Title

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Authors

Elkind, Mortimer M.
Beam, Carl A.

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EFFECTIVENESS OF RADIATION ON HAPLOID YEAST

Contents

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Mortimer M. Elkind and Carl A. Beam

Donner Laboratory of Biophysics and Medical Physics, and
Radiation Laboratory, Department of Physics,
University of California, Berkeley, California

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ABSTRACT

The radiosensitivity of haploid Saccharomyces cerevisiae, SC-7, has been studied in the region of exponential survival using 50-kv x-rays and 3.4-Mev alpha particles (Po^{210} source). Cells harvested from YED agar (1/2% yeast extract, 1% dextrose) were used to inoculate a 30° C liquid YED medium (0.45% YE, 0.91% D). After about 3 to 4 generation times, log phase cells were harvested, washed, and either used to inoculate a starvation medium (5 gm % dextrose in M/20 KH_2PO_4) or used for a fresh-cell survival study. The criterion of biological effect was visible colony formation from irradiated cells plated on YED agar.

Although resistance to alpha-particle radiation remained relatively constant for starvation periods up to 9 days, in the same interval the x-ray resistance increased by a factor of 2.5. The relative biological effectiveness (RBE) during this same interval varied from 0.66 to 2.0 with most of the RBE and x-ray radiosensitivity variation occurring within the first three days of starvation. In addition to the RBE and radiosensitivity variation of the exponential portion of the survival curves, it is shown that alpha particles, as do x-rays, display a multiple-hit portion of survival attributable to the budding population, and that the multiple-hit portion can have an RBE > 1 while the single-hit portion has an RBE < 1.

VARIATION OF THE BIOLOGICAL EFFECTIVENESS OF X-RAYS AND ALPHA PARTICLES ON HAPLOID SACCHAROMYCES CEREVISIAE

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Donner Laboratory of Biophysics and Medical Physics, and Radiation Laboratory, Department of Physics, University of California, Berkeley, California

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INTRODUCTION

The concept of the relative biological effectiveness (RBE) of various radiations compared to x-rays has been intimately associated with considerations of the rate of energy loss (REL)** of the irradiating particles compared. In the pertinent literature of the past two decades, reviewed recently by Boag[1] and Zirkle,[2] there are numerous examples of both increasing and decreasing RBE's with REL. Unfortunately, the wide variety of techniques, criteria of effectiveness, and biological systems employed in these studies often hampers evaluation of the contrasting observations. The results to be reported here will provide another example of both increasing and decreasing effectiveness with REL, but under circumstances that relate the variation in RBE to the controlled manipulation of the biological system, cells of a haploid strain of Saccharomyces cerevisiae.

Although in previous work with this organism criteria of biological effect ranging from inhibition of division to inhibition of visible colony formation have been used, the initial portion of the survival curve generally has been found to be exponential. Recent work at the Donner Laboratory with x-rays[3,4] has shown that the initial region of exponential survival is followed by a region of "multiple-hit" survival displaying considerably greater radioresistance (Fig. 4). In addition, Beam and co-workers[4] correlated the multiple-hit portion or "tail" of the survival curve with a population moiety consisting of budding cells only. This was done (a) by extrapolating the tail of the survival curve to zero dose, (b) by observing that the level of the tail could be varied over several decades of survival as a function of starvation of the cells, and (c) by correlating the zero-dose

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* From the National Cancer Institute, National Institutes of Health; Bethesda, Maryland; on temporary assignment to the Donner Laboratory.

** Customary units of REL are kev/μ.

survival fraction of the tail with the budding fraction of the population observed microscopically. The correlation was found to obtain only for those buds appearing after starved cells were spread on a nutrient agar medium. Recently, Burns[5] has observed that only those budding cells in which the daughter cell is less than half the diameter of the mother cell display a radiosensitivity characteristic of the tail of the survival curve, and those cells in which the daughter is larger than half the diameter of the mother display a radiosensitivity expected from two cohering single cells. The observations of References 4 and 5 are consistent, since the budding cells in Ref. 4 had small buds.

At this writing, all previous RBE studies of haploid yeast have concerned themselves with the initial or exponential portion of the survival curve. Because it was observed that the level of the tail could be reduced as a function of starvation, it was found that the initial survival region could be conveniently observed for two or more decades if the population received a starvation pretreatment before irradiation. For instance, tail suppression was achieved by Sayeg[6] by starting with a 48-hour culture harvested from a yeast extract dextrose (YED) agar surface and then incubating these cells in a buffered dextrose medium at 30° C. A progressive reduction of the bud count can also be expected when cultures on solid medium are incubated beyond their point of active growth. Zirkle and Tobias[7] incubated their cells at room temperature for 4 to 6 days on potato dextrose (PD) agar. As a result, their cells were probably also starved to some degree. Starvation is known to vary the metabolic state of yeast cells. Recent discussions[6, 7] of RBE have not considered the possible influence of the metabolic state of the cell. In what is to follow, it will be shown that for haploid yeast the RBE of Po^{210} alpha particles compared to 50-kv x-rays is not independent of the metabolic state of the cell, and is dependent, moreover, on the state of division of the yeast cell. Therefore, questions of radiobiological theory must take these pertinent biological conditions into account.

RADIATION SOURCES AND DOSIMETRY

The physical arrangement for alpha-particle irradiation is shown in Fig. 1. The source* consisted of a thin layer of Po^{210} electrodeposited onto a nickel disc of about 0.005-inch thickness. With the aid of the 3/16-inch-diameter hole in the source-holding collar, the alpha-active area could be centered to within a few thousandths of an inch. The tygon window (0.00025-inch thick) helped confine the radioactive material. A series of concentric circles scribed into the head of the adjustable screw permitted the transparent agar block, which carried the cells, to be centered visually to within about 1/32 inch. By sighting across the vertical positioning collar while turning the adjustable screw (1/32-inch pitch), one could reproduce the source-to-cell spacing to within ± 0.004 inch. Samples were irradiated by manually sliding the lucite drawer to a position determined by a stop, then pulling it out again after the proper interval timed by a stop watch.

If the average diameter** of a haploid yeast cell is 5.0 microns, the diameter of a cell corresponds to a large fraction, about 1/3, of the total range of a 5.30-Mev alpha particle emitted by Po^{210}. Because of this, and because of increasing air absorption as the air path is increased, the effect of increasing the source-to-absorber separation is to increase the exit- to-entrance dose ratio of a cell as the peak of the Bragg curve is approached at about 1.0 Mev. Though increasing the source-to-absorber distance also increases range straggling, it decreases over-all dose variations due to (a) differences in the equivalent air path of all alpha particles reaching a given point on the absorber, and (b) differences in the local energy absorption across the absorber. Since optimum dosimetry requires that the dose be as uniform as possible across the diameter as well as throughout the thickness of the absorber, a compromise between the preceding factors was sought. The arrangement shown in Fig. 1 represents a practical solution of this problem.

