ELECTRON BOMBARDMENT OF BIOLOGICAL MATERIALS

II. The Rate of Death of Fungus Spores Bombarded in Vacuum with Cathode Ray Beams from 4 Kv.–15 Kv.

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INTRODUCTION

The profound effects which can be brought about in biological materials when they are subjected to beams of ionizing radiation, and in particular to x-rays, have long been well known. Such phenomena form the extensive basis of work in x-ray cancer therapy, and in the production of mutations by x-rays. It is an equally familiar fact that the total energy which need be absorbed from such a beam by the biological material in order to bring about marked changes within it may be extremely small. Such considerations led Dessauer in 1922 and 1923 (1) to propound his "point-heat" hypothesis, which assumed that the biological action of x-rays was localized to very restricted, discrete points within the irradiated material, where the quantity of energy absorbed per unit volume would indeed be high, although if integrated over the material as a whole it might be very low. Dessauer thought entirely in terms of thermal agitation. Later, as the concepts of ionization under x-ray bombardment became clearer, the idea of discrete energy absorption from the x-ray beam was retained, but the concept of a discontinuous absorption of heat energy in the material was replaced by that of ionization consequent upon the shower of secondary electrons released by the x-ray beam. Crowther (2), Condon and Terrill (3), Holweck and Lacassagne (4), Curie (5), Glocker (6), Wyckoff (7), Mayneord (8), and others have further developed this idea, especially in relation to the concept of the "sensitive volume" for various biological effects. It was further demonstrated by Stadler (9), Wyckoff and Rivers (10), Haskins and Moore (11), and others that high voltage cathode rays are qualitatively identical in their biological effects with x-rays. All of the considerable body of evidence now accumulated indicates clearly that the biological effects of x-rays are due primarily, not to the radiations themselves, but

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to the shower of photo- and Compton recoil electrons to which they give rise, and to the ionization which these electrons produce.

For the interpretation of the biological action of x-rays it is therefore of interest to investigate directly the effect of the corresponding electrons upon living cells. Such a procedure appears to have several advantages in the direct quantitative study of x-ray biological effects in general, and of x-ray cancer therapy in particular. The showers of electrons produced in tissue when irradiated with even a monochromatic x-ray beam, are far from homogeneous either in velocity or distribution, due to scattering and energy degradation suffered by the beam, to the combined presence of photo- and recoil electrons, to the wide spread of energies delivered to the recoil electron, and to the slowing down of all the electrons. There will, to be sure, be a maximum velocity for each x-ray wavelength, but the velocity distribution will be a very broad one. With cathode rays, however, it is easily possible to produce beams of a high degree of homogeneity in both energy and distribution.

A careful investigation of the relative mortality of unicellular organisms—and especially of carcinomatic tissue cells—under equivalent dosages of electron irradiation, as a function of electron velocity over a wide range of velocities, might be expected to provide interesting information as to the relative lethal efficiency of x-rays over a correspondingly wide wavelength band. A comparative study of differential killing as between cancerous and non-cancerous tissue would be especially interesting. Again, studies of the variation of survival ratio for single cells under bombardment by cathode rays with varying rates of delivery of identical dosages should yield very useful data in confirming or disproving the validity of the Bunsen-Roscoe reciprocity relationship for x-rays. The method has the advantage that a cathode ray tube has more flexibility for this purpose than semi-standard x-ray equipment. Third, it is possible to so design a cathode ray tube that the electron beam shall penetrate the cells to any desired depth, thus acting as a sort of blunt-ended probe (because of the high release of energy at the end of the electron track) wherewith to determine the relative sensitivity to ionization of various parts of the living single cell. Again, studies of the phenomenon of mutation under ionizing radiation, already observed very many times with x-rays and with high velocity cathode rays, can be investigated much more quantitatively by the use of low velocity, low current electron beams. And lastly, work of this sort can be made directly comparable with studies of the biological effects of proton beams and their corresponding neutrons.

