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SUMMARY

We have studied the radiation dose responses of two human fibroblast lines: cells from a patient with Ataxia telangiectasia (AT-2SF) and an established line of human T-1 cells. Aerobic and hypoxic 225 kVp X-ray survival curves were used as controls to the heavy ion exposures. Nearly monoenergetic accelerated neon and argon ions were used at the Berkeley Bevalac with various residual range values. The LET of the particles varied from 30 keV/μm to over 1,000 keV/μm. For some of these studies the particle fragment contamination was controlled to low levels of a few percent.

All Ataxia survival curves were exponential functions of the dose. Their radiosensitivity reached peak values at 100 to 200 keV/μm. Human T-1 cells have effective SLD (sublethal damage repair) as has been evidenced by split dose experiments, and they are much more resistant to low LET than to high LET radiation. At high LET their radiosensitivity approached that of the Ataxia cells.

The repair-misrepair model has been used to interpret these results. According to this model, the molecular repair processes culminate either in eurepair or in misrepair. We have obtained mathematical expressions that describe the cross sections and inactivation coefficients for both human cell lines as a function of the LET and the type of particle used. The results suggest either that high-LET particles induce a greater number of radiolesions per track or that heavy-ions at high LET induce lesions that kill cells more effectively and that are different from those produced at low LET. We assume that the lesions induced in T-1 and Ataxia cells are qualitatively similar and that each cell line attempts to repair these lesions. The result in most irradiated Ataxia cells, however, is either lethal misrepair or incomplete repair leading to cell death. T-1 cells have efficient repair mechanisms at

low LET, and the repair-misrepair model suggests that at high LET the T-1 cells can still efficiently repair individual lesions, but that as the lesions become closely spaced along the tracks, the probability of misrepair increases. The Ataxia cell line appears to misrepair damage produced by radiation of any LET.

INTRODUCTION

Ataxia telangiectasia cell lines are fibroblasts derived from patients with a genetic disease characterized by progressive neurovascular degeneration, and immunological deficiencies and it frequently culminates in the appearance of malignancies (for a review see Paterson et al., 1982). These cells have been shown in vitro to be hypersensitive to radiation damage. Paterson et al. (1980, 1982) studied the radiobiological responses of cultured Ataxia cell lines and cells derived from individuals assumed to be Ataxia heterozygotes and found several complementation groups. The exact mechanism of this radiosensitivity is still not completely known, but many laboratories are currently investigating multiple characteristics of the cell lines including the notable absence of repair of PLD (potentially lethal damage) (Weichselbaum et al., 1978; Cox et al., 1981; Arlett and Priestley, 1983).

We were interested in examining the LET dependence of the inactivation of Ataxia cells by accelerated heavy ion beams to test current models of cellular inactivation using data from human cells having normal and abnormal repair mechanisms. Although our work does not specify the exact mechanism, it does suggest that misrepair of low-LET damage may explain the great radiosensitivity of Ataxia homozygotes, and that the degree of misrepair occurring for both Ataxia and T-1 cells is increased with damage from high LET radiations.

MATERIALS AND METHODS

Cell Lines, Growth Conditions, and Survival Measurements

Human T-1 Cells. The culturing techniques used for the aneuploid human T-1 cells have been published (Blakely et al., 1979), and were nearly identical to those described below for the Ataxia cells. Under these conditions, the doubling time of the T-1 cells was measured to be approximately 22 hours. The T-1 cells were originally obtained from G.W. Barendsen (see Blakely et al., 1979).

Approximately 24 hours before irradiation, an appropriate number of cells was plated onto 35-mm glass petri dishes so as to reach a density of 5×10^5 at the time of irradiation. After exposure, the cells were trypsinized, resuspended, counted, plated with appropriate cell numbers for each dose to yield approximately 100 clones per flask, and incubated at 37°C for 12 days. Colony forming ability was scored by staining the cultures with 1% methylene blue in 30% ethanol, and the clones containing at least 100 cells were scored as survivors. Sixteen unirradiated flasks were used to establish the plating efficiency which was usually 60 to 80% for each survival curve. Four flasks were plated for each irradiated sample. Each experiment was repeated twice.

