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THE BACTERICIDAL ACTION OF X-RAYS, NEUTRONS AND RADIOACTIVE RADIATIONS

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(With 6 Figures in the Text)

INTRODUCTION

THIS paper contains the results of an investigation of the lethal action upon certain strains of bacteria of the α-, β-, and γ-rays of radium, X-rays of four different wave-lengths, and neutrons. The primary object of the investigation has been to elucidate the mechanism of the bactericidal action of radiations. In the section devoted to a discussion of the experiments, an interpretation is put forward which affords a consistent explanation of all the experimental facts, and which is rendered plausible by a comparison of the action of radiations on other biological systems. The principal experimental facts upon which this interpretation is based are as follows:

(i) The effect of a given dose of radiation is independent of the temperature; it is independent also of whether the radiation is given at high intensity for a short time, or at low intensity for a prolonged time.

(ii) During an irradiation at a steady intensity the death-rate remains constant, i.e. the number of organisms killed* in each minute's irradiation is a certain fraction of the number viable at the beginning of that minute, which fraction remains the same throughout the exposure. Thus if a count is made of the number of organisms viable at intervals of, say, 1 min., the numbers obtained diminish in geometrical progression. If their logarithms are plotted against the time, a straight line is obtained. In this paper the experimental results are shown by plotting the natural logarithm (to base e) of the ratio of the count of the exposed sample to the count of the control sample. The correctness of the statement that the death-rate is constant is tested by seeing whether the experimental points plotted thus lie on a straight line, or show a curvature. From the graph the dose which reduces the logarithm by unity may be read off; this is defined as the "mean lethal dose".

* The terms "killed", "lethal action", etc., are used in this paper to mean inability of the organism to give rise to a visible colony when plated out on nutrient agar. Motility, ability to metabolize and so on are not necessarily destroyed at the same time, so that no assumption is made about the destruction of any property of the cell other than viability.

(If the surviving number of bacteria in place of its logarithm is plotted against the dose, the curve obtained is exponential, and the mean lethal dose is that dose which reduces the number surviving to 37 % of the initial number.)

(iii) The mean lethal dose is not constant for different radiations, but (for vegetative bacteria) increases with change of radiation in the following order: β- and γ-rays, hard X-rays (short wave-length), soft X-rays (long wave-length), neutrons, α-rays. All the different radiations dissipate their energy in the material through which they pass by ionizing atoms. (The ionization of an atom normally causes the decomposition of, or chemical change in, the molecule in which it is situated: the dosage of radiation, being measured in terms of the ionization it produces, is closely related to the chemical change it is potentially capable of producing.) The ionization is caused by an electron, proton, or other fast particle which travels through the material leaving a trail of ionized atoms marking its path. All the radiations have the property of producing their ionization in small compact clusters of, 1, 2, 3, or sometimes more, ionizations. They differ, however, in the distances by which the successive clusters in the track of a particle are separated. For β- and γ-rays the distances are comparatively large (~ 300 mμ): for neutrons and α-rays they are much smaller (~ 1 mμ). The order of radiations given in the beginning of the paragraph turns out to be the order of diminishing separation between consecutive ion clusters. Thus the statement may be translated to the form: *The mean lethal dose is correlated with the ionization density of the radiation, being greatest for those radiations which produce their ionizations closest together.*

The results of the experiments have been thus briefly anticipated in order to make it clear what conclusions the experiments sought to test: in particular it was to establish the third conclusion, which plays a vital part in the interpretation, that many different radiations, covering as wide a range of ion density as possible, have been used.

The technical ease of the experiment, and the accuracy of the results obtained, vary considerably with different radiations. Thus experiments with γ-rays and hard X-rays are comparatively straightforward, since the bacteria can be irradiated in aqueous suspension and the source of radiation can be kept constant. On the other hand, experiments with soft X-rays are difficult on account of the low penetrating power of the radiations. (Absorption is serious in 10μ of matter of the density of water in some cases.) We have been at considerable pains to obtain the bacteria in thin films for these experiments, so that despite the low penetration of the radiations there should be no systematic error caused by some of the bacteria not being irradiated at the full intensity. This complication leads to an increase of the random error of the experiments, so that the shape of the survival curve cannot be determined with the same precision as with the more penetrating radiations. The

demonstration that the survival curves are accurately exponential thus rests mainly with those radiations (γ-rays, neutrons, and hard X-rays) with which the technical difficulties are least. (It is to be understood, however, that with the other radiations the survival curves are exponential within the error of the experiment.)

Bacteriological methods

Where the penetrating power of the radiations permitted, i.e. with γ-rays, neutrons, and hard X-rays, the bacteria were irradiated in aqueous suspension at a concentration usually of about 10^6 per c.c. Samples were taken at intervals during the irradiation and suitable dilutions made and plated in the usual fashion.

A suspension of vegetative bacteria was made up freshly as required from an agar slant incubated for 18 hr. at 37° C. The suspension was shaken vigorously and subsequently examined under dark field illumination to ensure that no appreciable proportion of the cells were in clumps or chains of two or more cells. Counts were made of the concentrated suspension in a Thoma-Hawksley counting chamber under dark field illumination to facilitate the preparation of a suspension of known concentration for irradiation.

