THE EFFECT OF X-RAYS ON THE SURVIVAL OF BACTERIA AND YEAST

I. A Comparative Study of the Dose-Survival Curves of *Azotobacter agile*, *Escherichia coli*, *Pseudomonas fluorescens*, *Rhodopseudomonas spheroides*, and *Saccharomyces cerevisiae* Irradiated in the Resting State[1]

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The variety of metabolic and morphologic characteristics found in microorganisms offers an opportunity to study the influence of these factors on cellular sensitivity to X-rays that has not been fully utilized. A review of the literature for bacteria showed that the survival curve and LD_{50} had been determined accurately for members of only nine genera: *Achromobacter*, *Aerobacter*, *Escherichia*, *Micrococcus*, *Pseudomonas*, *Salmonella*, *Sarcina*, *Serratia*, and *Shigella*. Seven of these are gram-negative rods and five belong to the *Enterobacteriaciae*. *Escherichia coli* was the species most frequently used. Because of differences in the biological and physical methods employed by the various investigators, many data were unsuitable for comparative purposes. It was therefore of interest to undertake additional comparative studies.

So that the differences in the survival curves would reflect differences in genetic constitution, the plans for each strain in the present experiments were drawn in an effort to meet three requirements. First, at the time of irradiation a fixed set of environmental conditions should be employed for all strains, whose physiological and morphological states should be comparable. Second, methods that do not differ significantly should be used for the culture and preparation of the various strains prior to irradiation and for the subsequent determination of their survival. Third, radiological technique should follow the approved recommendations for dosimetry.

The survival curves of six strains of microorganisms representing five genera were determined, using 250-kv X-rays. Two of the genera (*Azotobacter*, *Rhodopseudomonas*) had not been studied radiologically before. The strains were selected because of differences in cell size, motility, type of cell division, ploidy, and metabolism, and because of their suitability for the type of experiment planned. In order to maintain the cells in a resting state during irradiation, they were suspended in a buffered, inorganic solution in equilibrium with air, in which the viable cell count of the unirradiated controls remained constant for at least three hours at room temperature. For different strains the solution differed only in buffer concentration. The strains were cultured and were tested for survival on media which differed only in glucose content. Insofar as possible with such a varied group of organisms, identical conditions were used for all. Later studies will deal with the effect of altering basal state by various means.

MATERIALS AND METHODS

Microbiological. The strains employed and their sources were as follows: *Azotobacter agile* strain M.B.4.4, *Rhodopseudomonas spheroides* strain ATH 2.4.1 (C. B. van Niel, Stanford Univ.), *Escherichia coli* strain W-1485 (E. M. Lederberg, Univ. of Wisconsin), *Pseudomonas fluorescens* strain A.3.12 (R. Y. Stanier, Univ. of California, Berkeley), *Saccharomyces cerevisiae*, haploid, strain 93.1C (α) and diploid strain 93 (C. A. Tobias, Univ. of California, Berkeley). In the case of *Saccharomyces*, the original Lindegren strain numbers are used. Zirkle and Tobias (1953) called the haploid and diploid strains SC-7 and SC-6, respectively.

E. coli, *P. fluorescens*, and *R. spheroides* were routinely grown and plated on yeast agar containing NH_4Cl, 0.1 per cent; K_2HPO_4, 0.1 per cent; $MgSO_4 \cdot 7H_2O$, 0.05 per cent; yeast extract

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(Difco), 0.5 per cent; agar, 1.5 per cent. Yeast agar supplemented with 1 per cent glucose was used for the growth of *S. cerevisiae* and *A. agile*. All media were adjusted to pH 7 before autoclaving. All cultures were incubated at 30 C in the dark.

Cells to be irradiated were obtained from agar plates incubated 16 to 17 hours after inoculation with a suspension from a 24-hour slant culture. Stock cultures were stored at approximately 5 C and were passed through two or three serial subcultures before inoculation of the plates. The organisms were washed twice (15 to 20 ml/plate/washing), resuspended in buffer (5 ml/plate), and shaken with glass beads for five minutes to disperse clumped cells. This treatment reduced the number of cells present in groups of two or more to less than 10 per cent in all cases. Clumps of more than three or four were rarely found. The cell concentration was estimated with a spectrophotometer and adjusted to 2×10^4 or 2×10^5 cells/ml. In a few experiments, 2×10^6 cells/ml were employed. Under the conditions of these experiments, changing the cell concentration from about 10^2 to 10^7 cells/ml did not change the LD_{50} or the shape of the survival curve (Gunter and Kohn, 1955).

The medium for washing and suspending the cells was potassium phosphate buffer, pH 7, supplemented with $MgSO_4 \cdot 7H_2O$ at a final concentration of 0.002 M. The potassium phosphate concentration was that found most favorable for each organism (Gunter, 1954). The buffer concentrations employed were: 0.1 M, *P. fluorescens*; 0.05 M, *E. coli*, *S. cerevisiae*; 0.025 M, *R. spheroides*; 0.01 M, *A. agile*. Concentrated solutions of the buffer mixture and magnesium sulfate were autoclaved separately and mixed before use to prevent precipitation.