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* The Po^{210} source was made by Mound Laboratories; Monsanto Chemical Co.; Miamisburg, Ohio. The source activity was periodically checked through the courtesy of Dr. H. P. Robinson, Radiation Laboratory, University of California. The activity was determined to ± 2% through the use of a calibrated "good geometry" scintillation counter.

** The diameter, 5.0 microns, is an average value determined from microscopic measurement of members of many populations.

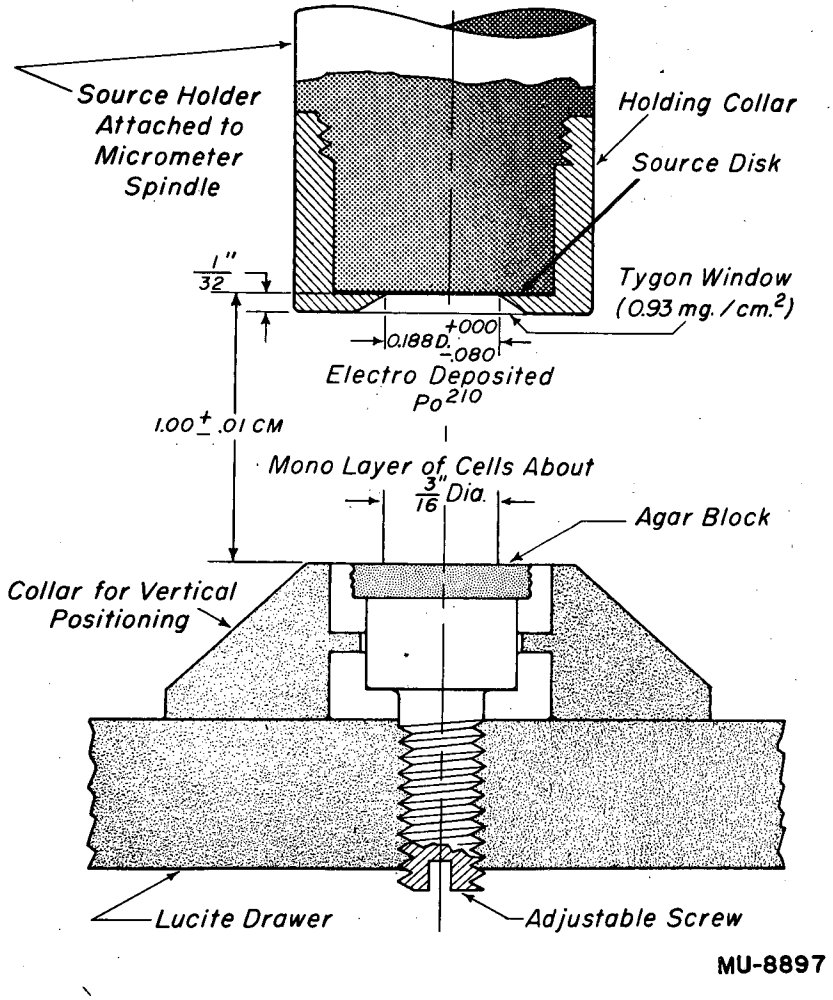

Fig. 1. Apparatus for alpha-particle exposure. The parts shown above are enclosed by a lucite box which in turn is kept in a "gloved box" to contain the radioactivity. The lucite box was designed by Sayeg.[6]

The alpha-particle dose rate was computed with the aid of the stopping power and range-energy data obtained from Bethe[8] and Livingston and Bethe.[9]

The expression for the dose rate D' is

$$D' = s_m \sigma_a \frac{N}{A_a} \frac{n}{4} \bar{G}, \qquad (1)$$

where s_m is the mass stopping power* of the absorber relative to air, σ_a is the absorption cross section per atom of air and has the units of energy times area, N is Avogadro's number, A_a is the equivalent atomic weight of air, and n is the alpha-particle activity per unit area of emitter. In Eq. (1), $\bar{G}$ represents the average of G over the absorber area where G is a geometry factor given by

$$G = \ln\left(\sqrt{\left(\frac{1}{2} - \frac{R^2}{2a^2} + \frac{r^2}{2a^2}\right)^2 + \frac{R^2}{a^2}} + \frac{1}{2} + \frac{R^2}{2a^2} - \frac{r^2}{2a^2}\right), \qquad (2)$$

in which R and r are the radii of emitter and absorber respectively, and a is the perpendicular distance between emitter and absorber. Equations (1) and (2) are based on the assumption that the differences in the equivalent air paths of all alpha particles reaching the absorber are negligible. A derivation of Eq. (2) for $r = 0$ is given in Reference 6.

The alpha-particle dose rate was computed on the assumption that the average cell composition was the same as the composition of wet tissue suggested by Lea[10], and that the average cell density was 1.05. These assumptions were the same as those used in Refs.6 and 7. The average alpha-particle energy at the center of a 5.0-micron-diameter cell was computed to be 3.4 Mev, for which $s_m = 1.25$ and the REL per alpha particle = 130.8 kev/micron. Possible effects of chemical binding were not considered. These values are consistent with those given in Refs.7 and 10. The dose rate per millicurie was computed to be 59.9 rep/sec. For the geometry shown in Fig. 1, D' at a radius of 3/32-inch from the absorber center was 5% less than its value at the center; and D' along a diameter

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* $s_m = (A_a/A_t)s$, where A_t is the equivalent atomic weight of the absorber, and s is the atomic stopping power of the absorber relative to air.

of a 5.0-micron cell was computed to be 4% less at the entrance and 7% more at the exit than at the center of the cell. Since it is likely that the sites sensitive to alpha particles are somewhere near the center of a cell, the over-all uncertainty in alpha-particle dosimetry was probably within ± 7%.

The x-ray source consisted of a Machlett OEG-60 tube powered by a Picker full-wave rectifier. The tube, which had a 1.0-mm beryllium window, was operated at 50 kv and 25 ma. As in previous work performed with this machine,[3-7, 14, 15] the dose rate was measured with an extrapolation ionization chamber; the beam entered the chamber through an electrode made from 0.002-inch beryllium foil. Samples were irradiated at a distance of 15.9 cm from the tube target, and at this distance the dose rate was 252.2 rep/sec. A comparison of survival curves obtained with 1/2-cm-diameter samples as against 9-cm-diameter samples showed that there was essentially no transverse variation in dose over the latter diameter. Details of the x-ray apparatus as well as the conversion of air dose to tissue dose can be found in Ref. 3. The over-all uncertainty in x-ray dosimetry was probably within ± 4%. The alpha-particle dose rate during the course of this work varied from 0.9 to 0.5 the x-ray dose rate.

BIOLOGICAL MATERIAL

The yeast used in this study, haploid Saccharomyces cerevisiae strain SC-7, was used in many investigations;[3-7, 11, 14, 15] its lineage is given by Zirkle and Tobias.[7] A subculture was stored at 4° C; from it inocula were taken for the growth of experimental material, either upon YED agar or in liquid medium of similar composition as described below.

