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John T. Lyman

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ABSTRACT

The results of heavy-ion irradiation of mammalian cells cultured in vitro are summarized. The radiations that most effectively inhibit colony-forming ability and produce chromosome aberrations are those that have rates of energy loss between 1000 and 2000 MeV-cm^2/g. The effect of modifiers such as oxygen, dose fractionation, chemical protection (cysteamine), and chemical sensitization (5-iododeoxyuridine), which affect the radiosensitivity of the cells exposed to lightly ionizing radiations, fail to modify the response, or modify it less when the cells are exposed to densely ionizing radiations.

The dose-effect curves for mammalian cells exposed to radiations of differing rates of energy loss are not all necessarily the same shape; therefore the RBE values derived from the curves are generally a function of the level of damage being considered.

Estimates of RBE values at low levels of radiation exposure are given for cells that receive acute exposures of densely ionizing radiation. Exposures at low dose rates may result in RBE values 3 to 4 times the values given.

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INTRODUCTION

The radiation environment encountered in the vicinity of a modern particle accelerator is a very complex mixed radiation field. Generally, it is the fast neutron component of this field that makes the greatest contribution to the total rem dose.[1] Significant contributions also come from slow and thermal neutrons, high-energy particles, and γ rays. The relative proportions of these radiations found at a particular site depend upon the type of accelerator, the location of the site with respect to the accelerator, and how adequately this area is shielded from the primary and secondary radiations. Consequently, the hazards associated with different accelerators will most likely be different, because of differences in the radiation fields. It has been known for many years that equal doses of different radiations do not always produce the same effect. It is therefore of value to attempt to evaluate the hazards associated with the various components of these radiation fields. Some of the differences in effects produced by different radiations may be understood in terms of the different rates at which ionizing particles lose energy along their tracks. It is therefore convenient to evaluate the radiosensitivity of various biological systems to radiations of varying rates of energy loss or linear energy transfer (LET). (LET values are for the LET_{∞}, and this is taken as equal to the total particle-energy loss or stopping power. The results of such studies form the basis for constructing models to predict a biologic response to exposure in a complex radiation field, such as is encountered around a high-energy particle accelerator.

Fortunately, one of the high-energy accelerators is an excellent source for producing radiations over a wide range of LET values. Shown in Fig. 1 is the stopping power of water for various heavy ions as a function of the energy per atomic mass unit (amu). At any given energy, the stopping power or the LET of the various ions is a function of the square of the effective charge carried by the ion. The heavy-ion linear accelerator (Hilac)[2] is capable of accelerating these ions to an energy of 10.4 MeV/amu. Therefore it is possible to irradiate biological samples with heavy charged particles that have LET values from 40 MeV-cm^2/g to more than 20 000 MeV-cm^2/g. If the sample is thin compared with the range of the heavy ions, then the mean LET in the sample is easily determined, since these heavy-ion beams are nearly parallel, are monoenergetic, and lose only a small fraction of their total kinetic energy when passing through the sample. Furthermore, if the irradiations with the different heavy ions are done at the same energy per amu, and therefore the same velocity, then the fractions of energy transferred to electrons which are capable of further ionizations (delta rays) are the same for the different heavy-ion beams and the energy spectra of these delta rays will also be the same. This facilitates making calculations designed to correct for the effect of the δ rays.

Results with Mammalian Cells

Techniques for culturing mammalian cells in vitro and assaying for reproductive capacity[3] in the same manner as is routinely done for microorganisms have been perfected only within the last ten years. During this time tissue culture cells have been studied under varying physical, chemical, and physiological conditions. These studies have indicated that the physicochemical basis of radiation sensitivity in mammalian cells is not unlike that of microorganisms when loss of colony-forming ability is used as the end point.

Several types of mammalian cells, grown in tissue culture, have been exposed to heavy-ion beams accelerated by the Hilac.[4-6] The cells used have been Chinese hamster cells, human cancer cells, and human kidney cells. The results obtained for survival of the colony-forming ability when these cells are irradiated in a monolayer by the various heavy-ion beams are very similar. The results obtained with the human kidney cells (T1) are used here to demonstrate the radiosensitivity of cultured mammalian cells to ionizing radiations and also the modification of the response by various chemical and physical conditions. Figure 2 shows the survival curves for the T1 cells when exposed to highly filtered 50-kV x rays. The cells have been irradiated under both aerobic and anoxic conditions. Below the survival curves is a growth curve for the unirradiated cells. This curve, which shows the average number of cells per colony after different incubation intervals, is used to evaluate the condition of the cells and the growth media. The amount of protection afforded the cells by the removal of oxygen from the irradiation atmosphere is illustrated by the additional radiation necessary for the same inhibition of the colony-forming ability. The radiation doses must be about 2.7 times as great in the absence of oxygen.

Another important aspect of the survival curves is that at low doses, the curves have an initial negative slope, as is shown in Fig. 3. It has been suggested[6-12] that this may indicate that a portion of the lethal action of this radiation is due to an exponential killing. If this is an irreversible lethal inactivation, then at low doses, where this exponential killing predominates, only the initial slopes of the survival curves will be significant.

Survival curves of the T1 cells exposed to various heavy-ion beams are shown in Fig. 4. Again, cells were exposed under aerobic and anoxic conditions. Aerobic x-ray survival curves were also obtained as a control on the radiosensitivity of the cells; also growth curves are given for unirradiated cells for the different experiments. The energy of the heavy ions passing through the cells was approximately 6.58 MeV/amu, and the average effective charge of each heavy-ion beam is shown within the parenthesis.

The main features of the survival curves are that at low LET (i.e., with the lighter ions) the curves are of a multi-hit type, and again they have an initial negative slope. As the LET is increased, the radiosensitivity of the cells increases and the exponential component begins to dominate the compound curve. When the LET is in the range of 1000 to 2000 MeV-cm^2/g (i.e., boron and carbon irradiations) the curves are essentially exponential and the radiosensitivity reaches a maximum. Further increases in LET

show a lessening of the radiosensitivity. In this region it appears that one densely ionizing particle passing through the nucleus of the cell deposits more than enough energy to interfere with the reproductive capacity of the cell.