Ataxia Telangiectasia Cells. Human Ataxia telangiectasia (AT-2SF) skin fibroblast cells (obtained from Dr. R.B. Painter of the University of California, San Francisco) were grown and maintained at 37°C in monolayer in Eagle's minimum essential medium with Earle's salts supplemented with 12.8% fetal calf serum, 0.25 g/l glutamine, and the antibiotics potassium penicillin G (0.04 g/liter), streptomycin sulphate (0.04 g/l), gentamycin (0.09 g/l), and fungizone (0.002 g/l). The atmosphere was humidified and maintained with 5% CO_2. Under these conditions, the mean population doubling time for exponentially growing cells was about 28 hours.

Approximately 48 hours before each experiment, the cells were trypsinized from plastic 75 cm^2 tissue culture flasks and seeded at a density of 2 to 3 x 10^5 per 35-mm glass petri dish. After irradiation, cells were trypsinized and resuspended in fresh medium, counted, plated into 25-cm^2 T-flasks at appropriate cell numbers for each dose to yield approximately 30 clones per flask, and incubated at 37°C. In a typical experiment, 32 unirradiated control flasks were used to establish a plating efficiency for each survival curve and 8 flasks were counted for each irradiated sample. The cultures were fed every 4 to 6 days with fresh growth medium. After 14 to 16 days the colonies were stained with 1% methylene blue in 30% ethanol, and the clones containing at least 100 cells were scored as survivors. Because of beam time limitations, we are still accumulating data and not all experiments at all residual range points have been duplicated. Plating efficiencies ranged from 2.6% to 7%. Cells from passage numbers 14 to 17 were used for the experiments in this report.

Irradiation Procedures

X-irradiation of the cells was performed using a Philips 250 kV generator operated at 225 kVp and 15mA, with a filtration of 0.35-mm Cu and a half value layer of 1.00-mm Cu. The monolayer samples were irradiated in a vertical position perpendicular to the beam. All irradiations were completed at room temperature. The dose rate was usually 2.7 Gy/min as measured with a calibrated Victoreen condenser 250 R-meter at a target distance of approximately 24 cm.

The procedures for the heavy-ion exposures have been described (Blakely et al., 1979). The dosimetry was based on parallel-plate ionization chambers. The monolayer samples were irradiated at room temperature in the vertical position with a horizontal beam normally incident on the sample at a dose rate

between 1.0 to 5.0 Gy/min. Beams included in this study were neon (225, 425, 670 MeV/amu) and argon (330 and 570 MeV/amu).

The Bragg ionization curves are given in Figure 1. Mean primary beam dose-averaged LET (linear energy transfer) values have been calculated for the residual range positions studied. The exposure chamber and gas delivery methods used to measure survival for all cellular studies under hypoxic conditions have been described (Blakely et al., 1979).

Data Analysis

Computer-assisted curve fitting was done for all survival data using the linear quadratic model (Chadwick and Leenhouts, 1973) and also the repair-misrepair (RMR) model (Tobias et al., 1980b). To examine the LET dependence of derived quantities such as RBE (relative biological effectiveness) and OER (oxygen enhancement ratio), we obtained least-squares fits to the survival data using the linear quadratic model, and then calculated the appropriate ratios[1] at several dose levels using the best fit α and β parameters. Values of RBE and OER at 10% survival are reported.

We have calculated confidence limits for the inactivation coefficients based on the principle that when a least-squares fit of the data is made using the logarithm of survival as a weighting function, the Chi-square function near the minimum behaves as a paraboloid. At a given value of Chi-square, determined by the desired probability confidence limit, the values of α and β arrange themselves on an error ellipse (Blakely et al., 1979). For RMR coefficients, where these are used in functional relationships (e.g., δ vs. LET), we use coefficients from most probable fits.

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[1]Aerobic RBE = D_{10} X ray/D_{10} heavy ion; OER = D_{10} hypoxic/D_{10} aerobic.

