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Author
Dobson, R. Lowry.

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LETHAL RADIATION EFFECTS OF X-RAYS, DEUTERONS AND ALPHA PARTICLES ON THE BACTERIUM ESCHERICHIA COLI

R. Lowry Dodsen

Division of Medical Physics and Radiation Laboratory
University of California, Berkeley, California

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Introduction

Certain advantages present themselves in working with microorganisms in investigations of mechanisms mediating radiation effects, chief among which are that (1) enormous numbers of individuals may be used in single experiments thus giving immediate statistical advantage, and (2) the physical, physiological and biochemical conditions are less complicated than in higher forms, and may be more readily controlled experimentally. Most studies of the effects of radiation on bacteria have centered on lethal actions since changes in numbers of viable organisms are in general readily determined by standard bacteriological techniques.

In 1930 Wyckoff (1, 2, 3) proposed, from the slopes of survival curves obtained with Escherichia coli subjected to irradiation with cathode rays and x-rays of various wave lengths, that a single event process in each bacterial cell was responsible for death of the bacterium. This interpretation of the exponential survival curves obtained in irradiation experiments was more precisely developed into the target hypothesis by Lea, Haines and Coulson (4) in 1936. They presented theoretical and experimental evidence for the so-called target theory mechanism of action of ionizing radiations in producing lethal effects in bacteria. The target theory was subsequently discussed further by others (see for example Timofeef-Ressovsky (5)) and perhaps most completely expounded by Lea in 1946 (6).

This theory, in the one-hit form, postulates a specially sensitive and strategic volume or site within the protoplasm of the cell in which, or in the immediate vicinity of which a single event--usually assumed to be an ionization--will produce death of the individual cell. Such a first order reaction mechanism leads quite naturally to the exponential survival curves which are obtained in lethality experiments with ionizing radiations on bacteria. Lea (6) examined reported deviations from exponential survival, and presented arguments for their being artifacts due to various technical phenomena which may occur in the conduct of such experiments. The target theory may be generalized to include the necessity of multiple hits in a target, multiple targets, spread of effect on an ionization, uncertain target boundaries, and other factors which alter the results to be expected on the basis of a single target--single hit mechanism (see Lea (6) for general discussion of these various aspects of the target theory).

Indirect action on the bacterial cell by way of active radicals and hydrogen peroxide resulting from ionization of water (7) has seemed to be unimportant in the killing of E. coli since survival curves are exponential and concentration of organisms has been reported not to alter the effectiveness of irradiation (6). However, the importance of these indirect actions in producing changes in enzymes in vitro (8, 9), would suggest on general grounds the possibility that they might act to modify the single hit mechanisms.

When Witkin in 1946 (10) described a naturally occurring radiation resistant mutant strain B/r of the colon bacillus Escherichia coli strain B, it seemed desirable to examine these two closely related bacteria with regard to the nature of the altered radiation sensitivity and thus perhaps gain additional insight into the mechanism of the radiation effects.

This paper deals with lethality studies on strains B and B/r irradiated with x-rays, deuterons, and alpha particles.

Methods

Bacteriological

Agar slant cultures of strains B and B/r were kindly supplied by Dr. Witkin. In all of the experiments reported here the organisms were handled in the following manner: sixteen to twenty-four hour aerated broth cultures were grown to their maximum titers of approximately 2×10^9 and 10^9 bacteria per cubic centimeter for B and B/r respectively; these were centrifuged and the organisms resuspended in an aqueous solution containing 0.1% NH_4Cl, 0.6% anhydrous Na_2HPO_4, 0.02% $MgSO_4$, and 0.05% NaCl; the resuspension titers were adjusted to about 10^{10} organisms per cubic centimeter; these preparations were kept at refrigerator temperature and used for periods of from two to six weeks without noticeable change except for a small initial fall in titer.

For irradiation, 0.002 ml of an appropriate dilution of the refrigerator suspension was loaded by means of a micropipette onto the surface of a small block of agar measuring 3 mm on each side. All irradiation procedures were carried out with such agar block preparations. After exposure each block was dropped into a test tube containing an appropriate quantity of the salt solution mentioned above, shaken well, and 0.1 ml quantities pipetted onto the surface of nutrient agar plates (in Petri dishes) and smeared evenly with a bent glass rod. The plates were incubated at 37° C. for 16 to 18 hours, and counts made of the colonies. Determinations of survival fractions were made by comparing the counts with those from control blocks handled in the same way but not irradiated. The assumptions were made that each colony represented a single surviving organism and that all survivors appeared as visible colonies. The accuracy of the block technique itself determined by repeated runs without irradiation was within 5%.

X-rays

Experimental procedure

The 200 KV x-rays used were generated by a "Maximar 220 Therapy Unit", manufactured by the General Electric X-ray Corp., operated at 200 KVP and 15 ma. In most instances 1 mm Al filtration was used in addition to the inherent filtration of the machine. Doses in r were measured with a Victoreen thimble chamber.

In the majority of experiments 2 to 4 agar blocks were placed in a cylindrical capsule made of lucite. The diameter of the capsule was 2.5 cm, the height 3 cm, the wall thickness 1.5 mm, the top thickness 1.5 mm and the bottom thickness 9 mm. Several arrangements were used during irradiation:

I. 6 to 12 capsules containing agar blocks were placed in a tight circle on a 2 mm thick plastic shelf at a distance of 17 cm from the anode. Doses were measured continuously during exposure by a thimble chamber which emerged vertically through a hole in the shelf at the center of the circle and which was located at the same distance as the blocks. This chamber was connected to an "Integron" from which dose was read;

II. a small circle of blocks was placed on the shelf without capsules. Doses were measured in the same manner as in I;

III. a circle of blocks was placed on a 2 mm thick plastic platform 30 cm distant from the anode. The platform was supported by a thin metal rack and rotated at 1 r.p.m. with an electric motor during exposure. Doses were determined by measuring the dose rate with a Victoreen thimble chamber in the position of the blocks and timing the exposures with a stop watch;

IV. same as III except that blocks were placed inside capsules.

In order to test the importance of radiation contributed by backscatter from the agar blocks, as discussed in the next section on dosage, experiments

were performed with the following arrangements:

V. a circle of blocks was supported on a 0.25 mm thick sheet of lucite 10 cm distant from the 1 mm Al filter (25 cm from anode). The lucite sheet 15 cm in diameter was cemented at its periphery to a supporting lucite ring to which a long lucite handle was attached. This was held by a clamp and a ring stand 2 feet above the floor and away from other objects. Doses were measured as in III:

VI. similar to V except that agar blocks were placed in a small snug lacunae in a more extensive mass of agar held on the thin plastic sheet so that the bacteria were surrounded in all directions by at least 3 mm of agar.

In order to irradiate organisms in atmospheres of gasses other than air, agar blocks were put into capsules as described previously. The capsule however now had a small hole drilled in the side wall. This was closed with a small but tightly fitting vial type rubber stopper. The desired gas was led from a tank into the capsule by means of a hypodermic needle piercing the vial cap. A second needle served as outlet, and the gas was flushed through the capsule. When the needles were removed the gas tight capsule was conveniently handled and irradiated.

Dosage

When a beam of x-rays enters an absorbing medium, e. g. water or tissue, the density of ionization increases until a depth in the medium--the transition thickness--is equal approximately to the maximum range of secondary electrons in the medium (11). With the x-rays used in these experiments, where the maximum photon energy is 200 KeV and the photon energy emitted in greatest intensity is approximately 100 KeV (λ= 0.125 Å), about 98% of the energy of secondary electrons belongs to Compton recoil electrons with maximum and mean energy equal approximately 87 KeV and 43 KeV respectively. The maximum range of these 87 KeV secondaries in water is about 100μ (6). Hence radiative

equilibrium is reached after a thickness of 100μ of agar or tissue is traversed by the beam, and the dose, D, in rep* received by organisms exposed under such a layer will be numerically equal to D_r where D_r is the dose in r measured in air with the thimble chamber. This is true since at this point the factor 773, which is the density ratio of tissue and air, comes into full play. However in the case where bacteria are spread on the face of an agar block, and when there is no thickness of agar or lucite above them or adjacent filter in which secondaries are generated, D_{rep} might be expected to be somewhat lower than D_r since the width of each bacterium is of the order of 0.5μ --a small fraction of the transition thickness. This complication in the dosimetry of the problems turns out to be fortuitously negligible as will be seen from the results of experiments employing arrangements V, VI, VII and VIII (see Results); and this must be interpreted to mean that the backscatter from the agar block is not negligible as has been suggested (13), but indeed is so great as to put the surface effectively beyond the transition thickness, and justify the relationship $D_{rep} = D_r$ in all of the experimental arrangements previously described.

The other form of dose which has been calculated from D_r is the actual energy absorption in the bacterial cell

$$E_v = \frac{773\ D_r \times 32.5 \times 10^{-12}}{4.8 \times 10^{-10}} \quad eV / \mu^3$$

where 10^{-12} is the number of cm^3 / μ^3, 32.5 is the number of electron volts required to produce an ion pair, and $4.8\ 10^{-10}$ is the electronic charge in e.s.u.

* 1 rep = 83 ergs/gm (12).

Deuterons

Experimental Procedure

The deuterons were accelerated in the University of California 184 inch cyclotron to an energy of 190 MeV. The particles passed through an evacuated tube, having deen deflected out of the acceleration chamber, and emerged through an Al foil window into the air as a collimated beam. Physical measurements of the beam were made with ionization chambers and a Faraday cage as described by Tobias et al (14, 15). The energy of the particles to be used for bombardment was readily varied by introducing aluminum absorbers into the beam in front of the preparation to be irradiated. Deuterons corresponding to two portions of the Bragg curve were chosen for these experiments. The initial flat portion of the curve, spoken of in this paper as point A, was represented by the unfiltered 190 MeV particle beam; and the peak of the curve, spoken of as point C, was represented by the deuteron beam emerging from a thickness of aluminum (approximately 6.4 cm) such that the density of ionization produced in air was at a maximum. The average energy of the particles at point C was 26 MeV (14). In this way particles with the greatest difference in specific ionization were selected in order to compare their relative effectiveness in killing the two strains of bacteria.

The organisms were exposed on the surface of small agar blocks as in the x-ray experiments. In this case, however, 6 to 12 blocks were placed in a circle of outside radius 1/2 or 3/4 inch in a shallow lucite capsule. The inside diameter of the capsule was 2.5 cm and its height 2.2 mm. The top and bottom were each 0.6 mm in thickness. During irradiation the capsule was centered in the beam and rotated at 4 r.p.m.

by means of a small electric motor to assure equal dosage to all of the blocks.

Dosage

The dose, D in rep, was given by the amount of ionization in an accurately known volume of air in the monitor chamber. Now the energy loss in air of a 190 MeV deuteron, that is at Point A, is

$$A\left(-\frac{dE}{dx}\right)_{air} = 8.27 \times 10^3 \text{ eV/cm} \qquad (16)$$

and since at the peak of the Bragg curve, point C where the low energy irradiation was carried out, the 60% of the beam particles which remain (14) produce 3.7 times as much ionization per cm^3 air as at point A (where 3.7 is the average ionization ratio obtained at points C and A in these experiments) the average energy loss per deuteron in air at point C is given by

$$C\left(-\frac{dE}{dx}\right)_{air} = \frac{3.7}{0.6} \times A\left(-\frac{dE}{dx}\right)_{air} = 5.10 \times 10^4 \text{ eV/cm.}$$

From the above considerations, it is evident that the number of deuterons passing through each cm^2 of the sample, N, is given by

$$N = \frac{D \times 6.8 \times 10^{10}}{\left(-\frac{dE}{dx}\right)}$$

where 6.8×10^{10} is the factor required to convert energy absorption given in units of 1 rep = 83 ergs/gm to units of eV/cc of air.

