Ultraviolet Radiation and Streptomycin-Resistant Mutants. (19798)

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The origin and nature of antibiotic-resistant bacterial variants has been the subject of considerable discussion. A recent report has cast doubt upon a considerable amount of experimental data which has been presented in many reports concerning the enhancement of resistance to antibiotics following ultraviolet irradiation(1). These workers reported that they obtained no enhancement in resistance to streptomycin or penicillin following ultraviolet irradiation of *Micrococcus aureus,* although such was observed following irradiation of *Escherichia coli.* It was highly desirable to reinvestigate this matter, and the present report covers some of the data obtained.

Experimental. Micrococcus aureus PCI was grown in nutrient broth at pH 7.2 and 18 ml of 12-hour cultures were beaten for 30 seconds in a Waring Blendor as described below, and then irradiated for 240 seconds in sterile Petri dishes which were gently agitated to insure uniform irradiation. A GE germicidal lamp, emitting about 90% 2537 Å, was used at a distance of 3 inches from the irradiated culture. Appropriate dilutions were plated upon nutrient agar (Difco) containing varying concentrations of streptomycin sulfate for determination of spontaneous and zero point mutation incidences. Flasks of nutrient broth (Difco) were inoculated with 5 ml of the irradiated culture for determination of mutation incidences after periods of growth. Streptomycin was added to the sterile media (45°C) and the agar was then thoroughly stirred. Irradiation, dilution and plating procedures were carried out rapidly in a darkened room to minimize the various recovery phenomena, *e.g.,* photo- and heat-reactivation, recovery upon standing in dilution fluid, etc. Nonirradiated aliquots of each culture were treated similarly for controls. Plating was carried out in triplicate except at levels of streptomycin above 6/ml, where 6 plates each were used. All plates were incubated 6 days for final counts.

The pronounced clumping tendencies of *M. aureus* present a problem of considerable magnitude in interpreting the results of viable count determinations. A single resistant cell in a clump of 20 will yield exactly the same results upon plating as would have been the case had all 20 been resistant. To determine the relation of resistant cells to clumps, aliquots of each group were placed in a sterile Waring Blendor and beaten for 30 seconds with the motor cut to half speed by means of a variable rheostatic control. Average cells/clump were also determined microscopically in each case by counting all cells and clumps in 30 fields.

Results. Fig. 1 shows the streptomycin-resistance distribution of *M. aureus* before and after irradiation. Only spontaneous and end-point mutants are shown (24 hours incubation) since the radiation dose used (240 seconds) killed 99.998% of the cells. This reduced the number of viable organisms to such an extent that it was possible to determine the zero-point mutation incidence for only the very low levels of streptomycin(2). The percentages indicated in the ordinate are the percent survivors referred to the total number of viable cells at the time of test. Treatment in the Waring Blendor increased the colony count 3-fold although average clump size as determined by microscopic count was reduced from 6.4 to 1.4 cells/clump. The colony counts upon streptomycin agar were not increased by the treatment.

Discussion. The data presented support the several reports that there is an increase in incidence of resistant organisms following a heavy dose of ultraviolet radiation. The failure of Saz, *et al.*(1), to observe such an increase was undoubtedly due in large part to the low dosage used, since only 60% of their *M. aureus* cells were killed(3). With *E. coli,*

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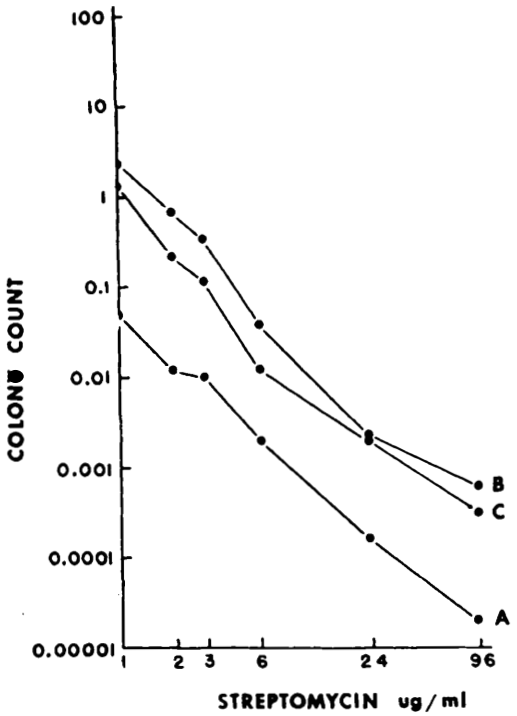

FIG. 1. Comparison of resistance to streptomycin of irradiated and nonirradiated *Micrococcus aureus* PCI. Colony count is referred to control without antibiotic as 100. Curve A: Preirradiated culture with clumps broken so as to yield clumps containing 1.3 cells; total organisms, 2.74 billion/ml. Curve B: End point mutants, 24 hr incubation following irradiation which killed 99.998% of the culture reported in Curve A; inoculum, 5 ml containing 47,000 viable cells/ml into 250 ml; total viable organisms (clump size 6.4 cells), .508 billion/ml. Curve C: Same culture as that reported in Curve B except clumps broken; total organisms after beating, 1.41 billion/ml.

where a dose sufficient to kill 99% of the culture was used, they obtained an increased incidence of resistant organisms.

The problem of clumping is crucial in making determinations of this nature. A large clump may consist entirely of resistant cells, or only one in the clump may be resistant. In either case, it will appear on the streptomycin agar plate as a resistant clone, and may thus drastically alter results obtained. In the experiments reported here, average clump size was 6.4 cells/clump. Following brief beating, it was reduced to 1.4. The treatment apparently reduced viability of the suspension, since the plate counts showed an increase of only 3-fold. The influence of this treatment, however, upon the actual number of resistant organisms was negligible. This would seem to indicate that resistant cells were either not contained in large clumps, or that the clumps formed were very tightly bound, and therefore, resistant to being broken by the beating. Irradiation of an organism which may form some clumps containing from 50 to 100 cells, cannot be uniform since those in the interior would receive very little radiation, even following prolonged treatment.

It is of interest that following irradiation there was a proportional increase in numbers of resistant organisms at all levels tested. Many factors besides clumping have been shown to markedly influence the actual number, and incidence, of antibiotic resistant mutants(4). The influence of several of these is indirect, such as the pH employed with antibiotics which show different activity as the pH changes, or where a considerable part of the antibiotic is absorbed non-specifically by the colloidal particles of the medium.

Summary. Data have been presented confirming earlier reports of an increased incidence of streptomycin-resistant organisms following a heavy dose of ultraviolet radiation. The importance of clumping characteristics in such determinations is emphasized by the fact that treatment designed to break the clumps results in an increased viable count, but not in an increase in actual number of resistant organisms.

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3. Demerec, M., and Latarjet, R., *Cold Spring Harbor Symposium Quant. Biol.,* 1946, v11, 38.

4. Mefferd, R. B., Jr., Doctoral Dissertation, University of Texas, Austin, Texas, 1951. *Problems in Bacterial Genetics and Related Physiology,* 141 pp.

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