

A STUDY OF THE BACTERICIDAL ACTION OF ULTRA VIOLET LIGHT

II. THE EFFECT OF VARIOUS ENVIRONMENTAL FACTORS AND CONDITIONS

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The first paper of this series (1) dealt with the reaction of an 18 hour culture of *Staphylococcus aureus* to monochromatic ultra violet energy, and it was shown that the course of the reaction was the same at each wave length studied. A consideration of certain factors, such as age and metabolic activity, in the resistance of individual bacteria gave a partial explanation of the course of the reaction among large numbers of organisms. But very different total incident energies were required at different wave lengths to produce these similar effects and an examination of energy relationships and the spectral limits of the bactericidal region was reserved for later consideration.

Before taking up the relation between incident energy and the coefficient of light absorption at different wave lengths, a relation essential to an analysis of the structural elements in bacteria which are affected by light, and to the nature of the resulting reactions, it seems desirable to estimate the effect on the reaction of various conditions of experiment and certain factors in the environment for which due allowance must be made.

The present paper, therefore, will deal with:

- (1) The relation between the intensity of the incident energy and the time required for bacterial destruction (the Bunsen-Roscoe Law).
- (2) The spectral limits of bactericidal action.
- (3) The temperature coefficient of the bactericidal reaction.
- (4) The effect of the hydrogen ion concentration of the substrate.
- (5) The effect of polarization of the ultra violet radiation.

The Bunsen-Roscoe Law

Within the bactericidal zone examined in these experiments ($\lambda 238$ to $\lambda 302 \text{ m}\mu$) widely different incident energies were required at different wave lengths to produce similar effects, and since the available source intensities at these wave lengths differed considerably among themselves, it became necessary to know the effect of different intensities on total energies; *i.e.* to determine the validity for these experiments of the Bunsen-Roscoe reciprocity law of photochemistry (2) that when the product of intensity and exposure-time is constant a constant photochemical reaction results. The law does not hold with exactness in certain reactions, and has been modified by Schwartzschild (3) for photographic blackening. Coblenz and Fulton (4) studying the bactericidal action of ultra violet light and employing source intensities in the ratio of $\frac{1}{4} : \frac{1}{16} : \frac{1}{64}$ found that with low intensities a "proportionate increase in the time of exposure falls short of bringing about an equal killing effect." An intensity reduction to $\frac{1}{64}$ required an increase of $\times 75$ in the exposure time to obtain a comparable reaction. This corresponds to a Schwartzschild exponent for the bactericidal reaction of 1.25. When such low intensities and correspondingly long exposures are used with living test objects like bacteria, the fact that the organisms may undergo metabolic or genetic changes during the exposure period must be taken into account, for such changes might themselves modify the reaction. Such wide differences in intensity as 1 and 50 did not enter into the present study, and Chart 1 illustrates the difference in effect of the extremes of intensity involved.

In the irradiation of *S. aureus* at $\lambda 266 \text{ m}\mu$ six series of plates were exposed to an average intensity of 21.6 ergs per mm.^2 sec. and four to an intensity of 5.6 ergs per mm.^2 sec. for proportionately longer periods. The averages of these determinations from smoothed curves of each series of bacterial counts (Chart 1*) show that at the lower incident intensity fewer bacteria were killed during most of the reaction period. But it is also evident that the curves for low and high incident intensity approach each other as the reaction progresses and the total energies involved in complete destruction are the same.

* The absence of points on the curves is explained in the first paper.

