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M. S. Matheson, Argonne National Laboratory

AT–LARGE

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A. P. Casarett, Cornell University

CONTENTS OF VOLUME 64

NUMBER 1, OCTOBER 1975

SYMPOSIUM ON THE DISTRIBUTION OF SECONDARY ELECTRONS FROM IONIZING COLLISIONS

NUMBER 2, NOVEMBER 1975

Number 3, December 1975

RADIATION RESEARCH 64, 245–255 (1975)

RBE as a Function of Neutron Energy[1]

I. Experimental Observations

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HALL, E. J., NOVAK, J. K., KELLERER, A. M., ROSSI, H. H., MARINO, S., AND GOODMAN, J. RBE as a Function of Neutron Energy. I. Experimental Observations. *Radiat. Res.* 64, 245–255 (1975).

The survival of Chinese hamster cells in culture was used as a test system to determine the RBE of neutrons over a wide energy range. The Radiological Research Accelerator Facility (RARAF) at Brookhaven National Laboratory was used for experiments involving nine neutron energies between 110 keV and 15 MeV; for all but the lowest energy the beams were essentially monoenergetic. Additional experiments were performed with high energy cyclotron-produced neutrons at the Naval Research Laboratory, Washington, D. C., and at the Texas A&M Variable Energy Cyclotron (TAMVEC). In both cases broad neutron energy spectra were involved. In each experiment, survival curves were obtained for one neutron energy and compared with 250 kVp X rays, using cells from the same suspension and common controls. In this way a detailed study was made of the relation between RBE and neutron absorbed dose for each neutron energy. At any given cell survival level, RBE varies with neutron energy. Neutrons at 350 keV are biologically the most effective, the RBE falling off for both higher and lower neutron energies.

INTRODUCTION

The radiobiological properties of neutrons are of interest from several viewpoints. First, there is concern about the effectiveness of small absorbed doses of neutrons to which large sections of the public may be exposed from fission reactions. Second, neutrons are already in use at a number of centers for the treatment of cancer patients. Third, experiments with neutrons may help to shed light on the basic mechanisms of the action of radiation, because they are relatively densely ionizing and yet able to penetrate to considerable depths in absorbing material.

The relative biological effectiveness (RBE) of neutrons, and the manner in which it varies with neutron energy, has been studied by a number of workers. The widest interest concerns effects on mammalian cells, and the now-classical studies were reported by Broerse, Barendsen, and van Kersen (*1*) and by

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Berry (*2, 3*). These experiments were constrained by the limited range of neutron energies available, and their interpretation was greatly complicated by the fact that most of the beams used involved a broad spectrum of neutron energies. Other investigations, reported by Dennis and Boot (*4*) and by Underbrink and Sparrow (*5*), utilized largely monoenergetic neutrons but concerned somatic mutations in Tradescantia, which are scored as color changes in the stamen hairs. It is not certain how these effects relate to mammalian cell killing. It was against this background that the present study was mounted, to investigate RBE as a function of neutron energy using mammalian cells in culture.

The study was made possible by the availability of the Radiological Research Accelerator Facility (RARAF), a unique source of monoenergetic neutrons covering a wide range of energies. RARAF is a joint enterprise between the Radiological Research Laboratory of Columbia University and the Medical Research Center of the Brookhaven National Laboratory. It consists of a large van de Graaff accelerator, capable of accelerating protons or deuterons up to an energy of 4.2 MeV. By employing suitable targets, and using neutrons emitted at an appropriate angle from the incident charged particle beam, essentially monoenergetic neutron beams can be generated, ranging in energy from 15.4 MeV down to 220 keV. In addition, a lower energy neutron beam is available which consists of a wide spectrum of energies described as the "110 keV spectrum."

This facility has been used by a number of investigators, and radiobiological findings have been reported in the literature (*6–9*). The present paper describes experiments in which the relative biological effectiveness (RBE) was studied over the entire range of neutron energies available at RARAF, using mammalian cells in culture. In addition, using the same biological test system and compatible dosimetry, visits were made to the Texas A&M Variable Energy Cyclotron (TAMVEC) and to the Naval Research Laboratory (NRL) Cyclotron. These machines generate neutron beams by bombarding beryllium targets with deuterons; the neutrons have a wide range of energies with maxima of 50 and 35 MeV, respectively, for the two facilities.

