

While control figures are not available on the number of natural immunes present in this community, my impression is that the infectivity of such preserved blood up to 8 days is fully equal to direct transfer of blood from patient to patient.

I wish to thank Dr. H. R. Unsworth, Psychiatrist of the DePaul Sanitarium of New Orleans, for his co-operation by making available the majority of the patients used.

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Photodynamic Action of Certain Dyes on the Inactivation of
Staphylococcus Bacteriophage.

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In previous notes the inactivation of staphylococcus bacteriophage by methylene blue (Schultz and Krueger¹) and by toluidine blue (Clifton and Lawler²) was reported.

Various samples of methylene blue in concentrations of 0.1 and 0.01% were mixed with antistaphylococcus bacteriophage, exposed to sunlight for different intervals of time, incubated for 24 hours at 37°C. and then tested for lytic activity. No inactivation was noted when the dye and 'phage were mixed, incubated, and tested in the dark. However, an exposure of 5 minutes, or even less than this time, to indirect sunlight before incubation resulted in complete inactivation of the bacteriophage.

When the methylene blue was added to the bacteriophage suspension in an atmosphere of nitrogen, or in a tube from which the air had been evacuated, no inactivation was noted. This was also true after 30 minutes exposure to direct sunlight and subsequent incubation of these oxygen-free systems. The leucobase of methylene blue likewise did not inactivate this bacteriophage.

Several indophenols having a higher oxidation potential than methylene blue were also tested. They are not photosensitive, did not inactivate the bacteriophage, and were reduced by other oxidizable substances present in the broth.

¹ Schultz, E. W., and Krueger, A. P., *PROC. SOC. EXP. BIOL. AND MED.*, 1928, **26**, 101.

² Clifton, C. E., and Lawler, T. G., *PROC. SOC. EXP. BIOL. AND MED.*, 1930, **27**, 1041.

Baumberger, Bigotti and Bardwell³ have likewise reported inhibition in the coagulation of plasma as a result of the photodynamic action of methylene blue and similar photodynamic actions have been reported for various dyes on different systems. In this note evidence is presented that this inactivation is due to a photodynamic action of the dye on the oxidation of the bacteriophage.

Similar results were obtained with toluidine blue although the results are not as strictly reproducible as with methylene blue.

The workers referred to above also observed that certain readily oxidizable substances such as resorcin, casein and tyrosine, will protect the clotting system of plasma against the photodynamic action of methylene blue and of eosin. Zinsser and Tang⁴ and Long and Olitsky⁵ have also reported that traces of cysteine favor the survival of certain viruses by tending to maintain anaerobic conditions in the suspension.

In our studies cysteine hydrochloride was added to the 'phage suspension to give a concentration of 0.01% and the mixture was then treated with methylene blue (0.01%). No inactivation of the 'phage was noted even after 30 minutes of exposure to sunlight, followed by 24 hours' incubation at 37°C. The controls were completely inactivated.

Tyrosine, even in higher concentrations, did not act as a protective agent.

These results, therefore, indicate that the inactivation of staphylococcus bacteriophage by methylene blue is due to an oxidation of this agent by photosensitized methylene blue in the presence of oxygen.

³ Baumberger, J. P., Bigotti, R. T., and Bardwell, K., *Am. J. Physiol.*, 1929, **90**, 277.

⁴ Zinsser, H., and Tang, F., *J. Immunol.*, 1929, **17**, 343.

⁵ Long, P. H., and Olitsky, P. K., *Proc. Soc. Exp. Biol. and Med.*, 1930, **27**, 380.