Petri dishes containing YED agar (0.5% Difco yeast extract, 1.0% dextrose and 2.0% Difco Bacto agar) were streaked with cells and incubated in the dark at 30° C. After 9 to 18 hr of growth, the cells were harvested, suspended in chilled water, and used either directly in survival studies, or, when a more physiologically uniform population seemed desirable, as an inoculum for growth in liquid YED. For the latter, the cell density was adjusted to $\sim 7.0 \times 10^7$/ml; 15 ml of this suspension was then used to inoculate a liquid YED medium consisting of 75 ml of 1.0% Difco yeast extract plus 75 ml of 2.0% dextrose (autoclaved separately) contained in a 250-ml centrifuge bottle. The bottle was fitted with a stopper containing aeration tubes through

which filtered air was bubbled at a rate sufficient to maintain a uniform suspension. A water bath provided an incubation temperature of 30 ± 1° C.

After a lag phase of ~90 min, the cell density increased exponentially with a generation time of 85 ± 10 min. When a density of ~7.0 x 10^7 cells/ml was reached, the cells were chilled to ~5° C in an ice bath and centrifuged for 10 min at about 600 g. The growth medium was decanted and the cells were resuspended for washing in ~250 ml of chilled neutral buffer* and again centrifuged as before. After a total of three such washes, a final suspension, of the desired cell density, was made in chilled neutral buffer. Cell densities and compositions of populations (percentage buds and single cells) were determined from hemacytometer counts. No tendency to form clumps was found.

In contrast to cells grown on YED agar, populations harvested from liquid YED could be characterized by their generation time and composition. These reproducible features led to criteria for the acceptance of a batch of cells which were (a) a generation time during log phase growth of 85 ± 10 min; and (b) a population composition after washing consisting of 30% cells with small buds, 30% cells with large buds, 38% to 40% single cells, and less than 2% cells with two buds (a mother cell with apparently two daughter cells). As opposed to "starved" cells to be described, cells grown in the foregoing way were called "fresh" and, as measured by the percentage of small buds, were the freshest cells obtainable in suitable quantities without recourse to division synchronization.[4] That this procedure successfully standardized the populations used was attested by the reproducibility of the fresh-cell survival curves. For x-rays, for instance, the lethal dose for 50% survival (LD_{50}) of the total population was consistently found to be 2.9 krep.

The preparation of starved cells was by a procedure refined from one previously described,[4] and involved the use of the apparatus shown in Fig. 2. The nitrogen-free starvation medium (5.0 g % dextrose in 0.05 M KH_2PO_4 but no fixed nitrogen source) was inoculated with about 5.0 x 10^9 fresh cells, and a continuous flow of medium was maintained at about 30 ml/hr by adjusting

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* The buffer composition used was obtained from Gunter[11] and consisted of: 2.65 g/l KH_2PO_4; 5.32 g/l $K_2H\ PO_4$; and 10 ml/l of 0.2 M $MgSO_4$ (autoclaved separately).

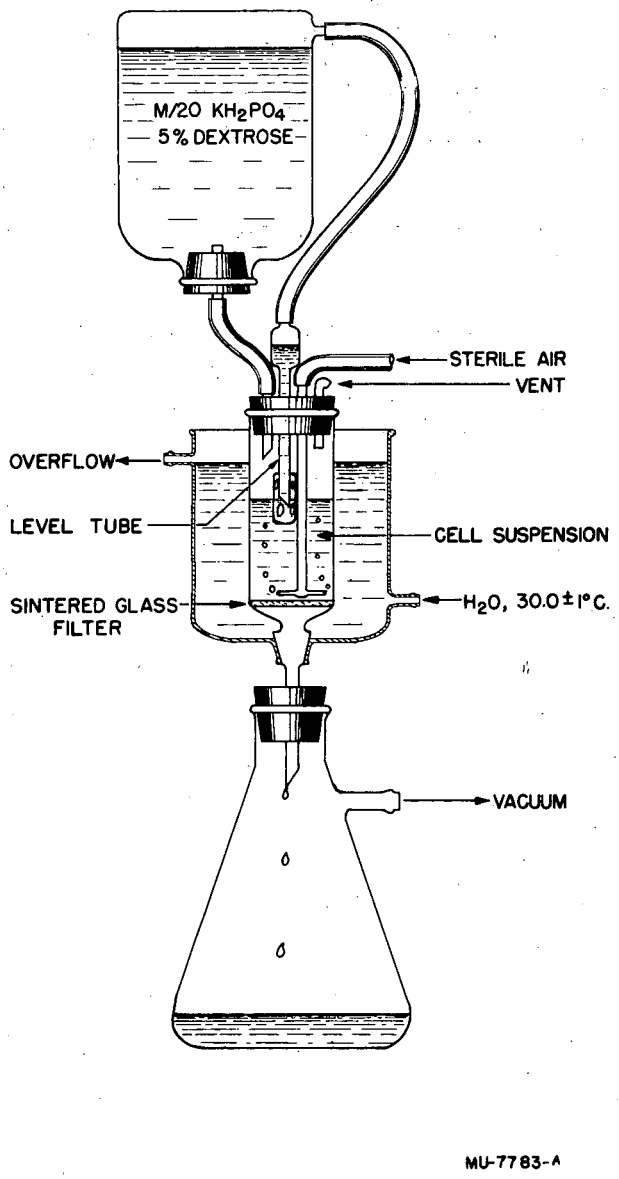

Fig. 2. Starvation apparatus for yeast. Suspension volume about 150 ml; starvation-medium flow rate about 30 ml/hr.

the pressure in the collection flask. The level tube maintained the suspension volume at about 150 ml. The air stream was sufficiently strong to prevent the collection of an appreciable number of cells on the filter. At prescribed intervals (i.e. 2, 5, and 9 days), about 1/3 of the cells were extracted and prepared for use through the same washing procedure used for fresh cells. Although the cell concentration was reduced with each extraction, the flow rate of the starvation medium was great enough to insure essentially constant conditions. Fresh- or starved-cell stock suspensions or their dilutions were maintained at about 5° C for the course of the experiment.

EXPERIMENTAL PROCEDURE

The irradiation procedure was planned so that the x-ray and alpha-particle irradiations could both be performed within the same 24-hour period. To check on possible effects due to the time course of the experiments, the x-ray irradiation sometimes preceded the alpha-particle irradiation-- and vice versa. No effect of the order of irradiation was found. From the descriptions to follow, it is seen that the techniques used for the two irradiation procedures were somewhat different. To insure that the results obtained did not reflect differences in technique, x-ray survival curves for the same stock suspension were obtained using both techniques. The results were statistically indistinguishable.

In both procedures, survival ratios were determined from counts of visible surface colonies formed on YED agar in petri dishes after 36 to 48 hours of incubation at 30° C. In both cases, the colonies were uniform in size and about 1 mm in diameter. Control samples received the same treatment except for irradiation. Experiments were planned to give from 100 to 500 colonies per petri dish, and both x-ray and alpha-particle irradiations were conducted at room temperature.

