RELATION BETWEEN THE DEPTH DISTRIBUTION OF IONIZATION AND THE LETHAL EFFECTS ON BACTERIA

Thesis for the Degree of Ph. D.
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J. Leon Newcomer
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Relation between depth distribution of ionization and the lethal effects on bacteria.

presented by

J. Leon Newcomer

has been accepted towards fulfillment of the requirements for

Ph.D. degree in Agricultural Engineering

Carl W. Hall
Major professor

Date Feb. 25, 1957

RELATION BETWEEN THE DEPTH DISTRIBUTION OF IONIZATION AND THE LETHAL EFFECTS ON BACTERIA

by

J. Leon Newcomer

AN ABSTRACT

Submitted to the School of Advanced Graduate Studies of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Agricultural Engineering

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Approved by Carl N. Hall Jr. 25 '57

ABSTRACT

Numerous investigators have shown that cathode rays exhibit a lethal effect on bacteria. The lethal action is attributed to ionization occurring within the substance irradiated. Since the rays are able to penetrate matter, it has been proposed that cathode rays be used to sterilize foods.

The distribution-in-depth of the ionization within a substance irradiated by cathode rays, determined by absorption methods, is known to be non-linear.

The investigation was conducted to ascertain the distribution-in-depth of the lethal effect of one million electron-volt cathode rays on bacteria and to compare the characteristics of the distribution with the ionization-in-depth curve obtained by absorption methods. The test organism used was Serratia marcescens.

The depth distribution of the lethal effect was found to be non-linear. The general features of the lethal curve were comparable to the absorption curve. It was shown that the lethal effect of one million electron-volt rays was disproportionate to the dose administered. The absorption curve alone is not a reliable measure of the lethal effect within a substance.

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ACKNOWLEDGMENTS

Although bibliographies and footnotes indicate many of the sources of information, such devices can not do justice to all persons who have been both instrumental in guiding our thinking and helpful throughout the project. It is a pleasure to note a few of these persons here by name.

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candidate for the degree of

Doctor of Philosophy

Final examination, February 25, 1957, 10:00 A.M., Conference Room, Agricultural Engineering Building

Dissertation: Relation between the Depth Distribution of Ionization and the Lethal Effects on Bacteria

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INTRODUCTION

It has been observed that α and β particles, γ-, X- and cathode rays and ultraviolet light exhibit a lethal effect on biological systems. These radiations penetrate matter to varying depths and cause ionization within the substance. The lethal effect is attributed to the ionization.

It was observed early that although X-rays exhibited a lethal effect on biological systems, the efficiency was only approximately 5 percent. Ninety-five percent of the energy of the electrons was dissipated in the form of heat from the target. By removing the target and permitting the electron beam, (cathode rays) to impinge upon the substance, not only were there lethal effects but also considerable improvement in beam utilization.

Insofar as cathode rays have been proposed as a means for killing bacteria within foods and food products, it becomes significant to evaluate their effectiveness as a lethal agent.

Two aspects of the problem must first be briefly reviewed, namely, 1) the manner in which bacteria respond to a lethal agent, and 2) the depth distribution of ionization within a substance irradiated by energetic electrons.

When bacteria are subjected to any lethal agent, such as heat, disinfectants, X-rays, cathode rays etc., they do

not all die at once, but a constant fraction of those present dies in each increment of time. The fraction of the number initially present which survives at any given time is called the survival ratio. The fraction killed is one minus the survival ratio. The survival ratio is an exponential function of the time of exposure and the intensity of the lethal agent. It takes the form

$$N = N_o e^{-KIt} \qquad \text{.....(1)}$$

where N_o = the number of bacteria initially present

N = the number surviving at time (t)

t = the time of exposure to the lethal agent

K = a constant

I = the intensity of the lethal agent

The effectiveness of the lethal agent is accordingly expressed in terms of the dose required to produce a given survival ratio. A dose of $I \times t = 1/K$ reduces the exponent of e to -1 , and the corresponding survival ratio is $1/e = 0.368$ or 36.8 percent. The dose required to give a survival ratio of 36.8 percent is called the mean lethal dose, lethal exposure, or inactivation dose. Usually, the whole number, 37 percent, is used.

Within wide limits the Bunsen-Roscoe reciprocity law (13) applies to the killing of bacteria. That is, a given dose results in a given survival ratio, regardless of whether the dose consists of a low intensity for a long time or a high

intensity for a correspondingly shorter time. For bacteria, this law has been found to apply over a thousandfold range in intensity. Probably the failure of the law at very low intensities is because the exposure time becomes so long that bacteria reproduce during the time of exposure.

There is no dispute that ionizing radiations can produce a lethal effect on bacteria but there is considerable disagreement among biologists regarding the exact mechanism of the cause of death. A number of theories have been proposed and presently a preponderance of experimental evidence supports the "single hit" theory. It is characterized by the following: 1) the survival curve is exponential, 2) destruction is independent of dose rate, 3) destruction is independent of temperature, and 4) the concentration of organisms does not effect the percentage survival.

Rahn, especially, treated the theoretical aspects as well as the experimental evidence in a comprehensive manner and concluded that so far as single-celled organisms were concerned, the single hit theory is the only plausible explanation for the fact that the survival curve is exponential.

Although this work is not especially concerned with the exact mechanism of death, some attention must be given to the shape of the survival curve insofar as it indicates that the death of a bacterium is evidently due to the energy dissipated by the radiation in the bacterium itself and is not an

an indirect action due to the dissipation of energy in the surrounding media.

For this purpose it will be more convenient to express equation (1) in the following form:

$$N/N_o = e^{-D/Do} \quad \text{.....(2)}$$

where N/N_o = the survival ratio

N_o = the original number of bacteria

N = the number of survivors of dose D

D_o = dose at which there is an average of one effective hit per organism.

Cathode rays consist of parallel beams of high velocity electrons, having attained their high velocity from being accelerated through a high electric potential. Thus, they possess kinetic energy and are able to traverse matter. The depth of penetration (range) of an energetic electron is a function of its initial kinetic energy and the density of the substance.

In their passage through matter, cathode rays (primary electrons) ionize atoms along their paths. The electrons which are ejected in this process, called secondary electrons, may themselves possess sufficient energy to ionize still other atoms. If the substance is sufficiently thick, i.e., equal to or greater than the maximum range of the electrons the primary electron plus all its secondary electrons will eventually lose energy and be absorbed within the substance.

The mass of the electron is very small compared to the mass of the atoms with which it collides, consequently, its path is a tortoruous one. Not all electrons travel the maximum range but dissipate their energies at different depths throughout the substance. Therefore, the depth distribution of ionization within a substance being bombarded with cathode-rays would not be expected to be uniform.

The usual procedure for determining the actual depth distribution of ionization is to place varying thicknesses of an absorber between a source of cathode rays and an ionization chamber and to relate the measured activity to the thickness. This relation is called an absorption curve. Absorber thicknesses are usually reported in terms of areal density (grams per square centimeter, etc.) instead of actual thickness. This is done partly because it is easy to find areal density by weighing thin foils but more importantly because thicknesses so expressed are roughly independent of the nature of the absorber.

Figure 1 shows the distribution of ionization-in-depth in aluminum produced by mono-energetic cathode rays of different initial energies. It will be observed that the relative ionization increases from approximately 60 percent at the surface of the absorber to a maximum of 100 percent and then falls off rapidly to zero at the maximum range for the particular energy level. Although the maximum range for an

electron is determined by its initial kinetic energy, the ionization distribution depends upon the irregular paths of the primary and secondary electrons.

A resume of the foregoing discussion focuses attention upon the following observations: 1) cathode rays act as a lethal agent on bacteria, 2) the lethal action is associated with ionization, 3) the depth of penetration of cathode rays is a function of their initial kinetic energy and the density of the absorber, and 4) the distribution of ionization within an absorber is non-uniform.