The differences in the curves would indicate, as suggested above, that it is the younger, metabolically or genetically active organisms which show the greater differences in response to differences in intensity. Since this difference in response varies continuously during the

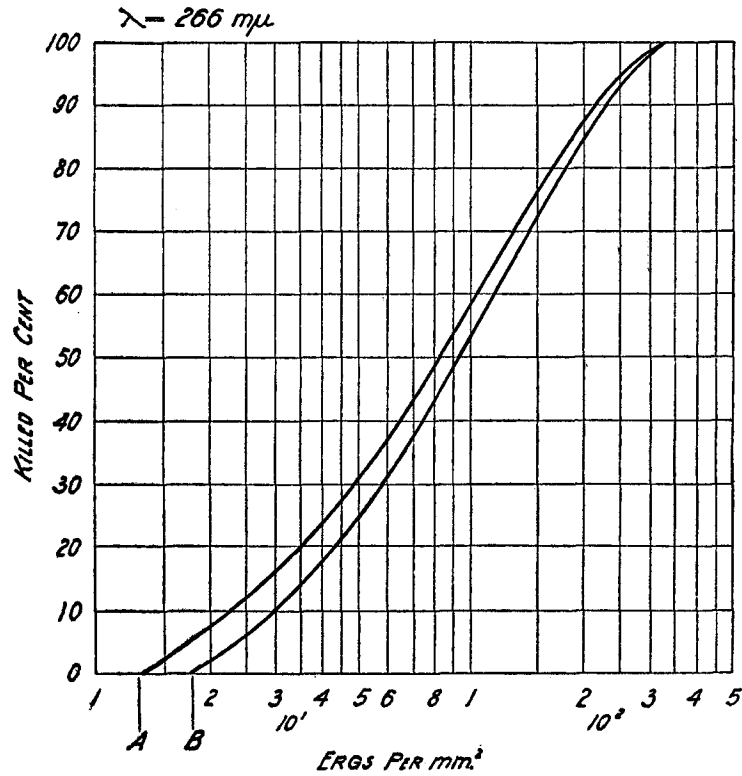


CHART 1. Effect on the bactericidal reaction of different intensities of incident radiation.

A = 21.6 ergs per mm.² sec.

B = 5.6 " " " "

course of the reaction, decreasing as more and more organisms are killed, it is not possible to determine a Schwartzschild exponent except for one point on the curve. Thus at 10 per cent destruction the Schwartzschild modification ($I^q T = K$) of the Bunsen-Roscoe equation requires as an exponent, $q = 1.12$ while for 50 per cent destruc-

tion differences in intensity must be raised to the 1.08 power in order to determine a time factor that will give the same effect. Except for $\lambda 302 \text{ m}\mu$ the intensities did not differ at the various wave lengths as widely as those illustrated in this experiment, so it is doubtful if greater accuracy would have been attained by adherence to the use of similar intensities at each wave length in the bactericidal range.

The Wave Length Limits of Bactericidal Action

The wave lengths $238 \text{ m}\mu$ and $302 \text{ m}\mu$ noted in the first paper do not define the limits of the bactericidal region of ultra violet light, but simply bound the zone in which complete curves for the lethal action were obtained in the present study. Early observations indicated some bactericidal action at $\lambda 313 \text{ m}\mu$ also, but in later experiments in which stray reflected light was more rigorously excluded even very large energies had no appreciable effect at $\lambda 313 \text{ m}\mu$. Thirty minute exposures to the $334 \text{ m}\mu$ and the $366 \text{ m}\mu$ lines also failed to reduce subsequent colony formation in the exposed areas, and because of other factors, such as metabolic changes in the bacteria, which vitiate quantitative energy determinations with such long exposures, the investigation was not pursued farther in this direction. Other investigators have variously estimated the longer wave length limit of bactericidal action from $295\text{--}6 \text{ m}\mu$ (5, 6) to $350\text{--}366 \text{ m}\mu$ (7, 4) and even into the visible region. Exposures measured in hours (8), however, are of questionable significance, and it seems improbable that in such instances the death of the organisms is due to the direct action of the ultra violet light.

So also with the shorter wave lengths of the far ultra violet, no attempt was made to find and measure a limit to the bactericidal zone. A few experiments with the weak mercury arc lines at $\lambda 234$, 230 , and $225 \text{ m}\mu$ established only the middle portion of the curves of lethal action, for the low intensities available at these wave lengths prolonged exposures and so increased the difficulty of obtaining accurate results.

