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Repair of neoplastic transformation damage following protracted exposures to ^{60}Co γ-rays

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Running title: Repair of neoplastic transformation

Summary The incidences of neoplastic transformation induced by ^{60}Co γ-rays in exponentially growing mouse embryo 10T1/2 cells were measured following acute and protracted exposures. Delivery of ^{60}Co γ-rays at a low dose rate (0.1, 0.5, 2.5 rad/min) compared with a high dose rate (100 rad/min) results in appreciable, dose rate dependent reductions in cell killing and, independent of the effect on cell survival, reduces significantly the incidence of neoplastic transformation. Exposure of exponentially growing 10T1/2 cells to a dose of γ-rays in five equal daily fractions also significantly reduces transformation frequency, compared with delivery in a single dose, throughout the dose range examined (25-300 rads). The initial parts of the induction curves are fitted quite well by a linear dose dependence. The slopes of the regression lines for multifractionation delivery or irradiation at 0.1 rad/min, are one-third and one-half, respectively, of those for single exposures at a high dose rate. Increasing the interfraction interval up to 48 hours, or reduction of the dose per fraction further reduce incidence of neoplastic transformation. We conclude that protracted exposures of low LET radiation result in a net "error-free" repair of subtransformation damage.

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DISCLAIMER

Introduction

It is well established that delivery of low LET radiation at reduced rates is less effective than acute irradiation in tumor induction in animals (Upton et al., 1970; Ullrich & Storer, 1979). We reported earlier that two-dose fractionation of X-rays reduces neoplastic transformation in 10T1/2 cells (Han & Elkind, 1979) and, consistent with this observation, we also found that the incidences of transformation were appreciably reduced when ^{60}Co γ-rays were delivered at a low dose rate (Han et al., 1980). Our results thus provide a cellular basis for understanding and interpreting dose rate dependent changes in tumor induction by radiation in vivo. The results of in vitro experiments can provide important insights concerning the biological mechanisms of radiation action, and are relevant to the basic concepts used in the assessment of the carcinogenic hazard of radiation exposure.

Materials and methods

The experiments were performed with mouse embryo-derived C3H/10T1/2 cells developed by Reznikoff and co-workers (1973a). Cell culture stocks were routinely grown at 37°C in large plastic T-flasks (75 cm^2) in Eagle's basal medium (Gibco) supplemented with 10% heat inactivated fetal calf serum. (Sterile Systems, Inc.). Details of our experimental techniques for the assay of transformation and methods used to calculate the frequencies have been published (Han & Elkind, 1979; Han et al., 1980). Neoplastic transformation in 10T1/2 cells is expressed by the appearance of densely piled up colonies of cells showing disorganized crissed-crossed pattern of growth (Reznikoff et al., 1973a); cells from these foci produce fibrosarcomas in mice (Reznikoff et al., 1973b; Han & Elkind, 1979).

To assess the effect of protracted exposures to ^{60}Co γ-rays on neoplastic transformation cells were exposed to various total doses, delivered in a single exposure at a high dose rate (50 or 100 rad/min) or at a low dose rate (0.1, 0.5, or 2.5 rad/min). Also, total doses from 25 to 300 rad were delivered in five equal fractions separated by 24 or 48 hours, or in 10 equal daily fractions. In all experiments involving protracted irradiation, cells were inoculated at low density and were allowed to grow for at least 48 hours before the beginning of the irradiations to ensure that the cells were in exponential growth. For single or fractionated delivery at a high dose rate, irradiations were carried out at room temperature (~ 20°C) and the cells were kept at 37°C between irradiations. For exposures at low dose rates, cells were kept at 37°C in an incubator throughout irradiations. Immediately after irradiation, suspensions of cells were prepared by trypsinization; after appropriate dilutions, between 150-250 viable cells were inoculated per 90-mm dish for determination of neoplastic transformation (Han & Elkind, 1979). Survival was measured by colony formation and for any given radiation protocol, transformation and survival were determined in the same experiment.

