THE LETHAL ACTION OF SHORT ULTRAVIOLET RAYS ON SEVERAL COMMON PATHOGENIC BACTERIA

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INTRODUCTION

The inhibitory and lethal effects of certain of the shorter ultraviolet rays on bacteria and fungi have been known for about fifty years. Recently, interest has been revived in problems concerning bacteria floating in the air especially in relation to the sterilization of air as a protection against air-borne infections. (Wells and Wells, 1936 and 1938, Hart, 1937 and 1938, and Sharp, 1938). Since a quantitative evaluation of the resistance to radiation of most of the bacteria against which ultraviolet-ray protection is needed has not been made, such comparative study seems desirable.

Careful quantitative estimates of the energy lethal for various species of bacteria have been made by Coblentz and Fulton (1924) on *Escherichia coli*, Gates (1929 and 1930) on *Staphylococcus aureus* and *Escherichia coli*, Wyckoff (1931) on *Escherichia coli*, Ehrismann (1931) on *Escherichia coli*, *Staphylococcus aureus*, *Serratia marcescens*, etc., and Hollaender and Claus (1936) on *Escherichia coli*. In the last paper, the results of the previous work are summarized. Through a comparison of the data obtained by different experimental methods, attempts are made to reduce all the results to the common denominator of lethal energy per bacterium. It is notable that, while all the studies cited include some of the same species of bacteria, the absolute lethal energies given for a particular wave length differ substantially.

Studies of spore-forming bacteria (*Bacillus subtilis* by Duggar and Hollaender, 1934 and *Bacillus megatherium* by Hercik, 1936) indicate that approximately twice as much energy is necessary to kill the spores as is needed for the vegetative forms.

In none of the studies in which absolute energy values have been measured has more than slight attention been given to the common pathogenic bacteria. The work of Dreyer and Campbell-Renton (1936) gives lethal curves for several pathogens but the light intensities were measured in arbitrary units and are therefore not directly comparable with other quantitative work.

The experiments reported here were concerned with the determination of the relative resistance of several pathogens, notably the more important pathogenic air contaminants, as well as *Staphylococcus albus*, *Escherichia coli*, and *Serratia marcescens*, to the unfiltered rays of a low-pressure mercury glow lamp[1] of the type used for sterilization of air in operating rooms. Its characteristics have been described elsewhere (Sharp, 1938) and will be reviewed only briefly here.

METHOD

The highly pathogenic nature of several of the species of bacteria studied makes measurement in air suspension dangerous. Previous work (Sharp, 1938) has shown that in the instance of *S. albus* the amount of energy necessary to kill 100 per cent in air suspension (presumably saturated air) is of the same order of magnitude as that given by the plate method. It was considered advisable to employ the latter method.

Standard nine-centimeter Petri plates containing solid media suitable for the growth of the organism under observation were seeded uniformly with bacteria in the following manner: A twenty-four-hour broth culture was diluted with normal saline solution until it contained about fifteen thousand organisms per cubic centimeter. Five cubic centimeters of this were pipetted on to the agar surface of each plate and kept in motion for twenty

[1] This lamp is manufactured by Westinghouse Electric and Manufacturing Co. under the name of "Sterilamp."

seconds; the plate was then inverted for about forty seconds to drain off the excess liquid. By this means 600 to 1,000 evenly distributed colonies could be obtained on each plate (fig. 1).

Prepared plates were exposed to ultraviolet rays in a frame (fig. 2) at a distance of 35 inches from a pair of radiation tubes and

