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Influence of Linear Energy Transfer on the Radioresistance of Budding Haploid Yeast Cells.
Radiation Res. Vol. 51 pp 310 (1972)

X-ray survival curves of haploid Saccharomyces cerevisiae consist of two components, a simple exponential portion at low doses followed by a multihit more radioresistant tail at high doses (1-3). It was established by Beam et al. that the radioresistant component could be correlated to the cells with small buds (4, 5). Studies by several investigators have shown that yeast cells with small buds are undergoing DNA synthesis (6). Consequently, these findings are in general agreement with recent observations on the age response of mammalian cells in culture--that the period of DNA synthesis is the most X-ray-resistant portion of the cell cycle (7).

The effects of different types of ionizing radiations on the survival of haploid yeast have been studied with the radiosensitive component (8). In these studies the cultures have been starved to minimize the resistant component. Elkind and Beam (5), however, have studied the effect of starvation time on the sensitivity of both components of the population, using ^{210}Po α particles and X rays.

They showed that although the budding cells were also more resistant than nonbudding ones to killing by α particles, the magnitude of the difference was smaller than with X rays. They further showed that the budding cells become more resistant to X rays after starvation in phosphate-buffered dextrose solution. The resistance to α particles, however, was not significantly affected by starvation.

In view of the importance of physiological determinants of cellular radiosensitivity we have investigated these effects over a greater range of LET_∞. In the experiments reported here the effect of LET_∞ on the survival curves of growing haploid yeast cells was studied with the use of radiations whose LET_∞ values ranged from 40 to 17000 MeV $g^{-1}cm^2$.

MATERIALS AND METHODS

The haploid strain SC-7 has been used in a number of investigations (4, 5, 9-11). It has been shown that when the cells are grown in a nitrogen-free starvation medium the budding cell population falls to about 1% (4). By growing the cells in YEPD medium until they reach a stationary phase, the percentage of small buds was found to be about 10%. The method used by Elkind and Beam to obtain fresh cells has been found to yield about 30% cells with small buds, the maximum obtainable without recourse to division synchronization (5). Therefore in the experiments reported here, the technique of Elkind and Beam was followed. To describe the method briefly, cells from a subculture stored at 2° C were used as inocula for growth in liquid YEPD medium. After about 10 hours of growth at 30° C the cells were harvested and the concentration was adjusted to 2.5×10^7/ml. The cell concentration and the percentages of buds and single cells were determined from hemacytometer counts. Samples for exposure were prepared by pipetting 20 µl of this cell suspension onto 9-mm-diameter buffered agar disks.

The X-ray exposures were carried out with a 150-kV 15-mA machine at a dose rate of 6 krads/min. The irradiation with ^{7}Li, ^{11}B, ^{12}C, and ^{40}A ions was done at the heavy-ion linear accelerator (Hilac). The energy of the ions covered an LET_{∞} range of 420 to 17 000 MeV $g^{-1}cm^{2}$. The delta-ray contribution was approximately constant for all the above ions. In order to see the effect of different δ-ray contributions on the sensitivity of budding cells, the ^{7}Li ions were degraded in energy by means of aluminum foils to correspond to LET_{∞} values of 1200 and 1550 MeV $g^{-1}cm^{2}$, and the ^{11}B ions to 2080 MeV $g^{-1}cm^{2}$. Doses for all the heavy ions were measured with a very thin ionization chamber positioned a few millimeters ahead of the sample. Details of the experimental arrangements for exposing the samples at the Hilac have been described elsewhere (12).

After exposure the cells were resuspended in 2 ml of sterile water. Suitable dilutions were made and approximately 200 surviving cells were plated onto each of a set of four YEPD plates. The plates were incubated at 30°C along with a set of controls. After 2 days the colonies were counted, and the plates were examined for any additional colonies after 5 to 6 days.

RESULTS AND DISCUSSION

A typical X-ray survival curve for haploid yeast cells taken from a growing culture is shown in Fig. 1. The curve has been resolved into two components by extrapolating the resistant-budding-cell component to zero dose and subtracting it from the total curve. The resulting exponential curve approximates the survival curve for the sensitive, nonbudding cells. Owing to the great errors inherent in small differences, we have used only the low-dose points, which fit an exponential survival curve. The LD_{50} values for growing nonbudding cells and for budding cells are less than the previous results by about a factor of two (4, 5). This change in sensitivity could be due to the differe-ences in techniques used and to possible changes in the culture.

