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INTRODUCTION

It has long been recognize that the metabolic stability of living cells depends upon a balance between biochemical breakdown and repair processes. Furthermore, at many levels of organization, homeostatic mechanisms produce responses to environmental stresses that tend to preserve the integrity of the organism. A number of recovery phenomena associated with radiation injury have recently been attributed to the enzymic repair of DNA structural defects. This might be regarded as an example of a "homeostatic" mechanism operating at the macromolecular level.

In view of the importance of recovery processes in radiobiology, it is essentail to determine what types of radiation damage are susceptible of repair in various kinds of cells. In diploid yeast (Saccharomyces cerevisiae), viability is increased if, instead of being plated immediately after irradiation, the cells are incubated in a nonnutritive suspension for a few hours prior to being plated (1). A problem in terminology arises in the description of these observations. It is both tempting and convenient to use the word "recovery" to denote such an increase of viability.

However, by this usage we do not wish to imply that the phenomenon is necessarily based upon the repair of macromolecular defects (e.g., as in enzymic photoreactivation or dark reactivation in E. coli); that is, we cannot rule out other mechanisms such as the prevention of expresion or the "bypass" of the damage.

There is considerable evidence that densely ionizing particles produce a greater proportion of irreversible damage than X rays (2, 3). Thus, it is of some interest to know whether or not recovery can occur in yeast irradiated with heavy ions, since it is not implausible to imagine that irreparable macromolecular damage might be produced within the columns of dense ionization formed along the particle tracks. However, we found that after heavy-ion irradiation, recovery occurs to the same extent as is observed after X irradiation. Also, it would appear that recovery is additive to the increased viability arising from glycercol protection, and is not due to sporulation during the postirradiation incubation period (4).

MATERIALS AND METHODS

The physical and biological techniques used in these experiments have been described elsewhere, and only a brief summary of the most important points is given here (4).

The X-ray source was a Norelco OEG-60 beryllium-window tube operated at 50 kV and 25 mA without any external filtration. Under these conditions the dose rate to the cells was 15 krads per minute.

The source of heavy-ion radiation was the Berkeley Hilac. Stripped nuclei of helium, carbon, and neon with energies of approximately 9 MeV

per atomic mass unit were used. In each experiment, the radiation doses were measured with a thin, transmission-type ionization chamber placed in the beam about 1 mm before the sample of cells. The dose rates employed varied between 6 and 15 krads per minute in various runs.

The mass stopping power $(\frac{1}{\rho}\frac{dE}{dx})$ of tissue for the ions is given in Table I. This simple measure of the collisional energy losses of heavy charged particles is sufficient: for the purpose of this paper. We restrict the use of the quantity LET (linear energy transfer) to cases in which it is necessary to distinguish between the energy losses along the tracks of the particles and the losses associated with those δ electrons which are scattered beyond the primary track "core" (5, 6). Thus, the calculation of $\frac{1}{\rho}\frac{dE}{dx}$ includes all the δ-electron losses even though part of this energy is deposited some distance from the primary tracks.

Since the range of the particles is much greater than the thickness of the monolayer of irradiated cells, the maximum energy loss (Bragg peak) occurs after the particles have passed through the cells. Thus, the energy loss in the cells for the 40-MeV helium ions is much lower than that commonly associated with α particles, and in fact is close to that of 50-kV X-rays (7). The RBE (relative to X-rays) for the inactivation of yeast goes through a maximum as the energy loss of the ions increases. The carbon ions, for which the energy loss is similar to that for the α-particle Bragg peak, are associated with the maximum RBE; the helium and neon ions fall on either side of this maximum (8, 9).

Subcultures of the following strains of the yeast Saccharomyces cerevisiae were used: X841, a diploid; X841p, a diploid petite; and XJ4, a diploid homozygous mating type (αα). All were grown in liquid YEPD

medium (1% yeast extract, 2% peptone, 2% dextrose) for 48 hours (96 hours for the petite strain) after which time the population is in stationary phase. The cells were harvested, washed three times, and resuspended in double-distilled water. Microscopic examination of the suspension usually revealed less than 3% budding cells, no asci, and no clumps.