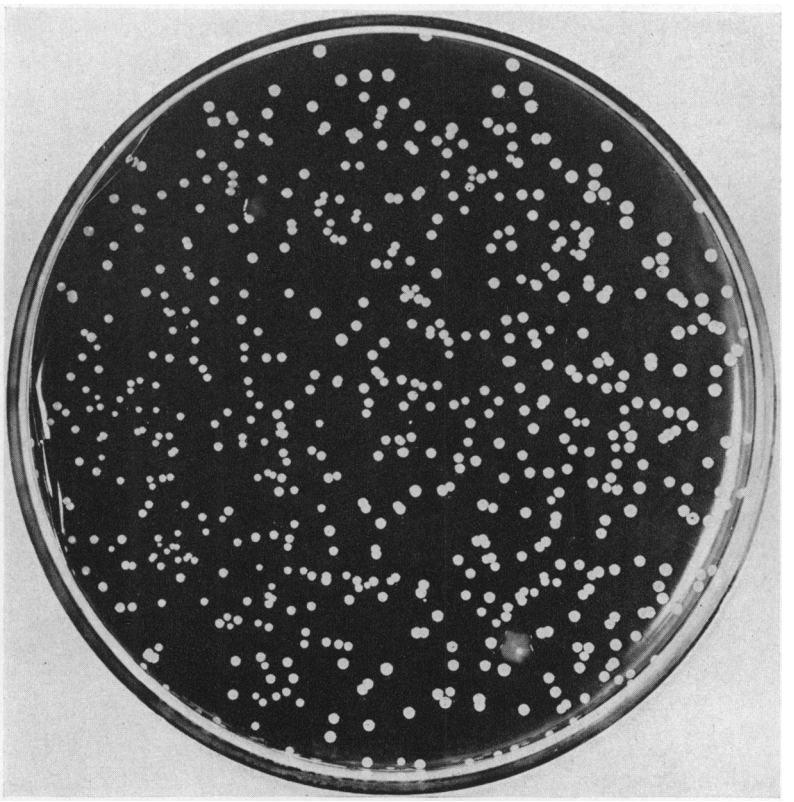


FIG. 1

in a plane normal to the rays. The tubes were 30 inches long, operated at about five degrees above room temperature and consumed 5 to 10 watts power each. They are particularly well adapted to this work since over 85 per cent of the total radiant output is in the resonance 2537 Å line. There are no other lines in the region below 3000 Å strong enough to have appreciable

bactericidal effect so that it is safe to assume, for present purposes, that the unfiltered rays are monochromatic and as such, comparable in their effect with those usually isolated with a monochrometer. This allows a direct comparison of results obtained here with those of other observers.

Immediately below the plates (fig. 2) and in a similar position with respect to the lamps was mounted a special ultraviolet dosimeter (Rentschler, 1930) consisting of a tantalum-target photoelectric cell and relay apparatus suitable for metering the energy received. This device recorded the actual amount of

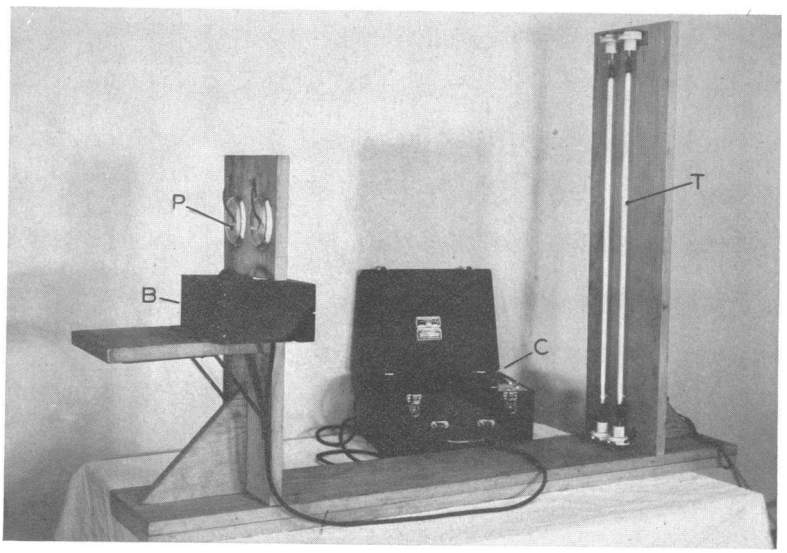


FIG. 2

energy received over a given length of time and so was free from errors that might have been caused by variation in lamp intensity. Standardization was effected by comparison with a bismuth-silver vacuum thermopile and National Bureau of Standards radiation constant. All energy values are given directly in incident ergs per square millimeter of exposed surface.