The rate of energy loss per deuteron in tissue $\rho = 1$ will be greater in air by the reciprocal of the density of air, hence

$$A\left(-\frac{dE}{dx}\right)_{tissue} = \frac{8.27 \times 10^3}{1.293 \times 10^{-3}} = 6.4 \times 10^6 \text{ eV/cm;}$$

and

$$C\left(-\frac{dE}{dx}\right)_{\text{tissue}} = \frac{5.0 \times 10^4}{1.293 \times 10^{-3}} = 3.95 \times 10^7 \text{ eV/cm};$$

and the energy absorption in the substance of the bacterium will be

$$E_v = N\left(-\frac{dE}{dx}\right)_{\text{tissue}}$$

Alpha Particles

Experimental Procedure

The 5.3 MeV α-rays emitted by naturally radioactive Po^{210} were used for irradiation. The source consisted of 1.3 mc or Po^{210} plated on the surface of a small disc of Ni.* The disc was mounted on the lower end of a threaded brass screw which traveled down through the top of a plastic housing making possible accurate adjustment of the distance between the source and the agar block surface by means of a knurled knob. The agar block, bearing bacteria on its upper surface, was placed on an adjustable platform mounted in a drawer near the bottom of the housing. In this way the upper surface of the block was adjusted by means of a screw to the zero level. Beginning of irradiation was taken with a stop watch as the instant the drawer was closed bringing the block directly beneath the source disc. A shoulder in the drawer immediately above the block level received thin aluminum filters so that α-particles of various energies could be chosen by selecting suitable filter thickness and distance between source and sample. The α-particle energy as a function of filter thickness and distance from

* The Po^{210} source was obtained and prepared by the Eldorado Mining and Refining (1944) Limited of Ottawa, Ont.

the sample was immediately obtained from experimentally obtained Range-Energy relationships (17).

Dosage

The total activity, a, of the source was determined by counting with an impulse chamber the number of α-particles emerging through a thin window from an evacuated chamber of known geometry. The source was located in the chamber at the end opposite from the window. a_o was measured on June 16, 1950 to be 3.67×10^9 disintegrations per minute.*

Since the source of Po^{210} α-particles was a disc of radius R = 2.38 mm, and the perpendicular distance between source and sample was usually small (5 to 16 mm), it could not be considered a point source obeying the simple inverse square law. The number of particles hitting unit area of sample per minute was calculated, after Zirkle (18) as follows:

For a sample area small in comparison to πR^2 located d mm away from the center of the source, the probability of an α-particle originating in the source element 2π rdr hitting unit area of the sample, A, is

$$P = \frac{A}{4\pi (d^2 + r^2)}$$

Hence the flux, or the total number of particles from the summation of such elements which strike unit sample area per minute is given by

$$\Phi = \int_0^R \left(\frac{A}{4\pi(d^2 + r^2)} \right) n\, 2\pi\, r\, dr = \frac{nA}{2} \int_0^R \frac{r\, dr}{d^2 + r^2}$$

* I am indebted to Mr. A. Ghiorso of the University of California Radiation Laboratory for the measurement of a_o.

when n is the activity of the source per unit area, $a/\pi R^2$;

$$n_o = \frac{a_o}{\pi R^2} = \frac{3.67 \times 10^9}{\pi(2.38)^2} = 2.06 \times 10^8 \text{ a/mm}^2 \text{ min.}$$

The above expression for ϕ integrates to

$$\phi = \frac{nA}{4} \times 2.3 \times \log_{10} \frac{d^2 + R^2}{d^2}$$

For the purposes of these experiments ${}^d\phi_0$ was computed for convenience in terms of a/μ^2 min. As an example, at d = 10 mm,

$${}^{10}_{0}\phi = \frac{(2.06 \times 10^8) \times 10^{-8}}{4} \times 2.3 \times \log_{10} \frac{100 + 5.66}{100}$$

In this manner ${}^d\phi_0$ for any d was readily computed. It was then corrected for radioactive decay of the source to the date of experiment by taking into account the half life of 138 days. The number of particles which passes through unit area of the sample, N, is then obviously given by the product of the flux and the duration of exposure, $N = \phi t$.

The energy absorption in the bacterial cell may be calculated from N and the rate of energy loss of the α-particle in tissue. This energy loss may be obtained from experimentally determined Range-Energy relationships or may be computed from Bethe's stopping formula (11). For the fast polonium alphas the two are equivalent, but when filters are interposed and low energies used, the experimental values are more reliable. The rate of energy loss at d = 10 mm, spoken of as point A of the Bragg curve, is 1.07 MeV/cm air and at point C, the peak of the Bragg curve where d = 33.7 mm, the value is 2.04 MeV/cm air (taken from data given by Livingston and Bethe (17)).

The energy absorption in the bacterial cell is given by

$$E_v = N \left(- \frac{dE}{dx} \right)_{\text{tissue}}$$

In certain experiments where low energy α-particles were required, it was convenient to interpose an aluminum filter between source and sample in order to avoid too great a distance d and hence a low intensity of radiation. Filter thicknesses of 0.25, 0.50, and 0.75 mil were used. Since the relative stopping power of aluminum compared to air for the α-rays is 1.67×10^3 (19), a path length of 0.25 mil in aluminum is equivalent to 10.6 mm in air. On this basis the effective range in air and hence the energy of the bombarding particles could be selected at will, and the relative effectiveness at different portions of the Bragg curve studied.

Results

X-rays

In table I are given the data from a typical experimental determination of survival curves for strains B and B/r with x-rays; arrangement III (see Methods) was used in this particular case. The experimental data appearing in the table are the dose in kilorep, the dilution which was transferred to the agar block by means of the micropipette, the number of ml of saline into which each block was dropped after irradiation, and the number of bacterial colonies counted. From these data the percent survival was calculated; this also appears in the table. Fig. 1 shows graphically that the curves are exponential since the logarithm of the surviving fraction bears a linear relationship to the dose. In similar fashion twelve determinations were made in the case of B and sixteen in the case

of B/r. Table II presents these data together with the experimental arrangements used. In each instance the logarithm of the surviving fraction was plotted as a function of dose, and straight line curves of best fit were drawn through the experimental points. The mean slope, $\bar{m}$, was then computed for B and for B/r; from this figure the dose required to reduce the bacterial population to 1/e, or 36.8%, was determined. This dose is designated by D_o, and appears in the table. Fig. 2 drawn from $\bar{m}_B$ and $\bar{m}_B/r$ compares the relative sensitivity of the two strains of bacteria to x-rays.

Experiments performed to compare the survival of strain B organisms when irradiation was carried out with arrangements V, VI, VII, and VIII are analyzed in tables III and IV. The mean numbers of colonies, M, given in table III represent survivals of 36%, 37%, and 36.7% following doses of 2,500 r with bacteria (1) on the upper surface of single agar blocks, (2) sandwiched between pairs of blocks and (3) on blocks inside plastic capsules respectively. The differences between means in this case are not significant. However, after 5,000 r doses given with bacteria (1) on the upper surface of agar blocks and (2) on agar blocks placed in snug lacunae in a larger mass of agar, the difference between mean survivals indicated in table IV, 1149 - 1001 = 148 is statistically significant. This is seen by comparing the actual difference with the product of σ_{dM} and the t' ratio at the 1% level. For the 8 degrees of freedom in this case t' = 3.36, hence for a difference greater than $t'\sigma_{dM} = 3.36 \times 42 = 141$ there is less than one chance in one hundred that it could be so great by random sampling. There is reason to believe therefore that the dose in rep received by bacteria on an agar block surface irradiated in air

is somewhat lower than D_r. But from this last experiment it is seen that the ratio of mean survival is 1001/1149 = 0.88, a difference of only 12%. Now the dose is proportional to the logarithm of survival, so the difference in dose would be of the order of 2% and may be neglected. These results, as pointed out in the section on methods, indicate the justification of pooling results of experiments using arrangements I, II, III and IV in determining $\bar{m}$ and the mean D_o. From this latter quantity the value of $(E_v)_o$ is computed and appears in table XVIII.

Deuterons

Survival curves of both strains of E. coli were determined with high and low energy deuterons. Experimental data from the four experiments are given in tables V to XI. From figs. 3 to 9 it is seen that exponential curves were obtained as with x-rays since a strictly linear relationship holds between the logarithm of the surviving fraction and the dose. It will be noted in the tables that logarithms of surviving fractions have in many cases been adjusted to higher orders for convenience in calculation. The straight lines shown on the graphs represent best fits determined by the method of least squares. The equations of the lines are given in the tables.

Again as with x-rays B/r is more resistant than B to the lethal action of the radiation. The other striking feature which presents itself is that slopes of Point C curves are steeper in all cases than those of Point A curves. This indicates that the low energy particles are more efficient in killing the bacteria than the high energy particles. The slopes taken from the least squares fits are gathered together in table XII together with the ratios of slopes (m_C/m_A). The ratio

Table I

(Experiment 229)

			B		B/r	
Dose in Kr	Stock Dilution	ml per Block	No. Col. Counted	% Survival	No. Col. Counted	% Survival
0	.0001	2	1700	100	1588	100
2	.0001	2	803	47	1103	70
4	.0001	2	381	22	682	43
6	.0001	2	219	13	421	27
8	.0001	2	133	7.8	263	17
10	.001	2	527	3.1	1531	9.6
12	.001	2	233	1.4	1049	6.6
14	.001	2	112	.66	893	5.6
16	.001	2	46	.27	716	4.5
18	.01	2	274	.16	1207	2.3
20	.01	2	143	.084	778	1.5
22	.01	2	112	.066	1000	.94
24	.01	2	54	.032	1079	.68

MU1328

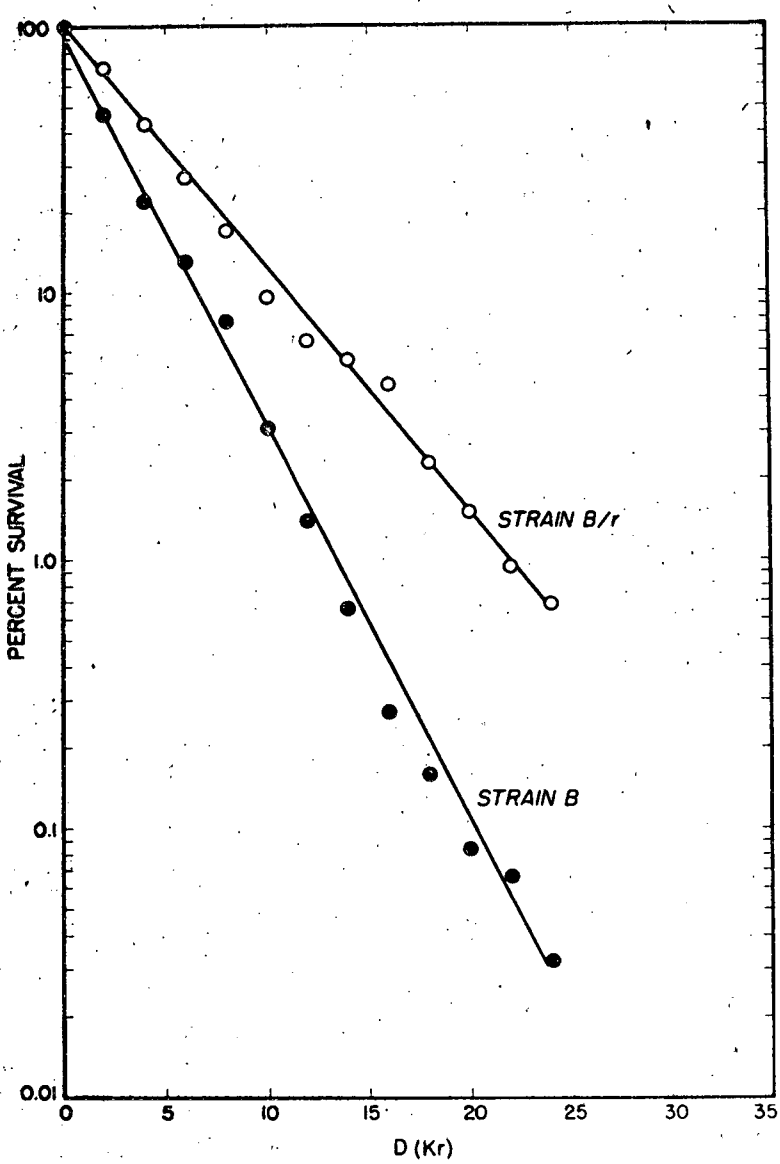

FIGURE 1.

