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● *Original Contribution*

THE RELATIVE BIOLOGICAL EFFECTIVENESS OF 160 MeV PROTONS—II†

BIOLOGICAL DATA AND THEIR INTERPRETATION IN TERMS OF MICRODOSIMETRY

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The radiobiological effectiveness of 160 MeV protons was measured relative to ^{60}Co γ-rays, using Chinese hamster cells cultured *in vitro*. Separate experiments were performed with cells irradiated in suspension, or attached to plastic tissue culture flasks. Proton irradiations were performed in the incident plateau of the depth dose profile and with the Bragg peak spread out to cover 10 cm. In all cases the relative biological effectiveness (RBE) for protons relative to gamma rays was 1.2 for doses in excess of about 200 rad. The attached cell experiments indicate an increasing RBE at low doses, which is consistent with the microdosimetric measurements.

Protons, Bragg peak, Microdosimetry, Relative biological effectiveness.

INTRODUCTION

The dose deposited by a proton beam increases with depth in the exposed medium and reaches a sharp maximum in the region of the Bragg peak, close to the end of the particles' range. Beyond the particles' range, the dose becomes very small. By using a proton beam of suitable energy (and therefore range), a tumor volume located at any depth in the body can, in principle, be irradiated precisely and selectively to a high dose, with a minimal dose to the intervening normal tissues and a negligible dose to the deeper tissues. This attractive feature of heavy charged particles, and its potential application to radiotherapy, was recognized early by Wilson.[9]

Much of the early clinical use of protons involved pencil beams to treat the pituitary gland, first at the 184 in. Berkeley cyclotron, and later at the Harvard cyclotron. The Harvard cyclotron has an energy of 160 MeV, which means that the unmodified beam would have a sharp Bragg peak at a depth of about 18 cm in unit density material. The sharp Bragg peak associated with monoenergetic protons is of little use in broad beam radiotherapy. However, the Bragg peak can be spread out to cover any hypothetical tumor dimension, certainly up to 10 cm, by the use of a moving absorber of variable thickness.[6]

In preparation for a clinical trial of proton radiation therapy, the Harvard group invited a number of outside investigators to make measurements of the relative biological effectiveness (RBE) of protons compared with ^{60}Co γ-rays. The radiobiological experiments described in the present paper were the result of this invitation, and the microdosimetric studies which followed were motivated by the need to understand the results that were obtained.

Robertson *et al.*[7] had performed prior radiobiological studies with the Harvard proton beam, using cells in culture, an RBE close to unity was reported. In the literature, there is a wide spread in RBE data for various proton beams in a multitude of biological systems, but few relevant to the broad beam radiotherapy situation.

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METHODS AND MATERIALS

V79 Chinese hamster cells were used for the biological intercomparisons, cultured according to standard techniques, and grown in F10 culture medium (Grand Island Biological Company), supplemented with 10% fetal calf serum and antibiotics. Two series of experiments were performed, one with cells irradiated while attached to Falcon plastic tissue culture dishes, and one with cells irradiated in suspension.

For the attached cell experiments, cells were harvested by trypsinization, counted in a Coulter electronic counter and appropriate numbers seeded into small (25 cm^2) Falcon tissue culture flasks in 10 ml of medium. After overnight attachment at 37°C, the flasks were removed from the incubator, filled brimful with tissue culture medium and maintained at 17°C for the trip to Boston. Plates were randomized into two groups and irradiated with graded doses of protons at the Harvard cyclotron or with ^{60}Co γ-rays at the Massachusetts General Hospital. Six replicates were used for each dose level, and each type of radiation. Following irradiation, the flasks were transported back to Columbia University, New York and incubated at 37.5°C for 8 days to allow colony formation, after this, the cells were fixed and stained, and the number of colonies per flask counted by a projection technique.