Survival curves obtained with a variety of accelerated charged particles have been resolved into sensitive and resistant components in the same manner. These curves, normalized to 100% survival for zero dose, are shown in Fig. 2.

The growth condition prior to exposure were selected to yield the maximum percentage of budding cells. Consequently the data do not permit precise analysis of the initial exponential part of the survival curve. With these reservations, however, the results are of interest because previous studies of the radiosensitivity of nonbudding haploid yeast as a function of LET_∞ have all used starved cultures (11, 13). Different LET_∞ dependences for starved and for nonstarved cells are expected from the results of Elkind and Beam (5).

The normalized survival curves for budding yeast cells (Fig. 2B) exhibit no significant change of shape over the range of LET_∞ values studied. The curves can be superimposed by applying appropriate dose-reduction factors. It is therefore valid and expedient to represent the results by a sensitivity measure calculated as the reciprocal of the dose that yields 50% survival ($1/LD_{50}$).

Reciprocals of the LD_{50} for budding and nonbudding cells are plotted against LET_∞ in Fig. 3. The values obtained by Sayeg *et al*. (11) for starved cells are also plotted, for comparison.

The relationship shown for the nonbudding cells from the growing culture is surprising, for there appears to be little, if any, significant increase of radiosensitivity with increased LET_∞. All previously examined radiation effects in both haploid and diploid yeast have shown an increase in sensitivity with increasing LET_∞, with a maximum sensitivity in the neighborhood of 1000 MeV $g^{-1}cm^2$. The effects studied have included haploid and diploid viability, (11, 13, 14) back mutation, suppressor mutations, and mitotic crossing-over and gene conversion (15). All these studies, however, have used non-growing cells.

By contrast, the relationship for the budding cells shows a large increase in sensitivity with increasing LET_∞, with a maximum sensitivity more than 4 times the X-ray sensitivity. At even greater LET_∞ the sensitivity declines only slowly. In this respect the LET_∞ dependence closely parallels the relationship obtained for induction of diploid lethality.

The ratios of the LD_{50} for budding cells to the LD_{50} of the non-budding cells are plotted as a function of LET_∞ in the Fig. 4. Owing to the differences in shape of the survival curves for the two components of the population, the absolute values of this ratio depend on the survival level chosen for comparison. Only the relative values are significant. This ratio, which is a measure of the variation of the radiosensitivity throughout the mitotic cell cycle, shows a strong negative LET_∞ dependence between approximately 50 and 2000 MeV $g^{-1}cm^2$. At higher values of LET_∞ the ratio is constant at a value of approximately 15% of the value for X rays.

The results on the radiation responses of growing haploid yeast cells are in agreement with the findings by Elkind and Beam. They further establish the LET_∞ dependence of the age response of radiation-induced lethality in haploid yeast. The cell-age dependence demonstrated by these results is remarkably similar to that which has been described for Chinese hamster cells in tissue culture with respect to the nature of both the age dependence and the LET_{∞} dependence. The highly resistant stage of the cell cycle, in both cases, is the period of DNA synthesis. Recent studies by Bird and Burki (16) show that the variation in sensitivity in cell cycle of Chinese hamster cells is small and essentially constant for LET_{∞} greater than 2000 MeV $g^{-1}cm^2$. The magnitude of the variation is strongly LET_{∞} dependent at values between 50 and 2000 MeV $g^{-1}cm^2$.

The preliminary results for budding-yeast cells exposed to degraded beams of lithium and boron indicate the following. Degradation of lithium to an energy of 50 MeV to yield an LET_{∞} value of 1200 MeV $g^{-1}cm^2$ does not lead to sensitivity different from that expected for a full-energy beam of the same LET_{∞}. However, the degradation of lithium to an energy of 35 MeV to yield an LET_{∞} value of 1550 MeV $g^{-1}cm^2$ results in a slightly higher radiation sensitivity. In contrast, a significant decrease in the effectiveness of the beam was observed when the Boròn was degraded to an energy of 54 MeV, corresponding to an LET_{∞} value of 2080 MeV $g^{-1}cm^2$ (which corresponds approximately to that of the full-energy ^{12}C ion). These results indicate that the qualitative effect of degraded beams due to changes in δ-ray energies depends on whether the LET_{∞} of the degraded beam is less than or greater than the most effective LET_{∞} (≈ 1300 MeV $g^{-1}cm^2$).

