THE EFFECT OF CATHODE RAYS UPON CERTAIN BACTERIA

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PLATE 30

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A study of the rate of killing of bacteria by cathode rays is a step towards finding out what changes these radiations and X-rays bring about in cells. The great interest to biology of X-rays, and probably also of cathode rays, lies not in the fact that they are lethal, but rather in their ability to produce permanent and inheritable changes in cell properties. Nevertheless, because they are deadly it is obvious that an understanding of the conditions under which cells are killed is a necessary preliminary to any thorough-going study of how they are changed. Bacteria have been used in the following experiments partly because they are, on the whole, the simplest cells to work with, partly because much has already been done on the lethal action of X-rays upon them, and partly because through their small size they provide the best introduction to the examination of the effects of these radiations upon the still smaller and imperfectly understood viruses.

Experimental Procedure

In the experiments to be described in this paper, single bacteria spread upon an agar plate are bombarded with a known number of cathode rays and, after incubation, the number of survivors is determined through counts of the colonies of bacteria. From these data and various well known physical characteristics both of the rays and of the bacteria, a satisfactory picture can be gained of many details of the destruction of these cells.
The cathode rays were obtained from a Coolidge type electron tube \(^1\) leased from the General Electric Company. This tube is essentially a large hot cathode X-ray tube with a hollow anode covered by thin metal foil. Many electrons proceeding from the cathode pass through this anode and foil, and, emerging into the air, constitute the cathode ray beam. The velocity of these electrons and, in general, the transparency of matter to them increases with the voltage applied to the tube. For these experiments the voltage was approximately 155 K.V. The velocity of the emitted electrons is of the order of 0.8 of the velocity of light.

The absorption of an electron in inanimate matter is attended by the release of a large number of ions within a very small volume. A 150 K.V. electron will liberate about \(10^4\) ions within less than 0.001 mm.\(^2\) It is natural and in accord with existing knowledge to associate the changes brought about in a bacterium by cathode rays with the absorption of electrons and the consequent shower of ions freed within it. Together with this ionic shower, X-rays are emitted as a consequence of electron absorption. The passage of cathode rays through air is likewise attended by the formation of much ozone and it might be argued that the observed destructive action on bacteria was due either to these X-rays or to ozone. We have ascertained by suitable experiments that this is not the case.

Several conditions must be fulfilled if a statistical analysis of the killing of bacteria by cathode rays is to have any physical significance. In the first place, the absorption of these rays is so great that the bacteria must be exposed upon the surface of an agar plate rather than held in a suspension if the dose they receive is to be satisfactorily measured. It is likewise necessary that they be spread upon this surface with sufficient uniformity so that colony counts in selected standard areas can be taken as measures of the numbers of irradiated bacteria. Not only must the bacteria be spread as single organisms, but their multiplication must be prevented until irradiation is completed. This is evident when it is realized that if several organisms are associated together in a clump, a single survivor will produce a countable colony. Many experimental procedures were tried with several types of bacteria before one was found which gave a satisfactory spread of single organisms. The highly motile \(B. \text{aertryke}\) gave excellent results with 200 to 300 organisms per square inch and almost as satisfactory data were provided by \(B. \text{coli}\) in somewhat greater dilution. Repeated trials, however, failed to produce as good spreads of single organisms of \(Staphylococcus \text{aureus}\). This bacterium has frequently been used in

\(^1\) Coolidge, W. D., \(J. \text{Franklin Inst.},\) 1926, 202, 693.
studies of the action of X- and ultraviolet rays. Our experience makes it seem probable that single cell spreads were not ordinarily obtained in these experiments.

The bacteria were prepared for irradiation in the following way. Broth cultures, from standards provided by Dr. L. T. Webster of this Institute, were carried for at least 3 days through daily transplants before use. The final 20-hour old tube was diluted an hour before irradiation using either physiological salt or Locke's solution. This dilute suspension, so chosen as to give the desired number of bacteria per plate, was kept in an ice bath until actually used in order to prevent cell multiplication. In seeding, 1 cc. of this suspension was run evenly over the agar surface of a poured 10 cm. Petri dish. After vigorous shaking to remove the excess fluid, the plate was allowed to drain 10 minutes and its edges wiped free from adhering liquid. A known area was then immediately irradiated and another marked as standard. After incubation overnight, counts were made of the colonies developing in each region. Approximately 100 plates were irradiated for each experiment. In some experiments the plates themselves were refrigerated before being inoculated. This proved to be unnecessary and had the disadvantage that the attendant condensation of moisture on the agar surface often destroyed the bacterial distribution.