If foods are to be sterilized by irradiation with cathode rays, then the significance of such non-uniform distribution of the lethal agent becomes evident. This investigation was undertaken to determine the depth distribution of the lethal effect of cathode rays within an absorber.

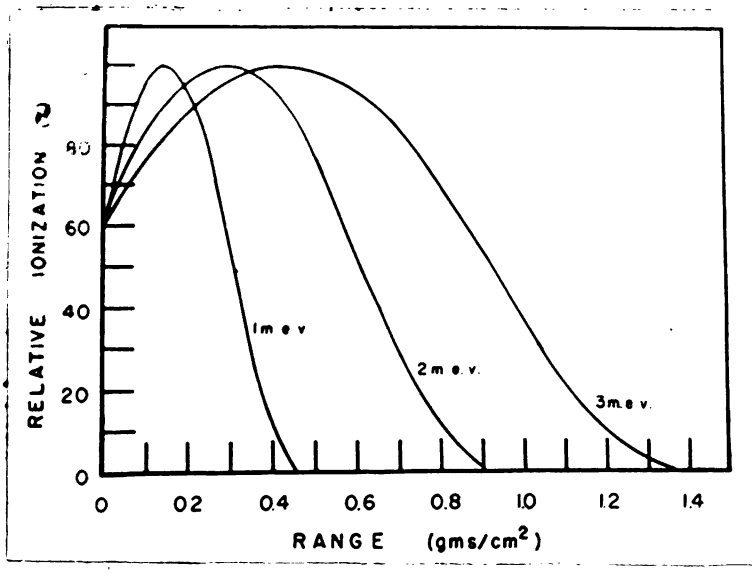

Fig. 1. Relative ionization densities of 1, 2, and 3 mev. electrons in matter (23).

REVIEW OF LITERATURE

The bactericidal effects of ultraviolet light were observed as early as 1903 (Rentschler et al., 20), but they were not studied with any degree of quantitative accuracy until recent years. Gates (8, 9) showed that the wavelength for germicidal action was 2660 Å. He showed, further that bacteria exhibited widely different resistances to ultraviolet radiation, depending on the different stages in their life cycles. A sublethal dose was shown to retard the rate at which colonies developed after irradiation.

Soon after the discovery of radium and X-rays, investigators in the field of bacteriology became interested in the effect of these radiations on microorganisms (Pacinotti and Porcelli, 1898); Prescott, 1904). Schmidt (21) studied the effects of radioactive phosphorus (P^{32}) on E. coli. He found that the β radiations of P^{32} had a lethal effect on E. coli and that, in general, this effect was related to the initial concentration of the P^{32}.

X-rays have been of interest to the engineer, the chemist, the bacteriologist, and the food technologist almost since their discovery by Roentgen in 1895. Dunn et al. (6) determined the effects of high voltage X-rays on a number of

bacteria, yeasts, and molds. They found that these microorganisms could be destroyed by X-rays and that the dosage necessary for destruction varied with different types of microorganisms.

The mechanism of X-ray destruction of bacteria is explained according to the "direct hit" theory, also called the "target" or "Treffler" theory. This theory, which is thoroughly discussed by Lea (15), states that bacteria are destroyed by direct hits of the photons on the cells. On the assumption that the destruction of bacteria is brought about by direct hits, the survival curve would be exponential and therefore a straight line when plotted on semi-logarithm paper. According to Lea (15) and Rahn (19) the destruction of bacteria by photons is based on the mathematical probability of a direct hit taking place.

Proctor, Van de Graaff, and Fram (18) found that ground meat could be sterilized by irradiation with high voltage X-rays. Since X-rays produced at high voltage are able to sterilize food materials without raising the temperature to an appreciable extent, this might appear to be an efficient method of "cold" sterilization. However, approximately 95 percent of the electron energy goes into heat in the X-ray equipment when it strikes the target interposed between the electron source and the object irradiated and only approximately 5 percent into the production of X-rays. Hence, interest has

more recently been directed to high speed electrons, usually called cathode rays.

In recent years, several types of apparatus for producing high voltage cathode rays have been developed. To name a few: Van de Graaff Electrostatic Generator (Trump et al., 1948), Capacitron developed by Brasch and Huber (3), and the resonant transformer type (Charlton et al., 1940) which shall be more fully described later.

Coolidge and Moore (5) report that as early as 1926 cultures of S. aureus, B. coli, B. subtilis and B. prodigiosus were killed by short exposures to cathode rays. Wyckoff and Rivers (25) reported that the absorption of a single 155 kilovolt electron was sufficient to cause the death of B. coli and B. aertryke and that all, or nearly all the electrons were lethal to the bacteria. Dunn et al. (6) showed that energetic electrons are capable of destroying bacteria.

According to Dunn, Campbell, Fram and Hutchins (6) high energy cathode rays offer an effective means of producing biological, bacteriological, and even chemical changes on a practical scale. They report that these changes are brought about by excitation and ionization of constituent atoms of the absorber.

Several investigators have studied the effect of temperature upon irradiation. Rentschler et al. (20) report temperature has little if any effect on radiation between 5°C. (41.0°F.) and 37°C. (98.6°F.). Bachofer (1) experimented

in the same temperature range and concluded that temperature has no effect on the dose inactivation constant of E. coli by X-rays.

Koller (13) reported that when bacteria are subjected to any lethal agent, such as heat, disinfectants, X-rays, ultraviolet light, and β rays they do not all die at once but a constant fraction of those present dies in each increment of time. The fraction of the number initially present which survives at any given time is called the survival ratio (N/N_o). The survival ratio is an exponential function of time of exposure and the intensity of the lethal agent. It takes the form:

$$N/N_o = e^{-KIt} \qquad .....(3)$$

where N_o = the number of bacteria initially present

N = number surviving after time (t)

t = the time of exposure to the lethal agent

K = dose inactivation constant

I = the intensity of the lethal agent

The dose is the product of the intensity of the radiation and the exposure time. Koller (13) further reports that within wide limits the Bunsen-Roscoe reciprocity law applies to the killing of bacteria. The Bunsen-Roscoe reciprocity law states that a given dose results in a given survival ratio, regardless of whether the dose consists of a low intensity for a long time or a high intensity for a correspondingly shorter

time. For bacteria, this law has been found to apply over a thousand fold range in intensity.

Lea (15) concluded that the survival of bacteria is not dose-rate dependent. This fact has been confirmed with E. coli over a dose range of 8,000-fold (2). These results are similar to those reported by Hollaender, et al. (12) for X-rays.

The inactivation of bacteria by ionization is not clearly understood. Although first regarded as actual killing of the organism, it is now generally regarded to be a process which prevents the multiplication of the cells so that normal dying occurs before multiplication has taken place. This theory is thoroughly discussed by Lea (15) who considers the lethal action (in single-celled organism, e.g., bacteria) to be a "lethal mutation." Since it had been determined that the survival of bacteria is not dose-rate dependent, Lea (15) and others have emphasized that not only is the survival curve exponential but that consequently the "single hit" theory is valid.