Mashimo (5) using an iron spark before a spectrograph in which bacteria were exposed on nutrient agar plates found that with long exposures (150–300 minutes) the limits of bactericidal action on *B. coli* were $\lambda 2948\text{--}86$ and $\lambda 1856 \text{ \AA}$. u., the limit of air transmission. Lyman (9) had already shown a bactericidal effect of radiations below $\lambda 186$

$m\mu$ and Bovie (10) had extended the lower limit to $\lambda 125 m\mu$ by the use of a fluorite window before a hydrogen discharge tube. Although no energy measurements were undertaken he found these Schumann waves highly destructive to protoplasm, so that only short exposures were required for a lethal effect.

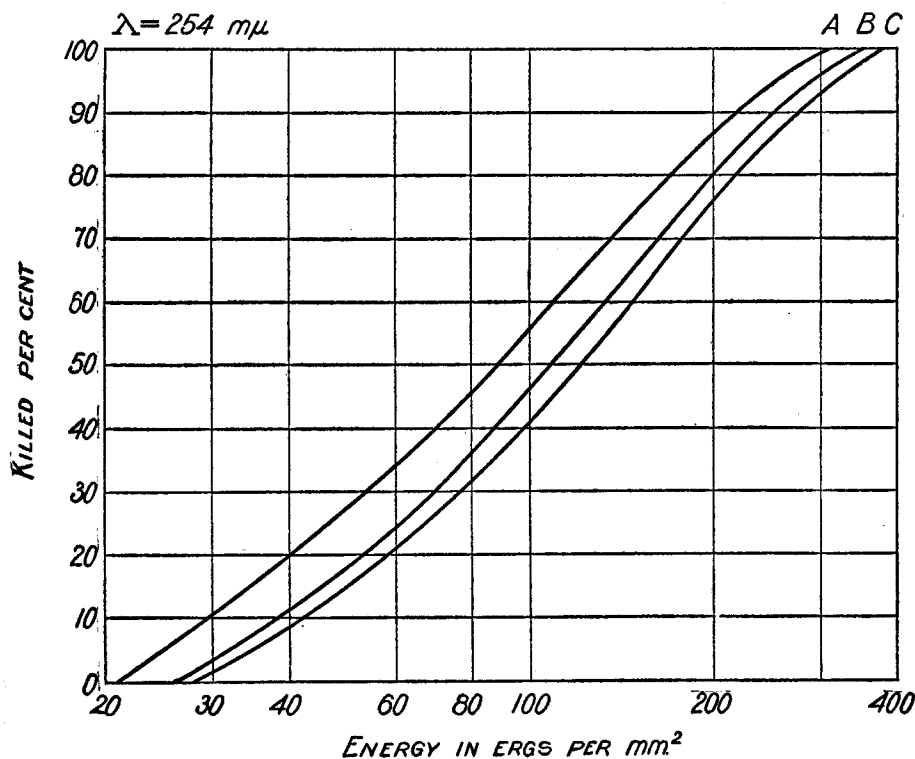


CHART 2. Temperature coefficient of bactericidal action.
 A at 36°C.
 B at 21°C.
 C at 5°C.

The Temperature Coefficient of the Bactericidal Reaction

Of primary importance in determining whether the bactericidal activity of ultra violet light is physical or chemical in nature is the temperature coefficient of the reaction. For although, as Cohen (11) points out, the generalization that the temperature coefficient of physi-

cal reactions is about 1 and of chemical reactions usually above 2 rests on a purely empirical basis, enough experimental evidence has accumulated to warrant a deduction when the observations are clearcut and striking. Even when, in biological experiments, reactions with different temperature coefficients may be progressing simultaneously (12), an observed coefficient for the sum of the reactions which approaches 1 rather than 2 stresses the physical side of the complex. Such was the finding in the present study.

