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REPAIR OF RADIATION DAMAGE IN MAMMALIAN CELLS: ITS RELEVANCE TO ENVIRONMENTAL EFFECTS

by

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Production of energy is of public concern not only because it is essential for human activities, health and perhaps life itself, but also because of its consequences to the environment. Hence the need to identify environmental problems associated with energy production, to assess and analyze the possible adverse effects is quite obvious. Assessment of the potential biological hazards associated with energy production technologies will clearly involve the quantitation of risk on the basis of "dose-effect" dependencies, from which, it is hoped, some safety guidelines can then be developed.

Cultured mammalian cells have been useful tools in the past decade in the studies and analysis of adverse effects of ionizing and nonionizing radiations. Our current day knowledge of biological importance of damage/repair processes stems by and large from radiation studies which clearly demonstrate that cellular response to radiation (1,2,3) depends upon the ability of cells to repair the damage. Apparently, the same is true for cellular response to different chemical agents (4).

Drawing upon our experiences from radiation studies, in this review we try to demonstrate the relevance of ongoing repair processes, as evident in the studies of radiation induced cell killing and neoplastic transformation, to the type of risk estimates that might be associated with the hazards from energy production technologies.

Influence of the repair of potentially lethal damage on survival response

We consider first the effect of repair of cell survival. Cell viability is of importance in connection with mutation and malignant transformation since cell viability must be maintained in order to express

altered phenotype. Consequently, if repair of induced damage affects cell survival, it may influence the expression of other cell properties or phenotypic changes.

Detection of survival changes in the region of large doses, e.g., for doses that produce more than 90% lethality, is facilitated due to the fact that the noted effects are relatively large. This is primarily true for changes in survival as a consequence of the repair of sublethal (5) or potentially lethal damage (6-9). In the region of small doses, however, it is generally difficult to examine the effective changes in survival due to repair of sublethal or potentially lethal damage for the following reasons. As the radiation dose is smaller, the amount of sublethal damage that may be repaired is less and it is becoming more difficult to demonstrate the repair of sublethal damage as the first dose approaches zero. For potentially lethal damage, whose enhanced expression may be reflected in a change of D_0, the survival differences become progressively less as the radiation dose is smaller. The aforementioned difficulties obviously apply to any given agent whose deleterious effects as a function of dose are being estimated. It is, therefore, generally difficult to establish if the conventional interpretation of an exponential dose response (or linear) relationship is really a consequence of no repair condition. Nevertheless, the effects of repair in the region of small doses can be explored by the use of an extrapolation procedure.

Studies of the potentially lethal damage demonstrated, that when changes in survival are influenced by the changes in the growing conditions of cells after irradiation, one may infer the involvement of potentially lethal damage.

Increases in survival after single doses of radiation were demonstrated when growing conditions after irradiation favorable for repair and suboptimal for growth were used (6-9). Stationary or plateau phase cell cultures are often used in these studies and the findings may not be directly applicable to actively growing cells. Following up on an initial report by Raaphorst and Kruuv (10), Utsumi and Elkind showed (11,12) that a brief incubation of actively growing cells in anisotonic phosphate buffered saline after irradiation results in significant reduction of survival, with no concomitant effect of anisotonic treatment on viability of unirradiated cultures. Survival curves for X-ray and neutron irradiation with, and without, post-treatment consisting of 0.5 M NaCl in PBS are shown in Fig. 1. The data show large changes in survival in the mid-to-high-dose regions, i.e., in the region where a large contribution to cell killing is from the damage accumulation factor (11,13,14). It is clear that after both X-rays and fission-neutrons, although less after neutrons, posttreatment enhances what would otherwise be a potentially lethal damage. Hence, it appears that under normal postirradiation growing conditions, cells repair accumulated damage which can be potentially lethal as demonstrated by the brief postirradiation hypertonic treatment. However, to make an estimate if the initial part of the survival curves in Fig. 1 are affected, the following approach can be used. Plotting the survival ratio as a function of dose for each radiation, the plot would have a zero initial slope if the initial part of either survival curve is unaffected by the posttreatment, indicating that in a region of small doses killing is not enhanced by the change in tonicity. It is clear from Fig. 2, which shows these plots, that neither curve shows an initial region of dose in which

survival ratio is unity. The initial slopes of both curves in Fig. 2 are apparently greater than zero. Thus, the analysis of the experiments with hypertonic PBS posttreatment suggest the existence of potentially lethal damage repair process which is effective in actively growing cells under normal assay conditions (11,12) in respect to both, damage accumulation mode of cell killing and so-called "single-hit" mode (13,14).