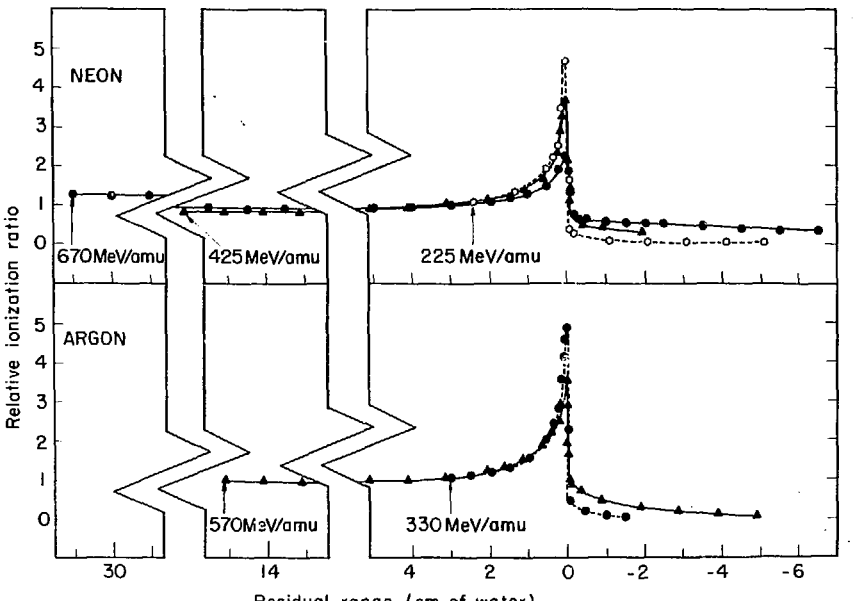

FIGURE 1. Bragg ionization curves for neon and argon beams of various initial energies, normalized at 3 cm residual range. The portion of dose due to fragmentation is lower for beams at lower extraction energy as indicated by the ionization beyond the stopping region of the primary particles (to the right). (XBL 839-3991)

RESULTS

X-Ray Survival and the Oxygen Effect

Aerobic and hypoxic X-ray cell survival studies have been conducted in simultaneous experiments with the T-1 and Ataxia cell lines. The dose-survival data are shown in Figure 2. The aerobic dose at 10% survival (D_{10}) of cells plated on a plastic surface was about 6.2 Gy for the T-1 cells and was about 1.2 Gy for the Ataxia cells. Using 95% confidence limits, the OER_{10} measured on glass was 2.8 ± 0.5 for the T-1 cell, and lower (2.5 ± 0.6) for the Ataxia cell line.

Heavy-Ion Survival and the Oxygen Effect

The Ataxia survival curves were essentially exponential for X-rays as well as for all of the heavy ion exposures. The particle data are shown in Figure 3. When these curves are compared to T-1 survival data (most of which appeared in Blakely et al., 1979), the Ataxia cells are more sensitive than T-1 cells at the LET values studied. The results of 225 and 670 MeV/amu neon ion exposures are presented in Figure 4. The Ataxia cells are more sensitive than T-1 at all LET values reported here.[2]

Calculated values of RBE_{10} and OER_{10} for both human cell lines studied are plotted as a function of the primary beam LET values in Figure 5. The

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[2] In another investigation, a preliminary set of survival curves is available for each cell line with a uranium beam at L = 15,700 keV/μm (E. Blakely and G. Kraft, private communication). Data from both cell lines fall on approximately the same exponential survival curve, indicating that if there is a difference in the cross section for inactivation between these two different cell lines, it is likely to be smaller than 20%.

