A QUANTITATIVE METHOD OF DETERMINING THE LETHAL EFFECT OF ULTRAVIOLET LIGHT ON BACTERIA SUSPENDED IN AIR

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INTRODUCTION

The use of ultraviolet rays to reduce the number of infections from air-borne bacteria in the operating room (Hart, 1936, 1937a and b; Hart and Gardner, 1937) has stimulated interest in the development of a technique to measure quantitatively the minimal dose of a particular kind of radiation necessary to sterilize air containing pathogenic bacteria. A great amount of work has been done on bacteria using various kinds of rays and exposing the organisms on solid media. The work of Gates (1929) on *Staphylococcus aureus* and other organisms gives percentages killed as a function of the energy used. He plotted a series of these curves for different wave lengths of light and found that 2660 Å was the most efficient wave length, 88 ergs per square millimeter being necessary for 50-per cent killing of *Staphylococcus aureus* on plates. In these experiments the bacteria were sprayed on a solid medium, exposed to radiation and the plates incubated to determine growth. This technique brought up several questions concerning the mechanism of the killing reaction. That bacterial death is a secondary effect brought about by photochemical changes in the media has long been discussed and not entirely agreed upon at present. Furthermore, aside from the possible changes in hydrogen peroxide concentration, pH, etc., that might be contributing factors there is another variable of a purely mechanical nature. No matter how uni-

formly one sprays a culture of bacteria on an agar surface there is a chance of screening one organism by another. This effect might be of considerable magnitude where soft, non-penetrating rays are used on organisms that normally develop clusters in culture media.

Some of these difficulties have been avoided in the method to be described in which the rays exert their effect on bacteria suspended in the air. Death of all exposed organisms has been taken as the end point.

FIGURE I APPARATUS FOR DETERMINATION OF LETHAL EFFECT OF ULTRA VIOLET RADIATION ON AIR BORNE BACTERIA

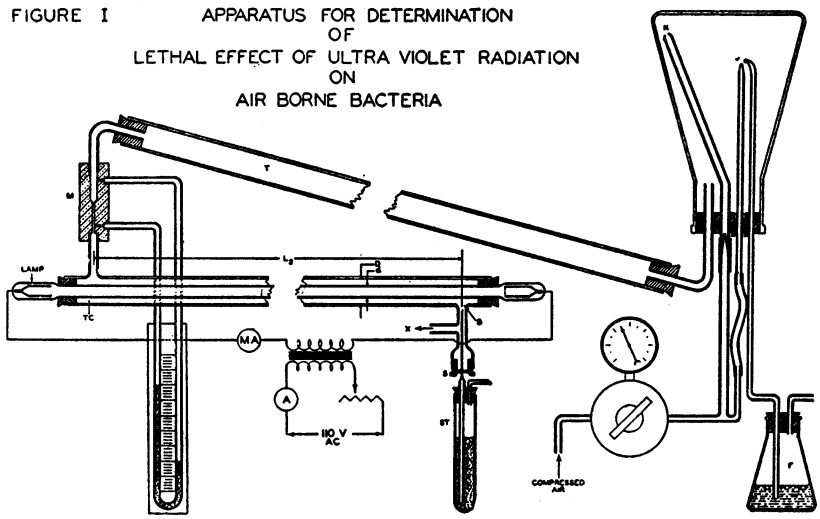

FIG. 1. DESCRIPTION OF APPARATUS

APPARATUS

General

The principle involved in this apparatus (fig. 1) is to drive a mixture of air and viable bacteria at constant rate through a test chamber where it is treated with a known amount of radiant energy. At the point where this mixture leaves the test chamber a method of sampling is provided. An aliquot part of the treated air is bubbled continuously through sterile broth during the entire length of the experiment. The broth is then incubated under optimum growth conditions for the organism involved.

Control is accomplished by taking similar samples without the radiation.

Spray system

A broth culture of bacteria is diluted in sterile saline solution and placed in flask (*F*) (fig. 1), which is connected to the spray chamber. Compressed air is then admitted through the control valve shown and passes at high speed through the jets (*j*) drawing with it this saline suspension of organisms and atomizing it into the chamber. This is accomplished at the rate of only about 10 cc. per hour, the liquid jet being small to prevent large droplet formation.

