# REPAIR KINETICS OF RADIATION-INDUCED MITOTIC DELAY

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ABSTRACT The recovery from radiation-induced mitotic delay in asynchronous sarcoma-180 (S-180) ascites tumor cells has been analyzed in a manner analogous to the repair of sublethal damage. 200-R increments were separated by various fraction intervals (not exceeding the time necessary for mitosis to return to control levels) for total exposures up to 1600 R. The accumulated mitotic delay after the last exposure increment, in percent of an equivalent single exposure, decreased exponentially with overall treatment time in a bimodal fashion. An initial repair process displayed a half time of 2.3 h of overall elapsed time and was followed by a slower process with a half time of 15.1 h. Such a bimodal recovery provides an explanation of why fractionation intervals long with respect to the amitotic period resulting from a single 200 R exposure enhance mitotic delay over that of equivalent single exposures, while shorter fractionation intervals diminish it. It also predicts that mitotic delay vs. dose curves should bend toward the abscissa as the exposure time is increased with large single exposures and large fractionated exposures given over short fraction intervals.

## INTRODUCTION

A previous report (Evans et al., 1971) detailed the effect of fractionation on radiation-induced mitotic delay in a transplantable mouse sarcoma irradiated in vivo. Separating 200-R exposure increments by various time intervals profoundly influenced the end result of the total exposure. After an exposure of 200 R, mitotic figures were absent for a period of 4 h. It was found that fractionation intervals short (i.e., 0.5–1.0 h) with respect to an amitotic period of 4 h resulted in less mitotic delay than the equivalent single exposure. Fraction intervals long (i.e., 3.0–4.8 h) with respect to the amitotic period, however, resulted in longer delay than that from the single exposure.

The present report presents an analysis of the recovery kinetics of mitotic delay after fractionated exposures. This was accomplished by considering the duration of mitotic delay after the last exposure fraction of 200 R for different modes of fractionation and different total exposures.

## MATERIALS AND METHODS

S-180 ascites tumor cells were propagated in male Swiss mice from a colony maintained in this laboratory for the past 12 yr. The tumor cells have a generation time of about 15 h and all experiments were performed on exponentially growing cells 4 days after inoculation. The experimental techniques have been described in detail previously (Evans et al., 1971). Briefly they were as follows.

Tumor-bearing animals were irradiated with either single exposures or fractionated exposures given as 200-R increments separated by intervals of 0.5, 1.0, 3.0 h, or the time necessary for the mitotic index to recover to four to eight mitotic figures per 1000 cells. The return of the mitotic index to four to eight was taken as the point for giving the next 200 R fraction in the later case because it was a reliable and readily discernable index of complete recovery. In all experiments this time was very close to 4.8 h. The fraction of cells in mitosis was determined by periodic aspiration of a few drops of the cellular suspension from the peritoneal cavity with a syringe. Samples were taken in sequence from five animals at each dose-fractional period. The duration of mitotic inhibition was determined by the intercept of the extrapolated ascending curve of mitotic index with the time axis. Each total exposure and fractionation pattern combination was performed three times. The physical factors of irradiation were: 250 kVp; half-value layer (HVL), 1 mm Cu: distance from target to midline of mouse 48 cm; and exposure rate of 165 R/min. The cells were irradiated in vivo.

## RESULTS

The following results could be derived in part from a previous publication (Evans et al., 1971) but are presented here in extended form to facilitate detailed analysis

