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ASSESSMENT OF TUMOUR RESPONSE IN A RAT RHABDOMYOSARCOMA

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Summary.—A rhabdomyosarcoma in a WAG/Rij rat with capacity for colony growth after tumour excision and enzymatic dissociation has been used to study response to high and low LET radiation. End points are tumour volume response, $TCD_{50/180}$, clonogenic capability after tumour irradiation *in situ*, and *in vitro* cell survival after irradiation in both the well-oxygenated and the hypoxic conditions. Experience has shown that sublines with different growth rates, radiosensitivity and plating efficiency can arise from the same frozen stock. The conclusions that can be drawn from an analysis of the data obtained to date are as follows:

1. **There is no correlation between the doubling times of our two cell lines growing in culture and in the animal.**
2. **RBE values obtained from growth delay and from TCD_{50} end points are in fair agreement.**
3. **Cell survival curves obtained after *in situ* irradiation show the cells to be more radioresistant than cells irradiated in suspension *in vitro*.**
4. **RBE values for cell survival after *in situ* irradiation compare favourably with those obtained both for tumour growth delay and *in vitro* cell survival.**
5. **Not enough is known at present about radiation-altered cell kinetics to develop a self-consistent model of tumour response after irradiation.**

THE R1 rhabdomyosarcoma tumour system developed originally at Rijswijk (Reinhold, 1965; Barendsen & Broerse, 1969) has been in use for several years at the Lawrence Berkeley Laboratory. The major characteristics of this tumour system are as follows.

(a) It is adapted for growth both *in vitro* as a monolayer culture and *in vivo* as easily measurable spherical tumours in inbred WAG/Rij rats.

(b) The tumours are homogeneous and free of large necrotic areas until they exceed 3 g.

(c) Growth properties are reproducible, and there is no evidence of an immune response by the host animal.

(d) Cell suspensions obtained by enzymatic dissociation of excised tumours can be grown *in vitro* with acceptable plating efficiencies, thus permitting cell survival curves to be obtained for tumours irradiated *in situ*.

Two different cell lines, designated R1/LBL and R2D2, have been used in our experiments. The R1/LBL line was originally obtained from Rijswijk and has been used for the majority of our volume response (growth delay) and tumour cure (TCD_{50}) experiments. However, its plating efficiency after growing as a tumour in the WAG/Rij rat is unacceptably low ($< 1\%$) making it unsuitable for *in vitro* assay of cell survival after irradiation *in situ*. The R2D2 cell line was subsequently obtained from Rijswijk out of the same frozen stock. It is clonable after growth in the animals (plating efficiency $\sim 18\%$) and has been used for cell survival studies after irradiation *in situ*, as well as for a limited number of tumour regrowth studies. The R2D2 cell line has also been used for all of our *in vitro* studies of cellular radiation response. The plating efficiency is $\sim 65\%$ for this cell line maintained *in vitro*.

A comparison of the growth rates of these two cell lines both in the animal and in culture is of interest. Although the R1/LBL line grows faster *in vivo* than the R2D2 line (T_D ranging from 4·8–7 days over a 20 day period cf 5·8–10 days) the reverse is true *in vitro* where the cultured R2D2 cells have a shorter doubling time, 14 h cf 18 h for R1/LBL. Thus, there is no correlation between the two cell lines in their growth rates *in vivo* and *in vitro*.

Studies have recently been reported on the growth delay observed for tumours of the R1/LBL line after helium-ion and neon-ion irradiation (Curtis *et al.*, 1978). Fig. 1 shows the volume response after various doses of 220-kV X-rays as an example of the type of data obtained. The radiation-induced growth delay is determined from the tumour volume response data by calculating the difference between the times for the irradiated and the control tumours to double in volume.