MATERIALS AND METHODS

Culture of the Cells

V79 Chinese hamster cells were cultured by standard techniques and grown in nutrient medium F10 supplemented with 10% fetal calf serum (*10*), L-glutamine, and antibiotics. Because of the limited dose rates at the RARAF accelerator it was essential to expose cell samples close to the neutron-producing target. To achieve this end without sacrificing dose uniformity, it was necessary for the cells to occupy a small volume during irradiation. This was achieved in the following way.

For each experiment, an actively growing, partly confluent stock bottle was chosen, the cells were removed by trypsinization (2 min at 0.25% trypsin), and prepared into a suspension in complete growth medium consisting of 1.5

TABLE I

CHARACTERISTICS OF NEUTRON BEAMS STUDIED

Facility	*Energy*	*Maximum energy spread*	*Neutron production process*	*Average dose rate*
TAMVEC	50 MeV max	0–50 MeV	d → Be	70 rad/min
NRL	35 MeV max	0–35 MeV	d → Be	65 rad/min
RARAF	15 MeV	± 4%	d → T	1500 rad/hr
	5.8 MeV	± 8%	d → D	475 rad/hr
	2.0 MeV	± 7%	p → T	450 rad/hr
	1.0 MeV	+15 −12	p → T	625 rad/hr
	680 keV	±13	p → T	550 rad/hr
	430 keV	±15	p → T	300 rad/hr
	340 keV	±15	p → T	250 rad/hr
	220 keV	+28 −25	p → T	130 rad/hr
	110 keV spectrum	0–110 keV	p → T	85 rad/hr

$\times 10^4$ cells/ml. One-third of a milliliter of this suspension was pipetted into each of 90 small plastic vials. The vials were fabricated from Falcon 1-ml disposable pipets in the following way. The pipets were cut into 7-cm lengths and heat-sealed at one end. The internal diameter of each vial is about 2 mm. After being filled with the cell suspension, the open end of each vial was heat-sealed, and then the vials were gently centrifuged so that the cells settled out into a small volume at one end of each vial. In this way the cell samples occupied a small volume and could be located accurately close to the neutron-producing target. Plating efficiencies were in excess of 80%, and did not decrease significantly for the experiments involving the longest exposure times.

For each neutron energy studied, four vials at each of seven absorbed doses were used. On each occasion that a neutron experiment was performed, a parallel series of vials, filled from the same cell suspension, was exposed to graded doses of 250 kVp X rays. This plan was followed because it was assumed, and subsequently confirmed (*11*), that variations *within* a given experiment are smaller than *between* separate experiments. Consequently each experiment was a self-contained comparison of one neutron energy versus 250 kVp X rays. All irradiations were performed at room temperature.

Following the completion of a set of irradiations, each vial was agitated in a mechanical vibrator to resuspend the cells, after which various fractions of its contents were seeded into petri dishes containing fresh growth medium. Following an 8-day incubation period, the cells were fixed and stained, and the number of visible colonies per dish counted.

By comparison with unirradiated controls, the fraction of cells surviving each absorbed dose of X rays or neutrons was calculated.

Methods of Irradiation

At the RARAF Facility nine neutron energies were studied. They were 15.4, 6, 2, and 1 MeV; 680, 440, 340, and 220 keV; in addition, a spectrum with