Whenever the sensitivity of a system increases with increase in LET_{∞} the cooperative effect of the energy deposition in the track core and that contributed by δ rays is not known. However, when the LET_{∞}

of the radiation is within the LET at which peak efficiency occurs, it is known that the higher the local energy density, the greater the efficiency of inactivation. On this basis, degradation of ^{7}Li ions (with consequent increase in the energy density along its track and surrounding it) is expected to increase the sensitivity relative to that of the full-energy beam corresponding to the same LET_∞. This in fact was observed for ^{7}Li ions of LET_∞ value of 1550 MeV $g^{-1}cm^2$. Degradation to yield 1200 MeV $g^{-1}cm^2$ did not show any effect due to change in the δ-ray contribution, which implies that a significant reduction in the velocity of the particle is necessary to alter the spatial distribution of energy around a heavy-particle track (17).

In contrast, when the LET_∞ of the radiation was beyond the LET_∞ corresponding to the peak sensitivity, the sensitivity was found to decrease with increase in LET_∞. This implies that even with the spatial distribution associated with the full-energy beam, energy is wasted with increase in LET_∞. Therefore with degradation of ^{11}B ion, which results in a larger energy density, more energy is wasted for the same total LET_∞ and hence the sensitivity falls. It appears that on this side of the sensitivity-versus-LET_∞ curve, no great degradation is necessary for the δ-ray effect to become apparent. More work in this area would help one to understand the effect of δ rays.

In conclusion, the maximum radiosensitivity for budding cells occurs at approximately the same LET_∞ (1300 to 1500 MeV $g^{-1}cm^2$) as that for nonbudding cells. This is the same value that has been reported for starved cells. We interpret these results to define a component of radiosensitivity in haploid yeast that is either repaired, or does not occur, in cells undergoing DNA synthesis. This component is greatly reduced at the most effective LET_∞ (1300 to 1500 MeV $g^{-1}cm^2$), indicating that at this higher LET_∞ most of the damage is not repairable by this mechanism.

SUMMARY

The radioresistant component of haploid yeast cells (SC-7) was established by previous investigators to be due to budding cells, and cells with small buds are known to be undergoing DNA synthesis.

The response of budding cells of haploid strain SC-7 (saccharomyces cerevisiae) as a function of LET was studied in this investigation by use of heavy ions (in the LET_∞ range 40 to 17000 MeV $g^{-1}cm^2$) from the Berkeley heavy-ion linear accelerator.

The maximum sensitivity for budding cells occurs at approximately the same LET_∞ (1300 to 1500 MeV $g^{-1}cm^2$) as that for nonbudding cells. The radiosensitivity as a function of LET, of budding haploid yeasts relative to nonbudding cells, is found to be remarkably similar to that of S-phase Chinese hamster cells in culture relative to cells not undergoing DNA synthesis.

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KEY WORDS:
Saccharomyces cerevisiae
Haploid yeasts
LET
Age response