Another feature of these experiments is that when the lightest ion is used, the anoxic protection due to irradiation in a nitrogen atmosphere is the same as is observed with x irradiation. As the LET is increased the anoxic protection is reduced, until it is apparently no longer present when radiations with LET values about 3000 MeV-cm^2/g or more are used.

Since the survival curves change shape as the LET is increased, it is not proper to assign a single RBE value to a particular LET value without specifying the survival level where the comparison is made. This is illustrated in Fig. 5. The end points considered for these curves were 80, 50, 10, and 1% survival. The RBE values derived from the doses related to the 80 and 50% survival levels are very similar because the x-ray and low-LET survival curves have the initial negative stage previously mentioned. Therefore at high survival levels (i.e., the low-dose region), where this exponential term dominates, the RBE is independent of the survival level.

The shoulder of the low-LET survival curves has generally been interpreted to be due to the accumulation of sublethal injury. It has been shown that this kind of damage is not inherited, but is rapidly repaired,[9] as indicated by the higher survival obtained when the radiation is given in two fractions separated by several hours, when compared with the survival following single dose equal to the two fractions. When there is an exponential survival curve, it is not expected that there will be accumulation of this type of sublethal damage and therefore there should be no increase in survival if the radiation dose is fractionated. It has been demonstrated that this is indeed the case for carbon ions when the two fractions are separated in time by as much as 8 hours.[13]

Chemical agents such as cysteamine have been used to decrease the radiosensitivity of T1 cells exposed to 200-kVp x rays. But this chemical protection works only slightly, if at all, when the cells are irradiated with α particles[14] having a dE/dx $\approx$ 1700 MeV-cm^2/g, as is shown in Fig. 6.

Chemical sensitization of the cells has been accomplished by the incorporation of thymidine analogs,[15] such as 5-iododeoxyuridine (IUdR) into the DNA of the cultured cells. Figure 7 shows the survival curves following 50-kVp x rays and helium and carbon ions.[16] For low and intermediate LET values there is sensitization, but not for fast carbon ions (dE/dx = 2200 MeV-cm^2/g).

Chromosome aberrations induced by ionizing radiations have been reported for Chinese hamster cells.[17] The induction of chromatid exchanges by various ionizing radiations is shown in Fig. 8. When the cells are irradiated with x rays or low-LET heavy ions, the number of chromatid exchanges appears to be produced by a two-hit process. For cells irradiated by the more densely ionizing particles, the production of chromatid exchanges increases linearly with the dose. Shown in Fig. 9 are the dose-

effect curves for the incidence of abnormal metaphases. These curves differ from those for the chromatid exchanges, since the proportion of cells with abnormal metaphase must not exceed 100%. Since the curves for the chromosome aberrations change shape as the LET is increased, it is again necessary to express the RBE as a function of the level of damage. This is shown in Fig. 10. Estimates of RBE values that might be of significance in low-dose exposures can be obtained by extrapolation to low levels of damage. The risk involved in such an extrapolation depends upon the validity of the function chosen to represent the experimental data. It is certainly possible that in the low-dose region the chromosome aberrations might increase linearly with the dose, in which case the RBE values at low dose would be independent of the level of damage, analogous to the survival curves at low dose.

DISCUSSION

Recovery of the cells from the sublethal damage caused by low-LET radiations stresses the distinction between acute and chronic (or fractionated) exposures. There is ample evidence that in mammalian systems, acute low-LET irradiations are more effective than chronic. For instance, chronic gamma irradiation is only one-fourth as effective as is acute irradiation for life shortening in mice[18] and for producing chromosome aberrations in mouse liver cells.[19] With high-LET radiations, mammalian cells in vitro show no recovery between fractionated doses, which for these cells equates the effects of acute and chronic exposures. This has also been demonstrated in mice following fast-neutron irradiation, for which it has been shown that chronic irradiation is just as effective for producing life shortening and chromosome aberrations as acute irradiation.[18, 20] This means, when one is concerned with low exposure rates, that RBE values may be three to four times as great as were shown in Figs. 5 and 10.

The major component of the radiation background encountered in the vicinity of a high-energy particle accelerator consists of the fast neutrons. The biological hazards associated with these particles are evident when one considers the methods by which these particles transfer energy to tissue. The 14.1-MeV neutrons from the reaction lose most of their energy (69.5%) in elastic collisions with hydrogen atoms.[21] The resulting recoil protons, when they come to rest, have rates of energy loss approaching that required for the greatest RBE. The remainder of the energy is lost primarily to carbon, nitrogen, and oxygen atoms, mostly by means of inelastic collisions. Occasionally the energy of the excited nuclei is carried away by α particles that are associated with the highest RBE values.[22, 23]

SUMMARY

The effects of irradiation on mammalian cells in vitro by heavy charged particles have been discussed. Radiation modifiers such as oxygen, dose fractionation, chemical protection, and chemical sensitization, which are effective with low-LET radiations, fail to modify or modify less when used in conjunction with densely ionizing radiations. The change in the shape of the dose-effect curves which accompanies an increase in the LET of the radiation results in RBE values which depend upon the level of injury, being maximum for low levels of injury. The differences between chronic and acute exposures for lightly and densely ionizing particles increases the hazards associated with chronic exposures to densely ionizing particles when compared to chronic x-ray exposures.

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*This work done under the auspices of the U. S. Atomic Energy Commission.

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FIGURE CAPTIONS

Fig. 1. Stopping power of water for various heavy ions as a function of energy.

Fig. 2. Survival curves for T1 cells irradiated by 50-kVp x rays under aerobic (open circles) and anoxic conditions. Growth curves for unirradiated cultures are presented with the data as an evaluation of the cells and medium. From reference 6.

Fig. 3. Survival curve for T1 cells exposed to low doses of 50-kVp x rays. From reference 6.