The alpha-particle procedure used was the following: Samples of about 4.8×10^5 cells were pipetted onto the surface of a 1-mm-thick agar layer with a 0.003 ml pipette. After the suspending buffer solution was absorbed into the agar, it was found that about half of these samples had monolayer diameters of 3/16-in. or less. The latter were cut out and irradiated as shown in Fig. 1. Following irradiation, the cells were resuspended in 10.0 ml of neutral buffer. Aliquots of 0.1 ml of this resuspension, or a 1:10 dilution of it, depending upon the expected survival, were plated on the surfaces of

YED agar in 9-cm petri dishes and then incubated. On the average, about six samples were taken per dose point and five platings made per sample.

The x-ray procedure consisted of making serial 1:10 dilutions of the same stock suspension used for a given alpha-particle experiment and plating 0.1-ml aliquots of the appropriate dilutions onto the surfaces of YED agar in 9-cm petri dishes.* Five to ten minutes after plating, the suspending neutral buffer solution was absorbed into the YED agar. The cells were irradiated (with the petri dish cover removed) and then were incubated. From 10 to 20 plates were used per dose point.

EXPERIMENTAL RESULTS

Figure 3 is a semilog plot of the alpha-particle and x-ray survival curves obtained after starvation for 0.91 day. The uncertainties of the plotted points in the figure, as well as the other survival curves to be presented, represent standard deviations of the means. It is clear, from this figure, that the exponential region of the curves down to 2.5% survival is not influenced by the radioresistant, multiple-hit component due to budding cells. This means that the budding population was reduced to less than about 0.1% in 22 hours of starvation. Because of this, the survival curves shown in Fig. 3 indicate that the level of the alpha tail, if any, cannot be much greater than the level of the x-ray tail.

It is clear in Fig. 3 that the RBE is > 1, since the alpha curve lies below the x-ray curve. The actual value of the RBE can be obtained from ratios of the slopes or lethal doses at a given percent survival. However, the concept of a quantitative RBE is not restricted to cases of exponential survival. A general definition can be given as follows: If S_x and S_p stand for the survival fractions of a biological system exposed to x-rays (i.e. 220 kv) and another type of particle respectively, then a meaningful RBE exists only when the survival curves are of the form

$$S_x = f(D) \tag{3}$$

$$S_p = f(KD) \tag{4}$$

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* Irradiation on nutrient agar had no detectable effect, as shown in a previous report[4] and by control experiments performed as part of this study.

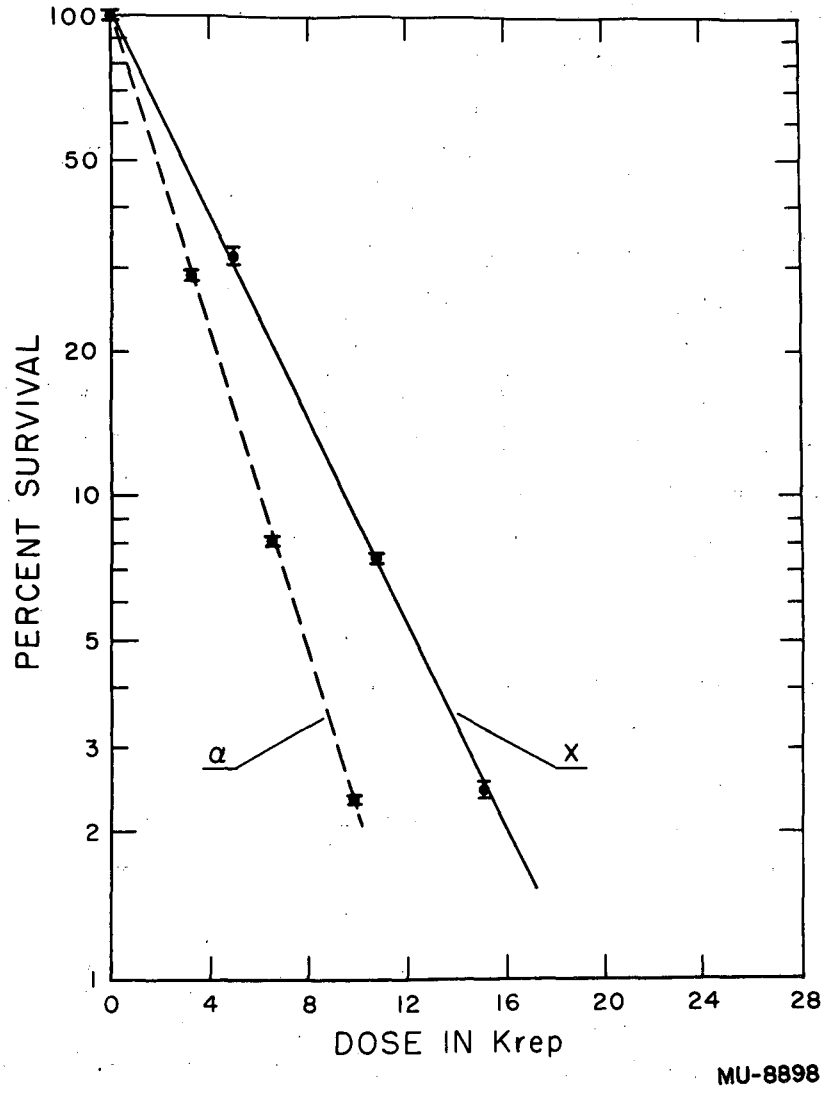

Fig. 3. Alpha-ray and x-ray survival curves for SC-7 starved for 0.91 days at 30° C in liquid medium consisting of 5.0 g % dextrose in 0.05 M KH_2PO_4. The uncertainties shown are standard deviations of the means.

In Eqs. (3) and (4), f (D) means a given function of the dose D, and K is a constant or dose-reduction factor. These equations require only a functional similarity between the survival curves for the two particles; when that similarity exists, RBE = 1/K.

Figure 4 shows the alpha-particle and x-ray curves through four decades of survival for cells harvested after about 6 hours of growth on YED agar surfaces. The x-ray survival curve is typical of those previously obtained with such cells.[4] Since the cells used were not grown in the standard way established for this work, RBE and radioresistance values are treated only qualitatively. However, several points of interest can be deduced from Fig. 4. Firstly, it is clear that the alpha curve has a tail, as well as the x-ray curve. Secondly, it appears that both the alpha and x-ray tails extrapolate to about the same percentage survival at zero dose, about 16%. And thirdly, it is clear that the RBE is not constant for the entire survival curve, but has at least two values. In particular, it appears that the RBE of the initial survival region is < 1.0 (about 0.7), and that RBE of the tail region is > 1.0 (about 2.0). This fact reinforces the conclusion that there are two moieties in a relatively fresh population of haploid yeast cells. The important questions -- namely, are RBE, radiosensitivity, and the slopes of the asymptotes of the tails also dependent upon starvation?-- will be the subject of a later work.