It is evident from the foregoing that in the region of small doses repair of damage relative to cell lethality is of importance in estimating the magnitude of effect.

Effect of repair on the incidence of neoplastic transformation

Aside from the cytotoxic effects in terms of cell killing, one of the greatest concerns associated with the energy production is the potential of a given technology,or its effluents,to produce cancer. From the public hazard standpoint it is most probable that our environmental exposure to any given carcinogen will be essentially a fractionation or protraction experience. It is therefore of importance to estimate the probabilities or frequencies of effect, i.e., to quantify the risk, in this context of damage registration and possible effect of repair on damage expression.

By using appropriate mammalian cell systems in culture, it has been generally established that exposure of normal cells in culture to a variety of known carcinogens, including low and high LET radiations, results in neoplastic transformation, i.e., the conversion of normal cells that exhibit a high degree of contact inhibition and oriented growth pattern into cells that lose the growth control, begin to pile up and grow at random (15-19). In contrast to normal cells, transformed cells induce neoplasms upon inoculation into appropriate host (17-19).

We have demonstrated that in mouse embryo derived cells, C3H/10T1/2 fractionation of a dose of X-rays results in substantial reduction in the frequency of neoplastic transformation per surviving cell as a consequence of repair of the first dose subtransformation damage (19). The analysis of these data further suggested (19,20) that the repair of sublethal and subtransformation damage does not involve the same repair processes. This notion was further supported by the studies of neoplastic transformation at low dose rates of ^{60}Co γ-rays.

It is well established that, for low LET radiations, exposures at low dose rates result in a reduction in cell killing as a result of repair of sublethal damage. Survival curve of mammalian cells exposed at low dose rates exhibits reduced shoulder with decreased final slope, and if the dose rate is sufficiently low the survival curve could become exponential (21). Figure 3 illustrates the survival changes for C3H/10T1/2 cells exposed to ^{60}Co γ-rays at high and low dose rate, 100 rad/min and 0.5 rad/min, respectively. The final slope of the ^{60}Co survival curve at 0.5 rad/min is reduced 5-fold with about 43% reduced shoulder width, as compared to the survival curve following acute exposure. This reduction in cell killing is due to repair of sublethal damage at low dose rate (21,22). The changes in the incidence of neoplastic transformation per surviving cells following exposures to various doses of ^{60}Co γ-rays at acute and low dose rate are shown in Fig. 4. As can be seen, the protracted exposures of cells to ^{60}Co γ-rays results in a significant reduction in the frequencies of neoplastic radiation. If the observed changes in the frequency of neoplastic transformation were only the consequence of the repair of sublethal damage, then we would have expected quantitative changes in the incidence of neoplastic transformation to reflect primarily

the changes in cell survival. For instance, following protracted exposure (0.5 rad/min) the survival levels at total doses of 375 and 600 rads are higher by factors of 1.5 and 1.9, respectively, compared to that at acute dose rate (100 rad/min), and if the repair of sublethal damage is the only contributing factor, we would expect the transformation frequencies to decrease by the same factors. This is clearly not the case; the observed changes are greater than expected since at both total doses there is a 4-fold reduction in transformation frequency following protracted exposure as compared to the frequency for exposures at acute dose rate.

Animal experiments generally indicate reduced tumor incidence at reduced dose rates (23,24) and following fractionation of a dose of low LET radiation (25-27). Our observations with C3H/10T1/2 cells in culture are in good agreement with the animal data and furthermore lend a direct evidence for hypothesis proposed by Upton and coworkers (23) that reduced tumor incidences at low dose rates of radiation could be due to the repair of induced damage.

In conclusion, we note that from public hazard standpoint, the exposure to noxious agent associated with various energy producing technologies, including nuclear power, are almost certain to be in the region of small doses delivered in fractions or as protracted exposure. The evidence that the initial part of survival curve and the induction of neoplastic change are influenced by the repair processes should be taken into consideration when the frequencies and probabilities of the effects associated with risk estimates are made.