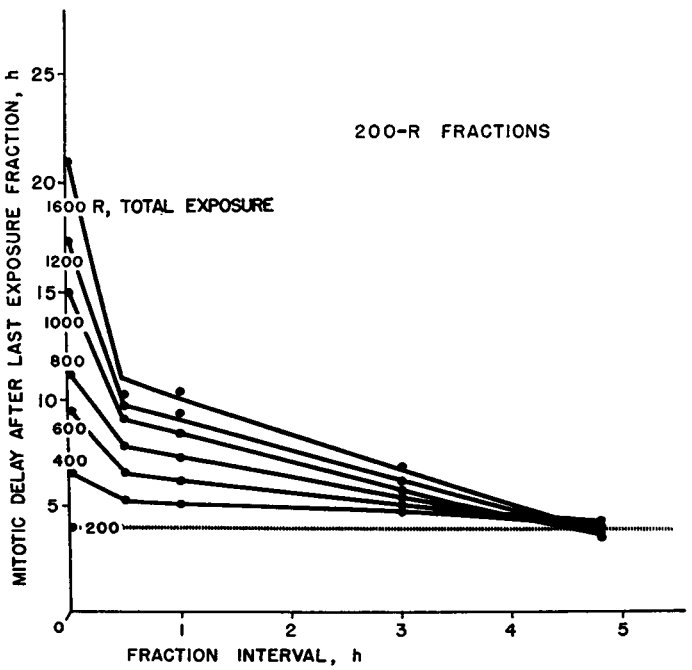


FIGURE 1 The duration of mitotic inhibition measured from the last 200 R fraction for various total exposures presented as a function of the hourly spacing between the 200-R increments. The stippled line is the mitotic delay resulting from a single exposure to 200 R. A fraction interval of zero hours is termed a single exposure.

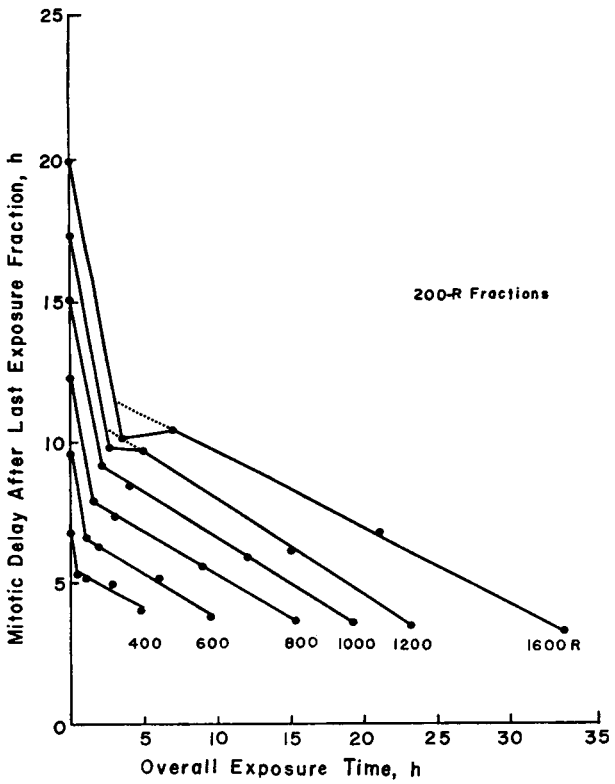


FIGURE 2 The duration of mitotic inhibition measured from the last 200 R fraction for various total exposures presented as a function of the overall elapsed time for the respective treatment mode. A fraction interval of zero is termed a single exposure.

of recovery. The sensitivity to mitotic delay was found to increase linearly with the time between 200-R exposure fractions at the rate of 0.2 min/R per h (Evans et al., 1971). The mitotic delay coefficient resulting from a single exposure, however, was the same as that from 200-R exposures spaced 1.9 h apart. Intervals between 200-R fractions of less than 1.9 h resulted in less mitotic delay than from a single exposure while fraction intervals longer than 1.9 h yielded a greater delay. A fraction interval longer than 4.8 h would not have meaning in the present context since cell division would have resumed indicating complete recovery.

