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Application of the constant exposure time technique to transformation experiments with fission neutrons: failure to demonstrate dose-rate dependence

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Abstract. A direct comparison of the effectiveness of fission neutrons at high (11·0–31·3 cGy/min) or several low dose-rates (0·14–3·2 cGy/min) was carried out under identical conditions. Monolayers of exponentially growing C3H/10T½ cells were exposed at 37°C to reactor-produced neutrons (fluence-mean energy $E_n = 0{\cdot}68$ MeV, $\leqslant 5\%$ γ component, frequency mean linear energy $y_F = 21$ keV/μm, dose mean lineal energy $y_D = 42$ keV/μm in an 8-μm spherical cavity). Survival or transformation induction were studied at five doses from 10·5 to 94 cGy. In low dose-rate irradiations, these doses were protracted over 0·5, 1, 3 or 4·5 h, resulting in 17 different dose-rates. Up to six experiments were performed at each of five exposure times. Concurrently with transformation we studied cell proliferation in control versus cells irradiated at 40 cGy (acute and a 4·5-h protraction) and found no evidence of a shift in the cell cycle distribution among these cells. At a given dose and dose-rate, the effect of dose protraction on survival or transformation was assessed by the dose-rate modifying factor (DRMF), defined as the low:high dose-rate effect ratio at the same dose. Survival or transformation induction curves were nearly linear with initial slopes, respectively, of about $6{\cdot}5 \times 10^{-3}$ or $6{\cdot}2 \times 10^{-6}$ cGy^{-1}. Consistent with dose response curves, DRMFs were independent of the dose and dose-rate. The mean values of the DRMF with their uncertainties and 99% confidence intervals, based on measurements in individual doses and dose-rates for survival or transformation were, respectively: $1{\cdot}01 \pm 0{\cdot}03$ (0·92, 1·09) or $0{\cdot}98 \pm 0{\cdot}04$ (0·83, 1·08) indicating a similar precision in determining DRMF for survival or transformation, and no dose or dose-rate influence on these end points.

1. Introduction

Numerous theoretical and experimental studies have been conducted with the objective of improving our knowledge of dose–response relationships for radiation carcinogenesis at high dose-rate or protracted doses of medium and high linear energy transfer (LET) radiations. Among these studies several neutron fields, including that at the Armed Forces Radiobiology Research Institute (AFRRI) TRIGA reactor, have been used to irradiate C3H/10T½ cells. Exposure of these cells to putative carcinogens, including ionizing radiation, may convert a cell with normal growth and morphologic characteristics to one with the characteristics of a tumour cell, a process termed neoplastic transformation. It is well established experimentally in the C3H/10½ system that high-LET radiations have greater biological effectiveness than low LET radiations. However, the effects of protracted or split-dose exposures continue to be a subject of controversy; for the literature background on dose protraction studies with low and high LET radiations, see Balcer-Kubiczek and Harrison 1991c, Brenner and Hall 1990, 1992, Elkind 1991 and Cao *et al.* 1992. In the case of fission neutrons, the need for additional transformation measurements at doses $\leqslant 0{\cdot}5$ Gy and dose-rates $\leqslant 0{\cdot}5$ cGy/min has been identified (Brenner and Hall 1990, 1992, Hall 1991, Redpath *et al.* 1991).

All the previous dose-rate experiments with C3H/10T½ cells, including our transformation studies with TRIGA neutrons (Balcer-Kubiczek *et al.* 1988, 1991, Balcer-Kubiczek and Harrison 1991a), used a constant dose-rate approach in which the total dose is proportional to the exposure time. Consequently, different irradiation times are needed to accumulate different doses, a procedure which makes the analysis of the protracted data conceptually complicated especially when intervals of enhanced sensitivity within the cell cycle, the division delay, repair or similar kinetic and time- or dose-dependent phenomena, singly or in combination, are suspected to affect the induction process (Rossi and Kellerer 1986, Brenner and Hall 1990, 1992, Elkind 1991, Hall 1991, Cao *et al.* 1992, Watt 1992).

The objective of the present series was to re-evaluate the effect of fission neutron dose-protraction on neoplastic transformation of C3H/10T½ cells by using a constant exposure time approach in which dose-rates are proportional to the total dose (Kellerer

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and Rossi 1972). Consequently, different doses are accumulated over the same exposure time. An additional advantage of the constant exposure time technique is that contributions of the total dose and dose-rate can be separated, so consequently questions of time-, dose- and/or dose-rate-dependent windowed responses can be addressed directly by a suitable choice of doses and exposure durations (Sykes and Watts 1989). In our present experiments, total doses of about 10, 25, 40, 60 and 90 cGy were protracted over $\tau = 0{\cdot}5$, 1, 3 or 4·5 h, resulting in 17 dose-rates from 0·14 to 3·2 cGy/min. Our previous constant exposure time experiments with X-rays showed that a moderate protraction ($\tau \leqslant 5$ h) of doses in the dose range from 25 cGy to 4 Gy, can produce a significant, statistically discernable reduction of cell killing and transformation in the C3H/10T½ assay (Balcer-Kubiczek *et al.* 1987).

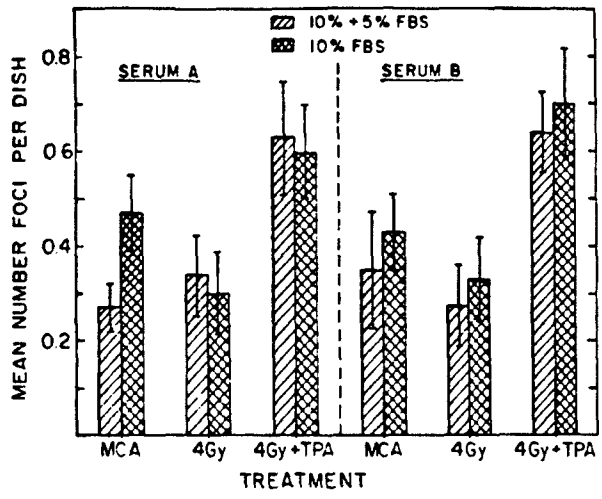

Figure 1. Comparison of transformation induction in C3H/10T½ cells by X-rays or chemicals with carcinogenic activity (methylcholanthrene, MCA; 12-*O*-tetradecanoyl phorbol-13-acetate, TPA) to demonstrate the effect of serum quality and concentration on transformation or clonogenic responses (data not shown). Other abbreviations have the following meaning: 10%+5% FBS, indicates a protocol with the first four media changes performed using medium containing 10% of foetal bovine serum (FBS), and the remaining three media changes performed using a 5%-serum content; 10% FBS, high-serum medium-change protocol.

2. Materials and methods

2.1. *Cell culture and media*

C3H/10T½ cells from the line established by Reznikoff *et al.* (1973a, b) were maintained as described previously (Balcer-Kubiczek *et al.* 1988, Balcer-Kubiczek and Harrison 1990, 1991a c) in conformity with published guidelines for this assay (IARC/NCI/EPA Working Group 1985). Our quality control of experiments with C3H/10T½ cells is described in the reports cited above. New batches or lots of serum are routinely prescreened using procedures and criteria summarized in the IARC/NCI/EPA Working Group (1985) report. Serum lots are characterized as being suitable for the transformation assay by ascertaining compliance with survival and transformation expectations after 4 Gy X-rays, after 4 Gy X-rays with post exposure to 0·1 μg TPA/ml, or after 2·5 μg MCA/ml. Results for two lots, matched with respect to the concentration of 11 natural serum components, tested for use in the present study are shown in Figure 1; a single lot, denoted serum B in Figure 1, was used in the experiments reported here.