For experiments involving cells in suspension, cells were harvested by trypsinization from a partly confluent, actively growing stock flask, and prepared into a suspension in complete growth medium. After counting in a Coulter electronic counter, suspensions were prepared containing various concentrations of cells from 600 to 3×10^4 cells/ml. Series of small plastic vials were filled, each with one-third of a milliliter of these various cell suspensions. These vials, fabricated from Falcon plastic disposable 1 ml pipets, were 6 cm long, with inside and outside diameters of 2 and 4 mm respectively. The ends were sealed by the application of heat. It has been found that cells may be stored in this way at a temperature of about 17°C for periods of at least 24 hr without loss of plating efficiency (which characteristically is greater than 80%) and without any change in the age distribution of cells within the mitotic cycle. This has been described in previous publications.[1,2]

The vials were prepared and filled with cells in New York, and transported to Boston where half were irradiated with protons and half with ^{60}Co gamma rays at the Massachusetts General Hospital. Vials containing appropriate numbers of cells were irradiated with graded doses; eight replicates were used for each dose level and type of radiation. After irradiation, the vials were transported back to New York and agitated vigorously on a vortex mixer to resuspend the cells, before they were opened and the contents of each vial transferred to a Falcon culture flask containing fresh growth medium. After 8 days incubation at 37°C, the cells were fixed and stained; the number of macroscopic colonies per flask were counted by a projection technique. The cells were in the vials for a total of 12 hr including transportation and irradiation; and during this time they were kept at about 17°C in an insulated carrier.

Dosimetry

Dosimetry was provided by the Harvard group (Dr. Michael Goitein and Dr. Andy Koehler) as it was used for the clinical applications of the protons and ^{60}Co γ-rays. In the case of the γ-rays, absorbed dose was computed from measurements with a Farmer 0.6 cm^3 ionization chamber, using a calibration factor determined by comparison with a Farmer chamber calibrated by the National Bureau of Standards. The dose delivered by protons was determined from measurements with a 0.1 cm^3 ionization chamber which had been intercalibrated against a Faraday cup in a monoenergetic proton beam.

RESULTS AND DATA ANALYSIS

Two visits were made to Boston. On each occasion separate experiments were performed with attached cells and cells in suspension.

On the first occasion, survival curves were obtained for cells irradiated at the center of the spread-out Bragg peak (position A in Fig. 1), and compared with ^{60}Co γ-rays. On the second occasion, cells were irradiated again at the center of the spread-out Bragg peak, but also in the plateau (position B in Fig. 1). Survival curves also were obtained for ^{60}Co γ-rays. Figure 2 shows the data for attached cells in the first and second experiment. The data points are plotted, together with their standard deviations.

These data were analyzed in two different ways.

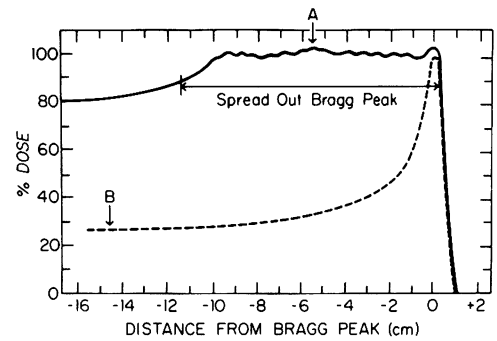

Fig. 1. Depth dose profiles for 160 MeV protons at the Harvard cyclotron. Curve A—Spread out Bragg peak. A moving absorber of variable thickness is used to spread out the Bragg peak to cover 10 cm. Curve B—Unmodified beam with a sharp Bragg peak at a depth of about 16 cm.

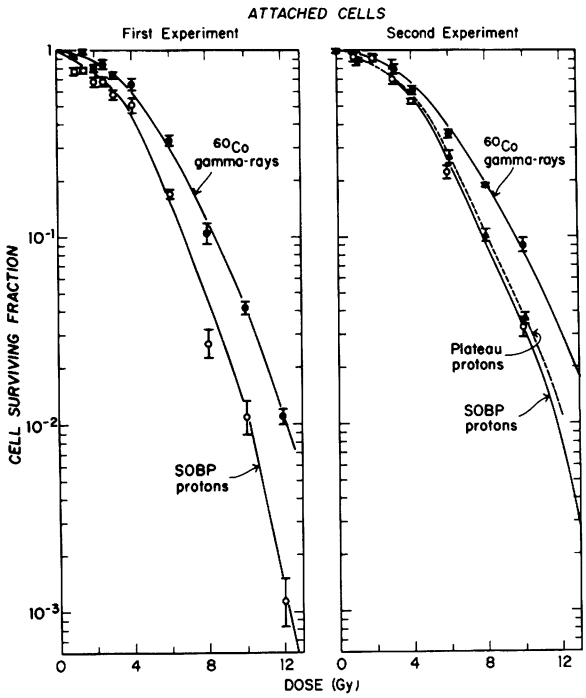

Fig. 2. Survival data for V79 Chinese hamster cells, irradiated attached to Falcon tissue culture flasks and irradiated with ^{60}Co γ-rays or with protons in the spread-out Bragg peak (SOBP) or plateau positions of the depth dose profile. (Positions A and B respectively in Fig. 1). Two separate experiments were performed separated by several months. The curves were fitted to the data by a method described in the text.