These reasons have prompted the initiation of a research program which

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has been partly described elsewhere (12). It envisages a careful study of the biological effects of cathode ray irradiation by beams of electrons at energies ranging from 20 to 150,000 electron volts and current densities from 10^{-7}–10^{-4} amperes per square cm. upon single cells, upon cancer tissues, and upon other biological materials which appear to be of particular interest.

When using cathode rays of energies lower than about 20,000 electron volts, it is necessary that all work shall be done in high vacuum, of the order of 10^{-5} mm. of mercury, in order to provide adequate insulation and to prevent undue scattering of the electron beam. For this energy range, therefore, it has been necessary to select biological objects which are capable of withstanding these low pressures. For this purpose, spores of the ascomycete fungi *Aspergillus niger* and *Penicillium sp.* have proven most suitable. The present paper deals entirely with the effects of cathode ray beams of currents from $1 \times 10^{-7} - 3 \times 10^{-6}$ amperes per square cm. and energies from 4-15 electron kilovolts on such spores, inactivation of the spore being taken as the end-point.

PREVIOUS WORK

A number of investigations of the biological effects of high voltage cathode rays have been made by various workers. Thus Wyckoff (10) investigated their lethal effect upon bacteria, Stadler (9) studied the mutation of various grains under the radiations from a Coolidge high voltage cathode ray tube, and Haskins and Moore (11) studied the inhibition of growth in pollen grains and mold spores when bombarded from a similar source, operated at 250 kvp.

Very much less work, however, has been attempted with low velocity cathode ray beams, comparable in energy with the electronic showers released by x-rays. In 1929, D. A. Wells, at the University of Cincinnati (13), designed a tube to produce such beams, and used them in preliminary studies of the effects of electron beams of high current density and very low voltage on the bacteria *Staphylococcus aureus* and *Bacillus coli*. This work, unfortunately, was discontinued rather shortly after its inception. No further work, so far as is known to the authors, has been attempted in this field.

Source and Character of Cathode Ray Beam

The cathode ray tube designed and used for this work has been more fully described elsewhere (12). It consists essentially of an electron gun, built of glass, designed to produce the cathode beam, connected to a raying box of brass which contains the equip-

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ment for holding and manipulating the spores. The electron gun comprises a straight bare-wire tungsten filament mounted vertically, accelerating electrodes, and a pair of deflecting plates, by which the mechanically collimated cathode ray beam is bent at 90° in an electrostatic field, to eliminate any effect of radiant heat upon the irradiated material. After being bent through a 90° arc, the beam from the electron gun is further accelerated and a portion of it passed through a narrow slit (0.351 mm. by 6.31 mm.) with its long axis vertical, into the raying box. Steady D.C. voltage for this acceleration is provided by means of a General Electric kenetron testing set and a capacitor. Within