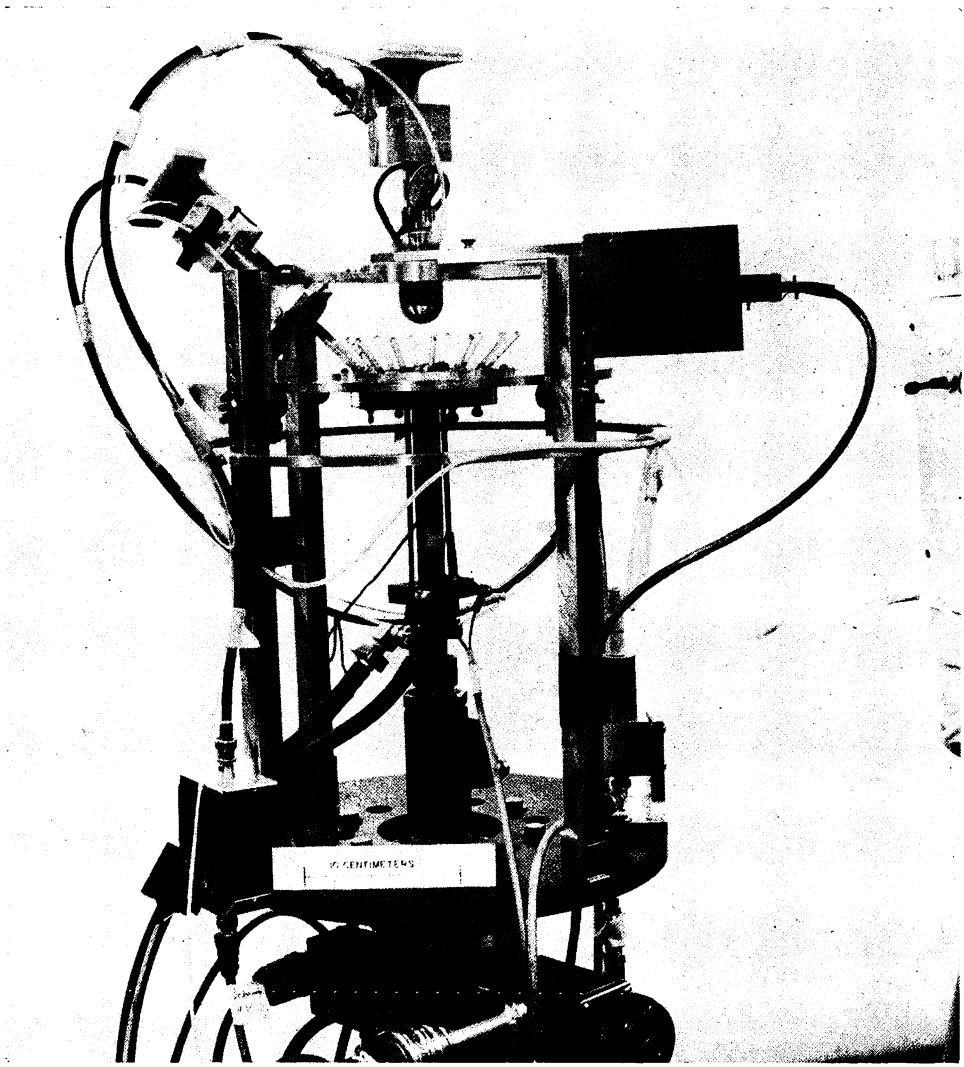

FIG. 1. Arrangement used to irradiate Chinese hamster cells with neutrons of various energies at the Radiological Research Accelerator Facility (RARAF). A vertically upward charged particle beam was used to bombard the target. Cells were contained in small sealed plastic vials, and were gently centrifuged to one end. The cell samples were arranged around the circumference of a circle. The diameter of the circle and its position with respect to the target varied with neutron energy. The vials were rotated about the axis of the charged particle beam to average out small fluctuations in absorbed dose rate. They were also rotated about their own axes to achieve a more uniform absorbed dose. The 6-mm diameter tissue equivalent ionization chamber used for dosimetry is shown in place of one of the vials. The monitor ionization chamber used for all irradiations can be seen above the array of vials.

