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MASTER

THE EFFECT OF RADIATION QUALITY AND REPAIR PROCESSES ON THE INCIDENCE OF NEOPLASTIC TRANSFORMATION IN VITRO

by

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THE EFFECT OF RADIATION QUALITY AND REPAIR PROCESSES ON THE INCIDENCE OF NEOPLASTIC TRANSFORMATION IN VITRO

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INTRODUCTION

Studies of the effect of radiation dose fractionation on survival of mammalian cells show that X-ray dose fractionation results in a substantial increase in net survival due to the rapid repair of sublethal damage [1,2]. For high LET radiations, however, the magnitude of any net survival increase is appreciably less and may be essentially absent [3,4,5). Thus the capacity of cells to repair sublethal damage is an important factor in considering the effect of fractionation radiation exposure in the potential of surviving cells to produce cancer.

The carcinogenic potential of dose fractionation has been studied in animals (e.g., 6,7) and in recent years it has been extensively studied in vitro [8,9,10]. Using mouse embryo derived C3H/10T1/2 cells we have demonstrated that X-ray dose fractionation results in appreciable repair of cumulative damage related to transformation, i.e., subtransformation damage [10]. Considerably less reduction in neoplastic transformation is observed after fission-spectrum neutron dose fractionation [10]. This paper presents new information demonstrating that reduction in neoplastic transformation following dose fractionation is a result of repair of subtransformation damage, rather than repair of sublethal damage.

NEOPLASTIC TRANSFORMATION FOLLOWING DOSE FRACTIONATION

The effect of X-ray and fission-spectrum neutron dose fractionation on survival and frequency of neoplastic transformation is shown in Figs. 1 and 2. The effect of dose fractionation on survival shown in these two figures is qualitatively in accord with data obtained with other cell lines *in vitro* [1,3,4] and clonogenic cells *in vivo* [2,5]. Fractionation of the X-ray dose (Fig. 1) results in prompt increase in survival due to repair of sublethal damage, while fractionation of the fission-spectrum neutron dose indicates no repair of sublethal damage (Fig. 2). The number of transformants per surviving cell decreases significantly with time between X-ray exposures (Fig. 1), but only slightly with time between the two neutron exposures (Fig. 2). Frequency of neoplastic transformation after X-irradiation declines steadily with increase in time between fractions up to about 12 hr resulting in about 5-fold reduction in transformation frequency. In comparison, fractionation of neutron dose results in about 1.7-fold decrease in transformation per survivor, at most.

Since the fractionation of X-ray dose results in substantial increase in survival due to repair of sublethal damage and that of neutron dose in essentially no change in net survival because of the absence of repair of sublethal damage, it is likely that repair of sublethal damage is important, if not the only, contributing factor in the observed changes in the incidence of neoplastic transformation following X-ray and neutron dose fractionation. However the biophysical analysis of the changes in transformation frequency following low LET dose fractionation strongly suggests appreciable repair of the first dose subtransformation damage as well as sublethal damage [10,11]. Repair of first-dose transformation damage and no repair of sublethal damage are suggested by the data of fission-spectrum neutron dose fractionation.

NEOPLASTIC TRANSFORMATION AT LOW DOSE RATES OF ^{60}CO γ-RAYS

To extend, and to elucidate better our results with fractionation of X-ray dose in relation to repair of subtransformation damage, we have measured survival and incidence of neoplastic transformation at low dose rate of ^{60}Co γ-rays. For low LET radiations, exposures at low dose rate result in a reduction in cell killing as a result of sublethal damage repair, decline [12] and mutation frequency [13]. Relative to the survival curve of mammalian cells at low dose rates the shoulder on the curve is reduced, the final slope of the curve is decreased, and the survival curve could become exponential if the dose rate is sufficiently low [12]. The survival curve of 10T1/2 cells exposed to ^{60}Co γ-rays at high and low dose rate is shown in Fig. 3. The ^{60}Co survival curve of 10T1/2 cells for acute dose rate differs mainly from that reported for 50 kV X-rays [10] in that the shoulder is 1.8-fold wider. However, the D_o for both curves are about the same. At 0.5 rads/min, the final slope of the ^{60}Co survival curve is reduced by 5-fold with 43% reduction of shoulder width, as compared to the survival curve following acute exposure (100 rad/min). This reduction in cell killing is due to repair of sublethal damage during the exposure at low dose rate [12,14]. The incidence of neoplastic transformation per surviving cell following a range of single exposures of ^{60}Co at acute and low dose rate is shown in Fig. 4. As can be seen, exposure of cells to ^{60}Co γ-rays at low dose rate results in a significant reduction in the frequencies of neoplastic transformation. The single dose transformation curve for acute exposures of ^{60}Co γ-rays and 50 kV X-rays [10] are the same within the uncertainties of available data thus far.