In order to examine the transition region between the initial and tail portions of the survival curves, the data shown in Fig. 5 were obtained. Here too, radiobiological quantities are only approximately determined, because the cells used were harvested after 9 hours' growth on YED agar surfaces. Although Fig. 5 clearly shows that the initial portions of the curves display an RBE of about 0.76 (the curves are functionally similar down to ~ 30% survival), it indicates, as in Fig. 4, that the RBE for the tail region is > 1.0, because the alpha curve falls off more rapidly than the x-ray curve. Figure 5 also shows that the tails of both curves could have the same zero-dose intercept, although the two initial tail portions between zero and about 16 krep are probably not superimposed. It therefore appears likely that the alpha-radiation tail intercept can also be correlated with the percentage of small buds in the population, and that the initial portions of the curves have only a limited range of similarity because the alpha tail falls off more rapidly than the x-ray tail.

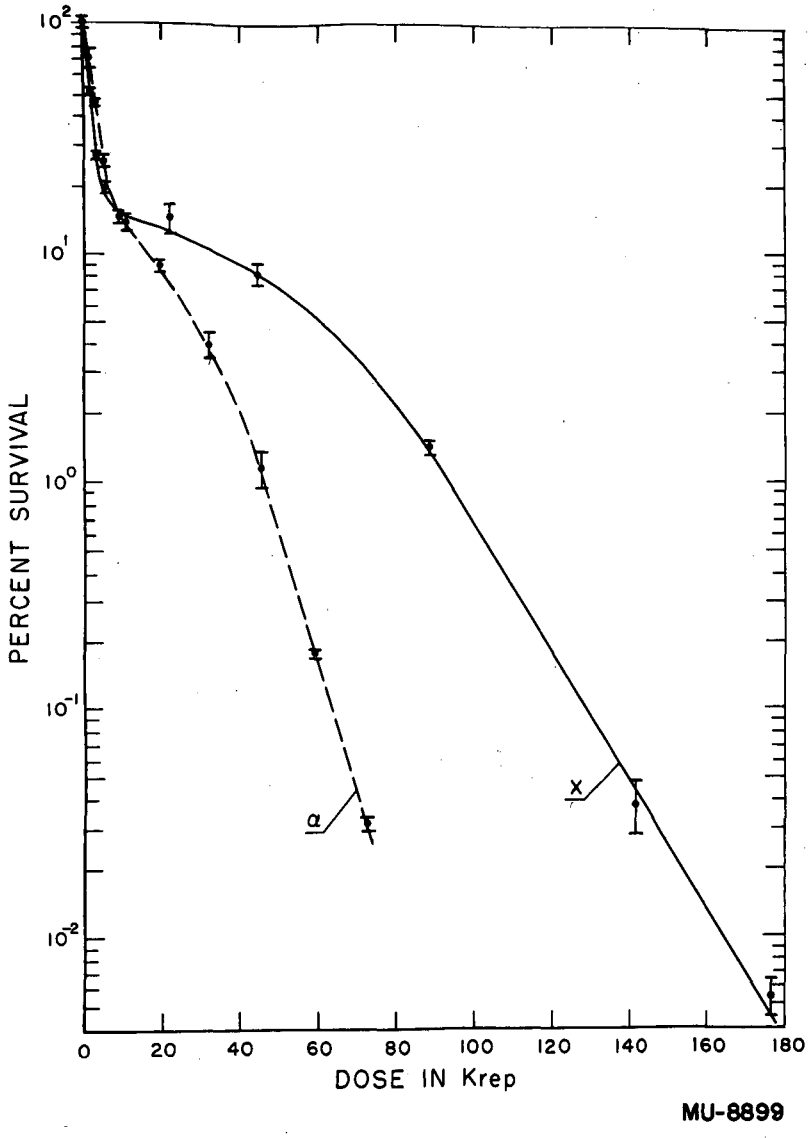

Fig. 4. Alpha, x-ray survival curves for SC-7 harvested after about 6 hours' growth at 30° C on YED agar surfaces. The uncertainties shown are standard deviations of the means.

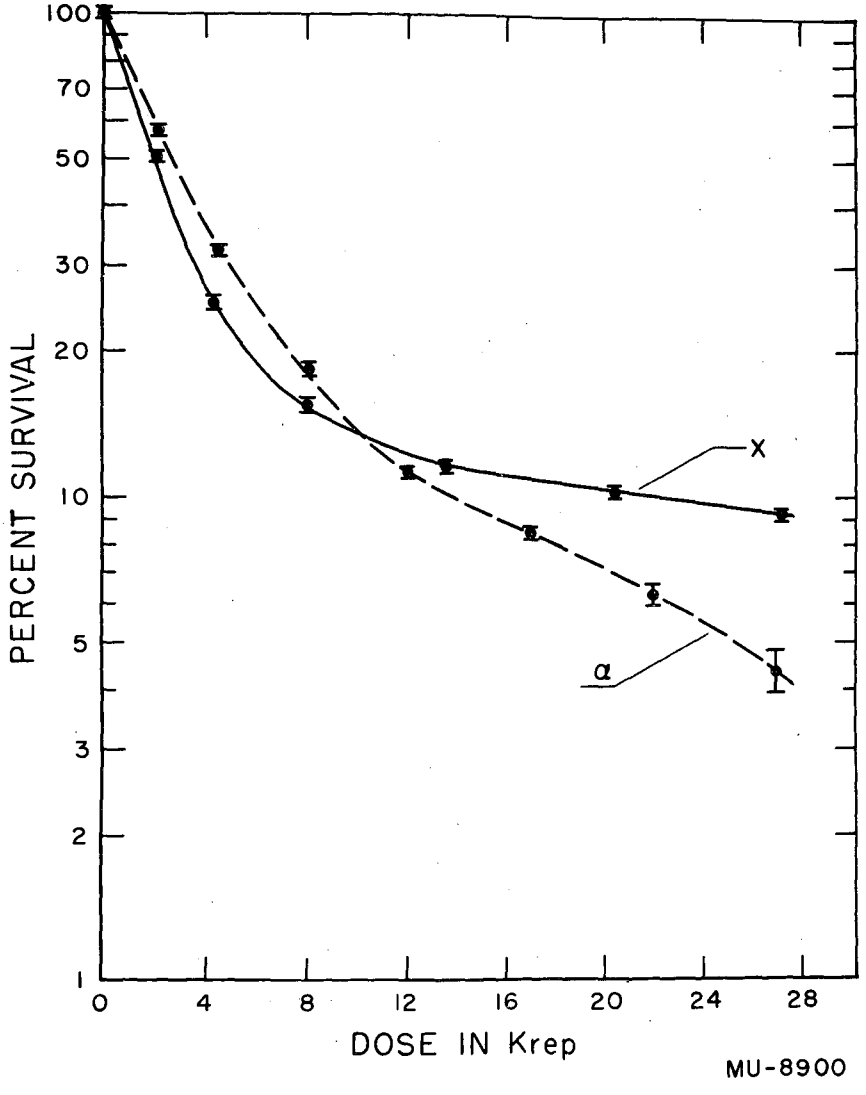

Fig. 5. Alpha, x-ray survival curves for SC-7 harvested after 9 hours of growth at 30° C on YED agar surfaces. The uncertainties shown are standard deviations of the means.