RESULTS

For each organism used, two separate groups of 12 to 15 plates were exposed on different days. Exposures were selected to give

the best possible determination of the amount of energy necessary to reduce the number of surviving bacteria to 10 per cent of the original number. No attempt has been made to examine closely the extremes of the reaction, namely; near 0 per cent and near 100

TABLE 1

ORGANISM	MEDIUM (pH 7.4)	INCIDENT ENERGY ERGS/MM² FOR 90 PER CENT KILLING (10 PER CENT SURVIVAL)	OTHER OBSERVATIONS
Bacillus anthracis (mixed spores and veg. forms)	Beef-extract agar	452	
Corynebacterium diphtheriae	Beef-infusion blood agar	337	
Staphylococcus aureus-hemolyticus	Beef-extract agar	260	218 ergs, Gates (1929) 600 ergs, *Ehrismann and Noethling (1931)
Escherichia coli	Beef-extract agar	245	150 ergs, *Ehrismann and Noethling (1931) 640 ergs, Wyckoff (1931)
Serratia marcescens	Beef-extract agar	220	82 ergs, *Ehrismann and Noethling (1931)
Streptococcus hemolyticus	Beef-extract blood agar	216	
Eberthella typhosa	Beef-extract agar	214	
Streptococcus viridans	Beef-extract blood agar	200	
Staphylococcus albus	Beef-extract agar	184	
Shigella paradysenteriae	Beef-extract agar	168	

Table showing incident energy at 2537 Å necessary to reduce several species of bacteria to a survival ratio of 10 per cent (90 per cent killed).

*Values based on 90 to 100 per cent killing.

per cent survival. This has been done by others, (Hollaender and Claus, 1936) for non-pathogenic bacteria, and it was not considered advisable to repeat it here since the exposure of many more plates would have been necessary.

Figures 3 to 12 show the logarithm of the survival ratio plotted against the incident energy in ergs per square millimeter and the

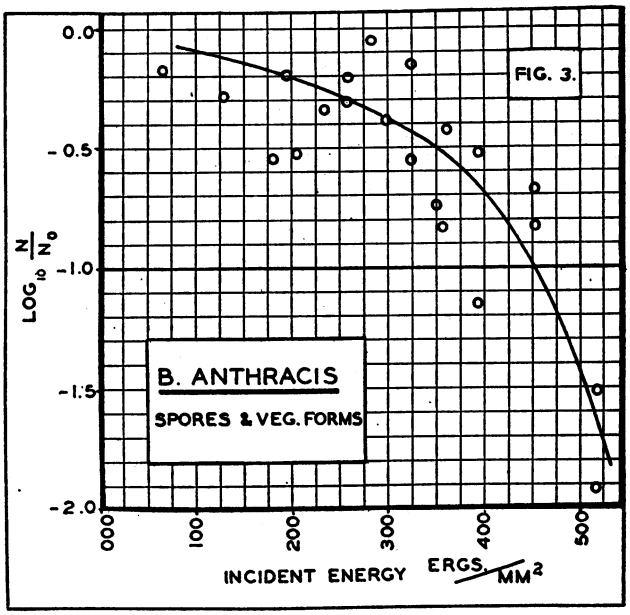


FIG. 3.
B. ANTHRACIS
SPORES & VEG. FORMS
0.0
-0.5
-1.0
-1.5
-2.0
$\log_{10} N/N_0$
000
100
200
300
400
500
INCIDENT ENERGY ERGS/MM²