Stock suspensions of spores free from vegetative cells were prepared in the manner described in an earlier paper (Lea, Haines & Coulson, 1936) and kept at 0° C., where they remained unchanged almost indefinitely, and used as required.

For the experiments with radiations of small penetrating power (soft X-rays and α-particles) the organisms were prepared in thin gelatine films by the following procedure (cp. also Lea *et al.* 1936). A suspension of bacteria in 10 % gelatine was kept molten at 27° C. in a hot box. By immersing a loop of platinum wire 9 mm. in diameter vertically in the gelatine and slowly withdrawing it, a thin film was formed on the loop. This was dried over P_2O_5 in a horizontal position for 5 or 10 min., when most of the water evaporated and the bacteria remained embedded in the gelatine film, which, although extremely thin, was reasonably robust. After exposure of the film to radiation, the central portion of the film (to a diameter of about 5 mm.) was dissected out with the aid of a sterile cataract knife. The dissection was performed on a sterile cover-slip which was then dropped into water at 37° C. carrying the film with it. The film immediately dissolved, and dilution and plating were then carried out in the usual fashion. The portion of the film dissected out in this way had a thickness of about 1μ, so that even with the least penetrating radiation (Al K-radiation) absorption was negligible. With the radiations which were a little more penetrating (α-rays and Cu K-radiation) it was not necessary to dissect out the centre of the film, and the whole platinum loop was dropped into water at 37° C. after irradiation.

The complicated technique necessary with soft radiations increased the random errors of the experiment. By a sufficient number of repetitions however (usually about fifteen films were exposed and an equal number used as controls for each point on a survival curve) reasonable accuracy was achieved. We prefer this procedure to that used by most authors who have worked with soft radiations, namely, of spreading the organisms on the surface of nutrient agar, on which they are irradiated and subsequently incubated. Using the latter technique, it is difficult to be certain (*a*) that the organisms really remain on the surface of the agar, (*b*) that they are not in part shielded by a film of water, (*c*) that the lethal action is not in part an indirect one due to the production in the nutrient agar of some substance toxic to the bacteria, and (*d*) that the radio-sensitivity of the cell is not altering as changes occur in it preparatory to division. We have evidence that all these factors play a part; and while, working with soft X-rays under suitable precautions, the results agreed well with those obtained by the gelatine loop method, the results with α-particles in general gave too rapid a rate of death.

Radiological data

The method of making the exposures to α- and β-rays has been described in an earlier paper (Lea *et al.* 1936). For the α-ray measurement the gelatine film technique was used, the film being exposed at a distance of about 2 mm. from the surface of a silver button on which polonium was deposited. These were the earliest experiments made and since the theory rests heavily upon the evidence they provide, the precaution has been taken of repeating them after an interval of about 3 years. Satisfactory confirmation was obtained. We are indebted to Dr Feather for the loan of a polonium source used in these repetition measurements. The description of the β-ray experiment has been given elsewhere (Lea *et al.* 1936) and need not be repeated.

The exact disposition used in the γ-ray experiments varied on different occasions according to the amount of radium available, and the number and shape of containers in which it was distributed. Usually a total quantity of up to 1 g. was used, the various units being arranged at a distance of about 2 cm. from the suspension of bacteria to produce as nearly uniform a distribution of radiation as practicable, the intensity being about 25 roentgen per minute. The intensity was measured by means of a thimble ionization chamber either of graphite or of "aerion" (Zimmer, 1936), it being known that a saturation ionization current of 1 e.s.u./c.c. at N.T.P. in such a chamber corresponds within 1 or 2 % to a dose of 1 r.

Exposure to hard X-rays was made with the aid of a filament tube of the therapy type, operating at about 160 kV. 7 mA. and with a filtration of 0·7 mm. Cu + 1·2 mm. Al. This radiation had a half-value thickness of 1·0 mm. of Cu corresponding to an effective wave-length of 0·15 A. The intensity was

about 25 r./min. and was measured with a thimble ionization chamber either of "aerion" or of graphite. Aerion was assumed to be "air walled" (Zimmer, 1936); the graphite chamber was calibrated against a free air ionization chamber. The two methods of measurement agreed satisfactorily.

The neutrons were provided by the one million volt generator of the Cavendish Laboratory. They were emitted from a target of lithium metal bombarded by deuterons of 0·9 million volts energy. The average current of the magnetically analysed deuteron beam was 100 μA. The reaction responsible for the neutron production is $Li^7 + H^2 = 2He^4 + n^1$, and the strength of the source corresponds to about 800 g. radium-beryllium mixture. The energy spectrum of the neutrons is continuous, with a maximum energy of about 14·5 MV. and a mean energy of about 3·9 MV. The mean energy of the protons

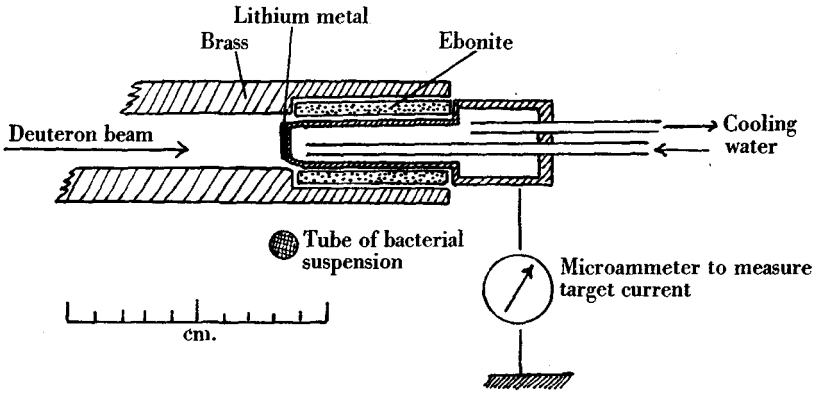

Fig. 1. Target for production of neutrons with a beam of artificially accelerated deuterons.

responsible for the ionization in the irradiated material is 2 MV. The construction of the ion tube in the vicinity of the target is shown in Fig. 1.