MU1329

Table II

	B			B/r		
	Exp. No.	Exp. Arr.	Slope m	Exp. No.	Exp. Arr.	Slope m
1	339	I	2.21	341A	I	1.35
2	338	I	1.82	341B	I	1.16
3	336	IV	2.06	341C	I	1.27
4	335	I	2.43	336	IV	0.9[illegible]
5	334	I	1.98	335	I	1.13
6	300	I	2.01	334	I	0.91
7	282	I	2.12	300	I	1.10
8	272	II	1.98	283	I	1.10
9	246	II	2.25	282	I	1.12
10	233	III	1.86	279	I	1.36
11	230	III	2.24	272	II	1.21
12	229	III	1.83	268	I	1.30
13				233A	III	1.26
14				233B	III	1.22
15				230	III	1.33
16				229	III	1.14

$\bar{m}$ = 2.05

σ = 0.182

σ_M = 0.055

D_o = 2,650 r

$\bar{m}$ = 1.18

σ = 0.126

σ_M = 0.0325

D_o = 4,610 r

MU 1330

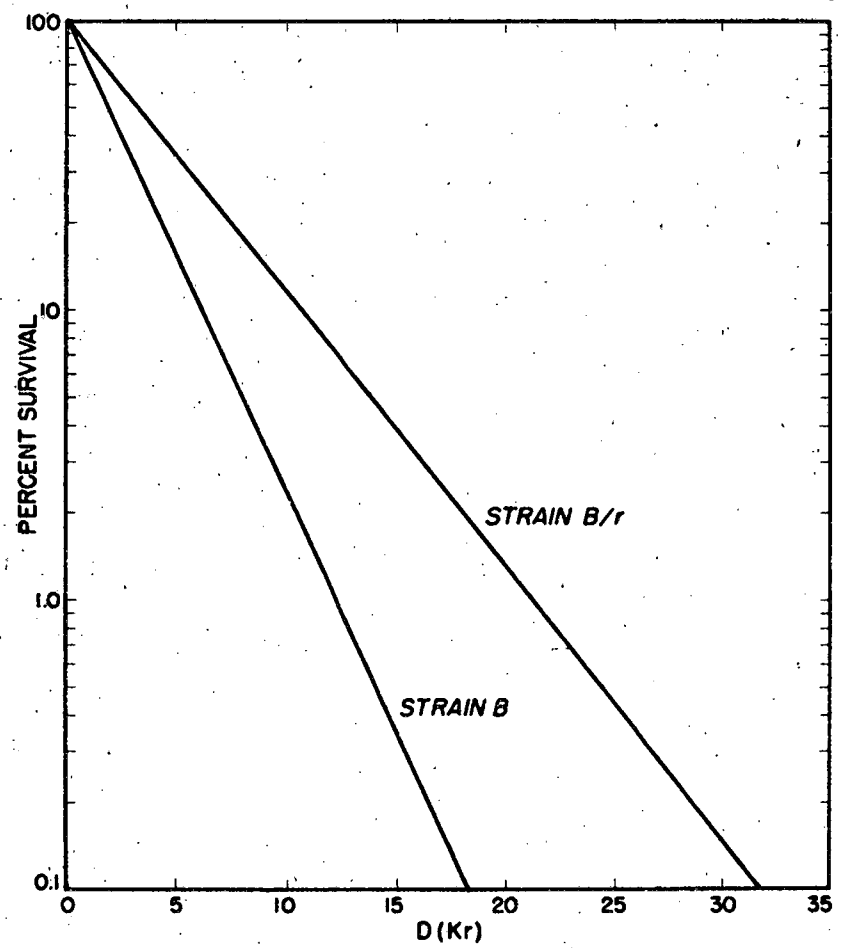

FIGURE 2

MU 1331

Table XIX
(Experiment 360)

	Single Blocks Art. V	Double Blocks Art. VI	Capsules Art. VII
1	488	477	555
2	541	571	571
3	523	585	518
4	525	578	
5	606	544	
$\bar{x}$	536	551	548
σ	53.8	33.7	22.1
σ_M	19.4	19.3	19.7

σ_{dM} for single blocks and capsules = 25

MU 1332

Table IV
(Experiment 364)

	Single Blocks Arr. V	Blocks in Agar Arr. VIII
1	1379	1046
2	1163	1074
3	1162	852
4	1134	1014
5	908	1017
M	1149	1001
σ	149	77.5
σ_M	37.3	19.4

$$\sigma_{dM} = 42$$

m_C/m_A serves as a measure of the relative effectiveness of low energy and high energy deuterons. The significance of this ratio is greater than unity may be demonstrated by multiplying $\sigma_M = 0.068$ (table VII) by $t = 3.71$, selecting fiducial limits to include 99% of cases where the degrees of freedom are $N-1 = 6$. Thus $t\sigma_M = 0.252$. Since this is less than 0.33, the difference between 1.33 and unity, the probability is well over 0.99 that $\frac{m_C}{m_A} > 1$.

From m_A and m_C for B and B/r given in table XII the dose, D_o, necessary to produce 36.8% survival was calculated. Values of D_o obtained in this way are as follows:

B_A , $D_o = 3,060$ rep;

B_C , $D_o = 2,439$ rep;

B/r_A, $D_o = 5,080$ rep;

B/r_C, $D_o = 3,750$ rep.

From these values N_o and $(E_v)_o$ were calculated as indicated in the section on dosage. These values are listed in table XVIII.

Alpha Particles

Survival curves for both strains of organism were determined experimentally by maintaining a fixed distance between source and sample and timing the various doses with a stop watch. The α-particle flux, $^d\phi$, for any distance, d, between source and sample is known from calculation (see Methods). The dose, N, in terms of α/μ^2 is thus given by $^d\phi_t$. In tables XIII and XIV the surviving fractions for graded doses are given. The straight line curves of best fit, determined by the method of least squares and drawn through the points, are shown in figs. 10 and 11. The equations appear on the

Table V-a
(Experiment 332)

B/B'_A

Dose in Krep	No.Col. Counted #1	No.Col. Counted #2	Stock Dilution	ml per Block	% Survival #1	% Survival #2	Mean Survival	Log Surv. Fraction	Dose Increment
0	458	458	.001	10	100	100	100	2.000	1
3.03	581	627	.001	5	63.3	79.2	71.3	1.853	2
6.06	825	800	.001	3	53.9	52.3	53.1	1.725	3
			.001	1.5					
12.12		1600	.01	10		34.9	34.9	1.543	5
15.15	2350	2520	.01	5	25.3	27.5	26.4	1.422	6
18.18	3195	2057	.01	3	20.9	13.5	17.2	1.235	7

$y = -0.119 x + 2.12$

MU 1334

Table V-b
(Experiment 332)

B/r_C

Dose in Krep	No.Col. Counted #1	No.Col. Counted #2	No.Col. Counted #3	No.Col. Counted #4	Stock Dilution	ml per Block	% Survival #1	% Survival #2	% Survival #3	% Survival #4	Mean Survival	Log Surv. Fraction	Dose Increment
0	458	458	458	458	.001	10	100	100	100	100	100	2.000	1
3.03	632	615	541	588	.001	5	68.8	67.0	58.9	64.1	64.7	1.811	2
6.06	789	835	613	792	.001	3	51.6	54.6	40.1	51.7	49.5	1.695	3
9.09	969		825	816	.001	1.5	31.7		27.0	26.6	28.4	1.453	4
12.12	1155	1175	1080		.01	10	25.2	25.6	23.5		24.7	1.393	5
15.15	1819	1266	1551	965	.01	5	19.8	13.8	16.9	10.5	15.2	1.182	6
18.18	2113	1557	1674	1454	.01	3	13.8	10.2	10.9	9.5	11.1	1.045	7

$y = -0.158\ x + 2.14$

MU 1335

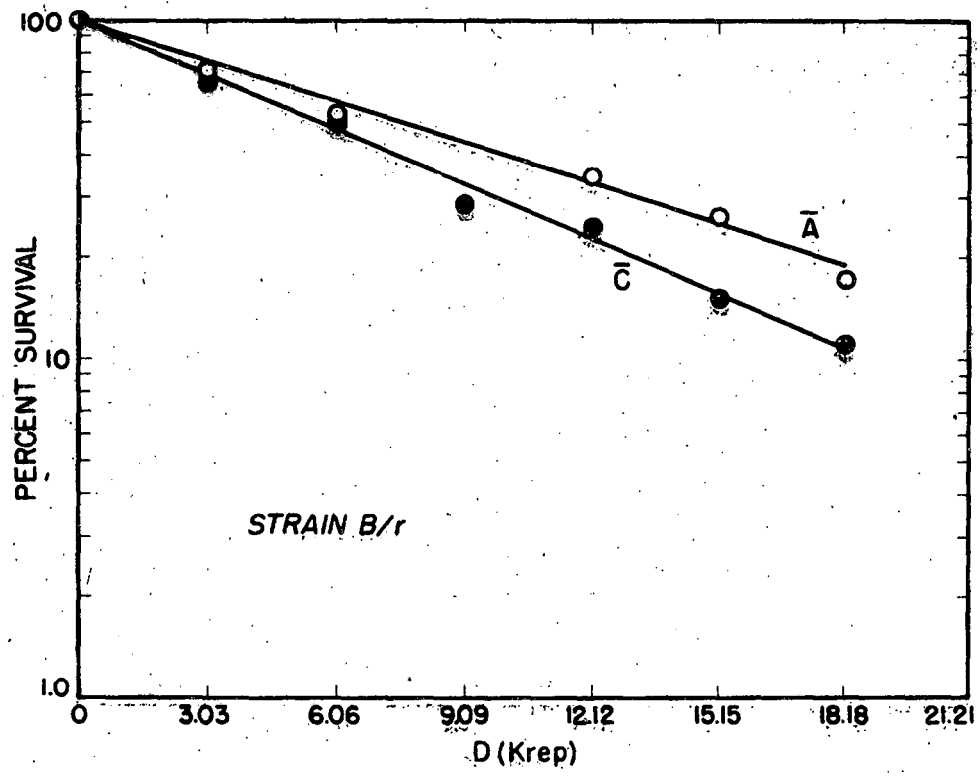

FIGURE 3

MU 1336

Table VI
(Experiment 253)

	Dose in Krep	No. Col. Counted	Stock Dilution	ml per Block	% Survival	Log Surv. Fraction	Dose Increment
	0	1900	.001	4	100	4.000	0
	3.03	623	.001	2	16.4	3.215	1
	6.06	377	.001	1	4.94	2.694	2
B_A	9.09	564	.01	2	1.47	2.167	3
	15.15	363	.1	3	0.142	1.152	5
	18.18	150	.1	2	0.040	0.597	6

$y = -0.547\ x + 3.86$

	0	1900	.001	4	100	4.000	0
	3.0	558	.001	2	14.7	3.167	1
	6.0	330	.001	1	4.32	2.635	2
B_C*	9.09	267	.01	2	0.699	1.844	3
	12.12	206	.01	1	0.276	1.441	4
	15.15	125	.1	3	0.049	0.692	5

$y = -0.652\ x + 3.94$

* Figures represent averages of two separate runs.