In Fig. 2 tumour growth delays are plotted as a function of dose for carbon-, neon- and argon-ion beams, and are compared with the growth delay curve obtained following exposure to X-rays. RBE's for different growth delays *e.g.* 50 days (RBE_{50} values) and their standard deviations are easily calculated from these

TABLE I.—*RBE's for growth delay and tumour cure*

Radiation modality	Initial energy	Tumour position	RBE_{20}	RBE_{50}	$RBE_{TCD50/180}$
^{12}C	400 MeV/u	4 cm extended peak	2·8±0·7	2·3±0·4	2·3±0·3
^{20}Ne	400, 425 MeV/u	4 cm extended peak	2·9±0·7	2·6±0·5	3·1±0·6
^{40}Ar	570 MeV/u	4 cm extended peak	3·0±0·6	2·5±0·3	—
^{12}C	400 MeV/u	plateau	1·3±0·3	1·3±0·2	—
^{20}Ne	400, 425 MeV/u	plateau	1·7±0·4	1·8±0·3	—
15 MeV neutrons*			3·3	3·1†	2·8‡

* From Barendsen & Broerse, 1969.
† Extrapolated value.
‡ RBE for TCD 90/120, calculated from extrapolation of cell survival data.

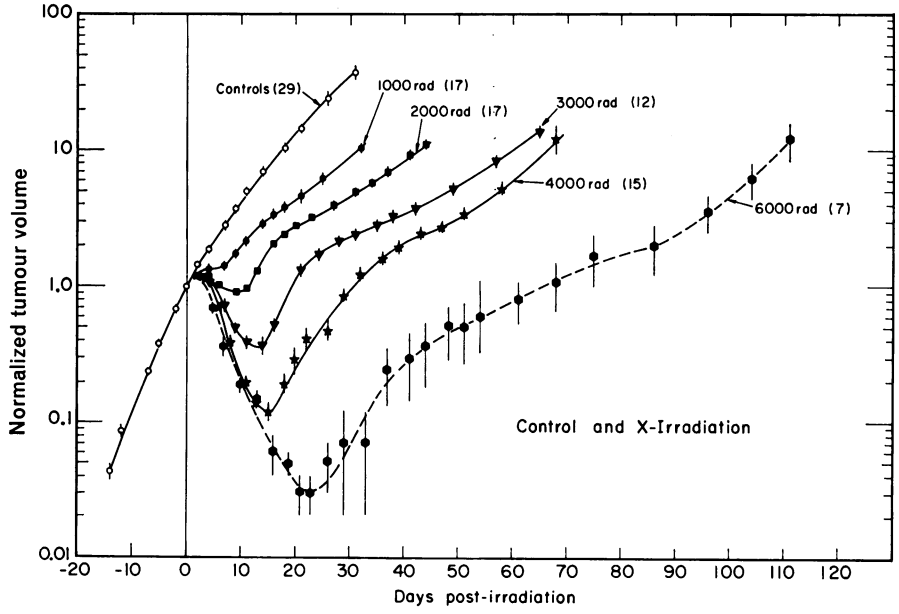

FIG. 1.—Volumes of R1/LBL tumours are plotted as a function of time for controls and for tumours receiving graded doses of 220-kV X-rays. The volumes have been normalized to unity on the day of irradiation. Numbers in parentheses represent the number of tumours exposed to each radiation dose. Error bars represent one standard error of the mean.

