A STUDY OF THE BACTERICIDAL ACTION OF ULTRA VIOLET LIGHT

III. THE ABSORPTION OF ULTRA VIOLET LIGHT BY BACTERIA

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In this study of the bactericidal action of ultra violet light the first paper (1) described the reaction of an 18 hour culture of *Staphylococcus aureus* to monochromatic radiations. It was shown that the course of the reaction among large numbers of organisms was approximately the same at each wave length studied but that widely different incident energies were required at different wave lengths to produce these similar effects.

The second paper (2) discussed the limits of the bactericidal zone, showed that the reaction had a low temperature coefficient, (approximately 1.1), gave evidence that within the variations of the methods used no significant errors were introduced by differences in the measured intensity of the source or in the hydrogen ion concentration of the medium, and indicated that plane polarization of the incident light had no effect upon the reaction.

The present paper deals with the absorption of ultra violet light by intact bacteria. A final paper in this series will discuss structural and chemical units of bacterial protoplasm that may prove to be involved in the reaction which results in the organism's death.

Incident Energy Relationships at Various Wave Lengths

Text-fig. 1, reproduced from the first paper of this series, shows that although the course of the bactericidal reaction was approximately the same at each wave length studied, these similar curves were found at very different incident energy levels at different points in the ultra violet spectrum.

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For example, if the incident energy required to kill half the exposed staphylococci be taken as an index, this energy requirement ranged from 3,150 ergs per mm.² at 302 m. μ , a wave length near the limit of bactericidal action, to 88 ergs at 266 m. μ . At other wave lengths, either above or below 266 m. μ , more incident energy was required. The destruction of 50 per cent of the microorganisms is chosen as the index because it is in the most accurately determined part of the curves where the mortality rate is least affected by variations in individual resistance.

If the incident energies involved in 50 per cent destruction be plotted and joined by a continuous line the resulting curve appears as in Textfig. 2A. Parallel experiments on an 18 hour culture of *Bacillus coli* gave bactericidal energy curves similar in trend to those for *S. aureus*, and although complete statistics were not obtained on this bacterium the middle or exponential portion of the lethal reaction curves was determined by repeated observations at each wave length studied. Textfig. 3A shows the incident energies involved in the destruction of 50 per cent of the exposed *coli* organisms. Its essential similarity to the corresponding *aureus* curve is apparent. Both curves would probably be somewhat modified in detail if more wave lengths were available for study.

These characteristic curves (Text-figs. 2A and 3A) show clearly that less incident energy is required between 260 and 270 m. μ than in any other region of the bactericidal zone examined, and point toward a second minimum below 230 m. μ . The presence of a sharp peak in the energy requirement near 240 m. μ appears to be equally significant. Due apparently to the use of but a few wave lengths in the bactericidal zone, or to failure to measure spectral intensities, or to crude methods of estimating bacterial destruction, the occurrence of a minimum at about 266 m. μ , and of the peak in the curve near 240 m. μ has been overlooked by most investigators. Usually they have been content with the conclusion that the shorter the wave length the more marked the bactericidal action (3).

Bang (4) was apparently the first to observe regions of special bactericidal "effectiveness" or of corresponding bacterial susceptibility. Using a 30 ampere carbon arc through a spectrograph at $20 \text{ m.}\mu$ intervals, and estimating bactericidal efficiency mainly by relative exposure, he found two regions of maximal action, an

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"inner maximum" between 340 and 360 m. μ , and an "outer maximum" between 240 and 260 m. μ . The lethal exposure varied from 1920 seconds at 330 to 300 m. μ through 120 seconds at 300 to 280 m. μ to 4 seconds at 280 to 260 m. μ and 2 seconds at 260 to 240 m. μ . Then longer exposures were required. The zone from 240 to 220 m. μ needed 20 seconds, that between 220 and 210 m. μ , 30 seconds, and that between 210 and 200 m. μ required 120 seconds exposure to kill the organisms.



TEXT-FIG. 2. A. Curve of incident energies involved in the destruction of 50 per cent of S. aureus.

B. Curve of the reciprocals of 2A.

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These longer exposures at short wave lengths were evidently due to the rapid decrease in intensity of the source employed. Mme. Henri (3) thought that both of Bang's maxima should be ascribed to variations in intensity of his carbon arc.

Newcomer (5) exposed *B. typhosus* in quartz capillary tubes to narrow bands of the iron arc spectrum and counted surviving bacteria plated in agar, after exposures of 5 or 10 minutes. His figures, like Bang's, indicate a region of maximum effect

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between 254 and 268 m. μ , but his iron arc varied so much in spectral intensity that he did not find any significance in this peak of effectiveness. He concluded that "equal intensities produce equal effects in the regions 2100–2800. If there is a maximum in this region it is at most only slight and would be in the neighborhood of 2600."

When Mashimo (6) varied the exposure of bacteria in a spectrograph from 15 seconds to 80 minutes he found the first evidence of bactericidal action at $275 \text{ m.}\mu$.



TEXT-FIG. 3. A. Curve of incident energies involved in the destruction of 50 per cent of *B. coli*.

B. Curve of the reciprocals of 3A.

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With somewhat longer exposures the zone widened rapidly so that with a 3 minute exposure it extended from below 210 to above 280 m. μ . The marked action at 275 m. μ was evidently due to a relatively high intensity of his source at this wave length, as an examination of his published photograph shows.

Thus variations in spectral intensity, with no adequate methods of measurement or control, made it difficult to interpret the maxima found by Bang, Newcomer, and Mashimo, and these authors laid no stress upon them.

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