As already stated, most of the exposures were carried out at room temperatures between 20 and 22°C., a range of variation which proved

TABLE I

The Temperature Coefficient of Bactericidal Action λ 254 m μ

Killed	Energy in ergs per mm. ² required		Reciprocal of energy ratio for 31°C.	Reciprocal for 10°C. (temperature coefficient)
	At 5°C.	At 36°C.		
<i>per cent</i>				
20	58	40	1.45	1.13
40	98	70	1.40	1.11
60	148	110	1.35	1.10
80	220	172	1.28	1.08
100	380	316	1.20	1.06
				1.096

to have no demonstrable effect upon the bactericidal reaction. This observation indicated a low temperature coefficient and pointed to a basic reaction physical rather than chemical in nature. But as further evidence, a special series of experiments was made over a much wider temperature range in order to obtain the coefficient for a rise of ten degrees in the environment.

Three series of observations at λ 254 m μ were run in parallel, with the agar medium on which the *S. aureus* were strewn maintained at 5°, 21°, or 36°C. during exposure. Thus three groups of bacteria were exposed to the same range of ultra violet energy, but underwent at different temperatures the reactions that resulted in their deaths. The smoothed curves summarizing the experiments are shown in Chart 2, and from these curves the temperature coefficient of the

bactericidal reaction may be figured, for the coefficient would be the reciprocal of the ratio of energies required to produce the same effects at a difference of 10° in the reaction temperature. The ratios of energies involved at 5° and at 36° are shown in Table I, and the reciprocal of these ratios for a change of 10° is found to vary between 1.06 and 1.13, a range of difference within the limits of experimental error. When averaged over the entire course of the reaction, the temperature coefficient is found to be approximately 1.1. Obviously this is the temperature coefficient of a physical (or purely photochemical) rather than a chemical reaction and it suggests that the lethal effect is a direct result of the absorption of ultra violet energy by some essential element, or elements, of the bacterial protoplasm.

The coefficient of 1.06 for the bactericidal reaction when all the organisms are killed (see Table I) is in agreement with the coefficient of 1.05 found by Bayne-Jones and von der Lingen (7) for bacteria exposed in a fluid medium to the total radiations of a zinc spark. They were unable to confirm the reports of Thiele and Wolf (13) and of Wiesner (14) that an increase in temperature extended the bactericidal zone in the direction of longer wave lengths. Cernovodeanu and Henri (8) found no effects of changes in temperature on the bactericidal reaction, but the methods used would not have revealed a coefficient of 1.05 or 1.06.

Hydrogen Ion Concentration

The nearest approach to a variation in the hydrogen ion concentration of the bacterial protoplasm was afforded by exposing the test organisms on agar media of different alkalinities.

The veal-peptone 2 per cent agar medium used uniformly in the series was made up without buffer, and flasks of it were titrated with $\frac{N}{2}$ HCl to pH 4.5 and 6.0,

estimated colorimetrically, and with $\frac{N}{2}$ NaOH to pH 7.5, 9.0 and 10.0 respectively.

Small Petri plates of these media were then washed with *S. aureus* and exposed in the usual manner to $\lambda 266 \text{ m}\mu$ of the mercury arc spectrum. After exposure all the plates were layered with buffered agar at pH 7.4 and incubated at 37.5°C . overnight. The buffered agar did not bring the hydrogen ion concentration of all the media to 7.4 but to values between 5.5 and 8.2, and within this range no appreciable difference was observed in the number of colonies in equal control areas.

Counts of 9 plates at each pH (in 3 parallel experiments) are averaged in Table II and the corresponding smoothed curves are shown

TABLE II
Bacteria Killed, Per Cent, at Different Hydrogen Ion Concentrations
 λ 266 $m\mu$

Energy in ergs per mm^2 : pH	100	150	200	250	300
4.5	43	63	73	85	95
6.0	44	62	77	89	96
7.5	42	67	77	87	96
Average 4.5, 6, 7.5.....	43	64	76	87	96
9.0	53	71	83	93	99
10.0	52	69	80	89	97

