str-d-3 might be connected with the experimental procedure in so far as the treated bacteria have been plated not on minimal but on complete medium. Preliminary results with another auxotrophic strain suggest a decrease of induced mutant frequency if the treated cells are plated on minimal medium supplemented with deoxyribosides as compared with plating on unsupplemented minimal medium.

Acknowledgments

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