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● *Symposium III—Radiobiology of Charged Particles*

MOLECULAR AND CELLULAR RADIOBIOLOGY OF HEAVY IONS

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Quantitative studies at the BEVALAC have demonstrated some of the physical and radiobiological factors that promise to make accelerated heavy ions important for the therapy of cancer. The measured physical dose-biological effect relationships allow the safe and effective delivery of therapeutic schedules of heavy ions. Among the charged particle beams available, carbon, neon and helium ions in the "extended Bragg peak mode" have optimal physical and biological effectiveness for delivery of therapy to deep seated tumors. The depth-dose profiles of these beams protect intervening and adjacent tissues as well as tissues beyond the range of the particles. For the treatment of hypoxic tumors, silicon and argon beams are being considered because they significantly depress the radiobiological oxygen effect in the region of the extended Bragg ionization peak. The depth-effectiveness of the argon beam is somewhat limited, however, because of primary particle fragmentation. Silicon beams have a depth-dose profile which is intermediate between that of neon and argon, and are candidates to become the particle of choice for maximizing high LET particle effects. Heavy accelerated ions depress enzymatic repair mechanisms, decrease variations of radiosensitivity during the cell division cycle, cause greater than expected delays in cell division, and decrease the protective effects of neighboring cells in organized systems. Near the Bragg peak, enhancement of heavy particle effects are observed in split dose schedules. Late and carcinogenic effects are being studied. With the newly developed Repair-Misrepair theory we can quantitatively model most observations.

Heavy-ion/charged particle beams, Carbon, Neon, Silicon, Argon ions, Cellular/molecular radiobiology, OER, OGF, RBE, Bragg peak, Cell age radiosensitivity, SLD and PLD repair, Cell progression effects, RMR model of cellular inactivation.

The two major advantages of heavy accelerated particles for therapy are that these particles produce biologically effective depth-dose distributions for the treatment of deeply seated tumors and that the differences in radiation sensitivity of aerobic and hypoxic cells are much smaller for heavy ions than for X rays.[31,33] Our current research has three major objectives:

1. Which heavy ion beams combine the greatest "oxygen gain factor" (OGF) with the best biologically effective depth dose distribution? These beams will become candidates for controlled therapy trials.
2. Are there additional radiobiological characteristics of heavy ion beams that would make them advantageous or disadvantageous for therapy?
3. What are the basic molecular and cellular mechanisms of heavy-ion injury; ultimately the full understanding of these mechanisms might lead to better methods for control of cancer cell proliferation and for reducing adverse normal tissue responses.

In the Berkeley Bevalac we can accelerate nuclei of almost any isotope in the periodic table to multibillion electron volts of kinetic energy.[14,17] In Table 1, we demonstrate some properties of the beams we have used in biomedical investigations. (For general reviews see refs. 12, 23, 24.) These beams can penetrate any depth in the human body at ample intensities for therapeutic use. Each of the beams has an intensity in the plateau of more than one gray per minute when spread to include a volume of one liter or more in the plateau of the extended Bragg peak.

Figure 1 shows a typical Bragg curve which includes the relative ionization dose ratio from the primary beam particles as they emerge from the accelerator. Also shown is the dose contribution of secondary particles which results from interactions of the primary particles with the nuclei of the absorber. The fragments accompany the beam at various velocities and create a tail to the depth-ionization curve that slowly decreases with depth of penetration.

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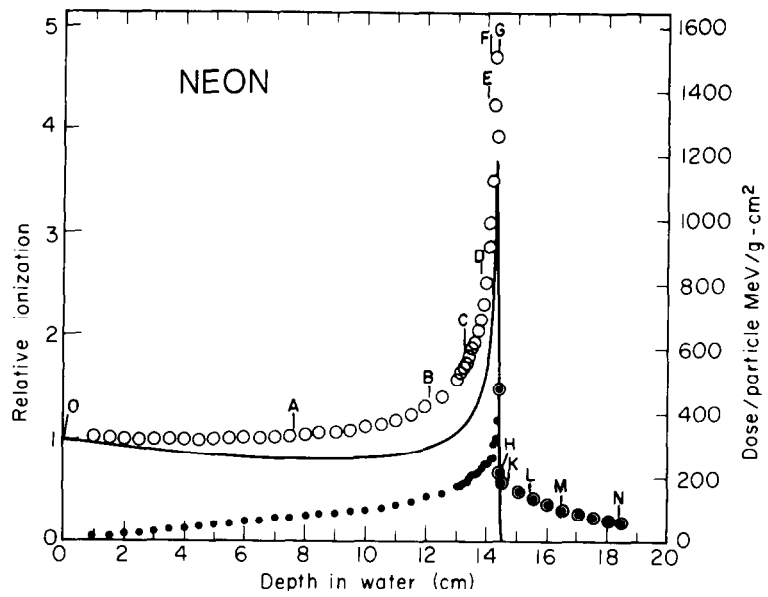
Table 1

BEVALAC	Carbon	Neon	Silicon	Argon	
Z atomic number	6+	10+	14+	18+	
Range in tissue	40	33	25	16	cm
Flux /pulse	10^9	4.10^8	2.10^8	6.10^7	
Plateau dose rate 10 x 10 cm Gray / min	5	3	2.5	1.5	

It was our initial task to characterize the biological effects of the various beams by exposing monolayers of cultured mammalian cells at various values of residual range shown in Fig. 1 as positions "O" to "N." Human T-1 cells as well as a variety of other cultured mammalian cells were used.[1-5] At high residual range values all the survival curves have shoulders; at low residual range values the survival curves are nearly pure exponential functions of dose. The oxygen effect, which represents the difference between the aerobic and hypoxic survival curves at the same level of survival, is substantial in the plateau but gradually diminishes as the residual range becomes small. The reduction of the oxygen effect is much more marked for the heavier than for the lighter ions. Figure 2 shows relative biological effectiveness (RBE), and oxygen enhancement ratios (OER) for two particles: carbon and argon. It is evident that carbon has low RBE and high OER for most of its range except near the Bragg peak, whereas argon has significantly reduced OER and high RBE throughout the Bragg curve.

In Figure 3 we show the LET dependence of RBE in aerobic and hypoxic cells for particle beams mixed with fragments. When the LET is greater than 100 keV/micrometer, the RBE values for neon and for argon are not described by a single-valued function. This is an indication that dose and mean LET in themselves are not sufficient to quantitatively describe the biological effects of heavy ions. The divergence between the two curves might be due in part to fragmentation effects and in part due to the fine structure of energy deposition in particle tracks.



Residual range (cm)	16.1	6.8	2.4	1.2	0.54	0.29	0.14	0.04	0	-0.2	-1.0	-2.0	-4.0
Letter designation	O	A	B	C	D	E	F	G	H	K	L	M	N
LET (keV/um)	32	38	54	71	100	139	234	419	531	22	18	13	11

Fig. 1. Dose vs. depth curves in water. Actual normalized ionization rations measured (O). Calculated ionization contributions from secondary fragments (●). Calculated Bragg curve (-) accompanying table provides residual range and LET values for beam ranges indicated alphabetically on measured Bragg curve (redrawn figure from Ref. 6).