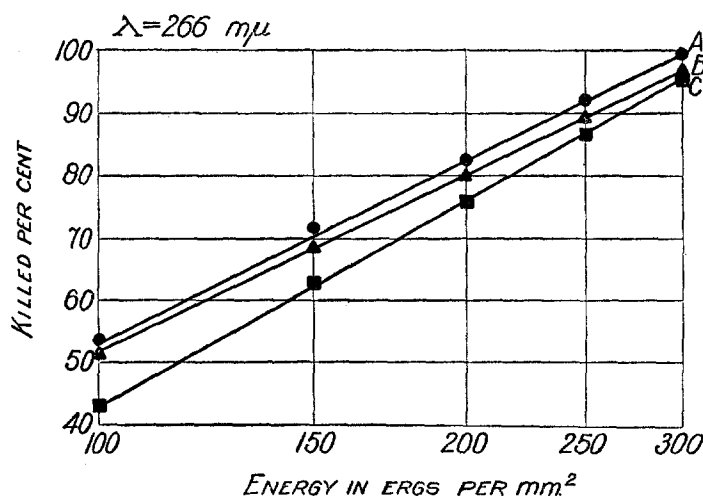


CHART 3. Bacteria killed per cent at various hydrogen ion concentrations of the medium.

- A = pH 9.0 ●
B = pH 10.0 ▲
C = pH 4.5, 6.0, and 7.5 (averaged) ■

in Chart 3, except that the points for pH 4.5, 6.0 and 7.5 are so nearly alike that they have been averaged and a single curve drawn through

them in the chart. The figures for pH 9 and 10 approach this average within the accepted limit of error of the method, but since they are uniformly higher, they may indicate a real difference, at these hydrogen ion concentrations, in the susceptibility of *S. aureus* to ultra violet light. Possibly the higher death rates are due to a less favorable environment for the subsequent recovery and multiplication of damaged cells. It is evident that the difference is not so great as to warrant positive deductions to be drawn from these experiments, and one may conclude, on the contrary, that with the methods employed, variations in the hydrogen ion concentration of the substrate between 4.5 and 7.5 would have no appreciable effect upon the bactericidal reaction. Within the range pH 4.5 to 9.0 these results are in accord with those of Bayne-Jones and von der Lingen (7) who found but slight variation in the time required to kill staphylococcus in an alkaline fluid medium. When acid media were used, however, the bactericidal action was greatly accelerated and at pH 2 all the exposed bacteria were killed in 2 seconds exposure, although at pH 6 to 8 the same radiations had required 22 seconds for the same effect.

The Effect of Polarization of the Incident Light

Unless test objects are crystals, or have some plane of symmetry in which they may be placed with respect to a plane of polarization it is difficult to see how polarized light can have a special and significant effect upon them. Yet many examples could be collected from folklore and from the literature of the alleged biological action of polarized, as distinguished from unpolarized light. This series of experiments afforded an opportunity to determine with measured energies under controlled conditions whether polarization made any difference whatever in the bactericidal action of ultra violet radiation.

After passage through the monochromatic illuminator, the line at $254\text{ m}\mu$ was polarized by reflection from a plane quartz surface at the polarizing angle so that no measurable energy traversed a Nicol prism set at 90° to the plane. After removal of the Nicol prism from the path, this polarized monochromatic light was measured in ergs per mm^2 sec. by means of the thermocouple and galvanometer, and then used to irradiate *S. aureus* spread on agar plates in the manner already described. Controls were obtained by "depolarizing" the light by a polished plate of crystal quartz. Rotation of a Nicol prism in the beam of depolarized light no

