Submitted to Radiation Research

UCRL-20161
Preprint

c.2

"INDUCTION OF HETEROALLELIC REVERSIONS AND LETHALITY IN SACCHAROMYCES CEREVISIAE EXPOSED TO RADIATIONS OF VARIOUS LET (^{60}Co γ RAYS, HEAVY IONS, AND π^- MESONS) IN AIR AND NITROGEN ATMOSPHERES."

M. R. Raju, M. Gnanapurani, B. Stackler, B. I. Martins,
U. Madhvanath, J. Howard, J. T. Lyman, and R. K. Mortimer

October 1970

AEC Contract No. W-7405-eng-48

TWO-WEEK LOAN COPY

This is a Library Circulating Copy which may be borrowed for two weeks. For a personal retention copy, call Tech. Info. Division, Ext. 5545

LAWRENCE RADIATION LABORATORY
UNIVERSITY of CALIFORNIA BERKELEY

UCRL-20161 c.2

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

"INDUCTION OF HETEROALLELIC REVERSIONS AND LETHALITY IN SACCHAROMYCES CEREVISIAE EXPOSED TO RADIATIONS OF VARIOUS LET (^{60}Co γ RAYS, HEAVY IONS, and π^- MESONS) IN AIR AND NITROGEN ATMOSPHERES."

M. R. Raju, M. Gnanapurani, and B. Stackler

Lawrence Radiation Laboratory, Berkeley, California 94720
and The University of Texas at Dallas, Dallas, Texas 75230

and

B. I. Martins, U. Madhvanath, J. Howard
J. T. Lyman, and R. K. Mortimer

Lawrence Radiation Laboratory
University of California
Berkeley, California 94720

October 1970

Number of copies: 3
Manuscript pages: 9
Tables : 2
Figures : 6

RUNNING HEAD:

Effects of Heavy Ions and π^- Mesons in Yeast

AUTHOR ADDRESS:

M. R. Raju
University of California
Lawrence Radiation Laboratory
Bldg. 29, Room 229A
Berkeley, CA 94720

ABSTRACT

Induction of heteroallelic reversion in diploid yeast is one of the most radiosensitive systems known. Reversion to arginine independence in a diploid strain BZ34 of Saccharomyces cerevisiae was used to measure the RBE of π^- mesons and heavy ions. Exposures were also conducted under anoxic conditions to obtain the OER for these radiations and for ^{60}Co γ rays. The LET's used covered a range from 3 to 5500 MeV/gm/cm^2.

The OER values obtained for ^{60}Co γ rays and π^- mesons were 2.5 and 1.9 respectively. The response of this yeast system was found to be nearly the same at dose rates of 40 rads/hr and 40 rads/min of ^{60}Co γ rayds. With increasing LET, the RBE rose to a maximum value in the region 2000 MeV/gm/cm^2, corresponding to that of carbon ions. The OER decreased with increasing LET, approaching unity for neon ions.

The study was extended to induction of lethality with the heavy ions. The maximum effectiveness still occurred at an LET of 2000 MeV/gm/cm^2, and no oxygen effect was found at 5500 MeV/gm/cm^2. The survival curves, unlike those for other diploid yeast strains reported, shifted from a sigmoidal to an exponential type as the LET increased. This is similar to the response of mammalian cell systems.

KEY WORDS:

Yeast, Heteroallelic Reversions, Lethality, RBE, OER, Heavy ions, and π^- mesons

INTRODUCTION

It is known that sublethal doses of X rays result in a large increase in reversion to wild type in diploid yeasts that are heteroallelic for mutations at a given locus (1, 2). This system is well suited for the measurement of oxygen enhancement ratio (OER) and relative biological effectiveness (RBE) with the presently available low-intensity π^--meson beams. A study of the influence of linear energy transfer (LET) and oxygen tension on the induction of heteroallelic reversions would be useful for elucidating the information obtained for π^- mesons. In the work reported in this paper, ^{60}Co γ rays and helium, carbon, and neon ions were chosen to cover an LET range of 3 to 5500 MeV/gm/cm^2.

Induction of lethality was also studied for X rays and heavy ions to see how the variation of radiosensitivity as a function of LET compares with the results obtained by using heteroallelic reversion as the end point.

MATERIAL AND METHODS

A diploid strain BZ34 of the yeast Saccharomyces cerevisiae described earlier by Fogel and Mortimer (3) was used in this study. Its genotype is

$$\frac{a}{\alpha}\ \frac{+}{\text{pet 1}}\quad \frac{\text{arg 4-4}}{+}\quad \frac{+}{\text{arg 4-17}}\quad \frac{\text{thr 1}}{+}\quad \frac{+}{\text{leu 1-12}}\quad \frac{\text{trp 5-48}}{\text{trp 5-48}}\quad \frac{+}{\text{met 1}}\quad \frac{\text{ura 3}}{+}$$

$$\frac{\text{his 5-2}}{\text{his 5-2}}\quad \frac{\text{lys 1-1}}{\text{lys 1-1}}\quad \frac{\text{ade 2-1}}{\text{ade 2-1}}\ ;$$

the arg 4-4 and arg 4-17 are a noncomplementing combination of mutant alleles of the arginosuccinase locus. This strain was chosen because the two arg 4 alleles are located far apart in the locus; hence small doses of radiation can be readily detected by an increase in reversion frequency(2).