Irradiations were carried out with a Gammabeam-650 irradiator (Atomic Energy of Canada, Ltd.). The source and dosimetry were previously described in detail (Han et al., 1980).

Results and Discussion

The survival curves of 10T1/2 cells exposed to ^{60}Co γ-rays at high (100 rad/min) and low (0.1, 0.5, 2.5 rad/min) dose rates are shown in Figure 1. Consistent with our finding of survival sparing following fractionation of X-ray dose in these cells (Han & Elkind, 1979), irradiation at low dose rates results in higher survival, and changes in the final slopes of the survival

curves are dose-rate dependent. The survival changes in Figure 1 are qualitatively in agreement with other studies reported for mammalian cells (see Fox & Nias, 1970, for review), and are a consequence of the repair of sublethal damage during exposure (Fox & Nias, 1970).

Reductions in dose rate result in significant reductions in the frequencies of neoplastic transformation per surviving cell (Fig. 2, panels B,C,D). Although the shapes of the induction curves at low dose rates are similar to that for acute exposures (Figure 2, panel A), the curves for low dose rates exhibit systematic reductions in the incidences of transformation as the dose rate decreases. When transformation frequencies are expressed on a "per surviving cell" basis, it is possible that decrease in the incidence is influenced by the repair of sublethal damage. However, increase in survival, at reduced dose rates, alone cannot fully account for the reductions in frequencies shown in Figure 2, because if this had been the case, we would have expected the quantitative changes in the incidence of neoplastic transformation to reflect primarily the changes in cell survival, and this expectation is clearly not supported by the data. However, it is important to distinguish between the effects of the repair of sublethal damage from the possible influence of the repair of subtransformation damage.

An analysis that enables such distinction is based on a proposal introduced by Gray (1965) that radiation tumorigenesis in vivo consists primarily of two concomitant processes: the tumor induction and the killing of target cells. Because the induction process dominates the low dose region where the survival is relatively high, and the killing process influences the high dose region due to the increased killing of target cells, the tumor induction curves typically would have a bell shape (e.g., Mole, 1983). When incidences of transformation are expressed on a "per exposed cell" basis,

i.e., when cell killing is not accounted for, and are plotted on linear coordinates as in Figure 3, the resulting curves indeed have shapes qualitatively similar to those for tumor induction in vivo, e.g., leukemia induction (Upton et al., 1970; Mole, 1983). Using the high dose rate induction curve (Figure 3A) and Gray's hypothesis, we can consider the qualitative influences of the repair of sublethal damage only, or that plus the repair of subtransformation damage on the incidences of transformation.

Tumor induction depends upon the number of cells at risk, but the fraction of cells in vivo that survive given radiation protocol is not known, whereas the survival curves of 10T1/2 cells at high and low dose rates were measured. Therefore we can estimate the absolute frequency of transformation by dividing observed frequency per exposed cell by the surviving fraction as a function of dose. The curve labeled absolute frequency in Figure 3A is derived in this way, and is the same as that in Figure 2A (except for the change in the scale of the ordinate). By this approach we can estimate the incidences of transformation if the only dose rate-dependent variable is the surviving fraction. Using the absolute frequency curve of the high dose rate, and accounting for cell survival at a reduced dose rate, we obtain the curves labeled (abs. freq.)·(sur. frac.) that lie below the absolute frequency curve (Figure 3B,C,D). These curves approximate the induction curves as a consequence of the repair of sublethal damage only. Thus, we may generalize that the induction curve should fall between the observed high dose rate curve and the absolute frequency curve if the incidence of transformation is influenced only by the sparing in cell killing. However, if subtransformation damage is repaired, in addition to sublethal damage, the low dose-rate induction curve should lie below the curve that traces the effect of sublethal damage repair only. It is clear that the low dose rate results

(Figure 3B,C,D) are consistent with the repair of subtransformation damage in addition to the repair of sublethal damage. The two repair processes, as indicated by our results published elsewhere (Han & Elkind, 1979), may not be entirely the same.