MU 1337

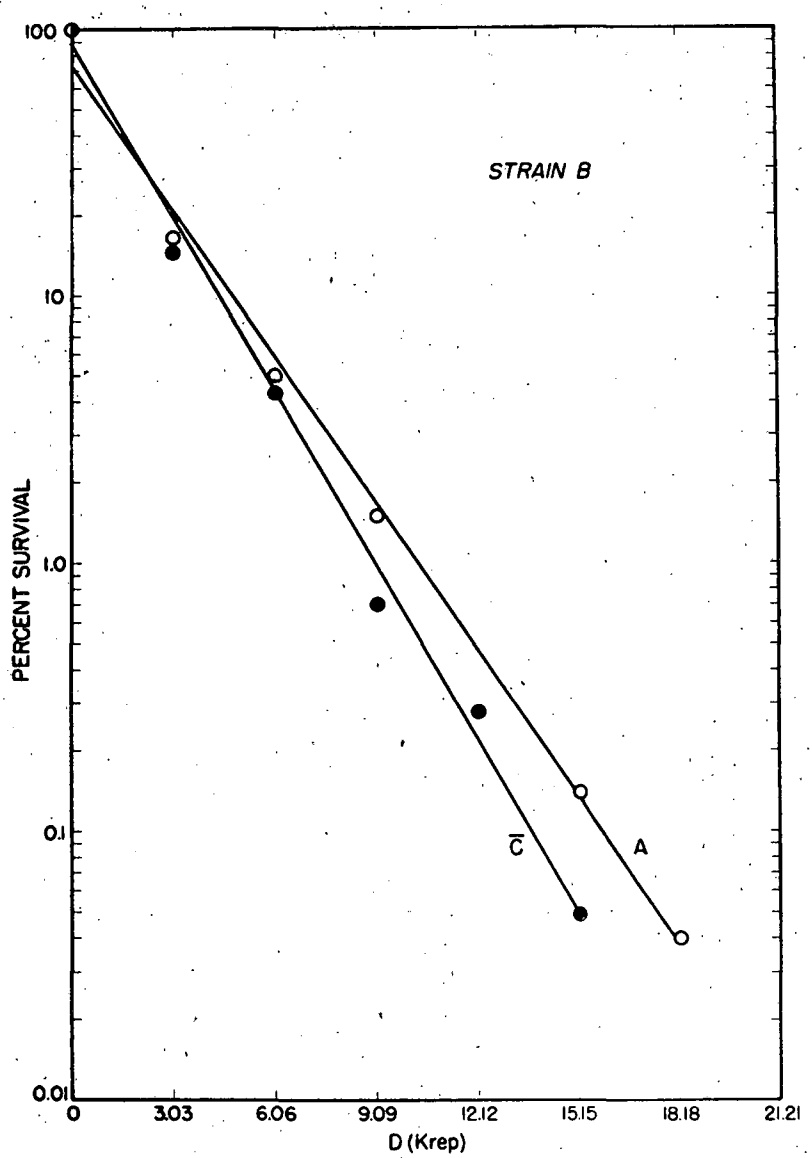

FIGURE 4

MU 1338

Table VII
(Experiment 253)

	Dose in Krep	No. Col. Counted	Stock Dilution	ml per Block	% Survival	Log Surv. Fraction	Dose Increment
	0	1840	.001	4	100	2.000	0
	3.03	1870	.001	2	50.8	1.706	1
	6.06	844	.001	2	22.9	1.360	2
B/r_A							
	15.15	825	.01	2	2.23	0.348	5
	18.18	403	.01	2	1.09	0.037	6
			$y = -0.331 x + 2.01$				
	0	1840	.001	4	100	3.000	0
	3.03	1674	.001	2	45.5	2.658	1
	6.06	586	.001	2	15.9	2.201	2
B/r_C*	9.09	524	.001	1	7.11	1.852	3
	12.12	544	.01	4	2.95	1.469	4
	15.15	450	.01	2	1.22	1.086	5
	18.18	124	.01	2	0.334	0.524	6
			$y = -0.403 x + 3.04$				

* Figures represent averages of two separate runs.

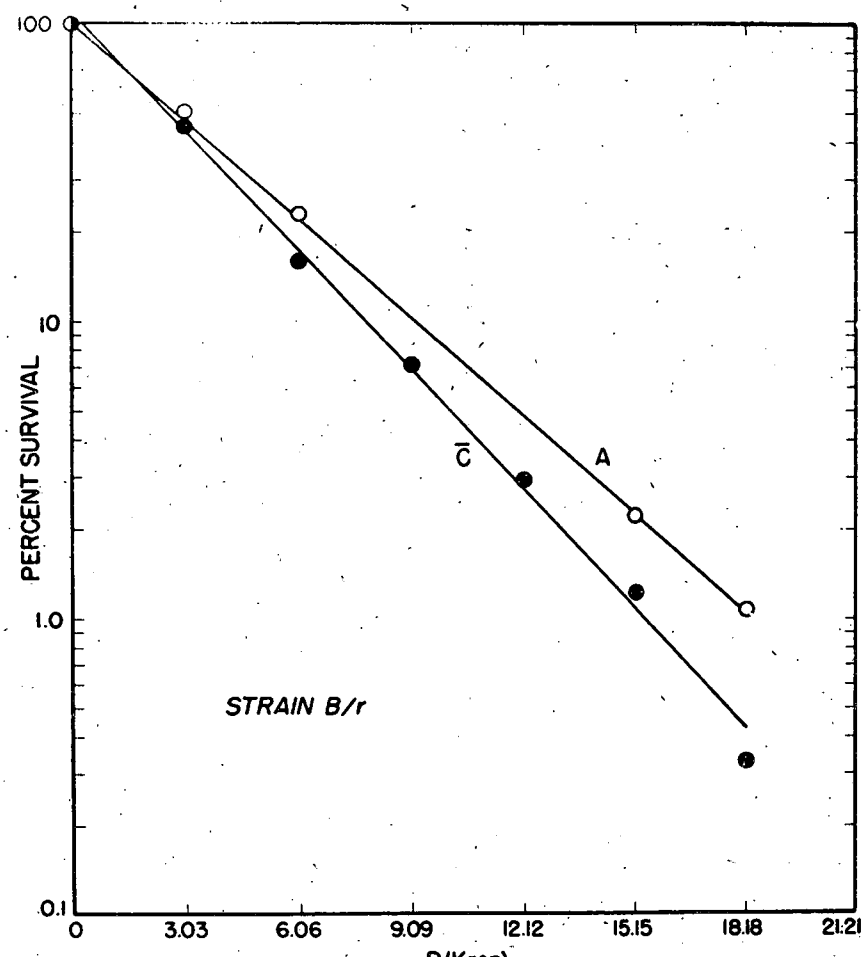

FIGURE 5

MU 1340

Table VIII
(Experiment 245)

	Dose in Krep	No. Col. Counted	Stock Dilu-tion	ml per Block	% Sur-vival	Log Surv. Fraction	Dose Incre-ment
	0	452	.001	4	100	3.000	0
	3.03	451	.001	2	49.8	2.697	1
	6.06	258	.001	1	14.2	2.152	2
B_A	9.09	239	.01	2	2.63	1.403	3
	12.12	248	.01	1	1.37	1.137	4
	15.15	387	.1	3	0.642	0.807	5
	18.18	496	.1	2	0.549	0.739	6

$y = -0.413\ x + 2.96$

	0	452	.001	4	100	3.000	0
	3.03	315	.001	2	35.0	2.544	1
	6.06	151	.001	1	8.41	1.925	2
B_C	9.09	192	.01	2	2.12	1.326	3
	12.12	143	.01	1	0.797	0.901	4
	15.15	286	.1	3	0.473	0.675	5
	18.18	228	.1	2	0.252	0.401	6

$y = -0.449\ x + 2.89$

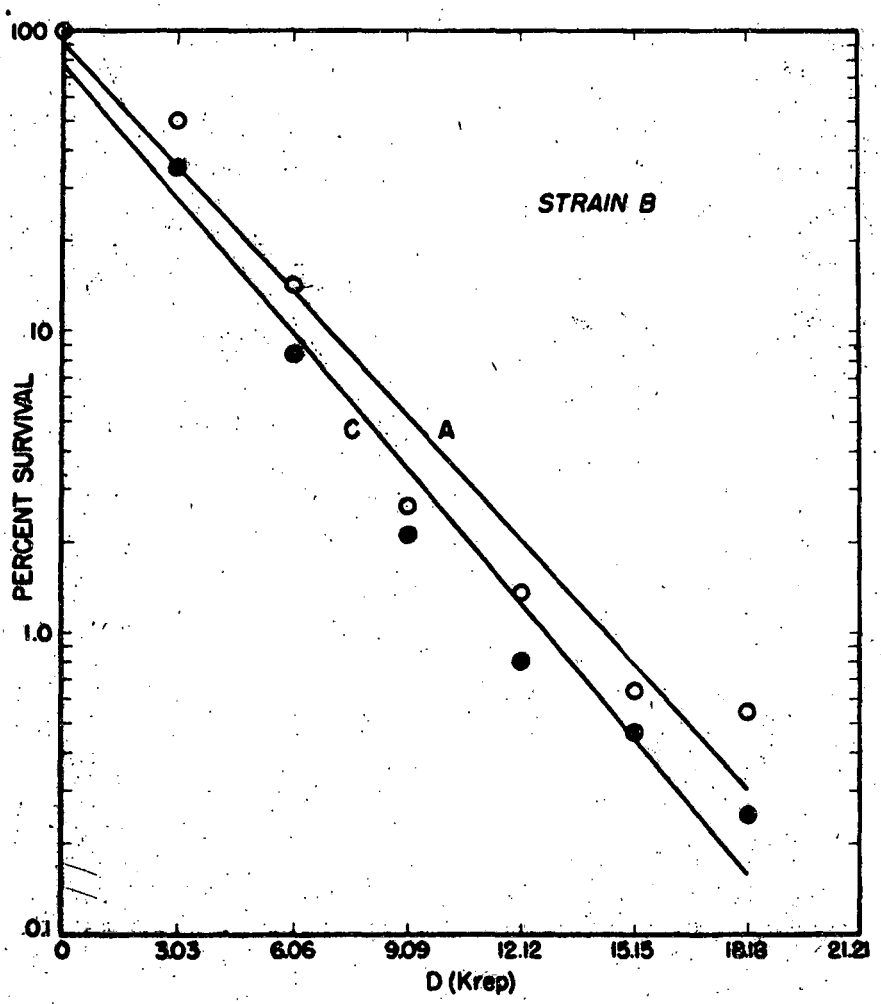

FIGURE 6

MU1342

Table IX
(Experiment 245)

	Dose in Krep	No. Col. Counted	Stock Dilution	ml per Block	% Survival	Log Surv. Fraction	Dose Increment
	0	600	.001	4	100	2.000	0
	3.03	878	.001	2	73.2	1.864	1
B/r_A	9.09	640	.001	1	26.7	1.427	3
	12.12	521	.01	4	8.68	0.939	4
	15.15	819	.01	2	6.82	0.834	5
	18.18	537	.01	2	4.47	0.650	6

$y = -0.239\ x + 2.04$

	0	600	.001	4	100	2.000	0
	3.03	918	.001	2	76.5	1.884	1
	6.06	501	.001	2	41.7	1.620	2
B/r_C	9.09	467	.001	1	19.5	1.290	3
	12.12	175	.01	4	2.92	0.465	4
	15.15	186	.01	2	1.55	0.190	5
	18.18	315	.01	2	2.63	0.420	6