Into the spray chamber is also fitted another tube (*K*) for admitting additional sterile air to make up the desired flow velocity and allow for adjustment. In this manner air can be mixed with the organism to be tested and both volume of mixture per unit time and number of bacteria per unit volume can be held constant or varied at will.

Lamp

The source of ultraviolet rays used in this work was made by Westinghouse Electric and Manufacturing Company and consists of a high transmission glass tube about 28 inches long by $\frac{1}{2}$ inch in diameter filled with a mixture of Argon and Neon with mercury and fitted with 50 milliampere cold electrodes. It operates on a small transformer from 110 volts A.C. mains and requires about 400 volts at 30 ma. At this rating it uses approximately 10 watts power and rises 5° or 6°C. above room temperature. Its output intensity is roughly proportional to the current input (fig. 2) and the spectral distribution may be seen in figure 3. From this figure it is seen that the only strong line in the bactericidal part of the spectrum is the 2537 Å line. Measurements have shown that 88 per cent of the total output of this lamp, as measured on a bismuth-silver vacuum thermopile with quartz window is in this single line. The remaining 12 per cent lies largely above 3000 Å or above the bactericidal range.

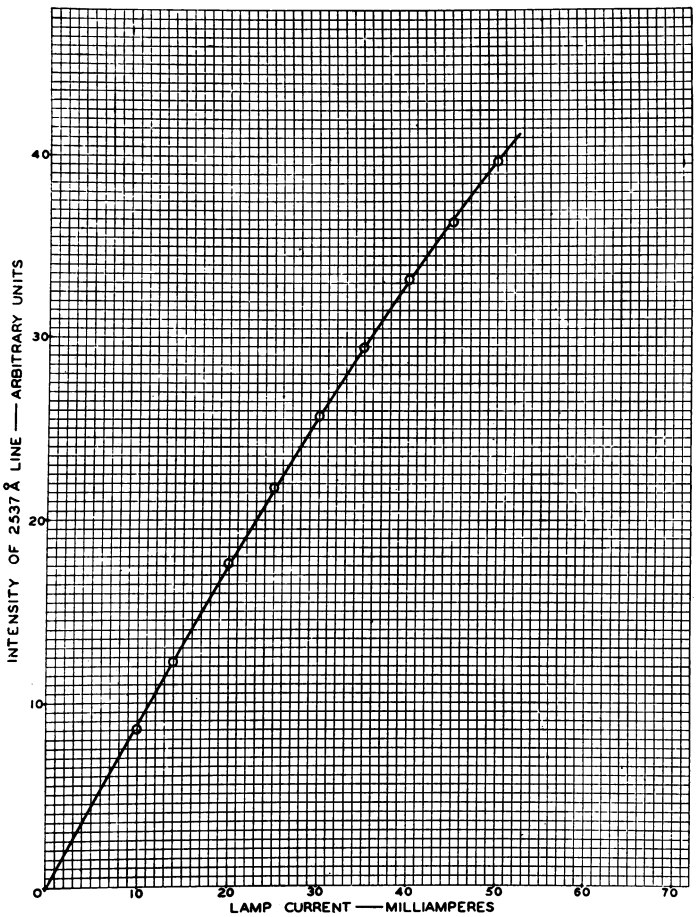

FIG. 2. GRAPH SHOWING RELATIONSHIP OF OUTPUT INTENSITY TO CURRENT INPUT

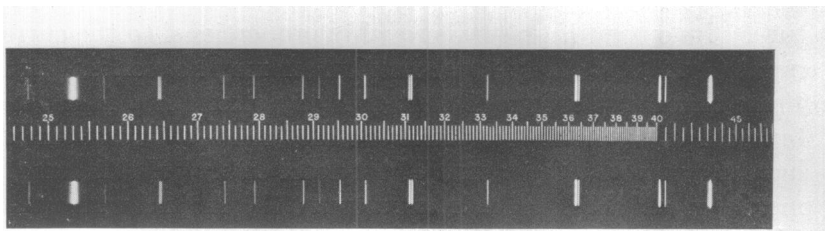

FIG. 3. SPECTROGRAM OF LAMP OUTPUT AT 27 MA. CURRENT

Venturi meter

The stream of contaminated air is passed through a Venturi meter (*M*) to measure the rate of flow through the apparatus in cubic centimeters per second. This instrument indicates the rate of air flow as a function of the water level in a manometer and is thus a direct reading instrument.