2.2. *Transformation and survival assays*

Cells in passage 10 were used for experimentation. Before neutron irradiation, cells were plated in several 25-cm^2 flasks at 5×10^4 cells per flask. Experiments were performed 2 days later to ensure that treated cells were in exponential growth phase (see §2·3). Except for neutron irradiation, flasks were kept in horizontal position at all times to facilitate attachment/reattachment of mitotic cells. A hypersensitivity of these cells to transformation was suggested (Elkind 1991, Brenner and Hall 1992, Cao *et al.* 1992). Temperature was maintained at 36·37°C, including during neutron irradiation. Cells were transported to the reactor in a 20-litre thermally insulated container, similar to that described previously (Balcer-Kubiczek *et al.* 1988, Balcer-Kubiczek and Harrison 1991c). At the reactor site, cell temperature before and after neutron irradiation was controlled by immersing culture flasks filled with warm medium in a constant-temperature water bath.

Each experimental run included a high dose-rate exposure group and, in most cases, more than one low dose-rate exposure group at the same dose. Neutron irradiations were arranged such that low dose-rate exposures always preceded high dose-rate exposure. Consequently, cultures that received low dose-rate irradiations were incubated in a horizontal position for up to 5 h before transporting them from Bethesda to our cell culture laboratory in Baltimore. There, cells were removed from flasks by trypsiniza-

Table 1. Pooled data from constant exposure time experiments with TRIGA fission neutrons

Mean dose $\pm\sigma$ (cGy)	Mean exposure time $\pm\sigma$ (min)	Mean dose rate $\pm\sigma$ (cGy/min)	$SF\pm\sigma$	N	Y	X	P	$X/N\pm\sigma$ (10^{-2})	$TR\pm\sigma$ (10^{-4})
10·68±0·74	0·94±0·08	11·35±0·77	0·994±0·059	692	671	23	280	3·3±0·7	1·10±0·24
26·22±1·32	2·37±0·15	11·06±0·46	0·780±0·176	365	342	24	297	6·6±1·3	2·19±0·46
41·47±1·60	1·39±0·08	30·02±2·34	0·538±0·063	191	165	30	334	15·7±0·9	4·15±0·86
62·28±1·84	5·68±0·37	11·01±0·78	0·431±0·100	513	453	65	259	12·7±1·6	5·68±0·37
91·30±4·45	2·92±0·13	31·29±1·28	0·266±0·057	333	249	89	303	26·7±2·8	9·58±1·05
10·23±0·21	29·87±0·19	0·34±0·01	0·947±0·083	293	283	10	278	3·4±1·1	1·25±0·39
25·38±1·75	29·34±1·09	0·87±0·06	0·804±0·198	272	248	24	346	8·8±1·8	2·67±0·55
40·20±0·35	28·16±2·30	1·44±0·11	0·527±0·075	359	332	27	298	7·5±1·5	2·63±0·51
61·43±2·17	30·10±0·07	2·04±0·08	0·546±0·067	187	159	29	245	15·5±3·9	6·62±1·27
91·95±1·85	28·84±1·16	3·19±0·06	0·260±0·097	76	52	24	371	31·6±6·5	10·24±2·10
10·50±0·61	57·56±2·18	0·18±0·01	0·948±0·087	311	302	10	256	3·2±1·0	1·15±0·38
26·20±1·25	57·83±2·18	0·45±0·01	0·733±0·175	228	211	18	289	7·9±1·9	2·68±0·65
41·50±1·38	61·48±2·55	0·68±0·04	0·585±0·050	264	234	33	291	12·5±2·2	4·15±0·76
60·50±0·50	59·15±0·00	1·02±0·01	0·499±0·119	79	67	12	333	15·2±4·4	4·95±1·43
90·00±0·00	62·53±2·15	1·44±0·05	0·341±0·051	100	67	33	360	33·0±5·6	11·13±1·95
25·92±1·30	179·3±6·6	0·14±0·01	0·806±0·145	239	224	17	307	7·1±1·7	2·11±0·54
40·53±0·91	186·4±4·8	0·22±0·01	0·491±0·072	221	199	31	312	14·0±2·5	3·37±0·72
61·34±1·37	178·3±1·0	0·34±0·01	0·408±0·053	125	111	17	234	13·6±3·3	5·08±1·36
90·32±0·27	180·6±4·3	0·50±0·01	0·232±0·093	362	291	77	246	21·3±2·4	8·86±1·05
40·40±0·60	251·8±3·8	0·16±0·00	0·661±0·079	218	203	19	263	8·7±2·0	2·71±0·70
59·95±0·05	258·8±2·8	0·23±0·00	0·432±0·008	185	158	32	280	17·3±3·1	5·63±1·09
90·45±0·90	271·5±3·7	0·33±0·01	0·250±0·022	353	279	80	299	22·7±2·5	7·86±0·92

SF, surviving fraction, plating efficiency of control was 55%; N, X, Y, P, total number of dishes, number of transformants of type 2 or 3 (Reznikoff *et al.* 1973b, IARC/NCI/EPA Working Group 1985), number of dishes without transformants, number of cells per dish corrected for plating efficiency of unirradiated cells, or surviving fraction and plating efficiency of irradiated cells; X/N, transformation rate per dish (IARC/NCI/EPA Working Group 1985) and its standard error, σ (Hieber *et al.* 1987); TR, transformation rate per survivor (Han and Elkind 1979) and its standard error, σ (Balcer-Kubiczek *et al.* 1987).

tion, counted, diluted, and plated at concentrations estimated to result in either 250 viable cells per dish for the transformation assay, or 50 viable cells per dish for the cell survival assay. As in previous studies the growth medium was renewed at weekly intervals (2 weeks for survival assay and 8 weeks for transformation assay). However, in the present experiments, medium with serum content reduced from 10 to 5% was used in the last three medium changes. As shown in Figure 1, this modified medium-change protocol has no effect on transformation frequency.

Cell survival was determined by colony formation, while neoplastically transformed foci were identified according to published criteria (Reznikoff *et al.* 1973a, b, IARC/NCI/EPA Working Group 1985). The end point of transformants per surviving cell was calculated by the null method of Han and Elkind (1979) with uncertainties determined according to our modified analysis (Balcer-Kubiczek *et al.* 1987). In addition, we determined the mean number of transformants per dish as recommended by IARC/NCI/EPA (1985) with uncertainties calculated according to published methods (Hieber *et al.* 1987).

2.3. *Proliferation studies of irradiated and control cells*

Concurrently with several of the above studies, experiments were performed to compare proliferation of cells taken to and from the reactor facility (90 and 40 cGy, acute or a 4·5-h protraction) with those continuously maintained in our laboratory. Up to four cultures per condition per experiment, established as described above for transformation assay, were used to establish growth curves and to perform flow cytometry measurement of the DNA content. Growth curves were initiated by plating 500 cells per 100-mm dish. Cell counts were obtained every 12 h for a period of 2 weeks. The results, summarized in Table 2, showed no difference in growth rates and in the cell cycle distributions between travelled and non-travelled cultures.