The exposures were made at a distance of about 4 cm. from the centre of the target, 2 ml. of suspension being exposed at a time and samples withdrawn periodically by means of a dropping pipette. At intervals of about $\frac{1}{2}$–1 hr. during the experiment (which usually lasted about 6 hr. in all) the tube of bacteria was removed and replaced by a thimble ionization chamber of volume 2·6 c.c., made of aerion, and having a 4 mm. gap between the electrodes. Aerion is a material specially suitable for the measurement of neutron dosage (Zimmer, 1936, 1938), since it contains a certain amount of hydrogen. Hence the ionization in a small aerion chamber is mainly due to hydrogen nuclei ejected from the walls. The problem of measuring neutrons in a unit which bears the same relation to the tissue ionization as does the international roentgen to the tissue ionization produced by X- or γ-rays has been considered by Zimmer (1938) and in greater detail by Gray (1939). Defining "1 roentgen

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of neutrons" to be that dose which produces the same tissue ionization as 1 r. of X- or γ-rays, they conclude that a saturation current of 1 e.s.u./c.c. in a small aerion chamber corresponds to a dose of 2 r. of neutrons. (Zimmer's figure is 1·8; Gray's 2·1. Here we assume 2·0.)

The soft-X-rays were generated in a laboratory-built continuously evacuated X-ray tube of the ionic type, having automatic control of the gas pressure. By changing the target, three different wave-lengths were made available, each nearly monochromatic, namely, 1·5 A. (Cu *K*-radiation), 4·1 A. (Ag *L*-radiation), and 8·3 A. (Al *K*-radiation). A description of the tube and the method of ionization measurement may be found elsewhere (Lea, 1940*a*).

In all cases the radiations have been measured in roentgens, the roentgen being a unit based on ionization in air. For the interpretation of these experiments it is necessary to know the ionization in water or tissue: the conversion factor depends on the relative absorption of the tissue or water and air for the radiation concerned. 1 r. corresponds to about 2×10^{12} ionizations/c.c. in water or tissue; the exact value varies a little for different radiations. In Table 1 the conversion factors for all the radiations used in this work are given, and also the "ion density" of the radiation, i.e. the number of ion clusters per cm. path in tissue or water, produced by the electron, proton or other particle responsible for the actual dissipation of energy.

Table 1. *Data of radiations*

				Soft X-rays				
Radiation	β-rays	γ-rays	Hard X-rays	Cu *K*	Ag *L*	Al *K*	Neutrons	α-rays
Wave-length (A.U.)	—	0·014	0·15	1·5	4·1	8·3	—	—
1 r. $\doteq \times 10^{12}$ ions/c.c.	1·74	1·74	1·70	1·30	1·54	1·52	1·72	2·63
Ion density $= 1/L$ ion clusters/cm.	2×10^4	5×10^4	$2{\cdot}1 \times 10^5$	5×10^5	9×10^5	$2{\cdot}4 \times 10^6$	$3{\cdot}5 \times 10^6$	$1{\cdot}3 \times 10^7$

Results

Exponential shape of survival curves

Survival curves of *Bact. coli* Escherich and spores of *B. mesentericus* exposed to α- and β-rays have already been published (Lea *et al.* 1936). Fig. 2 shows survival curves of *Bact. coli* exposed to γ-rays, hard X-rays and neutrons, and Fig. 3 to two wave-lengths of soft X-rays. Fig. 4 shows survival curves for spores of *B. mesentericus* exposed to two wave-lengths of soft X-rays. The curves obtained by plotting the logarithm of the fraction of organisms surviving irradiation against the dose are seen to be straight lines, which, as explained in the Introduction, means that the survival curves are exponential, or in other words that the death-rate is constant throughout the experiment. As is pointed out earlier, technical difficulties make it impossible to achieve

the same precision in the soft X-ray experiments as in the experiments with other radiations, and a certain amount of random deviation of the points from the curves thus occurs in Figs. 3 and 4. The demonstration of exponential