For irradiation, 1-ml samples in Teflon cups (1 mm thick, 1.3 cm deep, and 1.7 cm internal diameter) were placed within a sterile exposure box. After each exposure, two cups were removed and each cup was dropped into 9 ml of diluent. Aliquots were then taken for plating or further dilution. Viable cell counts were determined by the surface plating technique, using an aliquot of 0.1 ml per plate, 4 plates per cup, and therefore 8 plates per dose. The viable counts of unirradiated controls determined at the beginning and end of the exposures showed no consistent change and were averaged in each experiment for the calculation of per cent survival.

The plates used for the viable cell counts were incubated for the following periods of time: *E. coli* and *P. fluorescens*, 1 day; *A. agile* and *S. cerevisiae* (haploid), 2 days; *R. spheroides*, 3–4 days; and *S. cerevisiae* (diploid), 5 days. Longer periods up to 7–10 days did not increase the number of colonies (Gunter and Kohn, 1956).

The samples were irradiated in air at 25 to 27 C. Under the conditions described above, shaking the suspension or increasing the oxygen tension did not change the results (Gunter and Kohn, 1955).

Radiological. The cylindrical exposure box of 6-mm-thick aluminum (5 cm deep and 20 cm diameter, internal dimensions) was partially immersed in a water bath. The exposure box was oriented in the path of the X-ray beam by means of marks on its cover and plumb bobs hung from the X-ray tube housing. The Teflon cups were placed within fields inscribed on the floor of the box, in which the dose-rate was estimated to vary by less than 3 per cent.

The X-ray beam, from a 250-kv constant-potential, 15-ma unit, had a half-value layer (HVL) of 0.48 mm Cu (without added filtration), which increased to 0.5–0.6 mm Cu when filtered by the aluminum cover of the exposure box.

The doses stated in this paper are estimates of the *tissue dose* in the sense of the official definition (Glasser *et al.*, 1952; International Commission on Radiological Units, 1954). The dose-rate for each experiment was estimated from the readings of a Victoreen condenser r-meter whose 250 r or 100 r thimble ionization chamber had been exposed at the position of the Teflon cups within the exposure box. To allow for the increased scatter when cups with samples were present, the meter reading, corrected for temperature and barometric pressure, was multiplied by 1.03. The dose-rate varied from 100 to 400 r/min as the target-sample distance was decreased from 80 to 40 cm. No biological effect of dose-rate was noted.

Statistical. Each point in figures 1–8 represents the mean count for 7 to 8 plates from one experiment. The statistics of the regression lines fitted to these means were computed by the methods given by Snedecor (1946). The points for 100 per cent survival at zero dose (one per experiment) are not plotted and were omitted from the statistical calculations. The intercept of the regression line at zero dose, A_0, is stated in the graphs.

RESULTS

The effect of X-rays on survival may be described in terms of the curve obtained by plotting the fraction of survivors or its logarithm as a function of X-ray dose. When the quality of the X-rays is fixed and the biological conditions of testing are comparable, major differences in the shape of the survival curve suggest differences in the chain of processes leading to death. For organisms with the same kind of survival curve, the LD_{50} may be used as a relative measure of sensitivity to radiation.

Three types of survival curve were found which, for simplicity, will be called A, B, and C.

Type A. This curve, called single-hit and single-action, represents a simple exponential relation between survival and radiation dose that may be expressed in the linear form

$$\ln A = \ln A_0 - kD \qquad (1)$$

where

A = per cent of organisms surviving,
A_0 = per cent originally present (100 per cent),
D = radiation dose in kiloroentgens (kr), and
k = radiation-reaction constant, *i.e.* fraction of survivors killed per kiloroentgen, equal to the slope of the regression line or the reciprocal of the dose for 37 per cent survival.

The data for *P. fluorescens* and *R. spheroides* were fitted with type A curves and are presented in table 1 and figure 1. The LD_{50} values were 0.95 and 1.8 kr, respectively.

Comparative experiments by Schepmann and Flecke (1926), Sulkowitch (1929), Brown *et al.* (1933), and Fram *et al.* (1950) indicate that *P. fluorescens* and *P. aeruginosa* are similar in X-ray sensitivity and are among the less resistant bacteria. Moos (1954) reported an LD_{50} of 1.2 kr for *P. aeruginosa* exposed to 186-kvp

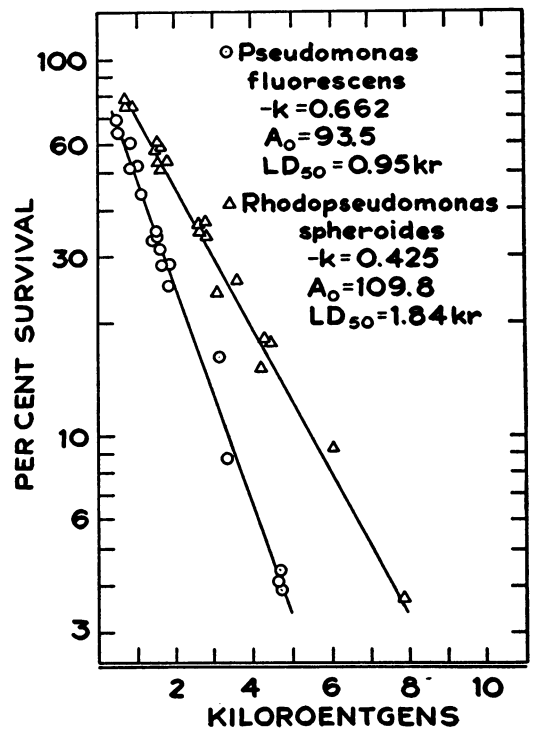

Figure 1. Per cent survival (logarithmic scale) as a function of X-ray dose. Type A survival curves for *P. fluorescens* and *R. spheroides*.