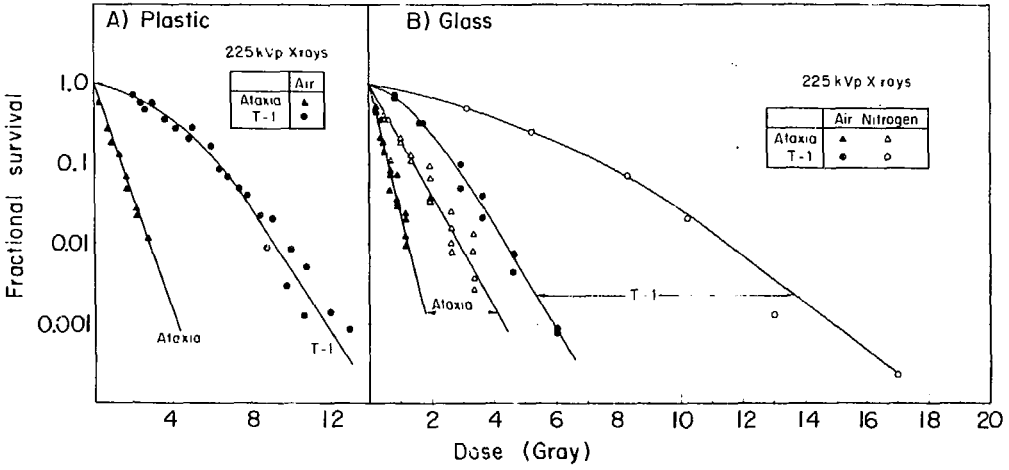

FIGURE 2. (Left) X-ray survival curves of Ataxia telangiectasia (AT-2SF) cells and T-1 cells irradiated in air on plastic petri dishes. (Right) X-ray survival curves of AT-2SF and T-1 cells irradiated in air and in nitrogen on glass petri dishes. For experiments measuring the oxygen effect, we irradiated cells grown on glass petri dishes in order to avoid the release of oxygen from plastic; however, the scattering of X-rays on glass surfaces contributes significant additional dose which is not measured by the Victoreen condenser R-meter. (XBL 837-3850)

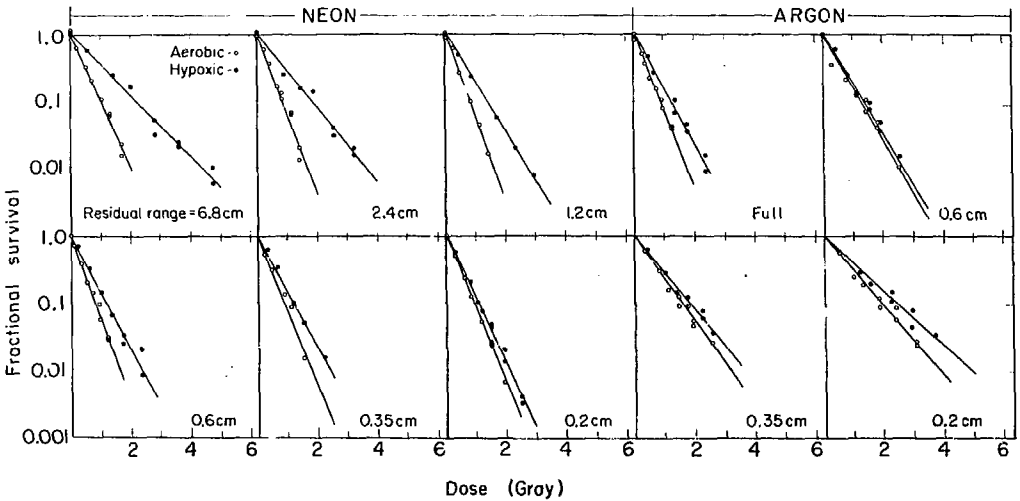

FIGURE 3. Aerobic and hypoxic survival curves of Ataxia cells exposed to 425 MeV/amu and 330 MeV/amu argon beams at various water-equivalent residual-range values. All survival curves are negative exponential functions of dose. The cells are more sensitive to lower speed particles with higher LET until a maximum is reached in sensitivity. The oxygen effect also declines with LET. (XBL 837-3851)