Figure 6 is an alpha, x-ray survival plot of the initial survival region investigated in detail. The cells in this case were harvested at log phase from growth in liquid medium, which accounts for the relatively high level of the x-ray tail. Curves A and B are the x-ray and alpha-radiation survival curves respectively, which display functional similarity down to approximately 45% survival. The RBE determined from A and B within this region is 0.66 ± .04; the uncertainty is a graphical estimate determined from the range of survival curves permitted by the uncertainties of the individual points. As previously stated, fresh cells (or zero starvation-time cells) consisted of 30% cells with small buds, 30% cells with large buds, and essentially 40% single cells (since the percentage of cells with two buds was usually small). The last three points on A are not significantly affected by the exponential portion of the curve; C, which is an extrapolation of these last three points, has a zero-dose intercept of 30%, in agreement with the percentage of cells with small buds. The points plotted below A and B result from subtracting C from A. D is a straight line fitted to these latter points by the method of least squares. Since the small-bud count incurred subjective estimation of bud size, the uncertainties of the points for D were arrived at (a) by constructing the extreme lines for C permitted by the uncertainties of the last three points on A; (b) by using the extremes of C for the uncertainties of the tail portion of the survival curve; and (c) by propagating the squares of the latter uncertainties with the variances of the points traversed by A as sums of squares. To account for the contribution of cells with large buds, the necessarily independent inactivation of both bud and mother cell must be considered. This would give a survival curve, after subtraction of the tail, of the form $c_1 e^{-kD} + c_2(2e^{-kD} - e^{-2kD})$ where c_1 and c_2 are the percentages of single cells and cells with large buds respectively. The straight line E represents the asymptote of the preceding expression from which k was determined.

Although Fig. 6 confirms the general observation that the initial portion of a haploid yeast x-ray survival curve (fresh or starved) is exponential, the value of the radiosensitivity cannot be very accurately determined because the actual shape of C could not be observed. Consistent with Burns's observation,[5] the radiosensitivity for the initial x-ray survival region was determined from E. However, D also fits the resolved points well. Since the actual shape of C could not be observed, the uncertainty in the x-ray

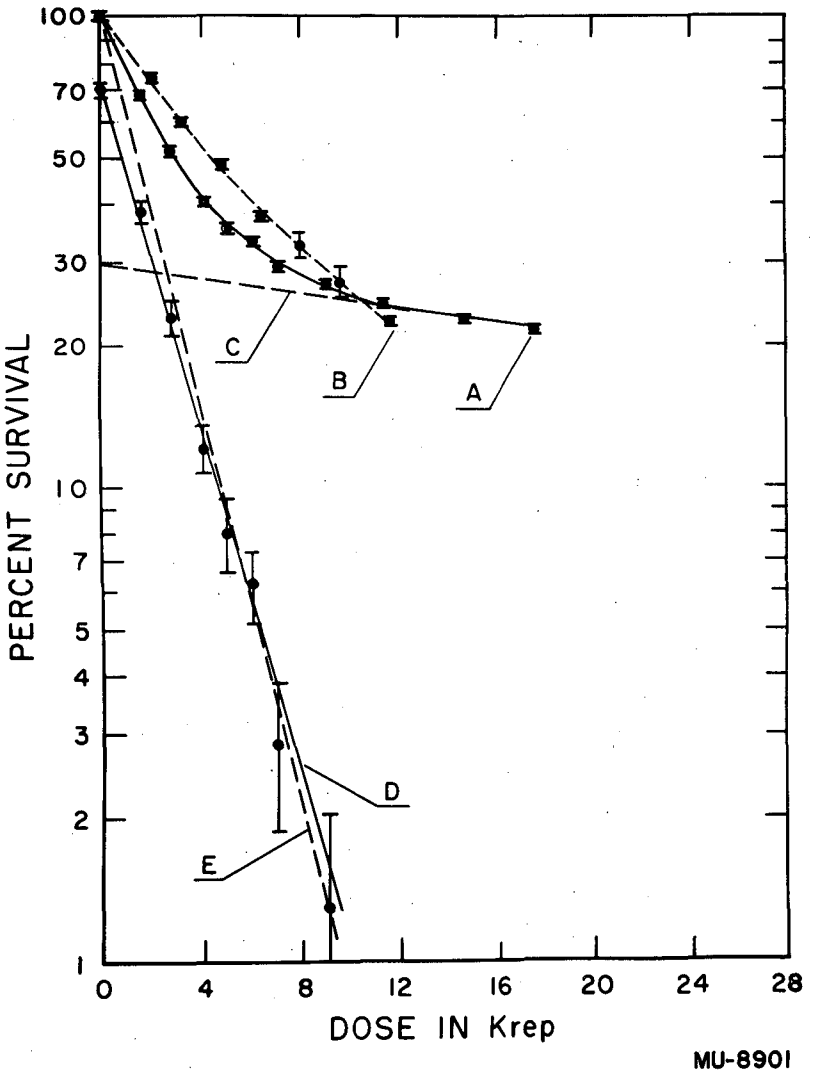

Fig. 6. Alpha, x-ray survival curves for SC-7 log-phase cells grown in liquid medium at 30° C. Curves A and B are the total x-ray and alpha-particle curves respectively; curve C is the extrapolated tail of A; curve D is the result of subtracting C from A; and curve E accounts for large buds (see text).

radiosensitivity* (LD_{10} in Fig. 7) was made large enough to include differences in the slopes of E and D resulting from possible ambiguities in the constructional procedure.

Because the alpha-particle curve falls off less rapidly initially and more rapidly finally than the x-ray curve, the preceding method could not be used to deduce the radiosensitivity to alpha particles. Instead, the following reasoning was applied. Starting with starved cells and progressing in the direction of fresh cells, one can induce that the initial x-ray survival region is always exponential.[4, 12] This is confirmed in Fig. 6 (D or E) because the "two-hitness" component asymptotically represented by E still basically corresponds to exponential survival. Now, since A and B are functionally similar down to 45% survival, and since within this region the composite curve A can be resolved into two curves C and E (or D), it follows that B is also composed of two curves which are functionally similar to C and E (or D) respectively, and related to them by the same RBE as B is to A. For instance, Fig. 6 shows that within the region of functional similarity A can be resolved into two components C and D. In terms of Eqs. (3) and (4), these relationships are

$$S_x = f(D) = f_1 e^{-k_1 D} + f_2 e^{-k_2 D}, \qquad (3a)$$

$$S_p = f(KD) = f_1 e^{-k_1 KD} + f_2 e^{-k_2 KD}, \qquad (4a)$$

where f_1 and f_2 are the fractions of the population corresponding to single cells and cells with small buds respectively, k_1 and k_2 are the radiosensitivities of D and C respectively, S_x and S_p stands for the survival fractions to x-rays and alpha particles respectively, and $\frac{1}{K}$ = RBE. A further consequence of the existence of functional similarity between A and B, which can be seen in the preceding equations, is that the fraction of the population correlatable with the x-ray tail can also be correlated with the alpha-particle tail. This is in agreement with observations already made in connection with Figs. 4 and 5. Hence, the alpha-particle tail also results from the population moiety having small buds and the initial exponential region represents the survival of single cells. Computation of the alpha-particle radiosensitivity was from the RBE of B to A and the determined x-ray radiosensitivity. The uncertainty in this latter value is even larger than the corresponding x-ray value because of the uncertainty

* The radiosensitivity k is related to LD_{10} by: $k = (2.30/LD_{10})$.

in the RBE. However, in spite of the fact that the fresh-cell data are relatively unprecise compared to starved-cell data, it can be seen that the conclusions to be drawn need only minor qualification.