TABLE 1

Type A and B survival curves

Organism	Population	Survival Curve	LD_{50} (kr)	A_0*	$-k$*	$s_{y\cdot x}$* (antilogue)
P. fluorescens	Homogeneous	A	0.95	93.5	0.66 ± .02	1.12
R. spheroides	Homogeneous	A	1.84	110	0.425 ± .011	1.09
S. cerevisiae (haploid)	Mixed ($S + R$)	B	2.1	—	—	—
	Nonbudding (S)	A	1.9	105	0.389 ± .011	1.16
	Budding (R)	C	>20.	—	—	—
E. coli	Mixed ($S + R$)	B	2.12	—	(0.295)	—
	S	A	1.15	101	0.605 ± .046	1.21
	R	A	3.52	100	0.197 ± .014	1.31

* Statistics for the regression line: $\ln S = \ln A_0 - kD \pm s_{y\cdot x}$, where $s_{y\cdot x}$ represents the standard error of estimate of the regression of ln survival on dose.

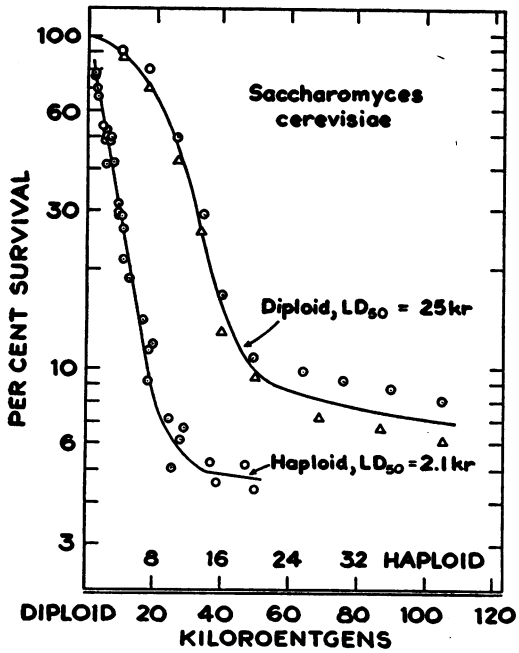

Figure 2. Per cent survival (logarithmic scale) as a function of X-ray dose. The haploid strain of *S. cerevisiae* has a type B survival curve; the diploid strain, a type C curve.

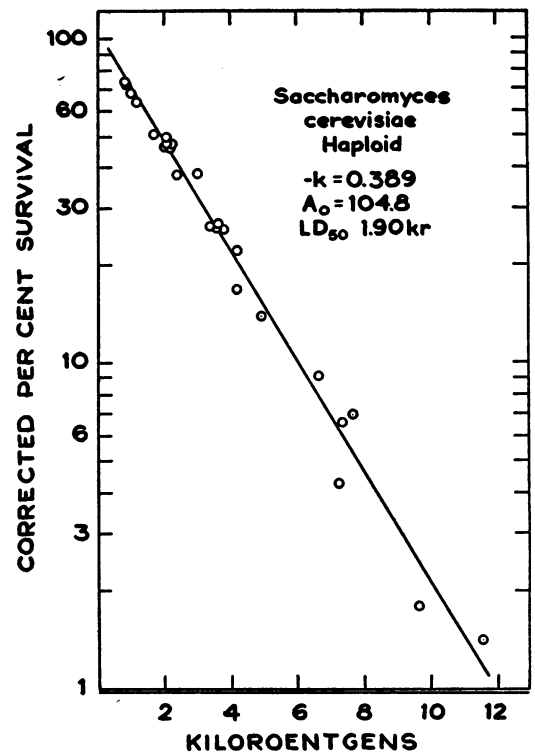

Figure 3. Corrected per cent survival (logarithmic scale) as a function of X-ray dose, *S. cerevisiae*, haploid.

X-rays. Radiological data for *R. spheroides* have not been reported.

Type B. This curve is concave when plotted arithmetically or semilogarithmically and involves the sum of a simple exponential function and some other function which may or may not be simple exponential. The fit of data by a type B curve suggests that at the time of irradiation the suspension contained two or more kinds of cells differing in radiological sensitivity.