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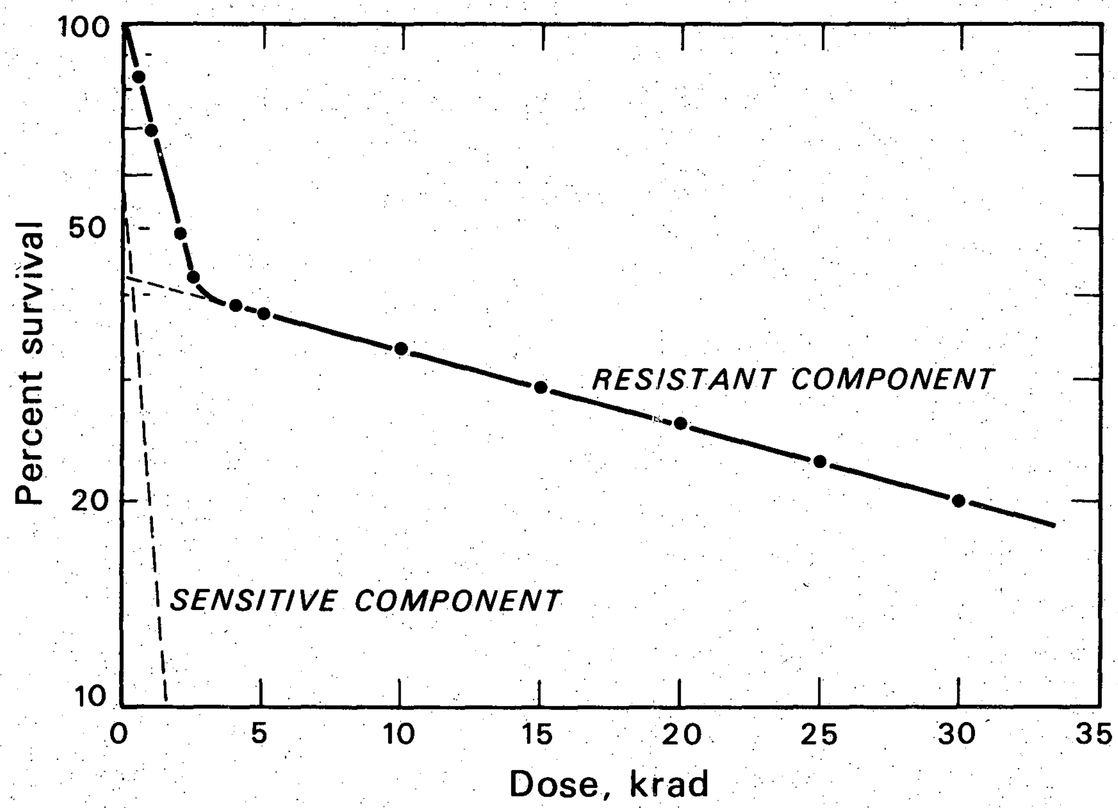
FIGURE CAPTIONS

Fig. 1. X-ray survival curve for haploid yeast cells (Sc-7).

Fig. 2. Survival curves. a) nonbudding