Figure 7 is a plot of the alpha-particle and x-ray LD_{10}'s as a function of starvation time. With the exception of the zero-starvation time points already discussed, the uncertainties of the points were computed from straight-line, weighted, least-square fits to the experimental data and represent standard deviations. Each pair of alpha, x-ray points at a given time was derived from survival experiments on the same population of cells. The points at 1.85, 4.91, and 8.93 days were obtained from the same initial inoculum in the starvation apparatus, Fig. 2; the points at 2.92 and 6.76 days were obtained from another inoculum, as were the points for 0.91 day. The smooth curves through these points indicate a good measure of control and internal consistency in the over-all experiment.

Figure 8 is a plot of the variation in RBE with starvation time. The curve in Fig. 8 is a ratio of the curves in Fig. 7, but the points in Fig. 8 and their uncertainties were obtained from corresponding pairs of points in Fig. 7.

Except for possibly a small initial increase in radiosensitivity to alpha particles, the results of this work show that for interdivisional cells the RBE varies by a factor of 3 mainly because the x-ray radiosensitivity varies by a factor of 2.5. The latter variation is approximately an exponential function of starvation time with a half period of about 17 hours.

DISCUSSION

During the course of the present study Wood[12] reported a 1.5-fold decrease in the x-ray sensitivity of SC-7 occurring as a function of culture age. Since his cultures were grown and maintained on potato dextrose (PD) agar at room temperature, his results should not be compared quantitatively with ours. The LD_{10}'s reported, 6.43 krep for a 2-day-old culture, and a constant 9.43 krep for from 6 to 23 days, lie, nevertheless, within the range reported here, and the change may well reflect the same underlying phenomenon as that produced by starvation. Our work may be related in a similar way to that of Zirkle and Tobias,[7] who employed cells from 4- to 6- day cultures on PD agar. Their high values (2.7 and 2.9) for the RBE of alpha particles of REL similar to those used in this study (110 and 190 Kev/μ

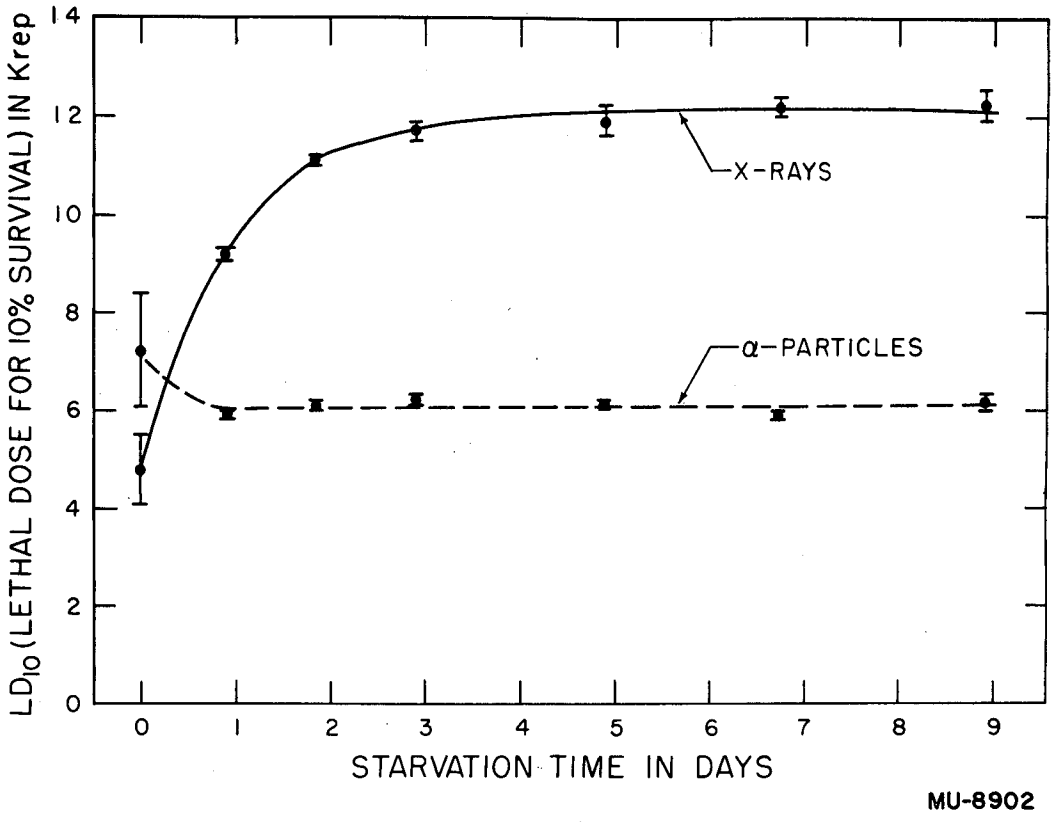

Fig. 7. Variation of LD_{10} of SC-7 as a function of starvation time in 5.0 g % dextrose in 0.05 M KH_2PO_4 at 30°C.