The plate ready for irradiation was placed in the holder C of Fig. 1 and its agar surface pressed against the cutting edge of the tube A. This tube is divided

<table>
<thead>
<tr>
<th>Time</th>
<th>Survival ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1) B. coli</td>
</tr>
<tr>
<td>4 sec.</td>
<td>0.698</td>
</tr>
<tr>
<td>5 sec.</td>
<td>0.610</td>
</tr>
<tr>
<td>8 sec.</td>
<td>0.455</td>
</tr>
<tr>
<td>12 sec.</td>
<td>0.311</td>
</tr>
<tr>
<td>14 sec.</td>
<td>—</td>
</tr>
<tr>
<td>16 sec.</td>
<td>0.294</td>
</tr>
<tr>
<td>20 sec.</td>
<td>0.061</td>
</tr>
<tr>
<td>24 sec.</td>
<td>0.250</td>
</tr>
<tr>
<td>28 sec.</td>
<td>0.026</td>
</tr>
</tbody>
</table>

Electrons incident per cm.²/sec.

1.65 × 10⁹ | — | 0.85 × 10⁹ | 0.96 × 10⁹ | 2.99 × 10⁹
lengthwise into two hemicylinders by a brass and lead partition. The electrons stream through the lower of these halves from the tube window, D, and strike the surface of the agar pressed into the cutting edge. The upper half, sealed with lead to exclude the electrons, provides a standard adjacent to the irradiated surface and of equal area. Cutting through the agar with the tube A has the double advantage of defining sharply the irradiated area and of marking permanently both it and the standard. The tube end was sterilized between irradiations by wiping with alcohol. The time of irradiation was controlled by operating a heavy lead shutter shown at B. In counting the number of electrons emerging from the tube, the measuring device to be described later replaced the plate, other conditions being the same as during irradiation.

In each experiment involving about 100 plates eight different times of exposure were commonly used and 10 or more plates were irradiated for each of these times. The ratios of the colony counts made after incubation upon their irradiated and standard areas were averaged for each time of exposure to give the typical results of Table I.

**Analysis**

These data can be analyzed with the help of the following considerations from elementary probability theory. If \( a' \) is the probability that an event will take place, by definition the probability of its not happening is \( b' = 1 - a' \). Furthermore, if \( e' \) is the probability that some other event will occur, then the probability that \( a' \) and \( e' \) will happen together is \( a' e' \). In the experiments of this paper many electrons are being shot at a few bacteria. If \( a \) is the probability that an electron will hit a bacterium and \( n \) is the number of electrons shot at it, the probability that one of these electrons will hit and that every other electron will miss it is

\[
a (1 - a)^{n-1}
\]

Since any one of the \( n \) electrons may hit, the probability of striking a bombarded bacterium only once is

\[
P_1 = n a (1 - a)^{n-1}
\]

Similarly, the probability of hitting a bacterium by each of two selected electrons is \( a^2 \) and the probability of doing it only twice and by these two out of the \( n \) electrons is

\[
a^2 (1 - a)^{n-2}
\]

If there are \( \binom{n}{2} \) ways of combining two out of \( n \) electrons, the probability that a bacterium is hit twice and only twice is

\[
P_2 = \binom{n}{2} a^2 (1 - a)^{n-2} = \frac{n (n - 1)}{2} a^2 (1 - a)^{n-2}
\]
In the same way, the probability that a bacterium will be struck \( r \) out of \( n \) possible times is

\[
P_r = \binom{n}{r} a^r (1-a)^{n-r} = \frac{n(n-1)(n-2)\ldots(n-r+1)}{r!} a^r (1-a)^{n-r}
\]

The number of electrons striking the irradiated surface per second is of the order of \( 10^{10} \). The number \( n \), therefore, may be expected to be very large compared to \( r \), the number of hits. If this is true, then

\[
(1-a)^{n-r} \approx (1-a)^n
\]

and

\[
n(n-1)(n-2)\ldots(n-r+1) \approx n^r
\]

and

\[
P_r \approx \frac{1}{r!} a^r n^r (1-a)^n
\]

Expanding \((1-a)^n\) by the binomial theorem gives

\[
(1-a)^n = 1 - an + \frac{n(n-1)}{2} a^2 - \frac{n(n-1)(n-2)}{3} a^3 + \ldots
\]

\[
= 1 - an + \frac{1}{2} (an)^2 - \frac{1}{3} (an)^3 + \ldots
\]

By MacLaurin's formula this is

\[
(1-a)^n \approx e^{-an}
\]

In this way we arrive at the Poisson law

\[
P_r = \frac{1}{r!} (an)^r e^{-an} \quad (1)
\]

in which \( an \), the average number of hits, can be calculated from the results of irradiation. If one hit is enough to kill a bacterium, the survival ratios as given