For ^{60}Co irradiation, 150- and 1500-Ci sources were used to obtain dose rates of 40 rads/hr and 40 rads/min. The details of dosimetry and the irradiation facility for π^- mesons at the 184-inch synchrocyclotron are given elsewhere(4). Experiments were done at the peak of the depth-dose distribution. Dose rates of 0.5 to 1 rad/min were obtained in this region.

Heavy-ion irradiations were carried out at the Berkeley heavy-ion linear accelerator (Hilac). The energies of the ions He, C, and Ne at the cell position were 40, 108, and 140 MeV respectively. The total LET's corresponding to these ions were 190, 2000, and 5500 MeV/gm/cm^2. The dosimetry and experimental arrangements for exposing the samples at the Hilac were described by Brustad(5). A 150-kVp 15-mA X-ray machine was also used for survival studies.

The yeast growth media and techniques employed have been described previously(6). The cells were grown in 30 ml of liquid growth medium YEPD. After 2 days of incubation at 30°C, the cells were centrifuged, washed, and resuspended in water to a concentration of 1.4×10^9 cells/ml. For irradiation, 9-mm-diameter buffered (nonnutrient) agar disks were prepared, and 15 μl of the cell suspension was pipetted onto each of these disks. It was possible to position six of these samples within the nearly uniform area of the pion beam. The sample disks were mounted in a 35-mm petri dish which in turn was fitted into a Lucite box. The box was cooled by circulating cold water. Precooled moist air or nitrogen was passed through the box during exposure. The temperature of the samples during irradiation was about 5°C. In some cases, the irradiation times were long. It was, hence, found necessary to keep the cells at a low temperature to avoid any increase in the frequency of arginine revertants due only to storage in nonnutrient

medium(7). Duplicate samples were exposed for each dose point. A control set of samples in identical boxes was maintained at the same temperature for both aerobic and anoxic conditions.

For exposures with heavy ions, 20-μl samples that contained 1.5×10^7 cells were pipetted onto the 9-mm-diam agar disks. The procedure for exposing samples to the external beam of the Hilac has been described previously(5).

After exposure, the cells were resuspended in sterile water and 0.2 or 0.3 ml was pipetted onto each of a set of arginineless (AR^-) plates. Serial dilutions were made for plating on YEPD plates to assay survival. Five AR^- and five YEPD plates were used for each sample. The doses given in this part of the experiment were sublethal. Cells exposed to higher doses of heavy ions were plated in appropriate numbers on YEPD plates. All plates were incubated at 30°C. An initial count of the colonies was made after 3 to 5 days. Plates exposed to high doses were recounted after 8 or 9 days.

RESULTS AND DISCUSSION

Induction of heteroallelic reversions by ^{60}Co γ rays and π^- mesons

To determine the frequency of revertants per survivor for a given dose, the number of colonies counted on the AR^- plates was divided by the number of colonies on the corresponding YEPD plates times the appropriate dilution factor.

Figure 1 shows the number of arginine revertants that arise during storage of samples at room temperature and at 5°C. Figure 2 is a plot of the frequency of revertants per survivor vs dose for the two dose rates of ^{60}Co γ rays. The number of revertants produced is proportional to the dose.

The slope β (revertants per survivor per rad) determined from least-squares analysis of the data was chosen as an index for the efficiency of reversion induction. The results indicate that the slopes are nearly the same for dose rates of 40 rads/hr and 40 rads/min. The OER for ^{60}Co γ rays for induction of heteroallelic reversion is 2.5.

Figure 3 shows the results obtained for irradiation with π^- mesons. There is a detectable increase in the number of revertants over that of the control at a dose of about 25 rads of π^- mesons; at 100 rads, the frequency is more than doubled. The results indicate an RBE value (aerobic) of 1.4 for mesons relative to ^{60}Co γ rays and an OER value of 1.9. Thus, a significant reduction in OER is obtained. The RBE for this system is found to be lower and the OER higher than for bean roots(4). These RBE and OER values should be valid for acute exposure to π^- mesons.

Induction of heteroallelic reversions by heavy ions

Figure 3 also shows the plots of the frequencies of revertants per survivor versus dose for irradiations with He, C, and Ne ions. The frequency of revertants was found to vary linearly with dose for all the ions used. Cells exposed to doses in the lethal range were also plated on AR^- plates. The results showed that the "revertants per survivor" remained a linear function of dose even up to 50 krads. Perhaps this is because the irradiated cell population is homogeneous with respect to sensitivity to reversion, as well as with respect to killing. However, when the X841 strain was plated on AR^- plates after exposure to neon ions, it showed a linear response initially and an upward bend beyond 20 krads. A similar pattern of response was found with this strain for induction of mutations(6).