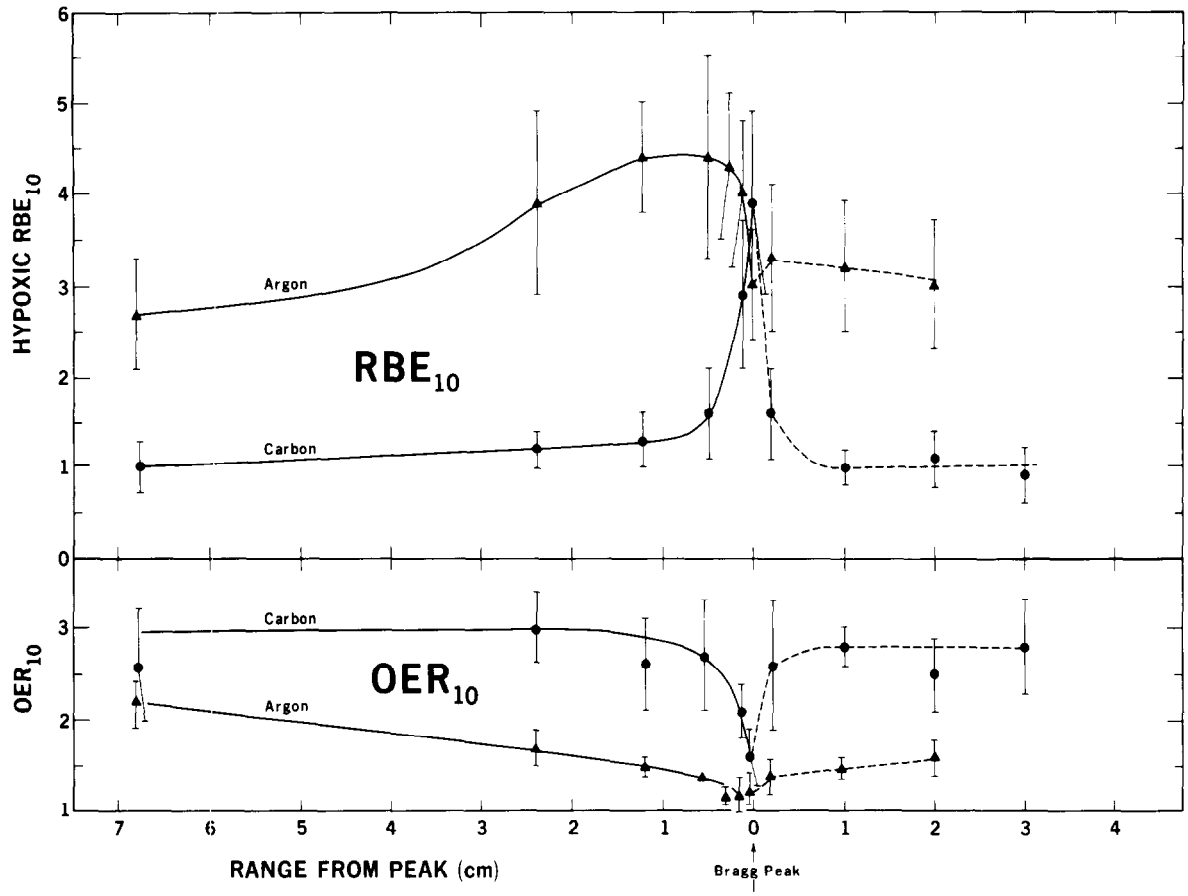


Fig. 2. OER and hypoxic RBE at 10% survival as a function of beam range: carbon (●); argon (▲). Error bars represent 95% confidence interval (redrawn from Ref. 5).

As fragments are produced, some primary beam particles are lost and the number of fragment particles with lower atomic numbers and LET values increases. Figure 4 shows differences in the ratio of the fragment dose to the primary particle dose between silicon and argon beams. Fragmentation should be reduced to a practical minimum in order to emphasize the advantageous properties of the primary beams. If the fragment dose is less than the primary particle dose, then usually the properties of the primary particles prevail.

For therapeutic applications, techniques were developed to spread the Bragg peak in such a manner that the relative ionization has a broad maximum over several centimeters depth before the beam comes to a stop, in order that the region to be treated is covered in depth as well as in width. Some Bragg peaks produced by interposing rotating ridge filters in the monoenergetic beams are shown in Fig. 5. These are calculated to produce an isosurvival effect in the extended peak. Figure 6 shows the depth-dose distribution for a 10 cm filter as well as the mean dose-average and track-average LET values as a function of depth for carbon, neon, silicon and argon beams of 28 cm range (calculated by S. Curtis). One should realize that at each depth there is a rather complex distribution of ionizations from primary beam particles of varying energies, as well as from an assortment of fragments. Figure 6 also illustrates the distribution of stopping particles for an extended neon 557 MeV/u Bragg curve showing that the majority stop in the distal peak.

Figure 7 shows typical aerobic and hypoxic survival curves at various positions in extended Bragg peaks of carbon and of argon beams. The diminished separation between aerobic and hypoxic curves for argon is clearly visible. The shape of the depth-dose curve for each ridge filter is designed for aerobic isosurvival on the basis of the previous biological studies. In Fig. 8 we summarize the cellular effects produced by three different ion beams at different depths: including the plateau region (24 cm residual range), the proximal peak (10 cm residual range), the midpeak (5 cm residual range), and the distal peak (1 cm residual range).

In Fig. 9 we relate physical parameters of extended Bragg dose distributions to biological effects. For the four particles, carbon, neon, silicon, and argon we show values of oxygen gain factor ($OGF = OER_x/OER_{ion}$) and RBE at 10% survival as a function of residual range. We interpret the patterns of Fig. 8 and 9 as follows: Carbon and neon produce relatively more significant aerobic cell killing effects at the proximal and distal peaks when compared with the plateau than silicon or argon. The reduction of the oxygen effect, however, is not impressive

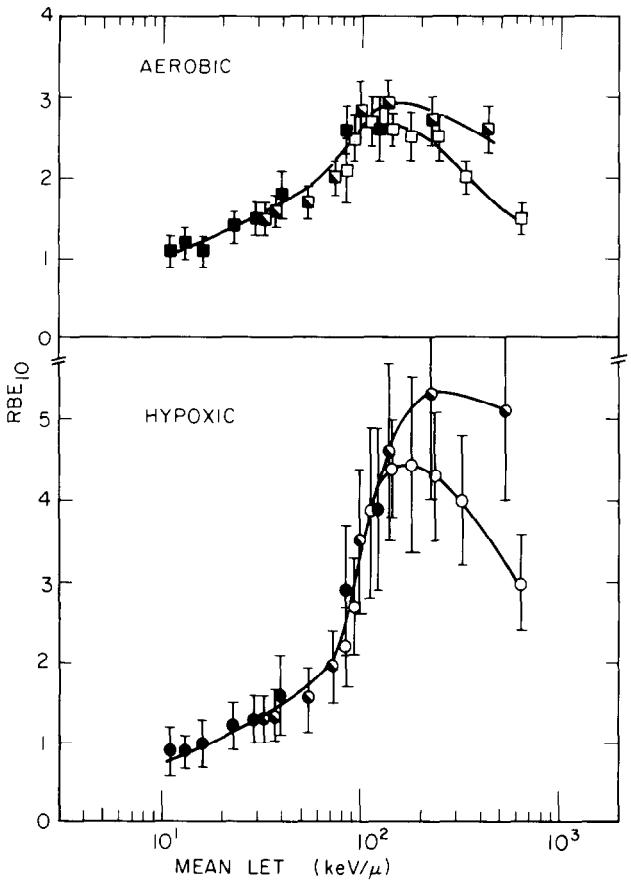


Fig. 3. RBE at 10% survival as a function of the average mean LET: carbon data (●), neon data (◐), and argon date (○). Error bars are for 95% confidence limits (figure from Ref. 5).