The survival curve of *S. cerevisiae* (haploid) was type B (figure 2). The deviation of the survival curve from the simple exponential type was attributed to the presence of a relatively small number of very resistant budding cells in addition to the sensitive interphase cells, as first noted by Beam *et al.* (1954). Since the deviation of the curve occurred at about 5 per cent survival, it was concluded that the mixed population consisted of 95 per cent sensitive cells, *S*, responsible for the initial steep part of the curve, and 5 per cent resistant cells, *R*, responsible for the final plateau. Microscopic examination showed that between 5 and 10 per cent of the cells possessed buds. The number of *R* cells was considered constant since their sensitivity was negligible relative to that of the *S* cells and the per cent of *R* cells was small. S^*, the number of surviving *S* cells, was calculated from the total number of surviving cells N^* by correction for the number of insensitive cells R^*:

$$S^* = N^* - R^* = S^*_0 e^{-kD} \qquad (2)$$

This was changed to the form of equation 1 for the calculation of the regression line for the *S* cells (figure 3). The statistics are given in table 1. The LD_{50} for the suspension containing both *S* and *R* cells was 2.1 kr; for the *S* cells alone it was 1.9 kr.

Wood (1953) using the same haploid strain of *S. cerevisiae* and methods similar to ours also found an LD_{50} of 1.9 kr for the nonbudding *S* cells. An LD_{50} of about 3 kr was reported by Beam *et al.* (1954), who employed somewhat different methods. Aging the cells either on agar plates (Wood, 1953) or in glucose buffer solution (Elkind and Beam, 1954) results in an increase in the LD_{50} determined with X-rays.

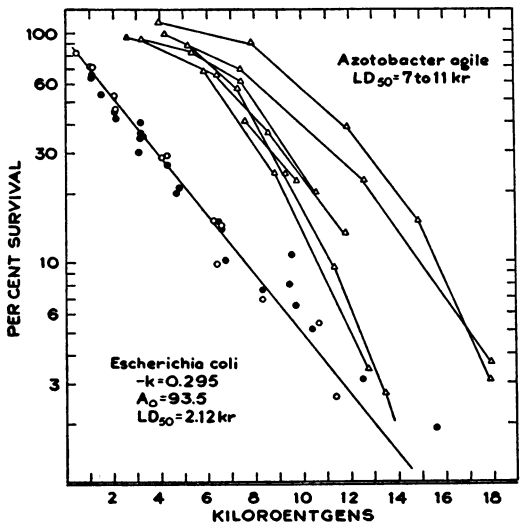

Figure 4. Per cent survival (logarithmic scale) as a function of X-ray dose. The survival curve for *A. agile* is type C. In the case of *E. coli*, the points of two experiments (open circles) are distributed at random about the regression line, but the points of the other six experiments (solid circles) tend to fall a little below the upper part of the regression line and a little above the lower part.

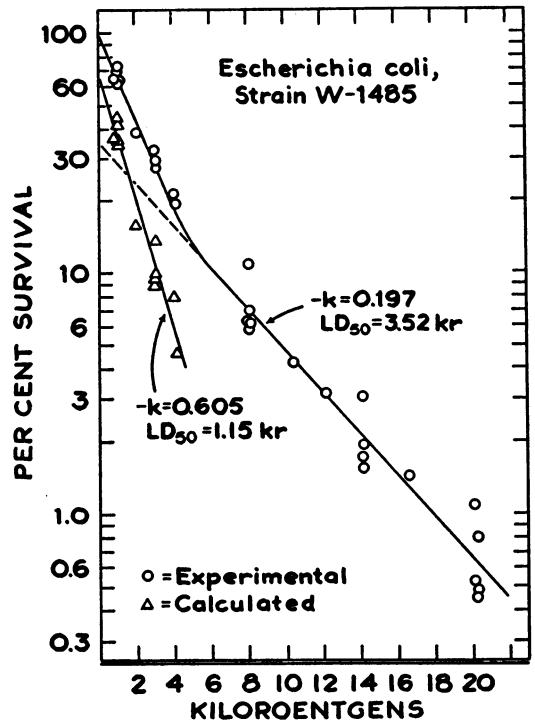

Figure 5. Per cent survival (logarithmic scale) as a function of X-ray dose. The type B curve of *E. coli* is the sum of two type A curves, one for sensitive, *S*, the other for resistant, *R*, cells.

The LD_{50} values (based on macrocolony formation) of other haploid strains of *Saccharomyces* were reported to be 1.8 kr (Latarjet and Ephrussi, 1949), 2.2 kr (Mortimer, 1952) and 4 kr (Lucke and Sarachek, 1953).