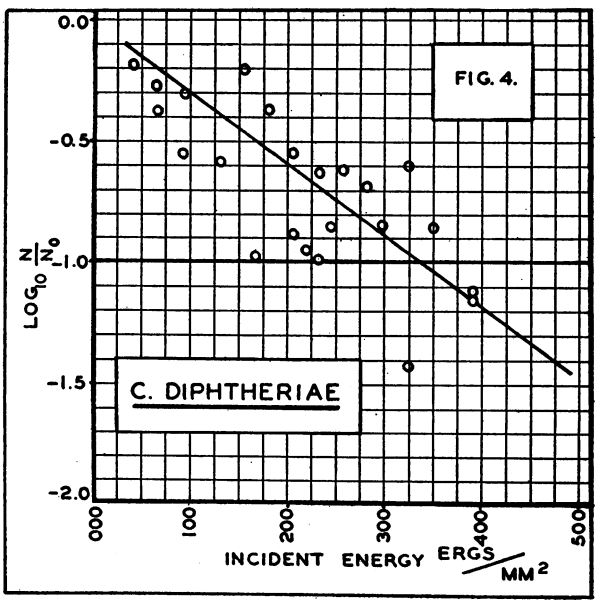
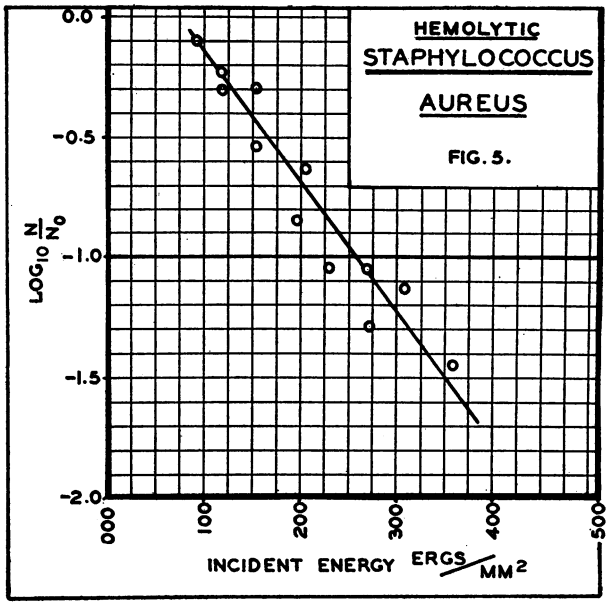
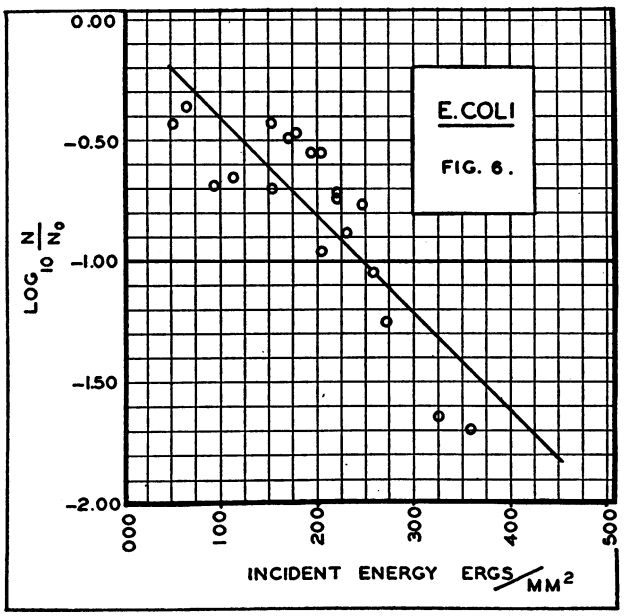
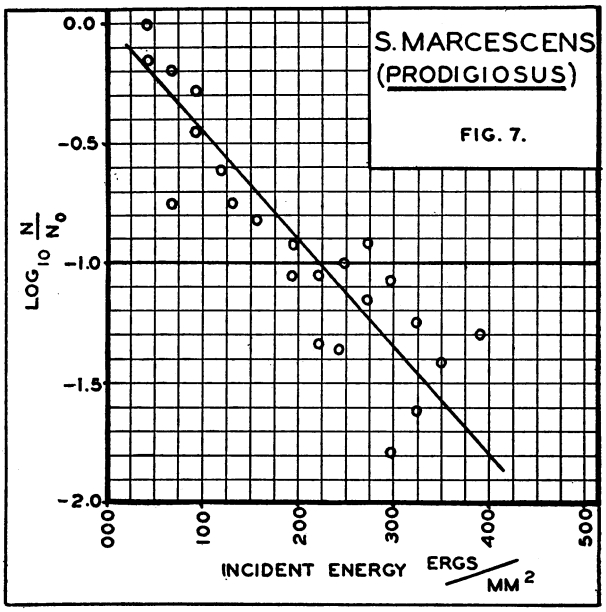
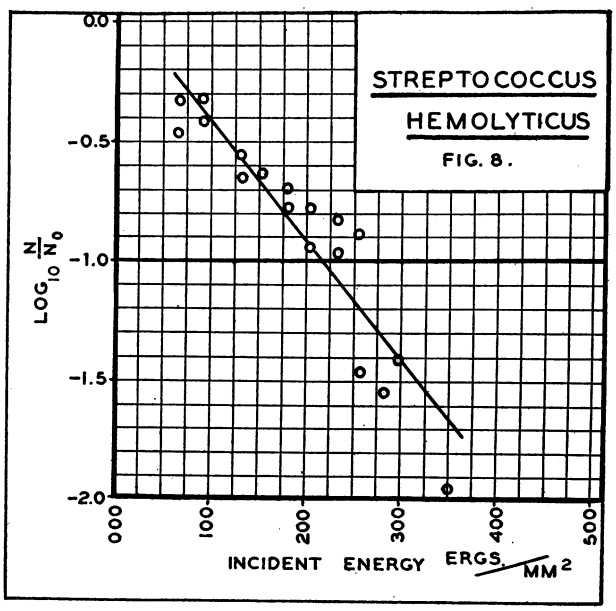


