Possible error-prone repair of neoplastic transformation induced by
fission-spectrum neutrons.

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Summary We have examined the effect of fission-spectrum neutrons from the JANUS reactor at Argonne National Laboratory, delivered either as acute or protracted irradiation, on the incidence of neoplastic transformation in the C3H 10T1/2 mouse embryo cell line. Acute exposures were delivered at 10-38 rads/min, protracted exposures at 0.086 or 0.43 rad/min. The total doses for both ranged from 2.4 to 350 rads. In the low dose region (2.4-80 rads), there was a large enhancement in transformation frequency when the neutrons were delivered at the low dose rates compared with the high dose rates, but the survival of the cells was not significantly different between the two exposure conditions. Analysis of the initial parts of the curves shows that the regression line for protracted doses is about 9 times steeper than that for single acute exposures. Finally, the possibility is discussed that an "error-prone" repair process may be causing the enhanced transformation frequency by protracted neutron exposures.

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Our earlier studies (Han & Elkind, 1979) compared single with fractionated doses of X-rays and fission spectrum neutrons at high dose levels. The work was stimulated in part by the need to determine the efficacy with which neutrons would induce transformation and in part in order to examine the role of repair in transformation induction in the light of the contrasting results of Borek & Hall (1974) and Terzaghi & Little (1976); the former authors reported an increase and the latter authors no change in transformation frequency when low doses of X-rays were fractionated. For the large doses (350 + 350 rads of X rays or 189 + 189 rads of fission-spectrum neutrons) used by Han & Elkind (1979) and Elkind & Han (1979), there was a significant decrease in transformation when the X-ray dose was fractionated but only a small decrease when the fission neutron dose was fractionated. Their interpretation was that repair processes were indeed involved in the X-ray case (Elkind & Han, 1979; Han & Elkind, 1979) but that in the fission neutron case, no repair of sublethal damage occurred.

These results and other considerations stimulated a study of the effects of low dose rate ^{60}Co γ -rays on the induction of transformation. Using dose rates from 100 rads/min down to 0.1 rad/min we clearly showed that although repair of sublethal damage occurred during protracted exposures causing an increase in survival, a significant decrease in transformation frequency per surviving cell occurred (Han et al., 1980). It was concluded that this decrease in transformation frequency could not be due to the repair of sublethal damage alone, but must also reflect the repair of subtransformation damage. These results led us to the present study in which we explore the effect of low dose rates and low doses of fission neutrons on transformation, particularly at doses where little cell killing occurs.

In a recent report it was shown that reduction of the dose rate of low doses of neutrons (10 to 150 rads) from 10-38 rads/min to 0.43 or 0.086 rad/min actually enhanced transformation frequency significantly, although cell killing was not significantly influenced (Hill et al., 1982). We have now extended these studies to lower doses, and have begun a multifraction experiment to examine further the role fission neutrons play in the induction of neoplastic transformation.

Materials and methods

Clone 8 of C3H 10T1/2 mouse-embryo-derived cells, developed by Reznikoff et al. (1973), were used throughout these studies. Cell cultures were grown at 37°C in small plastic T-flasks, or in plastic 100-mm Petri dishes, in Eagle's basal medium (Gibco) supplemented with 10% heat inactivated fetal calf serum (Sterile Systems, Inc., Utah) according to methods previously described (Han & Elkind, 1979; Han et al., 1980).

The JANUS reactor of the Division of Biological and Medical Research, Argonne National Laboratory, was used to expose cells to fission-spectrum neutrons (Han & Elkind, 1979). A special incubator, made of light plastic and styrofoam to minimize any changes in the quality of the neutron beam and the induction of radioactivity, was used for the low dose rate exposures at 0.43 and 0.086 rad/min. For the exposures, between 140 and 800 viable cells/cm² were seeded into small T-flasks and were grown at 37°C for about 48 hrs before irradiations were begun to ensure that the cells were in asynchronous, exponential growth. During exposure the cells were attached in plastic T-flasks filled with Eagle's supplemented medium and were kept at 37°C inside the special incubator. Irradiations at acute dose rates were done with T-flasks in air at room temperature both for neutrons (38.5 or 10.3 rads/min)

and for ^{60}Co γ -rays (100 rads/min). In all experiments, irradiated cells were removed from the incubator immediately after exposure, suspended by trypsinization, plated into 100-mm Petri dishes, and placed in conventional incubators for the 6 weeks necessary for transformed foci to develop (Reznikoff et al., 1973; Han & Elkind, 1979). As controls, cells were grown in the special incubator without irradiation and then assayed for transformation. No transformed colonies were found for these controls; that is, the transformation frequency per surviving cell was $< 8.7 \times 10^{-5}$. The background frequency of transformation from more than 2000 Petri dishes accumulated over the past several years is 1.03×10^{-5} per surviving cell. Dosimetry was performed inside the special incubator at the position of the cells and also in air, for acute dose rate irradiations, according to the method described previously by Han & Elkind (1979). Transformed foci were scored as previously described (Reznikoff et al., 1973; Han & Elkind, 1979) and neoplastic transformation frequency per surviving cell was calculated by the null method according to Han & Elkind (1979).