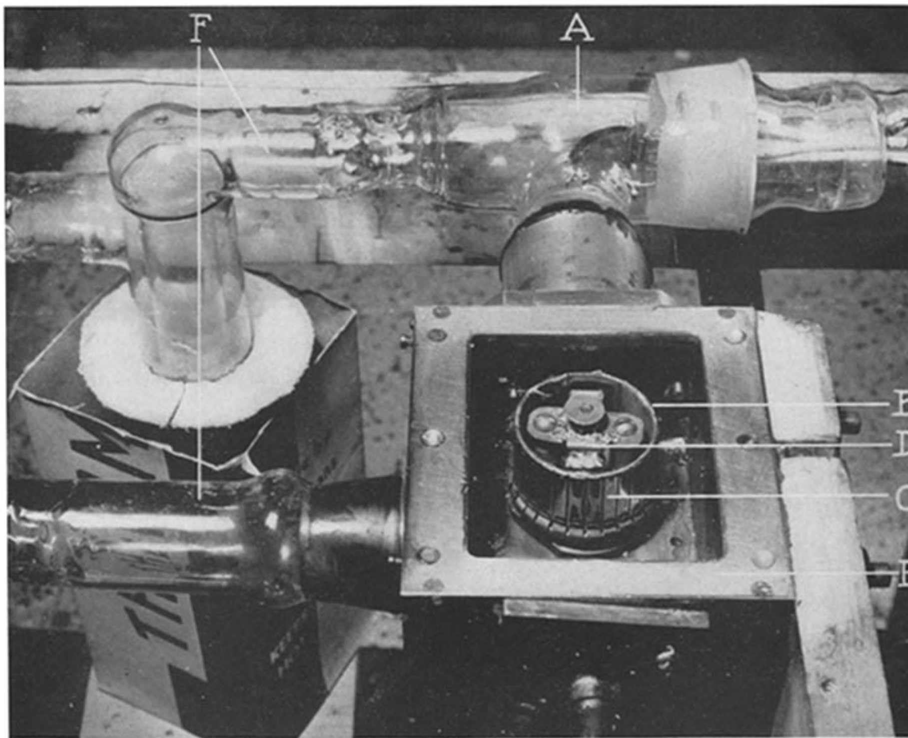

FIG. 1. Raying box with drum in position. A, electron gun; B, rotatable drum; C, specimen slides in clips; D, Faraday cage; E, raying box; F, pump connections.

the raying box is mounted a rotating drum, shafted vertically through a grease-packed vacuum seal in the bottom of the box, and geared to a telechron motor, so that it is possible to rotate it very uniformly. This drum is so mounted relative to the slit system connecting the electron gun and the raying box that it rotates very close to it. On the periphery of the drum are clipped twenty-three small slides, designed to hold the spores to be irradiated. These slides measure 2.5 cm. × 0.5 cm., are of brass, and have a heavy chromium-plated surface, highly polished.

A Faraday cage, connected to a D.C. amplifier for measurement of the electron current, is mounted in the center of the drum, and opens onto the drum surface through a slit large enough to pass the entire beam entering the raying chamber.

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As the drum is slowly revolved by the telechron motor, each slide on its periphery, and finally the opening of the Faraday cage, is brought opposite the slit to the electron gun, so that, when a beam is produced, a band of 0.631 cm. width is scanned across the center of each slide. Provision is made for disengaging the shaft of the drum from the telechron motor, so that the opening of the Faraday cage, or any slide, can be brought opposite the slit system at will during a run. A general view of the electron gun and the raying box, with cover removed and drum in position, is shown in Fig. 1.

This tube is capable of providing cathode ray dosages from 6×10^{-9} to 4×10^{-5} coulombs per square cm., at energies of 4 to 20 electron kilovolts, with electron beams which are highly homogeneous in both energy and distribution over the rayed area of the slide.

Materials and Methods

Mature spores of a species of the ascomycete fungus *Penicillium* were at first used in this work. It was found, however, that spores of the black mold *Aspergillus niger* were considerably more satisfactory because of the single, centrally located nucleus, of the uniform size, and, particularly important, that they are less adhesive and liable to clustering. All the work to be described here, therefore, was done with spores of *Aspergillus niger* taken from standardized cultures grown upon a potato-maltose-agar medium under standard conditions. The spores used were all taken from cultures which had been sporulating for at least 3 weeks. Frequent test counts were made to be certain that the percentage of germination in the controls remained consistently high.

The spores were spread, in a single layer, upon the chromium-plated slides already described. Before each irradiation these slides were carefully cleaned and sterilized by immersion in alcohol for at least 30 minutes. When the slides were dry, the spores were spread on them with a soft camel's-hair brush. The slides were then mounted in sterile clips and kept in sterilized Petri dishes until ready for use. In this condition they could safely be transferred from place to place and kept for some time before irradiation with no chance of contamination.

Immediately before irradiation, the spore-bearing slides were transferred to the irradiation drum, and the latter was mounted in position in the raying box of the tube. The system was sealed, vacuum pumps were started, and the tube was evacuated to a pressure of the order of 10^{-5} mm. of mercury. The time required for this procedure was of the order of 75 minutes. The actual irradiation then took place. Each slide was bombarded with a given dosage of electrons at an accurately known voltage according to a predetermined schedule. Normally two or three slides were not irradiated in each run, and were used as controls. After all slides had been irradiated, a procedure which usually took about 20 minutes, the system was allowed to stand under vacuum for 40 to 45 minutes to permit the mercury pumps to cool. Air was then admitted and the irradiation drum removed. Care was taken to admit the air into the vacuum chamber in such a manner as to prevent foreign bodies in the outside air from being swept into the chamber and onto the slides. To check the efficacy of the precautions taken against contamination at this time, blank slides were placed in the position of normally loaded ones and careful microscopic examination was made after a

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regular run, but no material has ever been found which had been swept in from the outside nor were spores carried from slide to slide.