Fig. 4. Response of the colony-forming ability of T1 cells to irradiation with heavy ions of equal velocity. The ion and its average charge are indicated on each plot. Plotted squares correspond to data obtained under anoxic conditions. Solid points are for x-ray irradiation, and open circles are for heavy-ion irradiations. From reference 6.

Fig. 5. Plots of RBE against - dE/dx for inhibition of colony formation by T1 cells for various levels of survival. From reference 6.

Fig. 6. Effect of 0.025 M cysteamine upon the response of T1 cells to irradiation by 200-kVp x rays and natural α particles. Redrawn from reference 14.

Fig. 7. Effect of IUdR pretreatment upon the response of T1 cells to irradiation by 50-kVp x rays, 26.3-MeV helium ions, and 79.0-MeV carbon ions. Growth curves are given for cells under both conditions. Composite drawing, based on a number of experiments. From reference 16.

Fig. 8. Dose-response curves for the production of chromatid exchanges in $CH2B_2$ cells after exposure to various heavy ions and x rays. From reference 17.

Fig. 9. Dose-response curves for the production of abnormal metaphases in $CH2B_2$ cells after exposure to various heavy ions and x rays. From reference 17.

Fig. 10. RBE values for chromosome aberrations in $CH2B_2$ cells after exposure to various heavy ions and x rays. Points are based upon doses from curves drawn by Skarsgard through his experimental points shown in Figs. 8 and 9.

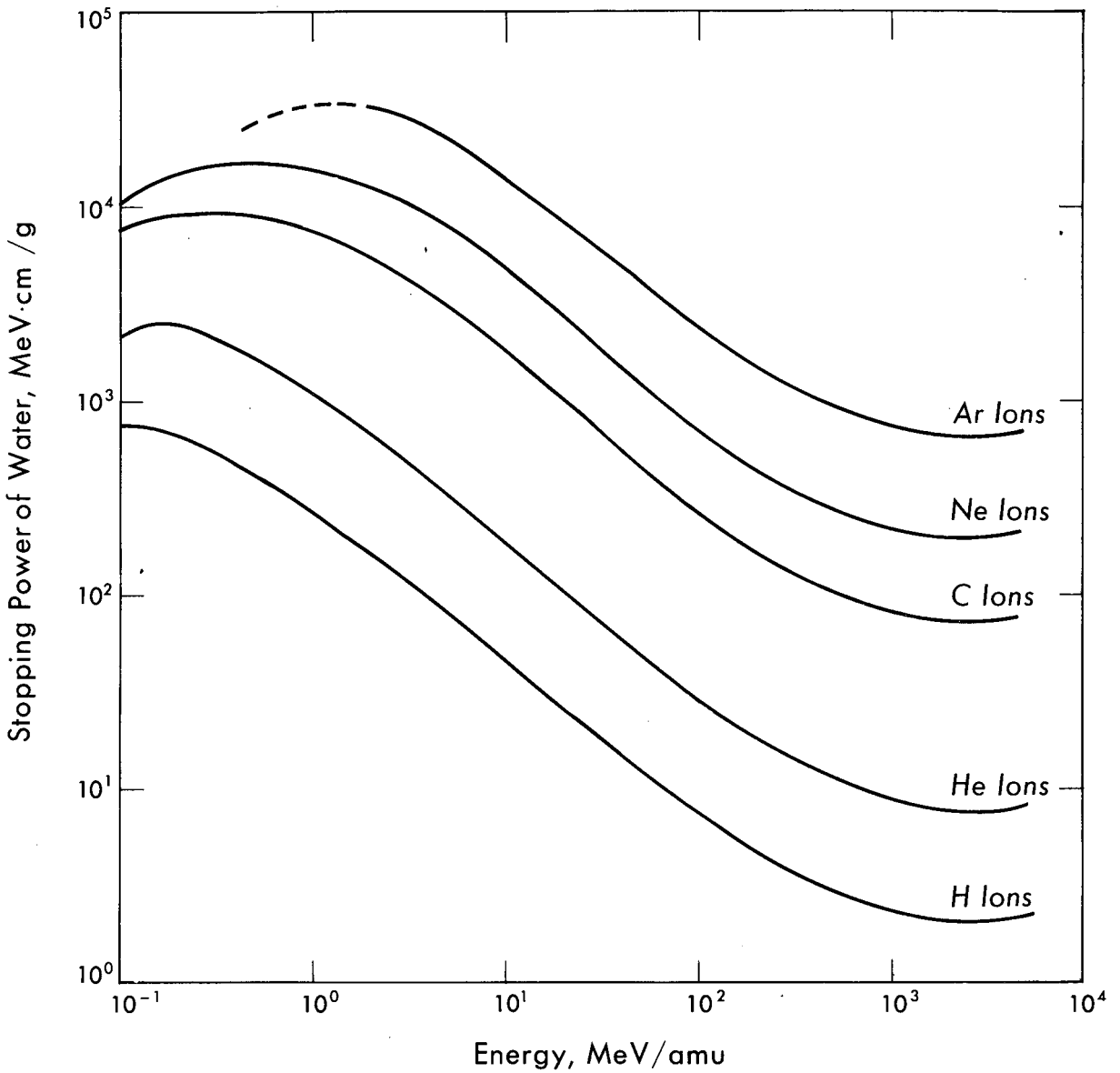
Stopping Power of Water, MeV·cm /g
10^5
10^4
10^3
10^2
10^1
10^0
Ar Ions
Ne Ions
C Ions
He Ions
H Ions
10^{-1}
10^0
10^1
10^2
10^3
10^4
Energy, MeV/amu
MUB-8334