Results and Discussion

In Fig. 1A are presented the results of a classical two dose fractionation experiment on cell survival comparing 50-kVp X-rays with fission-spectrum neutrons. The doses used were chosen to coincide with the plateau region of the transformation induction curves (Han & Elkind, 1979). A classic recovery of cell survival due to repair was observed when the X-ray dose was fractionated, but no increase in cell survival with fractionation was seen for the fission neutrons.

Figure 1B shows the transformation frequencies per surviving cell corresponding to the cell killing data in Fig. 1B. Again there was a clear

indication of repair for X-rays, shown by the significant decline in transformation frequency. However, little if any decline in transformation frequency occurred in the case of fission-spectrum neutrons.

In Fig. 2 we present a synopsis of our results at low dose rates. When the JANUS neutron dose rate was reduced to 0.086 rad/min, a quite unexpected result occurred. Although survival following exposures at low dose rates remained essentially unchanged, the transformation frequency was considerably enhanced, particularly at low doses, compared with the frequencies following the acute dose rate exposures. At high total doses, the low dose rate transformation data are essentially the same as the acute dose rate data (Hill et al., 1982). The comparisons in Fig. 2 between high and low dose rates show that, as expected, fission spectrum-neutrons delivered at high dose rate produce more transformations per surviving cell than γ -rays at high or low dose rate. However, the enhanced transformation frequency seen when fission-spectrum neutrons are given at low dose rate is unexpected and widens the difference in neoplastic transformation frequency significantly between the γ -rays at high or low dose rates and fission-spectrum neutrons.

Two important points need to be addressed regarding the enhanced transformation frequency. The first, of importance for occupationally exposed workers, is the question of the relative biological effectiveness (RBE) for transformation at low doses of low dose rate radiation. The second is the more basic question of what a dose rate effect with fission spectrum neutrons might be; classical radiobiological thoughts, at least for cell survival, suggest no repair of sublethal damage.

First, regarding RBE's we have already published data suggesting that the initial parts of the ^{60}Co γ -ray curves at high and low dose rates are linear (Han et al., 1980). Recent data for neutrons (to be published elsewhere) has

allowed us to make a similar analysis. Both sets of results, for low dose rates up to 10 rads and for high dose rates up to 40 rads, are well fitted by linear dependencies. However, both curves are not linear throughout the extended dose ranges studied. From the initial linear fits, RBE values for neoplastic transformation were determined based upon the ratio of initial slopes. In Table I we present these estimates using the acute ^{60}Co γ -ray dose rate as a reference (RBE=1) (Han et al., 1980). Fission-spectrum neutrons at the two low dose rates were about 9 times more effective than neutrons at acute dose rates. Also, the low dose rate fission-spectrum neutrons are about 20 times more effective than acute doses of ^{60}Co γ -rays. From an occupational hazard point of view, it might be more meaningful to compare low dose rate ^{60}Co γ -rays to low dose rate fission-spectrum neutrons. For such a comparison, the results from this study suggest an RBE of 50 or more.

Such a large variation in effect raises questions about mechanisms. This concern brings us back to the second question: how can a dose-rate effect be apparent with fission spectrum neutrons? Microdosimetric considerations alone might suggest that a dose-rate effect is not possible, and our results for survival are consistent with such a view since no measurable change in cell survival occurs with decreasing dose rate for neutrons. However, we have clearly demonstrated, at low cell killing doses, that transformation induction does vary with dose rate.