One of these employs the non-parametric method developed by Kellerer and Brenot[3] which permits the construction of an envelope of the RBE vs absorbed dose curve. The results are shown in Fig. 3(a–c). There is a strong indication that the proton RBE exceeds unity at all dose levels and that it increases at doses below a few hundred rad.

In addition, the data were fitted by a new method that also does not invoke any analytical expression

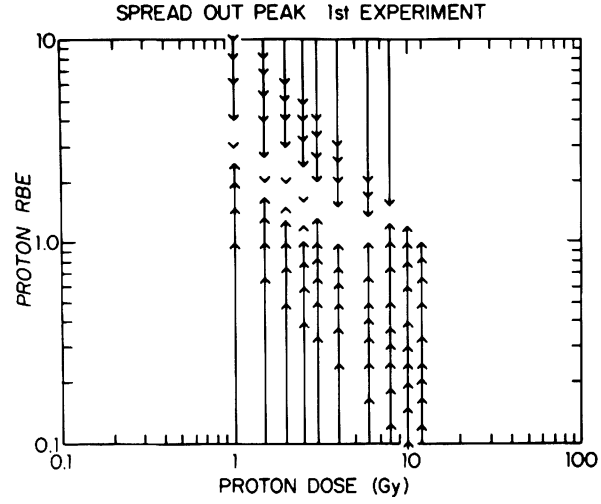

Fig. 3a.

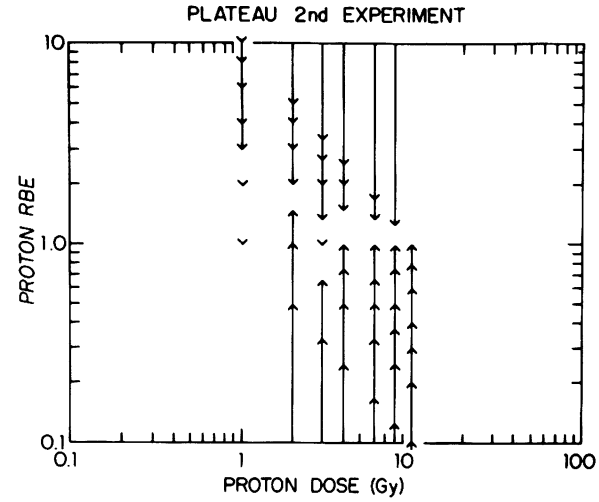

Fig. 3b.

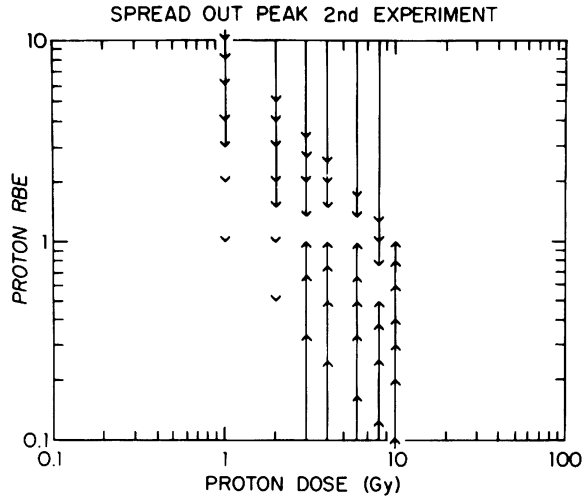

Fig. 3c.

