

the needle thrust in. On cooling of the metal a good contact is secured. Any defect in the rigidity at the contact is overcome by pouring minute drops of melted Woods metal with a fine glass pipette. After mounting the coated needles in this way thicker coats of silver or of any other metal can be deposited electrolytically. For non-polarizable electrodes a thicker coat of silver is desirable. The film of silver chloride is deposited electrolytically by making the electrodes the anode in a 5% KCl bath, the cathode being another piece of silver. With 0.5 volts across the terminals, and keeping the current on only for 30 to 40 seconds a good continuous coat of silver chloride film is obtained. The electrodes are washed and kept in distilled water for a few hours before use. Electrodes when broken are easily replaced by fresh ones by warming the Woods metal of the electrode holder.

When necessary the metallic film of the shank can be insulated by applying shellac solution with a fine brush. The insulation of the micro-tip end is done under a microscope. The tip pointed upward is illuminated in the dark field of the microscope and its shank is gradually lowered by rack and pinion into a small cup of shellac solution. By this method it is easy to insulate up to 10μ from the micro-tip. With care and by using a thicker shellac solution, insulation up to within 5μ can be obtained. The resistance of a pair of electrodes with a micro-tip less than 3μ in diameter has been found to be only 35 ohms. We have been using these electrodes for the last 6 months and they are found to be very satisfactory alike for measuring resistances, by the method previously described,⁵ and currents in single living cells and for stimulating single nerves and muscle fibers.

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Effect of Cathode Rays Upon Certain Bacteria.

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(Introduced by Peyton Rous.)

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The following experiments, which are intended as preliminary steps in a study of some of the effects of cathode rays upon cells,

⁵ Sen, B., *Roy. Soc. Proc.*, 1923, xciv, 216.

provide data for a statistical analysis of the rate of killing of cultures of *B. coli*, of *Staphylococcus aureus* and of *B. aertryke*.

The cathode rays have been obtained from a Coolidge type electron tube operated at a voltage of approximately 200 K.V. Small numbers of the organisms under investigation were evenly spread upon the surfaces of agar plates and known areas were exposed for different lengths of time to the electron stream. After incubation, counts were made of the numbers of colonies growing out in these areas and in similar standard areas shielded from radiation. The ratios of the bacterial colonies in these areas are survival ratios.

A picture of the physical consequences of an electron absorption in a bacterium will be provided by remembering that whenever a fast moving electron is absorbed in matter, some of its energy will be emitted as X-rays but the major part will produce a large number of charged ions within a very small volume. In the present experiments this volume is probably less than 0.001 mm.³ and within it an absorbed electron gives rise to upwards of 10,000 ions. It is natural to attribute the destructive action of cathode rays to the chemical and physical changes resulting from this ionic shower.

By estimating both the number of electrons which strike a bacterium in unit time and the absorption coefficient of these electrons in the bacterium, it is possible to analyse the observed survival ratios by the usual methods of probability theory. Such an analysis will show how many electrons may be stopped by a single bacterium before death results. It will also indicate whether absorption must take place in a particular portion of the organism in order to be lethal.

When the motile bacilli of mouse typhoid were spread on agar so sparsely that not more than about 200 cells were in an area of one square inch, the results agreed with the conclusion that one electron-hit is sufficient to kill. This simple result was only obtained, however, when the effects of cell multiplication were eliminated by irradiating quite immediately after seeding the plates. In order to give a similar one-hit-to-kill relation with the less motile colon bacillus, it was necessary to work with fewer bacteria per plate than with the bacillus of mouse typhoid. Even under the most favorable conditions the data from irradiations of *Staphylococcus aureus* required more than one hit. Microscopic examination of the suspension with which the plates were seeded showed that even at these great dilutions, the cocci were still for the most part associated together in clusters.

The detailed results of the experiments outlined above are considered to justify the following conclusions: (1) For *B. coli* and for

B. aertryke not only is one hit by a 200 K.V. electron sufficient to kill, but every electron absorbed is lethal. The same is undoubtedly true of *Staphylococcus aureus*. (2) These data indicate that the only differences that exist in the resistance of these organisms to 200 K.V. electrons are due to their relative sizes. Since the physical phenomena consequent upon the absorption of an electron are, except for the additional charge borne by the electron, identical with those following the absorption of an X-ray quantum of equivalent energy, it is likely that the specific resistances of these cells to 200 K.V. X-rays can also be quantitatively explained by taking account of their relative sizes. Experiments to bear upon this question are being made with X-rays. (3) These results prove that measurements will lead to valid conclusions concerning the mechanism of the action of the radiation only when extraordinary care is taken to eliminate clumping of cells and to prevent multiplication before raying. These requirements have not been met by the experimental procedures commonly followed in X-ray and ultraviolet research upon bacteria.

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The Distribution of Ergosterol Administered to Rabbits.

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Rosenheim's¹ recently announced specific color reaction for ergosterol has been applied to the analysis of tissue from rabbits fed on "Vigantol" (irradiated ergosterol in oil).

The reaction as described by Rosenheim consists in the addition of a saturated aqueous solution of trichloroacetic acid to a chloroform solution of the sterol. There is every reason to believe this reaction quite specific for ergosterol or structurally related sterols. We have found after testing many solvents that dichloroethylene (low boiling) is a more satisfactory solvent for the reaction than chloroform. Using this solvent, quantitative estimations of the extracted, partially purified sterols may be made.

Ten adult rabbits were fed "Vigantol" with a stomach tube in doses ranging from 250-870 mg. of irradiated ergosterol. Four

¹ Rosenheim, Otto, *Biochem. J.*, 1929, xxiii, 47.