LET dependence of reversion induction

The reversion-induction efficiencies of all radiations studied (β values from Figs. 2 and 3) are plotted as a function of LET in Fig. 4, and the results are summarized in Table I. The value of β increases with increasing LET of the radiations, reaches a maximum at an LET of 2000 MeV/gm/cm^2, and decreases slightly with further increase in LET. The RBE of various heavy ions with respect to ^{60}Co γ rays and the OER as a function of LET have been plotted in Fig. 5. The oxygen effect decreases continuously from a value of 2.5 for γ rays approaching unity at an LET of 5500 MeV/gm/cm^2. These results are similar to those reported for the variant colonies of X841 arising from induced mitotic crossing-over in that the efficiency of induction does not fall rapidly with increase in LET beyond the maximum, and the oxygen effect decreases with increase in LET(6).

When the values of RBE and OER obtained for π^- mesons are plotted onto the curves in Fig. 5, the following becomes evident. The "equivalent LET" corresponding to the measured RBE value for π^- mesons is found to be 4.5 MeV/gm/cm^2. However, the "equivalent LET" obtained in a similar manner from the OER-LET curve is 300 MeV/gm/cm^2. The LET spectrum of π^- mesons at the region where they stop is broad, and the dose contributed by the high-LET components is about 25%(8). Although this dose fraction is not enough to increase the RBE value appreciably, it is obviously responsible for a considerable reduction in the oxygen enhancement ratio. Similar results have been obtained with 14-MeV neutrons(9) and 910-MeV helium ions(10).

Induction of lethality by heavy ions

Figure 6 shows the survival curves for BZ34, for aerobic and anoxic conditions of exposure to various radiations. The survival curves are seen to change from a sigmoidal type to an exponential type as the LET of the radiation is increased. The result contrasts with the finding for the diploid yeast strain X841, which yielded sigmoidal survival curves for all radiations up to neon ions. In addition, the LD_{50} values are smaller by about a factor of four than those obtained for the homoallelic strain X841 studied earlier under identical conditions. The results are summarized in Table II.

The most striking property of this yeast strain seems to be the exponential survival curves for high-LET radiations. The survival curves of different diploid strains studied earlier were sigmoidal even for the high-LET radiations(6, 11). The radiation response of BZ34, with a shoulder that narrows as the LET increases and becomes exponential for high-LET radiations, is similar to the response of mammalian cell systems(12). Even though mammalian cell systems are an order of magnitude more radiosensitive than the BZ34 yeast strain, the peak efficiency for inactivation occurs for both systems at the same LET of about 2000 MeV/gm/cm^2 (that corresponding to carbon ions). Further, at this LET, the survival curves for mammalian cells become exponential and the oxygen effect disappears, whereas for the yeast system, the shoulder still persists and the OER is 1.25. The OER, however, reaches unity at an LET value of 5500 MeV/gm/cm^2, with concomitant disappearance of the shoulder. The absence of a shoulder-type response in BZ34 for high-LET radiations suggests their inability to repair sublethal injury produced by such radiations. It would, therefore, be of interest to examine this property of the BZ34

strain at a genetic level and see whether any information about the genetic control of repair mechanisms could be obtained. As an initial step towards this end, a hybrid was made by mating spores from the strains BZ34 and X841. This hybrid showed a response similar to X841 in the persistence of the shoulder for high-LET radiations. These preliminary results are consistent with the hypothesis that BZ34 is homozygous for a recessive mutation that blocks repair of high-LET damage. Additional hybrids must be studied to confirm this interpretation.

We are grateful to Dr. C. Richman, Dr. J. H. Lawrence, and Dr. C. A. Tobias for their continued interest and encouragement.

REFERENCES

1. R. K. Mortimer, Invited Discussion, Radiation Research, Supplement 1, 394-402 (1959).
2. T. R. Manney and R. K. Mortimer, Allelic mapping in yeast by X-ray-induced mitotic reversion. Science 143, 581-583 (1964).
3. S. Fogel and R. K. Mortimer, Informational transfer in meiotic gene conversion, Proc. Natl. Acad. Sci. 62, 96-103 (1969).
4. M. R. Raju, N. M. Amer, M. Gnanpaurani, and C. Richman, The oxygen effect of π^- mesons in *Vicia faba*. Radiation Res. 41, 135-144 (1970).
5. T. Brustad, Molecular and cellular effects of charged particles. Radiation Res. 15, 139-158 (1961).
6. Robert Mortimer, Tor Brustad, and Douglas Cormack, Influence of linear energy transfer and oxygen tension on the effectiveness of ionizing radiations for induction of mutations and lethality in *Saccharomyces cerevisiae*. Radiation Res. 26, 465-482 (1965).

7. J. T. Lyman, Dark recovery of yeast following ionizing radiations (Ph. D. Thesis), Lawrence Radiation Laboratory Report UCRL-16030, March 1965.

8. S. B. Curtis and M. R. Raju, A calculation of the physical characteristics of negative pion beams, energy-loss distribution, and Bragg curves. Radiation Res. 34, 239-255 (1965).