Table 2. Proliferation parameters of C3H 10T½ cells used in reported studies

Parameter†	Travelled cells	Laboratory control	40 cGy brief	40 cGy 4·5 h
Cell cycle distribution %				
G_2/M	12	10	14	12
G_1	69	74	70	68
S	19	16	16	20
Doubling time h	18·8 ± 0·3	19·2 ± 0·2	nd	nd

†Cells were processed immediately after return from the reactor facility. nd, Not done.

2.4. *Dosimetry and irradiation*

Experiments were performed at the Armed Forces Radiobiology Research Institute (AFRRI) TRIGA Reactor Facility with fission neutron field optimized for low dose-rate studies. The technical descriptions of the neutron source and of the exposure facility were published previously (Moore and Elsasser 1986, Balcer-Kubiczek *et al.* 1988, Redpath *et al.* 1991). The mean fluence-weighted neutron energy for this configuration is 0·68 MeV (Goodman 1985).

Since our previous experiments, the lead shielding arrangement around the point of cellular exposure was redesigned to reduce the turnover time for samples from the previous 45 min to about 1 min, and reduce the γ contamination by fully enclosing samples with 5-cm lead shielding and by increasing the source-to-target distance, measured from the central axis of the reactor core, from 1 to 2 m, thus providing favourable conditions for the multiple protracted exposures required in the present study. The latter modification, suggested by one of us (G.H.H.), permits achieving dose-rates of $<0·5$ cGy min at correspondingly higher reactor power, leading to an improved neutron-to-total dose ratio.

Dosimetric measurements were performed at the plane of cellular exposure prior to each of our 19 experimental runs using the paired ionization chamber technique (ICRU 1976, AAPM 1980, Goodman 1985, Zeman *et al.* 1988). A tissue-equivalent (TE)-wall, methane-based TE-gas filled 0·5-cm^3 Exradin chamber was used to measure γ and neutron events, whereas a 0·5-cm^3 magnesium-wall, argon-filled chamber was used to measure the γ events in the neutron-plus-γ field. From the measurements made by the paired chambers, the portions of neutrons and γ in the mixed field could be determined. For the present irradiations, the ratio of neutron to total dose was $0·95 \pm 1·4$% at all dose-rates. The instrumental uncertainties for dose and dose-rate determinations were $\pm 3·1$ and 3·6%, respectively.

Recently, the radiation quality of the TRIGA-reactor neutron field has been characterized in terms of microdosimetric spectra (H. Gerstenberg, manuscript in preparation). The spectrum was calculated from a TRIGA neutron spectrum based on both measurements and calculations (Verbinski *et al.* 1981a, b). Briefly, the mean values of lineal energy based on measured spectra using 1- or 8-μm diameter detectors, respectively, are: $y_F = 38·9$ keV/μm and $y_D = 70·5$ keV/μm, or $y_F = 21·1$ keV/μm and $y_D = 41·5$ keV/μm.

2.5. *Statistical analysis*

At the total doses used in this study the survival level were >20%. Accordingly, a linear quadratic equation $-\ln S = \alpha \cdot d + \beta \cdot d^2$ was appropriate to analyse survival data. In the case of transformation data, several radiobiologically realistic relationships were tried, such as:

$$T = a + b \cdot d \quad (1)$$

$$T = a + b \cdot d + c \cdot d^2 \quad (2)$$

$$T = b \cdot d + c \cdot d^2, \quad (3)$$

where T is the incidence of transformation, d the dose, a, b and c are regression coefficients for the induction of neoplastic transformation. The calculated best-fit curves were compared using several goodness-of-fit criteria. The coefficient of determination was used to estimate the fraction of the total variance accounted by a model, and the model selection criterion (Akaike 1976) was used to evaluate least-squares fittings to competing models. Calculations were performed using commercial software (PSI-Plot, Poly Software Inc., 1993).

Quantitative comparison of dose response curves for cell transformation from our five protraction protocols was performed by multiple-regression analysis. We used this procedure to test specifically whether the high dose-rate regression line and protracted irradiation regression lines could have come from populations with the same regression coefficients (Kleinbaum *et al.* 1988, Peixoto 1993). Commercial software (MathCAD version 2·5, MathSoft, Inc., 1989) was used for interactive programming of F-value calculations from transformation data sets in Table 1 (see Appendix).

In addition, the effect of dose protraction on survival or transformation was assessed by the dose-

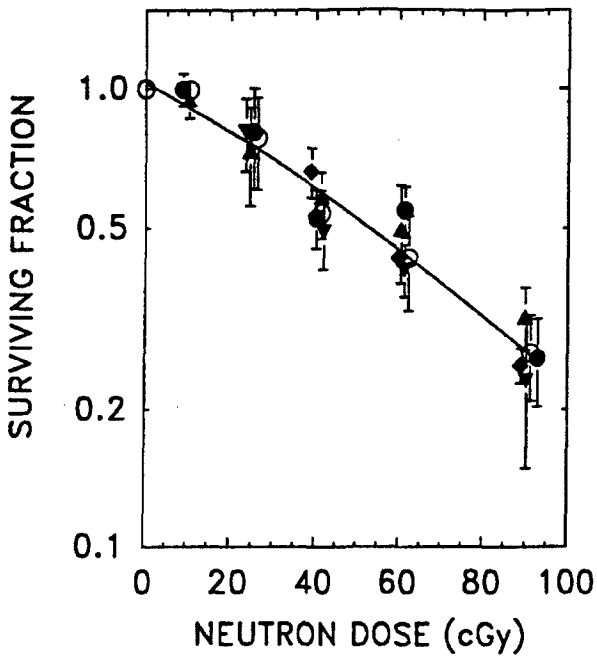

Figure 2. Survival of C3H 10T½ cells after irradiation with AFRRI TRIGA neutrons. Symbols correspond to the following patterns of exposure: ○, acute; and ●, $\tau = 0{\cdot}5$ h; ▲, $\tau = 1$ h; ▼, $\tau = 3$ h; and ◆, $\tau = 4{\cdot}5$ h; τ denotes irradiation times with the actual values listed in Table 1.

rate modifying factors (DRMF), defined as the low : high dose-rate effect ratio at the same dose. Standard formulas were used to calculate the uncertainties in DRMF estimates shown in Figures 4 and 5. The DRMF for survival or transformation were analysed by linear regression to determine whether or not they were independent of dose and dose-rate. The actual equations were:

$$DRMF = a + b \cdot d \quad (4)$$

$$DRMF = a + b \cdot \delta \quad (5)$$

where d the dose, δ the dose rate, a, b are regression coefficients in Table 3. The t-values obtained from data analysis by the above equations (4) and (5) were compared with the tabulated values, as described in the Appendix. Required calculations were performed using commercial software (MathCAD version 2·5, MathSoft, Inc., 1989).

3. Results

As shown in Table 1 and Figure 2, there were no significant effects of temporal dose distributions on cell survival. The shape of the curve was close to exponential with a linear coefficient $\alpha = (6{\cdot}48 \pm 1{\cdot}46) \times 10^{-3}\,\mathrm{cGy}^{-1}$ and a quadratic coefficient $\beta = (1{\cdot}03 \pm 0{\cdot}25) \times 10^{-4}\,\mathrm{cGy}^{-2}$. These results are in good agreement with observations from related experiments with high-LET radiations reported earlier by us (Balcer-Kubiczek *et al.* 1991, 1993, Balcer-Kubiczek and Harrison 1991a) as well as others (Han and Elkind 1979, Hill *et al.* 1982, Hieber *et al.* 1987, Saran *et al.* 1991, Goodhead *et al.* 1992, Komatsu *et al.* 1993).