cells, b) budding cells.

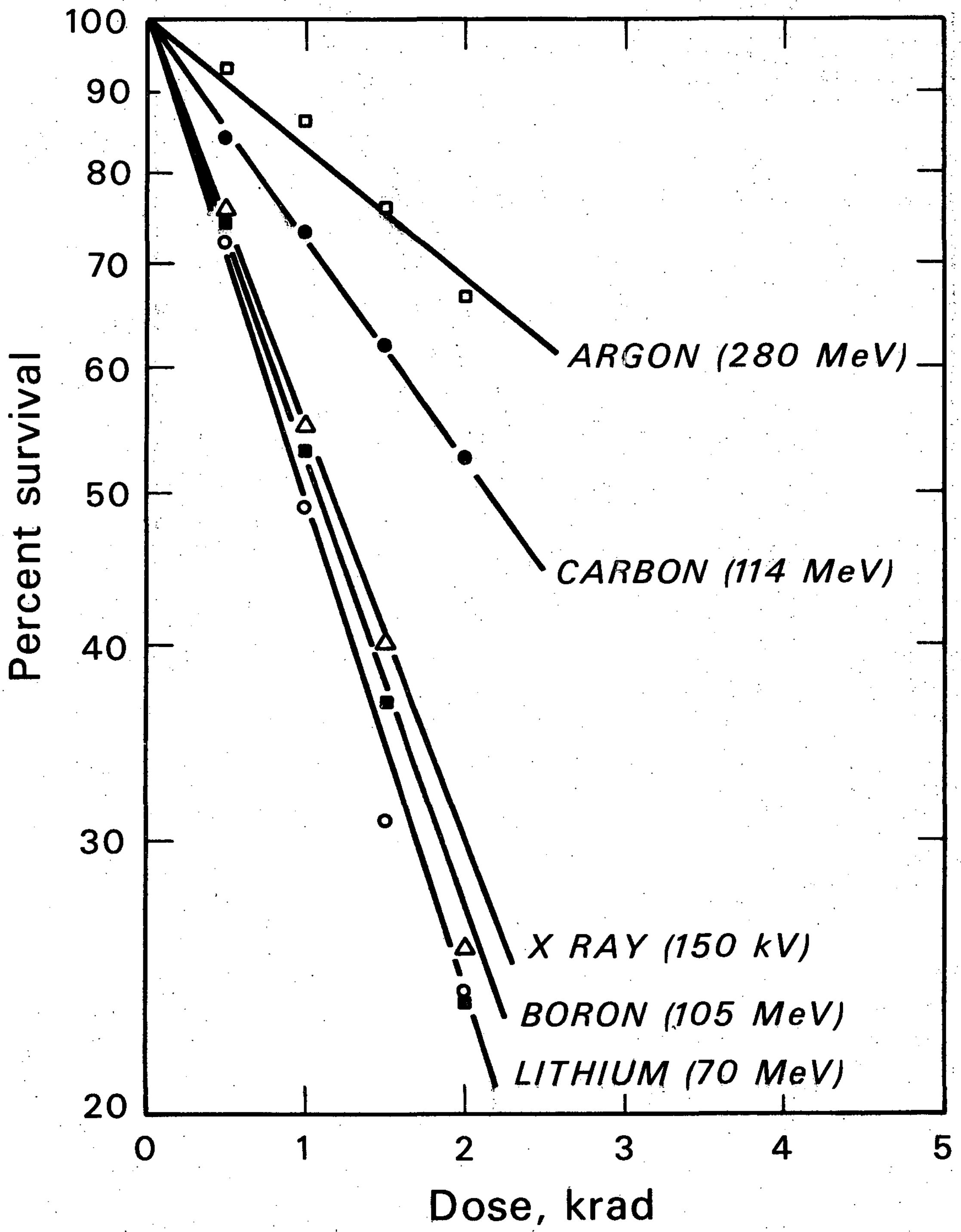
Fig. 3. The radiation sensitivity for inhibition of colony formation for growing haploid yeast cells (Sc-7) as a function of LET_∞.