9. G. J. Neary and J. R. K. Savage, Oxygen effect with 14-MeV neutrons. Nature 204, 197 (1964).

10. M. R. Raju, M. Gnanapurani, B. I. Martins, J. Howard, and J. T. Lyman, Relative biologic effectiveness and oxygen enhancement ratio at various depths of a 910-MeV helium ion beam. Acta Radiologica (in press).

11. W. Laskowski, Inaktivierungsversuche mit homozygoten Hefestämmen verschiedenen Ploidiegrades. Z. Naturforsch. 15B, 495-506 (1960).

12. G. W. Barendsen, T. L. J. Beusker, A. J. Vergroesen, and L. Budke, Effects of different ionizing radiations on human cells in tissue culture. Radiation Res. 13, 841-849 (1960).

Table I. Induction of heteroallelic reversion in relation to oxygen tension and LET.

Radiation	Irradiation in	Revertants per survivor per rad $\times 10^{-7}$	Oxygen effect
^{60}Co gamma	air	1.5	
	N_2	0.6	2.5
π^- mesons	air	2.1	
	N_2	1.1	1.9
Helium	air	6.6	
	N_2	3.3	2.0
Carbon	air	10.6	
	N_2	7.9	1.3
Neon	air	7.3	
	N_2	7.5	1.0

Table II. Induction of lethality in relation to LET and oxygen tension.

	Air		N_2			
Radiation	LD_{50}, (rads)	D_0, (rads)	LD_{50}, (rads)	D_0 (rads)	RBE	OER
X rays	14 000	10 500			1	
He ions	9 500	5 700	20 500	9 200	1.84	1.6
C ions	4 000	2 400	5 200	3 000	4.4	1.25
Ne ions	4 200	5 200	4 200	5 200	2.0	1

FIGURE CAPTIONS

Fig. 1. Frequency of revertants due to storage in a nonnutrient medium at room temperature and at 5°C.

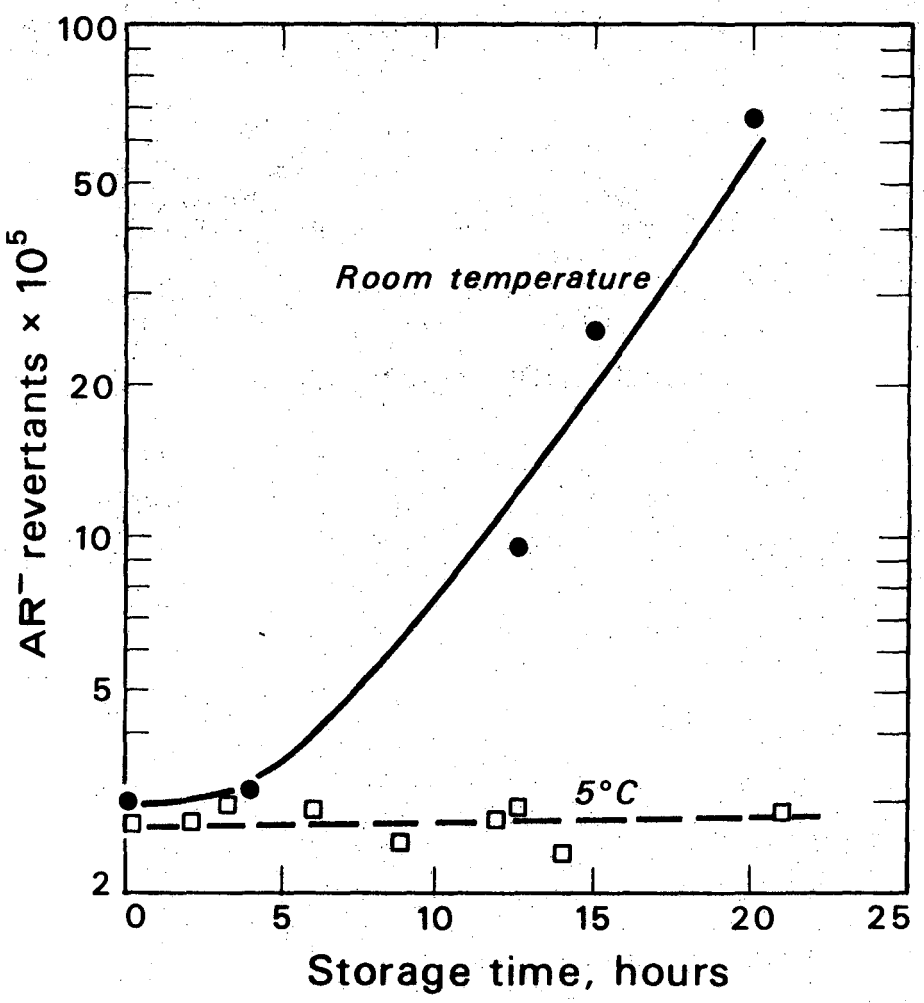
Fig. 2. Frequency of arginine-independent revertants as a function of ^{60}Co γ-ray dose in atmospheres of air and nitrogen, at dose rates of 40 rad/min and 40 rad/hr.