Fig. 3. Proton RBE as a function of proton dose computed by the non-parametric analysis of Kellerer and Brenot.[3] The analysis was carried out on the data in Fig. 2. Panel (a) refers to data obtained in the spread-out Bragg peak position in the first experiment. Panels (b) and (c) refer to data obtained in the plateau and spread-out Bragg peak in the second experiment. The vertical bars denote RBE values which are excluded at a confidence level of 95%, while the detached arrows indicate values that are unlikely at a lower level of confidence. The most likely RBE values fall in the space between the vertical bars. There is strong evidence that the proton RBE exceeds unity at all dose levels, and that it increases at doses below a few hundred rad.

for the dose–effect relationship. All that is assumed is that the curve is convex upwards, which assures a smooth fit. The three proton curves, one obtained at the first visit, and two at the second, were fitted with a curve of the same shape. A computer program searches for that convex curve which fits the three curves best. A simultaneous fit is made possible by allowing different dose modifying factors for the individual curves. Different vertical shifts are also

permitted for the two experiments to account for the possibility that the unirradiated controls are not necessarily the "correct" values; however, this effect is negligibly small since large numbers of control colonies were counted. Curves of the same shape (but different from the proton data) also were fitted to the survival data for the ^{60}Co γ-ray irradiations for the two experiments. The results of this fitting procedure are shown by the curves in Fig. 2. Based on these best-fit curves, the RBE of protons, compared with ^{60}Co γ-rays, can be computed as a function of dose for the attached cells; Fig. 4 shows the results. The RBE for protons is about 1.2 at high doses, but increases at low doses. There is only a small difference between the biological effectiveness of protons in the spread-out Bragg peak, compared with the plateau, the peak has a slightly greater effectiveness.

Figure 5 shows data for cells irradiated in suspension. The same method of curve fitting is applied as in the case of the attached cells. The data are subject to greater fluctuations than the attached cell data, and in particular, the γ-ray curves are rather ill-defined. Consequently, these data do not warrant an explicit analysis of RBE as a function of dose; however, they certainly support the conclusion that the RBE difference between plateau and extended peak protons is probably not significant, and that in both cases, the charged particle beam is more effective than ^{60}Co γ-rays by about 20%.

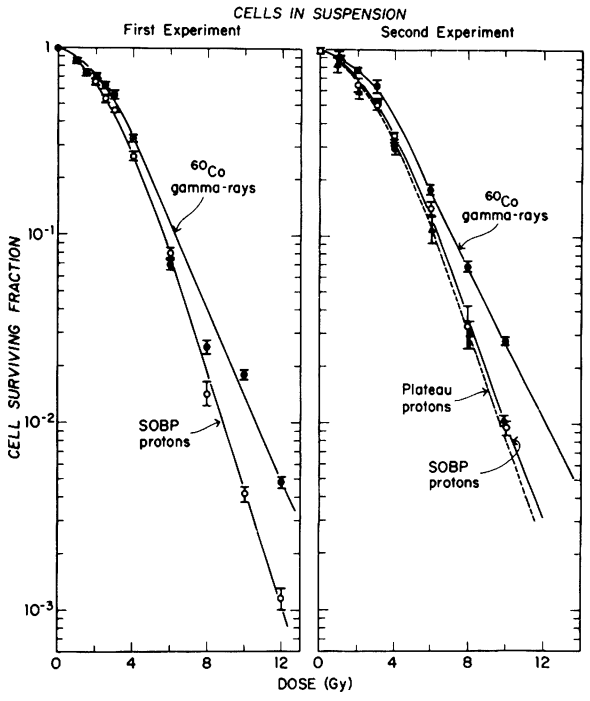

Fig. 5. Survival data for V79 Chinese hamster cells irradiated in suspension with ^{60}Co γ-rays or with protons in the spread-out Bragg peak (SOBP) or plateau positions of the depth dose profile (positions A or B respectively in Fig. 1). Two separate experiments were performed on different occasions. The curves were fitted to the data by a method described in the text.