As in the earlier studies with cycling or stationary cultures of C3H/10T½ cells and TRIGA neutrons (Balcer-Kubiczek *et al.* 1988, and references cited above), the dose–response curve for transformation induction was well represented by two- or three-parameter models, given by the equations (1), (2) or (3), whether exposure-time groups were considered separately or pooled. However, the amount of data in each dose response set was too small to assert any model conclusively by the usual curve fitting. Multiple-regression analysis of all the transformation data in Table 1 showed $F = 1{\cdot}59$, whereas $F_{0{\cdot}95}(4, 16) = 3{\cdot}01$ and $F_{0{\cdot}99}(4, 16) = 5{\cdot}24$ are

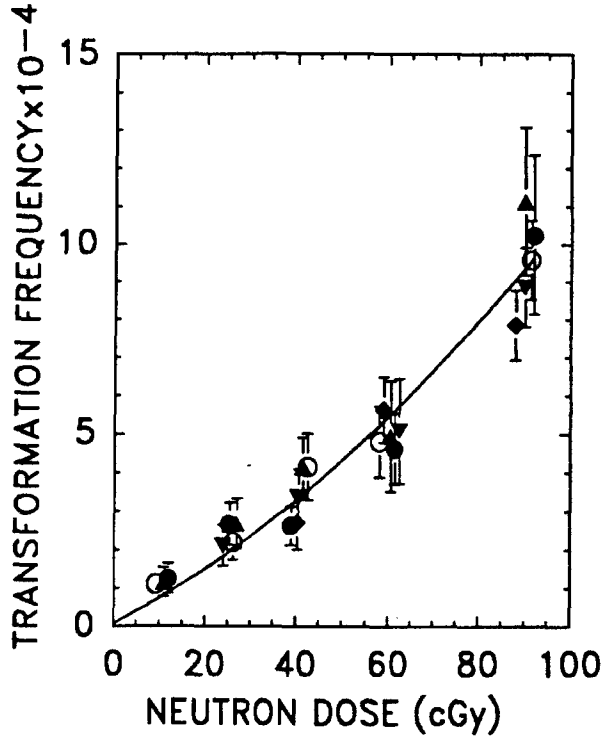

Figure 3. Neoplastic transformation of C3H/10T½ cells after irradiation with AFRRI TRIGA neutrons. Symbols correspond to the following patterns of exposure: ○, acute; ●, $\tau = 0{\cdot}5$; ▲, $\tau = 1$ h; ▼, $\tau = 3$ h; and ◆, $\tau = 4{\cdot}5$ h; τ denotes irradiation times with the actual values listed in Table 1.

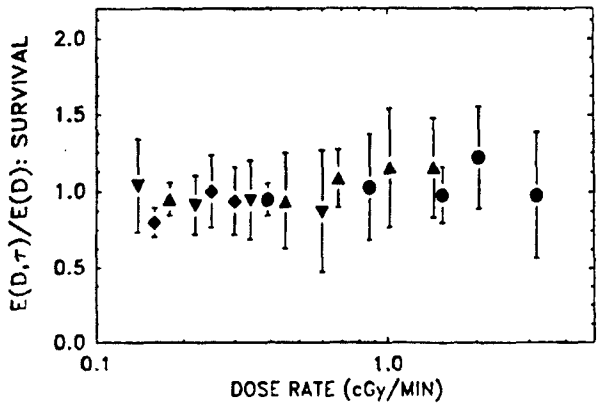

Figure 4. Dose-rate modifying factors for survival of C3H 10T½ cells plotted as a function of AFRRI TRIGA neutrons dose rate. Symbols correspond to the following patterns of exposure: ●, $\tau = 0{\cdot}5$ h; ▲, $\tau = 1$ h; ▼, $\tau = 3$ h; and ◆, $\tau = 4{\cdot}5$ h; denotes irradiation times with the actual values listed in Table 1.

required for differences among the means to be significant at the 5 and 1% levels, respectively. Thus, we concluded that the five dose response curves were satisfactorily homogeneous, and, consequently, that at a given dose there was no dependence of transformation response on dose protraction.

Accordingly, we were able to pool the data from our five exposure time groups in determining dose dependencies in transformation induction. For the models 1–3, the goodness-of-fit measures had similar values: coefficients of determination 0·93–0·95, model selection criteria 2·5–2·8, correlation coefficients 0·91–0·95. Predictably, the numerical

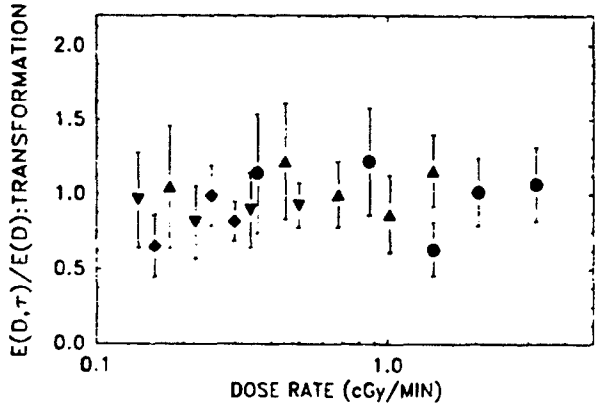

Figure 5. Dose-rate modifying factors for the induction of neoplastic transformation of C3H 10T½ cells plotted as a function of AFRRI TRIGA neutrons dose rate. Symbols correspond to the following patterns of exposure: ●, $\tau = 0{\cdot}5$ h; ▲, $\tau = 1$ h; ▼, $\tau = 3$ h; and ◆, $\tau = 4{\cdot}5$ h; denotes irradiation times with the actual values listed in Table 1.

Table 3. Linear regression coefficients and the test of independence for dose rate modifying factors DRMF

	a	b†	t
Survival			
DRMF versus dose	0·955	0·001	0·23
DRMF versus dose-rate	0·975	0·071	0·60
Transformation			
DRMF versus dose	0·961	-7×10^{-4}	0·18
DRMF versus dose-rate	1·014	0·052	0·45

a, intercept; b, slope; t value defined in Appendix.

†The unit to be used with factor b is: for the dose response relationships (cGy^{-1}), and for the dose-rate response relationships (min/cGy). Dose-rate modifying factors calculated from the data on survival or transformation (Table 1) are plotted, respectively, in Figures 4 or 5 as a function of dose-rate.

values of regression coefficients depended on the model. Our best estimate of the initial slope based on the pooled data is $5{\cdot}0\ 7{\cdot}5 \times 10^{-6}$ cGy^{-1}, and the mean weighted by the reciprocal of the variance is $6{\cdot}2 \times 10^{-6}$ cGy^{-1}. The solid line in Figure 3 represents the best fit to the linear quadratic equation (3), with the numerical solution $b = (7{\cdot}5 \pm 2{\cdot}4) \times 10^{-6}$ cGy^{-1}, and $c = (2{\cdot}4 \pm 1{\cdot}9\ \ 10^{-8}$ cGy^{-2}. Thus, if the statistical analysis is restricted to the initial part of transformation induction curve (<60 cGy) a linear fit is an adequate representation, since the contribution from the quadratic term is <10%.