Fig. 3. Frequency of arginine-independent revertants as a function of dose for π^- mesons and He, C, and Ne ions in atmospheres of air and nitrogen.

Fig. 4. Efficiency for induction of heteroallelic reversion as a function of LET in atmospheres of air and nitrogen.

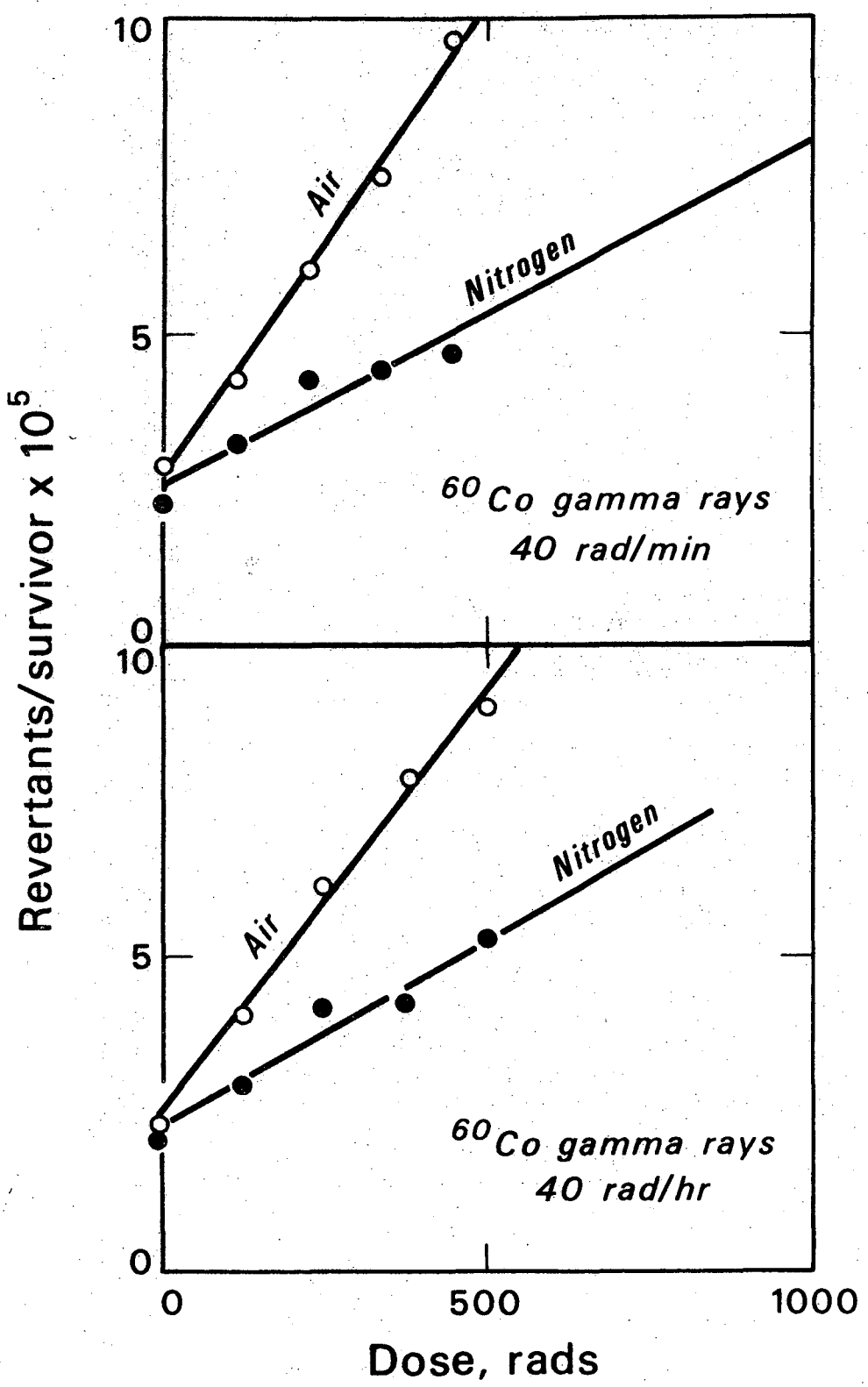
Fig. 5. Relative biological effectiveness (compared with ^{60}Co γ rays) for induction of arginine reversion as a function of total LET and the influence of oxygen on the effectiveness of different radiations.

Fig. 6. Survival (colony-forming ability) as a function of dose of different radiations in irradiation atmospheres of air and nitrogen.