The total length of time that the spores were subjected to high vacuum was about 2 hours and 20 minutes. Extensive study of evacuated controls has indicated that spores of *Aspergillus niger* of the age and type irradiated are capable of withstanding longer periods of vacuum than this without detectable effect.

After the drum was removed, the slides were placed again in the sterile clips, preparatory to transferring the irradiated spores to agar culture, or the transfer was made directly. The spores were transferred from the surface of the slide to that of an agar plate in a Petri dish by the simple procedure of laying the slides on the agar surface, leaving them in position for a few seconds, and then removing them. The spores then remained firmly fixed on the agar surface with very little derangement of their initial distribution.

Potato-maltose-agar was used throughout the work. Each run required at least three Petri dishes. A control group consisting of spores which had been subjected to treatment identical with the irradiated ones, except that they had been completely shielded from electrons, was always included in each dish. As a further control, a sowing was made for each run of a group of spores which had not been subjected to vacuum.

Counting of the spores began from 5 to 7 hours after the commencement of incubation, depending upon the end-point of effect being sought; and was completed in from 3 to 4 hours.

Four distinct effects were found, of which only the last will be considered in detail in this paper. At very low voltages a distinct shortening of germination time was observed, which is fully described elsewhere (15). At voltages from 11 to 15 kv., germination became very irregular and uneven at certain dosages and many spores swelled extensively without ever producing germ tubes. Cultures obtained from these spores have shown several mutations. Such mutations have been described elsewhere (14) and additional ones will be further described later. Marked retardation of growth with consequent stunting, was also found. And finally, the effect most generally observed, and fully described here, was that of complete inactivation of the cell. Two criteria can be used for this end-point. A cell invariably swells before the production of a germ tube, and the end-point for "killing" may be taken either as the swelling of a cell without the production of a mycelium, or as failure of the cell to swell, so that it is altogether inert. The form of the curves for both types of data has been found to be closely similar, but the abscissas will differ, since in each culture a number of spores swell but do not produce mycelia. The former criterion has been used for the data presented herein.

In counting spores, the stage of the microscope was fixed so that the field of vision was near the edge of the irradiated band. The stage was then moved so that the field of vision scanned a narrow strip across this irradiated band. Spores were counted as living if they had produced any

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sign of a germ tube at the time of counting, otherwise as dead. The number of spores counted for each slide ranged from 250 to 500.

As a precaution to eliminate any possibility of preconceived notions concerning the data, the worker counting a given slide was never informed until long after the count as to the conditions of irradiation of that slide. Even the order of slides during irradiation was often transposed, unknown to the worker who counted the spores from them, so that there was no possibility of any anticipation of results. The data were rearranged and calculated by the worker who had done the irradiation.

Survival ratios were computed for each group of spores cultured, and defined as the quotient of the percentage germination of the sample from the irradiated group divided by that from the control cultured with it. In order to increase the accuracy of the data and eliminate the effect of minor changes in the biological material a weighted average survival ratio from 15 to 20 slides irradiated with the same energy and density of cathode rays was taken in the following manner:
Let

$(L_r)_k$ = number living counted on the kth slide
$(D_r)_k$ = number dead counted on kth slide
$(L_r)_k + (D_r)_k = (T_r)_k$ = total number counted on the kth slide
$(L_c)_k$ = number living counted on the control for the kth slide
$(D_c)_k$ = number dead counted on the control for kth slide
$(L_c)_k + (D_c)_k = (T_c)_k$ = total number counted on the control for the kth slide

Then

$$\left(\frac{L_c}{T_c}\right)_k = \frac{1}{M_k} = \text{fraction living on the control for the } k\text{th slide}$$