The kinetics of the recovery from damage that results in chromosome aberration production and cell killing has been analyzed by separating two exposures by various time intervals and comparing their combined effect with that of the single total exposure. This has been termed the repair of sublethal radiation damage (Elkind and Whitmore, 1967, Dewey et al., 1971). If no recovery occurred during the interval, the effect of two fractionated exposures would be the same as their sum total given at once. A similar analysis of the cellular recovery from radiation-induced mitotic delay is presented graphically in Figs. 1–3. In Fig. 1, the duration of mitotic inhibition measured from the last 200 R fraction for the various total

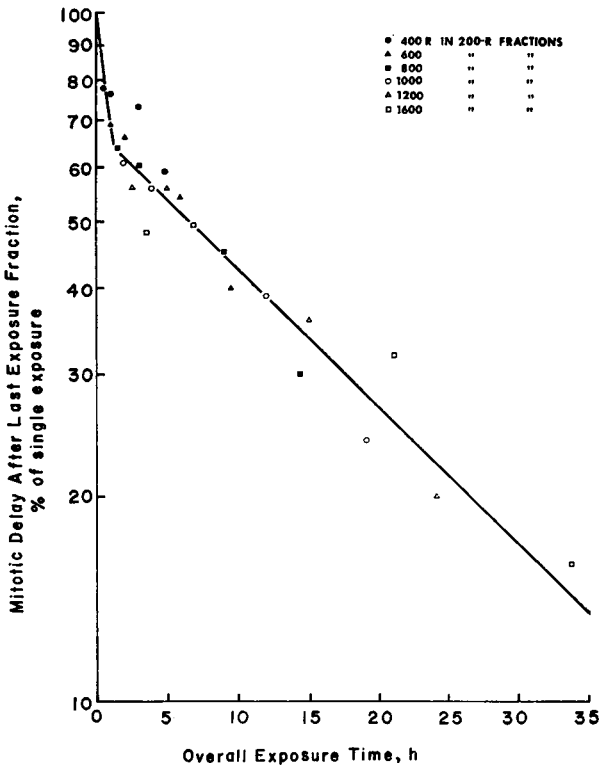


FIGURE 3 Mitotic delay remaining after the last 200 R exposure fraction in percent of the equivalent single exposure presented as a function of the overall elapsed time for the various total exposures. Note semilog axis. One-half of the damage is repaired initially every 2.3 h of overall exposure time (according to the formula $y = 96.8\ e^{-0.303t}$) while one-half of the existing damage is reduced every 15.1 h by the slower repair process (according to the formula $y = 68.0\ e^{-0.046t}$).

exposures is presented as a function of the hourly spacing between the 200-R increments. It is evident that the accumulated mitotic delay remaining from the previous exposures was reduced by longer fraction intervals. The curves intersect at a fraction interval of 4.8 h indicating that the only damage remaining was from the last 200 R test exposure. Thus, when 200-R fractions were separated by fraction intervals of 4.8 h, the total mitotic delay was a simple summation of the number of fractions given. The curves in Fig. 1 are biphasic which suggests the existence of two repair processes.

When the mitotic delay after the last exposure fraction is plotted as a function of the overall elapsed exposure time for different total doses, a family of parallel curves results reflecting the two components of the recovery process (Fig. 2). The curve for 400 R represents the situation where only two doses were separated by time. Much of the damage was repaired rapidly during the first ½ h and the rest at a much slower rate over the next 4 h until no damage remained, i.e., mitosis resumed.

The data of Fig. 2 are normalized and combined in Fig. 3 to show the decreased mitotic delay remaining after the last exposure increment in percent of the delay from a single exposure as a function of overall elapsed time between fractions. When the results from all dose-fractionation patterns were combined and sufficient data accumulated, both of the repair processes appeared to follow exponential kinetics. The prompt repair process reduced damage with a $T\frac{1}{2}$ of the 2.3 h of overall exposure time. The slower repair process reduced the remaining damage by one-half over 15.1 h of overall exposure time. Thus, for any total exposure up to 1600 R given as 200-R fractions before cell division can resume, 50% of the damage (relative to a single exposure) will be repaired by 6.6 h of overall elapsed time after the first exposure.

## DISCUSSION

The recovery from radiation-induced mitotic delay in S-180 ascites tumor cells has been analyzed in the manner analogous to the repair of sublethal damage as first described by Elkind and Sutton in 1960. If indeed, mitotic delay after the last exposure fraction is a measure of unrepaired injury, the bimodal nature of the family of curves in Figs. 1–3 provides an explanation of why mitotic delay is increased with longer and decreased with shorter fraction intervals. Intracellular repair of the radiation damage is rapid initially while much slower from 0.5 h after exposure until completion.