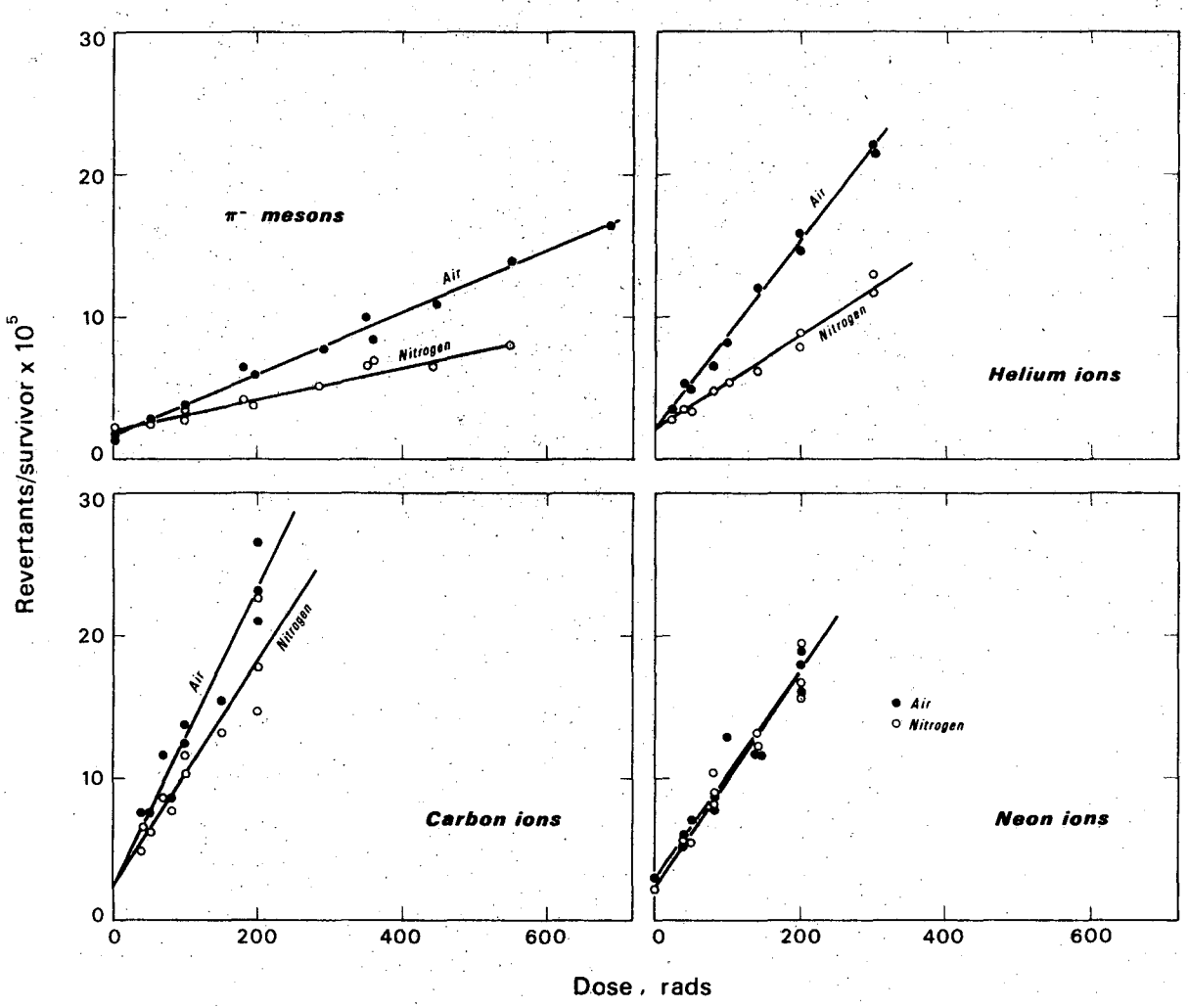
DBL 6911 5138

Fig. 1



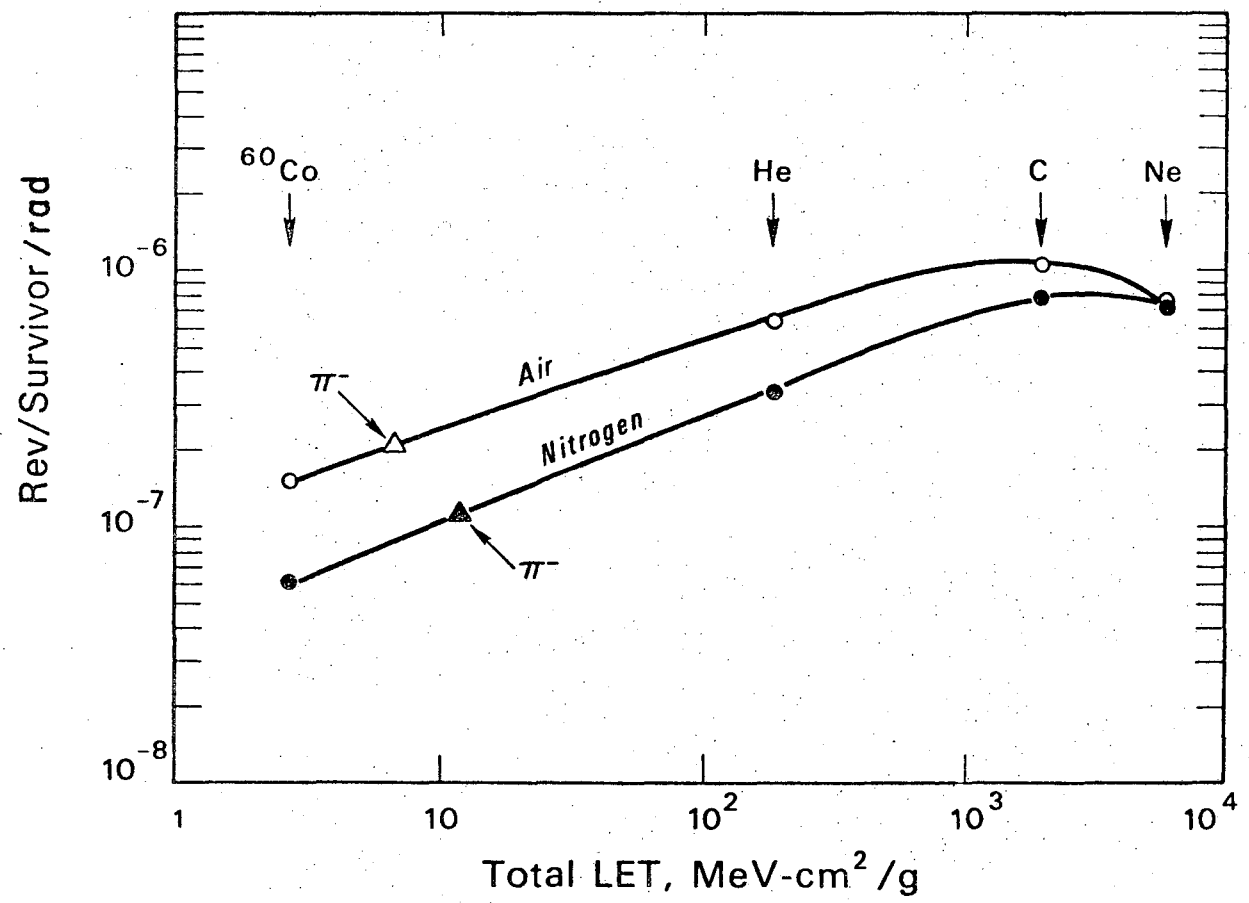
DBL 7011 5960

Fig. 2



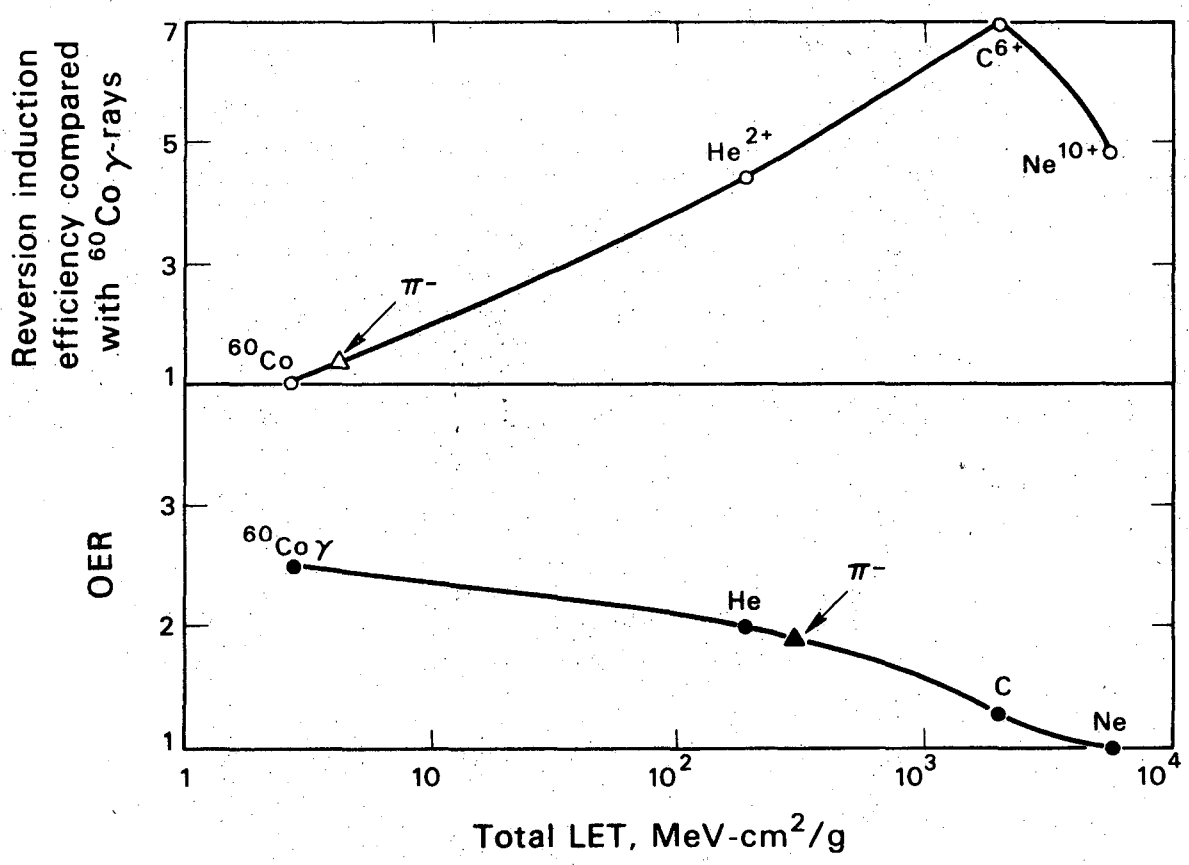
DBL 7011 5965

Fig. 3