Fig. 4. LD_{50} ratios of resistant to sensitive populations plotted as a function of LET_∞.



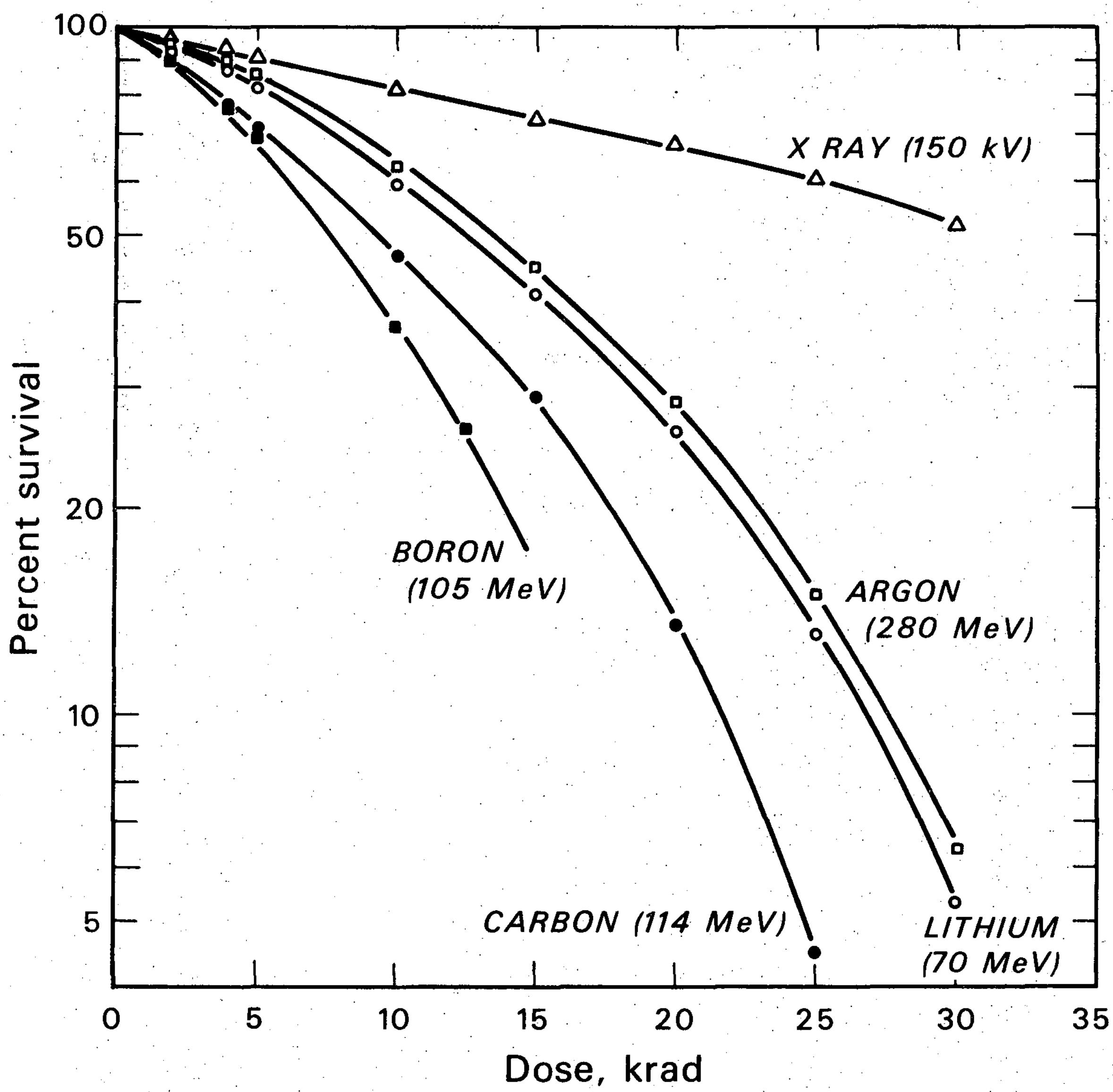
DBL 717-5929

Fig. 1



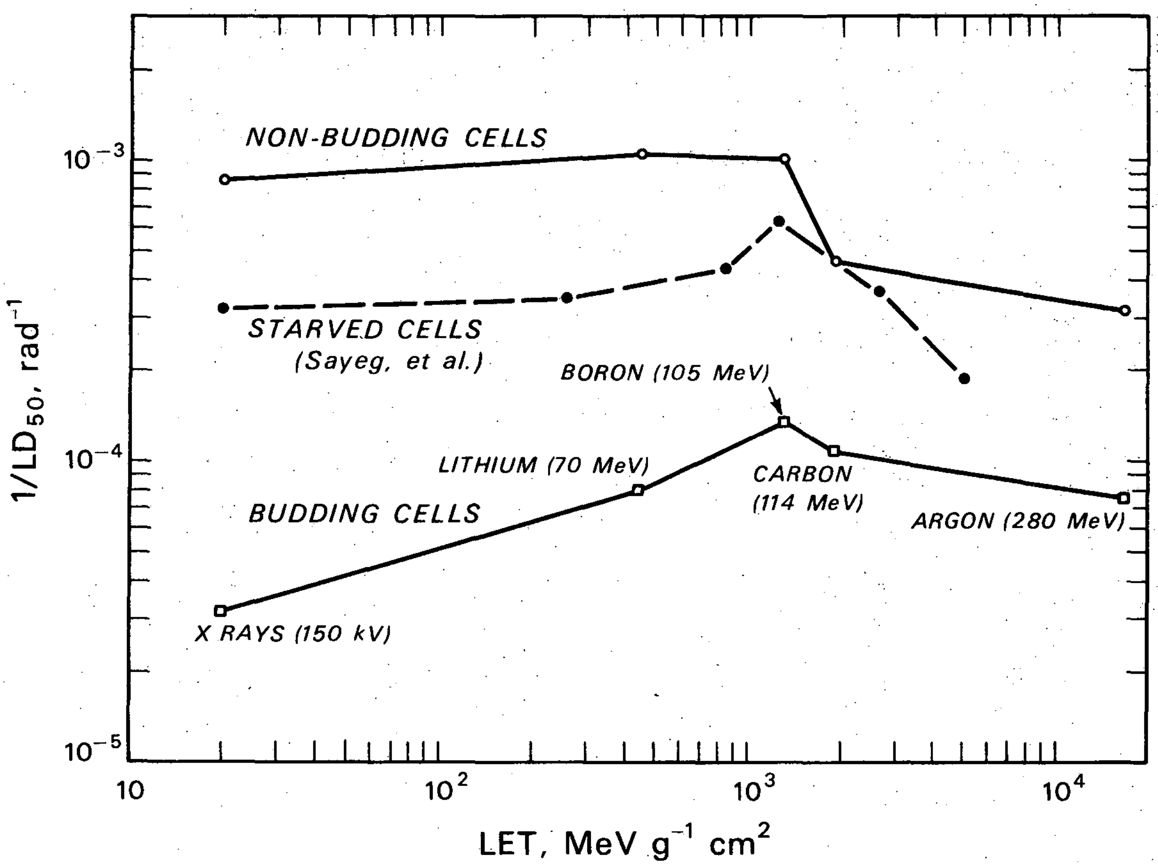
DBL 717-5925

Fig. 2a



DBL 717-5930 A

Fig. 2b



DBL 717-5928 A

Fig. 3