Irradiation at reduced dose rates may be approximated by radiation exposures to multiple fractions at a high dose rate. In addition to being of interest for further understanding of the cellular mechanisms of radiation tumorigenesis, the multifractionation mode of radiation delivery is also relevant to many epidemiological studies. Therefore, to explore further the effect of dose protraction on neoplastic transformation, 10T1/2 cells were exposed to total doses from 25 to 300 rads delivered in different numbers of fractions and with different time intervals between fractions. The main radiation protocol consisted of delivery of total doses from 25 to 300 rads in five equal fractions separated by 24 hour intervals. Figure 4 shows the incidences of transformation for cells given a single exposure at 0.1 rad/min (closed circles) and for cells that received a given dose at high dose rate either as a single dose (open circles), or in five daily fractions (open squares). The low dose data are replotted from the initial portion of the corresponding induction curve from Figure 2D to show more clearly the reduction of transformation in the low dose region (25-150 rads) at 0.1 rad/min. Throughout the dose range shown, and regardless of the protraction mode of radiation delivery, incidences of transformation are reduced and most clearly indicate significant repair of subtransformation damage.

As indicated by the correlation coefficients in Figure 4, all the data can be fitted quite well by a straight line. The slopes of the lines for low dose rate (0.1 rad/min) and for fractionated irradiation are about twofold and threefold, respectively, less steep than the slope of the line for single

exposures at 100 rads/min. The observed reductions in slope (Figure 4) indicate that repair of subtransformation damage can occur even in the dose range where transformation is linearly dependent upon dose. The linear dose dependencies, in general, lead to the inference that they are nonmodifiable by repair processes, and are dose-rate independent, due to the all-or-nothing character of single hit action. Our data for cell transformation clearly illustrate the potential role for repair processes in the dose range where the effect varies linearly with dose, as has been already noted on theoretical grounds (Elkind, 1977).

Even though the frequencies of neoplastic transformation are quite low after radiation exposures in five daily fractions, we have extended the protraction of the same total doses to five fractions separated by 48 hours (total dose delivered over 8 days), and to 10 fractions separated by 24 hours (total dose delivered over 9 days) to examine whether further decreases in transformation occur (Table I). The data for these two radiation protocols indicate further reduction in transformation frequencies from that shown in Figure 4 for five daily exposures. However, the observed numbers of transformed foci, thus far, are small and the errors are quite large, so that the calculated frequencies are indistinguishable from the background. Nevertheless, the general trend of the data (Table I) seems to suggest further decrease in transformation with increased protraction of radiation delivery.

Finally, from a radiobiological standpoint the data for low dose rate irradiation and fractionation are consistent with earlier work that shows reduced transformation frequencies as a result of splitting a large total dose into two fractions (Han & Elkind, 1979), and are also in agreement with several animal studies that show reduced effects of protracted low LET irradiation in tumor induction (e.g., Upton et al., 1970; Ullrich & Storer,

1979). The results also suggest that, for extended periods of radiation protraction and small doses per fraction, in contrast to the two-fraction data for 5-hour interfraction interval (Borek & Hall, 1974; Miller et al., 1979), a definite reduction in transformation occurs which is linearly dependent on dose to at least a total dose of 300 rads. This is consistent with the hypothesis of net error-free repair of the low LET subtransformation damage.