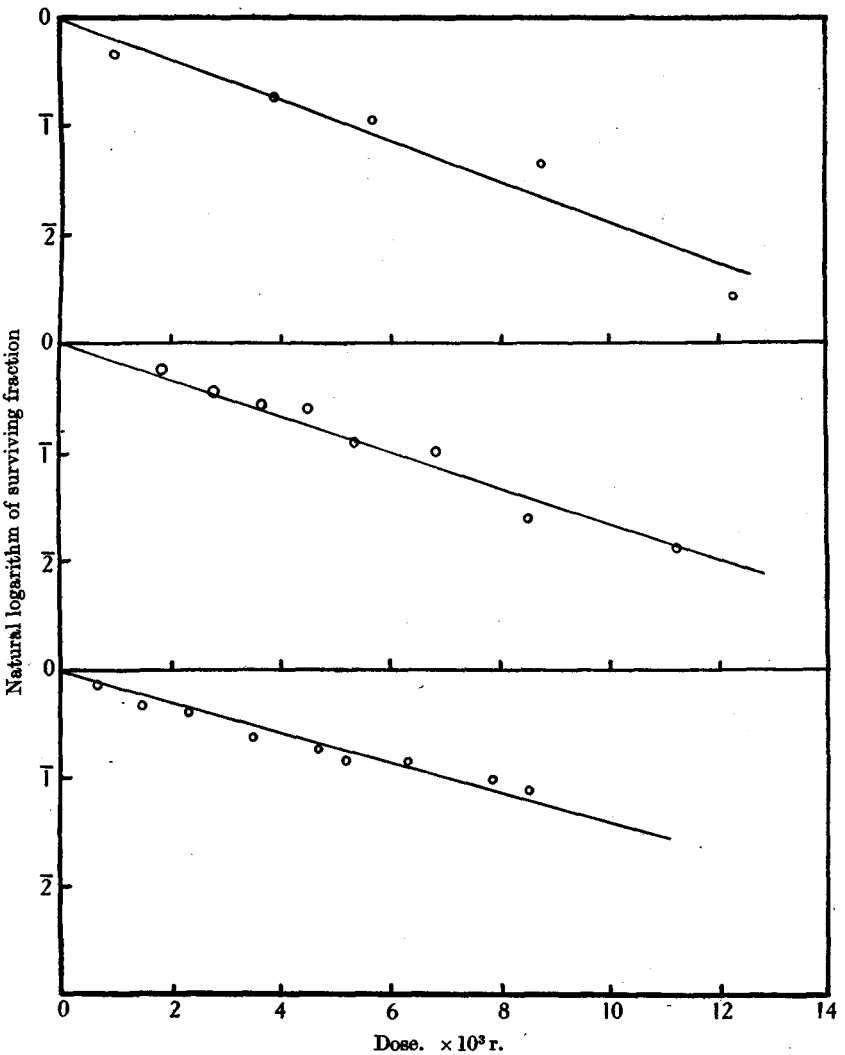

Fig. 2. Lethal action of γ-rays, X-rays and neutrons on *Bact. coli*. Upper curve γ-rays, middle curve X-rays, lower curve neutrons.

survival thus rests mainly on the three curves of Fig. 1 and on the curves previously published (Lea *et al.* 1936). (In the latter sufficient replication was possible to calculate standard deviations and hence to prove that the curves were accurately exponential over the whole range.)

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In Table 2 the experimental results on which the three curves of Fig. 2 are based are set out side by side with the theoretical values calculated, assuming an exponential survival curve. The theoretical values are calculated according to the equation *Surviving fraction* $= e^{-m}$, where $m =$ *dose administered divided by mean lethal dose.*

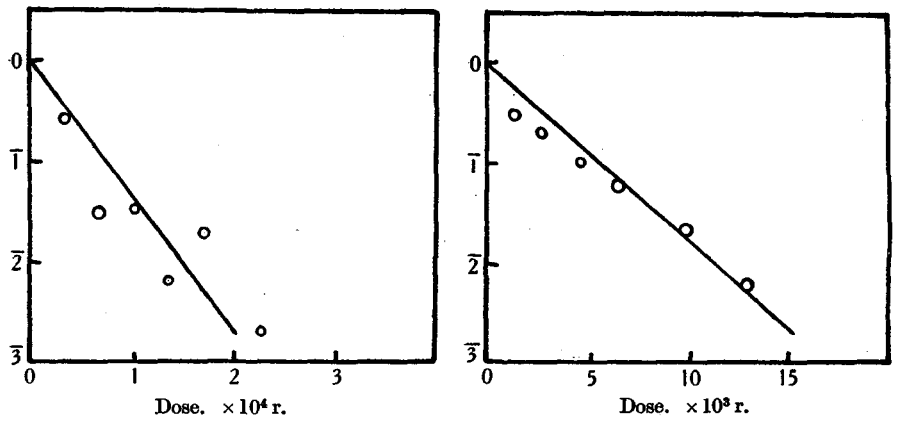

Fig. 3. Lethal action of soft X-rays on *Bact. coli*. (Ordinates natural logarithms of the fraction of organisms surviving.) Left: X-rays 8·3 A. Right: X-rays 1·5 A.