The observed reduction in transformation exceeds the decrease that one would expect if the repair of sublethal damage is the only factor contributing to the reduction of neoplastic transformation. If this had been the case, we would expect quantitative changes in the incidence of

neoplastic transformation to follow closely those in cell survival. For example, the survival levels at total doses of 375 and 600 rads are higher by a factor of 1.5 and 1.9, respectively, at low dose rate as compared to that at 100 rads/min. We would expect then, if the repair of sublethal damage is the only contributing factor, the transformation frequency to decrease at this dose levels to the same extent. It is clear that the observed changes far exceed this expectation. At both doses there is a 4-fold reduction in transformation frequency at 0.5 rad/min as compared to the frequency following acute exposures. Assuming that survival properties of normal and potentially transformed cells are essentially the same [10], the data strongly suggest appreciable repair of subtransformation damage. The repair process involved is very likely different from that responsible for repair of sublethal damage and the two processes seem to have different time dependencies.

The results described are also relevant to tumor induction in animals. Although the proportion of cells that survive a particular dose of radiation in given tissue is not known, animal experiments generally indicate reduced tumor incidence following dose fractionation of low LET radiation [6,7] or at reduced dose rates [15-17]. The ^{60}Co-induction curves for acute and low dose rate (Figs. 3 and 4) when expressed on a per irradiated cell basis (to be presented elsewhere) are qualitatively similar to data for induction of myeloid leukemia in mice [16].

Finally, since our environmental exposure to ionizing radiation is more likely to be a fractionation or protraction experience, rather than in a single dose, the results of this study and interpretations derived from them are also important from the radiation hazard evaluation standpoint.

ACKNOWLEDGMENT

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REFERENCES

1 ELKIND, M. M., SUTTON, H. A., Radiat. Res. 13:556 (1960)

2 WITHERS, H. R., ELKIND, M. M., Radiat. Res. 38:598 (1969)

3 ELKIND, M. M., Curr. Topics Radiat. Res. 7:1, North Holland Publishing Co., Amsterdam (1970)

4 NGO, F. Q. H., UTSUMI, H., HAN, A., ELKIND, M. M., Int. J. Radiat. Biol (1979) in press

5 WITHERS, H. R., OLIVER, G. D., GLENN, D. W., Radiology 103:741 (1972)

6 METALLI, P., SILINI, G., CASTILLO, S., COVELLI, V., Radiation Induced Cancer IAEA-SM-118/4 (International Atomic Energy Agency) 1969