Fig. 1

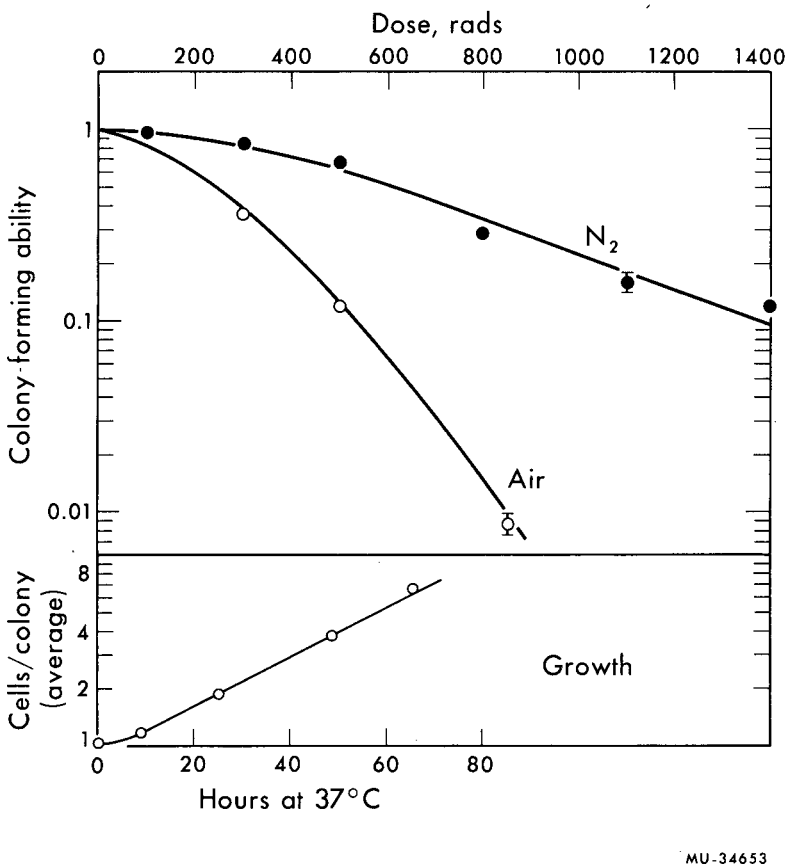
Dose, rads
0
200
400
600
800
1000
1200
1400
1
0.1
0.01
Colony-forming ability
N_2
Air
8
4
2
1
Cells/colony (average)
Growth
0
20
40
60
80
Hours at 37°C
MU-34653

Fig. 2

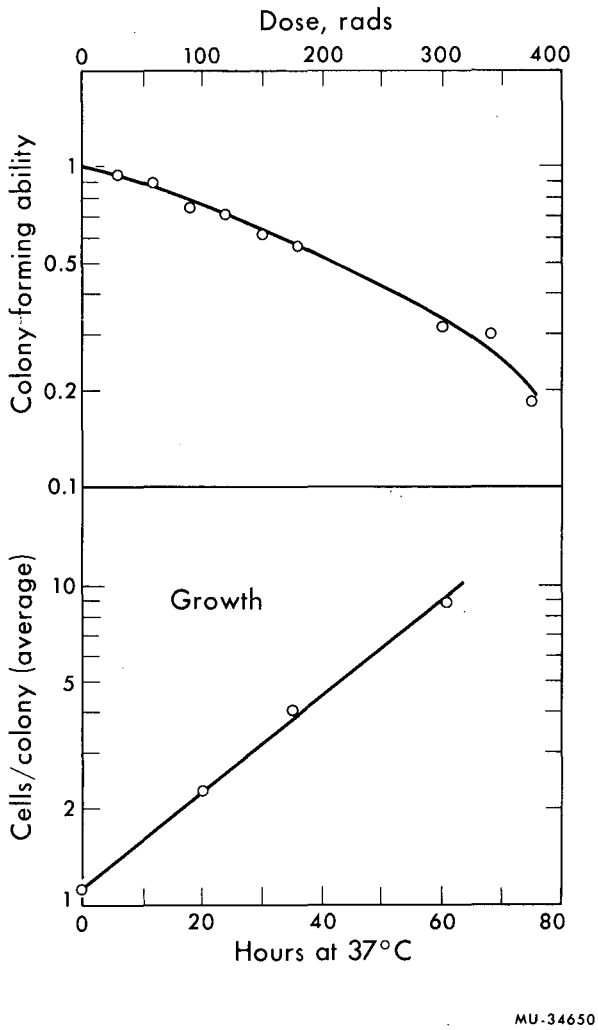
Dose, rads
0
100
200
300
400
Colony-forming ability
1
0.5
0.2
0.1
Growth
Cells/colony (average)
10
5
2
1
0
20
40
60
80
Hours at 37°C
MU-34650