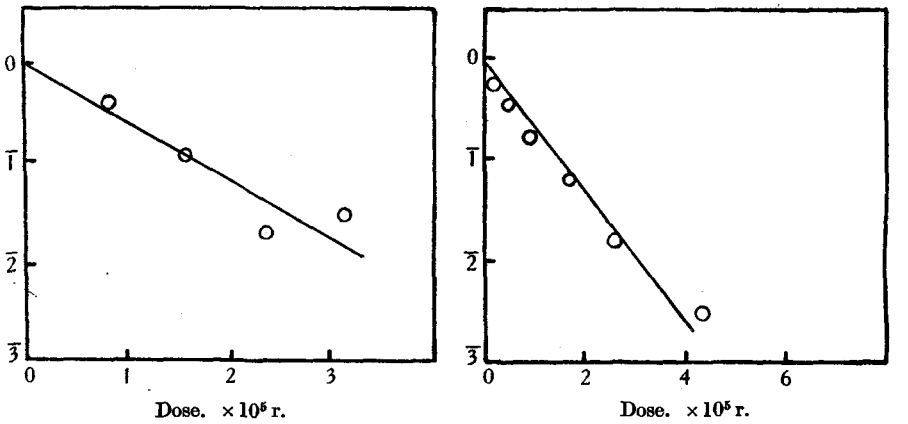

Fig. 4. Lethal action of soft X-rays on spores of *B. mesentericus*. (Ordinates natural logarithms of fraction of organisms surviving.) Left: X-rays 8·3 A. Right: X-rays 1·5 A.

It was not practicable with the intensity of neutrons we had available to determine the survival curve of spores of *B. mesentericus* exposed to neutrons. Axelrod, Aebersold and Spear (unpublished), however, have shown that it is exponential.

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γ-rays on *Bact. coli*. Mean lethal dose $= 5{\cdot}29 \times 10^3$ r.

Dose ($\times 10^3$ r.)	0·98	3·83	5·63	8·77	12·26
m = dose/5290	0·18	0·72	1·06	1·66	2·32
e^{-m} (theory)	0·83	0·48	0·34	0·19	0·10
Experimental surviving fraction	0·73	0·50	0·38	0·26	0·08

X-rays on *Bact. coli*. Mean lethal dose $= 6{\cdot}04 \times 10^3$ r.

Dose ($\times 10^3$ r.)	1·87	2·80	3·68	4·49	5·39	6·85	8·57	11·25
m = dose/6040	0·31	0·46	0·61	0·74	0·89	1·13	1·42	1·86
e^{-m} (theory)	0·73	0·63	0·54	0·48	0·41	0·32	0·24	0·16
Experimental surviving fraction	0·80	0·66	0·56	0·55	0·40	0·37	0·20	0·15

Neutrons on *Bact. coli*. Mean lethal dose $= 7{\cdot}09 \times 10^3$ r.

Dose ($\times 10^3$ r.)	0·68	1·52	2·33	3·56	4·70	5·20	6·34	7·83	8·58
m = dose/7090	0·10	0·21	0·33	0·50	0·66	0·73	0·89	1·10	1·21
e^{-m} (theory)	0·91	0·82	0·72	0·61	0·52	0·48	0·41	0·33	0·30
Experimental surviving fraction	0·87	0·71	0·67	0·54	0·47	0·43	0·43	0·36	0·33

Independence of effect upon intensity and temperature

In Table 3 are shown data for the spores of *B. mesentericus*, showing that the mean lethal dose is independent of intensity.

Table 3. *Independence of lethal dose of intensity*

Radiation	Intensity	Mean lethal dose
α-rays	164 r./sec.	$2{\cdot}3 \times 10^4$ r.
	1020 r./sec.	$2{\cdot}6 \times 10^4$ r.
Soft X-rays	104 r./sec.	$1{\cdot}5 \times 10^5$ r.
	1004 r./sec.	$1{\cdot}2 \times 10^5$ r.
8·3 A.	7830 r./sec.	$1{\cdot}7 \times 10^5$ r.

Data have already been published by Lea *et al.* (1936) showing that the effect of a given dose of α-rays on *B. mesentericus* spores is the same whether it is administered at +50, +20, 0, or −20° C., and that the lethal dose for *Bact. coli* exposed to γ-rays is the same at 37 and 0° C.

Dependence of lethal dose upon ion density of radiation

In Table 4 the various radiations are arranged from left to right in order of increasing ion densities. It is seen that for spores of *B. mesentericus* the lethal dose is least for the highest ion density, while for *Bact. coli* the reverse is true.

Table 4. *Lethal doses of various radiations*

				Soft X-rays				
Organism	β-rays	γ-rays	Hard X-rays	1·5 A.	4·1 A.	8·3 A.	Neutrons	α-rays
Bact. coli	4 $\times 10^3$	5·2 $\times 10^3$	6·0 $\times 10^3$	6·5 $\times 10^3$	—	7·5 $\times 10^3$	7·1 $\times 10^3$	24 $\times 10^3$
Spores of *B. mesentericus*	1·1 $\times 10^5$	1·3 $\times 10^5$*	—	1·3 $\times 10^5$	1·1 $\times 10^5$	1·5 $\times 10^5$	—	0·26 $\times 10^5$

* This figure is a new determination, the value 2×10^5 r. given by Lea *et al.* (1937) being in error on account of some mortality in the controls having occurred during the prolonged exposure.

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While *B. mesentericus* and *Bact. coli* are the only organisms for which full data are available, less detailed experiments on other organisms suggest that this contrast in behaviour of vegetative cells and spores is probably a characteristic difference.

The target theory

As most workers (Lacassagne & Holweck, 1934; Wyckoff, 1930; Heřcík, 1933; Pugsley, Oddie & Eddy, 1935; Lea *et al.* 1936) in this field have done, we interpret our experiments in terms of the target theory. The fundamental postulate of the target theory is that, although many ionizing particles pass through the bacterium before it is killed, its death, when it does occur, is due to one of these particles alone, which chances to pass through a specially sensitive region or "target" in the organism. On account of the small size of the target it is possible for many ionizing particles to pass through the organism without producing ionization in the target.