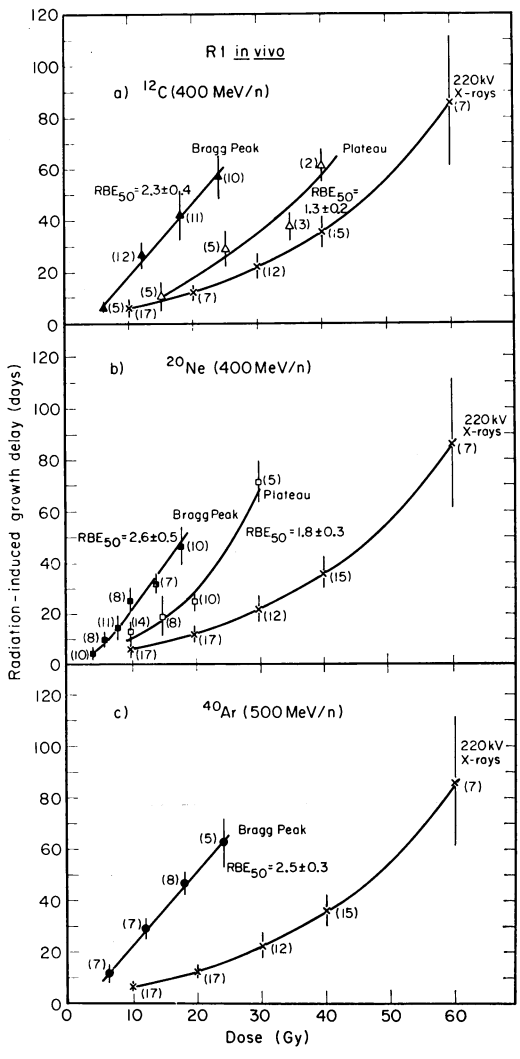

FIG. 2.—The radiation-induced growth delay (to twice the volume at irradiation) is plotted as a function of dose for R1/LBL tumours exposed to the following radiation modalities: 220-kV X-rays, plateau (high-energy) carbon and neon ions, and carbon, neon and argon ions in the distal position of a 4-cm extended peak. The RBE_{50} values were calculated as the ratio of the dose of X-rays to the dose of charged-particle radiation required to produce a 50-day growth delay.

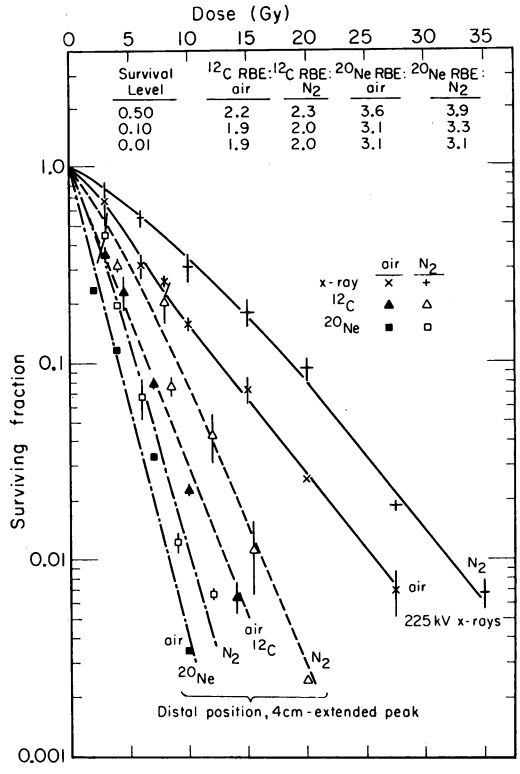

FIG. 3.—Cell survival curves are shown for R2D2 tumours irradiated *in situ* with 225-kV X-rays and with carbon and neon ions in the 4-cm extended peak region. Data are presented for tumours irradiated both in air-breathing rats and in rats asphyxiated with nitrogen gas to produce hypoxia.

data as shown in Fig. 2. A comparison of these RBE's with those obtained from tumour cure studies is shown in Table I. In the latter studies, the end point was tumour cure in 50% of the animals at 180 days post-irradiation. Two experiments, one in the carbon and one in the neon peak regions, yielded similar RBE values with the two techniques.

The R2D2 cell line was used to measure cell survival after heavy ion irradiation *in situ* (Tenforde *et al.*, 1980). Fig. 3 shows a composite of the survival curves obtained for peak carbon and neon ions and X-rays, for tumours irradiated in both air-breathing and nitrogen-asphyxiated animals. The hypoxic fraction calculated from the X-ray curves is 35%. Using this value of the hypoxic fraction and the survival curve for hypoxic tumours in asphyxiated animals, a survival curve for the oxygenated cell population in the air-breathing animals