When radiation is delivered as 200-R increments separated by 4.8 h, the damage from each previous increment has been completely repaired by the time the next is delivered and the total delay is essentially a summation process. Since recovery is nearly complete 3 h after a dose of 200 R, most of the difference in delay between the 3- and the 4.8-h fractionation patterns is due to the longer time interval separating each exposure in the 4.8-h series, less the small difference in repair between them. For example, if repair had been completed by 3 h after a 200 R exposure, then for a 1600 R total exposure one would expect a difference between the 3- and the 4.8- h modes of $7 \times (4.8 - 3) = 12.6$ h of delay. The actual difference was 11.1 h (Evans et al., 1971). This 1.5 h difference between expected and observed can be explained if part of the damage remaining 3 h after each exposure increment is repaired by the initially rapid repair process. Likewise with the shorter fraction intervals; if most of the accumulated damage is repaired rapidly by the initial process after each successive 200 R increment, while less and less time is allowed for the slower part of the repair process, then the total delay would be expected to decrease with shorter fraction intervals. Thus, it may be that the operant mode of repair is designated by the degree of residual damage, and not by qualitatively differing forms of accumulated injury.

Variations in stage sensitivity for mitotic inhibition (Terasima and Tolmach, 1963; Whitmore et al., 1967; Yu and Sinclair, 1967, Leeper et al., 1972) coupled

with the altered stage frequency distribution that may occur due to progression during the various modes of fractionation (Terasima and Tolmach, 1963; Brent et al., 1966; Sinclair, 1967; Walters and Tobey, 1970; footnote 1) could be factors influencing mitotic delay after fractionated irradiation in asynchronous populations. For example, if late S or $G_2$ cells suffered the greatest delay (Kim and Evans 1964; Leeper et al., 1972, footnote 1) all modes of fractionation would progressively increase the population of cells in $G_2$. The total delay, however, was less than that after an equivalent single exposure for 0.5 and 1.0 h fraction intervals, but was greater for 3- and 4.8-h intervals. In addition, the limited cyclic progression of cells which could take place in a period as short as 0.5 h, i.e., 3% of the population could hardly explain the profound alterations in mitotic delay observed at the lower total exposures (cf. Figs. 2 and 3). The most likely hypothesis remains intracellular repair with a minor contribution of cellular progression during the fraction intervals.

The rapid rate at which damage is repaired by the initial recovery process may help to explain why the curves of mitotic delay vs. dose for single exposures or short fraction intervals (less than 1.9 h) are linear up to about 1200 R and then bend toward the abscissa at higher exposures (Whitmore et al., 1967; Evans et al., 1971). With a constant dose rate and successively larger single exposures, more damage can be repaired during the exposure period by the rapid initial repair process. The same consideration would apply to total exposures utilizing short fraction intervals where successive exposure increments are delivered when there is still a great deal of damage to be repaired and when the rapid repair process is operative. Exposure increments spaced by longer fraction intervals are administered when there is little damage remaining, and the predominant repair process proceeds at a much slower rate.

The cellular damage responsible for radiation-induced mitotic inhibition and the related repair processes apparently occur at the level of translation and involve the loss or alteration of specific proteins necessary for division (Rustad and Burchill, 1966; Walters and Petersen, 1968; Doida and Okada, 1969; Bacchetti and Sinclair, 1970). Such a defect and its repair might very well fit the constraints of the logistic second-order model for mitotic delay described by Sacher (1968). In this tumor system, however, the delay vs. dose curves bend toward the abscissa above 1200 R for single exposures and short fraction intervals; and the repair processes, per se, are unaffected by irradiation. This would be more in accord with the first-order model presented by Lea (1955) whereby damage is accumulated linearly with dose and repaired exponentially with time. Hopefully, ongoing research will permit an attempt to relate the recovery process described in this report to specific intracellular responses.

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