The argument by which we are led to this interpretation of the experiments is as follows. Suppose it is experimentally found that to kill a typical bacterium it is necessary to fire through it, on the average, 100 α-particles (a figure which is of the correct order of magnitude for *Bact. coli*). On the surface, the obvious interpretation would be that the death is a cumulative process to which each particle contributes. There are, however, serious objections to this view. In the first place, if the effect were a cumulative one, requiring the co-operation of a large number of particles, a difference would be expected depending on whether the radiation were given slowly or quickly. Given slowly, for example, one might anticipate that a certain amount of recovery would occur for which there was not time when the radiation was administered quickly. Actually, however, as Table 3 shows, the effect of a given dose is independent of the time required for its administration.

A second argument is based on the exponential shape of the survival curve. If 100 is the number of α-particles required to cause the death of a typical bacterium, it might be expected that in a supposedly "uniform" bacterial population a certain amount of variation of radiosensitivity would be found, so that a few organisms might be killed by as few as, say, 70, and a few require as much as, say, 150. Under these circumstances the survival curves would be of the type shown in Fig. 5A. Practically none of the organisms would be killed by very small doses. Actually it is of type B, which demands an extremely skew distribution of resistance among the bacteria, some being killed by extremely small doses and some requiring very large doses. Moderately skew distributions are of course common among biological populations: such extreme skewness is, however, improbable. Where the lack of plausibility is most pronounced, however, is in the closeness with which the survival curve approximates to the exponential form: we have (on this view) not only to postulate an immoderate degree of skewness in the curve of distribution of

resistance, but also to assert that (for no known reason) it assumes with considerable accuracy a particular mathematical form.

According to the target theory the exponential survival curve becomes, instead of an unplausible coincidence, the basis of the argument, which runs as follows:

To say that the survival curve is exponential is merely a mathematical expression of the statement that during each minute the proportion of organisms killed is a fixed proportion of the number viable at the beginning of that minute: this proportion is the same near the end of the irradiation, when the few surviving organisms have already been heavily irradiated, as it was at the beginning. This is exactly what is to be expected on the target theory, since, on that theory, whether a given α-particle kills a bacterium is determined simply by whether it hits the target or not, and does not depend in the least on how many α-particles have previously passed through the bacterium and

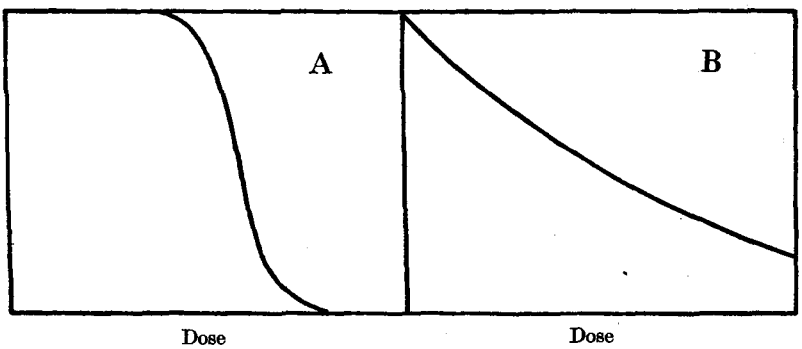

Fig. 5. Theoretical survival curves. See text. Ordinates: fraction of organisms surviving.

missed the target. If the size of the target is such that the chance that it will be hit in 1 min. irradiation is 1 %, then during each minute 1 % of the organisms will be killed, this death-rate being maintained throughout the irradiation.

The exponential survival curve is thus a necessary consequence of the target theory; so also is the independence of effect upon intensity. For, since we are postulating that when a bacterium is killed by, say, the hundredth α-particle which traverses it, the previous ninety-nine did not contribute at all to the death, it cannot have made any difference whether they followed one another at long intervals of time or short.

Since it explains naturally the two main experimental facts, which otherwise can only be regarded as unlikely coincidences, the target theory is clearly the acceptable interpretation, unless its postulates are themselves regarded as unplausible. Objection has indeed (e.g. Scott, 1937) been raised on this score. Before answering such objection we shall push the implications of the

target theory further and use the data of Table 4 to deduce the size of the target.

Interpretation of Table 4 shows that the lethal dose of *B. mesentericus* spores is less for densely than for less densely ionizing radiation. This means that the effectiveness per ionization increases when the ionizations are produced in close proximity with one another. It follows that the lethal action of radiation on spores is not due to a single ionization, but that more than one are required, or at any rate that several ion clusters are more effective than one. In contrast, for vegetative forms, the β- and γ-rays are most effective. Now these radiations produce their ion clusters at intervals of something like $0{\cdot}2\mu$, a distance which is large on an intracellular scale. It is unlikely therefore that a β-ray will leave more than one cluster when it passes through the target; if more than one were required, then, either β-rays would be less efficient than more densely ionizing radiations, or else the co-operation of several would be required, leading to a survival curve of non-exponential shape. We deduce therefore that for vegetative cells a single ion cluster in the target suffices to inactivate it. We can then immediately deduce the volume of the sensitive region by the consideration that if v is the volume, then the mean lethal dose must be that dose which produces on an average one ion cluster per volume v, i.e. it must be $1/v$ ion clusters/c.c. Now the average number of ionizations per cluster is 3, thus the mean lethal dose will be $3/v$ ionizations/c.c. in the tissue.