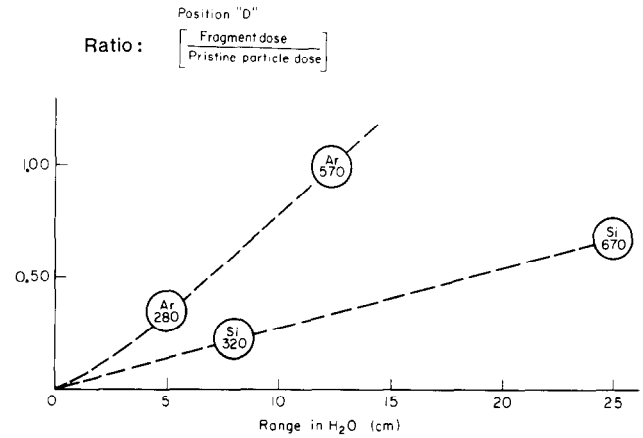


Fig. 4. Ratio of the dose from fragments to the dose from the primary particles of silicon and argon beams of two initial energies at a residual range of 0.54 cm of water. The numbers within each circle indicate the incident energies of the silicon or argon beams in MeV/u.

with carbon or neon. In contrast, silicon and argon depress the oxygen effect severely and increase the hypoxic RBE.

If the behavior of human T-1 cells in culture is accepted as representative of the behavior of cancerous cells in the body, then the most promising beam to be used for deep tumor therapy is the silicon ion (Z=14), or perhaps phosphorous, sulfur, or magnesium.

Curtis *et al.* and Tenforde *et al.* have been working on an entirely different system, the rat rhabdomyosarcoma tumor cell line, that can be grown *in vivo*[30] or cultivated *in*

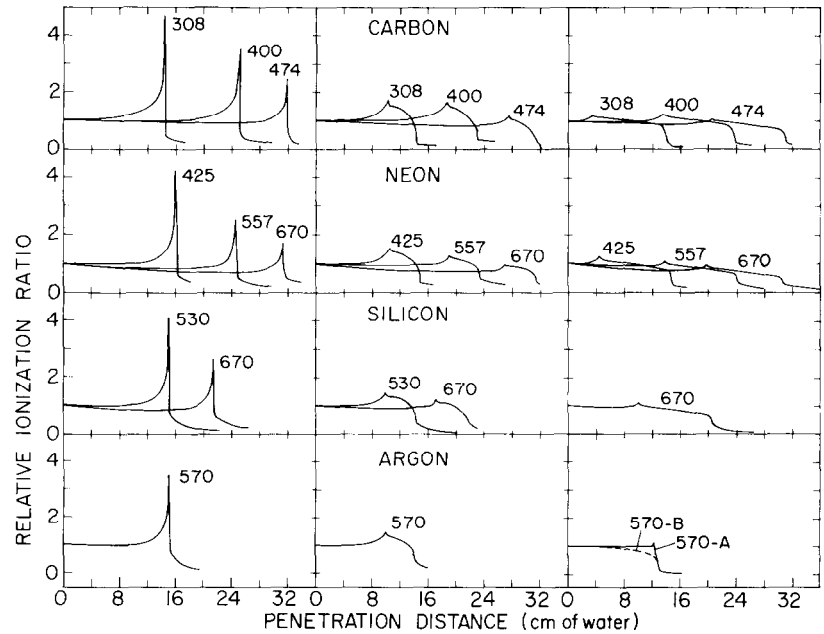


Fig. 5. Composite figure of Bragg curves of available Bevalac beams of carbon, neon, silicon and argon. Initial energies of each beam are indicated. (Left panels) unmodified monoenergetic Bragg peaks. (Middle panels) 4 cm extended Bragg peaks. (Right panel) 10 cm extended Bragg peaks, including two spiral ridge filter designs for argon. Thicknesses of between 0 to 1.8 g-cm^{-2} of lead scattering foils were used for these beams (redrawn from Ref. 3).