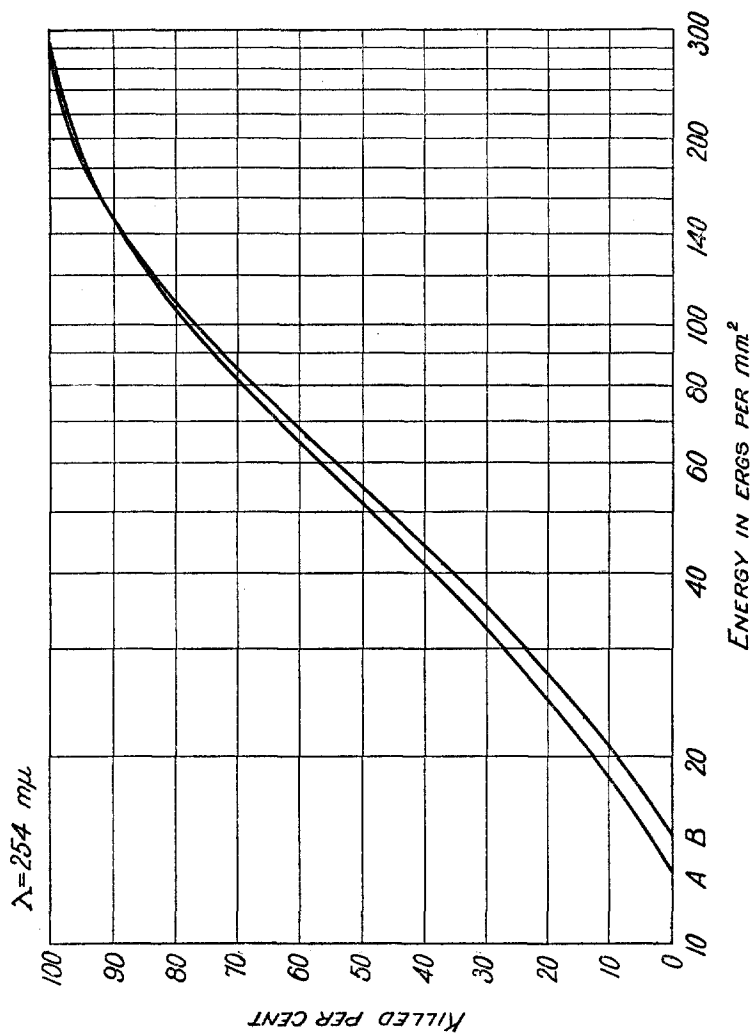


CHART 4. A comparison of the action of (A) depolarized and (B) polarized ultra violet energy.

longer varied its intensity appreciably. The energy incident on the agar plates was again measured with thermocouple and galvanometer so that a quantitative comparison between the bactericidal action of polarized and depolarized light could be made.

With this set-up eleven series of determinations were made, and the averages are shown in Chart 4. It is obvious that the effects of the polarized and depolarized light are identical, for the differences in the curves are well within the limit of error. That these experiments with polarized and depolarized energy are closely comparable with those done more than two years earlier with unpolarized light (Chart 2, Paper I) is further evidence that plane polarization has no observable effect upon the bactericidal activity of ultra violet radiation.

SUMMARY

1. Wide differences in the intensity of incident ultra violet energy are not accurately compensated by corresponding changes in the exposure time, so that the Bunsen-Roscoe reciprocity law does not hold, strictly, especially for bactericidal action on young, metabolically and genetically active bacteria. In the present series of experiments, however, the energies used at various wave lengths did not differ by so much as to cause a significant error in the reported reactions.

2. The longer wave length limit of a direct bactericidal action on *S. aureus* was found to be between 302 and 313 $m\mu$. The shorter limit was not determined because the long exposures required vitiate quantitative results. Bactericidal action was observed at $\lambda 225 m\mu$.

3. The temperature coefficient of the bactericidal reaction approaches 1 and thus furnishes empirical evidence that the direct action of ultra violet light on bacteria is essentially physical or photochemical in character.

4. The hydrogen ion concentration of the environment has no appreciable effect upon the bactericidal reaction between the limits of pH 4.5 and 7.5. At pH 9 and 10 evidence of a slight but definite increase in bacterial susceptibility was noted, but this difference may have been due to a less favorable environment for subsequent recovery and multiplication of injured organisms.

5. Plane polarization of incident ultra violet radiation has no demonstrable effect upon its bactericidal action.

In a third paper of this group the ratios of incident to absorbed ultra violet energy at various wave lengths and the significance of these relations in an analysis of the bactericidal reaction will be discussed.

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