In the initial experiments with *E. coli*, survival was followed down to about 3 per cent. The LD_{50} was 2.1 kr and the survival curve appeared to be type A (table 1, figure 4). Close examination of figure 4, however, showed that in six of eight experiments the points (solid circles) tended to fall a little below the regression line in its upper part and a little above it in its lower part. Six more experiments were then done to determine the shape of the survival curve down to about 0.5 per cent survival. Two of these were with the culture previously tested. In the other four, cultures obtained in the following way were used. Cell suspensions were exposed to doses of about 50 kr and then plated as usual. The per cent survival was in the range 2×10^{-3} to 2×10^{-4}. Four macrocolonies were selected from the plates and served as inocula for cultures from which suspensions were prepared for the determination of dose-survival curves. Similar type B survival curves were found in all of the six experiments, and the data were therefore pooled (figure 5). The population was assumed to be composed of two types of differentially sensitive cells, *S* and *R*, and the number of surviving cells

$$N^* = S^*_0 e^{-k\,D} + R^*_0 e^{-k_r D} \qquad (3)$$

The survival of the *R* cells was estimated from the final linear portion of the survival curve and from its extrapolation to zero dose (dashed line, figure 5). At doses below 7 kr, subtraction of the extrapolated values for the *R* cells from those determined experimentally for the whole population (circles) gave the survival of the *S* cells (triangles). The LD_{50} values of the *S* and *R* cells based on the regression lines fitted to the data were 1.2 and 3.5 kr, respectively (table 1). It was estimated that the population contained 66 per cent *S* cells and 34 per cent *R* cells.

Previous determinations of the LD_{50} for *E. coli* ranged from 1 to about 15 kr with the majority falling between 2 and 5 kr. The differences re-

sulted partly from the variety of methods employed and partly from inherent differences in the strains. For example, Magni (1952) irradiated six strains with 50-kv X-rays under the same conditions and obtained LD_{50}'s ranging from 1.7 to 5.2 kr. Stapleton (cited by Zelle and Hollaender, 1955) tested eight strains under comparable conditions and found the LD_{50} to vary from 2.4 to 6.9 kr. The lowest reported LD_{50} is 1 kr for strain B (Langendorff and Sommermeyer, 1953; Moos, 1954).

The survival curves were usually reported as type A (Wyckoff, 1930; Lea *et al.*, 1937; Dieckmann and Dittrich, 1950), but in the case of strain B (Witkin, 1946; Cavalli and Modrone, 1948) and strains 90 and L (Magni, 1952) the curve was type B. The latter was interpreted as representing the combined survival of a mixture of several types of cells differing in sensitivity. In retrospect, it seems probable that in studies with other strains, type B curves were missed in some instances because the shape of the survival curve was not precisely determined to survival well below 5 per cent.

Type C curves (see below) were reported for an unspecified strain of *E. coli* by Luria (1939), for B/r (s-) and B/r (p-) by Anderson (1951), for "young" HIG cells but not for "old" ones by Houtermans (1954), for late lag phase cultures of B/r (Stapleton, 1955), and for cultures of B/r grown or irradiated under special conditions by Hollaender *et al.* (1951). The cultures used by Hollaender *et al.*, according to one of the authors (cited by Birge and Tobias, 1954), may have been composed of long multinucleate forms or other forms that differ significantly in morphology from the cells generally employed.

TABLE 2

Type C survival curves

Organism	Population	LD_{50} (kr)	Model: $S(n, p/p_0, q/q_0)$
A. agile	Homogeneous	8.3 ± 1.4	$S(5\text{–}10, 1/1, 1/1)$ $S(1, 20/20, 1/1)$
S. cerevisiae, (diploid)	Mixed ($S + R$)	25	—
	Nonbudding (S)	24	$S(5, 1/1, 1/1)$ $S(1, 2/2, 1/100)$
	Budding (R)	>50	—

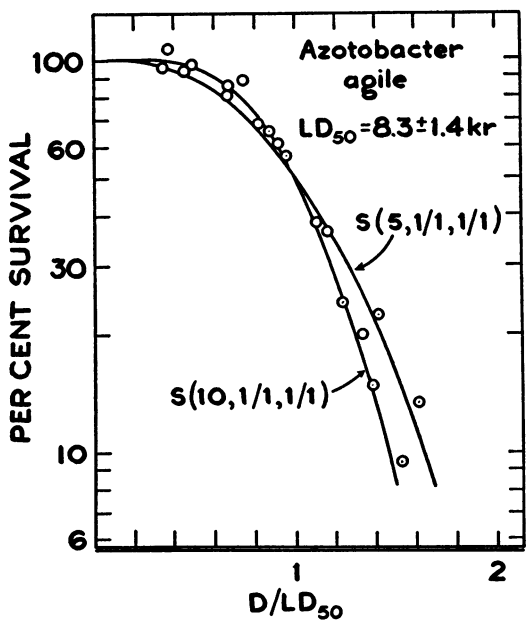

Figure 6. Per cent survival (logarithmic scale) of *A. agile* as a function of X-ray dose D divided by LD_{50}. The smooth curves are derived from the assumptions indicated.

Type C. This curve, sigmoid on an arithmetic plot and convex on a semilogarithmic one, may be derived as the product of two functions, one or both of which are exponential. Type C curves have been called sigmoid, multi-hit, and multiaction.

The data for *A. agile* are shown in figure 4 and table 2. Although the survival curve was always type C, the LD_{50} varied from 7.4 to 10.7 kr in five experiments (mean ± standard deviation, 8.3 ± 1.4 kr). The cause of the variation is not known. In order that the shape of the survival curve might be independent of such variations in LD_{50}, per cent survival was plotted against X-ray dose divided by LD_{50} (D/LD_{50}), a device suggested by Glocker (1932). The points then lay along a smooth curve from which their deviations were not striking (figure 6). It appeared that sensitivity (LD_{50}) varied from experiment to experiment, but the relation between survival and X-ray dose was relatively constant. Since *A. agile* has not been studied radiologically before, no data are available for comparison.