Fig. 3

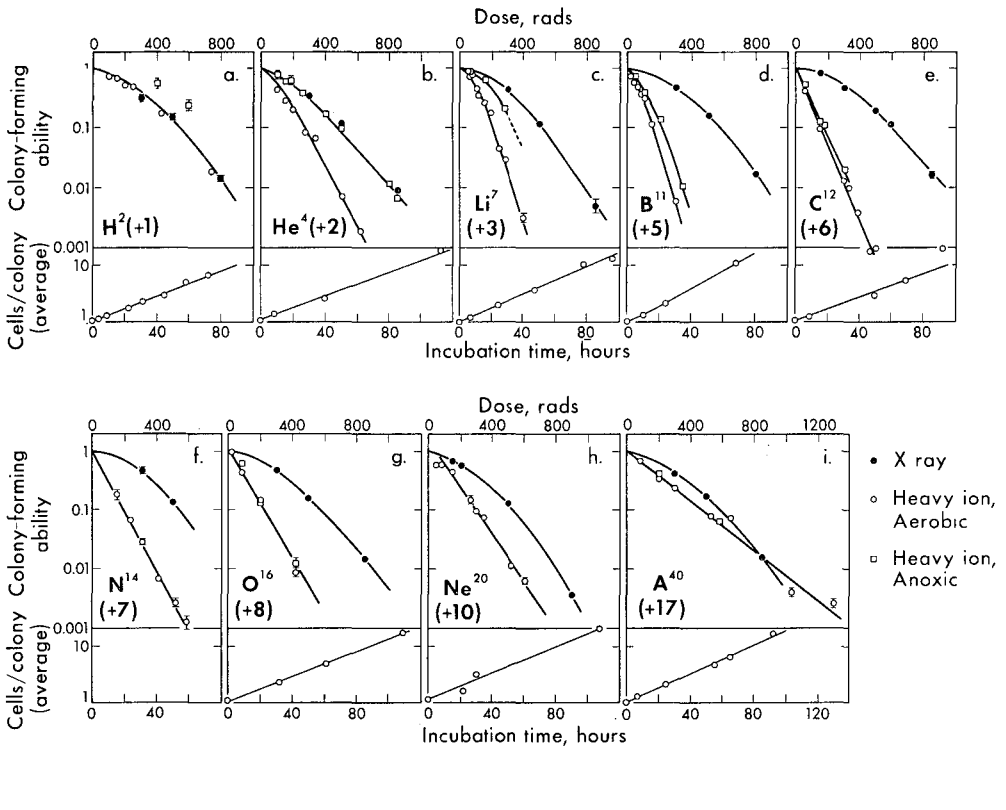
Dose, rads
Colony-forming ability
Cells/colony (average)
Incubation time, hours
a.
b.
c.
d.
e.
f.
g.
h.
i.
H²(+1)
He⁴(+2)
Li⁷ (+3)
B¹¹ (+5)
C¹² (+6)
N¹⁴ (+7)
O¹⁶ (+8)
Ne²⁰ (+10)
A⁴⁰ (+17)
X ray
Heavy ion, Aerobic
Heavy ion, Anoxic
MU-34655

Fig. 4

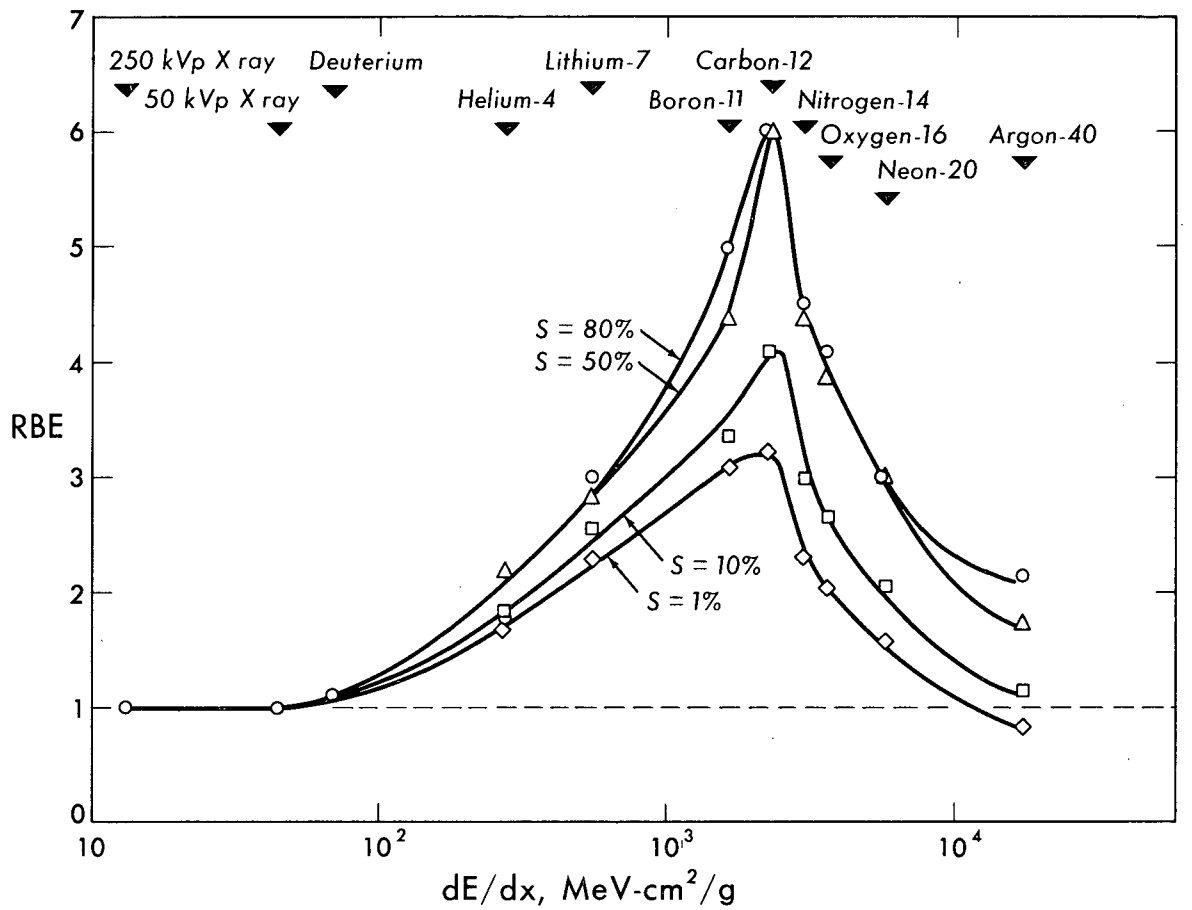
250 kVp X ray
50 kVp X ray
Deuterium
Helium-4
Lithium-7
Boron-11
Carbon-12
Nitrogen-14
Oxygen-16
Neon-20
Argon-40
S = 80%
S = 50%
S = 10%
S = 1%
RBE
dE/dx, MeV-cm²/g
MUB-6729

