Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 39. DECEMBER, 1938. No. 3.

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Photodynamic Action of Safranine on Gram-Negative Bacilli.

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In a previous study on the photodynamic action of various dyes on bacteria,[1] it was found that several dyes were lethal for gram-positive organisms but none was effective for gram-negative bacilli. Efforts have, therefore, been continued to determine the photo-sensitivity of gram-negative organisms with dyes hitherto

[1] T'ung, T., and Zia, S. H., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 326.

not used. After many trials, it was found that safranine was suitable for this purpose, and in the present communication, the result of an investigation of its photodynamic action on some common gram-negative bacilli is presented.

Saturated aqueous solution of safranine* was diluted to different concentrations with distilled water, the pH of which was adjusted to 8.0 by the addition of sodium hydroxide. Organisms grown on plain meat-infusion agar-slants for 24 hours were suspended in water of the same pH and the number of bacteria was adjusted to about 500 millions per cc. The technic previously employed[1] for mixing the dye and organisms and for exposing to light was followed. Both exposed and unexposed mixtures were plated on China-blue rosalic-acid medium and incubated for 48 hours. The maximal photodynamic action of safranine on most of the gram-negative organisms could not be obtained without prolonged exposure; the results of a 2-hour exposure are presented in Table I.

The effects of the dye on unexposed bacteria have been omitted from the table because it was found that safranine possesses no native bactericidal power in 2 hours' contact with all gram-negative bacilli tested, except for *S. dysenteriæ* at 1-2 and for *V. choleræ* and *B. alcaligenes* at 1-100 dilution. From the table it is clear that safranine has marked photosensitizing power on a large number of varieties of gram-negative bacilli but it varies even among closely related species. It was further noted that a prozone in the photodynamic action of safranine was present on a majority of these organisms even after prolonged exposure. It seems that the prozone is more marked in cases in which the organisms are less susceptible to the photodynamic action. It appears also that the presence of a prozone is a group-characteristic. For instance, marked prozone reaction was observed in 15 strains of *E. coli*, but not in 15 strains of *K. pneumoniæ*.

Because of our previous experience[2] with the enhancing power of hydrogen peroxide on the photosensitizing activity of eosin on gram-positive organisms, it was considered of interest to see whether or not the photodynamic action of safranine on gram-negative ones could be increased by the addition of same reagent. Since *E. coli* is less susceptible to the photosensitization of this dye and comparatively more resistant to hydrogen peroxide, it alone was chosen for bearing out this fact. It was found that while safranine in the dilution of 1-1000 failed to kill *E. coli* after 60 minutes of exposure

* Water-soluble safranine O made by the National Aniline and Chemical Company, U. S. A.

[2] T'ung, T., *Trans. X Congress, Far East. Assn. Trop. Med.*, in press.

TABLE I.
Growth in Exposed Mixtures of Bacteria and Safranine.

Bacteria	Final concentrations of the dye					
	1-2	1-10	1-10^2	1-10^3	1-10^4	1-10^5
V. choleræ	—	—	—	—	—	++++
B. alcaligenes	—	—	—	—	++	++++
Shigella						
amigua	—	—	—	—	—	++++
paradysen. F	—	—	—	—	—	++++
paradysen. Y	—	—	—	—	++	++++
dysenteriæ	—	—	—	—	++	++++
E. typhi						
"O" (901)	—	—	—	—	++++	
"H"	—	—	—	—	++++	
"Watson"	—	—	—	++	++++	
Prot.						
"O" (X-19)	—	—	—	—	++++	
"H"	—	—	—	++++	++++	
Salmonella						
paratyphi A	+	—	—	—	++++	
schottmuelleri	++	—	—	+	++++	
hirschfeldii	+++	++	—	++	++++	
enteritidis	++	—	—	—	++++	
suipestifer	+++	++	—	+++	++++	
aertrycke	+++	++	—	+++	++++	
K. pneumoniæ (S)	—	—	—	—	++++	
K. pneumoniæ (R)	—	—	—	—	++++	
E. coli	++++	+++	—	—	++++	
P. aeruginosa	+++	+	—	+	++++	
B. prodigiosus	++++	—	—	++	++++	

— = no growth; + = growth less than 5 colonies; ++ = growth less than 50 colonies; +++ = growth less than 100 colonies; ++++ = growth more than 100 colonies.

to light, the addition of hydrogen peroxide in a final concentration of 0.15% enhanced the photosensitizing power and inactivated this organism after the same length of exposure. Safranine together with hydrogen peroxide in the absence of light or hydrogen peroxide alone in the presence of light had no bactericidal effect. Moreover, *E. coli* suspended in the above-mentioned concentration of hydrogen peroxide for one hour, washed and resuspended in water and then exposed to light with the dye for the same length of time was not killed. The result of the reënforcement of an oxidizing reagent with safranine in photosensitizing this organism suggests that oxidation may play a rôle in photodynamic action.

Conclusion. Safranine was found to be an efficient photosensitizing dye on certain gram-negative organisms. Its effect is characterized by the low native bactericidal power and the frequent occurrence of the prozonal phenomenon. The addition of hydrogen peroxide to the dye-organism mixture enhances the photosensitizing activity of the dye.