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TABLE I

TRANSFORMATION FREQUENCIES OF 10T1/2 CELLS
EXPOSED TO SINGLE AND FRACTIONATED DOSES OF ^{60}Co GAMMA-RAYS

TOTAL DOSE RAD	TRANSFORMATION FREQUENCY PER SURVIVOR X 10^{-4}							
	SINGLE DOSE	RANGE 1 S.E.	5 FRACTIONS/ 4 DAYS	RANGE 1 S.E.	5 FRACTIONS/ 8 DAYS	RANGE 1 S.E.	10 FRACTIONS/ 9 DAYS	RANGE 1 S.E.
0	0.103	0.15 0.06	--	--	--	--	--	--
25	0.70	0.98 0.42	0 (<0.25)	--	0.26	0.52 0	0 (<1.0)	--
50	1.18	1.52 0.84	0.25	0.5 0	0.28	0.56 0	0 (<1.5)	--
75	2.37	3.11 1.63	0.56	0.93 0.17	0 (<0.30)	--	0 (<1.9)	--
150	4.96	6.56 3.36	1.37	2.16 0.58	0.72	1.23 0.21	1.66	3.20 0
300	12.4	15.6 9.2	2.37	3.55 1.19	0.71	1.42 0	2.57	4.38 0.76

Figure Legends

Figure 1. Survival curves of 10T1/2 cells exposed to ^{60}Co γ-rays at the high dose rate of 100 rads/min (●) and low dose rates of 2.5 (○), 0.5 (■) and 0.1 rad/min (□). Bars, S.E. of individual data points are shown where they are longer than the points; P.E., plating efficiency; $\overline{N}$, multiplicity. [From A. Han, C. K. Hill & M. M. Elkind (1980) Cancer Res., 40, 3328.]

Figure 2. Frequency of neoplastic transformation frequencies of 10T1/2 cel#s induced by acute (A) and low dose rate irradiation with ^{60}Co γ-rays. B, 2.5 rads/min; C, 0.5 rad/min; D, 0.1 rad/min. Bars, S.E. of the pooled data from 2-4 experiments; ----, induction curve for acute exposures from A. [From A. Han, C. K. Hill & M. M. Elkind (1980) Cancer Res., 40, 3328.]

Figure 3. Transformation frequencies of 10T1/2 cells expressed per irradiated cell exposed to ^{60}Co γ-rays delivered at high (A) and low (B,C,D) dose rates. ----, in B,C,D indicates the curve labeled absolute frequency from A (see text). Uncertainties are the same as in Figure 2, but are omitted for clarity. (abs. freq.) x (surv. frac.), absolute frequency x surviving fraction. [From A. Han, C. K. Hill & M. M. Elkind (1980) Cancer Res., 40, 3328.]

Figure 4. The induction of neoplastic transformation in 10T1/2 cells by ^{60}Co γ-rays: (○) single exposure at 100 rad/min; (●) single exposure at 0.1 rad/min; (□) five fractions separated by 24 hrs, at 50 rad/min. Bars indicate standard error.