It has been pointed out that the most convenient method for expressing the sensitivity of organisms to irradiation is as "mean lethal dose," i.e., the dose necessary to reduce the number of survivors to 37 percent of the original. The relation is expressed by

$$N/N_o = e^{-D/D_o} \qquad \ldots\ldots(4)$$

where N = number of survivors of dose D

N_o = original number of bacteria

D_o = dose at which there is an average of one effective hit per organism

Although Lea proposes the "single hit" theory he recognizes the possibility of other mechanisms as well. However Rahn (19) proceeds to bring considerable experimental evidence to bear in favor of the single hit theory in the case of one celled organism and relegates the other theories concerning the mechanism of death to multi-cellular organisms. He concluded that a logarithmic order of death can be obtained only if the death of the bacteria is brought about by the reaction of a single molecule. The logarithmic order of death is entirely impossible if more than one molecule must be inactivated to produce the death of the cell. The logarithmic order of death of bacteria can be established only by counting the survivors, and the only practical method in use is the plate count method which gives the number of colonies developing from a known volume of bacterial suspension. The number of colonies represent the number of original bacteria only when the bacteria are single. Clumping should be avoided.

It has been demonstrated by Sherman and Albus (22) that young bacterial cells succumb more readily to harmful influences than old cells. The term "old cells" is applied to cells of cultures which have nearly or completely reached the maximum population.

According to Rahn (19), another characteristic of the direct hit theory is the concentration of organisms does not effect the percent survival. Lea (15) states that the fraction of the organisms in an aqueous suspension which are killed by a given dose of radiation is independent of the concentration of the organisms in the suspension, indicating that the death of a bacterium is due to the energy dissipated by the radiation in the bacterium itself, and is not an indirect action due to the dissipation of energy in the water.

Occasionally survival curves deviating systematically from the exponential shape have been reported. Gates (8) explains that the deviation takes the form of the fraction of organisms killed by a given increment of dose being less at small doses than at large doses, so that a sigmoid survival curve is obtained. It is probable that exponential survival is the typical result, and that the occasional sigmoid curve is due to some disturbing factor. Rahn (19) mentions that one such disturbing factor is clumping of the organisms. If the proportion of organisms which survive a given dose is the exponential function e^{-x}, where x is proportional to the dose, then the probability that an individual organism be killed by this dose is $(1-e^{-x})$. But if the organism exists in clumps of n individuals, the probability of all n organisms of a clump being killed will be $(1-e^{-x})^n$. Therefore the proportion of the clumps which produce colonies after

a dose proportional to x will be $\left[1-(1-e^{-x})^{n}\right]$. This function represents a sigmoid curve, not an exponential one.

Trump and Van de Graaff (24) report that the photochemical and the biological reactions produced by the absorption of X-rays and cathode-rays are similar in their physical nature and require closely equivalent energies. In cathode-ray irradiation, electrons are directly projected into the material and produce ionization similar in its general characteristics to that produced by X-rays wherein the energetic electron originates within the absorber. Each high energy electron in the cathode-ray stream proceeds into the material losing energy by collision with atoms in its path. These primary electrons thus distribute the energy of cathode rays through the volume of the absorber. Many of the secondary electrons produced in these encounters may themselves possess sufficient energy to act as biological agents by the ionization of other atoms. They further report that in the case of initially parallel cathode rays of homogeneous energy, the maximum ionization density produced in an absorber occurs at about one-third the maximum range. This location of the region of maximum ionization toward the forward part of the maximum range for any given voltage is due to the naturally high scattering tendencies of electrons.

Absorption of ionizing radiations has been measured by numerous investigators using various absorbing substances. It is reported by Halliday (11) that electrons involved in

radioactive decay (0.05 to 10 mev.) are penetrating enough so that solid absorbers are convenient. Aluminum is the usual choice, although mica, cellophane or colloid films are useful at very low energies. The technique is to place absorber foils between a source and a thin-windowed detector, usually a Geiger counter or an ionization chamber. By relating the activity to the thickness of the absorber, for various absorber thicknesses, the relative distribution-in-depth can be determined. For electrons (cathode rays) there is a maximum absorber thickness, beyond which electrons will not penetrate. This thickness is called the "range." According to Glendenin (10) absorber thicknesses are often given in terms of an areal density (grams per square centimeter etc.) partly because it is easy to find this quantity by weighing thin foils and partly because thicknesses so expressed are roughly independent of the nature of the absorber.

The penetration (maximum range) of cathode rays into matter can be determined by the following equation (Evans, 1947).

$$R_{max} = 0.54E - 0.15 \qquad .....(5)$$

where R_{max} = the maximum range expressed in grams per square centimeter

E = the voltage expressed in megavolts

Cathode rays may be either monoenergetic or non-monoenergetic depending upon the characteristic of the accelerating

potential. In either case the distribution of energy within an absorber has been found to be non-uniform.

The non-uniform distribution of ionization with depth and the maximum range for an absorber (aluminum) is shown in Figure 1 by Trump et al. (23). Each of these curves represent monoenergetic, cathode rays at the three different accelerating potentials (1, 2 and 3 mev.). The range for each energy is found to agree very well with the empirical equation reported by Evans (7).

The resonant transformer type of electron accelerator, used in this investigation, produces non-monoenergetic cathode rays. The accelerating potential is supplied by a transformer having a 1 mev. peak voltage, 180 cycle per second, sinusoidal wave form. Figure 2 shows the ionization distribution-in-depth for this apparatus plotted from data furnished with the machine by the manufacturer. Their data were obtained by absorption procedure using aluminum foil absorbers.