To further confirm the above findings, dose-rate modifying factors for survival or transformation, shown in Figures 4 or 5, were tested for dependence on dose and dose-rate by the equations (4) and (5). Specifically, the dependence of DRMF on dose provides qualitative information about the basic form of dose response curve obtained for different dose protraction patterns, relative to the basic form of dose response curve obtained from a high dose-rate exposure at the same dose. The results of the numerical curve fitting of our four data sets to a straight line with the intercept a and the slope b (cGy^{-1} or min/cGy) are shown in Table 3. The last column in Table 3 lists t-values of about 0·2 for dependence of DRMFs on the dose and of about 0·5 for dependence of DRMFs on dose-rate. At $\alpha = 0{\cdot}05$, the critical $t_{0{\cdot}025}(15) = 2{\cdot}131$ and at $\alpha = 0{\cdot}01$, $t_{0{\cdot}005}(15) = 2{\cdot}947$. Therefore, we are required to conclude that, at the 5 or 1% levels of significance, the DRMFs were independent of both the dose-rate and dose; that is, the basic forms of dose response curves are the same. Intuitively, the same conclusion can be reached by observing that both the intercepts and the mean values of DRMFs are close to unity; for survival and transformation they were respectively: 1·01 (0·92, 1·09) and 0·98 (0·83, 1·08), where

the numbers in brackets are 99% confidence limits. By inspecting Figures 4 and 5, an additional observation can be made that the quality of the transformation data can be quite good, comparable with the quality of survival data. Thus, the scatter in transformation data sets should not be relied upon in justifying a wide range of model-based speculations (Brenner and Hall 1990, 1992, Hall 1991).

4. Discussion

In this report cell survival and the induction of neoplastic transformation were studied after fission neutron irradiations at up to five exposure durations per dose. Such an experimental design, the first reported for high-LET radiation studies, permitted direct examination of the dose-rate influence on these end points at multiple dose rates. The dose-rate range included single values of dose rate $\leqslant 0{\cdot}5$ cGy/min previously studied in this and other laboratories using the constant dose-rate approach. The reference acute irradiations were performed concurrently with protracted irradiations at the same dose. Identical protocols were used in all irradiations, including media composition, age and proliferative state of cultures, initial plating densities and the numbers of dishes, thus providing statistically acceptable conditions for testing the null hypothesis that, at the same dose, there was no difference in the effectiveness of fission neutrons due to differences in the temporal pattern of exposure.

Technical limitations in the present study precluded investigations of neutron doses of < 10 cGy, a limitation that also applies to most (Hill *et al.* 1982, 1984, Balcer-Kubiczek *et al.* 1988, Coggle 1988, Miller *et al.* 1990, 1993, Hill and Zhu 1991, Miller and Hall 1991, Saran *et al.* 1991) but not all *in vivo* and *in vitro* studies of radiation carcinogenesis (Ullrich 1984, Balcer-Kubiczek and Harrison 1991a). Nevertheless, two interesting and potentially important results emerged from the present investigations. First, we confirmed that the dose–response curves for survival and transformation at doses < 1 Gy are best described by a linear equation or, possibly, a linear quadratic equation with a weak positive quadratic term. Similar to previous results with exponential or plateau cultures of C3H/10T½ cells and TRIGA neutrons (Balcer-Kubiczek and Harrison 1991a) as well as with some other neutron sources (Coggle 1988, Saran *et al.* 1991, Komatsu *et al.* 1993), we did not observe 'supralinearity' in the dose–response curve for transformation, often associated with the so-called 'inverse dose-rate effect' reported for γ-, X-rays (Borek and Hall 1974, Miller and Hall 1978, Little 1979, Hall and Miller 1981) and fission neutrons (Hill *et al.* 1982, Ullrich 1984). Indeed, models by Rossi (1981), Rossi and Kellerer (1986), Burch and Chesters (1986), Brenner and Hall (1990) and Elkind (1991) show, either directly or by implication, that the enhancement of an effect due to protraction may occur in a dose range where a dose–response curve has a fine structure such, for example, has a locally negative (convex) curvature; 'bending down' segments of response curves after brief and/or protracted exposures to low and high LET radiations were reported for neoplastic transformation, animal carcinogenesis and, more recently, for mutation induction, but not cell survival or mortality (Miller and Hall 1978, 1991, Little 1979, Hall and Miller 1981, Hill *et al.* 1982, Ullrich 1984, Bettega *et al.* 1992, Tauchi *et al.* 1993). Thus, our second important result that, at a given dose, survival or transformation did not vary with a temporal pattern of exposure, can be seen as a direct consequence of the first result, just discussed above, that dose–response curves for neutrons are linear < 1 Gy. Similar conclusions were published by Coggle (1988) based on the analysis of rodent carcinogenesis data.

Recently, several interpretations of locally complex dose–response curves and the inverse dose-rate effect have been proposed (Rossi and Kellerer 1986, Dennis and Dennis 1988, Sykes and Watt 1989, Brenner and Hall 1990, Elkind 1991, Watt 1992). Most of these models are an extension of an idea (Oftedal 1968) that there is a time period in the cell cycle during which cells are more susceptible to transformation (or mutation; Tauchi *et al.* 1993) than at other times. A corollary is that for exponentially growing cultures with the same distribution of cells throughout the cell cycle at the beginning of exposure, different distributions and, therefore, different fractions of the hypersensitive cells are expected at the end of protracted versus brief exposures. At present, there is no agreement among the models as to a duration (Rossi and Kellerer 1986, Brenner and Hall 1990, Elkind 1991, Harrison and Balcer-Kubiczek 1992, Watt 1992) or a specific location of the sensitive period within the cell cycle (Brenner and Hall 1990, Elkind 1991, Cao *et al.* 1992, Komatsu *et al.* 1993, Miller *et al.* 1993, Tauchi *et al.* 1993), but periods of sensitivity lasting from a few seconds or less to about 1 or 2 h can be inferred, as discussed below. Although the present study was concerned with investigation of dose-rate effects of fission neutrons rather than with testing the biophysical models cited above, our measurements of the cell cycle distributions of control and irradiated cells (Table 2) argue directly against the validity of these notions. Independently, quantitatively similar results and conclu-

sions, based on experiments with fission neutrons (Saran *et al.* 1993) and with monoenergetic neutrons of several different energies (0·5, 1·0 and 6·0 MeV) (Pazzaglia *et al.* 1993), were recently reported for control and neutron-irradiated C3H/10T½ cells.

In experimental investigations of dose-rate or dose-fractionation effects, it is important to ascertain whether the variability of a response can be attributed to a pattern of exposure and not merely the scatter of data. Figures 4 and 5 show enhancements (and reductions) of less than a factor of 2 at several dose-rates, such as are reported by some investigators as the inverse dose-rate effect. However, in contrast with our present report, these other constant dose-rate studies were limited to, at best, two dose-rates (Hill *et al.* 1982, Hill and Zhu 1991, Miller and Hall 1991, Redpath *et al.* 1991) and/or a narrow range of doses, including measurements at a single dose (Ullrich 1984, Hill and Zhu 1991, Miller *et al.* 1991, 1993, Redpath *et al.* 1991); consequently, these results do not lend themselves to rigorous analysis by conventional statistical methods such as presented here, nor to the analysis by multiparameter models, such as those proposed for transformation data by Burch and Chesters (1986), Rossi and Kellerer (1986) or Brenner and Hall (1990).