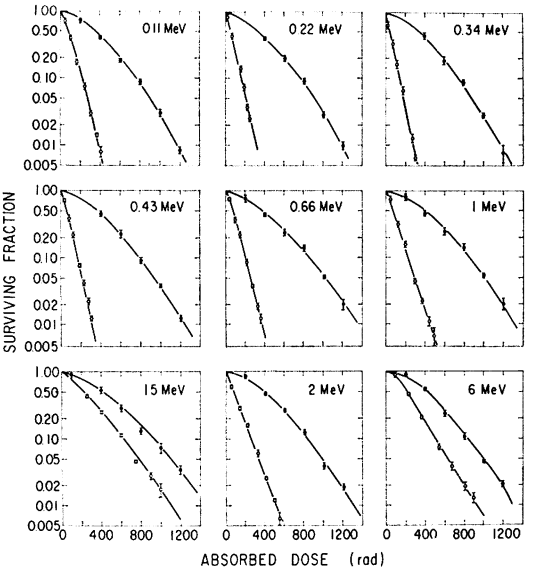

FIG. 2. Survival data for Chinese hamster cells exposed to neutrons of various energies at RARAF. In each experiment corresponding data were obtained for 250 kVp X rays.

a maximum energy of 110 keV was used. The characteristics of the beams used are summarized in Table I. To attain the required doses in exposures of reasonable durations, the plastic vials with the cells centrifuged to one end were positioned around the circumference of a circle coaxial with the charged particle beam and at 2.6 to 6.2 cm from the neutron-producing target, depending on the neutron energy desired. The vials were rotated around the beam axis and about their own axes to compensate for small variations in absorbed dose rate due to target asymmetrics, from inverse square law effects, and from self-attenuation. The fixture shown in Fig. 1 was designed to allow the ends of the cell vials to be positioned at specific angles with respect to the beam direction, ranging from 15 to 130°, to obtain the range of neutron energies employed. At each neutron energy, 16 vials could be irradiated simultaneously at a uniform absorbed dose rate; some were removed at various times and others inserted, so that in all, seven graded absorbed doses, with four replicate vials per absorbed dose, were available.

Dosimetry at RARAF was performed using a tissue equivalent, 6-mm diameter, spherical multiplication ionization chamber placed in one of the vial holes and centered at a position normally occupied by cells. These measurements were correlated to the response of a monitor ionization chamber used during all cell irradiations. Separate measurements with an energy compensated Geiger–Mueller dosimeter yielded gamma-ray absorbed doses ranging from 1.5 to 8% of the total absorbed dose, depending on neutron energy.

For comparison, the characteristics of the two high energy cyclotrons at which experiments were performed are also listed in Table 1. These machines are currently being used for neutron therapy of cancer patients (*12, 13*). The

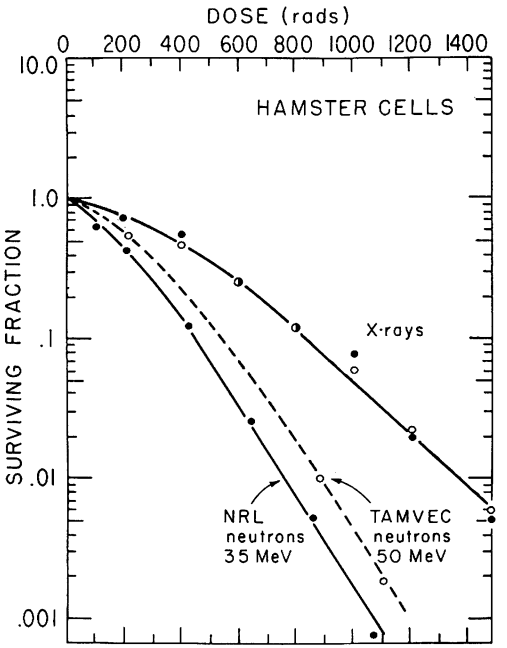

FIG. 3. Survival data for Chinese hamster cells exposed to high energy neutrons at TAMVEC and NRL. In each case corresponding data for 250 kVp X rays were obtained.

production of neutrons by bombarding a beryllium target with deuterons gives rise to a wide range of energies. The NRL cyclotron and TAMVEC use 35 and 50 MeV deuteron beams, respectively. The *maximum* neutron energies are slightly higher, and the *mean* energy of the neutrons is about 0.4 of the deuteron energy.