Then the unweighted average survival ratio will be

$$(S.R.)_{\text{unweighted}} \frac{1}{n} \sum_{k=1}^{n} \left(\frac{L_r}{T_r} \cdot M\right)_k$$

Slides on which a small total count had been made will have just as much effect in the average in this expression as slides with a large total count. An average which will take this fact into account can be easily obtained if the size of the total count be used as weighting factor. The expression for the survival ratio then becomes:

$$(S.R.)_{\text{weighted}} = \frac{\sum_{k=1}^{n} (L_r M)_k}{\sum_{k=1}^{n} (T_r)_k}$$

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RESULTS

The expression derived in the preceding section was used to determine the survival ratios of the spores for each point, several slides having been treated with the same dosage in each run. The same schedule, moreover, was repeated seven or eight times over a period of a week or more.

The dosage received by each spore sample was readily computed in the following fashion:

Let

D = the dosage
i = the current through the slit system, in amperes
v = the rate of travel of the slide past the slit = 0.018 cm./sec.
h = the height of the slit 0.631 cm.
e = the charge on the electron, in coulombs
A = the average cross-sectional area of a spore
E = the potential difference used to accelerate the electron beam, in volts
k = the number of ergs in an electron-volt

Then the dosage expressed as the current density in coulombs per square cm. incident on the slide is $D_c = \frac{i}{vh} = 88 \times i$, or the dosage expressed as the total number of electrons striking a single spore is $D_n = \frac{iA}{vhe} = 4.4 \times 10^{13} \times i$. Finally, the dosage expressed as the energy in ergs delivered to each spore is $D_e = \frac{kiA\ E}{vhe} = 7.06 \times i \times E$.

For each voltage the current was so chosen that the full range of survival ratios for the material was covered, this current range having been experimentally determined. The probable errors were computed for each point.

The results, for 3.88, 4.80, 5.80, 6.72, 7.60, 8.6, 11.5, and 14.4 kv. are shown in Figs. 2, 3, 4, and 5, the vertical bars indicating the magnitude of the probable error.

It is of great interest to consider whether the same total quantity of energy applied to a given spore produces the same percentage inactivation regardless of the velocity of the electrons. As a criterion of this, the curve shown in Fig. 6 has been constructed, in which the energy in ergs necessary to reduce the survival ratio to 50 per cent is plotted against voltage. It will be seen that the curve shows a marked dip. It is to be remembered, however, that this curve represents the incident rather than the absorbed energy. The absorbed energy, because of the geometry of the spore, complete penetration by the electrons, and other factors, may not

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vary in the same fashion. This point will be further elucidated in a theoretical interpretation of results soon to be published.