7 BURNS, F. J., VANDERLAAN, M., Int. J. Radiat. Biol. 32:135 (1977)

8 BOREK, C., HALL, E. J., Nature 252:499 (1974)

9 TERZAGHI, M., LITTLE, J. B., Int. J. Radiat. Biol. 29:583 (1976)

10 HAN, A., ELKIND, M. M., Cancer Res. 39:123 (1979)

11 ELKIND, M. M., HAN, A., Radiat. Res. (1979) in press

12 FOX, M., NIAS, A. H. W., Curr. Topics Radiat. Res. 7:71, North Holland Publishing Co., Amsterdam (1970)

13 RUSSELL, W. L., Recovery and Repair Mechanisms in Radiobiology. Brookhaven Symposia 20:179 (1967)

14 BEN-HUR, E., ELKIND, M. M., BRONK, B. V., Radiat. Res. 58:38 (1974)

15 SHELLABARGER, C. J., BOND, V. P., APOUTE, G. E., CRONKITE, E. P., Cancer Res. 26:509 (1966)

16 UPTON, A. C., RANDOLPH, M. L., CONKLIN, J. W., Radiat. Res. 41:467 (1970)

17 YUHAS, J. M., Radiat. Res. 60:321 (1974)

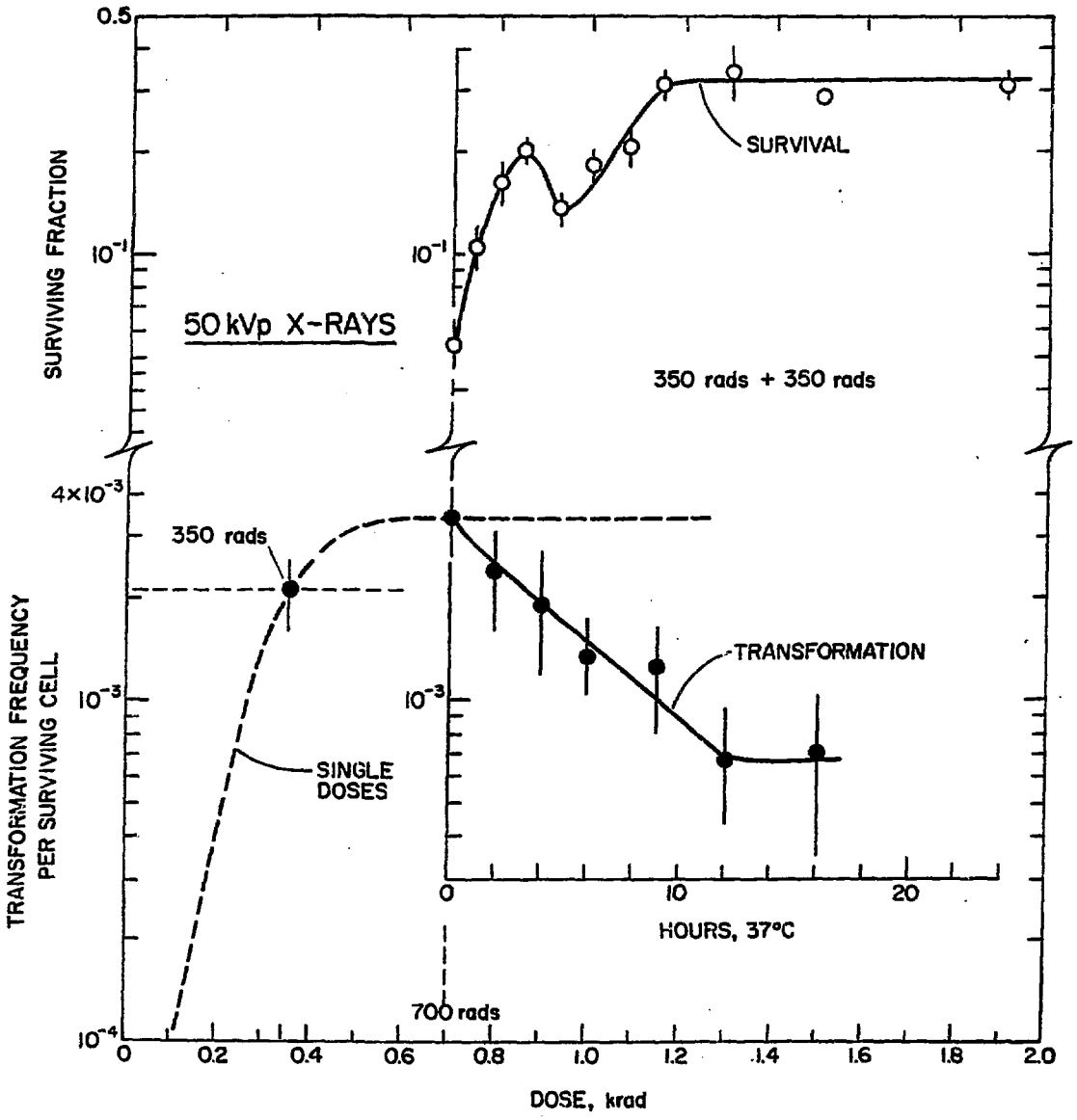
LEGENDS

Fig. 1. Changes in survival (○) and the frequency of neoplastic transformation per survivor (●) in C3H/10T1/2 cells exposed to fractionated doses of 50 kV X-rays. Error bars represent standard errors of the mean of the data pooled from different experiments. The dashed line indicates single dose induction curve (from 10). Inset abscissa, time at 37°C between the two exposures; the 0 fractionation time is plotted from the total dose of 700 rads. (From A. Han and M. M. Elkind, Cancer Res. 39, 123-130, 1979.)