Treatment chamber

After checking the velocity of flow the air is then passed through the treatment chamber (*T.C.*) which is a glass tube concentric with the ultraviolet lamp and forming a shell around it. The air passes between the lamp and this shell through a region where the intensity of the radiant flux can be accurately calculated. The product of this intensity by the length of time necessary to kill all the bacteria exposed is taken as a measure of the lethal dose.

Sampling tubes

In order to test the air issuing from the treatment chamber at (*B*) to determine sterility a tube of nutrient broth is used (*S.T.*). A $2\frac{1}{2}$ inch hypodermic needle (16 gage) is fastened to a glass capillary extending to the bottom of the tube. When this needle and tube with broth contents have been autoclaved it constitutes the sampling unit. Several of these are necessary in practice for a complete run.

The needle of the sampling tube is inserted through the serum bottle stopper (*S*) in the treatment chamber and a part of the treated air bubbles through the broth, the rest escaping through the side arm (*X*) which may be passed through an antiseptic solution when pathogenic organisms are used. After 48 hours incubation at 37.5°C. failure to cloud is taken as evidence of sterility of the tested air.

CALIBRATION

Lamp

A standard constant radiation lamp was obtained from the National Bureau of Standards and set up according to their

specifications to calibrate a bismuth-silver vacuum thermopile with quartz window. This thermopile was then used to measure the output of the ultraviolet lamp and 88 per cent of this value was taken as that which was active in the regions mentioned.

Venturi meter

This meter was of the common laboratory type calibrated with a gas meter checked on a National Bureau of Standards meter prover. It actuated a water manometer and gave a difference in water columns of about 6 cm. for an air velocity of the order of 100 cc. per second.

CALCULATIONS

Light intensity

The thermopile calibrated above was used to measure the output from 15 cm. of the lamp length at a point 40 cm. from the center of this length and perpendicular to it (fig. 4). Measurements could not be taken at a great distance from the lamp because of its low intensity. They could not be taken closer than a few centimeters because of the finite lamp size and consequent error. Thus it was necessary, for reasons to be discussed later, to calculate what the intensity would be at a point far from the lamp as a function of a reading taken at lesser distance. This value was then used to get the final result, the intensity in the treatment chamber (fig. 4).

If we choose a point, P_2, far from the lamp (fig. 4) the intensity at this point due to the whole length L_2 can be shown to bear the following relation to the intensity at point measured—P_1.

$$I_{P_2} = I_{P_1} \frac{S_1}{S_2} \sqrt{\frac{4S_1^2 + L_2^2}{4S_2^2 + L_2^2}} = I_{mh} \tag{1}$$

This value is known as the mean horizontal intensity and it has been shown (Kunerth, 1929) that the mean intensity I *m.s.* over a sphere about the lamp center and of radius large with respect to L_2 is given by the following formula:

$$I_{ms} = \frac{\pi}{4} I_{mh} \tag{2}$$

It has been assumed that Lambert's Cosine Law is valid for this source.

Integrating over the whole sphere we get the total radiant flux from the lamp at the current measured. For any other current the corresponding output can be obtained from figure 2 which shows, in arbitrary units, the variation of output radiant energy with lamp current. This flux is then divided by the area of the treatment chamber to give the treatment intensity IT.