$y = -0.332\ x + 2.12$

MU 1343

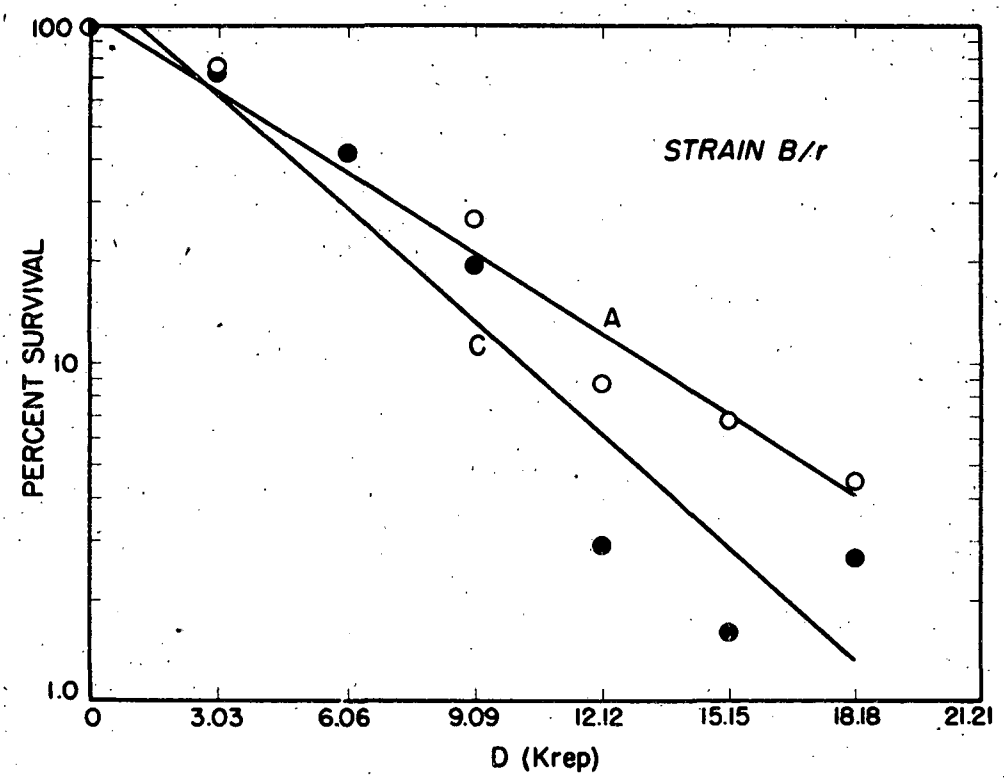

FIGURE 7 MU 1344

Table X
(Experiment 235)

	Dose in Krep	No. Col. Counted	Stock Dilution	ml per Block	% Survival	Log Surv. Fraction	Dose Increment
	0	728	.001	4	100	3.000	0
	3.03	348	.001	2	23.9	2.378	1
	6.06	381	.001	1	13.1	2.117	2
B_A	9.09	970	.01	3	10.0	2.000	3
	12.12	1025	.01	1	5.22	1.717	4
	15.15	910	.1	5	1.57	1.196	5
	18.18	1095	.1	2	0.75	0.876	6
			$y = -0.327\ x + 2.88$				
	0	728	.001	4	100	4.000	0
	3.03	327	.001	2	16.9	3.230	1
	6.06	186	.001	1	5.02	2.700	2
B_C	9.09	132	.01	3	1.12	2.050	3
	12.12	133	.01	1.5	0.59	1.770	4
	15.15	106	.1	5	0.116	1.220	5
	18.18	97	.1	2	0.062	0.793	6
			$y = -0.52\ x + 3.82$				

MU 1345

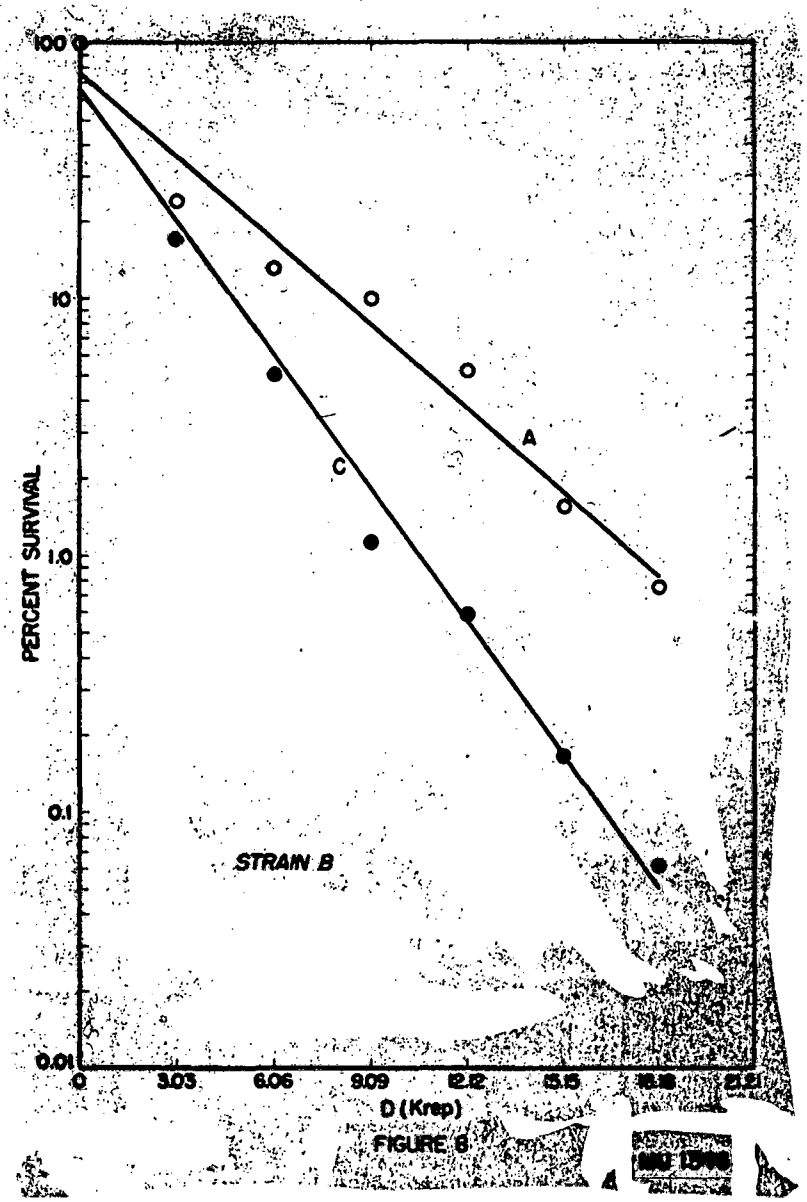
100
10
1.0
0.1
0.01
PERCENT SURVIVAL
A
C
STRAIN B
0
3.03
6.06
9.09
12.12
15.15
18.18
21.21
D (Krep)

FIGURE 8

Table XI
(Experiment 235)

	Dose in Kvap	No. Col. Counted	Stock Dilution	ml per Plate	% Survival	Log Surv. Fraction	Dose Increment
	0	543	.001	4	100	2.000	0
	3.03	650	.001	2	59.8	1.777	1
	6.06	592	.001	1	27.2	1.434	2
B/r_A	9.09	657	.01	5	14.7	1.167	3
	12.12	1047	.01	2.5	12.1	1.083	4
	15.15	1156	.01	1.25	6.65	0.823	5
	18.18	2040	.1	6.25	5.67	0.768	6

$y = -0.209\,x + 1.93$

	0	543	.001	4	100	2.000	0
	3.03	458	.001	2	36.6	1.563	1
	6.06	392	.001	1	16.2	1.210	2
B/r_C	9.09	317	.01	5	6.78	0.831	3
	15.15	308	.01	1.25	1.73	0.239	
	18.18	481	.1	6.25	1.36	0.135	6

$y = -0.317\,x + 1.90$

MU 1347

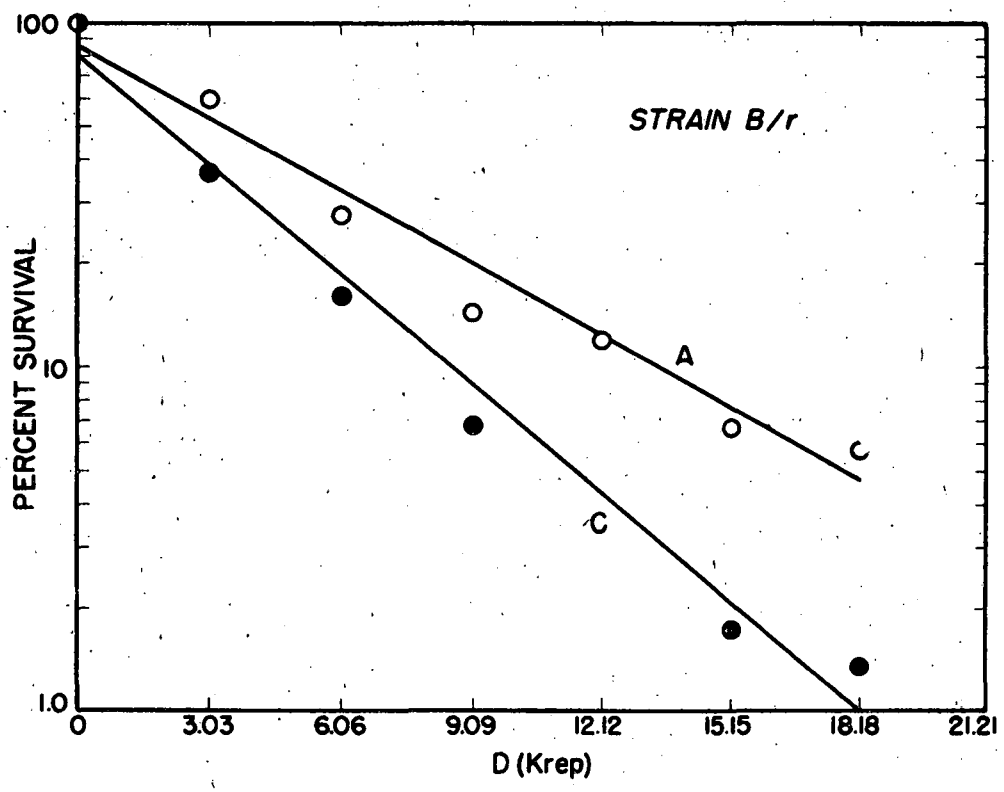

FIGURE 9

MU 1348

Table XII

Exp. No.	Ionis. ratio $\frac{C}{A}$	Strain	m_A	m_C	$\frac{m_C}{m_A}$
332	4	B			
		B/r	0.119	0.158	1.33
253	3.5	B	0.547	0.652	1.19
		B/r	0.331	0.403	1.22
245	4	B	0.413	0.449	1.09
		B/r	0.239	0.332	1.39
235	3.4	B	0.327	0.520	1.59
		B/r	0.209	0.316	1.52
Mean	3.7	B	0.429	0.540	1.33
		B/r	0.259	0.351	

For $\frac{m_C}{m_A} = 1.33$, $= 0.167$

± 0.068

MU 1349

tables. Again survival is exponential and strain B/r is more resistant to the α-radiation than strain B.

In order to test the efficiency of α-particles of various energies in killing the bacteria, irradiation was carried out at selected points along the particle range. By selecting suitable filter thickness and distance, d, survivals at various points on the Bragg curve were determined for fixed dose in terms of α/μ^2. Table XV gives survival fractions obtained in six experiments for B and B/r. The experiments 353, 354, 356 $^{10}N = 3\ \alpha/\mu^2$ and in experiment 363 $^{10}N = 2.7\ \alpha/\mu^2$. In both experiments on strain B, $^{10}N = 2.25\ \alpha/\mu^2$. These data in graphical form are shown in figs. 12 and 13. Here the percent survival is plotted as a function of the residual range in air in the same manner as the Bragg curve itself is a plot of specific ionization as a function of range. In the case of B/r it is apparent that the α-particles are more efficient at lower energy. The two experiments on B leave room for doubt. Therefore several determinations were made at d = 10.25 mm and at d = 31.45 for both strains. Table XVI gives data from these experiments which demonstrate a significant difference in the effectiveness at the two points on the Bragg curve in the case of both organisms, the low energy particles being the more effective in killing.

From all survival data gathered at d = 10 mm, the mean dose, $^{10}N_o$. resulting in 36.8% survival, was computed (table XVII). Curves drawn for B and B/r from these means are shown in fig. 14.