This calculation is valid for radiations which produce their ion clusters at such separations that more than one never fall in the target together. Now the more densely ionizing radiations produce their ion clusters closer together, and it may very well happen that several clusters fall in one target. Since one cluster suffices to cause the death of the organism, this radiation is prodigal of ionization, and *per ionization* is evidently less efficient than a radiation whose ionizations are more widely separated. The fraction (F) by which it is less effective is evidently simply the mean number of ion clusters left in the target by a radiation which leaves at least one. The calculation of this quantity is merely a geometrical problem if the size of the target and the mean separation of clusters (L) along the path of the ionizing particle are known. For simplicity of calculation we assume the target to be spherical of radius r and calculate F as a function of $\xi = 2r/L$. The mathematical calculation of $F(\xi)$ has been given elsewhere (Lea, 1940*b*), and its value may be read off for any value of $2r/L$ from the graph (Fig. 6). The mean lethal dose is thus $3F/v$ ionizations/c.c. If the target were a single sphere of radius r we could write $v = \frac{4}{3}\pi r^3$ in this formula. We speedily find, however, that no agreement between the theory and the experiment can be obtained in this manner, and it is necessary to assume that the sensitive region comprises a large number N of spheres, any one of which ionized leads to the death of the bacterium. Hence $v = \frac{4}{3}\pi r^3 N$, so that the mean lethal dose is $3F/\frac{4}{3}\pi r^3 N$ ionizations/c.c. By trial

we find the value of N and r which most nearly fit the experimental observations. In Table 5 we show together the calculated and theoretical values of

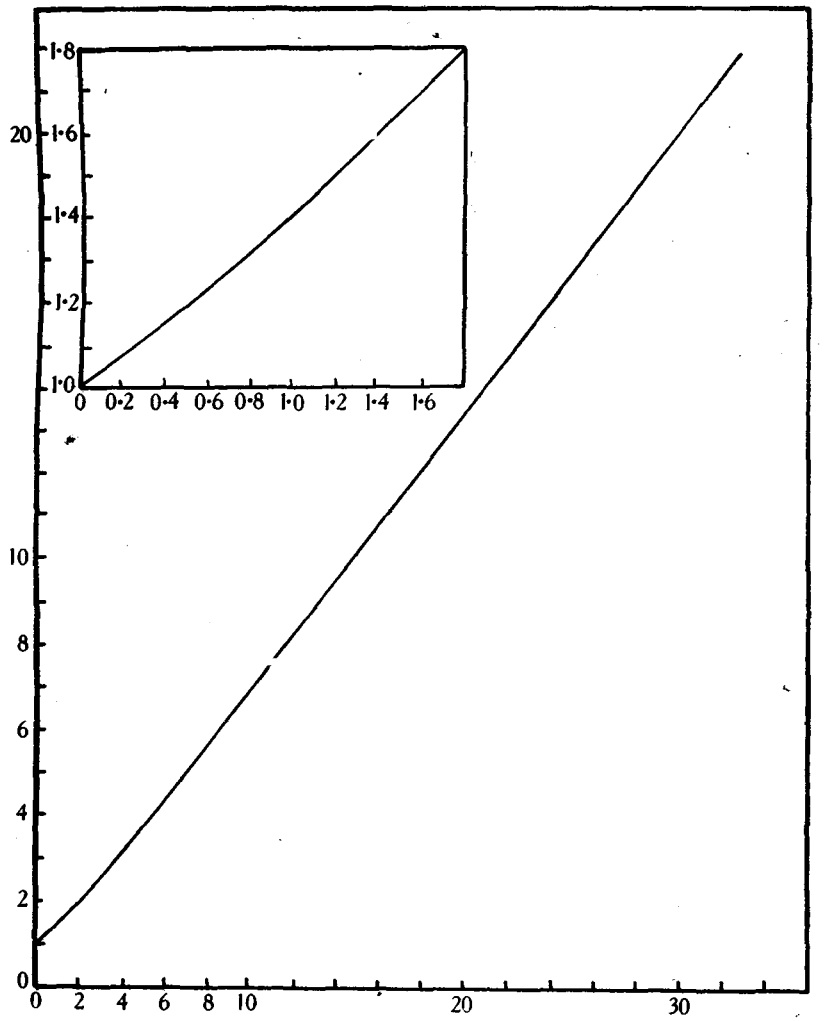

Fig. 6. The factor F. (Inset, early part of curve enlarged.)
Ordinates: F. Abscissae: $\xi = 2r/L$.