The survival data for *S. cerevisiae* (diploid) are shown in figure 2 and table 2. The type C survival curve ended in a plateau at 5 to 10 per cent survival. As in the haploid yeast, the plateau was attributed to the presence of a small fraction of insensitive budding cells by Beam *et al.* (1954). The LD_{50} taken from the curve drawn through the points in figure 2 was

25 kr. When the data were corrected for the plateau, the LD_{50} was 24 kr.

Type C curves have always been found with the diploid and polyploid strains of Saccharomyces and also with some other species of yeasts. Determinations with related haploid and diploid strains have consistently given a higher value for the LD_{50} of the diploid strain than for that of the haploid, as first described by Latarjet and Ephrussi (1949). The LD_{50} of 24 kr found in the present experiments with *S. cerevisiae* is close to the value of 22 kr obtained by Wood (1953) for aged cells of the same strain. The LD_{50} for other diploid strains has been reported as 8 kr by Latarjet and Ephrussi (1949), 12 kr by Lucke and Sarachek (1953), and 13 kr by Mortimer (1952). When survival was judged by the formation of microcolonies containing only two or three cells, the LD_{50} was very much higher than when judged by the formation of macrocolonies (Lacassagne, 1930; Frilly and Latarjet, 1944).

Since type C curves may differ significantly in shape even when allowance is made for differences in LD_{50}, the comparison of type C curves requires a knowledge of their equations. Various models have been proposed from which to derive such equations. Speaking in general terms, all of them involve some kind of multiplicity in the mechanism of radiological action—a sensitive unit must be hit several times before death occurs, or each of a number of units must be hit, or several sets of units must be hit. For present purposes, it will be simpler and more meaningful to compare the assumptions from which the equations have been derived rather than the equations themselves. The assumptions will be specified by using the following system of notation. Let $S(n, p/p_0, q/q_0)$ specify the three kinds of assumptions that determine the differences among the survival curves under discussion. In this, n is the number of radiological hits or decisive events necessary to inactivate one sensitive unit. There are p_0 such units in a *set*, and the set is inactivated when p of its units are destroyed. There are q_0 such sets of units in a *group*, and the group is inactivated when q of its sets are inactivated. Inactivation of the group leads to the death of the cell. The simplest model, that for the type A

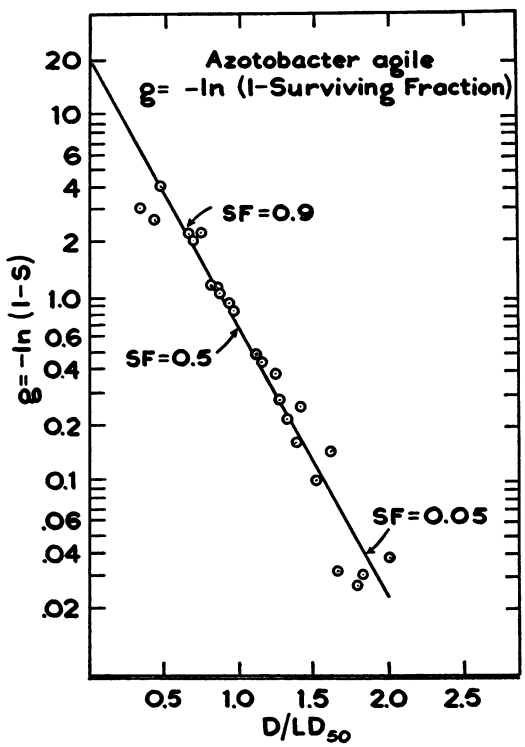

Figure 7. g (logarithmic scale) of *A. agile* as a function of X-ray dose D divided by LD_{50}. The value of the surviving fraction (SF) is indicated at various points along the line drawn through the points.

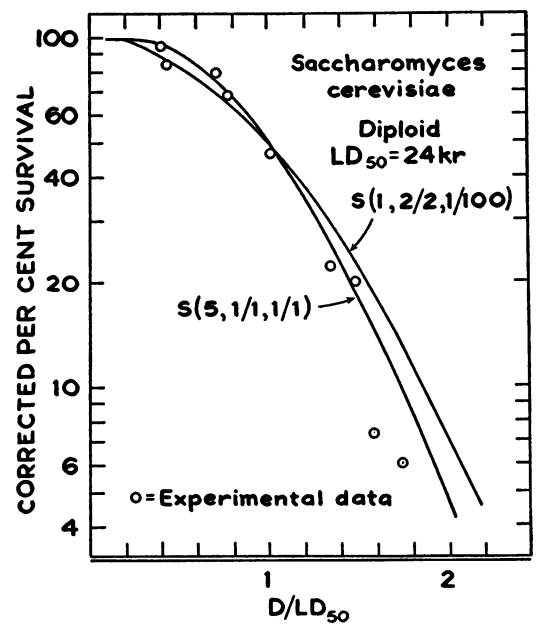

Figure 8. Corrected per cent survival (logarithmic scale) of *S. cerevisiae*, diploid, as a function of X-ray dose D divided by LD_{50}. The smooth curves are derived from the assumptions indicated.

