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Carotenoid Pigments and Photokilling by Acridine Orange

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It has long been known that certain organic dyes can photosensitize cells, leading to cellular damage or death. Recently, studies were performed on one such dye, Toluidine Blue, to determine the mechanism of its lethal photosensitization of bacteria (M. M. Mathews, J. Bacteriol. **85**:322, 1963). These studies showed that the cellular site of the lethal action was the cytoplasmic membrane and its associated enzymes, and that this destruction of membrane and enzymes, and the resulting cell death, can be prevented by the presence of carotenoid pigments (M. M. Mathews, J. Bacteriol. **85**:322, 1963; M. M. Mathews and W. R. Sistrom, Arch. Mikrobiol. **35**:139, 1960). It was also found that photosensitization with Toluidine Blue had some effect on the deoxyribonucleic acid (DNA) of the bacteria, as indicated by the increase in mutation rate to penicillin resistance (M. M. Mathews, J. Bacteriol. **85**:322, 1963).

Acridine orange is an organic dye capable of photosensitizing cells, and is known to affect cellular DNA (R. B. Uretz, Radiation Res. **22**:245, 1964). The purpose of the study reported here was to determine the cellular site of lethal photosensitization by this dye, and to observe whether carotenoid pigments could protect bacteria from the action of the dye.

The organisms used in this study were *Sarcina lutea* ATCC 9341a, and a colorless mutant obtained by ultraviolet irradiation of the parent strain. These organisms were used in previous bacterial photosensitization studies (M. M. Mathews, J. Bacteriol. **85**:322, 1963). The same methods of growing and enumerating the cells and of exposing them to light were used in the present work. General Electric photoflood lamps (250 w) were used as the light source.

Exposure of the wild-type and colorless mutant strain of *S. lutea* to visible light in the presence of 2.5×10^{-5} M acridine orange resulted in no difference in the rate of killing of the colorless mutant as compared with the wild type (Fig. 1). Thus, carotenoid pigments are unable to prevent the lethal action of acridine orange on bacterial cells in the presence of light. In those experiments in which the pigmented wild type was exposed to acridine orange and light, it was also found that colorless mutants appeared in numbers much greater than the spontaneous mutation rate. This indicated that exposure to acridine orange and light resulted in changes in the DNA of the exposed cells.

To determine whether photosensitization by acridine orange destroyed membrane enzymes, and to see whether carotenoid pigments could prevent this destruction if it existed, the following experiment was performed. Cells of both the wild-type and colorless mutant were grown, harvested, and then resuspended in 0.1 M *p*H 7 phosphate buffer to a concentration of approximately 1.5 mg (dry weight) per ml, and 8.3×10^{-5} M acridine orange was added to the cell suspension. Half of the cell suspensions of each organism were then exposed to visible light (2,000 ft-c) for 2.5 hr, and the remainder were kept in the dark for that time. After exposure, both light-exposed and dark-control cell suspensions were harvested, washed, centrifuged, and broken by grinding with alumina, and the ground material was extracted with the phosphate buffer. The cell extracts were then assayed for reduced nicotinamide adenine dinucleotide ($NADH_2$) oxidase and adenosine deaminase activity, the former enzyme being associated with the cell membrane, and the latter with the cytoplasm (M. M. Mathews and W. R. Sistrom, J. Bacteriol. **78**:778, 1959). The $NADH_2$ oxidase of the colorless cells was destroyed, whereas that of the pigmented cells was unharmed (Table 1). The soluble enzyme adenosine deaminase was destroyed to approximately the same degree in both colorless and pigmented cells. These results indicate that the carotenoid pigments were able to prevent the destruction of membrane enzymes by acridine orange, even though they could not prevent the lethal action of the dye (Fig. 1).

Thus, the effects of acridine orange on cells are similar to those of Toluidine Blue: destruction of both membrane-associated and soluble proteins as indicated by loss of enzyme activity,

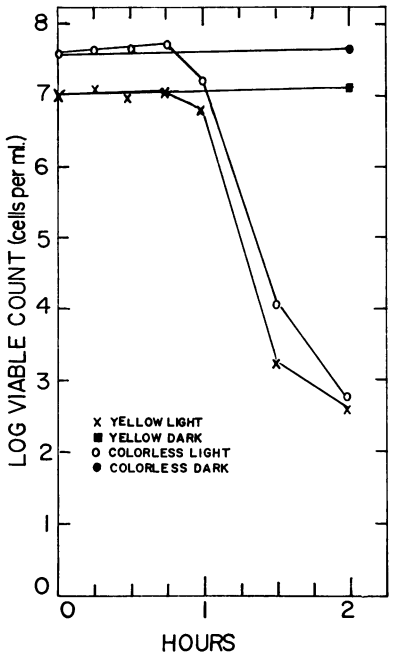

FIG. 1. *Effect of exposure of colorless and pigmented cells of Sarcina lutea to light from a tungsten lamp (1,000 ft-c) in the presence of 2.5 × 10^{-5} M acridine orange.*

and alteration of DNA as indicated by increases in mutation rate. As mentioned above, the primary mode of action of Toluidine Blue was found to be destruction of membrane proteins and enzymes, since carotenoid pigments which are also located on the cell membrane (M. M. Mathews and W. R. Sistrom, J. Bacteriol. **78**:778, 1959) can prevent the protein destruction and the lethal effect of the dye (M. M. Mathews and W. R. Sistrom, Arch. Mikrobiol. **35**:139, 1960). With acridine orange, however, the effect on protein seemed to be a minor one, since carotenoids were incapable of preventing cell death from photosensitization by this dye. However, the presence of a greatly increased number of colorless mutants in the experiments in which the pigmented wild type was exposed to acridine orange and light suggests that the alteration of the DNA of the cells by the dye may be the more likely lethal effect. It is indeed interesting to note that the carotenoid pigments, although incapable of preventing the lethal action of acridine orange on the cellular DNA, were still capable of preventing the destruction of membrane proteins, as in Toluidine Blue photosensitization.

TABLE 1. *Effect of exposure to acridine orange on the enzyme activity of extracts of pigmented and colorless Sarcina lutea cells*

Enzymes	Per cent activity remaining[a]	
	Pigmented cells	Colorless cells
Adenosine deaminase.....	27.3	24.5
$NADH_2$ oxidase.....	94	56

[a] Figures are per cent of activity of respective dark control remaining after exposure to acridine orange and light.

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