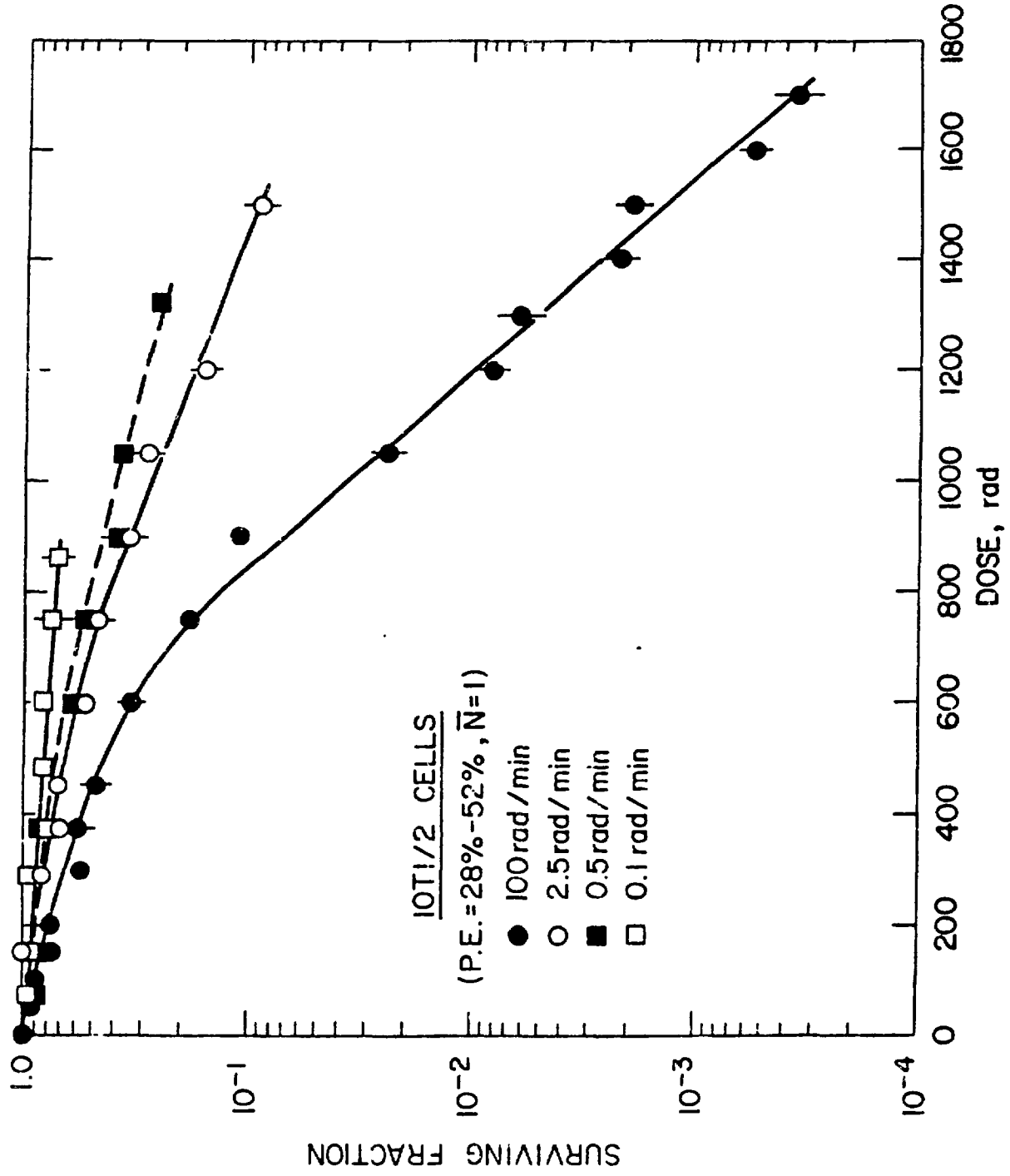
SURVIVING FRACTION
1.0
10^{-1}
10^{-2}
10^{-3}
10^{-4}
0
200
400
600
800
1000
1200
1400
1600
1800
DOSE, rad
IOT1/2 CELLS
(P.E.=28%-52%, $\bar{N}$=1)
100 rad/min
2.5 rad/min
0.5 rad/min
0.1 rad/min