Aliquots (50 λ) of the suspension were placed on sterile 13-mm-diameter Millipore filters (type HA). The suspending liquid was quickly absorbed by an absorbent pad placed under the filter, leaving the cells in a monolayer on the surface. The pad was then saturated with double-distilled water. Because of the high porosity of the filter, although the cells remained wet the water layer over the cells was not thick enough to appreciably reduce the beam energy. The cells were exposed to the air during irradiation, after which they were resuspended in double-distilled water. Samples were then plated on YEPD agar (immediately plating), and the remainder of the suspension was incubated for times up to 48 hours at 30°C. Further samples were plated at various times after irradiation in order to observe recovery (delayed plating). All plates were incubated for 5 days at 30°C.

RESULTS AND DISCUSSION

1. Recovery is Independent of LET

Immediate and delayed plating dose-survival curves for the diploid yeast strain X841 irradiated with 50-kV X-rays and He^4, C^{12}, and Ne^{20} ions are shown in Figs. 1-3. It is clear from these results that recovery upon delayed plating occurs even after irradiation with the very densely

ionizing Ne^{20} particles ($\frac{1}{\rho}\frac{dE}{dx}$ = 4920 MeV-cm^2/g). Furthermore, the magnitude of this recovery (measured as a dose-modifying factor: DMF ≈ 2) for all ions used is essentially the same as that observed for X-rays. A similar recovery after irradiation with Pu^{239} α particles has been reported in Saccharomyces vini (10).

The shoulder on the survival curves does not decrease with increasing dE/dx as has been observed in Aspergillus spores (11), Artemia eggs (12), and mammalian cells (3, 13). Clearly, yeast differs in this respect from other organisms of wide phylogenetic distribution. The reasons for this are by no means clear. However, it has been suggested that shoulders might be due to the saturation of or the inhibition of intracellular repair processes with increasing radiation dose (14, 15); recovery after C^{12} and Ne^{20} irradiation is at least consistent with the persistence of shoulders on the curves (9). Preirradiation treatment of diploid yeast with heat or ethyl methane sulfonate serves simultaneously to reduce the shoulder and to inhibit recovery (16). Thus, it is possible that recovery in yeast is related to, and perhaps an enhancement of a shoulder-producing repair process.

It has been shown that recovery after X-rays is unaffected by the presence or absence of oxygen during irradiation (1). A second line of reasoning may be invoked to relate this fact with recovery following densely ionizing radiation. The oxygen-dependent component of radiation damage has been attributed to the reaction of radicals produced by "small" ionization clusters, whereas larger clusters give rise to the oxygen-independent component (17, 18). If this idea is correct, then it follows

that the recovery process is equally effective in dealing with lethal damage produced by both "small" and "large" ionization clusters. Therefore, the predominance of "large" clusters associated with the heavy-ion tracks might not be expected to reduce recovery.

Recovery appears to be completely additive to glycerol protection at the time of irradiation. The protection afforded by 6 M glycerol is equivalent to a dose-modifying factor of about 2.6 and is independent of LET (8). Diploid yeast irradiated with either X-rays or helium ions benefits from this protection, and in addition exhibit a full normal recovery DMF, also approximately 2.6, upon storage in distilled water. Thus, nearly a sevenfold reduction in the effective radiation dose may be achieved by a combination of glycerol protection and delayed plating.

The general properties of recovery in yeast and the dark reactivation mechanisms in E. coli B/r are similar in that both appear to be enzymic, energy-requiring processes capable of repairing some fraction of the lethal damage produced by X-rays, 2537-Å ultraviolet light, or nitrogen mustard, but none of the damage produced by heat or acridine-sensitized photodynamic action (1, 15). However, a significant difference between the two processes is that whereas recovery in yeast is independent of dE/dx for ionizing radiation, this is not the case in E. coli. Preliminary studies indicate that the sensitivity ratio of the reactivating and nonreactivating coli strains B/r and B_{s-1} declines from a value of 3.0 for X-rays to 1.4 for Ne^{20} ions and Po^{210} α particles (19, 20). Thus, it would appear that damage caused by densely ionizing radiation cannot be repaired in E. coli B/r. These contrasting results might be explained

if recovery in yeast were based upon a "bypass" of the damage such as might occur during somatic recombination or sporulation.

2. Recovery Appears to be Unrelated to Sporulation

Since haploid and petite diploid strains are incapable of either recovery or sporulation, a common pathway might be shared by the two processes (1, 21). Furthermore, we have observed asci in stored suspensions of diploid cells. Despite these facts, the following observations tend to invalidate the suggestion that recovery is due to sporulation during the storage period:

(i) Sporulation does not occur under anoxic conditions and is depressed at oxygen concentrations above 40% (22). Recovery is only moderately depressed under anoxic conditions and is enhanced in the presence of 100% oxygen (16).