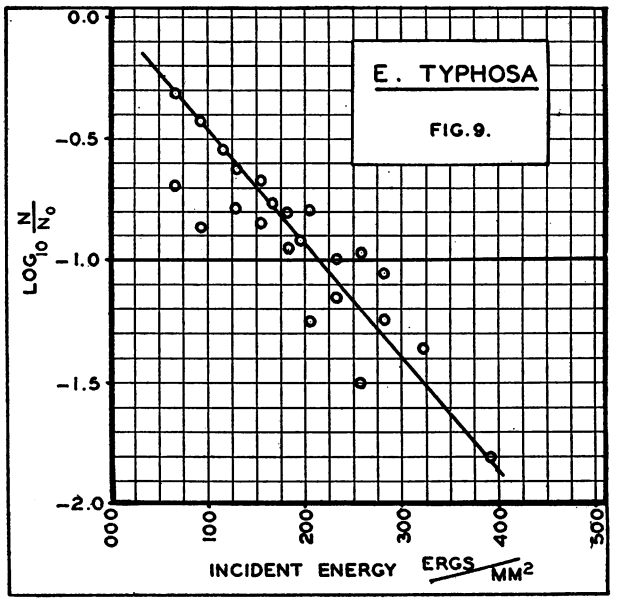
FIG. 4.
C. DIPHTHERIAE
0.0
-0.5
-1.0
-1.5
-2.0
$\log_{10} N/N_0$
000
100
200
300
400
500
INCIDENT ENERGY ERGS/MM²

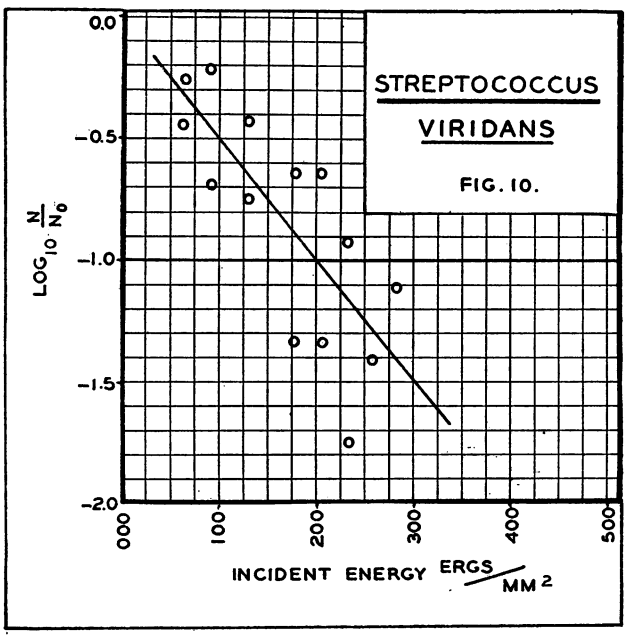

HEMOLYTIC
STAPHYLOCOCCUS
AUREUS
FIG. 5.
0.0
-0.5
-1.0
-1.5
-2.0
LOG10 N/N0
000
100
200
300
400
500
INCIDENT ENERGY ERGS/MM2