The greater efficiency of fission-spectrum neutrons in inducing neoplastic transformation when delivered at a low dose rate could be a consequence of two largely different mechanisms. One would be that a low dose rate delivery of neutrons facilitates the expression of subtransformational lesions. Such a mechanism might be similar to that involved in promotion of neoplastic transformation by a tumor promoter. The second mechanism might

involve a repair process. It has been shown that cells are apparently not able to repair sublethal damage induced by fission-spectrum neutrons. However, they are able to repair a portion of neutron-induced sublethal damage with which sublethal X-ray damage can interact (Ngo et al., 1977). Based on this result, it is possible that, at the reduced dose rates of fission-spectrum neutrons, a repair relative to neoplastic transformation does take place and its net effect is "error prone" (Elkind et al., 1982). Since the survival of cells is apparently neutron dose rate independent, we further infer that the net "error-prone" component of such a repair is independent of sublethal damage.

For a dose rate dependent repair (or misrepair) process to be possible in the low dose region, it is necessary that the end point in question result from two or more primary events. This is certainly the case for low doses of γ -rays but one might question whether or not transformation induced by a dose as small as 2.4 rads of fission-spectrum neutrons results from a multievent process.

Since the effective LET of a fission-spectrum neutron beam is quite high, the microdosimetry of energy absorption must be considered in the small dose region. If the smallest dose in the low dose region can only result, with high probability, from a single primary event (e.g., a single recoil proton), then it would not be likely that any dependence on dose protraction could be observed. The smallest dose that we have used at 0.086 rad/min is 2.4 rads. An analysis based upon the microdosimetric approach taken by Goodhead (1983) leads us to conclude that multiple primary absorption events could be involved in transformation induction at a dose as small as 1 rad of fission-spectrum neutrons when the following factors are taken into account: (1) The neutron energy distribution of the fission-spectrum beam (Williamson & Frigerio,

1972). (2) The average volume of a 10T1/2 cell, $\sim 10 \mu\text{m}$ diameter. (3) The flat shape of nuclei of cells during irradiation (Lloyd et al., 1979). (4) The likelihood of some gamma ray contamination of the neutron beam (Williamson & Frigerio, 1972). (5) The low expected frequency of cell transformation for doses in the low dose region (Fig. 2).

Lastly, we note that in the low region of neutron doses, e.g., 0-10 rads, very few cells are killed, and about 1 cell in 10^4 cells is transformed. The percentage of cells that are required to suffer multihits in order that a dose rate dependence would not be precluded on microdosimetric grounds is therefore 0.01% or less. Consequently, even at a dose of 1 rad it is likely that transformation could be the result of multiple primary events in a cell's nucleus. Alternatively, even if the target for transformation is only a fraction of the nuclear volume, the end point could still result from at least two primary events.

In summary, therefore, it can be stated that the transformation data for fission-spectrum neutrons show a significant enhancement at low dose rates where survival is largely unaffected. This result is consistent, based upon the limited data available, with a repair process occurring with respect to transformation damage that has a net "error-prone component." Finally, a repair-mediated dose rate effect is not ruled out from considerations of the microdosimetry of neutron delivery.

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TABLE I
RELATIVE BIOLOGICAL EFFECTIVENESS
(RBE)

<u>Radiation/delivery</u>	<u>Slope, freq./rad^a</u>	<u>Slope ratio</u>
⁶⁰ Co γ-rays		
100 rad/min	2.58×10^{-6}	1.0
0.1 rad/min	1.10×10^{-6}	0.43
high D ^b 5 frac./4 d.	0.80×10^{-6}	0.31
JANUS neutrons		
10.3-38 rad/min	5.96×10^{-6}	2.31
0.086, 0.43 rad/min	55.70×10^{-6}	21.60

^aSlope of neoplastic frequency/rad, calculated from the initial part of the neoplastic transformation frequency curves.

^bHigh dose rate (50 rads/min) delivered in 5 fractions over 4 days.

Figure Legends

Figure 1 (A) Changes in survival for C3H 10T1/2 cells exposed to fractionated doses of X-rays (O) or fission-spectrum neutrons (●). Abscissa, time at 37°C between the two irradiations; bars, S.E. of the pooled data for several experiments. (B) Changes in transformation frequencies for C3H 10T1/2 cells exposed to fractionated doses of X-rays (■) or fission-spectrum neutrons (●). Other details as in Fig. 1A.

Figure 2. The dose rate dependencies of the transformation of C3H 10T1/2 cells exposed to ^{60}Co γ -rays or to fission-spectrum neutrons. Transformation frequencies are expressed per surviving cell for fission-spectrum neutrons at 0.086 rad/min (■), fission-spectrum neutrons at 38 rads/min (□), ^{60}Co γ -rays at 100 rads/min (O) and ^{60}Co γ -rays at 0.1 rad/min (●); bars, S.E. of the pooled data from several experiments.

Fig. 1

Fig. 2