Fig. 1

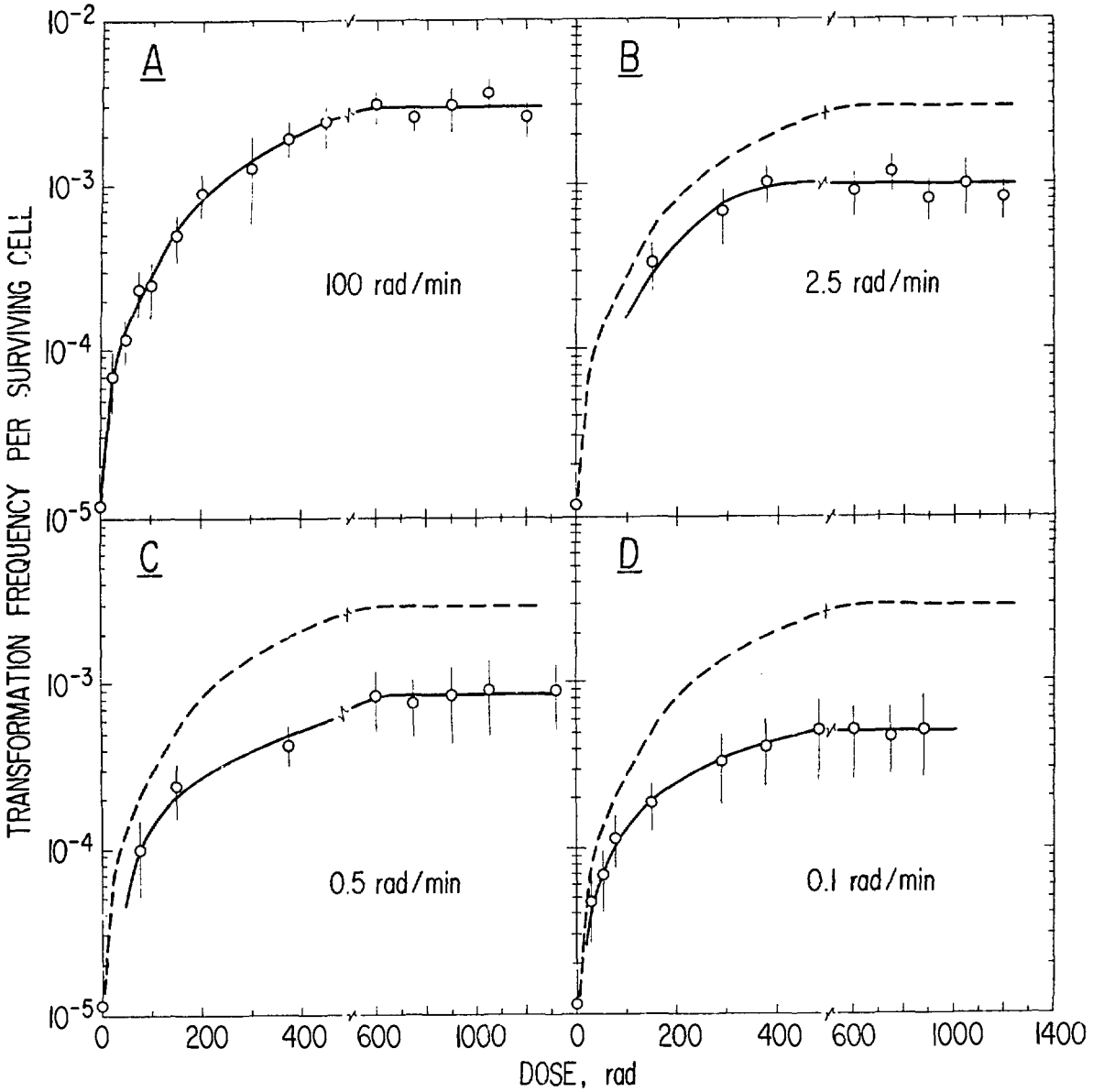
A
B
C
D
100 rad/min
2.5 rad/min
0.5 rad/min
0.1 rad/min
TRANSFORMATION FREQUENCY PER SURVIVING CELL
DOSE, rad
10-2
10-3
10-4
10-5
0
200
400
600
1000
1400