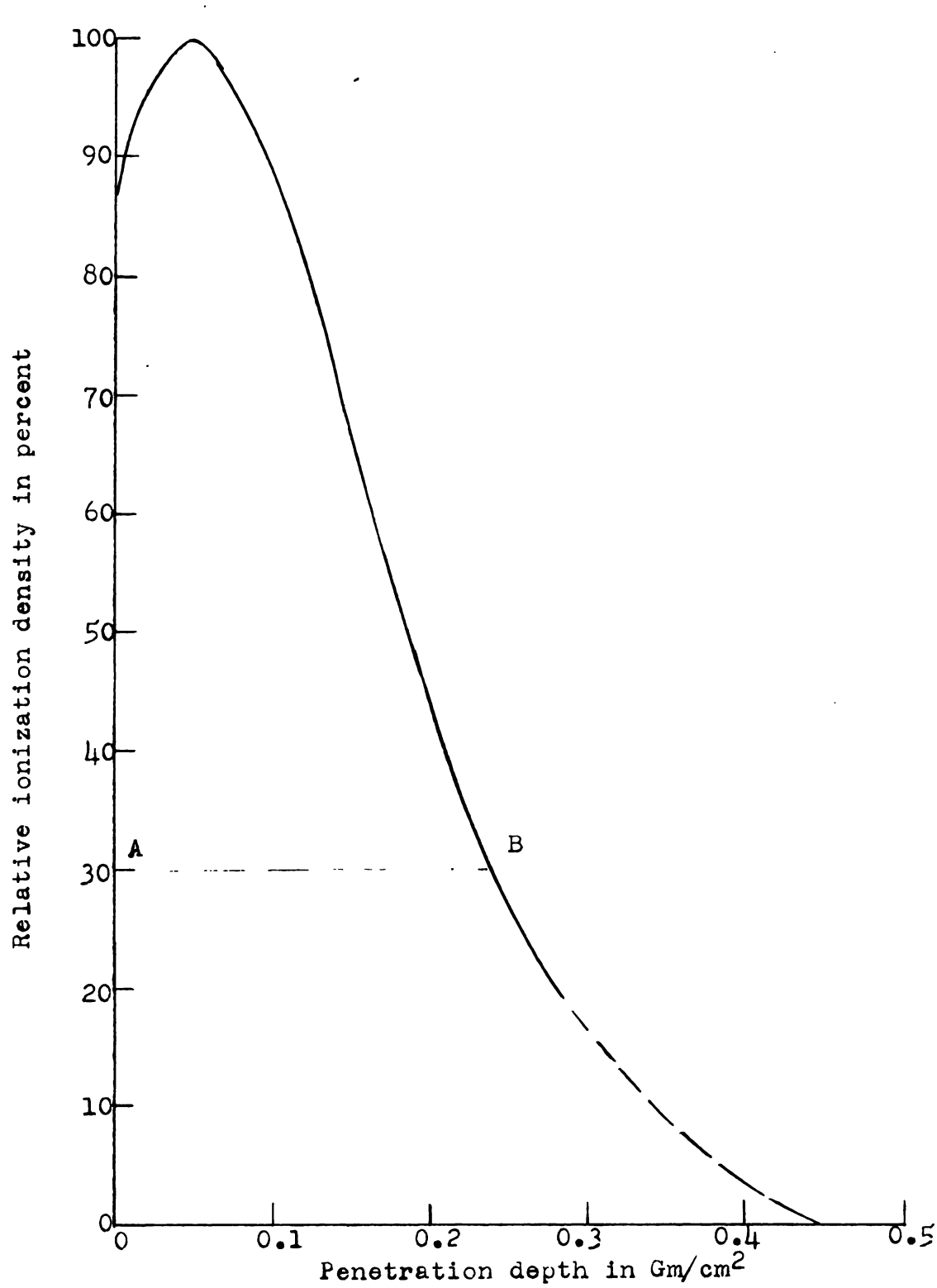

Fig. 2. Relative ionization density of 1 mev. non-monoenergetic cathode rays in matter.

OBJECTIVES

1. To determine the depth distribution of the lethal effect of one million electron-volt cathode rays on bacteria.

2. To compare the depth distribution of the lethal effect with the relative ionization-in-depth curve obtained by other methods.

APPARATUS

Cathode Ray Unit (Electron Beam Accelerator)

The source of cathode rays was a one million electron-volt, resonant-transformer type of electron accelerator, manufactured by General Electric Company. The apparatus has three basic electrical components: the transformer and tube unit, control stand, and motor-generator set.

Figure 3 shows a cutaway view of the transformer and cathode-ray tube unit assembled within the steel housing. Flat pancake coils form the transformer primary and the tuned secondary. The secondary coils are connected by pressure contacts through flat phosphor-bronze terminals; spacing allows for cooling, and decreases towards the top to maintain the required potential gradient. Each coil develops about 10,000 volts. Filament emission and current in the central cathode ray tube is controlled by a variable reactor in a coil that inductively couples the filament to the primary. The entire unit is mounted in a steel tank; shielded with overlapping silicon iron strips to prevent eddy current energy losses. The secondary (high voltage) circuit is tuned to resonance at 180 cycles per second. Power for the transformer is obtained from a synchronous motor-generator set. In the twelve-section, permanently evacuated, cathode ray tube, electrons are produced by a hot filament, accelerated through the tube by

intermediate electrodes connected to the terminals of the secondary, and focused by a coil into a beam that passes through a window to the outside air and irradiation area.

It was mentioned in the Review of Literature that cathode rays may be either monoenergetic or non-monoenergetic depending upon the nature of the accelerating potential. Electrons accelerated by a constant potential are monoenergetic. An absorption curve for these is shown in Figure 1.

The accelerating voltage in the cathode-ray apparatus used for this work varied sinusoidally, consequently the electron beam is non-monoenergetic. Figure 2 shows the relative ionization densities for one million electron-volt electrons. This curve was obtained by absorption procedure using aluminum foil absorbers.

Irradiation-Absorption Chamber

A chamber, shown in Figure 4, consists of a plastic (plexiglass) holding fixture (A) in which are assembled alternate layers of bacteria-laden filter discs (B) and thin sheets of polyethelene separators (C). This laminated stack of 20 filter discs and 20 separators form a cathode-ray absorber wherein ionization occurs. The total depth of the stack exceeds the maximum range for the most energetic electron. By depositing equal bacterial populations on each disc and keeping each disc isolated from adjacent ones by the polyethelene separators, the lethal effect of ionization at various depths could be determined.

The depth of the chamber in which the stack of filter discs and separators fit was 4.0 millimeters. The membrane filter discs shown in Figure 5, used to retain the bacteria, were 47.0 millimeters in diameter and 0.129 millimeters thick. When an aqueous suspension of bacteria was filtered through these discs, the organisms were retained. A grid is printed on the discs to facilitate colony counting. The separators, cut from sheet polyethelene, measure 47.0 millimeters in diameter and 0.0559 millimeters thick.

A stack of 20 discs and 20 separators weighs 7.703 grams, when the discs were saturated with aqueous suspension. The bulk density of the water saturated discs and separators, was 1.20 grams per cubic centimeter. The thickness of one separator and one saturated disc, when using areal density as a measure of thickness, was 0.02220 grams per square centimeter. The thickness of the entire stack of 20 separators and 20 filter discs then becomes 0.4440 grams per square centimeter. A chamber loaded with filter discs and separators is shown in Figure 6.

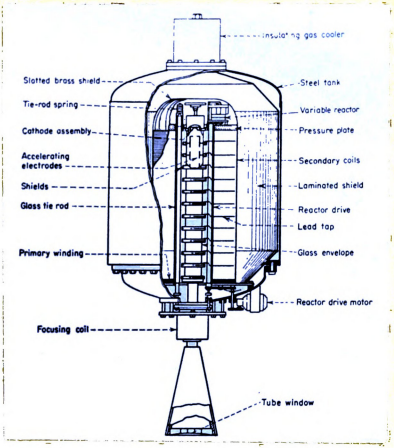

Fig. 3. Cutaway view of a 1 mev. cathode ray apparatus.*

*Permission to reprint this drawing was granted by General Electric Company by letter from their Milwaukee office, 1-21-57.