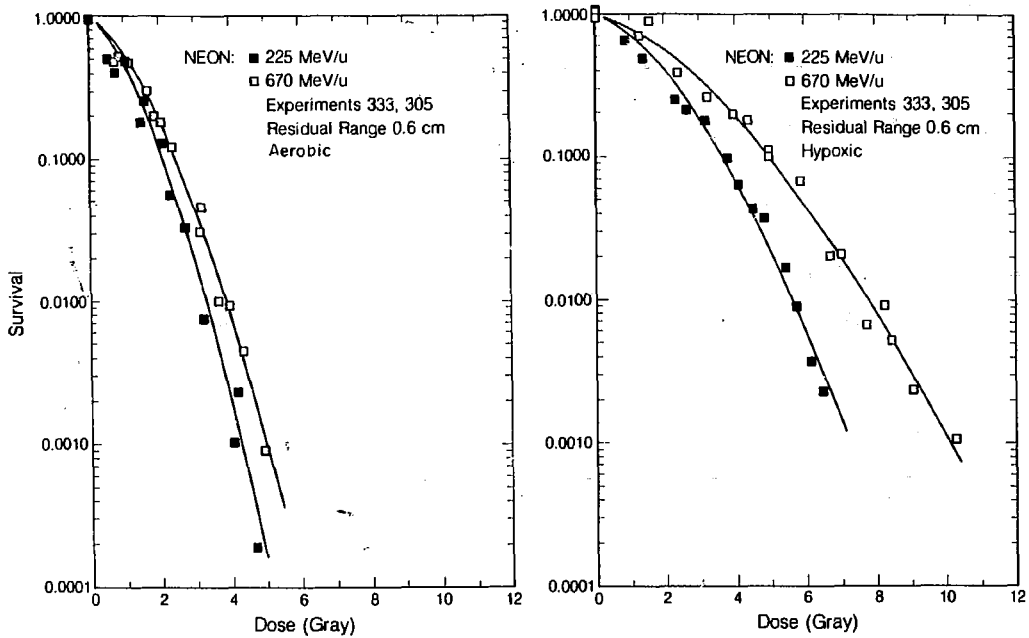

FIGURE 4. Typical survival curves of human T-1 cells irradiated with 225 and 670 MeV/amu neon beams at a primary ion beam LET of 118 keV/μm. Note that survival curves are not exponential, and the cells are more resistant than Ataxia cells. (XBL 838-3974)

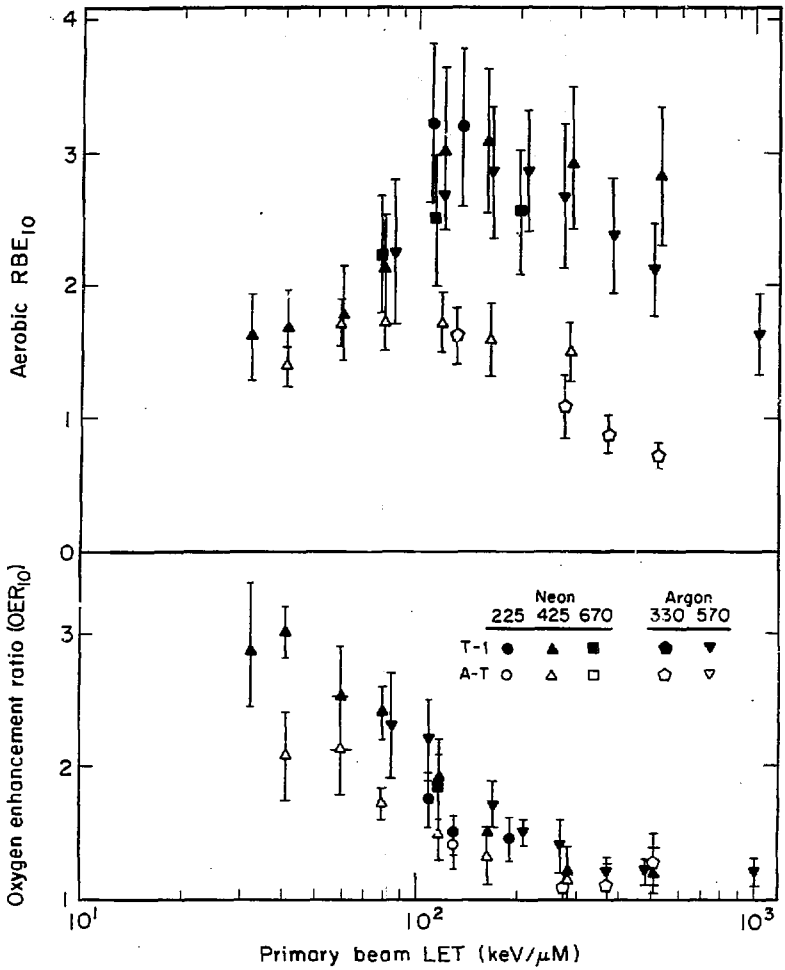

FIGURE 5. RBE_{10} relative to x-rays (top panel) and OER_{10} (lower panel) for human T-1 and Ataxia cells exposed to heavy ion beams (neon and argon) at various values of initial energy and LET. The OER_{10} of Ataxia cells is consistently but not significantly lower than the OER_{10} of T-1 cells. (XBL 838-3976)