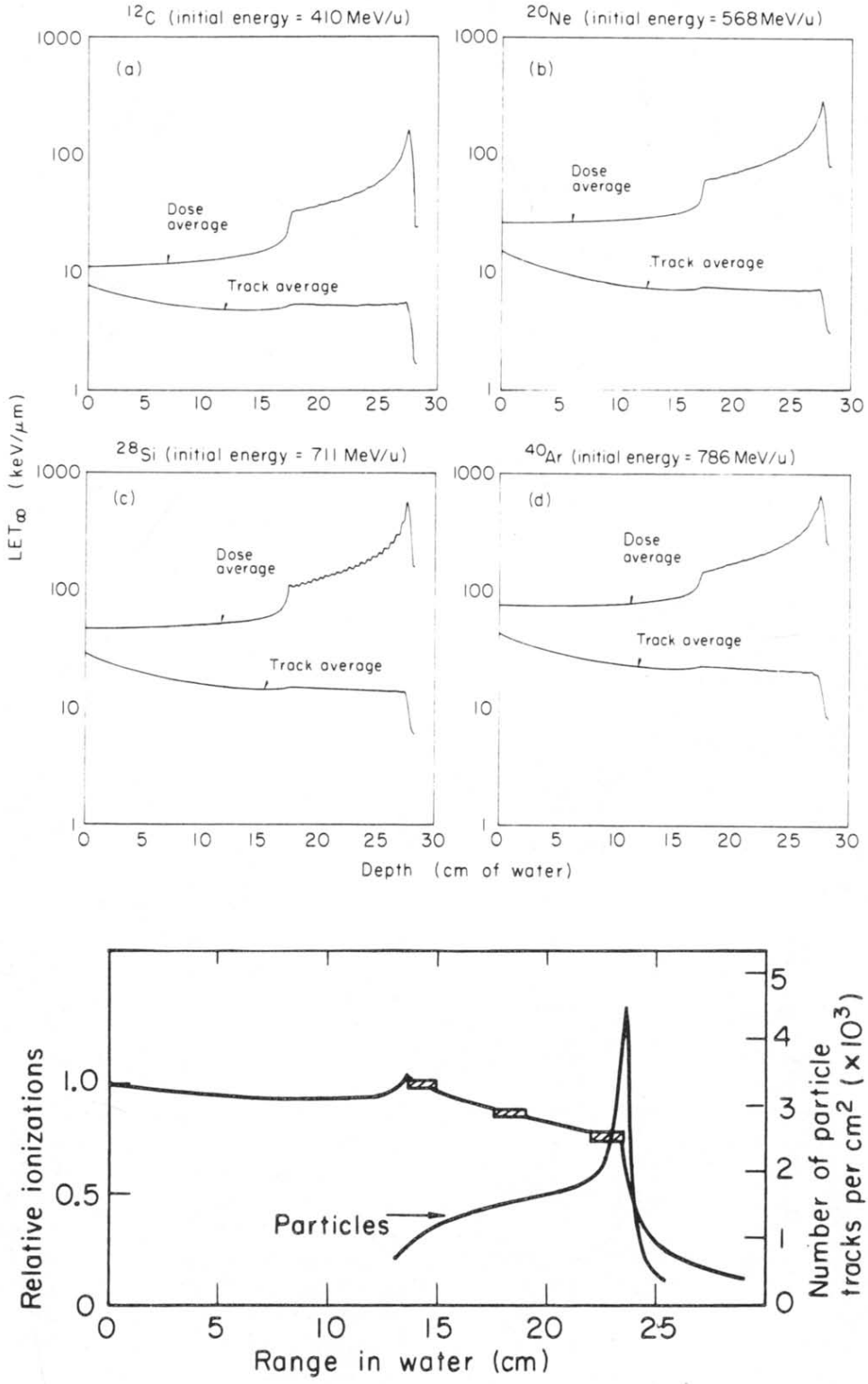


Fig. 6. Calculated track-average and dose-average LET values are shown throughout the Bragg curves of carbon, neon, silicon and argon beams with an identical range of 28 cm in water. For all of these beams, the Bragg peak ionization region has been extended to a width of 10 cm by means of a variable thickness absorber. The lower panel depicts the 10 cm Bragg ionization curve in terms of relative number of ionizations versus penetration distance in water. The flux of neon particles with an LET $\geq$ 75 keV/μm as a function of penetration distance is also shown. The 10 cm stack of plastic detectors was exposed to 1 rad (redrawn from Ref. 29).

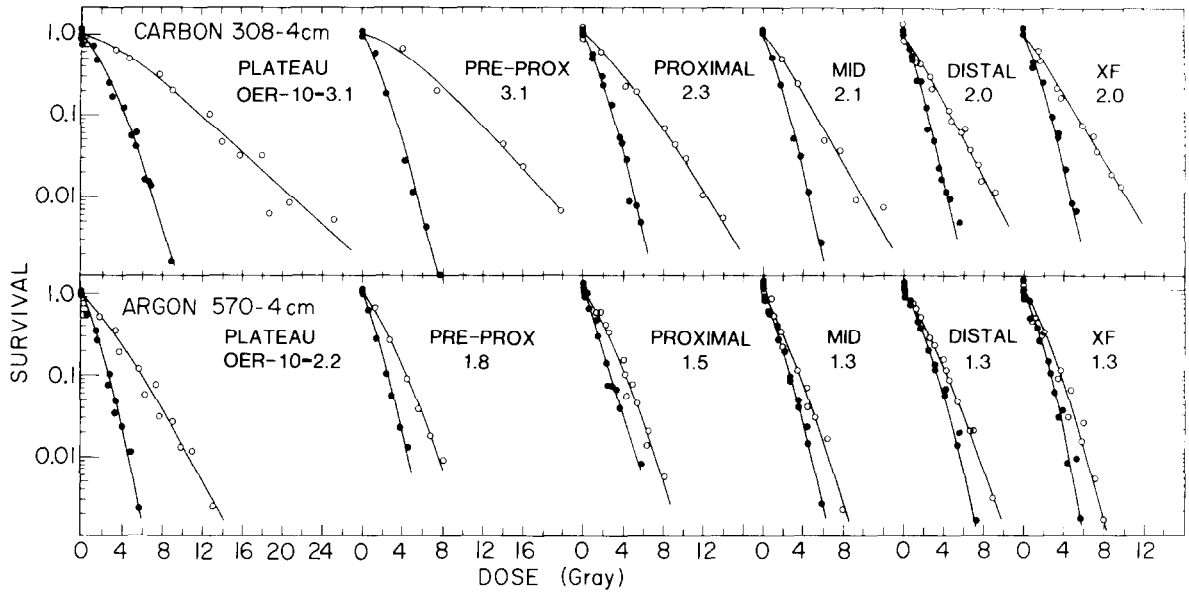


Fig. 7. Aerobic (●) and hypoxic (○) survival curves measured at various residual ranges of a 308 MeV/u carbon and a 570 MeV/u argon beam. The Bragg peaks had been extended to 4 cm. Survival was measured *in vitro* with human T-1 cells.

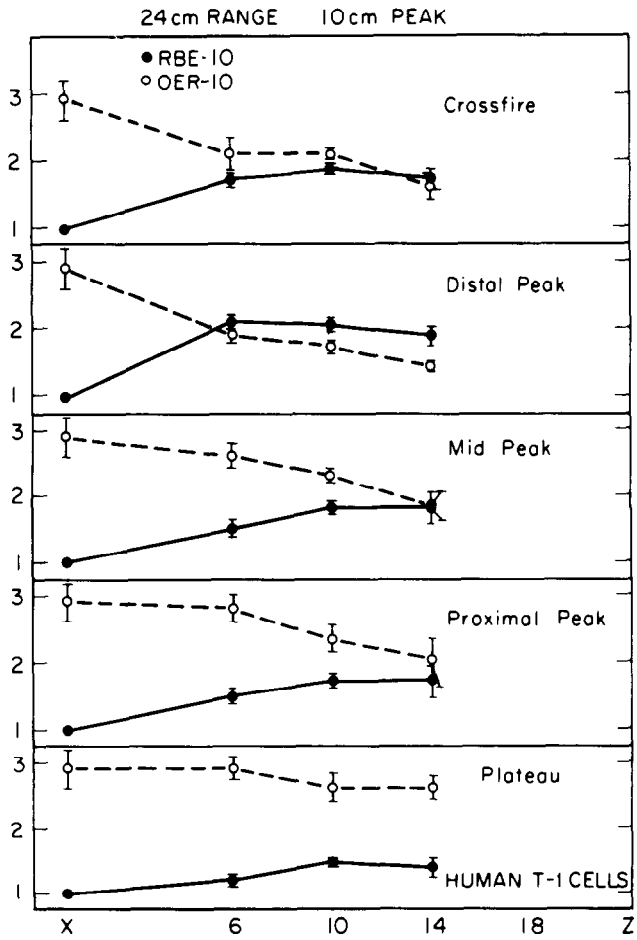


Fig. 8. Summary of Z dependence of aerobic RBE and OER of 10% human T-1 cell survival *in vitro* for Bevalac beams of approximately 24 cm range (with 10 cm extended Bragg peak). Data were measured at five different range positions: plateau, proximal peak, midpeak, distal peak and cross-fired peak. (Drawn from data described in Ref. 4).

vitro.[9] Their *in vivo* survival curves are shown in Fig. 10. Silicon reduces the survival of hypoxic cells in the tumor much more markedly than neon. Thus, this work also indicates the desirability of using silicon in solid tumor therapy trials, especially where hypoxic cell radioresistance is suspected. We wish to emphasize, however, that more biological research is necessary on cellular and tissue systems to reaffirm this conclusion.