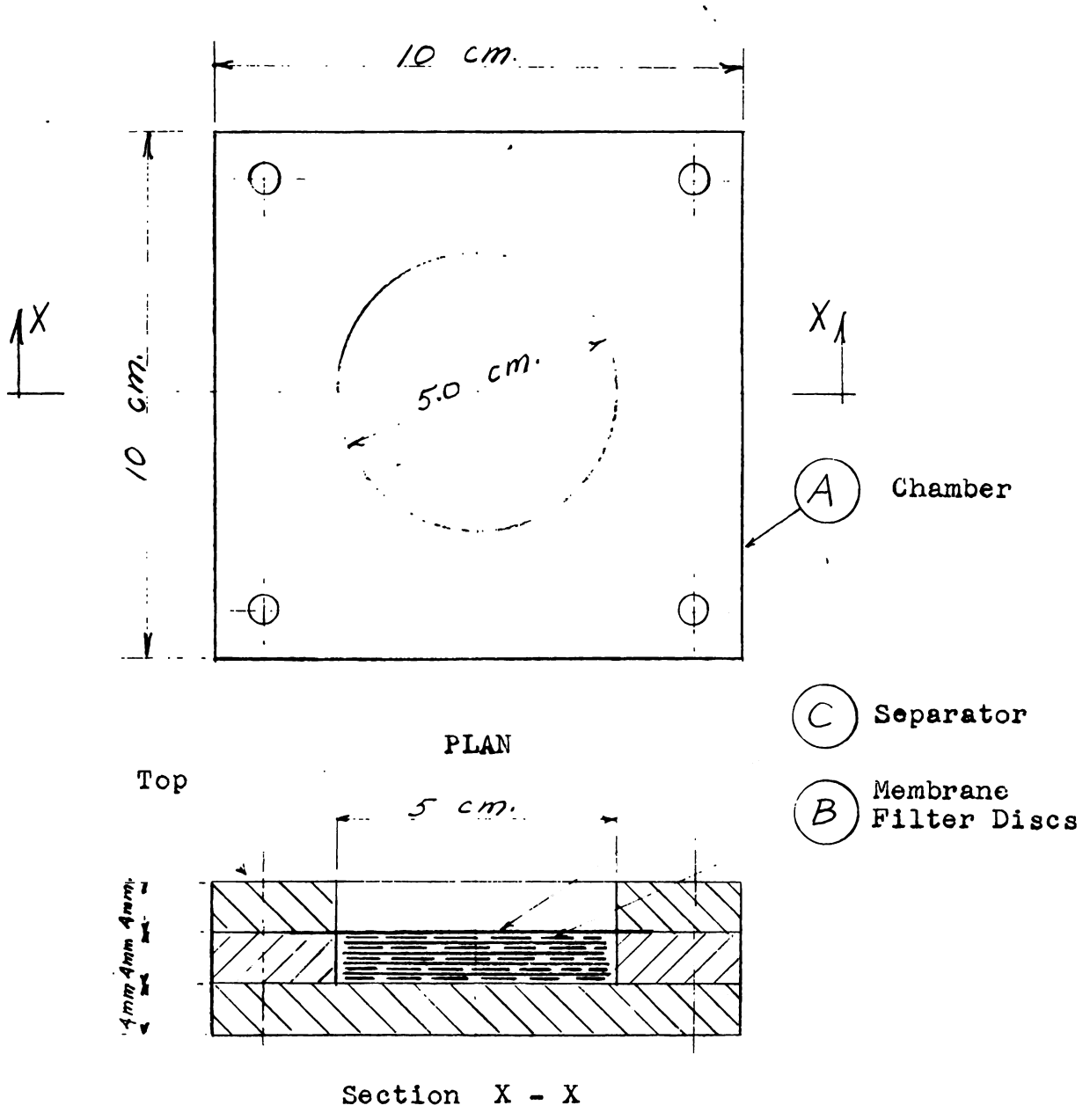

Fig. 4. Irradiation-absorption chamber

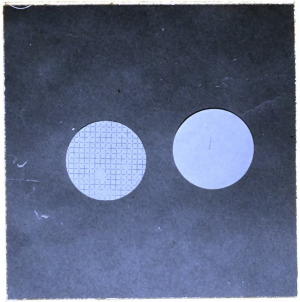

Fig. 5. Membrane filter disc.

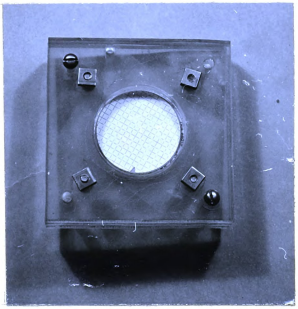

Fig. 6. Irradiation-absorption chamber loaded with bacteria-laden filter discs.

PROCEDURE

Determining the distribution-in-depth of the lethal effect involved four main steps: 1) preparing the culture, 2) preparing the irradiation-absorption chamber, 3) irradiating the organisms, and 4) incubating and counting the colonies.

Preparing the Culture

The test organism selected for this work was Serratia marcescens, which produces a red pigment. It was chosen because it is nonpathogenic and possesses a distinctive color. The numerous steps in the procedure made contamination of the filter discs a likely possibility. To obviate this difficulty the color characteristic was relied upon to distinguish the test organism from contaminants.

Organisms from refrigerated cultures were transplanted into 5 milliliters of nutrient broth in test tubes. These were shaken on a Burrell, wrist-action shaker at 28 to 39°C (82.4° to 86.0°F.) for 8 hours. One milliliter was transferred to another fresh test tube containing 5 milliliters of nutrient broth. This was shaken for 24 hours at 28-30°C. A one to 20 million dilution in phosphate buffer was used as the stock inoculum. This technique produced a bacterial population of approximately 80 to 120 organisms per milliliter of solution. Three milliliters of the stock solution were filtered

through the filter discs. Tests on the filtrate indicated 100 percent retention of the organisms on the filter disc.

Preparing the Chamber

Alternate layers of bacteria-laden filter discs and sterilized polyethylene separators were placed in the chamber. All filter discs were placed in the chamber with their inoculated surface towards the chamber window. In this position, the cathode rays were incident to the inoculated surfaces. The pressure was just sufficient to hold the laminae firmly together, yet not tightly enough to force solution from the discs. The back of the chamber was then fixed into place. Thus, the chamber when loaded with 20 filter discs and 20 separators constituted a bacteria-laden, cathode ray absorber. The depth distribution of the lethal effect of cathode rays could be ascertained by comparing the survivors in each layer to the number of bacteria in non-irradiated control discs. A loaded chamber is shown in Figure 6.

Irradiating the Organisms

The chamber was positioned in the cathode ray beam with the chamber window facing the beam source. In this position the cathode rays entered the laminae through a polyethylene sheet 0.0559 millimeters thick before impinging on the first disc. The chamber rested on a wooden block 1.0 inch thick to minimize any back scattering of electrons that might occur.

Several preliminary tests were made to ascertain the optimum dose. The ideal dose was that at which some organisms were killed throughout the stack except on the 19th and 20th filter disc which were farthest from the beam source and beyond the maximum range for one million volt electrons. The optimum dose was found to be 5000 rep.

This dose was administered by locating the chamber 47 centimeters from the cathode ray machine window. The central axis of the chamber was parallel and in alignment with the longitudinal axis through the cathode ray tube. In this position the beam was incident at a right angle to the face of the chamber and filter discs. The beam-out current was maintained at 10 milliamperes for 10 seconds. The tube potential was one million volts peak.

Incubating and Counting Colonies

After the chambers were irradiated, they were disassembled and the filter discs were placed in miniature Petri dishes on pads moistened with 2 milliliters of nutrient broth. The plates were incubated at 28-32°C for 48 to 72 hours. Colonies were counted at 48 hours and checked at 72 hours. The only noticeable difference at 72 hours was a more pronounced pigmentation and slightly larger colonies. Although a pigmented organism was selected to obviate contamination difficulties no such problem was encountered.

DATA AND DISCUSSION OF RESULTS

Ten irradiation-absorption chambers, each prepared as described in the Procedure were irradiated by one mev., non-monoenergetic catnode rays at a dosage of 5000 rep., at the incident surface. The colonies, which developed upon each filter disc were counted and related to the colony count of the controls. The percentage of the bacteria surviving on each disc within each chamber is reported in Table I. The ten experiments are noted as A through J. Since this work concerns the lethal effect at various depths within an irradiation-absorber, the depth from the face of the chamber to each inoculated filter disc is also given in Table I.

The observations are shown graphically in Figure 7. The experimental data are reported in percent of bacteria surviving. These were converted to percent destroyed and both the relative survivors and the relative bacteria destroyed were plotted against the depth.

In Figure 8 are shown 15 filter discs bearing bacterial colonies. The top left one is a top filter disc, the next one in the top row is the next disc below it in the chamber and so on, counting from left to right. Some of the discs in the bottom strata of the chamber are not shown since their appearance is similar to the last two or three in the series.

Two objectives were established, namely, 1) to determine the depth distribution of the lethal effect of one million electron-volt cathode rays on bacteria, and 2) to compare the depth distribution of the lethal effect with the relative ionization-in-depth curve obtained by absorption methods. These shall be discussed in that order.

In Figure 7 it is observed that the relation between the percentage of the bacteria destroyed and the depth is non-linear. At the surface, 95.5 percent were killed, increasing to a maximum of 96.5 percent at approximately one-fifth of the depth of the absorber, and thereafter the percentage killed gradually decreased to zero at the maximum depth.