Table 5. *Comparison of target theory with experiment*

			X-rays				
Radiation	β-rays	γ-rays	0·15 A.	1·5 A.	8·3 A.	Neutrons	α-rays
Ion clusters per cm. $1/L$	2×10^4	5×10^4	$2{\cdot}1 \times 10^5$	5×10^5	$2{\cdot}4 \times 10^6$	$3{\cdot}5 \times 10^6$	$1{\cdot}3 \times 10^7$
Value of F	1·00	1·01	1·06	1·16	2·00	2·46	7·65
Lethal dose calculated $\times 10^{15}$ ions/c.c.	7·8	7·9	8·3	9·1	15·7	19·3	60
Lethal dose experimental $\times 10^{15}$ ions/c.c.	7·0	9·0	10·2	8·4	11	12	63

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the mean lethal doses of *Bact. coli*. The lethal doses quoted as experimental are the figures of Table 4 converted into ionization/c.c. by use of the factors listed in Table 1. The calculated values are computed assuming there are 1150 targets each of diameter 8·6 mμ: the agreement reached is sufficient to suggest that the theory is on the right lines.

The bactericidal action of radiation interpreted as lethal mutation

We have seen that the analysis of the experiments with *Bact. coli* suggests that the organism contains about 1000 "targets" each of diameter 8·6 mμ, and that the organism is rendered incapable of forming a colony if an ionization is produced in any one of these "targets". A diameter of 8·6 mμ is not impossibly large for a molecule (molecular weight 2×10^5). If the targets are regarded as molecules, it is arguable that they must be all different or mainly so, since it is hardly likely that the destruction of 1 part in 1000 of any particular chemical constituent would render the organism non-viable. Now it is a fair assumption that the destruction of one molecule will not cause the immediate cessation of all metabolic processes. A dose sufficient to render a bacterium non-viable may still leave it motile, and it has been demonstrated for yeast (Lacassagne & Holweck, 1934) and for tissue cells (Tansley, Spear & Glucksman, 1937) that the death of an irradiated cell often does not occur until the first or second division and there is some evidence (Robinow & Lea, unpublished observations) that the same applies to bacteria. Presumably there is time for the destroyed molecule to be resynthesized; that this does not occur suggests that the organism is unable to manufacture the molecule *ab initio*. Originally therefore it must inherit it. The molecule is capable of being duplicated for the purposes of cellular division but cannot be created by the cell *ab initio*.

All these arguments, starting from the target theory, lead to a set of properties for the targets which seem fantastic until we realize that these are just the properties which current theory attributes to the gene. The existence of genes in bacteria has not hitherto been recognized, presumably because the fact that bacteria do not propagate bi-sexually precludes the possibility of establishing the existence of genes as Mendelian characters. However, bacteria have quite complicated functions and properties which are maintained intact for many generations, and there must be some mechanism for achieving an exact duplication of the essential hereditary factors at each division. If a gene is imagined as an entity apart from being merely a locus in a chromosome, then just the properties must be attributed to it which we have here found it necessary to attribute to the targets in the bacterium. Whether in bacteria the genes are separable or are organized in chromosomes as in higher cells, the radiation experiments give no information. Modern staining methods do show

up some Feulgen positive bodies in bacteria, and it is possible that these are chromosomes (cp. a paper in preparation, by C. Robinow; also Badian, 1933; Stille, 1937).

This interpretation of the experiments with bacteria is rendered more convincing by pointing out how close is the relation between these experiments and the production of lethal mutations in *Drosophila* by irradiation of the sperm. The three principal experimental facts on which the preceding theory has been based apply also to the experiments with *Drosophila*, namely, the constancy of the rate at which the sperms are affected throughout the irradiation; the independence of the effect of a given dose upon temperature and intensity, and the reduction of efficiency when densely ionizing radiations are used as compared with those less dense. A theory worked out exactly in the same manner as for bacteria enables an estimate to be made of the number of genes in the *X*-chromosome of *Drosophila*, the number obtained being of the right order of magnitude (Lea, 1940*b*,*c*).

We have now to consider the nature of the target in the case of the spores, in which there was evidence that although one ionization cluster may suffice to kill the spore, the efficiency is increased by the close proximity of two or more clusters. The following speculations may bear on the problem. Suppose that the target molecule in the spores consists not of one molecule, but of two or more identical molecules, i.e. that a spore differs from the corresponding vegetative cell in having each gene duplicated. The ionization of one of the several molecules in a target would not then result in loss of viability. However, if the ionization or ion cluster were suitably placed it would doubtless lead to the decomposition of all the molecules (the energy available being adequate) and so to loss of viability. The small probability of a single ion cluster being effective is thus a measure of the probability of the ion cluster produced at random anywhere in the target being suitably placed to cause the decomposition of several molecules. Or it could be supposed that while a single ionization would not cause decomposition of all the molecules, a cluster of several ionizations would; the small probability referred to would then be the probability of occurrence of a cluster of the required size. In either case, an α-ray traversing the target could be relied upon to produce ionization separately in all the molecules, and so inevitably lead to loss of viability.

Summary

Experiments are reported in which the bactericidal effect of α-, β- and γ-rays of radium, X-rays of various wave-lengths, and neutrons, has been observed, particularly on *Bact. coli* and spores of *B. mesentericus*. It is shown that the survival curves are exponential and that the lethal dose is independent of the intensity of the radiation and of the temperature during the irradiation. The lethal dose varies for different radiations, being, in the case of vegetative

bacteria, greatest for those radiations, particularly α-rays, which produce their ionizations close together.

These experimental facts can all be explained on the view that the "lethal" action of radiation is really the production of lethal mutations. It is deduced that the number of "genes" in *Bact. coli* is of the order of 1000.

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