Fig. 5

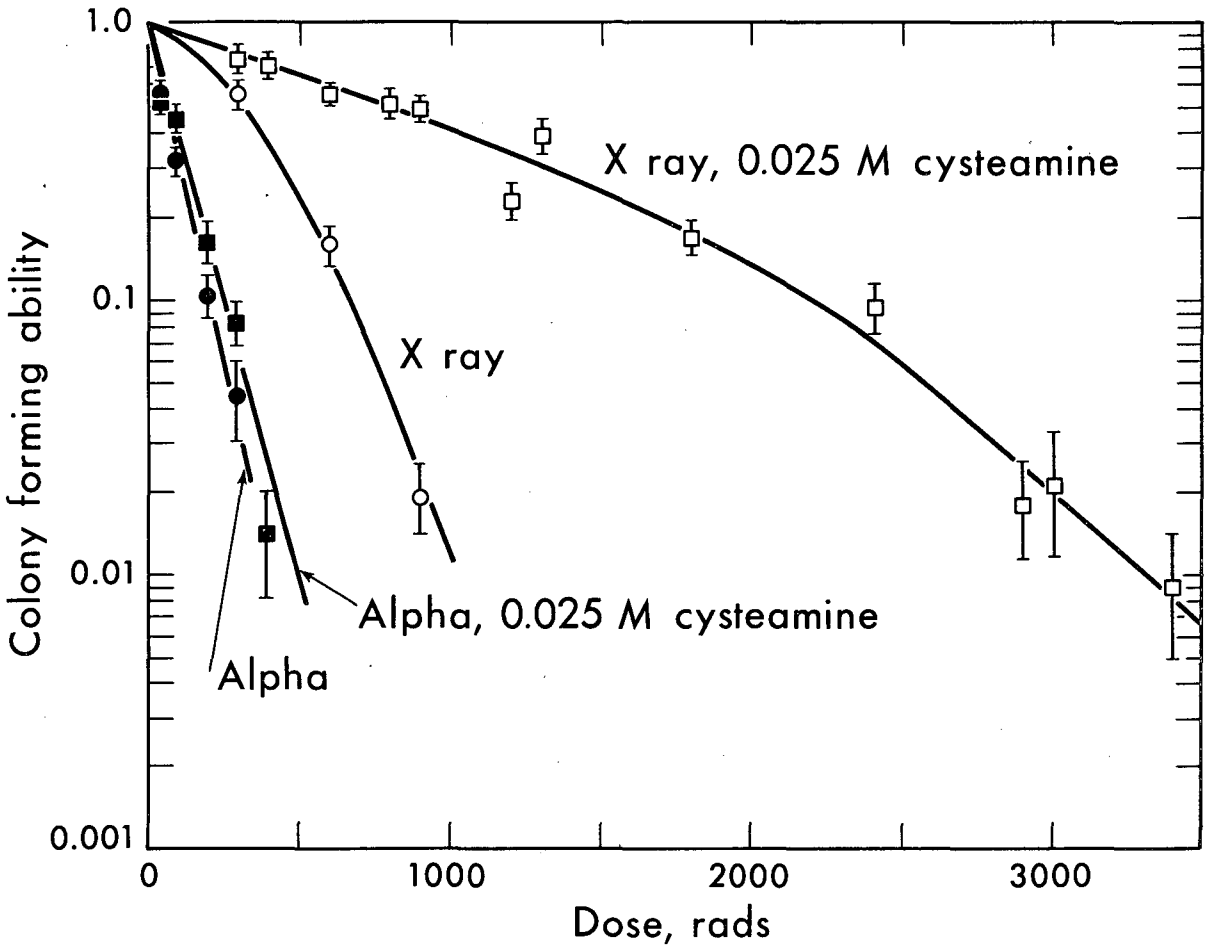

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Fig. 6

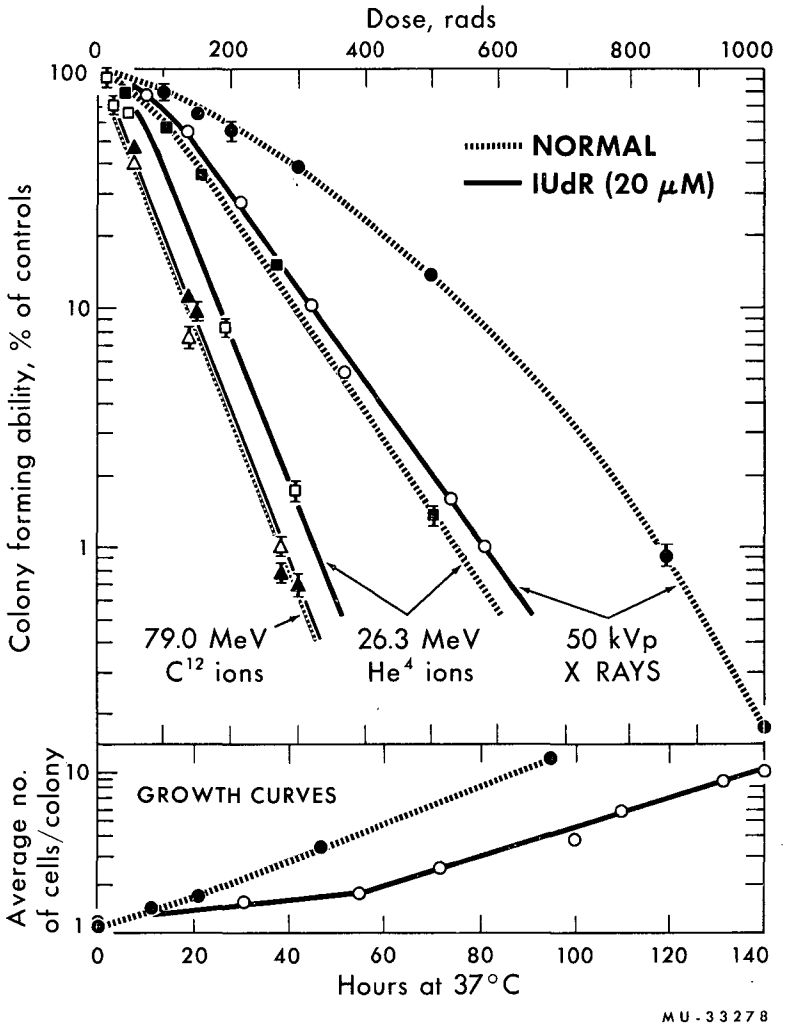
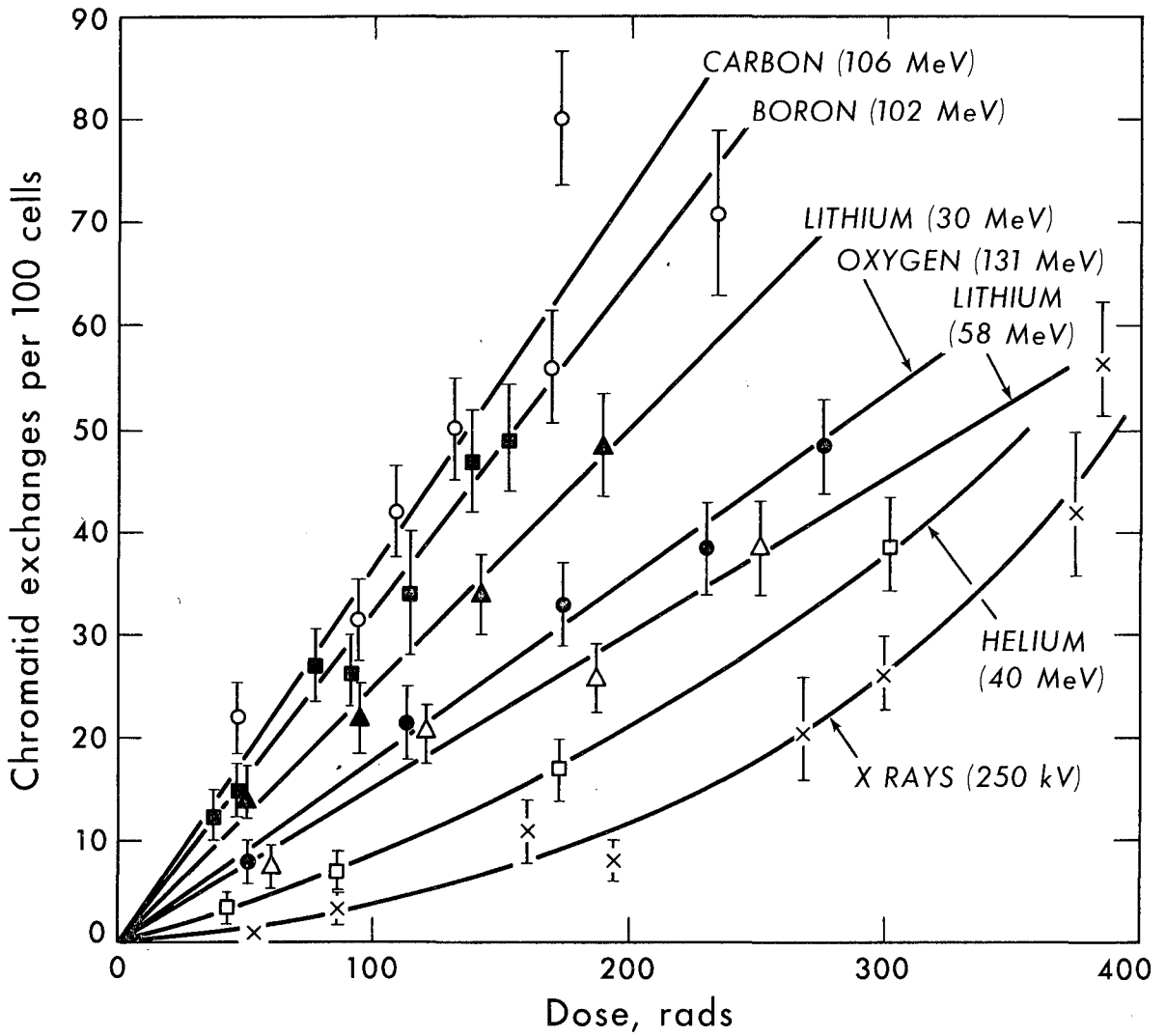

Dose, rads
0
200
400
600
800
1000
100
10
1
NORMAL
IUdR (20 μM)
Colony forming ability, % of controls
79.0 MeV
C12 ions
26.3 MeV
He4 ions
50 kVp
X RAYS
GROWTH CURVES
Average no. of cells/colony
10
1
0
20
40
60
80
100
120
140
Hours at 37°C
MU-33278