At these accelerators the absorbed dose rate was not a limitation; at a distance of 125 cm from the target the absorbed dose rates were 50 rad/min at the NRL cyclotron and 70 rad/min at TAMVEC. The cells, contained in the sealed plastic vials, were irradiated in a fixture fabricated of tissue equivalent plastic, with a thickness of plastic ahead of the cells approximately equal to the maximum range of the protons produced by the neutrons (1 cm at NRL, and 2.5 cm at TAMVEC).

Dosimetry at NRL and TAMVEC was performed using a tissue equivalent, 2.5-cm diameter, disc-shaped ionization chamber. These measurements were correlated to the response of the monitor ionization chambers at these facilities which were used during the cell irradiations. A separate measurement of the gamma-ray absorbed dose was performed at NRL using a Geiger–Mueller dosimeter.

RESULTS

The survival curves obtained for Chinese hamster cells exposed to neutrons of various energies at RARAF are shown in Fig. 2. The comparable data for the high energy neutron beams at TAMVEC and NRL are shown in Fig. 3.

With each neutron experiment, an X-ray survival curve was established using cells from the same initial suspension and common controls. These curves are included in Figs. 2 and 3. The repeated determination of the X-ray survival curve was necessary because of the possibility of changes in cellular sensitivity occurring over the 12-mo period during which the neutron data were accumulated. In addition, it was considered useful to obtain experimental data which would permit a statistical evaluation of such fluctuations in cellular sensitivity. The statistical analysis, presented in Part II of this study (*11*), does indeed lead to the conclusion that a systematic variation of cellular sensitivity occurred during the period over which experiments were performed. For this reason it is appropriate to derive the RBE of neutrons and its dependence on neutron absorbed dose from comparisons of each individual neutron dose–effect curve with the corresponding X-ray curve established on the same day.

The vertical bars shown in Fig. 2 represent the standard deviations, derived from a comparison of the surviving clones resulting from replicate vials of cells exposed to the same absorbed dose. Theoretical standard deviations, based merely on the Poissonian fluctuations due to the finite number of surviving clones at the various dose levels, have somewhat smaller values. However, it has been found (*11*) that the difference is small. The survival data for neutrons of intermediate energies are closely approximated by exponential functions of dose. At higher energies, involving protons which are relatively sparsely ionizing, or at very low energies where many of the secondary protons produced by the neutron beams have initial energies below 100 keV, the survival curves have sigmoidal shape.

The RBE values, plotted in Fig. 4, were obtained from the survival curves of Figs. 2 and 3 by determining dose ratios at 0.8, 0.37, 0.1, and 0.01 survival by interpolating between the two nearest data points. A detailed statistical analysis of the RBE as a function of dose and neutron energy will be given separately (*11*).

DISCUSSION

The neutron beams at RARAF having energies between 220 keV and 15 MeV are essentially monoenergetic (at least only a narrow range of energies exists). From 220 keV to 15 MeV, RBE varies as a function of neutron energy with a broad maximum near 340 keV (see Fig. 4). The low energy spectrum at RARAF and the cyclotron produced beams at NRL and TAMVEC contain a broad spectrum of neutron energies, and it is an arbitrary choice whether to plot RBE values against the maximum energy, the mean energy, or some more complicated function such as a dose-mean. In Fig. 4 *maximum energy* is used and so it is not surprising that these data points do not fall on the same smooth curve together with the monoenergetic neutron data.

The RBE maximum at around 340 keV is in good agreement with the data reported previously for *Vicia* seedlings irradiated at RARAF (*9*). Indeed, the overall *shape* of the dose–RBE relationship is much the same for these two

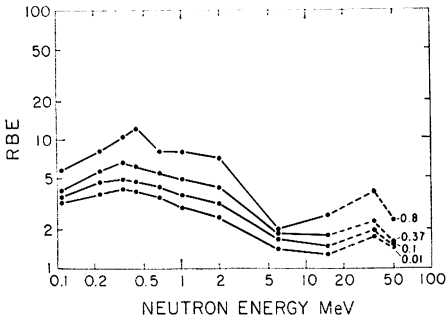

FIG. 4. RBE as a function of neutron energy. RBE values were calculated at four levels of cell survival, namely 80, 37, 10, and 1%. The curves result from interpolation without statistical analysis.

biological systems, although the absolute values of the RBE are much higher in the case of *Vicia* (Fig. 5).