The calculated maximum range for 1 mev. cathode rays, using equation (5), was found to be 0.39 grams per square centimeter, whereas the curve shown in Figure 7 indicates the range to be approximately 0.43 grams per square centimeter. There are three main reasons for this apparent discrepancy. Equation (5) is an empirical one and may not accurately give the maximum range for electrons having 1 mev. kinetic energy. Although the accelerating potential applied to the catnode-ray tube was reported as one million volts, this value can vary, plus or minus, 50,000 volts (5 percent) owing to uncertainties in reading the volt meter. Additionally there were chances for errors in counting colonies when the number of colonies was large. The latter reason is suspected to be of greater importance. Observe the scatter of the counts in Figure 7,

especially from point B′ to the point where the curve intersects the depth axis. There are at least two reasons for widely varying observation in this area, namely coincidence of multiple bacteria producing only one colony and starving of the bacteria. The former is obvious. The latter, although perhaps more subtle, is in evidence from Figure 8. Note that as the colony count increased beyond a certain population their size was diminished. The food supply was fixed by the quantity of nutrient capable of reaching each cell. When the population was large some organisms starved and did not develop into colonies.

The problem of accurately accounting for all bacteria was anticipated early in the experimental procedure and every precaution was exercised to minimize it. Such precautions were limited, however, since when the discs were inoculated with too few bacteria so few survived the maximum dose that inaccurate percentages resulted, yet when the initial population was large the difficulty referred to above was experienced. Since the main interest concerned the upper portion of the curve it was decided to tolerate the troublesome situation in the lower portion.

The curve in Figure 7 was drawn through the average of the observations at each depth. From the above discussion it becomes obvious that a curve falling somewhere below the drawn curve, in its lower portion, would more nearly represent the bacteria killed by cathode rays alone. Also such a proposed

displaced curve would probably intersect the depth axis at a point more nearly in agreement with the calculated maximum range. However, it was believed that the evident discrepancy would not effect the qualitative aspects of the work.

Before continuing with the discussion of the first objective it will be well to consider the second one. In Figure 2 is shown the ionization-in-depth relation for one million electron-volt, non-monoenergetic cathode rays emitted from the experimental apparatus. Observe that the percentage ionization increases from approximately 85 percent at the surface of the absorber to a maximum of 100 percent at near one-ninth of the maximum range, then decreases gradually to zero at the maximum range. The lethal distribution curve shown in Figure 7 possesses these same general characteristics, in that the lethal effect was less at the incident surface than at some distance within the absorber and thereafter decreased gradually to zero at or near the maximum range.

From a comparison of the general shape of the two curves it might be inferred that an absorption curve for a given cathode ray source is a faithful measure of the lethal distribution-in-depth to be expected within an irradiated bacteria-laden surface. There are, however, distinct differences between the two curves which cannot be overlooked.

The ionization curve exhibits a peaked upper portion while the lethal curve is much broader in that area. Though both curves diminish gradually from their maxima, the lethal

curve, as a consequence of its broader top, decreases more sharply. If the compensating curve proposed earlier were adopted the falling-off would be even more abrupt. It must be pointed out that the dashed line portion of the curve in Figure 3 was extrapolated beyond the available data. Here again this portion of this curve suffers from the same uncertainties as the experimental curve.

From the likenesses of the two curves it was inferred that the ionization curve was a measure of the lethal distribution, yet their differences, just pointed out, cast some doubt on such a conclusion. Superimposing one curve upon the other is meaningless and does not eliminate the dilemma. The lethal effect is related logarithmically to the ionization density (dose) by equation (2). The plot shown in Figure 9 was prepared, using equation (2) to relate the logarithm of the dose, taken from Figure 2 to the corresponding percent bacteria surviving, taken from Figure 7. The dose administered to the incident surface of the absorption chamber was 5000 rep. This is represented in Figure 2 as 85 percent relative ionization density. The dosages at other relative ionization density values were obtained. The following example is given to illustrate the procedure used to develop Figure 9. The dose represented by 30 percent relative ionization density in Figure 2 is 1765 rep. Line A-B was drawn on Figure 2. This dimension was transferred to Figure 7. The length of A-B fitted the curve in Figure 7 at A'-B' indicating that 37 percent of the

bacteria survived a dose of 1765 rep. The point representing 37 percent bacteria surviving for a dose of 1765 rep was plotted in Figure 9. This procedure was repeated at 5 percent relative ionization density intervals.

It is observed that this semi-logarithmic plot of dose versus percent bacteria surviving is not a straight line as predicted by the "single hit" theory. A number of reasons for this deviation must be explored.

Assume first that the "single hit" theory is a valid one. The digression from a straight line would occur if there were clumping of bacteria on the filter discs. In such a case when an ionization occurred within one organism its attached companion or companions might not experience a lethal influence. Even though a bacteria were destroyed a colony would develop and no accounting of the destruction would have been made. As a consequence the colony count would have been unduly great.

A direct hit supposedly destroys a bacterium. Bacteria are able to survive in a media which has suitable properties to sustain life. Consider what might happen when an ionization does not occur within an organism but in its near vicinity. Ionization can alter the properties of the environment. If this alteration changes the environment to one not conducive to the support of the bacterium it might die as an indirect result and not because of a direct hit. It could be argued that this too could be considered a "direct hit" in so far as one ionization destroyed one bacterium. But when two or

more bacteria, not clumped, but closely adjacent, were subjected to a poisonous environment and two or more were destroyed, the lethal effect would then be disproportionate to a "single hit" event. That is, the lethal effect would be unaccountably higher than predicted by the "direct hit" theory.

Consider next the "multiple hit" theory, wherein it is considered necessary that two or more ionization events must occur within a bacterium to produce death. It is obvious without belaboring the point that this would contribute to a colony count disproportionately low with respect to the single hit concept.

It was not an objective herein to lend support to or to take issue with any of the theories concerning the mechanism of death of bacteria subjected to the lethal action of cathode rays. The foregoing conjectures serve only to emphasize that there are evidently a number of subtle factors which can contribute to a disproportionate relation between the lethal dosage administered and the lethal results observed.

Regardless, the digression from a linear relation in Figure 9 implies that the ionization-in-depth curve obtained by absorption methods is probably not a reliable measure of the lethal distribution-in-depth inasmuch as this has been demonstrated for this test organism.

TABLE I

PERCENTAGE OF BACTERIA SURVIVING AT VARIOUS DEPTHS IN THE ABSORBER WHEN IRRADIATED AT A DOSAGE OF 5000 REP

Filter Disc Number	Depth to Face of Disc Grams/sq. cm.	A	B	C	D	E	F	G	H	I	J	Ave.
1	0.0104	5.8	6.3	10.0	5.2	8.2	9.0	3.4	5.4	4.9	7.2	6.54
2	0.0326	2.5	7.0	3.9	5.4	4.9	4.2	2.4	3.2	3.6	4.9	4.00
3	0.0548	3.9	4.2	3.2	3.9	2.8	4.0	1.2	1.7	1.2	2.5	2.86
4	0.0770	0	4.0	3.0	3.2	2.1	3.7	0	0	0.2	0.5	1.67
5	0.0992	2.5	2.7	3.5	2.2	1.2	3.5	0	2.4	0	0	1.80
6	0.1214	3.9	4.0	5.2	2.4	4.2	3.2	3.4	4.5	3.2	0	3.40
7	0.1436	2.8	5.5	4.4	3.5	---	3.9	3.2	5.9	3.2	4.9	4.14
8	0.1658	4.2	10.5	7.4	7.9	8.4	5.2	4.2	7.4	7.6	5.9	6.87
9	0.1880	6.4	11.8	12.1	8.6	10.0	8.7	6.4	3.1	12.2	9.6	8.89
10	0.2102	11.7	22.5	30.0	14.3	---	20.1	18.5	25.0	23.9	21.2	18.77
11	0.2324	10.6	22.5	30.0	---	21.2	32.0	19.6	30.5	27.4	24.0	24.20
12	0.2546	28.3	42.4	36.0	---	---	47.3	36.9	42.4	42.7	43.6	39.90
13	0.2768	24.0	68.5	44.0	50.1	52.0	61.2	46.0	58.1	46.5	50.0	50.04
14	0.2990	---	78.0	63.0	72.0	67.6	---	---	---	71.2	---	70.36
15	0.3212	46.6	82.0	72.0	80.0	83.5	---	---	---	---	---	73.22
16	0.3434	76.7	68.5	79.0	92.0	---	---	---	---	---	---	79.05
17	0.3656	88.8	100.0	75.0	---	---	---	---	---	---	---	87.93
18	0.3878	100.0	80.5	108.0	---	---	---	---	90.1	---	99.0	95.52
19	0.4100	99.0	99.0	78.0	---	---	97.0	81.0	97.5	101.0	102.0	94.31
20	0.4322	---	---	99.0	99.0	105.0	101.0	97.5	99.8	108.0	101.0	101.29