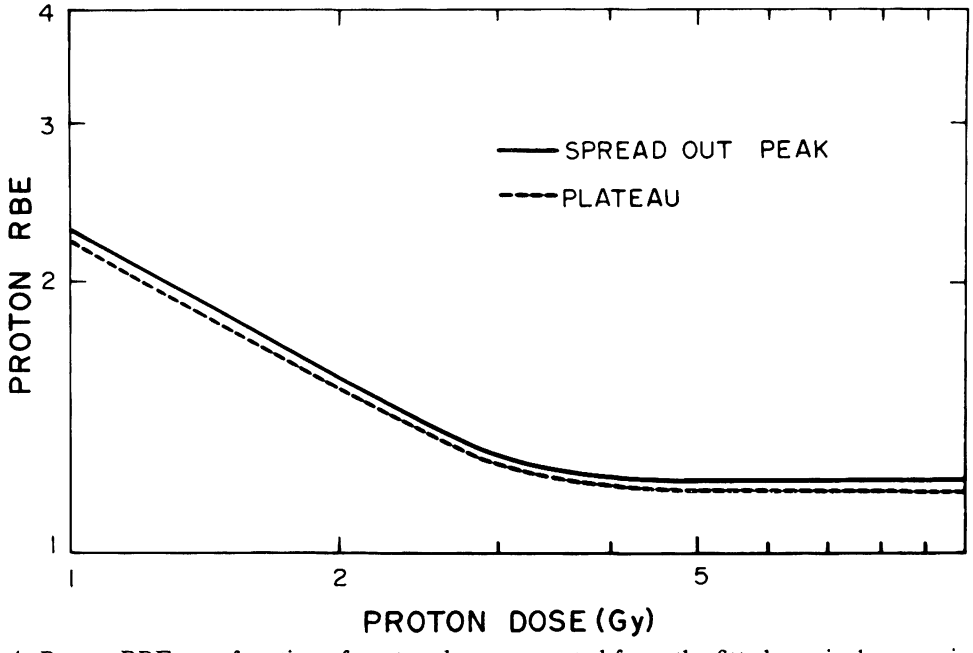

Fig. 4. Proton RBE as a function of proton dose, computed from the fitted survival curves in Fig. 2.

DISCUSSION

According to the theory of dual radiation action,[4] the rate of production of radiation induced lesions is given by:

$$E(D) = k(\zeta D + D^2) \ldots \quad (1)$$

where ζ is the quotient of the second and first moments of the specific energy deposited by single events, D is the absorbed dose, and k is a constant that is related to the effectiveness with which individual charged particles produce the sublesions that combine in pairs to form lesions.

According to eqn (1), the RBE at large doses should be equal to the ratio of the values $\sqrt{k}$ for the radiations under comparison. Although in an initial approximation k was considered to be independent of radiation quality, it was pointed out[4] that a dependence was likely to exist, and that it probably resulted from differences of energy deposition at the nanometer level.[8] In the present study the RBE of protons relative to ^{60}Co γ-rays is close to 1.2 at high doses. The value is similar to that observed for orthovoltage X-rays compared with γ-rays. This is consistent with the fact that both protons and γ-rays have dose lineal energies that are similar to one another, and significantly higher than the comparable figure for ^{60}Co γ-rays.

At doses that are comparable to, or less than, ζ for protons, the RBE must increase as the dose decreases in a manner indicated by eqn (1). At very low doses, that are significantly smaller than the ζ for either type of radiation, the RBE reaches a limiting value equal to $k_p\zeta_p/k_\gamma\zeta_\gamma$ where the subscripts p and γ refer to protons and γ-rays. Although values of ζ depend on the diameter of the site (i.e. the effective volume over which sublesions combine), their ratio is largely independent of site diameter and equal to the ratio of lineal energies. Values of $\overline{y^*}$, the dose average lineal energy, corrected for saturation, were reported in Kilauga *et al.*[5] The quantity is proportional to ζ; therefore, it may be substituted in the calculation of ratios. One obtains ratios of ζ's for protons and γ-rays that are about $5.7/1.9 = 3$ at the center of the spread-out Bragg peak and $4.8/1.9 = 2.5$ at the plateau. If $\sqrt{k_p/k\gamma}$ is taken as 1.2, the maximum RBE predicted is near 4.3 for the spread-out peak and about 3.6 for the plateau.

The data for attached cells in Figs. 2–4 show the rise of RBE with decreasing dose expected from the microdosimetric considerations outlined above. The limiting values are not reached because of the practical difficulties involved in obtaining biological data of sufficient statistical accuracy at doses below 100 rad. The data for suspended cells (Fig. 5) show little or no rise of RBE. However, an increase of RBE at lower doses cannot be ruled out. Such a displacement to lower doses conceivably could reflect a larger site diameter for suspended cells because of their different physiological state.

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