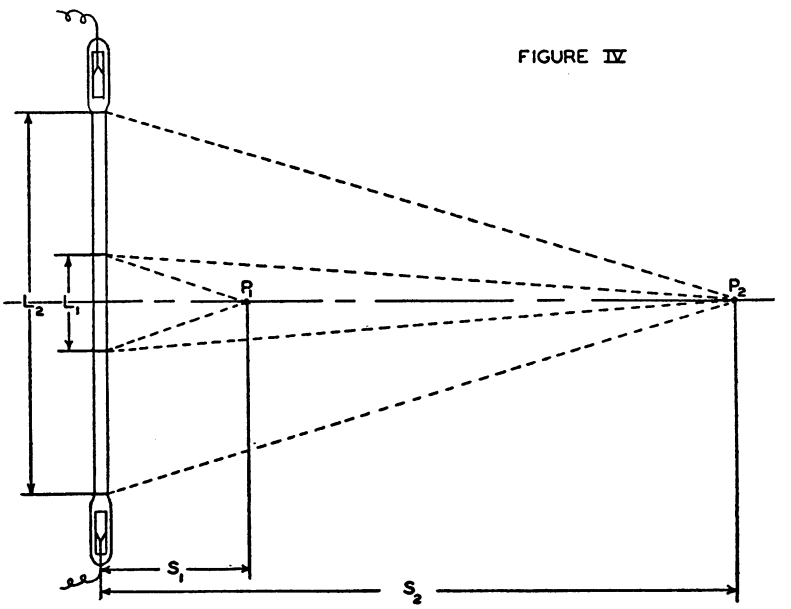

FIG. 4. DIAGRAM ILLUSTRATING THE BASIS OF CALCULATION OF INTENSITY
See text for explanation

Let $I_{P_1L_1}$ be intensity at P_1 due to length L_1 of lamp (calibration).

Let $I_{P_2L_2}$ be intensity at P_2 far from lamp $S_2 \gg L_2$.

$$I_{P_2L_2} = \frac{L_2}{L_1}\frac{S_1}{S_2}\sqrt{\frac{4S_1^2 + L_1^2}{4S_2^2 + L_2^2}} \times I_{P_1L_1}$$

$$I_T = \frac{\frac{\pi}{4} I_{P_1L_1} \frac{L_2}{L_1}\frac{S_1}{S_2}\sqrt{\frac{4S_1^2 + L_1^2}{4S_2^2 + L_2^2}} \times 4\pi S_2^2}{\pi D L_2}$$

$$I_T = \frac{\pi S_1 S_2 I_{P_1L_1}}{L_1 D}\sqrt{\frac{4S_1^2 + L_1^2}{4S_2^2 + L_2^2}}$$

but $S_2 \gg L_2$

so

$$I_T = \frac{\pi S_1 S_2 I_{P_1 L_1}}{2 L_1 D S_2} \sqrt{4S_1^2 + L_1^2}$$

$$I_T = \frac{\pi S_1 \sqrt{4S_1^2 + L_1^2}}{2 L_1 D} I_{P_1 L_1} \tag{3}$$

It should be noted here that in the calculations which follow, the diameter (D) of the treatment chamber has been taken to the outside of the chamber in order to give the weakest part of the field.

Exposure time

It has been assumed that the bacteria pass through the chamber at the same rate as the air. Although it is probable that they do not have the same rate of flow, at least their velocities will be grouped about this value and it has been taken as a first approximation. In order to calculate the exposure time (T) it is necessary to divide the volume of the treatment chamber by the air flow in cubic centimeters per second.

$$T = \frac{\pi L_2 (D^2 - d^2)}{4 \times \text{cc./sec.}} \tag{4}$$

Energy

The energy per unit area, to which a bacterium has been exposed, will be given by the product of the treatment intensity I_T and the treatment time T.

EXPERIMENTAL

A strain of *Staphylococcus albus* was isolated from the air and cultivated on beef-extract agar slants. Twenty-four hour broth cultures (37.5°C.) inoculated from these slants were used for each experiment and diluted 1 to 50 in sterile 0.85 per cent NaCl solution before placing in the flask (F).

Table 1 gives the results of three tests taken on different days. All tubes were incubated 48 hours at 37.5°C. and examined microscopically as well as macroscopically to determine sterility.