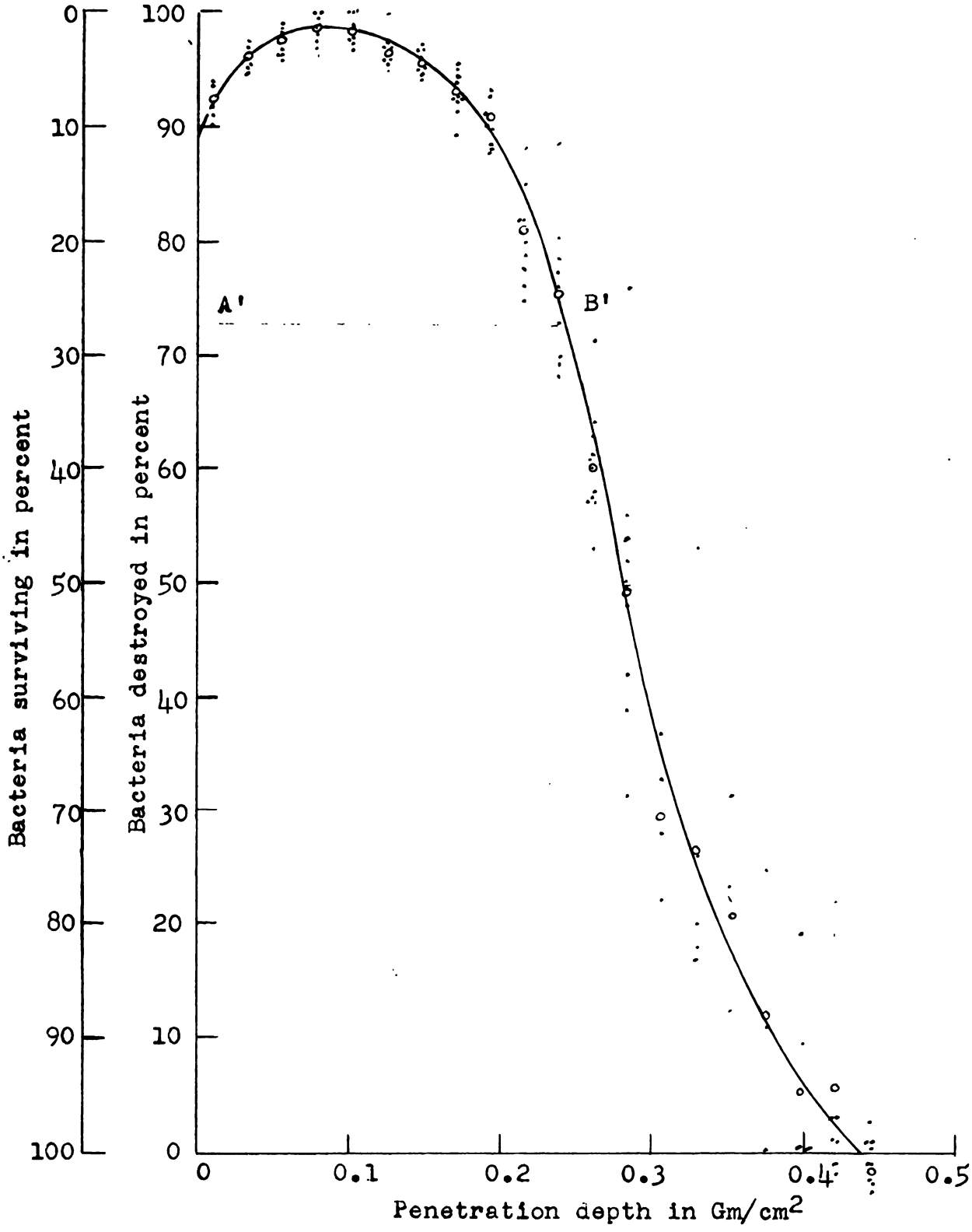

Fig. 7. Relative number of bacteria destroyed at various depths within the absorber.

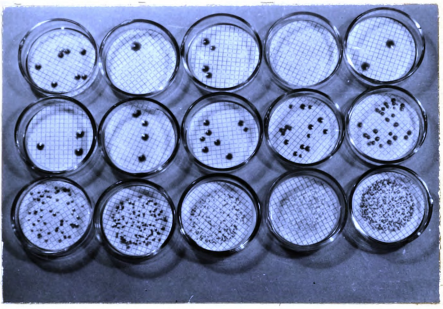

Fig. 8. Colonies growing on membrane filter discs. The number of colonies on each succeeding disc, reading from left to right and top to bottom, illustrates the relative survivors in adjacent increments of depth within the absorber.

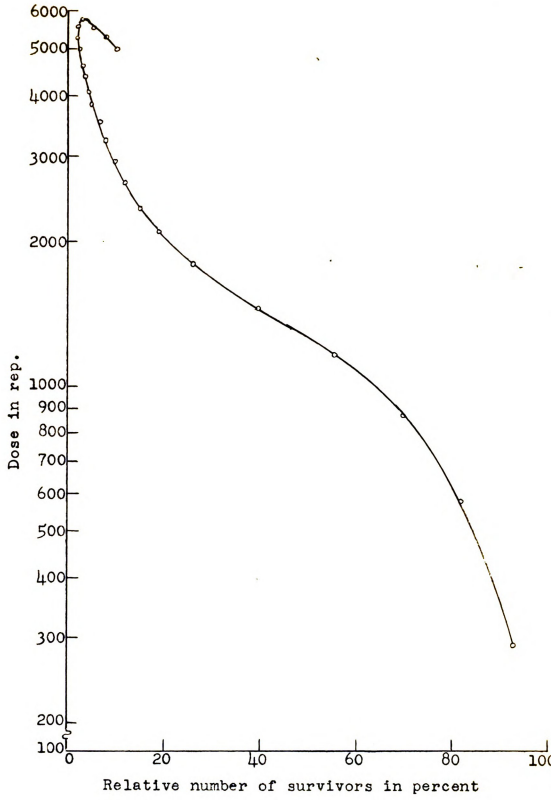

Fig. 9. Survival curve for S. marcescens irradiated with 1 mev. cathode rays.

CONCLUSIONS

1. One million electron-volt cathode rays exhibit a lethal effect on Serratia marcescens.
2. When a bacteria-laden substance is irradiated with one million electron volt, non-monoenergetic, cathode rays the depth-distribution of the lethal effect is non-linear.
3. The percentage of bacteria killed reaches a maximum at or near one-ninth the maximum range of the electron beam and then gradually decreases to zero at or near the maximum range for one million electron-volt cathode rays.
4. When the lethal distribution is compared to the ionization density distribution the lethal effect is disproportionate to the ionization density (dose).
5. Using S. marcescens for a test organism, it is indicated that a cathode-ray absorption curve alone is not a reliable measure of the relative lethal distribution within a bacteria-laden absorber.