The *Tradescantia* data of Dennis and Boot (*4*) and also of Underbrink and Sparrow (*5*), both of which cover a wide range of neutron energies and involve largely monoenergetic beams, are likewise similar in general shape to data in the present paper. It is more difficult to make a meaningful comparison of the present hamster cell data with mammalian cell data of Broerse *et al.* (*1*) and of Berry (*3*), since in these studies a much more limited energy range was covered and most of the beams involved a wide spectrum of energies. There is no marked conflict over RBE in the limited energy range for which comparisons are possible. All the studies previously reported in the literature are presented in Fig. 5 for purposes of comparison.

The characteristic RBE–energy dependence was predicted by Kellerer and Rossi (*14*) on the basis of microdosimetric data. Although the details of this analysis have been presented earlier, it may be useful to repeat the essential arguments in simplified form. From various experiments on higher organisms it was suggested that the cellular effect of ionizing radiations might depend on the square of the energy absorbed in sensitive sites which are of the size of the nucleus or somewhat smaller. At low doses of neutrons the radiation effect must be mainly due to single charged particles. According to the quadratic dependence on energy concentration, the RBE should then be proportional to the product of the mean event frequency per unit absorbed dose and the square of the energy transferred per event. In a simplified analysis which is based on the LET-concept this product can be shown[2] to be proportional to

[2] The event frequency, $\phi(o)$, per unit absorbed dose is inversely proportional to the track average LET, which is given by

$$\bar{L}_T = \int_0^\infty L l(L) dL,$$

where $l(L)$ is the distribution of LET in track length (ICRU Report 16). The expectation value, $\overline{\epsilon^2}$, of the square of the energy deposition, on the other hand, is proportional to the second moment

the so-called dose average linear energy transfer $\bar{L}_D$. The RBE of neutrons at small doses should therefore be proportional to this dose average LET.

In the microdosimetric analysis, LET is substituted by the quantity y, which is defined as the energy absorbed divided by the mean traversal length in a microscopic region. This quantity of lineal energy, y, corresponds closely to LET; the only difference is that y accounts for the actual statistical fluctuations of energy deposition along the tracks and for such complicating factors as the radial extension of the delta rays and the finite length of particle tracks. The distribution of the values of y, although it is in many cases closely related to the distribution of LET, is therefore a more accurate measure of energy deposition in critical sites in the cell. The dose averages of y which correspond to the dose averages, $\bar{L}_D$, of LET have been determined for various neutron energies and for spherical volumes of the order of several micrometers in diameter. These values are plotted in Fig. 5 (top panel).

There is one additional difference between the microdosimetric values y^* which are plotted in Fig. 5 and the dose averages of LET. This difference lies in the fact that the microdosimetric quantity has been adjusted for the saturation effect which occurs when a charged particle has such high stopping power that it deposits more energy in the cell then is necessary to inactivate the cell. A quantitative assessment of this correction which is based on data obtained by Barendsen (*15*) and by Todd[3] has been given earlier (*14*); it should be pointed out that the correction factor is small up to neutron energies of several mega-electron volts, while it is quite significant at the higher neutron energies, such as 14 MeV. This is due to the increased role of heavier recoiling particles at these high neutron energies. Finally, it should be noted that the microdosimetric quantity y^* does not strongly depend on the diameter of the region of reference. The values plotted in Fig. 5 refer to a site diameter of 2 μm; however, the values are not greatly changed if they are related to regions closer to the diameter of a cell nucleus.

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of the distribution of LET in track lengths:

$$\overline{\epsilon^2} \propto \int_0^\infty L^2 l(L) dL.$$

The effect per unit dose, and; therefore; the RBE at low doses, is equal to the product of event frequency and effect probability per event. If it is assumed (*14*) that the effect probability per event is proportional to $\overline{\epsilon^2}$, one has

$$\text{RBE} \propto \overline{\epsilon^2}/\phi(o) \propto \int_0^\infty L^2 l(L) dL \Big/ \int_0^\infty L l(L) dL.$$

The so-called *dose* average of LET is defined as

$$\bar{L}_D = \int_0^\infty L^2 l(L) dL \Big/ \int_0^\infty L l(L) dL.$$

Accordingly, the RBE at low doses is proportional to $\bar{L}_D$.