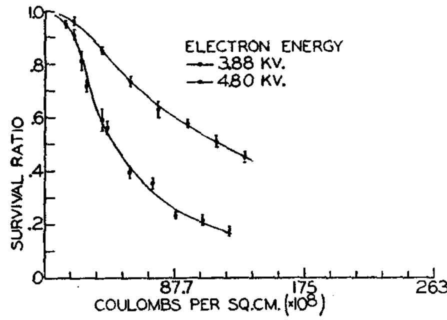

FIG. 2

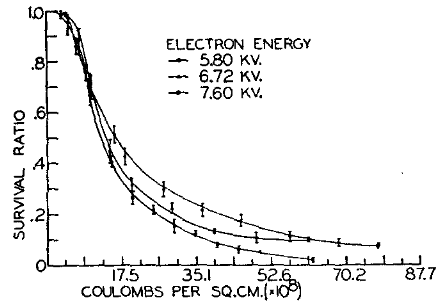

FIG. 3

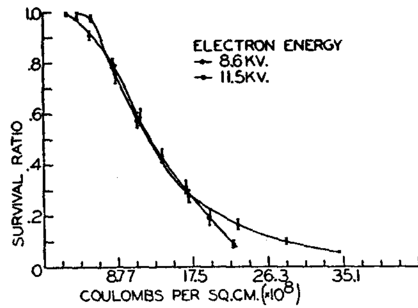

FIG. 4

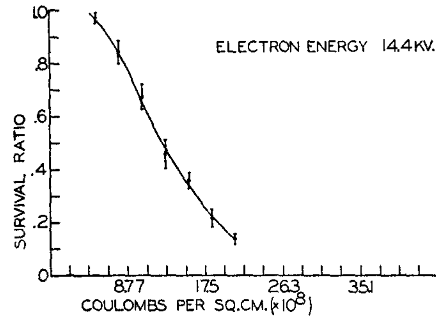

FIG. 5

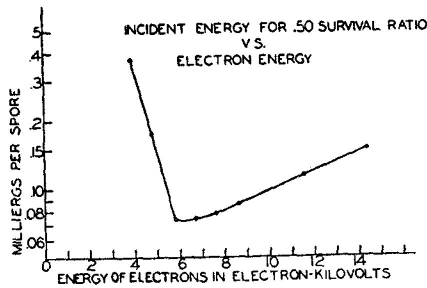

FIG. 6

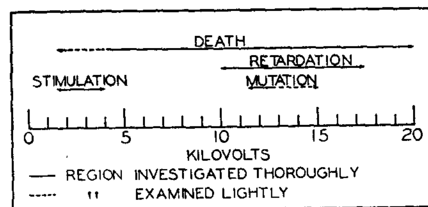

FIG. 7

In Fig. 7 is shown a diagram representing the range of cathode ray velocities which has been investigated, and showing the portion of that range within which each of four described effects was observed. These effects, as has been mentioned, have been or will be described elsewhere.

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SUMMARY

A study has been undertaken of the rate of inactivation of spores of the ascomycete fungus *Aspergillus niger* when bombarded in vacuum with homogeneous beams of cathode rays of energies from 4 to 15 electron kv. and current densities of 1×10^{-7} to 3×10^{-6} amperes per square cm. These velocities and densities are in the range of those of showers of secondary electrons produced in biological materials irradiated with moderately soft x-rays, and so may be made to serve as quantitative indicators of the mechanics of x-ray action. Four qualitative effects are described.

BIBLIOGRAPHY

1. Dessauer, F., *Z. Physik.*, 1922, **12,** 38; 1923, **20,** 288.
   Dessauer, F., *J. Radiology*, 1923, **4,** 411.
2. Crowther, J. A., *Proc. Roy. Soc. London, Series B*, 1924, **96,** 207; 1926, **100,** 390.
3. Condon, E. V., and Terrill, H. M., *J. Cancer Research*, 1927, **11,** 324.
4. Holweck, F., and Lacassagne, A., *Compt. rend. Acad. sc.*, 1928, **186,** 1316; 1929, **188,** 197; 1930, **190,** 524.
   Holweck, F., and Lacassagne, A., *Compt. rend. Soc. biol.*, 1929, **100,** 1101.
5. Curie, Mme. P., *Compt. rend. Acad. sc.*, 1929, **188,** 202.
6. Glocker, R., *Naturwissenschaften*, 1931, **19,** 20.
7. Wyckoff, R. W. G., *J. Exp. Med.*, 1930, **52,** 435.
   Wyckoff, R. W. G., *J. Exp. Med.*, 1930, **52,** 769.
8. Mayneord, W. V., *Proc. Roy. Soc. London, Series A*, 1934, **146,** 867.
9. Stadler, L. J., *Science*, 1928, **68,** 186.
10. Wyckoff, R. W. G., and Rivers, T. M., *J. Exp. Med.*, 1930, **51,** 921.
11. Haskins, C. P., and Moore, C. N., *Radiology*, 1934, **23,** 710.
12. Haskins, C. P., *J. Applied Physics*, 1938, **9,** 553.
    Cooper, F. S., Buchwald, C. E., Haskins, C. P., and Evans, R. D., *Rev. Sc. Instr.*, 1939, **10,** 73.
13. Wells, D. A., *Nature*, 1929, **124,** 983.
14. Whelden, R. M., *Mycologia*, 1938, **30,** 265.
15. Buchwald, C. E., and Whelden, R. M., *Am. J. Bot.*, in press.

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