(ii) Dextrose concentrations between 0.3 and 1% in the storage medium tend to depress sporulation (23), whereas recovery is largely unaffected. The recovery of X-irradiated cells upon storage in dextrose-phosphate buffer (0.5% dextrose) is shown in Fig. 4.

(iii) Sporulation depends upon cell concentration and is optimal at titers near 2×10^6 cells/ml (24). Recovery shows no such concentration dependence (4).

(iv) Sporulation is sharply inhibited at temperatures above 33°C (22), whereas recovery is still evident at 37°C (16).

(v) Diploid strains that are homozygous at the "mating type" locus are incapable of sporulation (25). We tested the homozygous strain XJ4 (αα mating type) and found that it was capable of recovery after X-irradiation (Fig. 5).

These facts make it clear that recovery cannot be attributed simply to sporulation during the storage period, although they do not serve to rule out the possibility that the two processes have some enzymic steps in common.

SUMMARY

A great enhancement of viability is observed if nonnutritive suspensions of diploid yeast, which have been irradiated with X-rays or heavy ions (He^4, C^{12}, Ne^{20}), are stored at 30°C in the dark for 4 or more hours prior to plating. Maximum recovery is usually observed after 24 to 48 hours; the survival curves obtained upon delayed plating are related to those for immediate plating by a constant dose-modifying factor. Several lines of evidence indicate that recovery is based upon enzymic postirradiation processes unrelated to the initial physicochemical reactions associated with absorption of the radiation. The magnitude of recovery is independent of such radiobiological modifiers as oxygen or glycerol, or track ion density. All these modifiers are thought to act by affecting the nature and distribution of the products of the initial radiochemical reactions. Thus, the recovery appears to be substantially independent of the precise chemical nature of the radiation-induced lesions. Very severe macromolecular damage is likely to be produced by the densely ionizing radiations. The ability of diploid yeast to recover after such irradiation suggests that a "bypass" rather than a direct repair mechanism may be involved. Segregation of the damage by sporulation would appear to be, a priori, a suitable bypass mechanism, but this hypothesis is ruled out by the results cited in this paper.

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Table I. Energy and stopping power for heavy ions used for irradiation of yeast strain X841.

Radiation	Energy (MeV/amu)	Stopping power of tissue ($MeV\text{-}cm^2/g$)
He^4	9.9	180
C^{12}	9.1	1765
Ne^{20}	8.2	4920

FIGURE LEGENDS

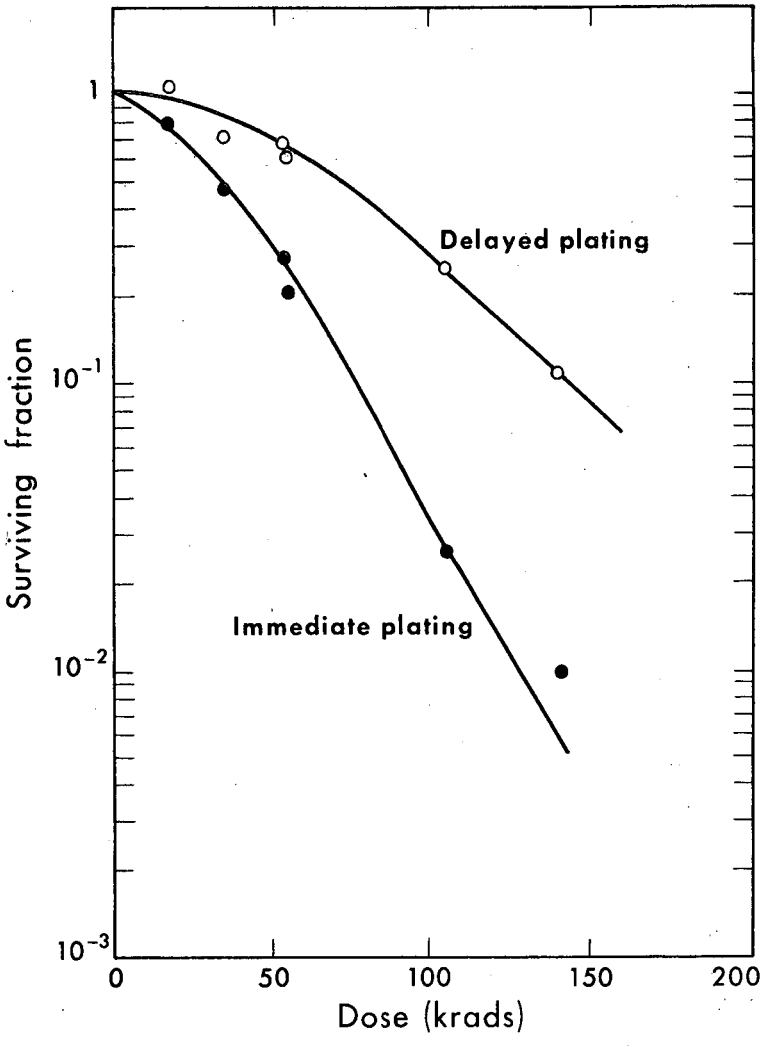
Fig. 1. Immediate and delayed (48 hours) platings of X841 following 50-kV X irradiation.