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2 This expression or its equivalent has been used several times in studies of the effects of X-rays on cells. See for instance Condon, E. U., and Terrill, H. M., J. Cancer Res., 1927, 11, 324; Crowther, J. A., Proc. Roy. Soc., 1926, B 100, 390; Holweck, F., Compt. rend., 1929, 188, 197.
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in Table I will be expressed by the probability that a bacterium will be missed every time. As the foregoing considerations have shown, this is

\[(1 - a)^n = e^{-an}\]

In other words

\[\frac{A_1}{A_0} = \text{survival ratio} = e^{-an}\] \hspace{1cm} \text{........................(2)}

If a bacterium could withstand one electron but were destroyed by the second, the survivors would be those which either escaped or were hit once, so that

\[\frac{A_2}{A_0} = e^{-an} + an e^{-an} = e^{-an} (1 + an)\] \hspace{1cm} \text{........................(3)}

Similarly, if \(r\) hits are needed

\[\frac{A_r}{A_0} = e^{-an} + an e^{-an} + \frac{1}{2} (an)^2 e^{-an} + \ldots\]

\[\ldots \frac{1}{r - 1} (an)^{r-1} e^{-an}\]

\[= e^{-an} \left[ 1 + an + \frac{1}{2} (an)^2 + \ldots \frac{1}{r - 1} (an)^{r-1} \right]\]

\[= e^{-an} \left[ \frac{1}{1 - an} \right]\]

\[\text{Standardization}\]

The average number of hits can readily be calculated from a knowledge of (1) the area of the irradiated surface, (2) the dimensions of a bacterium, (3) the number of electrons striking the irradiated surface and (4) the absorption coefficient of electrons in the bacterium.

The counting of the number of electrons under the conditions of these experiments cannot accurately be carried out. This is due partly to the enormous ionization produced in the air through which a cathode ray beam passes, partly to the fact that the absorption of the radiation from the tube in small thicknesses of matter is not linear and is accompanied by a considerable diffusion of the transmitted beam. Reproducible results which seem to be fairly accurate have, however, been obtained by the use of a counting chamber which consists of a

\(^3\)See also Thaller, R., Physikal. Zeit., 1928, 29, 841.
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brass disk enclosed, except for a \( \frac{1}{16} \) inch hole on one side, by a grounded and air-tight brass sheath. This sheath is a cylinder 2 inches in diameter and 1 inch high and is provided with a port for evacuation. The central disk is supported by a thin rod which, mounted in amber, provides electrical connection with one of the galvanometer leads. The current produced by the flow of electrons from the collecting plate to ground through a sensitive galvanometer has been taken to measure the number of electrons in the beam. This outfit has been placed directly behind the cutting tube A of Fig. 1 in the position of the Petri dish during irradiation and counts have been made at the conclusion of individual experiments. In use, grounded shielding tubes protect the emerging electrode of the collecting plate and the galvanometer leads pass through grounded copper sheathing. The shutter and cutting tube were grounded during both the irradiations and the counting measurements. Standardizations have been carried out (1) with the opening free and (2) with it covered by 0.001 inch aluminum foil and with the chamber evacuated. The fact that the two measurements were substantially identical after allowance had been made for absorption in the foil indicates that the potential developed between the collecting plate and the grounded case was insufficient to interfere seriously with the accuracy of measurement by drawing ions from the air in the chamber. The principal errors in the electron counts probably arise, therefore, from the reflection of electrons by the collecting disk and from inhomogeneity in the cathode ray stream over the irradiated area. No effort has been made to introduce corrections for reflection but shifts of the collecting chamber over the area of irradiation indicated that at the distance of the plate from the mouth of the tube, inhomogeneity of beam was unimportant.

Measured galvanometer currents were corrected for the relative areas of the irradiated surface and the opening in the collecting chamber and were expressed in electrostatic units. Taking one E. S. U. as equivalent to \( 0.21 \times 10^{10} \) electrons, the numbers of electrons incident per cm.\(^2\) of irradiated surface are recorded in Table I.