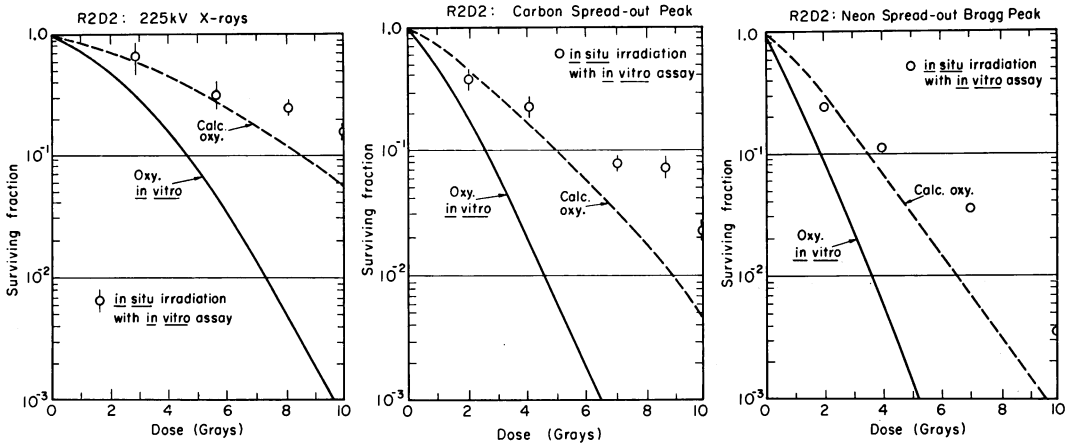

FIG. 4.—Oxic survival curves are compared for R2D2 cells irradiated *in vitro* as a suspension and *in vivo* within a solid tumour. The survival curve for oxygenated cells *in vivo* was calculated from the measured survival curves for tumours irradiated *in situ* in air-breathing rats (data points shown) and in nitrogen-asphyxiated rats.

can be calculated. These calculated curves are shown as dashed lines in Fig. 4, for tumours irradiated *in situ* in air-breathing animals. In each figure the oxic *in vitro* survival curve for this cell line irradiated in suspension is shown for comparison. For the carbon-ion and neon-ion beams, the suspension cultures were placed at the same position as the tumours in the extended peak region of each beam. It is clear that the survival curves calculated for oxygenated cells *in situ* imply less radiosensitivity (or more repair?) than the same cells irradiated in suspension. This finding is in accord with the observations of Durand & Sutherland (1972) on the lower survival of single cells as compared with cells growing in multicellular spheroids.

A small series of experiments was performed to obtain data on tumour growth delay for the R2D2 cell line, thus allowing a direct comparison of RBE's for growth delay and cell survival with the same cell line. This comparison is shown in Table II along with the results obtained earlier by Barendsen & Broerse (1969) for 15 MeV neutrons. The RBE's for 10% survival after culture *in vitro* and for regrowth delay *in situ* are closely similar.

TABLE II.—*RBE's for cell killing and growth delay*

Radiation modality	$RBE_{0.1}$	$RBE_{50\ days}$
Peak carbon ions	1.9 ± 0.3	2.3 ± 0.2
Peak neon ions	3.1 ± 0.6	2.9 ± 0.2
15 MeV neutrons*	2·7	3·1†

* From Barendsen & Broerse, 1969.
† Extrapolated value.

The general picture emerging from these data is that, for both of the heavy charged-particle beams tested, RBE's for the various end points (*in situ* and *in vitro* cell survival, TCD_{50} and growth delay) are quite comparable. On the other hand, there are significant differences between *in situ* and *in vitro* survival curves and the different cell lines used in these studies have widely differing clonogenicity and growth properties. It is concluded that there is currently not enough known of the post-irradiation cellular dynamics to predict the ultimate fate of tumours left in the animals following irradiation. Up until now, attempts to develop an all-inclusive model for the radiation response of the rhabdomyosarcoma tumour system based on cell-kinetic parameters and using *in vitro* survival curves have met with only limited success in predicting volume response (Curtis *et al.*, 1973). More must be learned about both the short-term

and long-term repopulation kinetics of the surviving and the doomed cells before a coherent model of tumour radiation response can emerge.

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