Fig. 2. Immediate and delayed (48 hours) platings of X841 following heavy-ion (C^{12} and He^{4}) irradiations.

Fig. 3. Immediate and delayed (48 hours) platings of X841 following Ne^{20} irradiation.

Fig. 4. Immediate and delayed (24 hours) platings of X841 following 50-kV X-rays. Cells were stored in dextrose-phosphate buffer (0.5% dextrose; M/15 phosphate; pH 6.4).

Fig. 5. Immediate and delayed (24 hours) platings of XJ4 following 50-kV X-rays.



MU-35519

Fig. 1

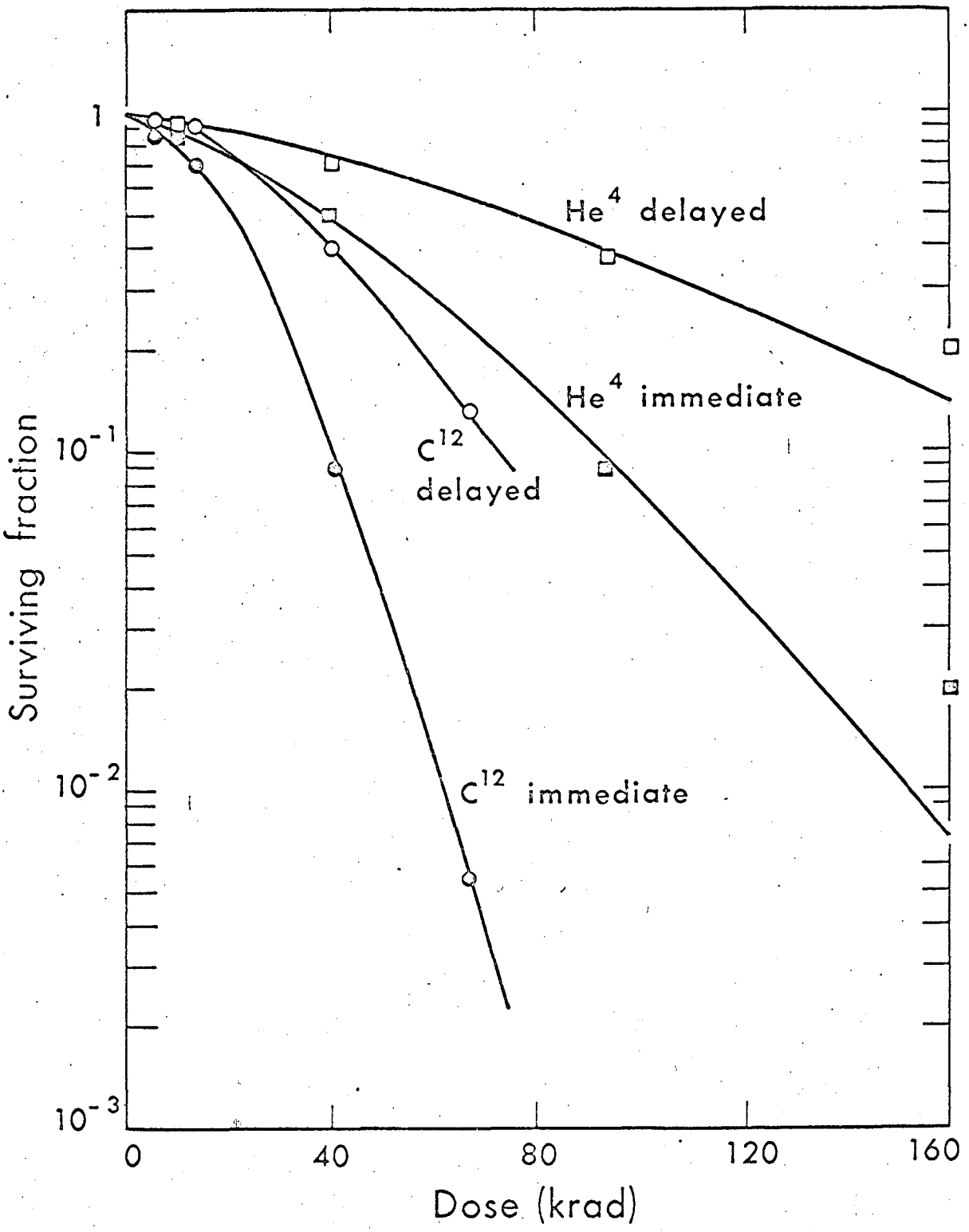


Fig. 2

MU-35520