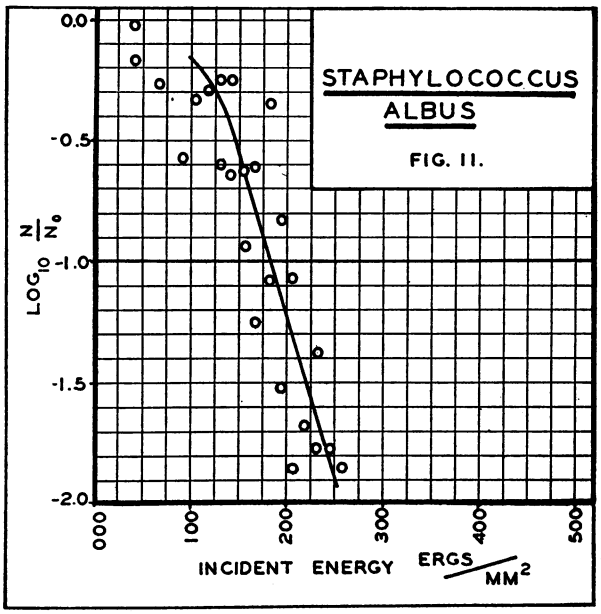

E.COLI
FIG. 6.
0.00
-0.50
-1.00
-1.50
-2.00
LOG10 N/N0
000
100
200
300
400
500
INCIDENT ENERGY ERGS/MM2

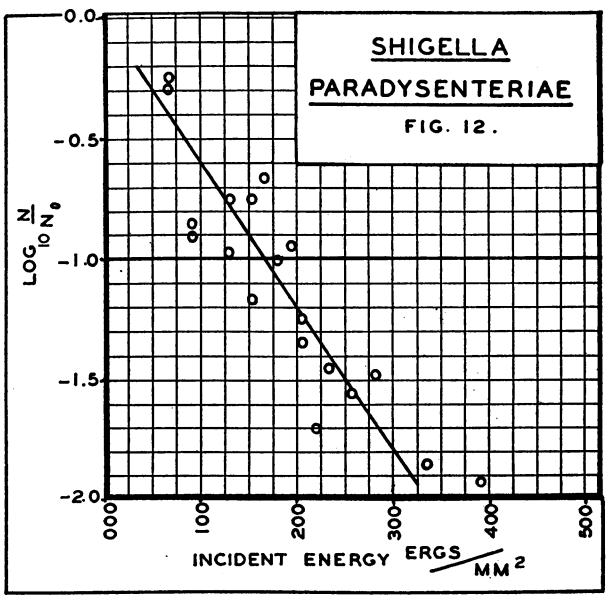

S. MARCESCENS
(PRODIGIOSUS)
FIG. 7.
0.0
-0.5
-1.0
-1.5
-2.0
LOG10 N/N0
000
100
200
300
400
500
INCIDENT ENERGY ERGS/MM2


STREPTOCOCCUS
HEMOLYTICUS
FIG. 8.
0.0
-0.5
-1.0
-1.5
-2.0
LOG10 N/N0
000
100
200
300
400
500
INCIDENT ENERGY ERGS/MM2


E. TYPHOSA
FIG. 9.
0.0
-0.5
-1.0
-1.5
-2.0
$\log_{10} \frac{N}{N_0}$
000
100
200
300
400
500
INCIDENT ENERGY ERGS/MM²


STREPTOCOCCUS
VIRIDANS
FIG. 10.
0.0
-0.5
-1.0
-1.5
-2.0
$\log_{10} \frac{N}{N_0}$
000
100
200
300
400
500
INCIDENT ENERGY ERGS/MM²


STAPHYLOCOCCUS
ALBUS
FIG. 11.
0.0
-0.5
-1.0
-1.5
-2.0
LOG10 N/N0
000
100
200
300
400
500
INCIDENT ENERGY ERGS/MM2


SHIGELLA
PARADYSENTERIAE
FIG. 12.
0.0
-0.5
-1.0
-1.5
-2.0
LOG10 N/N0
000
100
200
300
400
500
INCIDENT ENERGY ERGS/MM2

curves drawn through the points. The intersections of these curves with the ordinate line -1 (10 per cent survival) is the basis on which the bacteria are compared (table 1). The intensity was constant and such that the time required to give this amount of energy was about 2 minutes.

DISCUSSION

It has been shown for several types of bacteria, (Wyckoff, 1931, Hollaender and Claus, 1936, and Hercik, 1936), that the number dying per unit of incident energy is approximately in direct proportion to the number remaining alive in the culture, i.e.:

$$\frac{dN}{dE} = -k_1 N$$

E = energy incident on culture,
N = number of bacteria remaining after a given amount of energy has been applied.

$$\log \frac{N}{N_0} = -k_2 E$$

This, when integrated from energy $E = O$ to any given amount E, gives the corresponding survival ratio $\frac{N}{No}$.