Fig. 7



MUB-8335

Fig. 8

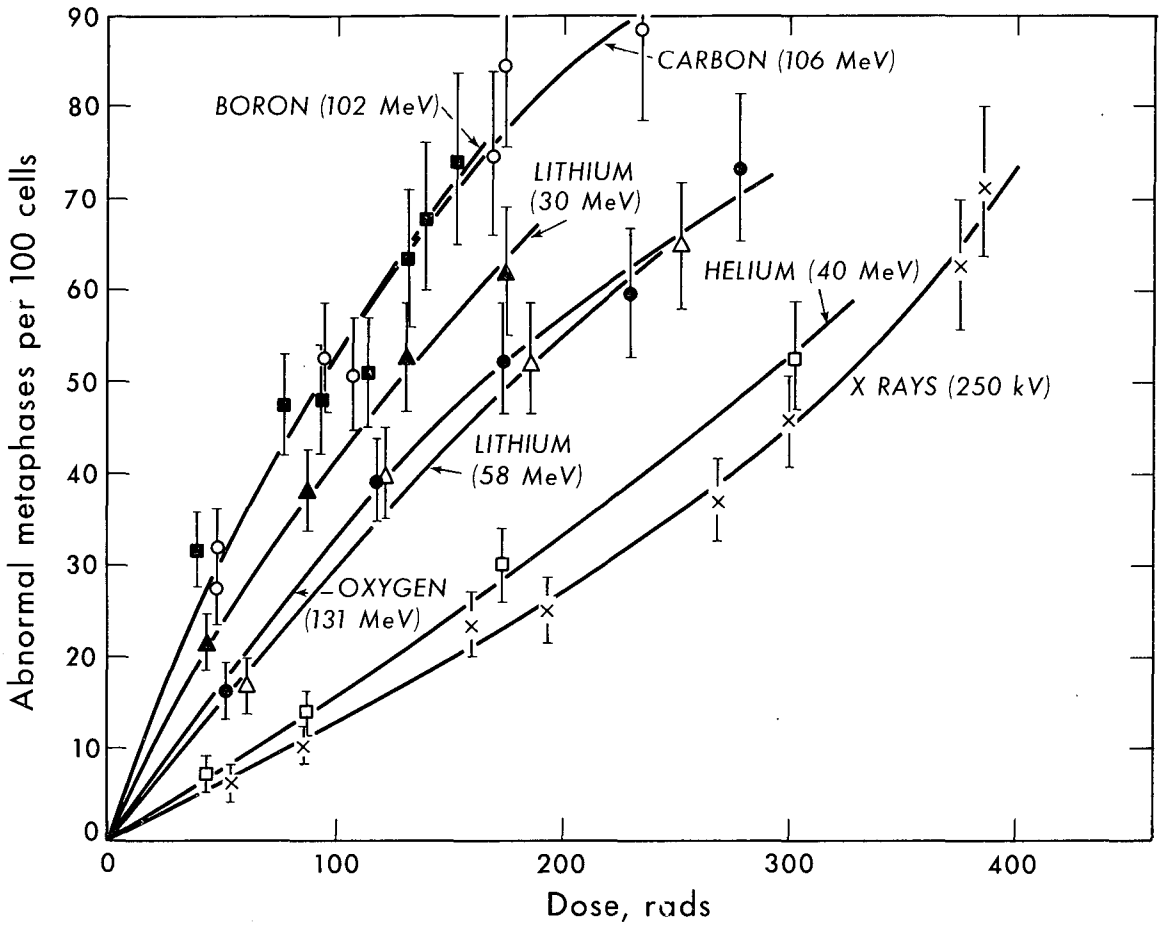

Abnormal metaphases per 100 cells
Dose, rads
0
10
20
30
40
50
60
70
80
90
0
100
200
300
400
CARBON (106 MeV)
BORON (102 MeV)
LITHIUM (30 MeV)
HELIUM (40 MeV)
X RAYS (250 kV)
LITHIUM (58 MeV)
OXYGEN (131 MeV)
MUB-8336

Fig. 9

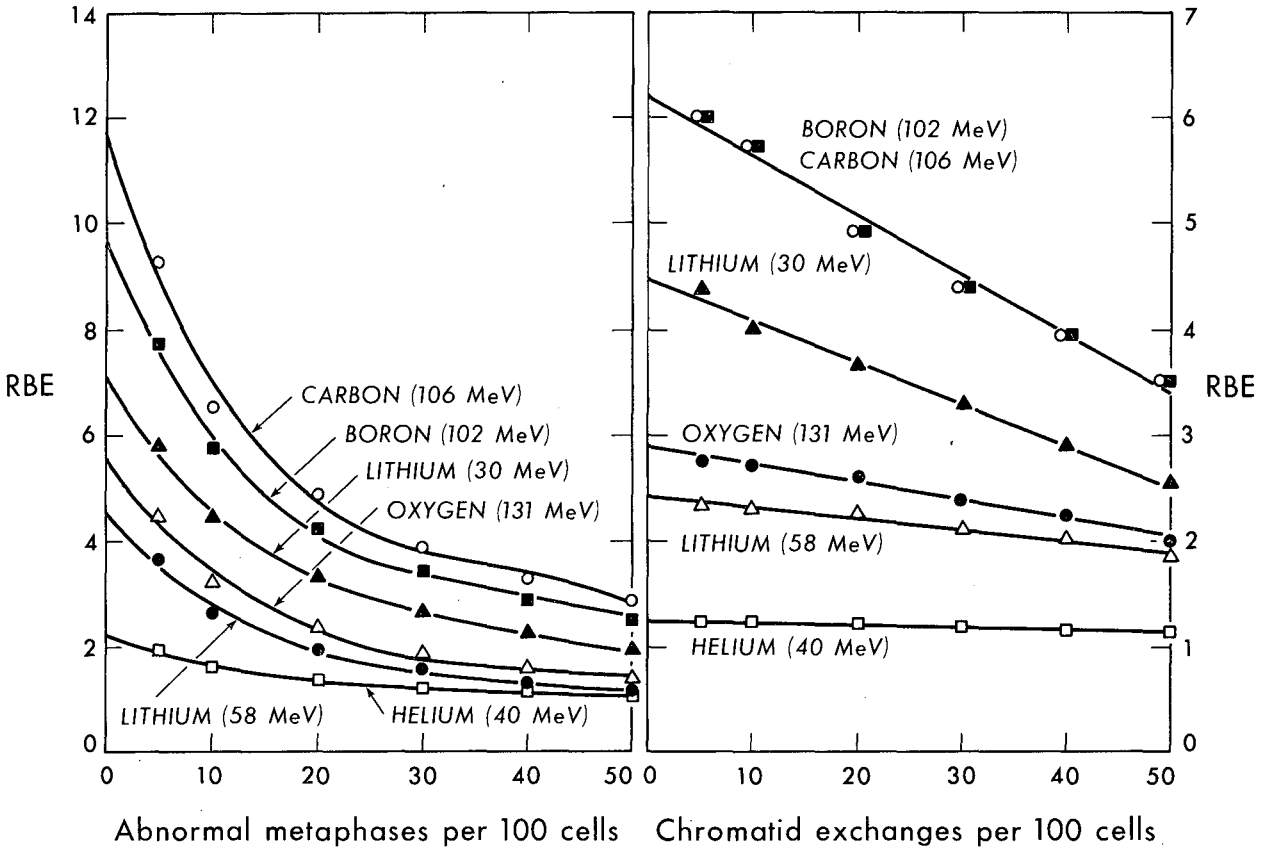


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Fig. 10