We examined the latter model analytically (Harrison and Balcer-Kubiczek 1992) with the intention of applying the model to our transformation data for TRIGA neutrons (Balcer-Kubiczek *et al.* 1988, 1991, Balcer-Kubiczek and Harrison 1991a, and this report) and found a lack of a single-valued solution for the duration of the postulated transformation-susceptible portion of the cell cycle for neutrons (Figures 1 and 2; Harrison and Balcer-Kubiczek 1992); this is in contrast with conclusions published by these authors that the model predicts a single sensitivity period of 1·02 ± 0·25 h. Our subsequent examination of the mathematical determination of model input parameters from experimental data (Balcer-Kubiczek and Harrison 1992) revealed an additional caveat, that the prediction of the inverse dose-rate for transformation by neutrons requires an unrealistically low value of frequency mean lineal energy, such as, $y_f = 1$ keV μm in an 8-μm diameter cavity for fission neutrons. As shown here, this assumption is out of line for TRIGA neutrons with $y_f = 21{\cdot}2$ keV μm. In the case of other neutron sources, for JANUS neutrons $y_f = 42$ keV μm, and for 6-MeV monoenergetic neutrons $y_f = 18{\cdot}7$ keV μm in an 8-μm site, which is similar to y_f for TRIGA neutrons (H. Gerstenberg, manuscript in preparation), whereas $y_f = 1$ or 15·7 keV μm were, respectively, assumed in the model calculations (Brenner and Hall 1990); thus, depending on neutron source, the model input variable y_f was *underestimated* by up to a factor of 40, so it follows that the magnitude of the inverse dose-rate for these sources was *overestimated* by correspondingly similar factors. Applied to our present data, this means that, based on the Brenner and Hall model, no enhancement can be expected in exponentially growing cultures of C3H/10T½ cells even at dose rates $< 0{\cdot}5$ cGy/min in the dose range examined. To further illustrate the impact of the higher y_f on this model, we note that no dose-rate effect can be predicted to occur in neutron doses and dose-rates relevant to radiation protection. Specifically, to use the example in Brenner and Hall (1990), no enhancement for fission neutrons can be expected at 0·5 cGy and a dose-rate $= 10^{-6}$ cGy/min. In fact, we calculated that in order to obtain the two-fold transformation enhancement for fission neutrons at this dose-rate, the total dose would have to be 50 cGy. Assuming a quality factor of 10, this corresponds to the dose which is 100 times the annual effective occupational dose permitted by current US standards.

In summary, it seems reasonable to conclude from the present direct comparisons of cell survival, transformation and age distributions of control and irradiated cells, as well as by examining assumptions in biophysical models of the inverse dose-rate effect, that there is no real, in a statistical sense, modification of these end points by extending the duration of fission neutron exposure or, equivalently, by lowering the fission neutron dose-rate.

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Appendix

The following methods are independent of any model assumptions for error calculation of surviving fractions, transformation frequency or derived qualities.

A1. *Test of independence*

One criterion of independence is that the mean DRMF is the same for each value of dose or dose-rate, which in the case of linear regression, requires that the slope equals 0. To test for independence of DRMF, we calculated:

$$t=\frac{(b-0)\cdot S_x\sqrt{n-1}}{S_{y\cdot x}},$$

where b is the slope, n the number of observations, and S_x and $S_{y\cdot x}$ have the usual meaning (Dixon and Masey 1957), and compared its value with the t-distribution. The null hypothesis: $b=0$ can be rejected at the α-level of significance, and the two variables are said to be dependent, if $t<t_{1/2\cdot\alpha}(n-2)$ or if $t>t_{1-1/2\cdot\alpha}(n-2)$.

A2. *Multiple-regression analysis*

This section will provide explicit formulas and mathematical procedures used in the multiple-regression analysis of transformation data graphed in Figure 3. They are also suitable for a similar analysis of any results, if the assumption can be made that all the curves have a coinciding intercept; in the present case, this is a reasonable expectation since the intercept, by definition, is the spontaneous transformation frequency.

We consider our exposure-time groups, i ($i=0, 1, 2, 3, 4$), where $i=0$ denotes an arbitrary reference group, chosen here to be the high dose-rate group, and separate independent variables x_k ($k=1, 2, 3, 4$), such that $x_k=1$, if group k, and $=0$ otherwise. The multiple-regression equation or

'full' model: $T=a+d\cdot b_0+x_1\cdot d\cdot b_1+\cdots+x_4\cdot d\cdot b_4$

has six independent multiple-regression coefficients to be found. A regression coefficient such as b_1, denotes the regression coefficient of T on variable x_1 that one would expect to obtain if other variables in the 'full' model equation x_2, x_3 and x_4 had been held constant experimentally. Thus, the 'full' model for group 1: $T=a+d\cdot b_0+x_1\cdot d\cdot b_1$, for group 2: $T=a+d\cdot b_0+x_2\cdot d\cdot b_2$, etc. The null-hypothesis is,

$$H_0: b_1=b_2=b_3=b_4=0,$$

and, accordingly:

$$H_0 \text{ model: } T=a+d\cdot b_0.$$

It should be noted that the above equation doses not imply that the actual relationship between transformation frequency and the dose is linear, but rather than a linear approximation explains a significant amount of variability in T over the range of d values, which is true in the case of our data.

The procedure compares the calculated F to the $F_\alpha(\mu_1,\mu_2)$ distribution with the appropriate numbers of degrees of freedom; the symbols have the following definitions: $\mu_1=(n-2)-(n-6)$, $\mu_2=n-6$ are degrees of freedom in the numerator and denominator, respectively, $\alpha=$ the cumulative probability, $n=$ the number of observations in all groups, and

$$F=\frac{[SSE(H_0 \text{ model})-SSE \text{ ('full' model)}]/\mu_1}{SSE \text{ ('full' model)}/\mu_2},$$

where $SSE=$ sum of squared sampling errors, defined just below.

Let $t_{ij}=$ observed transformation response of ith group at jth dose, and $d_j=j$th dose.

For H_0 model: $SSE=\Sigma[t_{ij}-(a+d_j\cdot b_0)]^2$,

where summation is performed over groups, $i(=0, 1, \ldots, 4)$ and data points in the combined set, $j(=1, \ldots, 22$ for our data set; Table 1).

For 'full' model: $SSE=\mathbf{T}^{\mathrm{T}}\cdot\mathbf{T}-\mathbf{T}^{\mathrm{T}}\cdot\mathbf{X}\cdot\boldsymbol{\beta}$,

where the matrix $\mathbf{X}$ is defined below, $\mathbf{T}$ denotes a vector constructed from t_{ij} values, and β denotes a vector constructed from multiple-regression coefficients found by solving the following matrix equation:

$$\begin{bmatrix} t_{0.1}\\ t_{0.2}\\ t_{1.1}\\ \cdot\\ \cdot\\ \cdot\\ \cdot\\ t_{4.1}\\ t_{4.2}\\ t_{4.3}\end{bmatrix} \equiv \begin{bmatrix} 1 & d_1 & 0 & 0 & 0 & 0\\ 1 & d_2 & 0 & 0 & 0 & 0\\ 1 & d_6 & 1 & 0 & 0 & 0\\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot\\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot\\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot\\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot\\ 1 & d_{20} & 0 & 0 & 0 & 1\\ 1 & d_{21} & 0 & 0 & 0 & 1\\ 1 & d_{22} & 0 & 0 & 0 & 1\end{bmatrix} \cdot \begin{bmatrix} a\\ b_0\\ b_1\\ b_2\\ b_3\\ b_4\end{bmatrix}.$$

$\mathbf{T}$ $\mathbf{X}$ $\boldsymbol{\beta}$

This equation has the solution:

$$\boldsymbol{\beta} = (\mathbf{X}^{\mathrm{T}} \cdot \mathbf{X})^{-1} (\mathbf{X}^{\mathrm{T}} \cdot \mathbf{T})$$

which is then used to determine *SSE* for 'full' model and, finally *F*. For the present data: $b_0 = -0{\cdot}433$, $b_1 = 0{\cdot}103$, $b_2 = 0{\cdot}222$, $b_3 = -0{\cdot}363$, $b_4 = 0{\cdot}354$, and $b_5 = -0{\cdot}981$. The sign change in multiple-regression coefficients indicates that both up and down adjustments in groups 1 ($\tau = 0{\cdot}5$ h), 2 ($\tau = 1$ h), 3 ($\tau = 3$ h), 4 ($\tau = 4{\cdot}5$ h) are required, consistent with small *F*-values which, in turn, corresponds to a high probability that the same curve holds for the data.