The major potential of heavy ions in therapy is to deliver effective depth doses to deeply-seated localized tumors while sparing surrounding and intervening normal tissues. Another equally important property is that in extended Bragg peaks the magnitude of the radiobiological oxygen effect is significantly reduced to levels of about 1.1 to 1.6. The oxygen effect is reduced more effectively with particles of higher atomic numbers, which would indicate the desirability of using the heaviest available particle. This argument is tempered by the fact that heavier nuclei produce relatively more fragmentation, which leads to the reduction of depth-dose effectiveness and to a reduction of the oxygen gain factor. On the basis of cellular studies, the optimal choice of particles for therapy depends on the size and depth of the tumor. At a relatively shallow depth, argon particles might be used. At a tissue depth of about 25 cm, silicon seems to have the most advantageous balance of properties. Neon particles might be used for even greater depths of penetration. These ideas are illustrated in Fig. 11, where the depth-dose effectiveness and the oxygen gain factor of various beams are compared.

Having briefly dealt with the basic rationale of using certain heavy ion beams in therapy, we now turn to some special effects: the knowledge of these is essential for a rational heavy ion therapy program.[23]

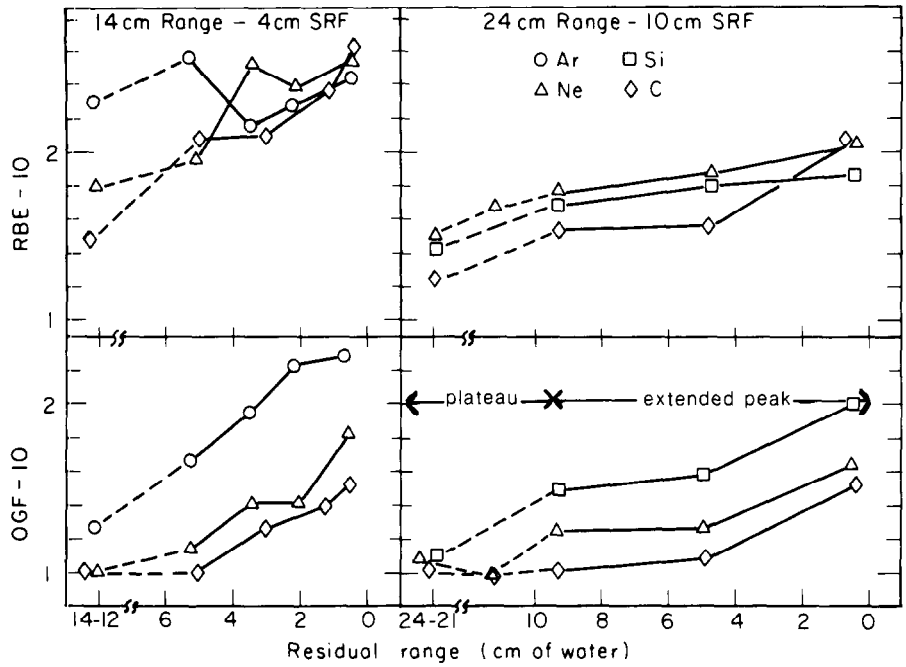


Fig. 9. Summary of range dependence of aerobic RBE and OGF at 10% cell survival *in vitro* for Bevalac beams of approximately 14 cm range (with 4 cm extended Bragg peak) and of approximately 24 cm range (with 10 cm extended Bragg peak). Data are from Refs. 5, 7, 8, 9, 13, 15, 25, 27 (figure from Ref. 3).

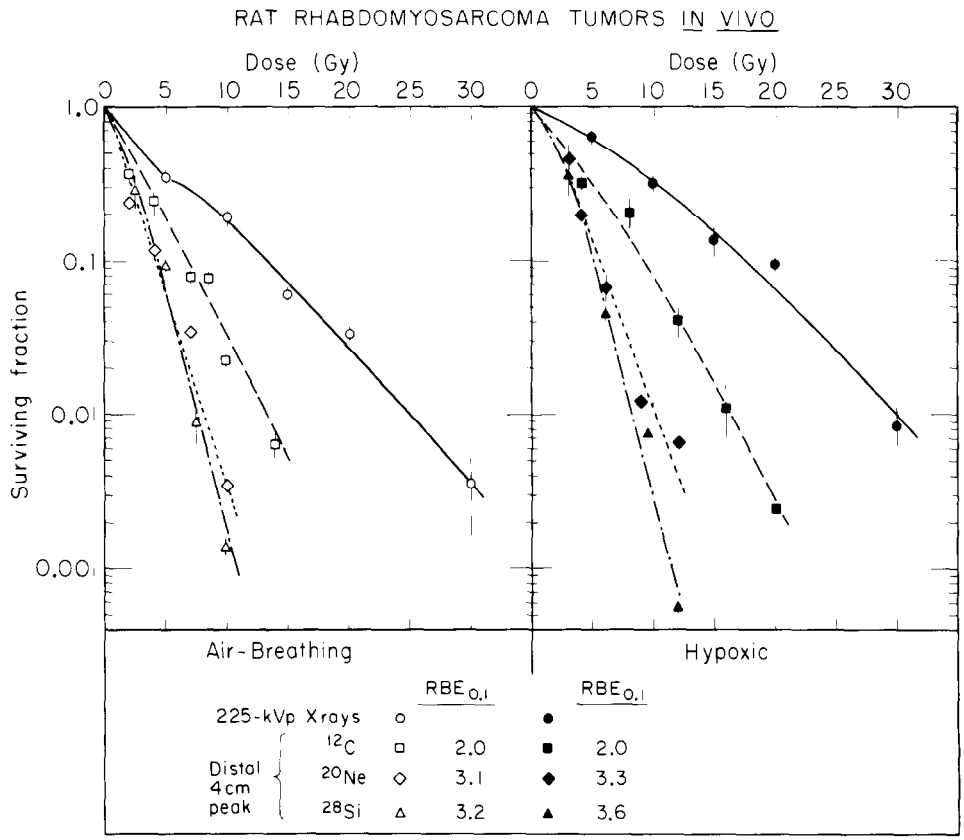


Fig. 10. Rat rhabdomyosarcoma tumor cell survival. Tumors are irradiated *in vivo* and assayed *in vitro*. Data were obtained for tumors irradiated with X rays and in the distal 4 cm extended Bragg peak of 400 MeV/u carbon, 425 MeV/u neon, and 670 MeV/u silicon ion beams.