The absorption of electrons in the bacteria themselves can scarcely be measured. Because this absorption, like that of X-rays, is not a function of the state of chemical combination it can, however, be estimated from measurements upon cellophane as a substance com-
posed only of light atoms and having approximately the density of bacteria. It is well known from studies of $\beta$-rays that the absorption of a stream of electrons possessed of different velocities rarely follows any simple law. Measurements of the absorption of the cathode ray beam used in these experiments in successive thicknesses of cellophane lie on a curve intermediate between a straight line and a simple exponential curve. Considering the extrapolation involved in estimating the absorption in a single bacterium from the absorption in a sheet of cellophane (0.0009 inch thick), it has not seemed significant to get more than the order of magnitude of this absorption by a linear interpolation from measureable thicknesses. This procedure has led to the conclusion that there will be an absorption of about 0.008 of the electrons striking either a cylindrical bacterium 0.5\(\mu\) in diameter and 2\(\mu\) long or a coccus having a radius of 0.4\(\mu\).

\textbf{DISCUSSION}

Whether more than one electron is needed to kill a bacterium can be told from the shape of the survival curves. Expression (2), being a simple exponential, will give a straight line as a graph on semilogarithmic paper. Plotted in this way, the equations for multiple hits yield curves which depart further from a straight line the larger the number of requisite absorptions (Text-fig. 1). Some of the data of Table I are shown graphically in Text-figs. 1 and 2. Within experimental limit the results of the best measurements lie on straight lines. From this fact alone it is to be concluded that the absorption of one electron is sufficient to kill a bacterium. The best obtainable data with \textit{Staphylococcus aureus} depart appreciably from a straight line through the origin. This departure is to be expected, however, for microscopic examination shows that even great dilutions contain many clusters of two or more individuals. The effect of a short incubation of \textit{B. coli} between spreading and irradiation is shown by the top curve of Text-fig. 1. Cell multiplication which has taken place gives a curve resembling but more extreme than that from the coccus.

\footnote{\textit{Cf.} for instance Rutherford, E., \textit{Radioactive Substances and their Radiations}, London, 1913, Chapter V.}
Although one hit kills, it is not necessarily true that every one does so. In Experiment (1) of Table I, \(1.17 \times 10^{10}\) electrons per second are incident upon the irradiated area of 7.07 cm.\(^2\) The average number of electrons absorbed per second by a cylindrical bacillus 0.5\(\mu\) \(\times\) 2 \(\mu\) is then

\[\text{Survival ratio} = \frac{N}{N_0}\]

\[\text{Survival ratios vs. Seconds}\]

**Text-Fig. 1.** The black circles of this figure are the survival ratios of Experiment (1) of Table I; the open circles refer to Experiment (5) with staphylococci.
and Equation (2) as applied to this experiment becomes

\[ \frac{A_1}{A_0} = e^{-0.132t} \]

The graph of this expression in Text-fig. 1 lies close to, though somewhat below, the experimental survival ratios. The curve of the corresponding equation if two hits are needed to kill (Equation 3) is shown as the thick dotted line of the same figure. Similar relations prevail in the other standardized experiments. The average number of absorptions per bacterium per second as calculated and observed are listed in Table II. In each instance the theoretical number exceeds the experimental by about 15 per cent. On its face this would mean that only 85 per cent of the bacteria which absorbed electrons were killed, though whenever death was brought about, one electron was sufficient to accomplish it. In view of the inaccuracy of the measurements involved in standardization, however, too much importance cannot be attached to this percentage of killed organisms. It is perhaps safe merely to conclude that the number of bacteria killed is of the same order of magnitude as the number which absorb electrons.

The similarity in the curves for B. coli and B. aertryke might be expected from their similarity in size and shape. Though none of the experiments using Staphylococcus aureus gave results as uniform as with
the two bacilli, the observed greater resistance of this bacterium is adequately accounted for by its smaller size (Table II).

![Text Fig. 2](image-url)

**Text-Fig. 2.** The data of this figure refer to Experiments (2) and (4) with *B. aertryke*.

**Conclusions**

1. For the two motile bacilli, *B. coli* and *B. aertryke*, the absorption of a single 155 K.V. electron is sufficient to cause death. Furthermore,
all, or nearly all, the electrons absorbed are lethal. The same is undoubtedly true of Staphylococcus aureus. In addition to providing a quantitative picture of the interaction of bacteria and cathode rays, these results suggest that radiation of the energy content used in our experiments is not suitable for altering the inheritable characteristics of bacteria.

2. The differences in sensitivity to cathode rays shown by the bacteria studied can be explained by the purely physical factor of size.

3. Counts giving significant conclusions concerning killing rates can be obtained only if there is no clumping of the cells when spread and only if the cells are not allowed to multiply before irradiation. Both these precautions seem rarely to have been met in the experiments that have in the past been made with X-rays and other forms of radiation.

EXPLANATION OF PLATE 30

Fig. 1. A photograph showing the anode end of the cathode ray tube and the apparatus used in irradiating the agar plates.
Fig. 1

(Wyckoff and Rivers. Effect of cathode rays upon bacteria)