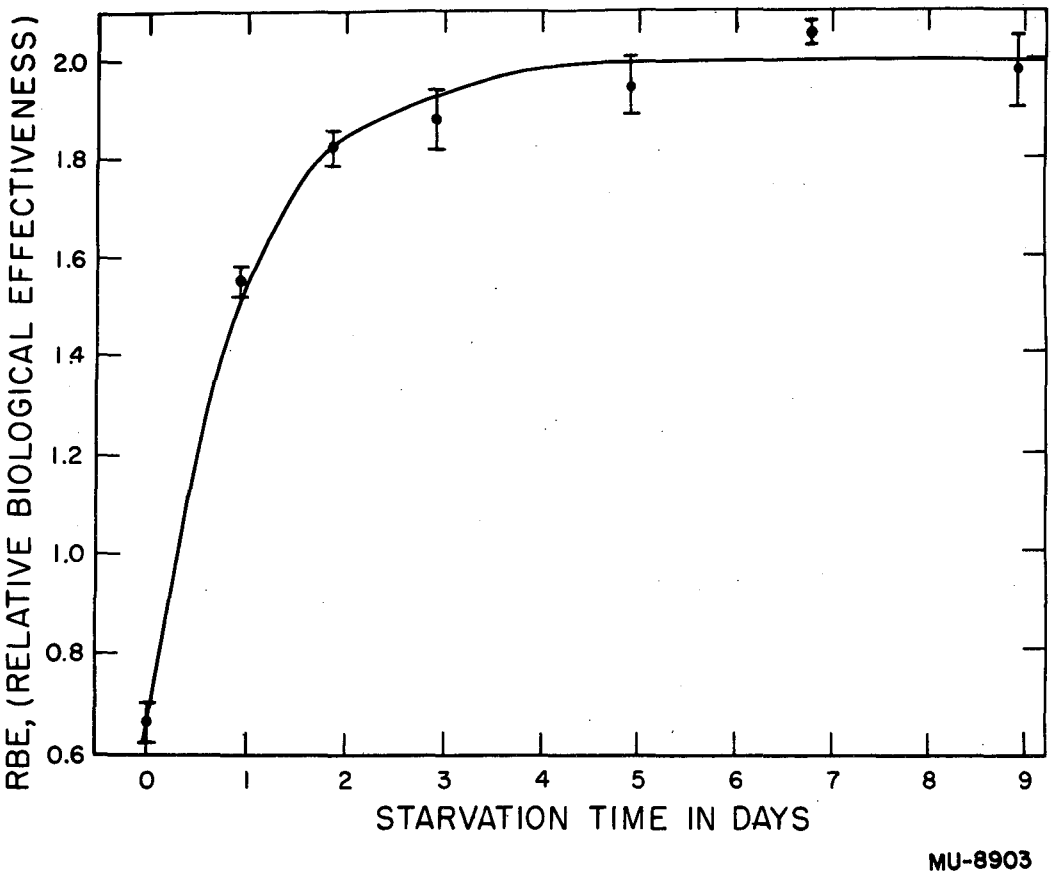

Fig. 8. Variation of RBE as a function of starvation time for SC-7 in 5.0 g % dextrose in 0.05 M KH_2PO_4 at 30° C.

respectively) may have resulted from their criterion of effectiveness (inability to undergo the second postirradiation cell division). In general, the preceding work with cells grown on solid medium suggests that prolonged incubation after the medium is depleted and growth has ceased produces radiosensitivity changes similar to those resulting from starvation.

The pertinent biological consequences of starvation are not known. That the effect upon radiosensitivity is physiologically mediated is indicated by the knowledge[12] that yeast cells from a 2-day PD agar surface (LD_{10} = 6.43 krep) agitated in M/15 KH_2PO_4 for 48 hours without dextrose were unaltered in sensitivity to 250-kv x-rays. A change in sensitivity of a biological system to an external agent could arise in either of two basic ways. It could result from shifts in the inherent sensitivity of the system to the radiation, or from changes that do not affect inherent sensitivity but which alter local energy absorption.

As previously mentioned, the computation of the alpha-particle dose was based upon an assumed gross average composition. It is possible that even if the gross composition remained constant, the local composition within the cell could vary with starvation. From Eq. (1), it is seen that changes in local composition would alter the local alpha dose rate through changes in s_m. It can be shown* that s_m varies approximately with $\Sigma\,(f/A)\,Z \log (C/Z)$, where f is the fraction, by weight, of a target constituent of atomic number Z and atomic weight A, and C is a constant for a given alpha-particle energy. On the other hand, the local 50-kv x-ray dose rate is a function of $\Sigma\,(f/A)\,Z$,[4] since such x-rays are absorbed mainly by the photoelectric effect.** The strong Z dependence of the x-ray dose rate suggests that the change in x-ray sensitivity reflects a decrease in the average Z of the x-ray sites. If the sites of alpha-particle damage were the same, one would expect a corresponding though smaller decrease in alpha-particle sensitivity. This was not observed (see Fig. 7).

Further evidence against a significant role of changes in local atomic composition in producing the observed variations in radiosensitivity comes from other studies employing 220–250-kv x-rays. Radiation of this quality delivers energy mainly through Compton absorption*** and therefore the

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* See Ref. 9, p 263.

** The photoelectric effect varies at least with the fourth power of Z. See, for instance, Ref. 10, p 347.

***See Ref. 10, p 346.

local dose would vary with Σ (f/A) Z. This Z dependence is about the same as that for the alpha-particle dose. If the observed dependence of x-ray sensitivity on starvation reflects a variation in the local Z of the sensitive sites, then the 1.5 variation reported for 250-kv x-rays[12] implies that a comparable variation in the sensitivity to alpha particles should have been observed in this work. This was not observed. Lastly, for cells similar to those used for Figs. 4 and 5[13] as well as for cells starved 48 hours,[6] the RBE of 50-kv to 220-kv x-rays was found to be 1.0. Hence, either the alpha-particle and x-ray sites are different, or the observed variation in RBE is not attributable mainly to variations in local composition with starvation.

In terms of target theory, changes in inherent sensitivity result from changes in target size. The relative constancy of the alpha-particle radiosensitivity requires that the product of the number of sites, m, and the average cross section per site, $\overline{A}$, is roughly constant. Further, for x-rays, it can be shown that the radiosensitivity is proportional to m $\overline{V}$ where $\overline{V}$ is the average volume of a site. Hence, $\overline{V}/\overline{A}$, which varies inversely with RBE, must decrease by about 3.0 with starvation. From experiments with diploid yeast, m appears to be between 20 and 64.[7] When we use m = 30, as reported by Wood,[12] the only reasonable target shape that fits the present data is a disc with a diameter of 2600 Å and a thickness varying from 5.4 to 1.8 Å with starvation.

While the preceding dimensions are only approximate, they do illustrate that for the target theory to explain the data a target of unlikely proportions is required. Figure 8 shows, however, that for fresh interdivisional cells alpha particles have an RBE <1, which is a qualitative requirement of the target theory. That this requirement did not previously appear to be fulfilled was considered a reason for invoking the diffusion model.[6,7] That is, with increasing REL, the diffusion model can predict RBE's >1 while the target theory requires RBE's <1. To explain these seemingly opposing requirements requires a theory more general than either the target or diffusion-model theory.

It is probably possible to construct a more general theory within the framework of the diffusion model because of the adjustable parameters available. For instance, the results of this work can be qualitatively

explained by assuming that one type of migrating intermediate is produced by particles of high REL, that another type is produced by particles of low REL, and that the production of the latter is a function of the metabolic state. A reasonable argument can also be developed in terms of diffusion lengths. It is, of course, possible that the sites of alpha-particle and x-ray damage are wholly or partially different and that the latter alone are influenced by starvation. Another possibility is that these results reflect the relative frequencies of dominant and recessive lethal damage known to occur in x-rayed yeast,[14, 15] and the effect of metabolic state upon the relative likelihood of their occurrence.

It is clear from this work that the radiosensitivity of haploid Saccharomyces cerevisiae is not an invariant property of a cell, but can be varied in two ways. Firstly, for interdivisional cells the radiosensitivity and RBE were shown to be strongly dependent on the metabolic state. And secondly, in relatively fresh cultures, it was shown that cells in the process of budding not only are much more radioresistant than interdivisional cells, but are also relatively more sensitive to alpha particles.

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