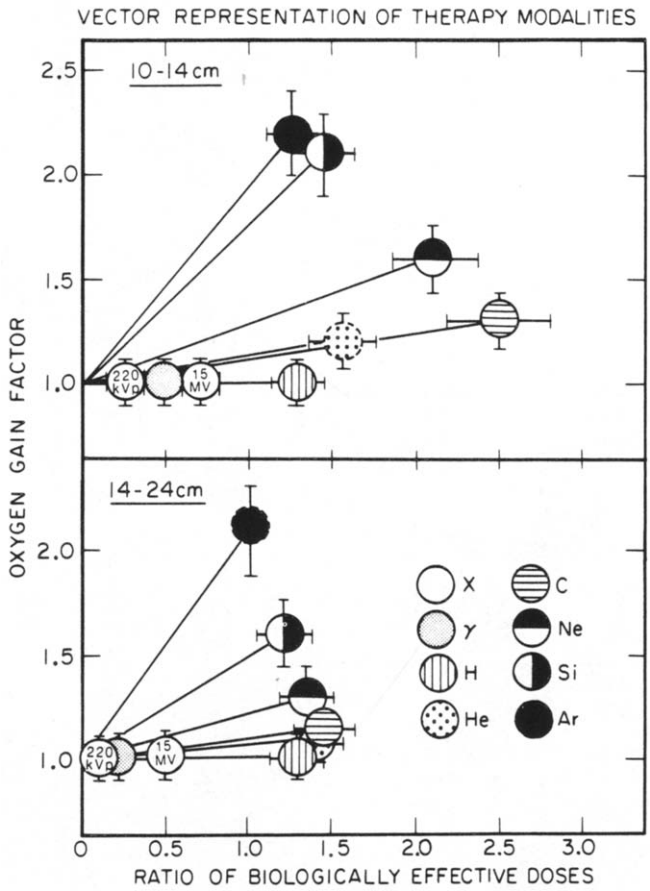


Fig. 11. Vector representation of low-LET and high-LET particle therapy modalities from treatment of: a small, shallow field (upper panel) and a large, deep field (lower panel). Ordinate: oxygen gain factor is the ratio of the oxygen enhancement ratio (OER) measured in a low-LET radiation to the OER measured in the high-LET radiation. Abscissa: ratio of biologically effective doses is the product of two ratios: 1) the dose to the mid target volume divided by the entrance dose and 2) the RBE at 50% cell survival measured in the mid-target volume to the entrance RBE-50 (figure from Ref. 3).

Sensitivity differences during the division cycle

The goal for tumor therapy is to effectively kill the maximum number of tumor cells while protecting normal tissues to an acceptable degree. This result is difficult to achieve with low-LET radiations, not only because of the poor depth-dose distribution or the high oxygen enhancement ratio, but also because of differences in radiation sensitivity through the cell cycle. The most resistant phase to X rays is the S-phase when DNA is synthesized. As shown in Fig. 12, argon ions effectively decrease the variations in radiosensitivity during the cell cycle and may thus make heavy-ion therapy more effective.

Studies on split dose repair

Repair at the cellular level is usually quantitated by measuring the degree of survival when radiation is administered in two split doses separated by varying time intervals. In our laboratory,[34] we have demonstrated that the degree of this repair is reduced by heavy ions.

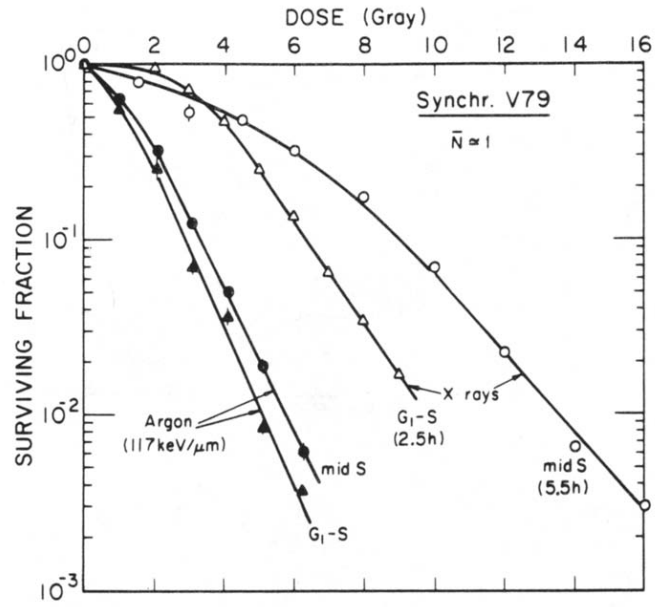


Fig. 12. Survival dose-response of synchronized G1/S (2.5 hr) and mid S (5.5 hr) phase Chinese hamster V-79 cells to X rays and Bragg plateau 570 MeV/u argon ions.

However, at very high LET the administration of split doses of heavy ions can be even more lethal than a single dose under conditions which show a sparing effect with X rays. This is illustrated in Fig. 13. V79 hamster cells synchronized in late G_1 phase were given split doses of neon Bragg peak ions. A lower cell fraction survived after split doses than after a single dose. It appears that the first

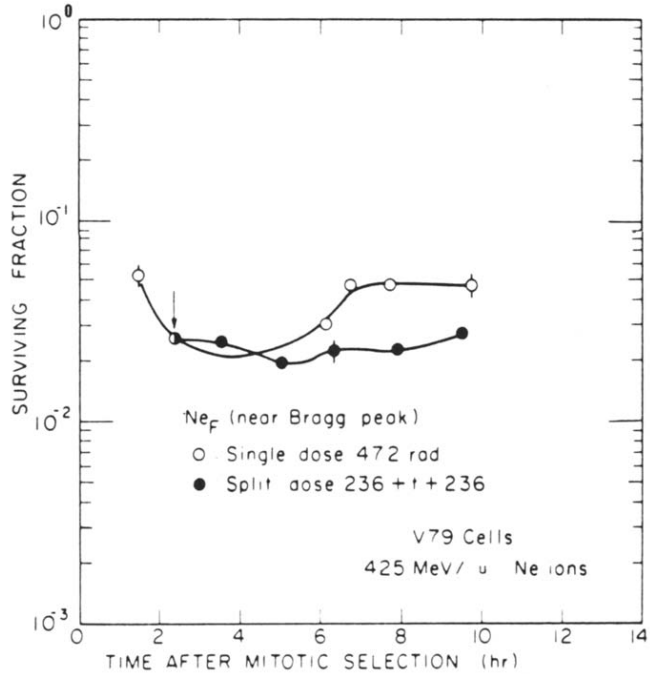


Fig. 13. Survival responses of V79 cells synchronized at mitosis and irradiated at various cell-cycle stages with single doses (open) or split doses (closed) of Bragg peak neon ions. The split-dose treatments started at 2.5 hr after mitotic selection, and the samples were kept at 37°C between exposures.

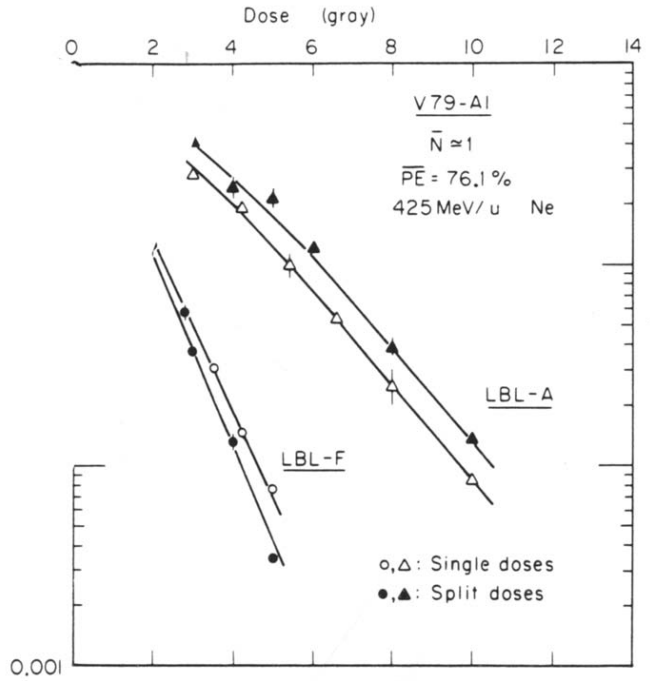


Fig. 14. Survival data for asynchronous V79 cells irradiated with singel doses (open symbols) or fractionated doses (closed symbols) of neon ions in the plateau (LBL-A) or Bragg peak (LBL-F) of a 425 MeV/u neon ion beam.

dose of high LET neon potentiated the effects of radiation injury caused by the second dose. Developing this subject further, in Fig. 14 we show the results of split dose irradiation with plateau and peak neon ions. In the plateau regions of the Bragg curve some of the neon ion-induced injuries were repaired, but at the Bragg peak the split dose schedule leads to decreased survival. The cellular mechanisms for the potentiation have been studied in detail.[21] A potentiation effect has been independently demonstrated in tissues,[16] and in carciongenic studies.[6] In the future the increased biological effectiveness as a result of potentiation must be taken into account in heavy ion therapy planning.