The rate of energy loss of α-particles at d = 10 mm, i. e. with residual range equal to 38.7 - 10 = 28.7 mm, is 1.07 MeV/cm (17). From this figure and from $^{10}N_o$ the energy absorption in the cell

$(E_v)_o$ is calculated as described in the section on Methods. The corresponding dose in rep, D_o, is then obtained by dividing $(E_v)_o$ by 51.9 since 1 rep = 83 ergs/gm corresponds to the absorption of 51.9 eV per cubic micron of tissue. These values appear in table XVIII.

Target Considerations

Assuming killing of the bacterium by radiation to be a target phenomenon of a one-hit type, the size of the target may be computed. If it is further assumed that the target is a spherical volume of radius, r, the simplest method of calculation for x-rays rests on the supposition that an ionization in any portion of the target volume results in cell inactivation.

Now the number of ion pairs formed per cubic micron in the bacterium, to produce inactivation, can be expressed by

$$j = \frac{D_o \times 773 \times 10^{-12}}{4.8 \times 10^{-10}} = 1.61\ D_o,$$

where D_o is the inactivation dose, 773 is the density ratio of water and air, 10^{-12} is the number of cubic microns per cubic centimeter, and 4.8×10^{-10} is the electronic charge in e.s.u. D_o for B and B/r determined experimentally are 2,650 r and 4,610 r respectively. Hence the corresponding j's are 4.27×10^3 and 7.43×10^3 ion pairs per cubic micron. Since inactivation now implies the average of one ionization per target, the target volume is equal to the reciprocal of j, or for B and B/r, $2.34 \times 10^{-4}\ \mu^3$ and $1.35 \times 10^{-4}\ \mu^3$ respectively. The target diameters and areas are determined from these values as in the following example: for B,

Table XIII
(Experiments 345, 346)

	Dose in α/μ^2	No. Col. Counted	Stock Dilution	ml per Block	% Survival	Log Surv. Fraction	Dose Increment
	0	1100	.001	8.0	100	3.000	0
	3.03	502	.001	2.5	28.5	2.455	1
B	6.07	274	.001	1.0	6.22	1.793	2
	9.10	190	.01	3.0	1.30	1.114	3
	12.1	329	.01	1.0	0.748	0.874	4
	15.2	455	.1	3.0	0.312	0.494	5
			$y = -0.513\ x + 2.90$				
	0	1289	.001	10	100	3.000	0
	5.96	242	.001	3	16.9	2.228	1
B/P	12.1	115	.01	8	2.14	1.330	2
	18.1	28	.01	2			
	24.1	3	.1	6			
	30.1	18	.1	1.5			
			$y = -0.833\ x + 3.03$				

MU 1330

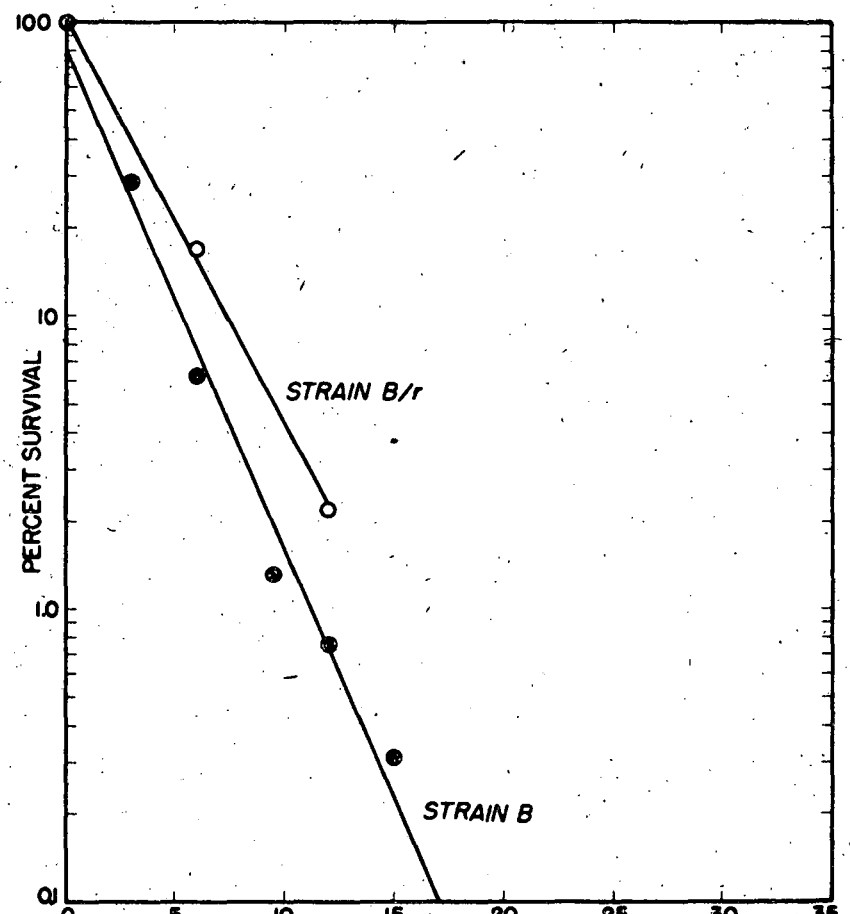

FIGURE 10

MU 1351

Table XIV
(Experiments 350, 352)

	Dose in a/μ^2	No. Col. Counted	Stock Dilution	ml per Block	% Survival	Log Surv. Fraction	Dose Increment
	0	9936	.001	8	100	4.000	0
	3.17	537	.001	2.5	27.0	3.431	1
B	6.32	228	.001	1	4.59	2.662	2
	9.48	257	.01	2	1.04	2.017	3
	12.6	52	.01	1	0.105	1.021	4
	15.8	94	.1	3	0.0568	0.754	5
	19.0	1182	.01	1	0.0238	0.377	6

$y = -0.638\ x + 3.97$

	0	1116	.001	10	100	5.000	0
	4.78	737	.001	2	19.8	4.297	1
B/r	9.56	702	.01	5	4.72	3.674	2
	14.3	327	.01	1	0.879	2.944	3
	19.2	355	.1	2	0.191	3.281	4
	23.9	477	1	5	0.0641	1.807	5
	28.8	328	1	1	0.0044	0.644	6

$y = -0.69\ x + 5.03$

MU 1352

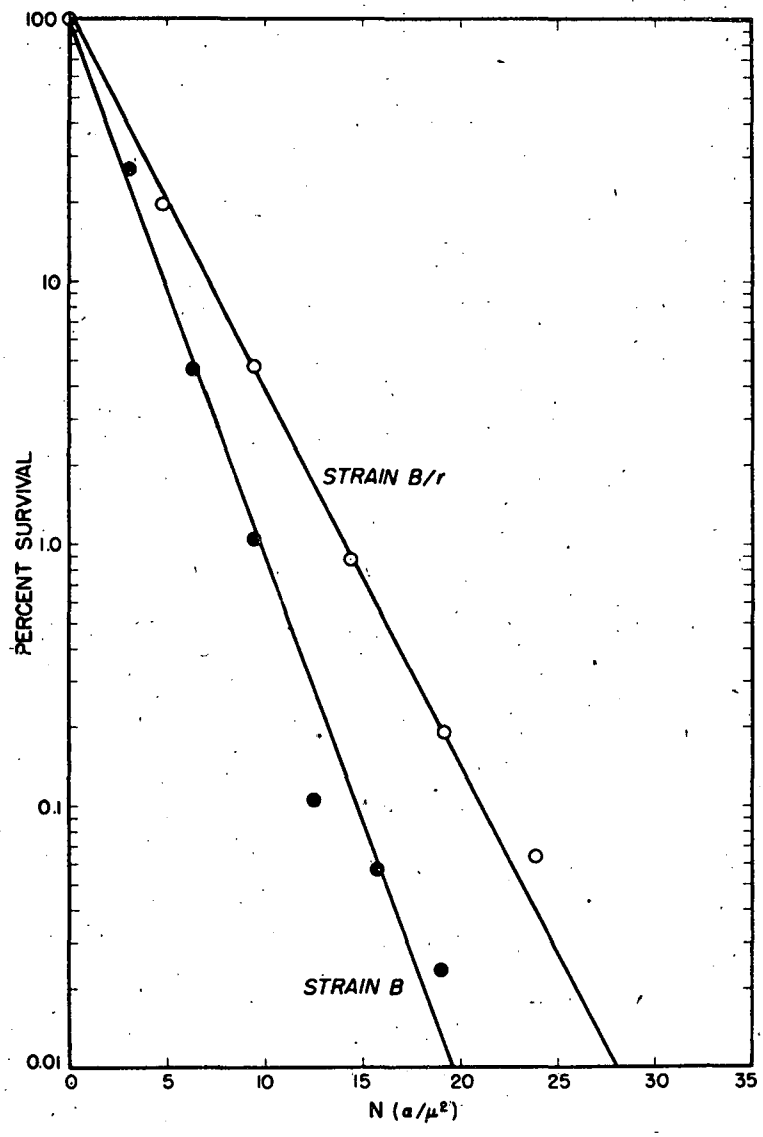

FIGURE 11

MU 1353

TABLE XV

					B/r					B		
ℓ	Range in Air	Al Filter (mil)	Source Distance (mm)	$d_o\Phi$	% Surv. (Exp. 353)	% Surv. (Exp. 354)	% Surv. (Exp. 356)	% Surv. (Exp. 363)	Mean Surv.	% Surv. (Exp. 357)	% Surv. (Exp. 362)	Mean Surv.
0	≤4	—	—	—	100	100	100	100	100	100	100	100
1	5	0	5	10.42	51.6	52.0	54.3	51.2	52.3	34.2	30.1	32.2
2	10	0	10	2.85	47.6		41.1	42.2	43.6	30.2	22.0	26.1
3	20.6	.25	10	2.85	45.5		39.2	39.4	41.4	28.8	29.5	28.2
4	28.1	.50	6.9	5.78		39.8	36.3	39.5	38.5	24.6	36.7	30.7
5	31.2	.50	10	2.85	21.3		33.7	38.6	31.2	35.4	40.8	38.1
6	33.2	..50	12	1.92	31.1	39.2	18.5	39.3	32.0	26.4	31.2	28.8
7	34.2	.50	13	1.66	26.7		36.9	39.5	34.3	27.3	26.3	26.8
8	35.2	.50	14	1.42	25.7		33.4	32.5	30.5	20.0	29.4	24.7
9	36.2	.50	15	1.23	43.0		40.6	40.8	41.5	27.4	31.2	29.3
10	37.2	.50	16	1.09	39.5	59.1	41.0	47.4	46.7	33.4	35.6	34.5
11	38.7	.75	6.9	5.78		93.6	50.5	79.8	74.6	36.8	80.8	58.8