Fig. 2. Changes in survival (○) and the frequency of neoplastic transformation per survivor (●) in C3H/10T1/2 cells exposed to fractionated doses of fission-spectrum neutrons. (Error bars and inset abscissa as in Fig. 1.) The 0 fractionation time is plotted from the total dose of 378 rads. (From A. Han and M. M. Elkind, Cancer Res. 39, 123-130, 1979.)

Fig. 3. Survival of C3H/10T1/2 cells exposed to different doses of ^{60}Co gamma rays at acute 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 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).


0.5
10^{-1}
SURVIVING FRACTION
50 kVp X-RAYS
SURVIVAL
350 rads + 350 rads
4×10^{-3}
350 rads
10^{-3}
SINGLE DOSES
TRANSFORMATION
TRANSFORMATION FREQUENCY PER SURVIVING CELL
0
10
20
HOURS, 37°C
700 rads
10^{-4}
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
DOSE, krad

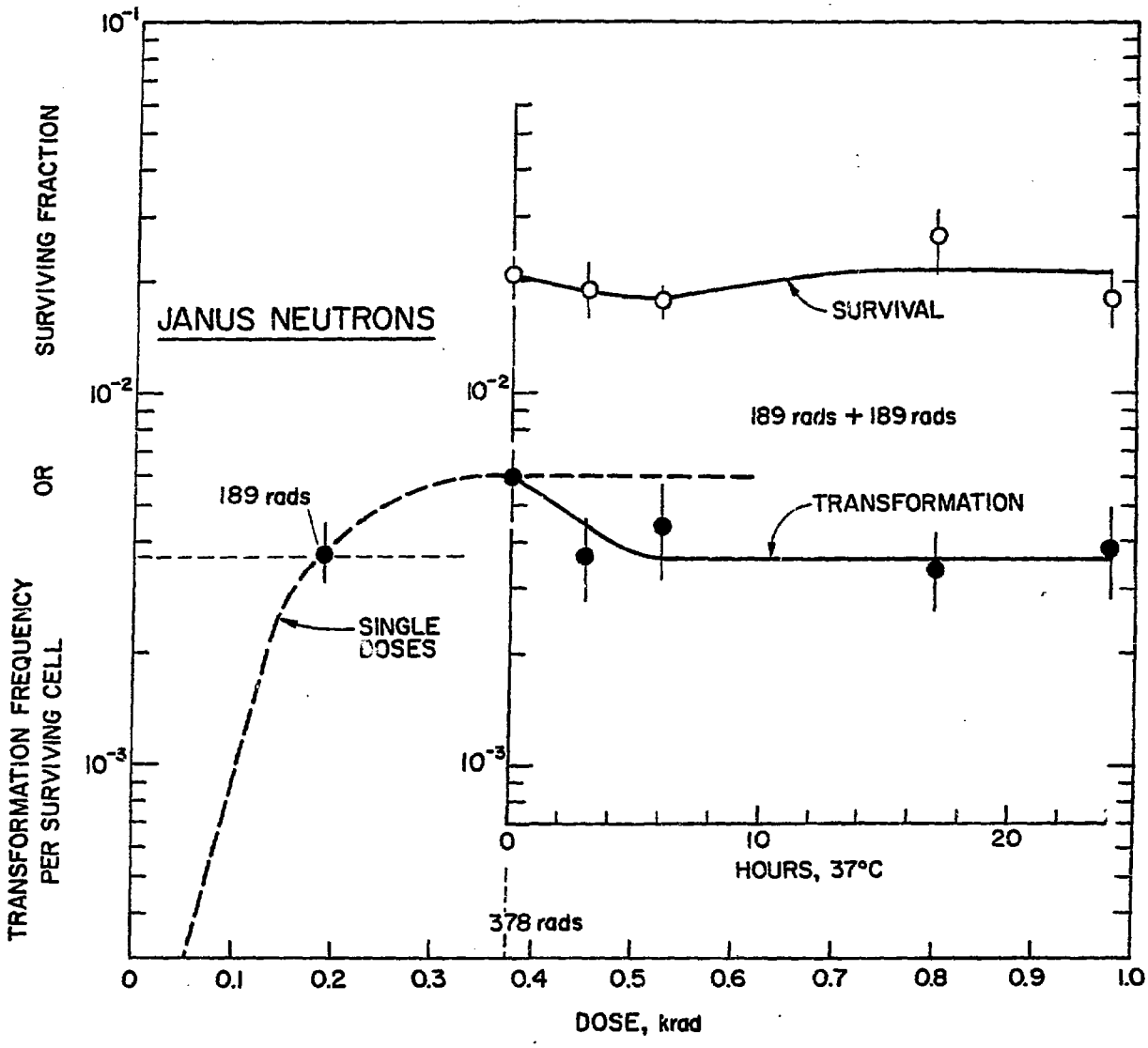

JANUS NEUTRONS
SURVIVING FRACTION
OR
TRANSFORMATION FREQUENCY PER SURVIVING CELL
189 rads
SINGLE DOSES
SURVIVAL
189 rads + 189 rads
TRANSFORMATION
HOURS, 37°C
378 rads
DOSE, krad

FIG. 3.