References

AAPM, 1980. Protocol for neutron beam dosimetry. Report No. 7 (New York, American Association of Physicists in Medicine, American Institute of Physics).

Akaike, H., 1976. An information criterion. *Mathematical Science*, **1**, 5–9.

Balcer-Kubiczek, E. K. and Harrison, G. H., 1991a. Lack of dose-rate modification 0·0049 versus 0·12 Gy·min⁻¹ of fission-neutron-induced neoplastic transformation in C3H 10T½ cells. *International Journal of Radiation Biology*, **59**, 1017–1026.

Balcer-Kubiczek, E. K. and Harrison, G. H., 1991b. Neoplastic transformation of C3H 10T½ cells following exposure to 120-Hz modulated 2·45-GHz microwaves and phorbol ester tumor promoter. *Radiation Research*, **126**, 65–71.

Balcer-Kubiczek, E. K. and Harrison, G. H., 1991c. '*Inter silvas Academi quaerere verum*': reply to Letter to the Editor by M. M. Elkind. *International Journal of Radiation Biology*, **59**, 1477–1482.

Balcer-Kubiczek, E. K. and Harrison, G. H., 1992. AFRRI-neutron transformation results viz. current models (Poster P-24-9, 40th Annual Meeting of the Radiation Research Society, Salt Lake City, UT, USA, 14–18 March).

Balcer-Kubiczek, E. K. and Harrison, G. H. and Hei, T. K., 1991. Neutron dose-rate experiments at the AFRRI nuclear reactor. *Radiation Research*, **128**, S65–70.

Balcer-Kubiczek, E. K. and Harrison, G. H. and Thompson, B. W., 1987. Repair time for oncogenic transformation in C3H 10T½ cells subjected to protracted X-irradiation. *International Journal of Radiation Biology*, **51**, 219–226.

Balcer-Kubiczek, E. K. and Harrison, G. H., Zeman, G. H., Mattison, P. J. and Kuxska, A., 1988. Lack of inverse dose-rate effect on fission neutron induced transformation of C3H 10T½ cells. *International Journal of Radiation Biology*, **54**, 531–536.

Balcer-Kubiczek, E. K., Hill, C. K., Harrison, G. H. and Blakely, W. F., 1993. Effects of WR-1065 and WR-151326 on survival and neoplastic transformation in C3H 10T½ cells exposed to TRIGA or JANUS fission neutrons. *International Journal of Radiation Biology*, **63**, 37–46.

Bettega, D., Calzolari, G., Noris Chiorda, G. and Tallone-Lombardi, L., 1992. Transformation of C3H 10T½ cells with 4·3 MeV α particles at low doses: effects of single and fractionated doses. *Radiation Research*, **131**, 66–71.

Borek, C. and Hall, E. J., 1974. Effect of split doses of X-rays on neoplastic transformation of single cells. *Nature*, **252**, 499–501.

Brenner, D. J. and Hall, E. J., 1990. Inverse dose-rate effect for oncogenic transformation by neutrons and charged particles: a plausible interpretation consistent with published data. *International Journal of Radiation Biology*, **58**, 745–758.

Brenner, D. J. and Hall, E. J., 1992. Commentary 2 to Cox and Little: Radiation-induced oncogenic transformation: the interplay between dose, dose protraction, and radiation quality. *Advances in Radiation Biology*, **16**, 167–179.

Burch, P. R. J. and Chesters, M. E., 1986. Neoplastic transformation of cells *in vitro* at low and high dose rates of fission neutrons: an interpretation. *International Journal of Radiation Biology*, **49**, 495–500.

Cao, J., Wells, R. L. and Elkind, M. M., 1992. Enhanced sensitivity to neoplastic transformation by ^{137}Cs γ-rays of cells in the G_2/M-phase age interval. *International Journal of Radiation Biology*, **62**, 191–199.

Goggle, J. E., 1988. Lung tumour induction in mice after X-rays and neutrons. *International Journal of Radiation Biology*, **53**, 585–598.

Dennis, J. A. and Dennis, L. A., 1988. Neutron dose relationships at low doses. *Radiation and Environmental Biophysics*, **27**, 91–101.

Dixon, W. J. and Massey, F. J., 1957. *Introduction to Statistical Analysis* (New York, McGraw-Hill), chapter 11.

Elkind, M. M., 1991. Enhanced transformation due to protracted exposures of fission-spectrum neutrons: a biophysical model. *International Journal of Radiation Biology*, **59**, 1471–1476.

Goodhead, D. T., Bell, M., Hill, A. J., Bance, D. A., Allen, L. A., Hall, S. C., Ianzani, F., Simone, G., Stevens, D. L., Stretch, A., Tabocchini, M. A. and Wilkinson, R. E., 1992. Direct comparison between protons and alpha-particles of the same LET. I. Irradiation methods and inactivation of asynchronous V79, HeLa and C3H 10T½ cells. *International Journal of Radiation Biology*, **61**, 611–624.

Goodman, L. J., 1985. A practical guide to ionization chamber dosimetry at the AFRRI reactor. AFRRI Contract Report CR85-1 (Bethesda, MD, Armed Forces Radiobiology Research Institute).

Hall, E. J., 1991. Weiss Lecture: The dose rate factor in radiation biology. *International Journal of Radiation Biology*, **59**, 595–610.

Hall, E. J. and Miller, R. C., 1981. The how and the why in *in vitro* oncogenic transformation. *Radiation Research*, **87**, 208–223.

Han, A. and Elkind, M. M., 1979. Transformation of mouse C3H 10T½ cells by single and fractionated doses of X-rays and fission spectrum neutrons. *Cancer Research*, **39**, 123–130.

Harrison, G. H. and Balcer-Kubiczek, E. K., 1992. Ambiguity of the Brenner Hall model. *International Journal of Radiation Biology*, **61**, 139–142.

Hieber, L., Ponsel, G., Roos, H., Fenn, S., Fromke, E. and Kellerer, A. M., 1987. Absence of a dose rate effect in the transformation of C3H 10T½ cells by α-particles. *International Journal of Radiation Biology*, **52**, 859–869.

Hill, C. K., Buonoguro, F. M., Myers, C. P., Han, A. and Elkind, M. M., 1982. Fission-spectrum neutrons at

reduced dose rate enhance neoplastic transformation. *Nature*, **298,** 67–68.