As an added check the following test was made. The lamp

was operated brightly enough to kill all the organisms coming through as evidenced by sterility in the exposed sampling tube. At the same time the air issuing from (*X*) was directed against a

TABLE 1

Venturi meter water column	6.6	cm.
Calculated air flow	113.0	cc./sec.
Calculated exposure time	1.06	sec.
Length of run per sample tube	7.0	min.
Volume air treated per sample	47.5	liters*

1. LAMP CURRENT	24-HOUR INCUBATION	48-HOUR INCUBATION
ma.		
00.0	Cloudy	Cloudy
50.0	Clear	Clear
33.4	Clear	Clear
27.3	Clear	Clear
21.2	Cloudy	Very cloudy
15.0	Cloudy	Very cloudy
10.0	Cloudy	Very cloudy
00.0	Not taken	Cloudy
29.3	Not taken	Clear
26.6	Not taken	Cloudy
24.2	Not taken	Cloudy
22.0	Not taken	Cloudy
19.8	Not taken	Cloudy
00.0	Cloudy	Cloudy
29.3	Clear	Clear
26.6	Clear	Clear
24.2	Clear	Clear
22.2	Cloudy	Cloudy
19.8	Cloudy	Cloudy

Maximum variation 21.2 ma. to 29.3 ma.

Output of lamp in 2537 Å line measured 40 cm. distance from center of a 15 cm. exposed length operating at 50 ma.—29.51 mw. per square centimeter.

* Twenty-five per cent of this air bubbled through the broth of the sampling tube (*ST*).

drop of sterile saline solution on a microscope slide. After thirty minutes operation the slide was stained and found to contain well-scattered gram-positive cocci in large numbers.

Taking 25.0 ma. lamp current (table 1) as the mean value from

the above data, using the lamp calibration cited with the data, and applying the equations developed under "calculations" it is seen that these organisms have been exposed to a minimum of 26,200 ergs per square centimeter of area. This energy was chiefly 2537 Å radiation and is only slightly lower than the value of 30,000 ergs per square centimeter obtained by Gates (1929) with *Staphylococcus aureus* on plates for the same wave length.

DISCUSSION

The radiant intensity is, of course, not constant throughout the treatment chamber. An effort has been made to minimize this variation by keeping the spacing between the walls as small as possible. However, as this intensity is naturally higher near the lamp at (d) than it is at (D), the outside wall, the latter value has been used in the calculations of (I_T). This, together with the exposure time, should give the minimum lethal dose of a given wave length for a given species of bacteria.

As has been pointed out, the lamp operates on low power and so the glass surface does not rise more that 5° or 6°C. above room temperature. Direct killing by heat should not then be an important factor in the action of this lamp.

In this method of measurement the bacteria are observed and treated under similar conditions of air suspension to those experienced in the operating room. This should make the data useful in any such air problem and render it free of certain common drawbacks of the usual method of plate exposure. There is no complication of the results by such issues as photochemical alteration of the supporting media.

Another phase of bacterial resistance to light and one which some authors (Wells and Wells, 1936) have considered of some importance is presented by the characteristic grouping of organisms. In the case of *Staphylococcus albus* the characteristic grape-like clusters which this organism forms have been said to contribute greatly to its resistance to ultraviolet rays of low penetrating power. In the present method of observation it seems that there are few clusters that are not broken up by the fine, high speed spray jets. The glass slide exposed to the

exhaust air caught only well scattered organisms alone and in pairs.

SUMMARY

Features of the experimental method:

1. Broth cultures of bacteria are atomized into the air.
2. The mixture of bacteria and air is passed through a tube where it is exposed to ultraviolet light.
3. The air is then passed through nutrient broth which can be plated out and count made or merely incubated and examined for growth.
4. Measurements are made of:
   (a) Lamp current—to calculate treatment intensity (I_T).
   (b) Air velocity—to calculate exposure time (T).
5. Energy received per unit area by the bacteria is given by the product of $I_T \times T$ = Energy.

Preliminary results using this method on *Staphylococcus albus* have shown the energy necessary to kill all of the organisms was approximately 26,200 ergs per square centimeter. This is in good agreement with the current work using the method of plate exposure.

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