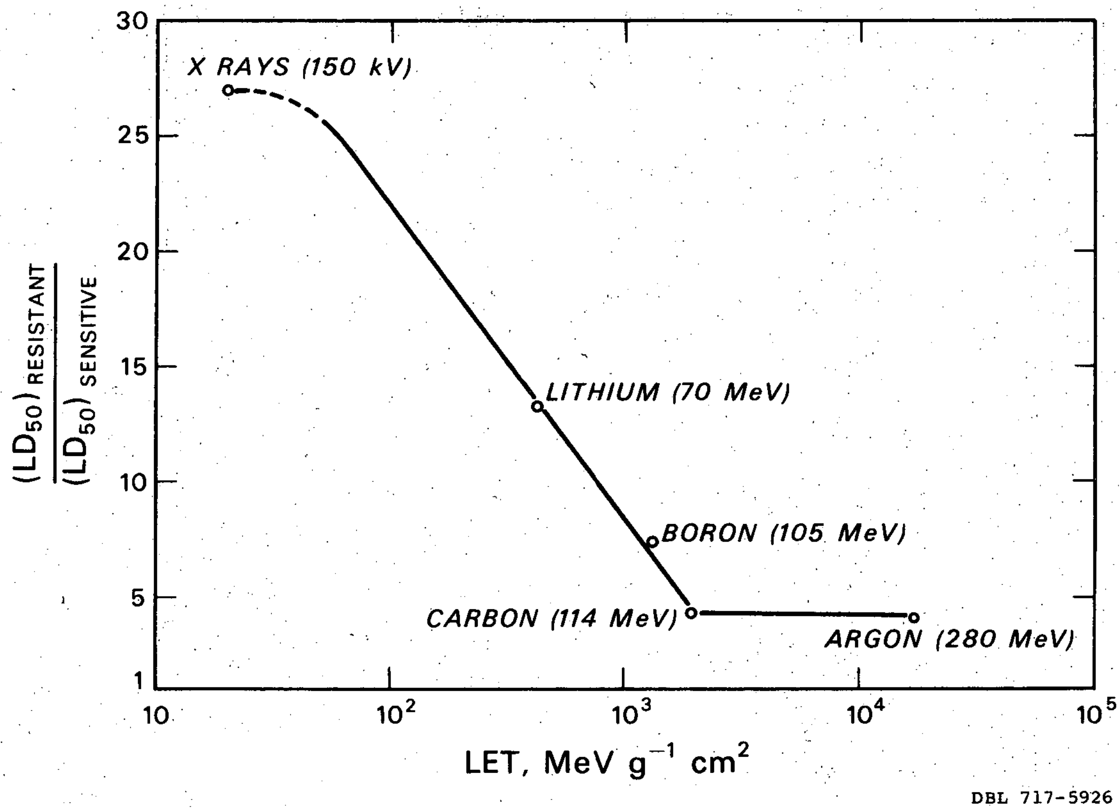

30
25
20
15
10
5
1
X RAYS (150 kV)
LITHIUM (70 MeV)
BORON (105 MeV)
CARBON (114 MeV)
ARGON (280 MeV)
(LD50) RESISTANT / (LD50) SENSITIVE
10
10^2
10^3
10^4
10^5
LET, MeV g^-1 cm^2
DBL 717-5926

Fig. 4

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