DBL 7011 5958

Fig. 4



DBL 7011 5959

Fig. 5

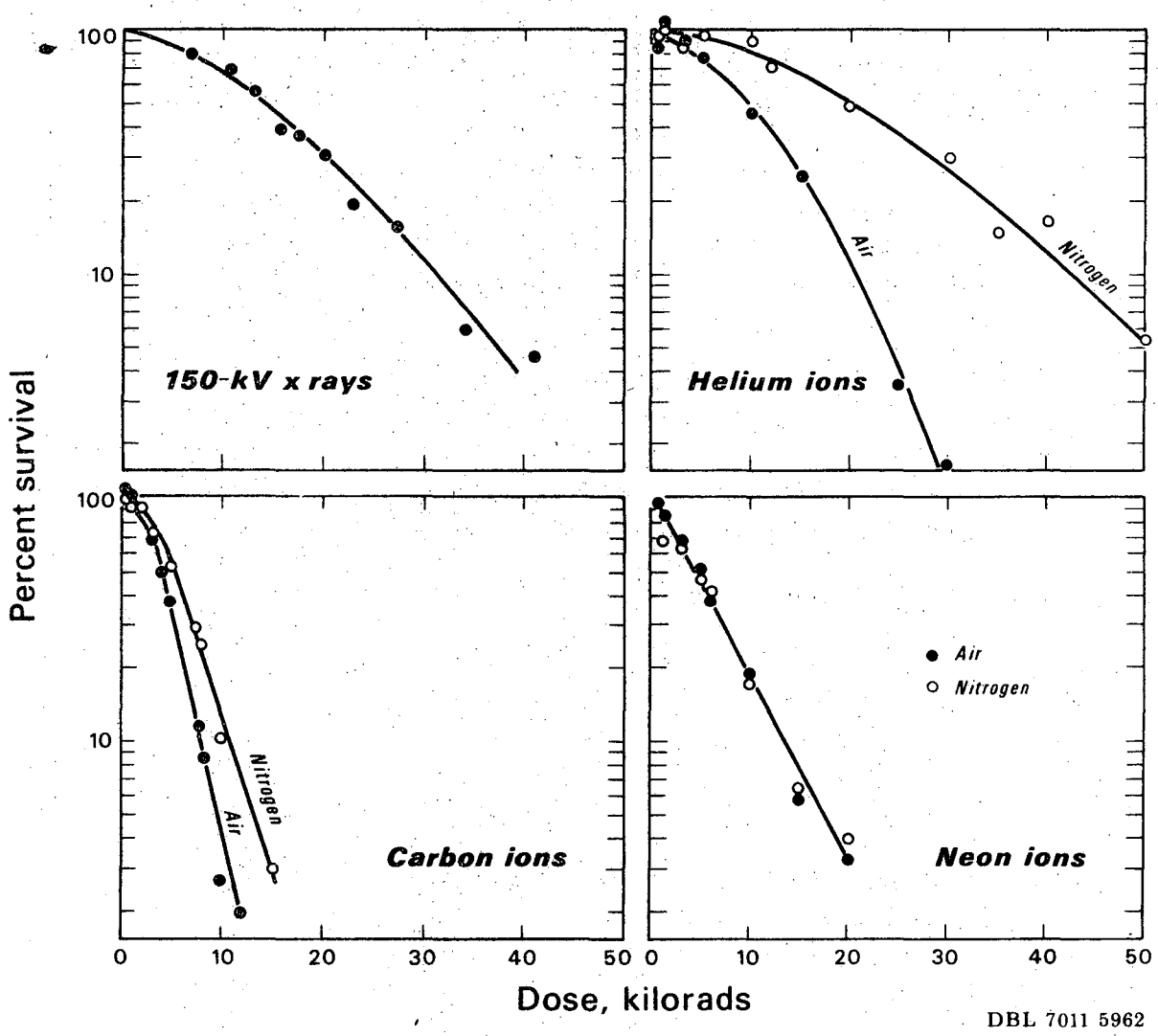

Percent survival
100
10
150-kV x rays
Helium ions
Air
Nitrogen
Carbon ions
Neon ions
Air
Nitrogen
0
10
20
30
40
50
Dose, kilorads
DBL 7011 5962

Fig. 6

LEGAL NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

TECHNICAL INFORMATION DIVISION
LAWRENCE RADIATION LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720