[3] P. W. Todd, "Reversible and Irreversible Effects of Ionizing Radiations on the Reproductive Integrity of Mammalian Cells cultured *in Vitro*," Thesis, University of California, Lawrence Radiation Laboratory UCRL 11614.

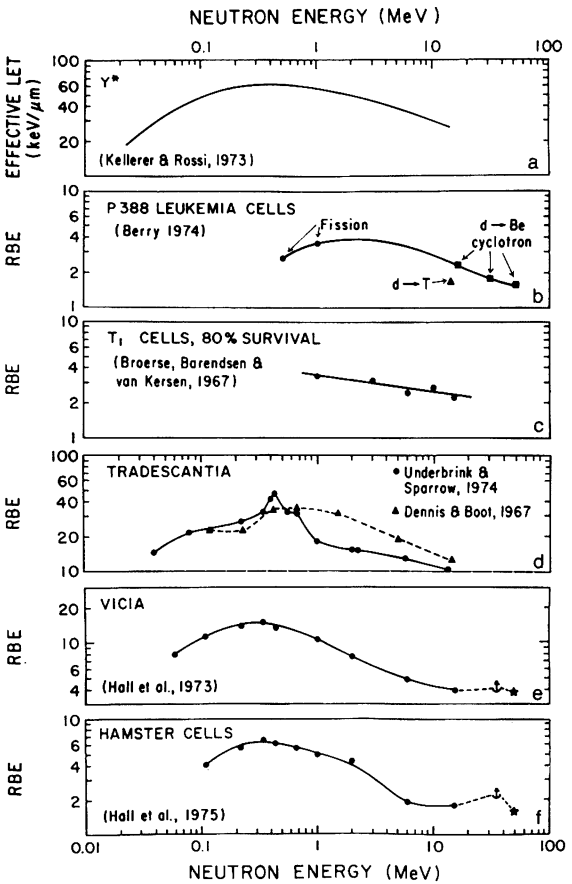

FIG. 5. The six panels, reading from top to bottom are: (a) Dose–mean energy density corrected for the saturation effect, (y^*) as a function of neutron energy. Redrawn from Kellerer and Rossi (*14*). (b) RBE as a function of neutron energy for P388 lymphocytic leukemia cells assayed *in vivo*. The d → T neutrons are monoenergetic at 14 MeV. The remaining energies are spectra, fission neutrons, or cyclotron produced d → Be neutrons. Data from (*3*). (c) RBE as a function of neutron energy for T_1 human kidney cells. The point at 15 MeV refers to monoenergetic d → T neutrons, the remainder to spectra of neutrons. Data from (*1*). (d) RBE as a function of neutron energy for the production of somatic mutations in Tradescantia, expressed as color changes in stamen hairs. Data from (*4*) and (*5*). (e) RBE as a function of neutron energy determined with *Vicia* seedlings, and calculated as the ratio of absorbed doses to produce 50% inhibition of root growth. Data from (*9*). (f) RBE as a function of neutron energy, determined with Chinese hamster cells and calculated as the ratio of absorbed doses to result in a surviving fraction of 37%. Data from present paper.

The results of the present experiments are consistent with earlier observations, for example, on bean roots (*9*), Tradescantia (*7*, *8*), and the murine lens (*6*), insofar as they agree with the overall shape of the theoretically predicted dependence of RBE on neutron energy. Certain deviations of the observed curves from the theoretical dependence may be due to the fact that the theoretical curve applies strictly only to the limit of very small absorbed doses,

while the experimental data have been obtained from an intermediate range of the survival curves where at least at very low and at very high neutron energies the interaction of several charged particles cannot be completely neglected.

A discussion of the dependence of RBE on absorbed dose will be given in the context of the more detailed statistical analysis of these data in Part II of this study (*11*).

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