Fig. 2

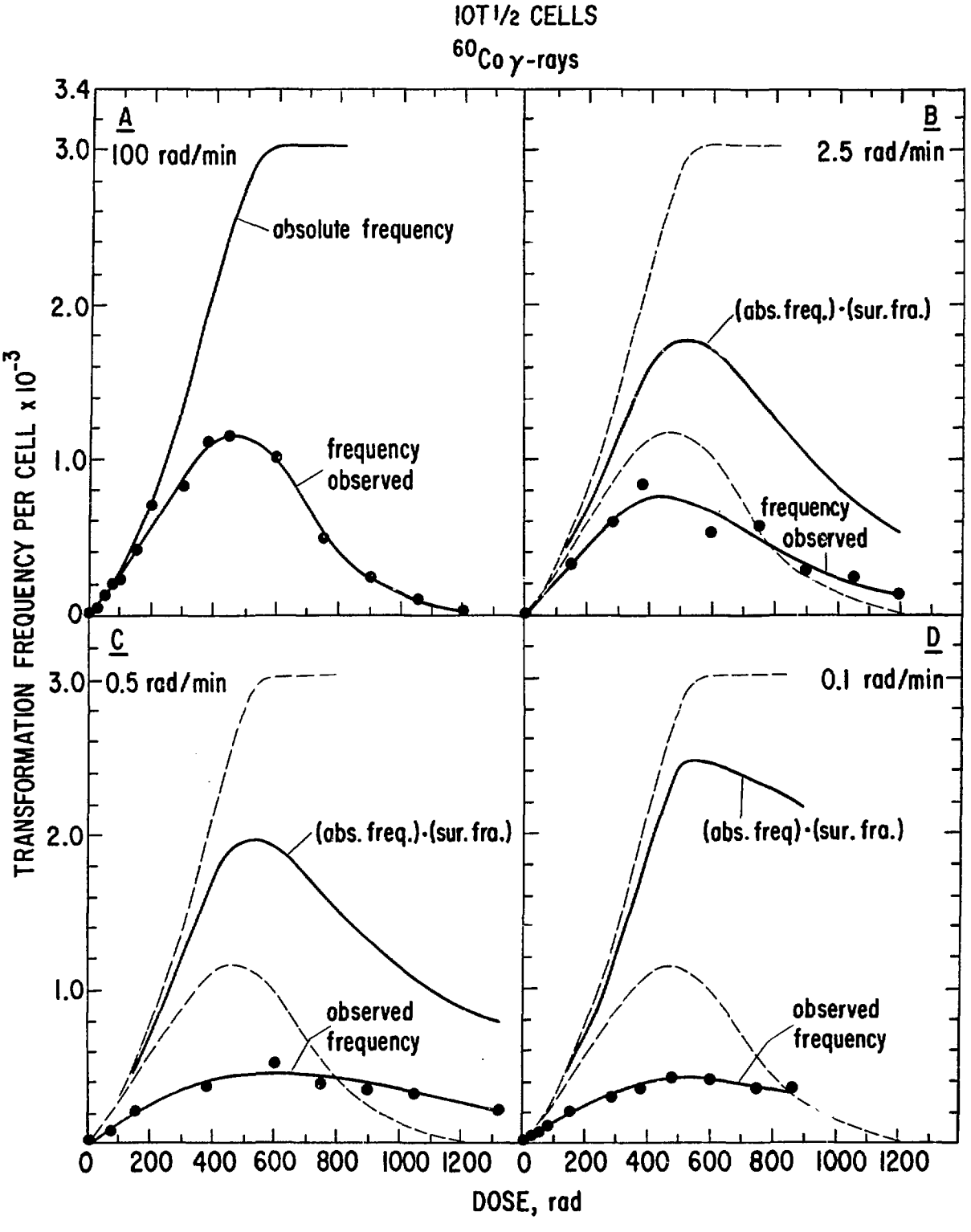
10T1/2 CELLS
60Co γ-rays
A
100 rad/min
absolute frequency
frequency observed
B
2.5 rad/min
(abs. freq.)·(sur. fra.)
frequency observed
C
0.5 rad/min
(abs. freq.)·(sur. fra.)
observed frequency
D
0.1 rad/min
(abs. freq)·(sur. fra.)
observed frequency
TRANSFORMATION FREQUENCY PER CELL x 10-3
DOSE, rad
0
1.0
2.0
3.0
3.4
200
400
600
800
1000
1200