TABLE 3

Models for type C survival curves of various strains of Saccharomyces

Strain	Reference	Radiation*	Endpoint for Survival†	Ploidy	LD_{50} (kr)	Model‡		
						n	p/p_0	q/q_0
S. ellipsoideus, var. Chambertin	Lacassagne (1930) and Holweck (1930)	1.83 A	>2 cells	—	27	4	1/1	1/1
		8.2 A	>2 cells	—	18	5	1/1	1/1
	Glocker *et al.* (1933)	0.56 A	>2 cells	—	42	5	1/1	1/1
		1.54 A	>2 cells	—	28	5	1/1	1/1
	Frilly and Latarjet (1944)	1.54 A	>9 cells	—	9	1	2/2	1/1
		8.33 A	>9 cells	—		1	2/2	1/1
S. species	Lucke and Sarachek (1953)	180 kv	Macrocolony	2	12	1	2/2	1/1§
		180 kv	Macrocolony	3	37	1	3/3	1/1§
		180 kv	Macrocolony	4	17	1	3.8/3.8	1/1§
S. cerevisiae, 93	Wood (1953)	250 kv	Macrocolony	2	22	1	2/2	1/30¶
	Zirkle and Tobias (1953)	200 kv	>2 cells	2	55	1	2/2	1/64¶

* Peak voltage of the X-ray unit, or the wavelength in Angstroms for monochromatic beams.

† Size of colony when plated on solid nutrient medium.

‡ Model: n = "hits" to destroy one unit; p_0 = units per set; p = threshold number of destroyed units to inactivate a set; q_0 = sets of units per cell; q = threshold number of inactivated sets to kill the cell.

§ A different value of k was employed for each ploidy.

¶ The haploid value of k was employed.

curve, is represented by $S(1, 1/1, 1/1)$. In this model one hit inactivates one sensitive unit, and there is only one unit per set and one set per group.

The survival curves derived from two models were tested for agreement with the data of *A. agile* (figures 6 and 7), and both appeared to fit the data equally well. In the first model, $S(n, 1/1, 1/1)$, there is only one sensitive unit per cell which is inactivated after n hits. The series of survival curves for values of n from 1 to 50 were published by Timoféeff-Ressovsky and Zimmer (1947). The data in figure 6 fell within the range bounded by the curves for $n = 5$ and $n = 10$.

In the second model, $S(1, p_0/p_0, 1/1)$, one hit inactivates one unit and each of the p_0 units in a set must be hit once to inactivate the set. Atwood and Norman (1949) devised the graphical method for determining the average value of p_0 for a given population, which we have applied to the data for *A. agile* (figure 7). The g-intercept of 20 for the straight line through the pooled data of all experiments indicated $p_0 = 20$. When the data of individual experiments were tested, the value of p_0 ranged from 10 to 50.[3]

[3] In the notation of Atwood and Norman (1949), p_0 would be n.

The survival curves predicted by three models were tested for agreement with the data of *S. cerevisiae* (diploid) which had been corrected for the plateau. The curve derived from $S(1, p_0/p_0, 1/1)$ did not fit, since a plot of g against D/LD_{50} was not linear. As shown in figure 8, the curves derived from $S(5, 1/1, 1/1)$ and $S(1, 2/2, 1/100)$ fitted the data fairly well down to about 15 per cent survival. The deviations below this were partly, if not largely, due to the uncertainty of the experimental points owing to the correction for the plateau. The curve derived from $S(1, 2/2, 1/100)$ was calculated using the value of the radiation-reaction constant $k = 0.39$ found for the haploid strain, as had been done by Zirkle and Tobias (1953).

Several models have been used in the past in deriving equations to fit the survival curves of yeast. These are summarized in table 3.

DISCUSSION

Since it was the purpose of the present experiments to make the differences in the survival curves depend primarily on differences in genetic constitution, identical or practically identical methods were used for the culture, preparation, and irradiation of the different species, and for the determination of their survival. Furthermore,

insofar as possible, at the time of irradiation all species were in a comparable physiologic and morphologic state, the resting state.

The resting state, as defined by us, is one that develops gradually and as a result involves limits that cannot be precisely set. When cells from a nutrient medium are washed and resuspended in a medium that is nontoxic and contains no utilizable substrate, they undergo a series of changes with time, namely, metabolism falls to a minimum, growth ceases, and in some cases cells that are in the process of dividing complete that division. During this period, before degenerative changes sufficient to cause death have occurred, the cells may be said to be in a resting state. This state could not be induced by chilling suspensions in nutrient broth, for example, since such a procedure would produce only a state of suspended animation. The precise condition of cells in the resting state therefore depends on the temperature and duration of their incubation in the non-nutrient medium, and also on their history prior to suspension in that medium. Furthermore, since the history of all cells in the suspension cannot be identical, especially when the cells are obtained from plate cultures, it is to be expected that the suspension will comprise at least some variety of resting types that may or may not differ radiobiologically.