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Figure Legends

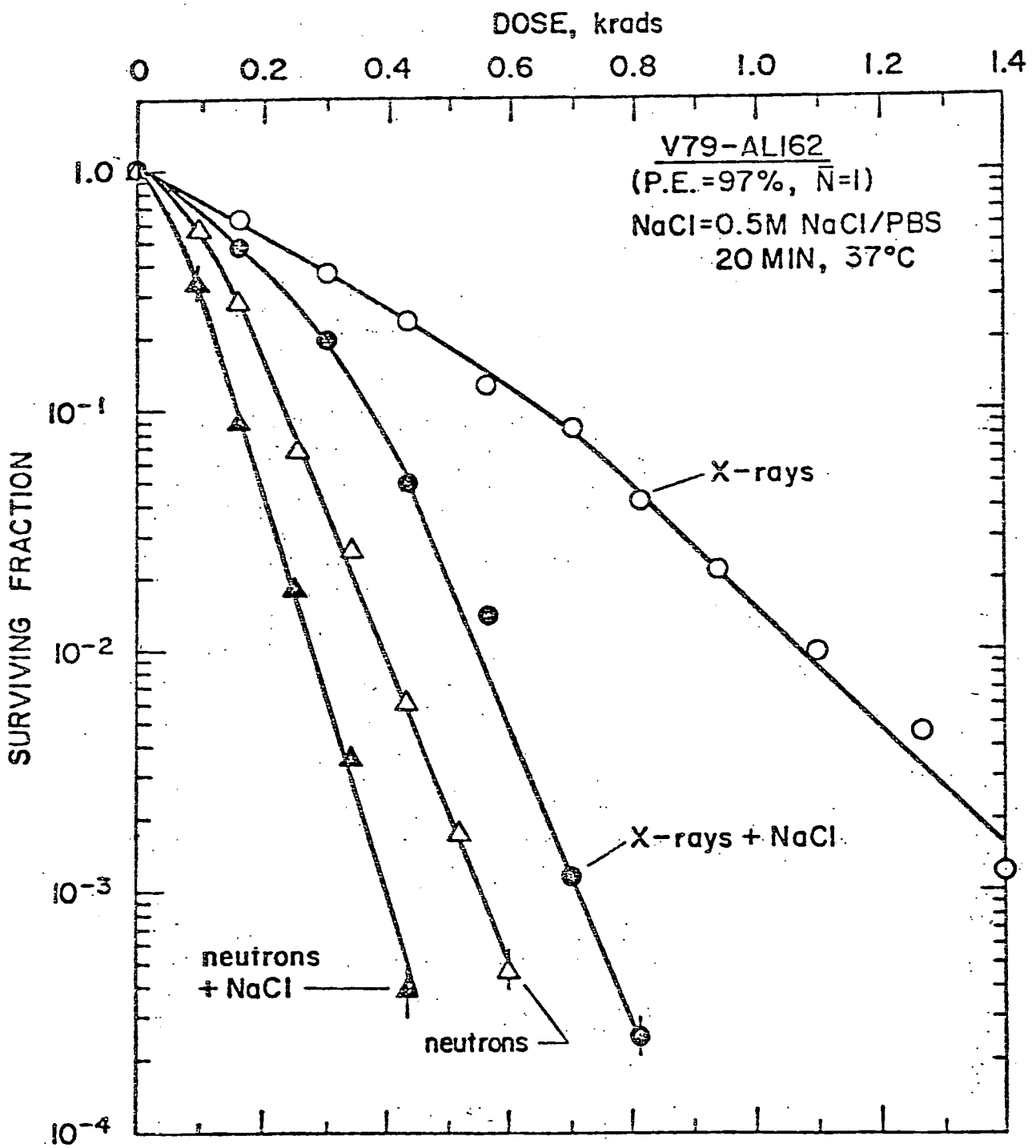
Fig. 1. Survival curves of V79-AL162 Chinese hamster cells following single doses of 50 kV X-rays or fission-spectrum neutrons. Immmediately after irradiation, cells were incubated in either isotonic PBS (○,△) or hypertonic (0.5 m NaCl PBS (●,▲). The survival curve parameters are: X-rays + isotonic PBS, D_o = 148 rad and D_q = 280 rad. X-rays + hypertonic PBS, D_o = 70 rad and D_q = 233 rad; neutron + isotonic PBS, D_o = 68 rad and D_q = 83 rad; and neutrons + hypertonic PBS, D_o = 48 rad and D_q = 53 rad. (From ref. .)

Fig. 2. Survival ratios as a function of dose for Chinese-hamster cells treated immediately after irradiation with isotonic versus hypertonic PBS. The survival ratios are obtained from the data in Figure 1. (From ref. .)