Delays in cell progression caused by heavy ions. A dose of radiation usually has a synchronizing effect on proliferating cell populations. Evidence is mounting that heavy ions are particularly effective in retarding cell processes as well as killing cells. Using the flow cytometric procedure,[18] evidence has been obtained that most cells in a population of V79 cells exposed to heavy ions are arrested in the G_2 stage just preceding cell division. The length of the S-phase is nearly normal after heavy ion exposure, whereas X rays also cause a delay in the S phase. The phenomenon has also been studied by the pulse-labeled mitoses method.[2]

In Fig. 15 the RBE for cell division delay is compared to that for cell survival. Let us assume that proliferating cells of a tumor might progress through various cell division stages faster than normal cells of the same tissue. The effect of a dose of heavy ions then would be to arrest many of the exposed tumor cells in G_2 stage prior to division. Normal cells would be less affected since these generally pass more slowly through the various cycle stages. If the next dose installment is given at the appropriate time, it might increase the preferential killing of tumor cells.

Interaction of the low and high LET radiation components

In most of the available high LET modalities, the high LET components of the radiation are mixed with lower LET components. There is a belief that the higher LET components of a given radiation field dominate the RBE as well as the oxygen effect. However, it is important to obtain quantitative data. In our laboratory,[22] we found that heavy ions produce sublethal lesions for X rays and

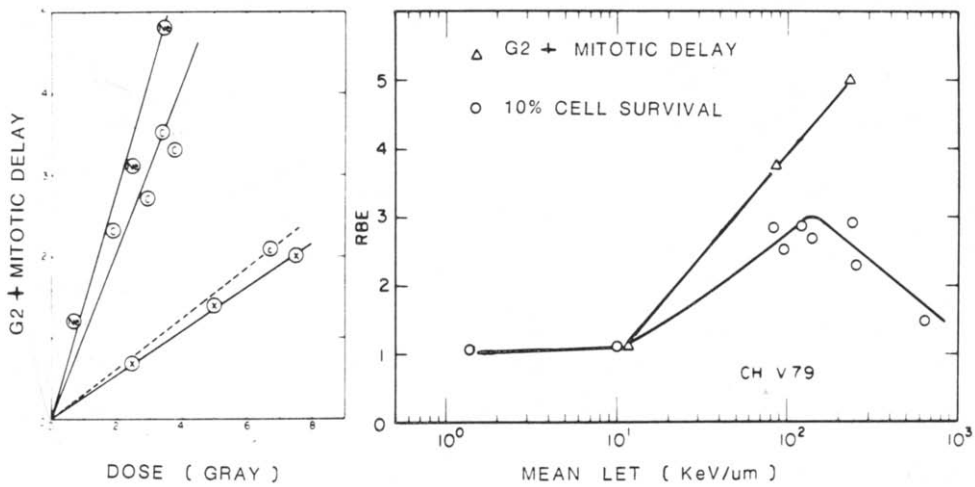


Fig. 15. (Left panel) Duration of G_2 + mitotic delay as a function of dose of 225 kVp X rays (x); Bragg plateau 400 MeV/u carbon ions (c); Bragg peak 403 MeV/u carbon ions (C); or Bragg peak 429 MeV/u neon ions (Ne). (Right panel) RBE for two end points (cell survival at 10%, and G_2 + M delay) vs. mean average LET (keV/μm). Data are taken from CHV-79 cell experiments with 400–403 MeV/u carbon, 425–429 MeV/u neon, and 570 MeV/u argon ion beams (figure from Ref. 3).

that X rays produce sublethal lesions for heavy ions (see Fig. 16). An important quantitative aspect of the interactions is the indication that the heavy ion component of a mixed beam should represent more than half of the total dose in order to have the high-LET component dominate.

Heavy ion lesions in DNA

Several years of research may be summarized by stating that the most likely lesions responsible for the high RBE of heavy ions are double-strand breaks in DNA generated in the high energy density core produced as heavy ions pass through genetic material. A single heavy ion appears to be capable of generating several double-strand lesions along its track across the cell nucleus. In contrast, X rays generate fewer double-strand scissions and many more single strand breaks. Single strand breaks are repaired by cells quickly and very efficiently in the course of a few minutes. The cells can also repair double strand lesions, albeit at a much slower rate, with a 50 to 100 minutes half life. Inability to repair all such breaks along a track, or misrepair and misrejoining, appear to be important causes of lethality. We have shown a relationship between the quantity of unrepaired double strand breaks and the RBE of heavy accelerated ions in human T-1 cells.[28]

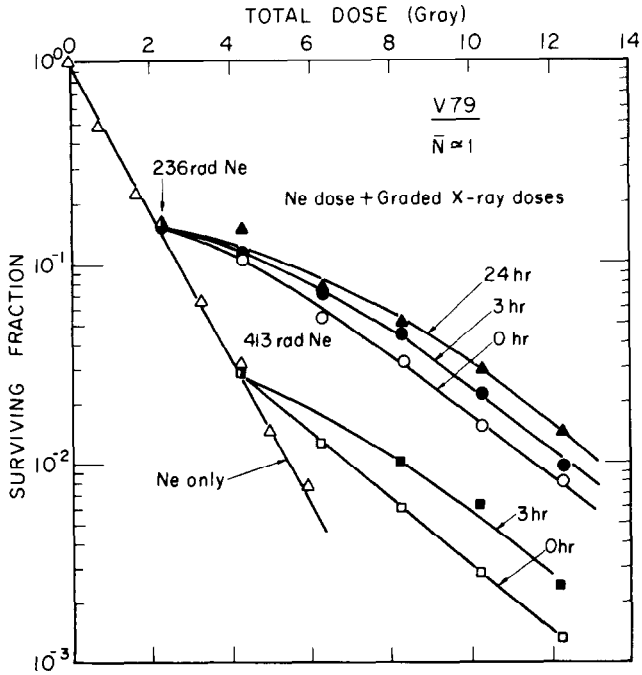


Fig. 16. Survival data of V-79 cells irradiated with neon ions alone or with graded doses of X rays at various time intervals after single doses of neon ions. The times indicated represent the incubation intervals at 37°C between the neon-ion and X ray exposures. For the 0 hr interval, the cells were incubated at ice temperatures shortly before neon-ion irradiation and between neon-ion and x-irradiation. The survival-curves are the best least-squares fits to the data points using a modified single-hit multitarget formula (figure from Ref. 22).