previously published T-1 cell RBE data for carbon (400 MeV/amu), neon (425 MeV/amu), and argon (570 MeV/amu) beams were reported relative to survival after 220 kVp X rays with a HVL of 0.75 mm Cu. The T-1 cell RBE values for these beams have been recalculated relative to survival after 225 kVp X rays with a HVL of 1.1 mm Cu so that comparisons could be made with X rays of identical radiation quality for both cell lines.

The linear (α) and quadratic (β) inactivation coefficients from the linear quadratic model are plotted against primary beam LET in Figure 6. From these figures it is obvious that a simple one-hit theory cannot explain Ataxia inactivation at high LET because it would not produce a peak in the RBE-LET relationship.

An Application of the Repair-Misrepair Model to Track Theory

For the special case of heavy-ion beams, we have proposed a modification of the general RMR model (Tobias et al., 1983b). In this paper only an outline of the arguments and some of the conclusions will be given. A brief statement on the RMR model is given in Appendix I.

A number of energy transfer events may occur along a track, which is usually characterized by its LET. The work of Blakely et al. (1979) suggested that LET alone may not quantitatively characterize all types of events induced by ionizing radiation in mammalian cells. Accelerated particles can be produced that have different atomic numbers but the same LET. The radial distribution of energy transferred is also different because such particles differ in velocities (Chatterjee et al., 1973). Two such tracks may produce somewhat different distributions of lesions in the very complex structure of the cell nucleus and chromatin.

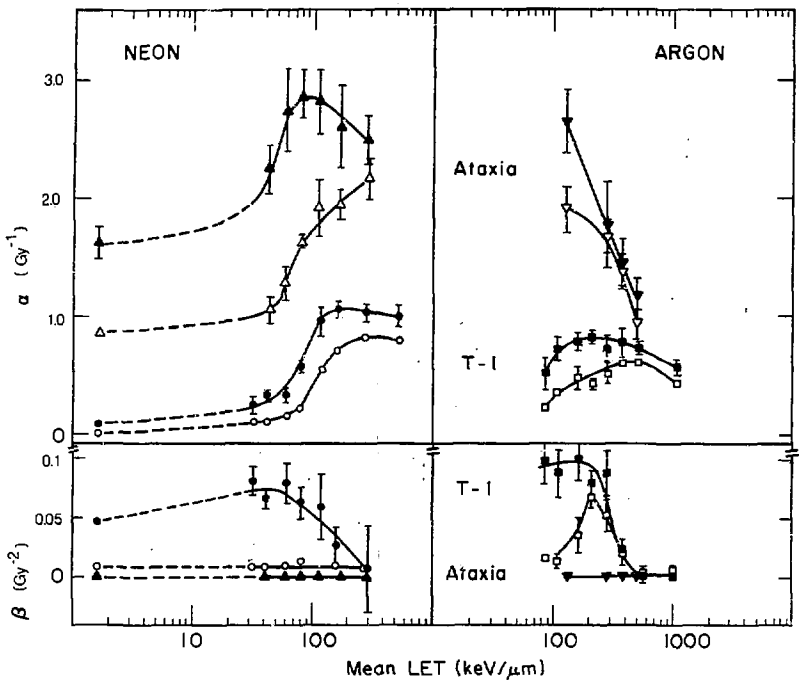

FIGURE 6. The linear quadratic model was used to obtain the LET dependence of values of the linear inactivation coefficient α and the quadratic coefficient β for Ataxia and T-1 cells irradiated with neon and argon ions. The values were obtained by a least-squares fit. We theorize that both T-1 and Ataxia cells are initially injured to a similar extent, measurable by α_{AT}, but the measured α values of T-1 cells are smaller because of the higher fidelity of intratrack repair. Closed symbols are aerobic results; open symbols are hypoxic results. Continuous lines through the points were drawn by eye. (XBL 837-3848)