HILL, C. K., HAN, A. and ELKIND, M. M., 1984, Fission-spectrum neutrons at low dose rate enhance neoplastic transformation in the linear, low dose region (0–10cGy). *International Journal of Radiation Biology*, **46,** 11–14.

HILL, C. K. and ZHU, L., 1991, Energy and dose-rate dependence of neoplastic transformation and mutations induced in mammalian cells by fast neutrons. *Radiation Research*, **124,** S48–S52.

IARC/NCI/EPA WORKING GROUP, 1985, Cellular and molecular mechanisms of cell transformation and standardization of transformation assays of established cell lines for prediction of carcinogenic chemicals: overview and recommended protocols. *Cancer Research*, **45,** 2395–2399.

ICRU, 1976, Neutron dosimetry for biology and medicine. Report No. 26. (Washington, DC, International Commission on Radiation Units and Measurements).

KELLERER, A. M. and ROSSI, H. H., 1972, The theory of dual radiation action. *Current Topics in Radiation Research Quarterly*, **8,** 85–158.

KLEINBAUM, D. G., KUPPER, L. L. and MULLER, K. E., 1988. *Applied Regression Analysis and Other Multivariate Methods* (Boston, PWS-Kent .

KOMATSU, K., SAWADA, S., TAKEOKA, S., KODAMA, S. and OKUMURA, Y., 1993, Dose-rate effects of neutrons and gamma rays on the induction of mutation and oncogenic transformation in plateau-phase mouse m5S cells. *International Journal of Radiation Biology*, **52,** 859–869.

LITTLE, J. B., 1979, Quantitiative studies of radiation transformation with A31-11 mouse BALB3/3T3 cell line. *Cancer Research*, **39,** 1474–1480.

MILLER, R. C. and HALL, E. J., 1978, X-ray dose fractionation and oncogenic transformation in cultured mouse embryo cells. *Nature*, **272,** 58–60.

MILLER, R. C., BRENNER, D. J., RANDERS-PEHRSON, G., MARINO, S. A. and HALL, E. J., 1990, The effects of the temporal distribution of dose on oncogenic transformation by neutrons and charged particles of intermediate LET. *Radiation Research*, **124,** S62–68.

MILLER, R. C. and HALL, E. J., 1991, Oncogenic transformation of C3H 10T½ cells by acute and protracted exposures to monoenergetic neutrons. *Radiation Research*, **128,** S60–64.

MILLER, R. C., RANDERS-PEHRSON, G., HIEBER, L., MARINO, S. A., RICHARDS, M. and HALL, E. J., 1993, The inverse dose-rate effect for oncogenic transformation is dependent on linear energy transfer. *Radiation Research*, **133,** 360–364.

MOORE, M. L. and ELSASSER, S., 1986, The TRIGA Reactor Facility at the Armed Forces Radiobiology Research Institute: a simplified technical description. AFRRI Technical Report TR86-1 (Bethesda, MD, Armed Forces Radiobiology Research Institute).

OFTEDAL, P., 1968, A theoretical study of mutant yield and cell killing after treatment of heterogeneous cell populations. *Hereditas*, **60,** 177–210.

PAZZAGLIA, S., PRISET, L., SARAN, A., ROBESSI, S., COPPOLA, M., DI MAJO, V., BROERSE, J. J., ZOTELIEF, H. and COVELLI, V., 1993, Neutron-induced neoplastic transformation of C3H 10T½ cells (Poster 17). International Symposium on Molecular Mechanisms of Radiation and Carcinogen-induced Cell Transformation. Mackinac Island, MI, USA, 19–23 September.

PEIXOTO, J. L., 1993, Incorrect F-formulas in regression analysis. *American Statistician*, **47,** 194–197.

REDPATH, J. L., SUN, C. and BLACKELY, W. F., 1991, Effect of fission-neutron dose rate on the induction of a tumor-associated antigen in human cell hybrids (HeLa × skin fibroblasts). *Radiation Research*, **128,** S71–74.

REZNIKOFF, C. A., BERTRHAM, J. S., BRANKOW, D. W. and HEIDELBERGER, C., 1973b, Quantitative and qualitative studies of chemical transformation of cloned line of C3H mouse embryo cells sensitive to postconfluence inhibition of cell division. *Cancer Research*, **33,** 3239–3249.

REZNIKOFF, C. A., BRANKOW, D. W. and HEIDELBERGER, C., 1973a, Establishment and characterization of a cloned line of C3H mouse embryo cells sensitive to postconfluence inhibition of cell division. *Cancer Research*, **33,** 3231–3238.

ROSSI, H. H., 1981, Consideration on the time factor in radiobiology. *Radiation and Environmental Biophysics*, **18,** 1–9.

ROSSI, H. H. and KELLERER, A. M., 1986, The dose-rate dependence of oncogenic transformation by neutrons may be due to variation of response during the cell cycle. *International Journal of Radiation Biology*, **50,** 353–361.

SARAN, A., PAZZAGLIA, S., PARISET, L., REBESSI, S., COPPOLA, M., DI MAJO, V. and COVELLI, V., 1993, Cell-cycle dependence of C3H/10T½ cell transformation by fission-spectrum neutrons and X-rays (Poster P-17-4). 41st Annual Meeting of the Radiation Research Society, Dallas TX, USA, 20–25 March.

SARAN, A., PAZZAGLIA, S., COPPOLA, M., REBESSI, S., DI MAJO, V., GARAVINI, M. and COVELLI, V., 1991, Absence of dose-fractionation effect on neoplastic transformation induced by fission-spectrum neutrons in C3H/10T½ cells. *Radiation Research*, **126,** 343–348.

SYKES, C. E. and WATT, D. E., 1989, Interpretation of the increase in the frequency of neoplastic transformations observed for some ionising radiations at low dose rates. *International Journal of Radition Biology*, **55,** 925–942.

TAUCHI, H., NAKAMURA, N. and SAWADA, S., 1993, Cell cycle dependence for the induction of 6-thioquanine-resistant mutations: G_2/M stage is distinctively sensitive to ^{252}Cf neutrons but not to ^{60}Co γ-rays. *International Journal of Radiation Biology*, **63,** 475–481.

ULLRICH, R. L., 1984, Tumor induction in BALB/c mice after fractionated or protracted exposures to fission-spectrum neutrons. *Radiation Research*, **97,** 587–597.

VERBINSKI, V. V., FERLICK, K., CASSAPAKIS, C. C., DAXON, E. and HAGAN, W. K., 1981a, Radiation field characterization for the AFRRI TRIGA reactor. Report No. 5793F-1, Vol. 1 (Washington, DC, Defense Nuclear Agency).

VERBINSKI, V. V., FERLICK, K., CASSAPAKIS, C. C., DAXON, E. and HAGAN, W. K., 1981b, Calculation of the neutron and gamma-ray environment in and around for the AFRRI TRIGA reactor. Report No. 5793F-2, Vol. 2 (Washington, DC, Defense Nuclear Agency).

WATT, D. E., 1992, Comment on mechanism of radiation effects with special reference to transformation. Letter to Editor. International Journal of Radiation Biology, **61,** 263–267.

ZEMAN, G. H., DOOLEY, M., EAGLESON, D. M., GOODMAN, L., SCHWARTZ, R. B., EISENHAUER, C. M. and MCDONALD, J. C., 1988, Intercomparison of neutron dosimetry techniques at the AFRRI TRIGA reactor. *Radiation Protection Dosimetry*, **23,** 317–320.