MU 1354

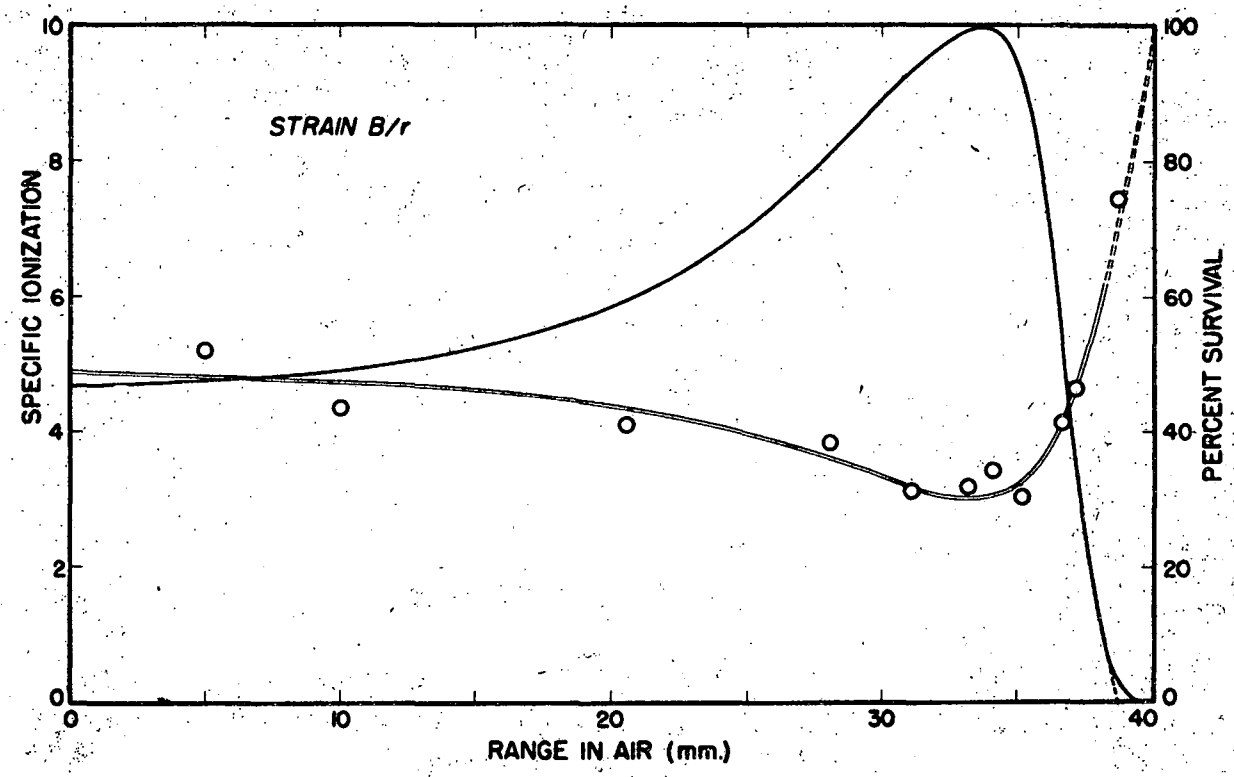

FIGURE 12

MU 355

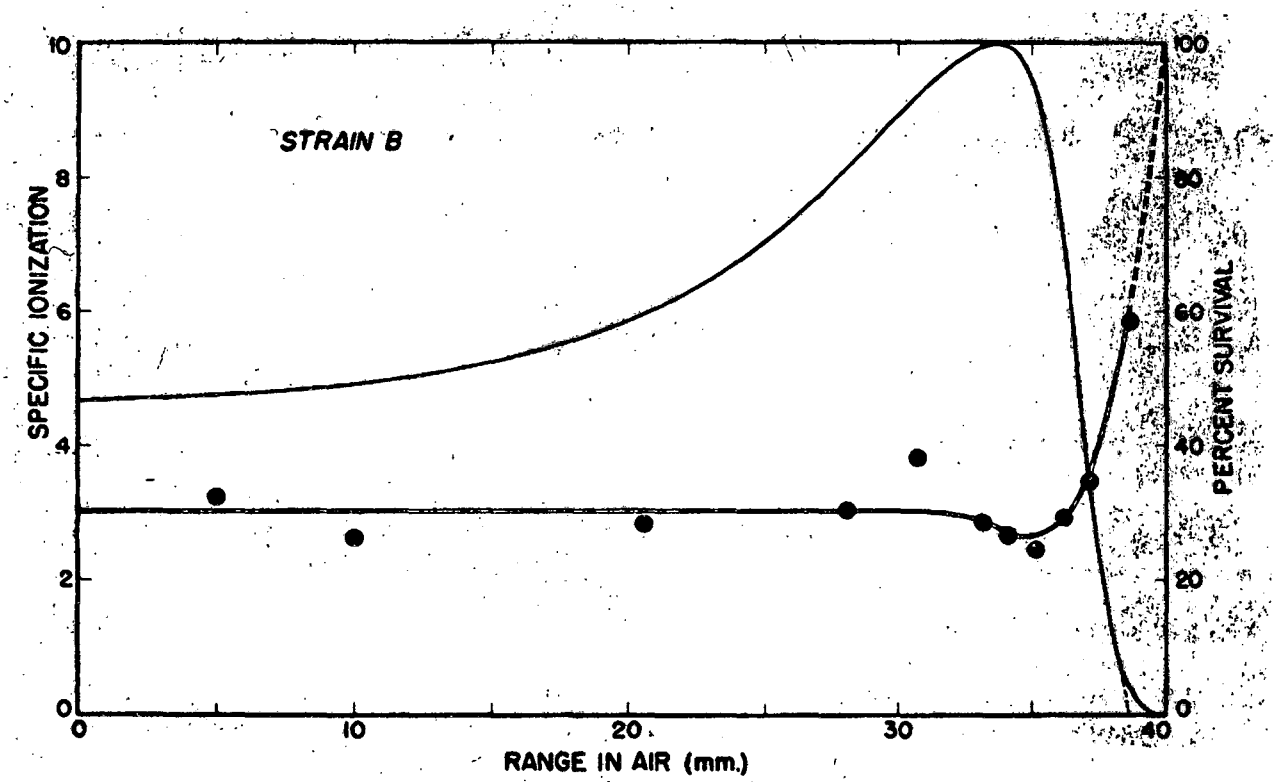

FIGURE 13

MU 1356

Table XVI
(Experiments 358, 359)

	B		B/r	
	A	C	A	C
	1753	1165	1160	1422
	1384	1212	1680	1645
	1426	1420	2045	1564
	1240	1202	1738	1570
	1436	1541	1560	1369
	1362	1143	1900	1946
	1310		1530	1635
N	1414	1281	1730	1593
σ	151	148	171	204
σ_M	25	29	29	34

$\sigma_{dM} = 38.3$

$t\sigma_{dM} = 2.20 \times 38.3 = 84.2$

$t^1\sigma_{dM} = 3.11 \times 38.3 = 119$

$dM = 1414 - 1281 = 133$

$\sigma_{dM} = 44.7$

$t\sigma_{dM} = 2.31 \times 44.7 = 103$

$t^1\sigma_{dM} = 3.56 \times 44.7 = 159$

$dM = 1730 - 1593 = 137$

Table XVII

Exp. No.	R_{10}/R_o	R/R_{10}
345	2.5	
346		3.2
350	2.2	
352		3.0
353		4.3
354		4.3
356		4.5
357	1.9	
358		6.25
359	3.2	
362	1.8	
363		3.0
366		3.5
367	3.2	
Σ	14.8	32.05
$\bar{x}$	2.5	4.0
σ^2	0.32	1.05
σ	0.56	1.02
$\sigma_{\bar{x}}$	0.09	0.13

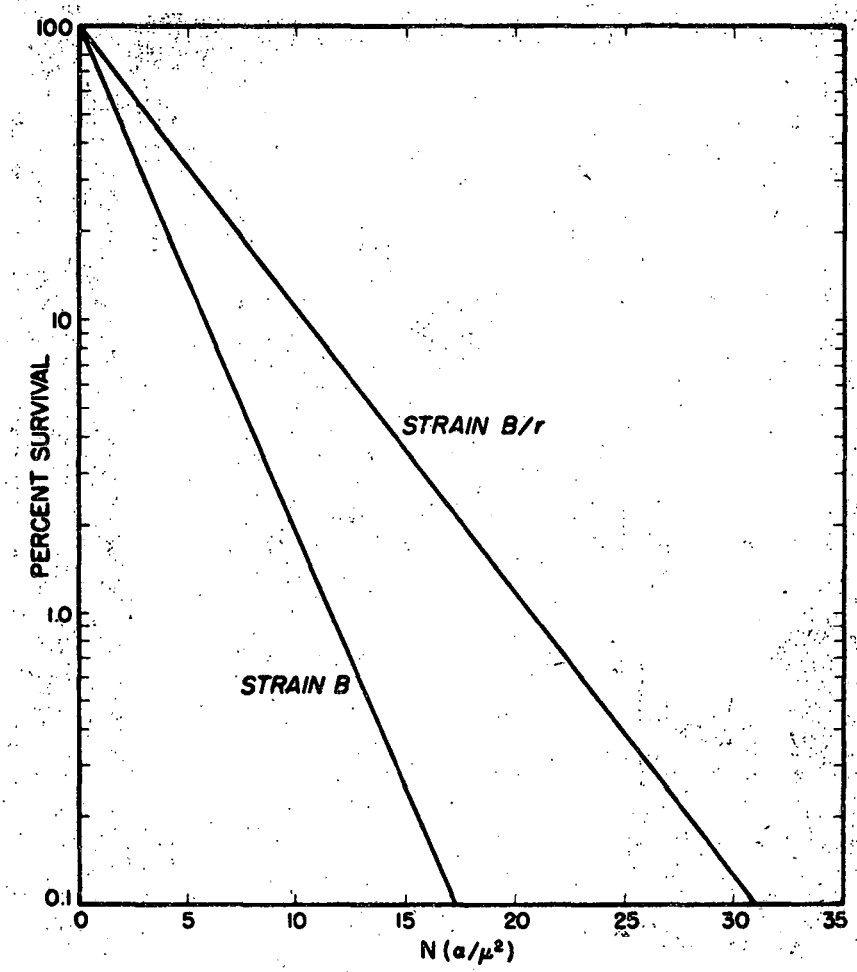

FIGURE 14

MU1359

$$v = \frac{4}{3} \pi r^3 = 2.34 \times 10^{-4} \ \mu 3.$$

Hence $r = (3/4 \times 1/\pi \times 2.34 \times 10^{-4})\ 1/3 = 38.3$ mμ,

and $2r = 76.6$ mμ.

The target area then is $\pi r^2 = .0046\ \mu^2$.

In considering target sizes as determined by deuteron and α-particle experiments, the assumption is made that if a particle path traverses any portion of the target volume inactivation results. This follows from the fact that density of ionization about these tracks is so great that ionization of the target is a certain consequence to its being hit by a particle. Therefore the target area is merely the reciprocal of N_o, the total number of particles passing through each μ^2 of the bacteria corresponding to the inactivation dose. The method of computing N_o was given in the section on Methods.

These values are summarized for the various radiations in table XVIII. It should be noted here that in the calculations as outlined no account was taken of overlapping ion clusters or of delta rays. When Lea's improved method of calculation (6) is used the target diameters for B and B/r with x-rays are approximately 190 mμ and 130 mμ respectively; for the fast deuterons these values become about 260 mμ and 210 mμ; for slow deuterons 320 mμ and 240 mμ; and for x-rays, 1020 mμ and 850 mμ. These improved values are also listed in the table.

It is readily seen that the apparent target size increases as one progresses from x-rays to α-rays. This can be explained according to target theory by a flattened shape of target--rather than a sphere as originally assumed--so that a greater ratio of area to volume makes it more readily hit by the densely ionizing particles. Or the target may consist of a number of parts, a hit in any one of which causes

death. However this reasoning demands that deuterons of low energy be less efficient than those of high energy in killing since one particle path intercepting the target area results in death. The same obtains in the case of α-particles. Results presented here however indicate the opposite to be the case, i.e., low energy deuterons are more efficient than high energy deuterons, and slow α-particles are more efficient than fast ones.

Mention should be made here of certain experiments where irradiation was carried out with the organisms in a nitrogen atmosphere rather than air, because this also effects the apparent target size. Table XIX contains data from such an experiment using strain B/r. The bacteria tolerated the radiation very much better while in nitrogen as can be seen in fig. 15 where the nitrogen curve is compared to the average curve in air. Essentially similar results have been reported by Hollander (21).

It may be noted here also that when bacteria were irradiated with x-rays in an atmosphere of argon steeper survival curves were found than when irradiation was carried out in air (22). By passing the x-ray beam through a layer of argon contained between drumheads of very thin nylon while the bacteria themselves were in air during irradiation, this effect was shown to be due to an increased dose of ionization caused by secondary radiation arising in the argon. In the case of α-irradiation, however, preliminary experiments indicate a degree of protective action from argon, at least in the case of strain B. Table XX and fig. 16 indicate that in spite of the increased dose of ionization demonstrated by the x-ray experiment, strain B tolerates α-bombardment in argon better than in air.