Fig. 3

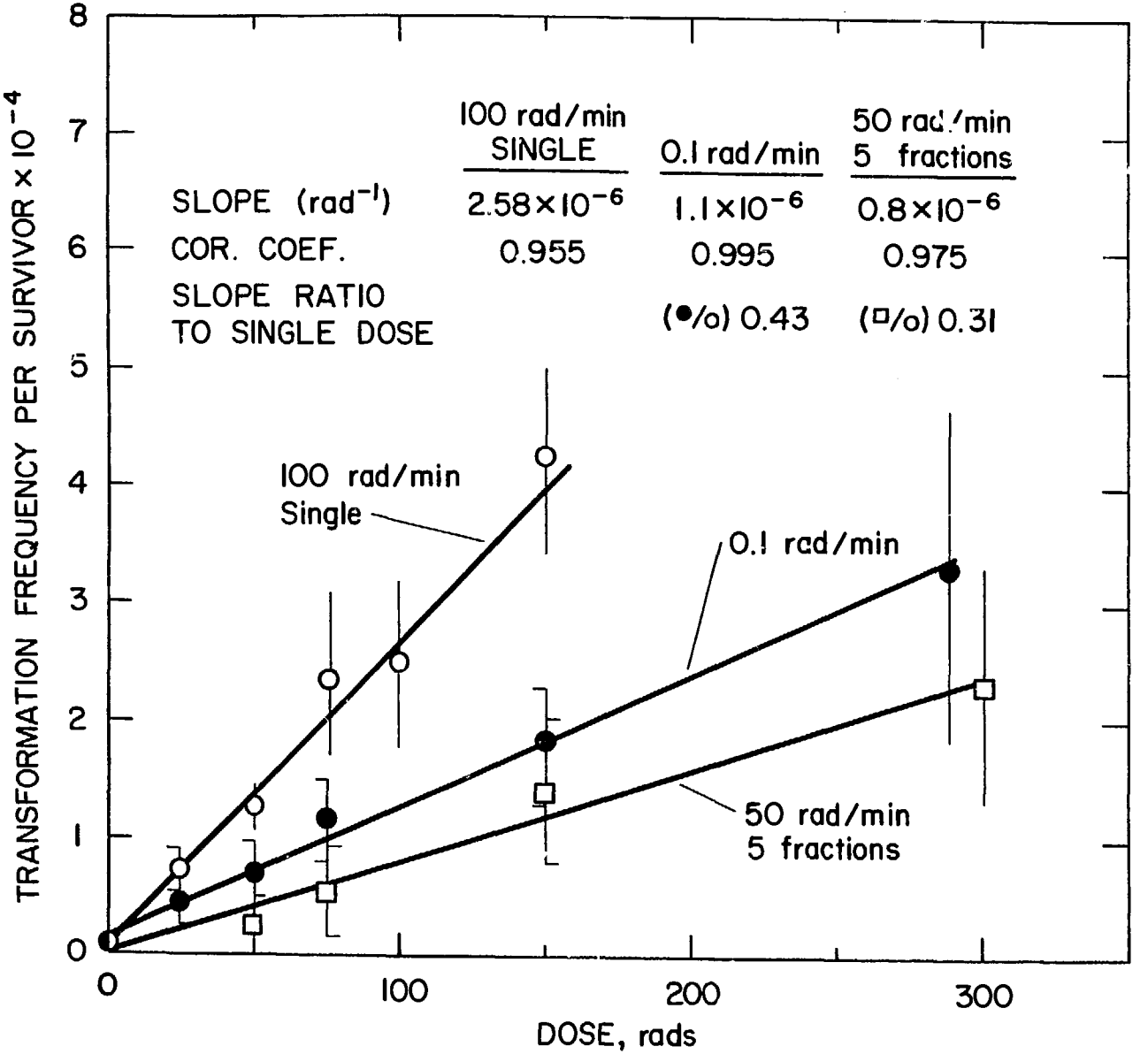
TRANSFORMATION FREQUENCY PER SURVIVOR × 10⁻⁴
DOSE, rads
0
100
200
300
1
2
3
4
5
6
7
8
100 rad/min SINGLE
0.1 rad/min
50 rad/min 5 fractions
SLOPE (rad⁻¹)
2.58×10⁻⁶
1.1×10⁻⁶
0.8×10⁻⁶
COR. COEF.
0.955
0.995
0.975
SLOPE RATIO TO SINGLE DOSE
(●/o) 0.43
(□/o) 0.31
100 rad/min Single
0.1 rad/min
50 rad/min 5 fractions

Fig. 4