Fig. 3. Survival of C3H/10T1/2 cells exposed to different doses of ^{60}Co γ-rays at high dose rate, 100 rads/min (○), or at low dose rate, 0.5 rad/min (●). Error bars, standard errors of individual data points, are shown where they are larger than the points.

Fig. 4. Frequency of neoplastic transformation of C3H/10T1/2 cells expressed on a per surviving cell basis as a function of ^{60}Co γ-rays dose delivered at high dose rate of 100 rads/min (○), or at low dose rate of 0.5 rad/min (●). Error bars represent the standard errors of the data pooled from different experiments (2-4 per point).

FIG. 1


DOSE, krads
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
V79-AL162
(P.E.=97%, N̄=1)
NaCl=0.5M NaCl/PBS
20 MIN, 37°C
1.0
10⁻¹
10⁻²
10⁻³
10⁻⁴
SURVIVING FRACTION
X-rays
X-rays + NaCl
neutrons
+ NaCl
neutrons

2

FIG. 2.

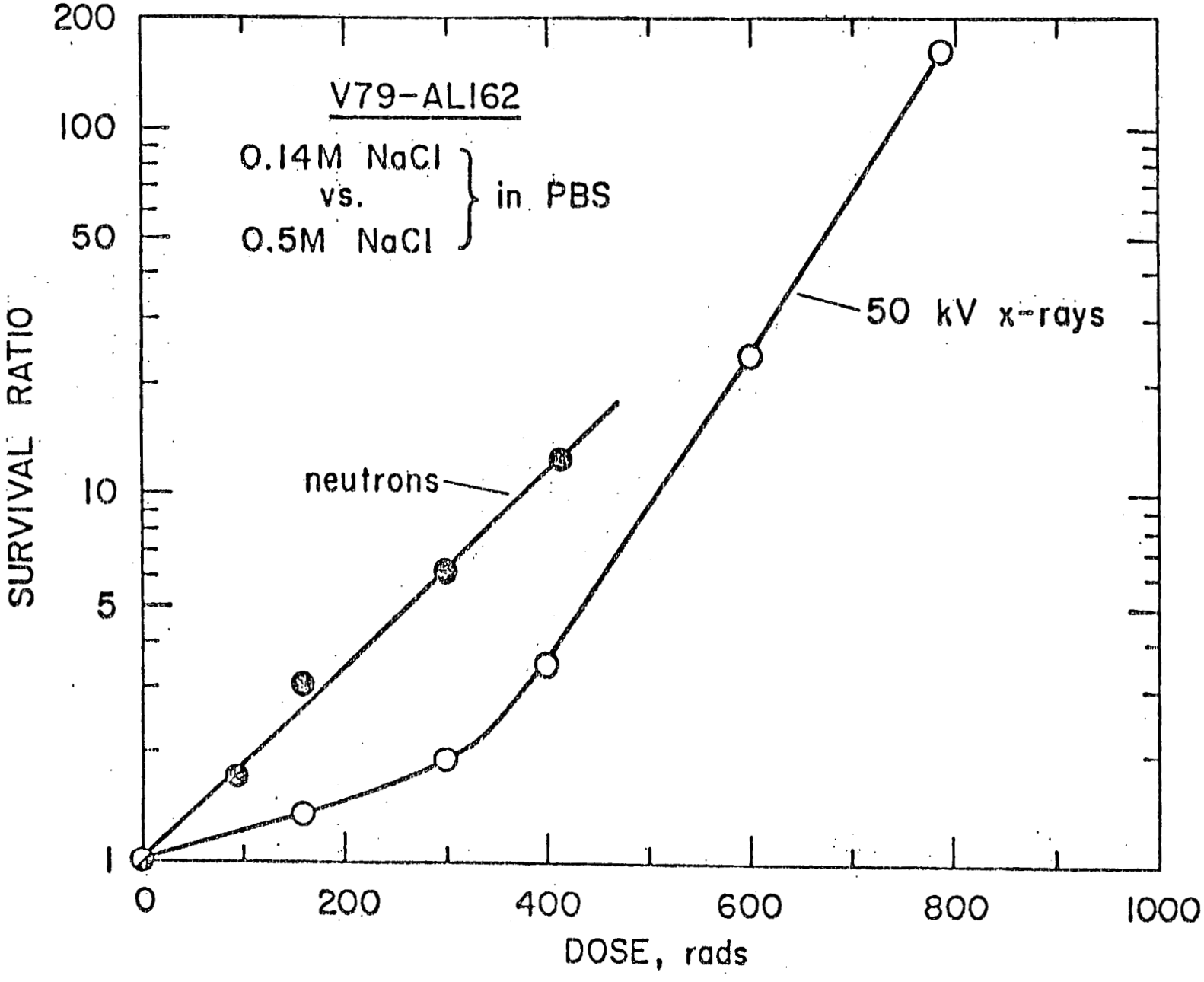

V79-AL162
0.14M NaCl
vs.
0.5M NaCl
in PBS
neutrons
50 kV x-rays
SURVIVAL RATIO
200
100
50
10
5
1
0
200
400
600
800
1000
DOSE, rads