PLD repair

Strand breaks in DNA are also suspected to result in lethal events, particularly when the repair process is inhibited. This appears to be the case in the repair of potentially lethal lesions (PLD). When PLD repair is allowed to proceed in the presence of high salt concentrations, the lethal effectiveness of low LET radiation markedly increases; the magnitude of the sensitizing effects of treatment with high salt decreases for heavy ions. This effect was not seen, for example with stopping argon ions of low initial energy.[34] Certain other experiments have been carried out on PLD repair in 10T-½ cell monolayer populations.[34] Most of these cells are in G_1 phase, and the cells are almost confluent with a very slow rate of cell division. The situation might be comparable to certain normal tissues where very few cell divisions are taking place; it might also relate to tumor cells that remain for long periods in G_1. Our finding is that PLD repair exists for heavy ions as well as for X rays. Whereas for X rays this type of repair is usually complete in 6 hours, for heavy ions it can persist for as long as 24 hours. Very high LET radiation, however, can apparently completely inhibit the PLD repair. Figure 17 illustrates these points.

The effects of cell density on cell survival

It has been demonstrated that cells in contact with each other are more resistant to lower LET radiations than cells that are isolated from each other.[11] We also know that certain mammalian tissues and density-inhibited stationary phase cells *in vitro* appear to have much greater resistance to radiation injury than single cells with minimal cell-to-cell contact.[10,20] It has been demonstrated that a feeder layer of cells previously killed by radiation increased the radioresistance of cell isolates in much the same way as cell contact acts.[27] This form of radioresistance is readily diminished by the application of heavy ions.[19,26]

Several of the effects discussed above might be quite important in relation to the ability of heavy ions to suppress tumor growth and to enhance the ability of normal tissues to repair. For example, assume that the cells of a deeply-seated tumor are proliferating more rapidly than the cells of the underlying normal tissue. If low-LET X rays are used, a considerable fraction of tumor cells, those in radioresistant S phase, would be spared by the daily dose installment of 2 Gray. These survivors would produce more tumor cells in the radioresistant S phase when later dose fractions are given. Relatively fewer normal cells are in S phase. When high-LET heavy ions (e.g., silicon or argon) are used, the selective advantage of radioresistant S phase tumor cells would largely disappear; instead, the cell division of many of these cells would be severely delayed; and, if the fractionation scheme is efficient, the next heavy ion installment might find tumor cells in the very sensitive G_2 stage.

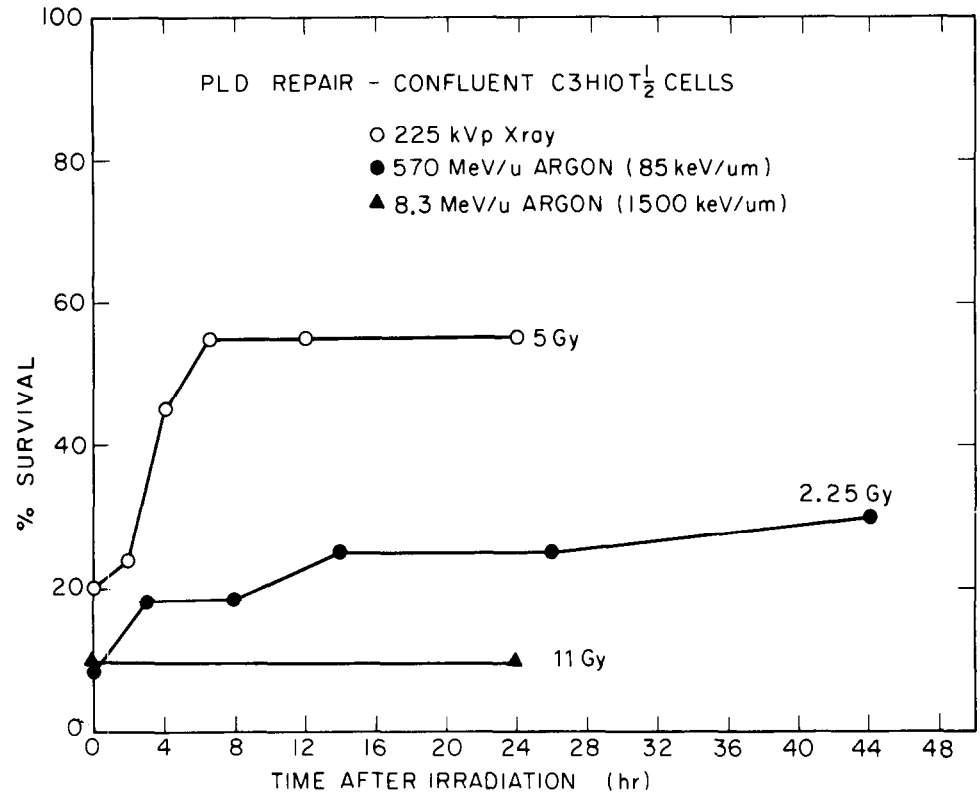


Fig. 17. Repair kinetics of potentially lethal damage in confluent C3H 10 T-½ cells irradiated with X rays or argon ions of either low (8.3 MeV/u) or high (570 MeV/u) initial energy as assayed by delayed plating.

The heavy ion effects are nearly additive or even superadditive in the extended peaks of silicon and argon ions; in the plateau, however, some repair capacity remains and helps intervening normal tissues to survive the therapy regimen.

One of the most interesting effects is PLD repair, a process that might spare normal and tumor cells in G_0 and G_1 states. Evidence is accumulating that β-DNA polymerase is necessary for the action of PLD repair. If we should succeed in inhibiting PLD repair by the combined use of heavy ions and enzyme inhibitors, the therapeutic efficiency of heavy beams would increase even further.

Fundamental approaches to the heavy-ion radiobiology of cells

It is important to understand at a fundamental level the spectrum of molecular and cellular responses to heavy ions. A practical result of such knowledge would be that one could intelligently model the responses to mixed beams and to protracted schedules. A general model of cellular inactivation, the repair-misrepair model (RMR), has been developed and appears to be suitable for a quantitative accounting of most observed effects.[32] This model treats the initial yield of heavy-ion-induced lesions in genetic material separately from the later modifications and enzymatic repair. Lethality and mutations are results of misrepair. Working with the model we realize that there is a potential for modifying cellular responses to heavy-ion irradiations; if we could understand the molecular mechanisms that regulate DNA polymerases, we might be able to influence cell and tissue response *in vivo*.

SUMMARY

Heavy ion radiobiology of mammalian cells is a rapidly developing field. The cellular responses depend on particle charge, fluence and residual energy. We find that good depth dose effectiveness and low oxygen enhancement ratios can be obtained with silicon beams of up to 25 cm range in water. Argon has an even lower OER, but less desirable depth-dose characteristics. Heavy ions also reduce the variation in radiation sensitivity through the cell division cycle. Furthermore, they initiate sublethal lesions that interact with X rays, and also produce potentially lethal lesions which are repaired albeit at a slow rate. With stopping low-energy argon beams of extremely high-LET, no potentially lethal damage repair was found. Neon and heavier particles applied in split doses produced an enhanced cell killing effect. The RBE for division delay is greater than the RBE for cell killing. These properties of heavy ion beams strengthen the justification for using heavy ions in therapeutic investigations. Initial Phase I and II therapy trials in our laboratory have verified the predictive value of cellular investigations in relation to human tissue tolerance.

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