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TERMINAL REPORT

August 15, 1968 to December 31, 1969

Host-Parasite Interactions and Genetics of Actinophages

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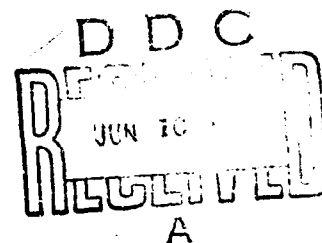
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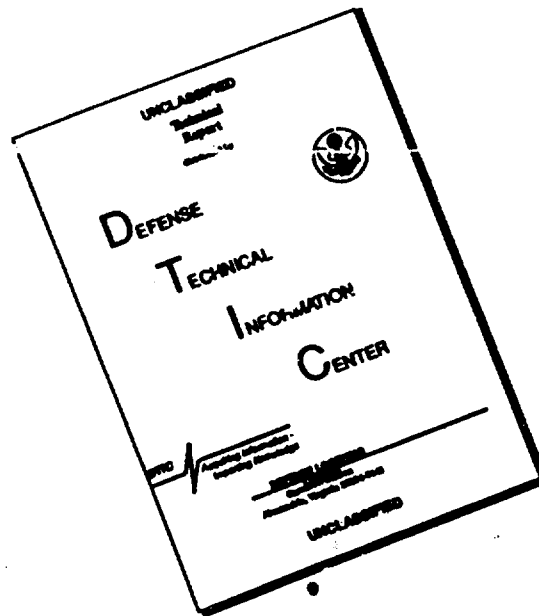


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Photosensitization. The heteroanthracenes proflavin, eosin Y, Janus green B, methylene blue, riboflavin, safranin and thionin, and the triphenylmethane dye crystal violet have been shown to photosensitize actinophages for Streptomyces, Nocardia and Mycobacterium. The extent of photoinactivation was determined by dye concentration, duration of exposure to light, ionic strength of the milieu and availability of air. Two of these eight dyes were chosen for further study because the previous work indicated that their mechanisms of photosensitization were different; the selected dyes were proflavin and methylene blue. The present data support the previous suggestion that these two dyes photoinactivate actinophage as a result of a photochemical attack on the DNA. Firstly, added nucleic acid protects the phage from photodynamic killing by the two dyes. Secondly, the conditions for photosensitization and for binding of the dyes to DNA are similar with respect to pH and ionic strength of the diluent. Three lines of evidence suggest that guanine residues are the photosensitive sites of methylene blue's action. Guanylate protects phage from photoinactivation by methylene blue; methylene blue photochemically destroys guanylate and guanosine; and methylene blue binds more extensively to DNA having 70% guanine and cytosine (GC) than to DNA with 50% GC. We have failed to detect photodestruction of tested nucleic acid components by proflavin. Proflavin is bound more extensively to DNA having 70% GC than with DNA having 50% GC. Binding of acridines to DNA, however, occurs in two ways. The initial binding involves intercalation and binding between the two polynucleotide chains. The second

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type of binding is on the exterior of the DNA helix. The extent of intercalation is proportional to the adenine and thymine content of the DNA. No change in molecular weight of DNA has been detected using light intensities and exposure times 100-fold greater than those that photoinactivated 90% of a population of free actinophage. In addition, liberation of ammonia could not be detected by an assay that would have revealed photodynamic deamination of 20% of the primary amino groups of the DNA. These findings indicate that neither photolysis of the DNA chain nor extensive deamination are responsible for photoinactivation of the free phage.

Synergistic toxicity. Enhanced toxicity in mice resulting from a synergistic interaction between bacterial endotoxin and the antitumor antibiotic sparsomycin or pactamycin was demonstrated previously by Karp and Bradley. Because this may have clinical significance, various agents have been tested for ability to prevent the enhancement. Inasmuch as steroids have been repeatedly referred to in the literature as effective protectants against the lethal action of bacterial endotoxins, this class of compounds was included in our screening procedure. Methylprednisolone (11 β ,17 α ,21-trihydroxy-6 α -methyl-1,4-pregnadiene-3,20-dione) when used prophylactically at 100 mg/kg, retarded the toxic effect of endotoxin in combination with sparsomycin or pactamycin. The antihistamine chlorpheniramine maleate (Chlortrimeton) and the anti-inflammatory agent phenylbutazone were ineffective against the pactamycin-endotoxin toxicity.

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Publications Not Previously Reported

1. R. D. Karp and S. G. Bradley. 1968. Effect of immunosuppressive agents on normal phage-neutralizing antibody in the mouse. J. Bacteriol. 96: 1931-1934.
2. W. C. Rose and S. G. Bradley. 1969. Retardation by methyl-prednisolone of the synergistic toxicity of endotoxin with sparsomycin or pactamycin. Proc. Soc. Exp. Biol. Med. 132: 729-731.
3. S. G. Bradley. 1970. Photodynamic inactivation of actinophages. (Paper presented at the III Internat. Sympos. Phage Typing of Mycobacteria on September 8, 1969.) Proceedings are to be published.