No = number of bacteria originally on the plate as given by the controls.

The constant K_2 depends on the absorption coefficient of the particular species and on the wavelength of the light. Although certain deviations from this straight-line relationship between Log $\frac{N}{No}$ and the energy have been reported, it serves as a convenient method of comparison of species resistance and is used here to facilitate comparison with other data.

A direct comparison can be made in the case of *Staphylococcus aureus* with the data of Gates (1929) which show 218 ergs/mm^2 necessary to kill 90 per cent of these organisms on agar surface. From our data (fig. 5) it is seen that 260 ergs/mm^2 were necessary

for our strain of *S. aureus*. The same author (Gates, 1930) found 110 ergs/mm^2 necessary to reach 50 per cent killing of *E. coli* and our data (fig. 6) would indicate 75 ergs/mm^2 for this organism. Wyckoff (1931), on the other hand, found 640 ergs/mm^2 necessary for 90 per cent killing while curve 6 shows only 245 ergs/mm^2. Only approximate comparisons with the studies of Ehrismann and Noethling (1931) are possible since their estimates were made on the broad basis of 90 to 100 per cent killing. Their figures have been entered in table 1, and it will be seen that large variations occur. No such wide differences in resistance have been found among the various species tested here. In fact, all the figures for 90 per cent killing of non-sporing organisms fall between 168 ergs/mm^2 (*Shigella paradysenteriae*) and 337 ergs/mm^2 (*Corynebacterium diphtheriae*).

Bacillus anthracis, which showed more resistance than any of the nonsporing species, was killed by about twice the energy necessary to kill *E. coli*. This was true even if complete sterilization instead of 90 per cent killing was considered as the point of comparison. The results of Hercik (1937) on *Bacillus megatherium* indicate, too, about twice as much energy required to kill spores as was required for vegetative forms. The curve (fig. 3) seems to indicate by its increasing rate of fall that there is an apparent decrease in resistance rather than the expected increase due to surviving spores.

The results summarized in table 1 indicate that the resistance to 2537 Å rays of the several species of bacteria was not only of the same order of magnitude but that the most resistant non-spore producer (*C. diphtheriae*) was only about twice as resistant as the least resistant (*S. paradysenteriae*).

It is to be expected that the ultraviolet ray resistance of these bacteria in air suspension would be subject to certain variables not present in this experiment. Wells (1936) has reported wide differences in bacterial resistance with changes in the relative humidity of the air. His findings indicate a reduced resistance in air of lower humidity. In the light of his observation our previous work on *S. albus* done in air (Sharp 1938) under conditions near saturation could be taken to indicate the maximum

resistance of the organism. The present data taken from bacteria on moist plate surfaces might be subject to the same considerations. The lethal energies in both experiments were found to be of the same order of magnitude.

SUMMARY

The following species of bacteria were tested for resistance to short ultraviolet rays, and the energy necessary to reduce each to a 10 per cent survival ratio is recorded: *Eberthella typhosa*, *Shigella paradysenteriae*, *Corynebacterium diphtheriae*, *Staphylococcus aureus* (*Hem*), *Staphylococcus albus*, *Streptococcus viridans*, *Streptococcus hemolyticus*, *Bacillus anthracis* (mixture of spores and vegetative forms), *Escherichia coli* and *Serratia marcescens*.

The mixture of the spore and vegetative forms of *Bacillus anthracis* was not more than twice as resistant as *Escherichia coli*. This was true even when 100 per cent killing or zero survival was considered as a reference point.

Unfiltered radiation (85 per cent 2537 Å) from a commercial low pressure glass mercury arc was used. Its degree of purity was sufficiently great to make a monochrometer unnecessary for bacterial work, and regular 9 cm. Petri plates can be conveniently exposed to a uniform field of rays.

A commercial ultraviolet ray dosimeter, calibrated in absolute units, was used to measure the energy. The sensitive agent is a tantalum-target vacuum photoelectric cell.

It was found that the extreme values of energy necessary to kill 90 per cent of non-sporing organisms were 168 ergs/mm^2 (*Shigella paradysenteriae*) and 337 ergs/mm^2 (*Corynebacterium diphtheriae*). For a mixture of spores and vegetative forms of *Bacillus anthracis*, however, 452 ergs/mm^2 were necessary. The results obtained are compared with the data of other observers.

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