In the cases of *S. cerevisiae* and *E. coli*, evidence was presented that two kinds of cells were present in the suspensions which could be distinguished by the marked differences in their radiation sensitivity. The survival curves of both the haploid and diploid strains of *S. cerevisiae* were affected by the presence of 5 to 10 per cent of resistant budding cells. However, by correcting the curves for the survival of these cells it was possible to obtain data relating only to the sensitive nonbudding cells.

In the case of *E. coli*, the irradiated population had a type B curve and it was hypothesized, without specific microscopic evidence, that it was composed of two kinds of cells, 66 per cent *S* cells and 34 per cent *R* cells, each with a type A curve. Similar hypotheses have been made to explain the nature of the survival curves of three other strains of *E. coli* (Magni, 1952; Cavalli and Modrone, 1949). Cultures derived from *R* cells were radiologically indistinguishable from cultures derived from mixtures of *R* and *S* cells. The relation of the *S* to the *R* cells and the basis of their differences in radiosensitivity are unknown.

The statistics for all type A curves (*E. coli*, *S* and *R*; *P. fluorescens*; *R. spheroides*; *S. cerevisiae*, corrected haploid) are collected in table 1. The values of the slope k varied over a threefold range, from about 0.2 to 0.66. The five values did not seem to be distributed at random, but fell within 10 per cent of 0.2, 0.4, and 0.6. Additional studies with other strains are needed to determine whether the periodicity of these values is real or coincidental.

Type C curves were obtained with *S. cerevisiae*, diploid, confirming previous work with this strain, and with *A. agile*. That of *A. agile* is of special interest since type C curves have rarely been found with resting vegetative bacteria except as a result of abnormal or uncontrolled experimental conditions. Several of these were eliminated as the possible cause of the present results. Although the LD_{50} varied from 7 to 11 kr, the shape of the survival curve was independent of the LD_{50} and was consistently type C (figure 6). From experiments with cells harvested after 10, 16, and 48 hours of incubation, it was concluded that neither the shape of the survival curve nor the LD_{50} depended on the age of the plate cultures from which the suspensions were prepared. Examination of the cell suspensions with the phase contrast microscope failed to reveal significant numbers of clumped cells, dividing cells, or abnormal forms such as giant cells or "snakes", which in the case of other species have type C survival curves (Pugsley *et al.*, 1935; Lea, 1955). Although *A. agile* cells, which are 3 to 4 μ in diameter, are much larger than the usual bacterial cells, their cytologic organization appears superficially similar to that of smaller bacteria. Chromatinic bodies which undergo orderly division and distribution to the daughter cells during cell division have been demonstrated by nuclear staining techniques (Pochon *et al.*, 1948; Eisenstark *et al.*, 1950), although the details of the nuclear structure are not fully understood (Jensen, 1954).

The interpretation of the type C curve is difficult. Type C data may be fitted equally well by curves based on multihit and multiunit models. This is shown by the results of the present investigation (figures 6, 7, and 8) as well as by the earlier studies with yeast summarized in table 3. More recently, investigators have favored models involving multiple sensitive units rather than multiple hits. In the case of both bacteria and yeast, knowledge is insufficient to provide a basis for a detailed cytologic inter-

pretation of the type C curve. The one generalization justified at present relates to the haploid and diploid strains of *S. cerevisiae:* haploid cells have type A curves, whereas diploid and polyploid cells have type C survival curves. This generalization, however, does not necessarily apply to other types of cells such as bacterial and animal cells.

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SUMMARY

Judging survival by macrocolony formation, the dose-survival curves for six selected strains of microorganisms irradiated with 250-kv X-rays were determined under standardized and comparable conditions. Washed cell suspensions of 10^4 to 10^5 cells/ml, prepared from 16 to 17-hour plate cultures were irradiated in potassium phosphate buffer, pH 7, supplemented with magnesium sulfate (0.002 M). The buffer concentration was optimal for the survival of each organism. The suspensions were in equilibrium with air during irradiation at 24 to 27 C. The bacteria were in a nondividing state, but the yeast included 5 to 10 per cent of very resistant budding cells for which the survival data were corrected.

The percentage survival was a simple exponential function of dose (type A or one-hit survival curve) in the case of *Pseudomonas fluorescens*, *Rhodopseudomonas spheroides*, and *Saccharomyces cerevisiae* (haploid); LD_{50} values of 0.95, 1.8, and 1.9 kr were found for these species, respectively.

The data for *Escherichia coli* strain W-1485 were fitted by a complex exponential function (type B) that was shown to be the sum of two simple type A curves. It was concluded that about 66 per cent of the cells (sensitive cells) had an LD_{50} of 1.2 kr and 34 per cent (resistant cells) had an LD_{50} of 3.5 kr. Subculturing either the resistant or a mixture of resistant and sensitive cells gave rise to the same kind of mixed population found originally.

The data for *Azotobacter agile* and *S. cerevisiae* (diploid) were fitted by complex exponential functions of dose called type C or multihit curves; the LD_{50} values were 8.5 and 24 kr, respectively.

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