SUMMARY

Numerous investigators have shown that cathode rays exhibit a lethal effect on bacteria. Cathode rays consist of parallel beams of high velocity electrons, whose high velocity was attained by having been accelerated through a high electric potential. Their maximum depth of penetration, called range, is a function of their initial kinetic energy and the density of the substance in which they are absorbed. The lethal property is attributed to ionization that occurs within the absorber. Because cathode rays possess these properties they have been proposed as a means for sterilizing foods.

When a high velocity electron traverses a substance, it ionizes atoms along its path and ejects other electrons, called secondary electrons, in the process. The secondary electrons may also possess sufficient energy to ionize still other atoms in their paths. The mass of an electron is very small compared to the mass of the atoms with which it collides, consequently it takes a zig-zag course within the absorber. As a consequence of a meandering path and secondary and tertiary ionization along the way the depth distribution of ionization in a substance being bombarded with cathode rays is non-linear.

The actual distribution of the ionization density has been measured by absorption techniques. The lethal property

of cathode rays is attributed to ionization and since the distribution of ionization within a substance is known to be non-uniform it becomes significant to determine the distribution-in-depth of the lethal effect and to compare the distribution with the ionization-in-depth distribution.

Thin filter discs were inoculated with known populations of bacteria. Twenty discs were assembled into a chamber with extremely thin plastic separators between each disc. These laminae thus constituted a bacteria-laden cathode-ray absorber. Ten chambers were assembled and irradiated with one million electron-volt cathode rays at an incident dosage of 5000 rep. The lethal effect was determined in each successive stratum by counting the bacterial colonies developing on each disc. By relating the number of colonies on each disc to the number of colonies on inoculated, unirradiated control discs, the percentage survivors (or percentage destroyed) were determined for twenty increments of depth. The data obtained by this procedure, when shown graphically, described the distribution-in-depth of the lethal effect of cathode rays.

The curve shows that the lethal effect is non-linear. The relative number of bacteria destroyed increased from a certain value at the incident surface to a maximum value near one-ninth the maximum range for one million electron-volt cathode rays and then gradually diminished to zero at or near the maximum range.

The distribution-in-depth of the lethal effect curve was compared with the distribution-in-depth of ionization curve. The likenesses between the two erroneously indicated that an absorption curve might be a reliable measure of the lethal distribution within a bacteria-laden substance.

The two curves could not be directly compared. It was necessary to graphically relate the dosage values taken from the ionization density curve to the corresponding percentage of bacteria surviving taken from the lethal curve, by using a logarithmic relationship. When this was done the relationship between the log dose and the relative survivors was shown to be non-linear. The digression from a linear relation became the discriminating criterion which denoted that the ionization density was disproportionate to the lethal effect. The cathode-ray absorption curve alone is not a reliable measure of the relative lethal distribution within a substance.

SUGGESTIONS FOR FURTHER RESEARCH

During the course of this work certain unanticipated technical problems developed. These were principally concerned with the bacteriological aspect of the undertaking. It was mentioned in the section on Procedure that an organism was selected which developed a characteristic pigmentation so as to preclude, what was at first believed to be, a potential contamination problem. However, during the investigation it appeared that another characteristic would have saved considerable trouble. This added characteristic should have been that the bacterium be highly heat resistant. There is evidence that bacteria which are highly heat resistant are also highly resistant to cathode rays. If this is true, then the bacterial suspensions would probably have been more stable, that is, the initial bacterial population used to inoculate the filter discs would probably have been kept constant more easily.

It is therefore recommended, that if this work is repeated, the researcher select a strain of bacterium which is highly heat resistant.

This reasoning leads to still a second recommendation; that, instead of using vegetative bacteria as a test organism, a spore-former organism be used. This might provide an even greater degree of stability.

GLOSSARY OF TERMS

Ionization

An atom consists of a positively charged nucleus and a surrounding constellation of negative electrons, the whole being electrically neutral. Ionization is the loss by an atom of one or more of these electrons. The principal means of energy dissipation by an ionizing radiation in its passage through matter is the ejection of electrons from atoms through which it passes. An atom so ionized is left positively charged, and is called an ion.

The electron which is ejected from an atom in the process of ionization eventually becomes attached to another atom and makes it a negative ion. As far as the physical measurement of ionization is concerned, the positive and negative ions are equally significant, thus one usually speaks of an ion-pair. But since the energy involved in the attachment of an electron to an atom to form a negative ion is usually even less than the energy of excitation, according to Lea (15) it is probably safe to neglect negative-ion formation as a factor of biological importance. Thus ionization refers to the production of a positive ion by the ejection of an electron, therefore it is the positive ions which are of biological importance.

Excitation

Radiations dissipate energy in matter by another process in addition to ionization, i.e., by excitation. This means the raising of an electron in an atom or molecule to a state of higher energy, but by an amount insufficient to free it from the atom or molecule. According to Lea (15) it appears probable that when dealing with an ionizing radiation, excitation may usually be neglected as a cause of biological effect by comparison with ionization.

Primary ion

The electron originating at the source. In the case of cathode-rays it is the electron which is accelerated in the cathode ray accelerator and hurled into matter.

Secondary ion

The ion formed as a result of ionization by the primary ion. In the case of cathode rays, it is the ion formed by the ionization of the electrons emitted by the accelerator as it passes through the matter. Secondary ions (electrons) may themselves have sufficient energy to ionize still other atoms. These resulting ions are called tertiary ions. In this work all ions except the primary electron shall be considered as secondary ions. Thus, secondary ions include tertiary ions as well.

Dose (dosage)

The radiation delivered to a specified area or volume, or to the whole body. The unit for dose specifications is roentgen for X- or γ-rays; and the unit used for β particles is rep (roentgen equivalent physical) (14).

Roentgen (r)

That quantity of X- or gamma radiation for which the associated corpuscular emission per 0.001293 gram of air produces, in air, ions carrying one electrostatic unit of electricity of either sign (14).

Roentgen equivalent physical (rep)

A unit to apply to doses of ionizing radiations not covered by the roentgen. There is some confusion as to its definition, but it is best taken as the absorption of 93 ergs of energy per gram of body tissue. The choice of 93 ergs per gram is not arbitrary but is made by assuming that all energy absorption is proportional to the electron density (electrons per gram) of the absorber. Electron density is essentially proportional to Z/A and hence for air = 1/2. For simplicity, we assume tissue to be equivalent to water for which Z/A = 10/18. Then since it has been shown that one roentgen is equivalent to the absorption of 83 ergs per gram of air we have energy absorption per gram of water

$$\frac{10/18}{1/2} \times 83 = 92.2 \text{ ergs.}$$

A more exact calculation for tissue yields 93 ergs per gram (14).

Electron-volt

Is the amount of work done when an electron is accelerated by a potential difference of one volt. One electron-volt is equal to 1.6018×10^{-12} erg.

Cathode rays

Are streams of electrons accelerated to high velocity, under the influence of an applied potential difference. At this point, the distinction between cathode rays and β rays should be clarified. Both are electrons. Their difference lies in their source. Beta rays are electrons emitted from radioactive substances while cathode rays are electrons induced to high velocity by man-made devices. Beta rays and cathode rays having the same energies exhibit the same properties in their passage through matter.

Death

Death cannot be defined by positive criteria; it can be characterized only by the absence of some property which is essential to life. The death of a cell is not always determined by the same method. Thus, a cell can be alive according to one definition, and dead according to another. The criterion almost universally used by bacteriologists to define death is the loss of reproduction. For purposes herein, a cell is dead when it is sterile, i.e., permanently unable to reproduce, and death shall be in evidence if a colony will not develop when a bacterium is transplanted to a suitable media (19).

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