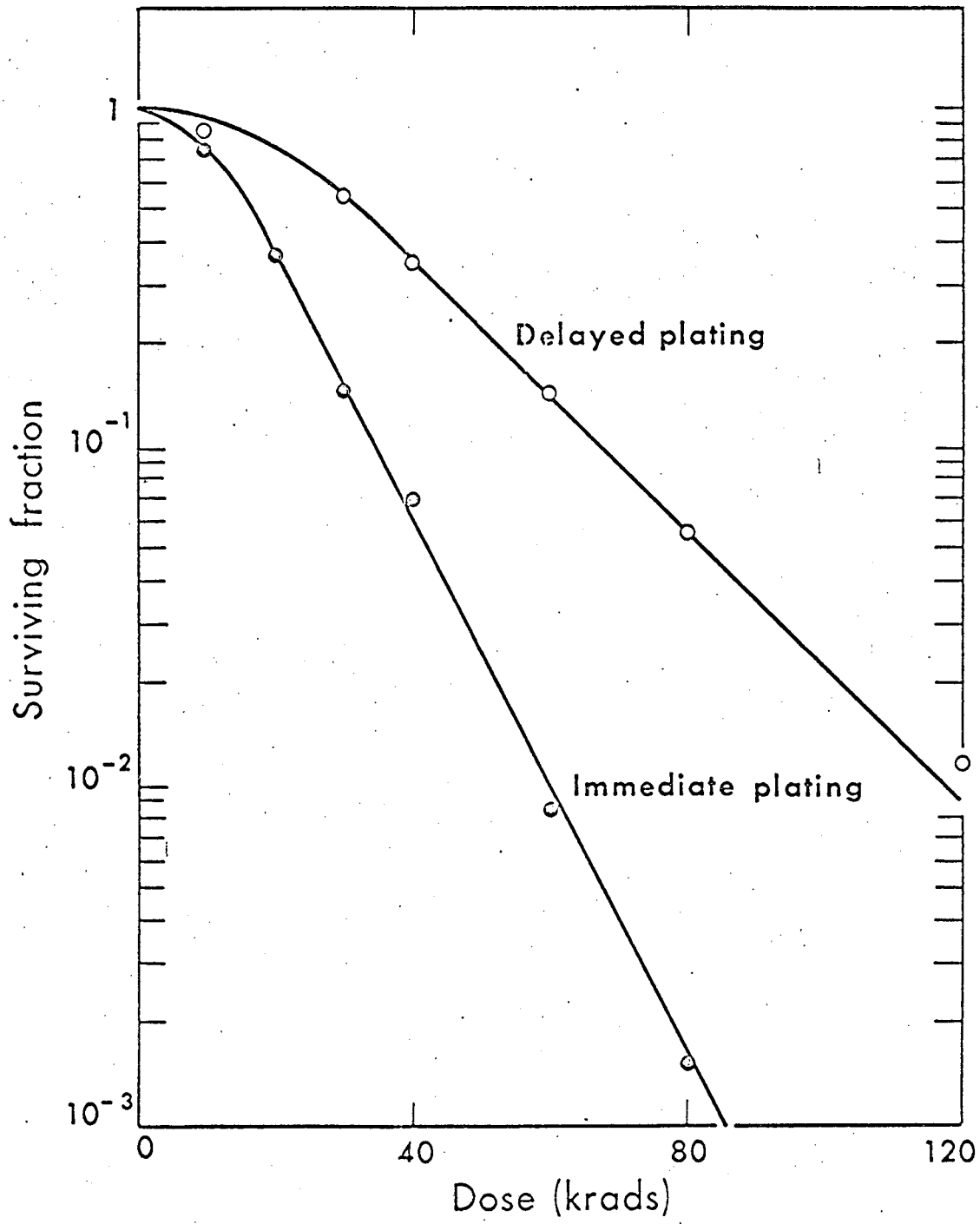


Fig. 3

MU-35521

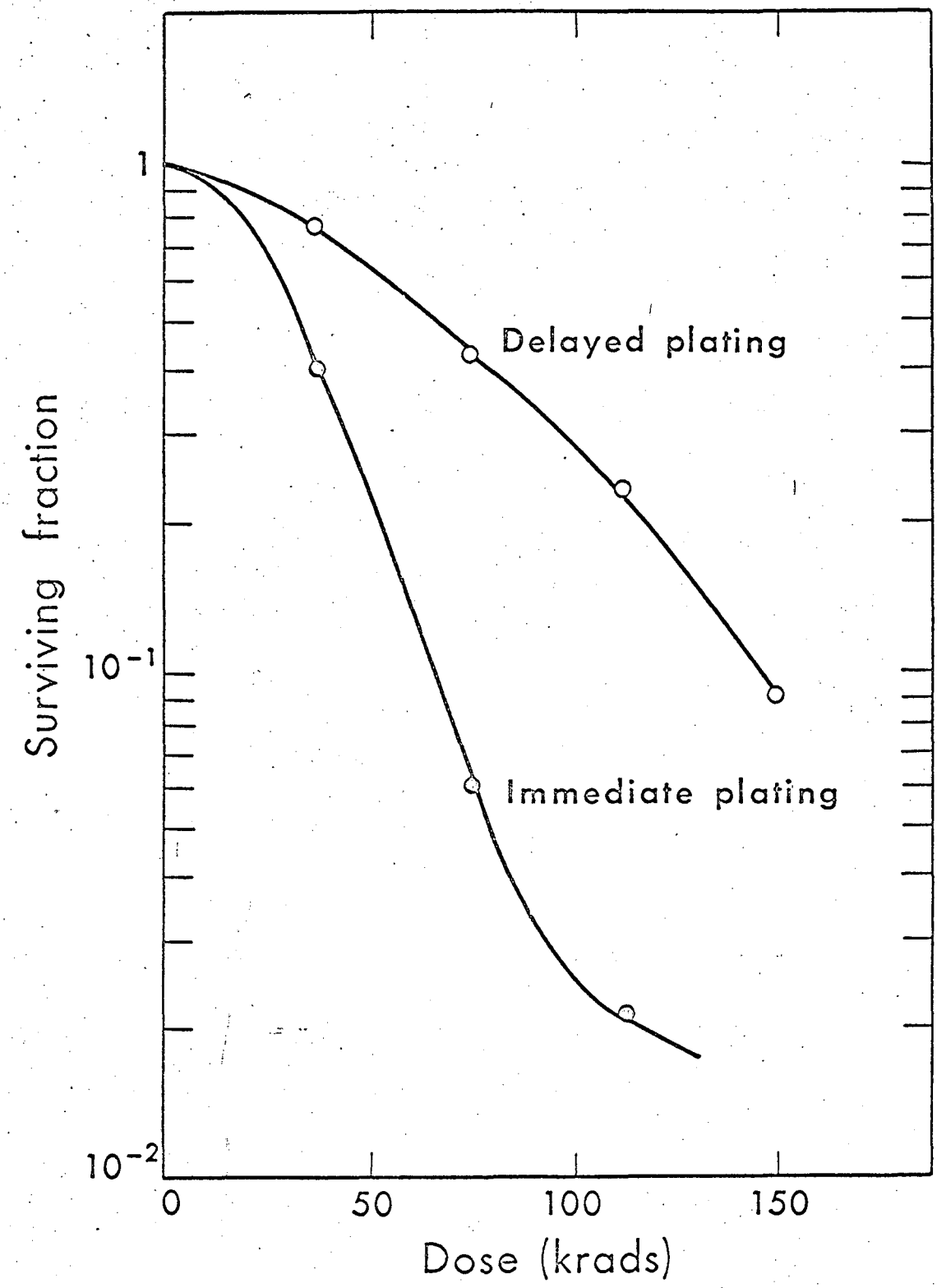


Fig. 4

MU-35524

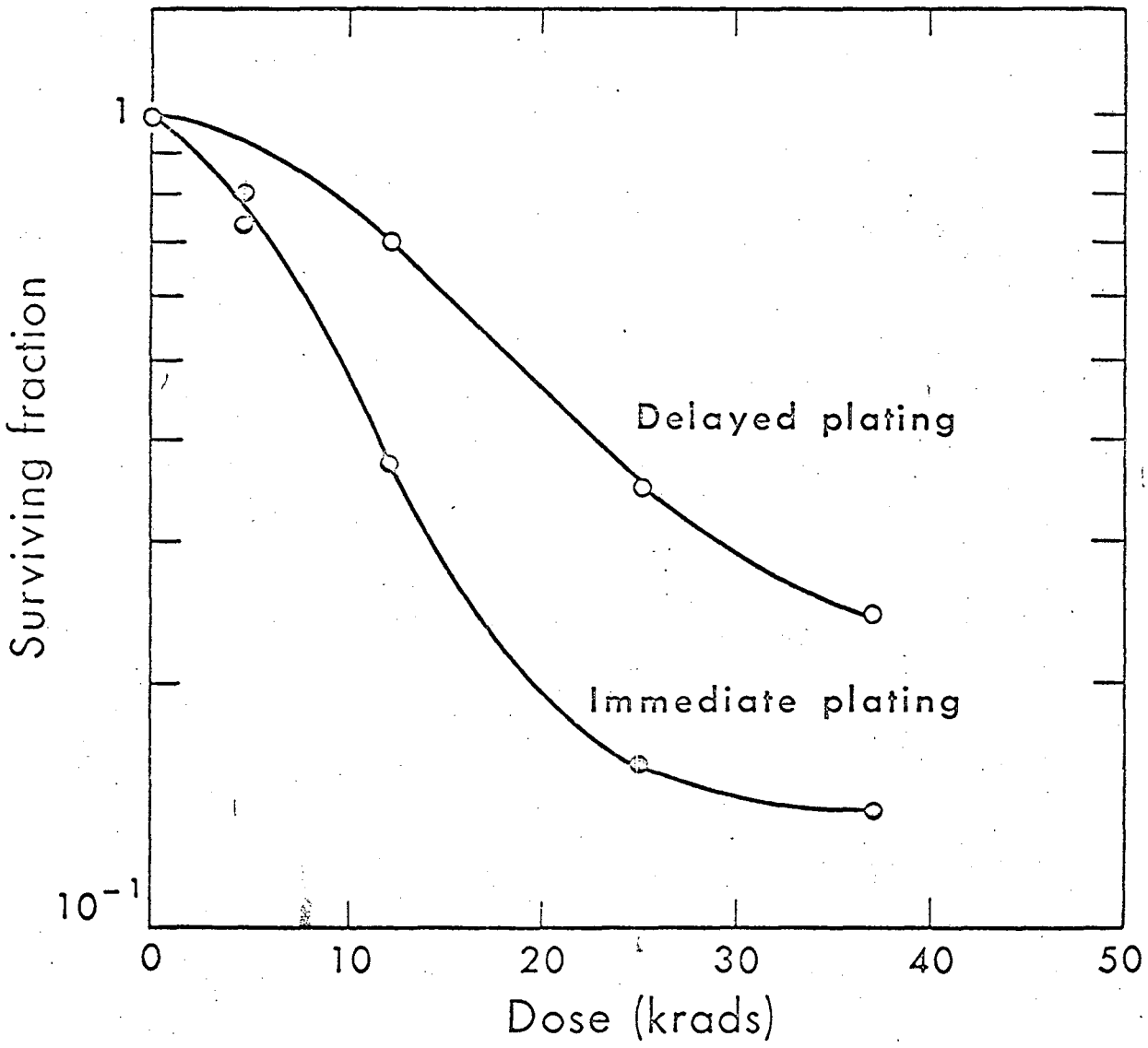


Fig. 5 MU-35525