FIG. 3

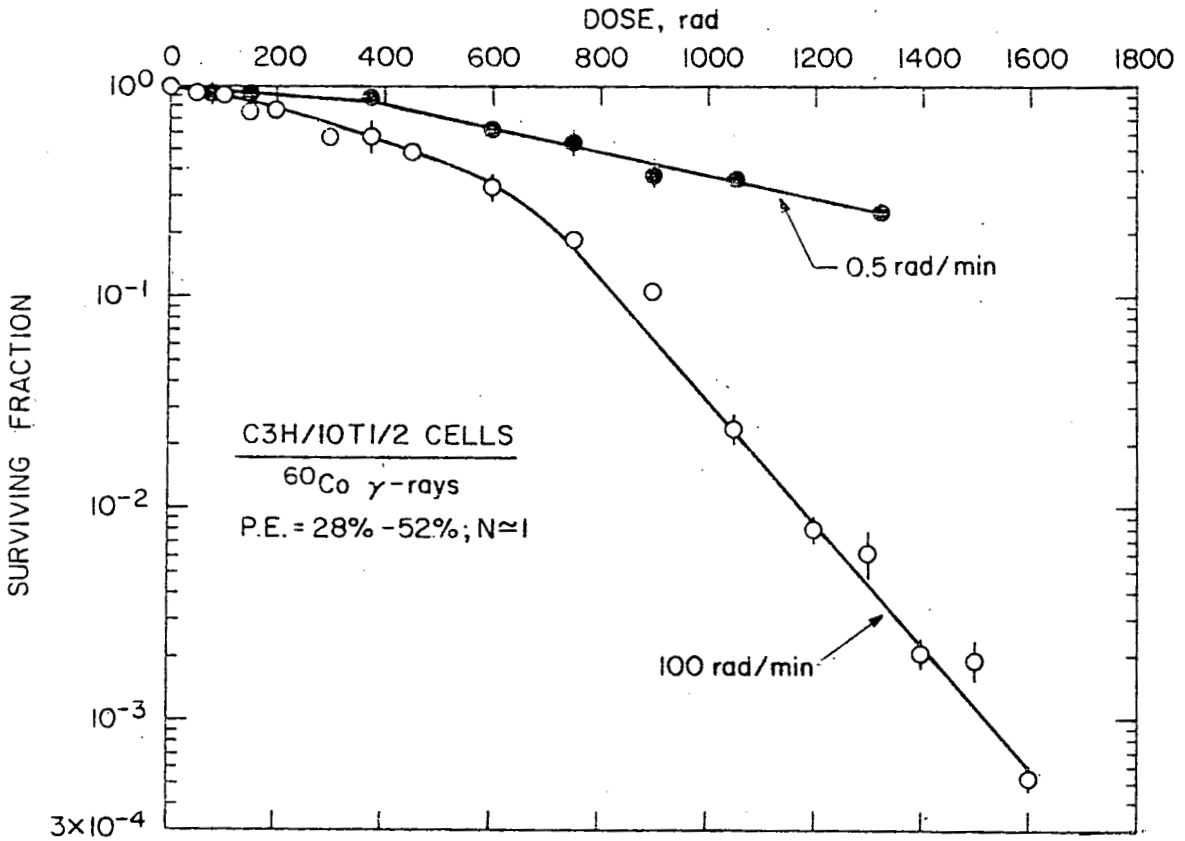
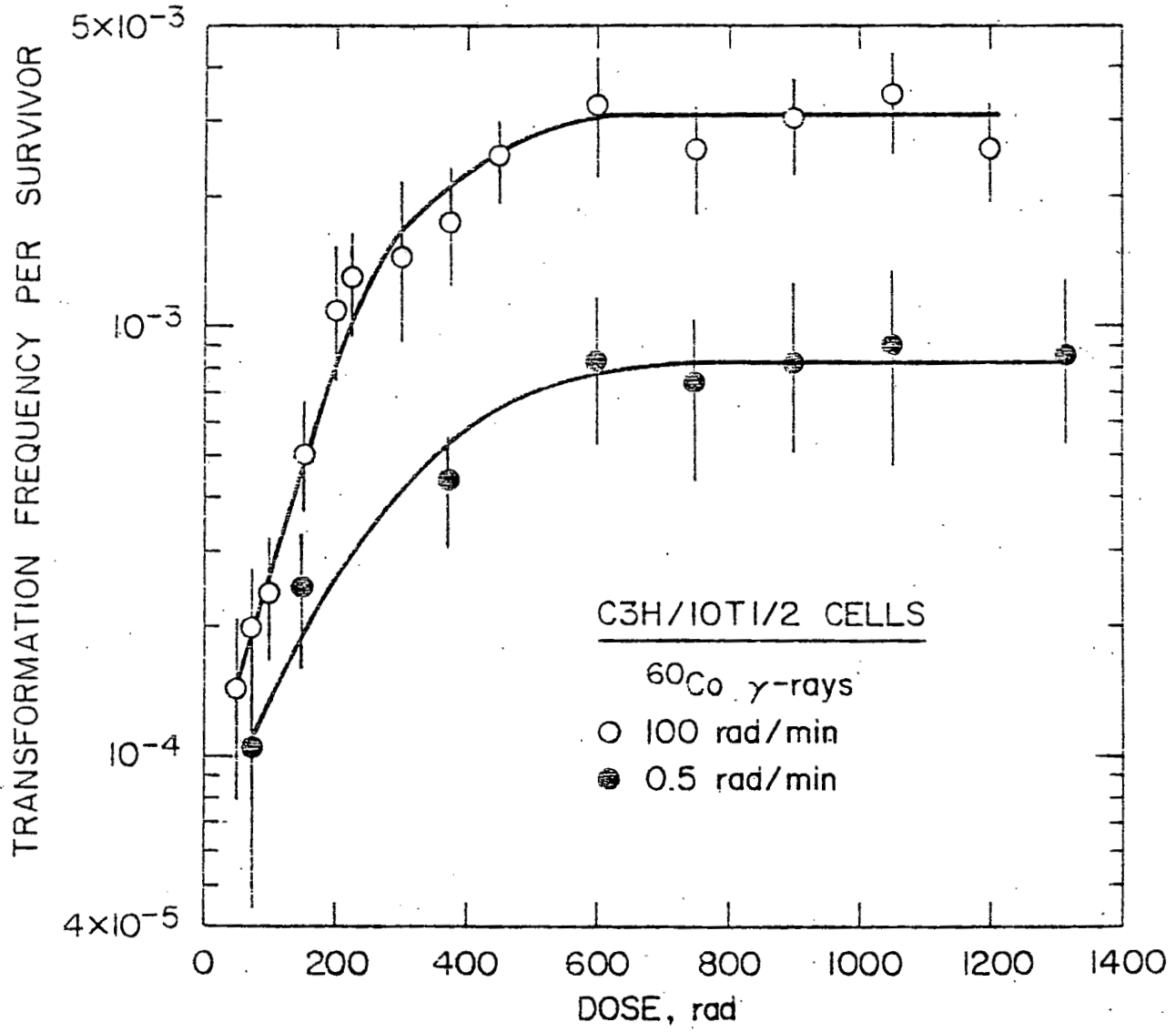

DOSE, rad
0
200
400
600
800
1000
1200
1400
1600
1800
10^0
10^{-1}
10^{-2}
10^{-3}
3×10^{-4}
SURVIVING FRACTION
0.5 rad/min
100 rad/min
C3H/10T1/2 CELLS
^{60}Co γ-rays
P.E. = 28% -52%; N≃1

FIG. 4


5×10⁻³
10⁻³
10⁻⁴
4×10⁻⁵
TRANSFORMATION FREQUENCY PER SURVIVOR
0
200
400
600
800
1000
1200
1400
DOSE, rad
C3H/10T1/2 CELLS
60Co γ-rays
100 rad/min
0.5 rad/min