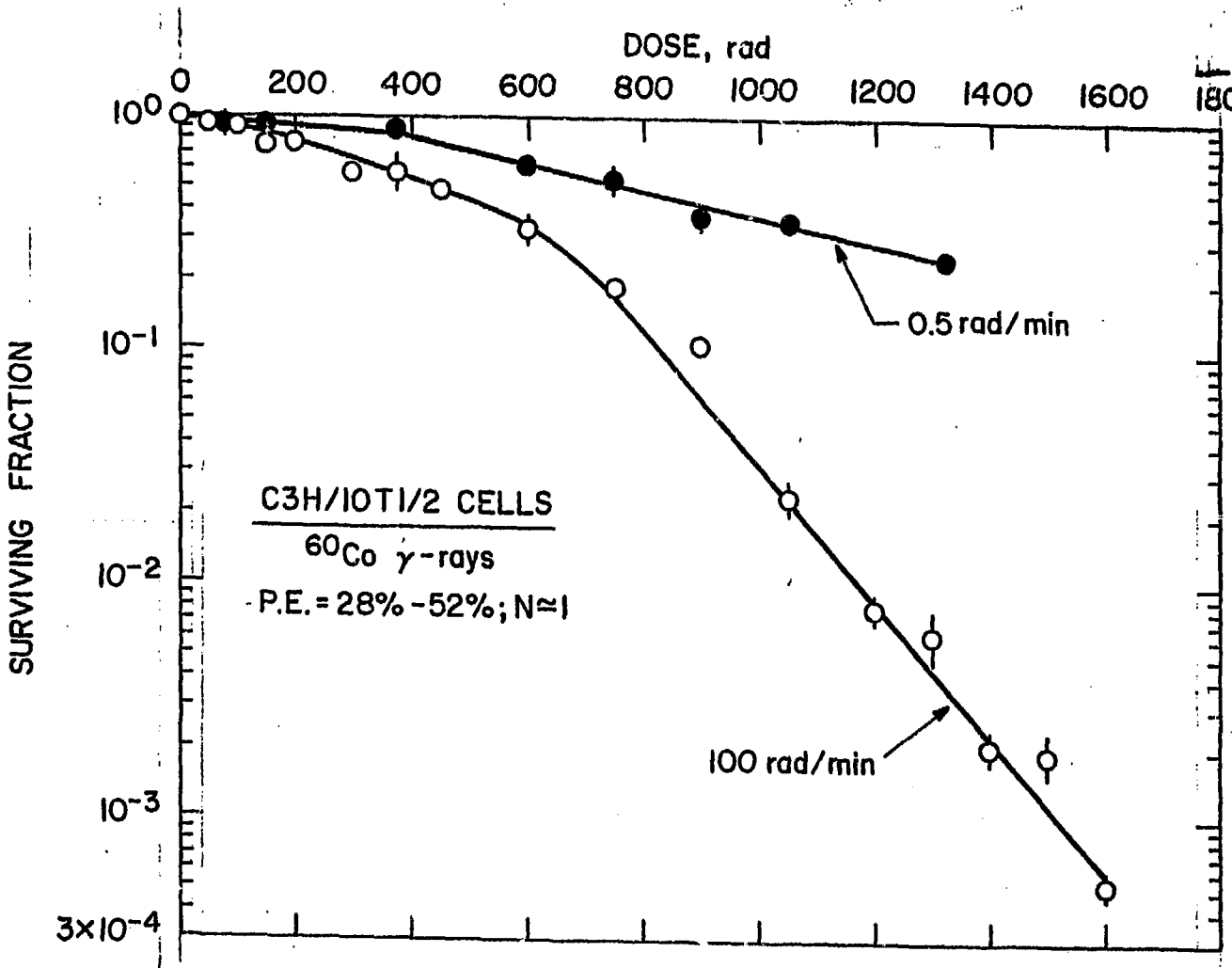
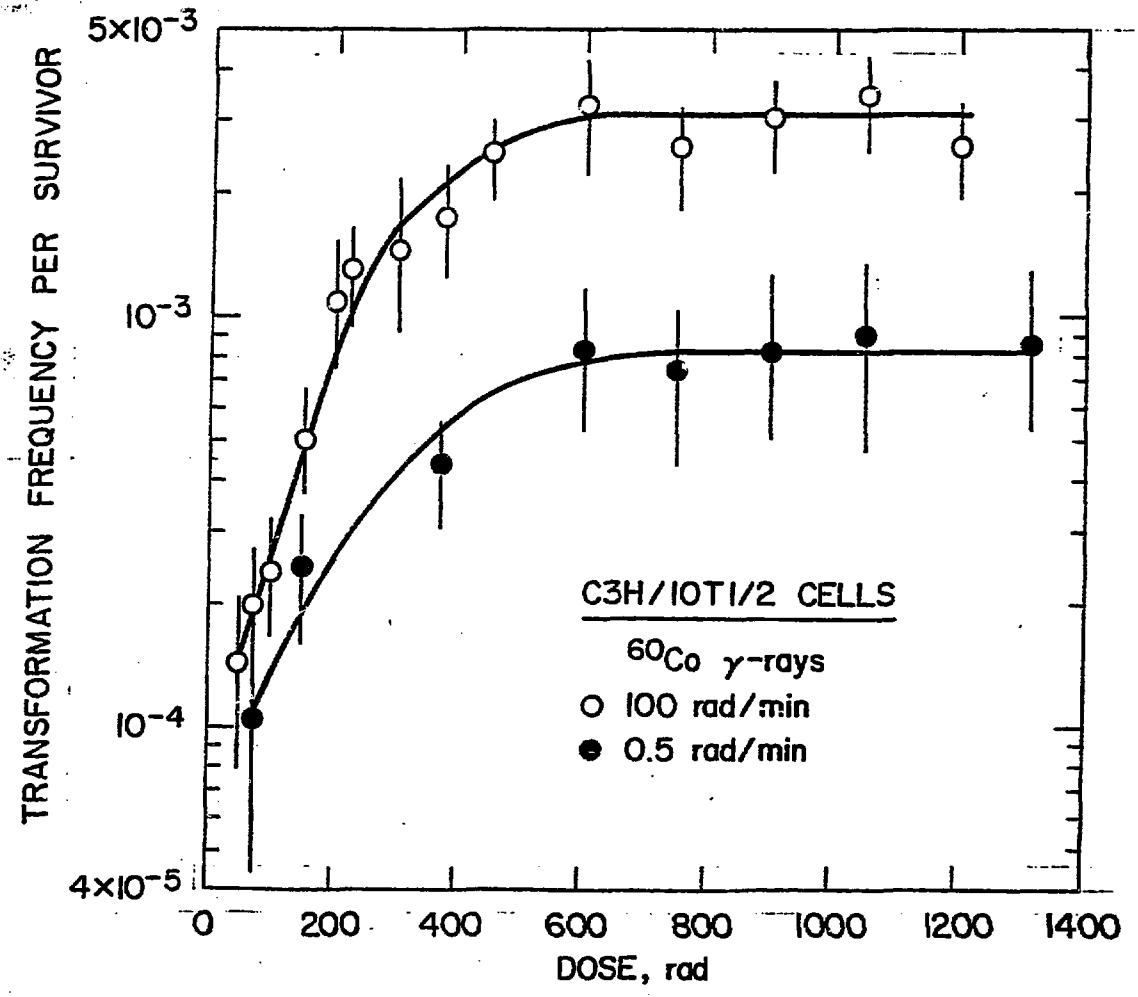

DOSE, rad
0
200
400
600
800
1000
1200
1400
1600
1800
SURVIVING FRACTION
100
10⁻¹
10⁻²
10⁻³
3×10⁻⁴
C3H/10T1/2 CELLS
⁶⁰Co γ-rays
P.E. = 28% - 52%; N≈1
0.5 rad/min
100 rad/min

FIG. 4.

A. Han


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