Table XVIII

		Inactivation Dose D (rep x 10^{-3})	Energy Absorption $(Ev)_o$ (eV/ μ^3 x 10^{-5})	Number of Particles N_o ($\#/\mu^2$)	Target Area (μ^2)	Target Diameter 2r ($m\mu$)	Target Diameter 2r ($m\mu$) Sensitive
	X-rays	2.65	1.39		.0046	76.6	190
R	Deuterons A	3.06	1.61	252	.004	71.4	260
	Deuterons C	2.44	1.29	32.6	.031	199	320
	Alpha-particles (at d = 10 mm)	3.99	2.07	2.5	0.400	712	1020
	X-rays	4.61	2.42		.0030	61.8	130
R/P	Deuterons A	5.08	2.68	418	.002	50.5	210
	Deuterons C	3.75	1.98	50	.020	150	210
	Alpha-particles (at d = 10 mm)	6.37	3.31	4.0	0.250	566	850

MU 1360

Table XIX

(Experiment 370)

Dose in Kr	Stock Dilution	Ml per Block	No. Col. Counted	% Survival
0	.0014	10	3680	100
5.9	.0014	7	466	88.5
11.8	.0014	5	490	67.3
17.7	.0014	3.5	540	51.6
23.6	.0014	2.5	619	42.2
29.5	.0014	1.8	685	33.5
35.4	.0014	1.3	771	27.2

MU 1361

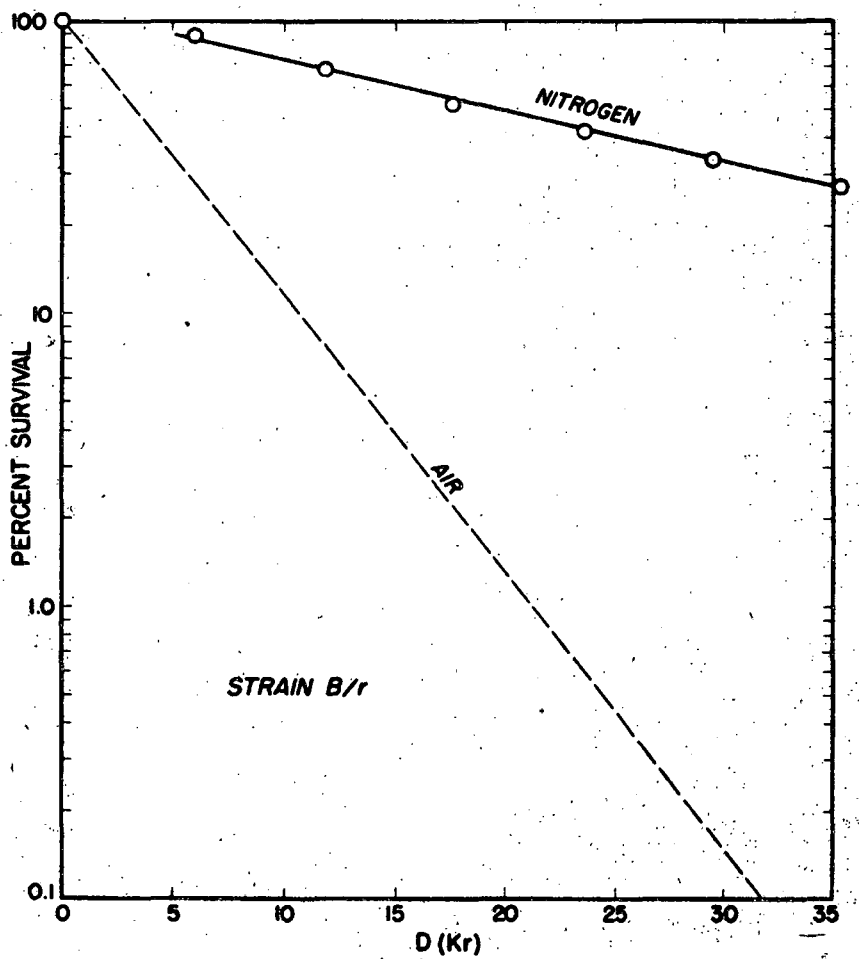

FIGURE 15

MU1362

Table XX

(Experiments 345, 346)

B			B/r		
Dose in α/μ^2	No. Col. Counted	% Sur-vival	Dose in α/μ^2	No. Col. Counted	% Sur-vival
0	1100	100	0	1289	100
3.03	635	36.1	5.96	479	33.4
6.07	638	14.5	12.1	433	8.06
9.10	765	5.23	18.1	465	2.16
12.1	1232	2.80	24.1	314	0.438
15.2	2618	1.79	30.1	204	0.0702

MU 1363

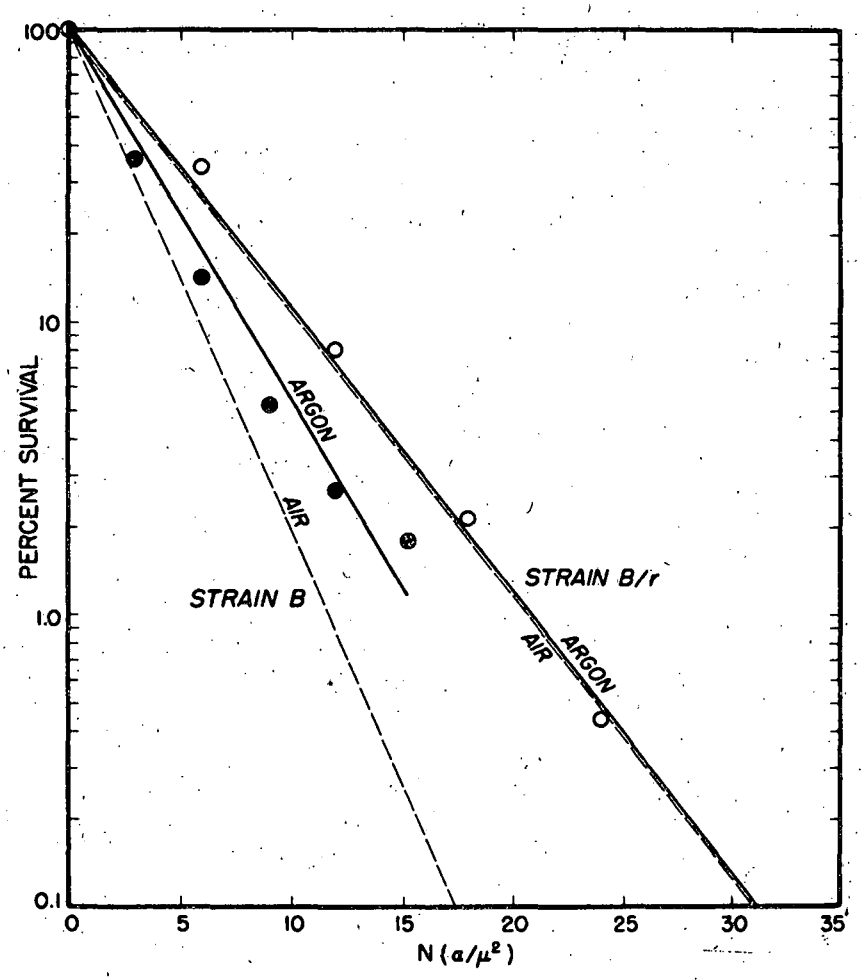

FIGURE 16

MU 1364

Discussion

In all the experiments reported in this study survival of the organisms is an exponential function of the dose, expressed by

$$\frac{n}{n_o} = e^{-kD}$$

where n is the number of bacteria surviving a dose D, n_o is the initial number of bacteria, and k is a constant of proportionality. This result agrees with previous reports (2, 4, 6, 10), and is evidence that some unit action is responsible for death of the bacterial cell. It also follows that when survival is 36.8% i.e. when

$$\frac{n}{n_o} = 0.368 = e^{-1}$$

$$D = \frac{1}{k} = D_o$$

D_o now represents that dose corresponding to an average of one effective unit action per cell. Interpreted according to target theory, this dose corresponds more specifically to an average of one hit per cell in the sensitive target; and k is a measure of the target size.

Another feature of the results obtained here is that D_o shows a graded increase as one progresses from x-rays to fast deuterons, to α-particles. This also is in agreement with previous results (2, 13, 6), and is to be expected according to target theory since any more than a single ionization or unit action, in the target, is unnecessary to produce death of the cell. Hence the more densely ionizing radiations, which deliver an overabundance of dose, are therefore less efficient in killing.

It will be noted however that as one goes from high energy

deuterons to low energy deuterons, i.e., to more densely ionizing particles, D_o decreases, as pointed out in the last section. This is also the case when irradiation with α-particles is carried out at points A and C of the Bragg curve. This result is directly opposed to target theory (6) unless modification is introduced. A somewhat similar result was reported by Pollard and Forro (20) when bacteriaphage was irradiated with deuteron of various energies.

Since the survival curves are indeed exponential, the conclusion seems inescapable that the probability of inactivating a sensitive "target" (with a given number of incident particles) increases with the density of ionization along the particle track, at least in the case of deuterons and in the case of α-particles. This phenomenon is seen with both B and B/r, and from these data appears to be the same in the two organisms. Furthermore since the apparent target size increases with the density of ionization, one is led quite naturally to the hypothesis that a spread of effect of ionization which varies with ion density is an important factor in inactivating the target. In other words if one postulates a cylinder of influence centered on the particle path, the radius of which increases with ion density, and which will inactivate the target if the cylinder and target overlap, a workable model is available. The fact that an approximately cylindrical volume containing ion pairs, excited atoms, and free radicals surrounds the particle path is well known (6); the only additional point needed here is that the distance from the track at which target inactivation may occur must vary with ion density.

The gas experiments indicate that oxygen may be important in this phenomenon since its exclusion during irradiation decreases the radio-

sensitivity of the bacteria. Hollander (21) has shown that the resistance to radiation may be even further increased by growing E. coli anaerobically and irradiating in the absence of oxygen. We have, then, evidence of a target mechanism which is influenced by factors usually thought of in connection with secondary effects.

The relative radiosensitivity of strains B and B/r has been discussed by Witkin (23). She offered evidence in support of the idea that a threshold for radiation effect has been increased in B/r. This threshold presumably lies in the energy range of ultraviolet photons and leads to a multiple hit curve with such irradiation. With x-rays it is supposed that the change alters the probability of a hit in the target being effective. Morse and Carter (24) found that cells of strain B/r contain three to four times as much desoxyribose nucleic acid as cells of the parent strain B. If nucleic acid is thought of as being intimately related to the target, this would suggest a larger target in B/r. However, we have shown the target in B/r to be smaller, or at least more difficult to inactivate, hence a further problem arises.

At the present time the model which we have suggested together with further possible secondary influences, such as local protective action of the nucleic acid, in B/r, would seem to be compatible with experiment.

Summary

Aerobically grown Escherichia coli strain B and its naturally occurring radiation resistant mutant strain B/r were irradiated in air with x-rays, deuterons, and α-particles. In all cases typical exponential survival surves were obtained. In this respect the

bacteria behaved as would be expected according to simple target theory. B/r was in all cases more resistant than B.

Contrary to expectations based on target theory, however, both strains were killed more efficiently by low energy deuterons than by high energy deuterons. This same reversal was found in the case of α-rays, low energy particles being more effective than high energy particles.

When x-irradiation was carried out in a nitrogen atmosphere, survival was increased over that in air. Such a protection was also demonstrated for argon in the case of strain B bombarded with α-particles.

In view of these departures from target theory predication, while at the same time survival remains a strictly exponential function of dose, one is led to a modified target hypothesis--that the "target" may be inactivated not only by an ionization occurring within its volume, but also by energy transferred from the track of the ionizing particle through a finite distance in the cell; and further, that the probability of this latter mechanism resulting in target inactivation at a given distance increases with ion density along the track.

That the presence of oxygen enhances the effectiveness of irradiation of these bacteria is also demonstrated.

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