Our current concept is that a given high-LET particle may produce a number of U lesions, perhaps of various kinds along its path. All components of living cells are at risk, but we assume that the crucial lesions are in DNA. Each time the track crosses chromatin it has the potential to make DNA lesions. For a number of years, there was a consensus that low and high LET particles produce qualitatively different lesions (Tobias, 1971; Goodhead et al., 1980) and at high LET there are quantitatively more double strand breaks per unit dose (e.g., Christensen, 1971). From the analysis of our available data, it appears that the nature of molecular lesions produced by high-LET particles is not materially different from those produced by electrons, except that there are more lesions produced with a degree of simultaneity along a single heavy ion track than along the tracks of electrons and the probability that some lesions will be misrepaired increases. In this context we are defining DNA strand breaks that do not rejoin after several hours of incubation as "misrepaired" (Roots and Kraft, 1982).

At very high LET, however, and in certain biological materials of low water content, the molecular effects seem to be very different from those produced by electrons, e.g., thermophysical lesions (Tobias et al., 1979; Nelson, 1980; Roots and Kraft, 1982). We assume in the work reported here that the number of DNA strands crossed by the particles limits the degree of injury that is achievable by a single track. The exact microstructure of the cell nucleus is of considerable interest in this regard, and thus the yield of U lesions is a special subject that must be approached experimentally. The Ataxia data in this paper, with some modifying assumptions, allowed us to calculate U lesion yields for neon and argon ions.

Because the lesions along a track might lie in close proximity, the RMR track model approaches the subject of cooperative repair by assuming that two

classes of cooperative repair exist: intratrack repair and intertrack repair. There are many other classes of lesions, e.g., single strand breaks and base damage in DNA, which are repaired by a self-repair mechanism that does not require a cooperative process. When only one track crosses the cell nucleus only intratrack (cooperative) repair may operate. When there are several tracks through the nucleus, intertrack repair interactions may also play a role. For example, we have shown experimentally that heavy ions produce sublethal lesions for X rays and vice versa (Ngo et al., 1981). When m identical tracks cross the cell nucleus and each produces a quantity of n_0 lesions, the mean number of initially uncommitted U lesions is $U_0 = mn_0$. According to the method usually used in RMR calculations, the rate of repair of U lesions is:

$$\frac{d(mn)}{dt} = \underbrace{-\lambda^* nm}_{\text{self-repair}} - \underbrace{k_i^* m \frac{n(n-1)}{2}}_{\text{intratrack repair}} - \underbrace{k_e^* n^2 \frac{m(m-1)}{2}}_{\text{intertrack repair}} \quad (1)$$

The first term is the rate of linear self-repair, with coefficient λ^*; the next two terms correspond to the cooperative repair process within each track (intratrack repair) with coefficient k_i^*, and between separate tracks (intertrack repair) with coefficient k_e^*.

Since the number of tracks crossing the cell nucleus, m, is not a function of time, we can modify the above differential equation for track repair:

$$dn/dt = -\lambda n - k(m)n^2, \quad (2)$$

where $\lambda = \lambda^* - (k_i^*/2)$ and $k(m) = (k_i^*/2) + (m-1)(k_e^*/2)$.

We may introduce the repair ratio, $\mu(m)$:

$$\mu(m) = \lambda/k(m), \tag{3}$$

and with the following procedure suggested by Tobias et al. (1980b), we can calculate the survival probability. In the formula below we assumed that all cooperative (quadratic) repair is lethal misrepair. The survival probability S(m) is given as:

$$S(m) = e^{-\sigma F}\left[1 + \frac{\sigma F\phi \cdot (1-e^{-\lambda t})}{\mu(m)}\right]^{\mu(m)} \tag{4}$$

where $mn_0 = \sigma F$, σ is the LET dependent cross section for production of U lesions, F is the fluence of heavy particles, and ϕ is the linear repair fidelity when $L \to 0$. The value of ϕ for T-1 cells is near 1, and we assume that for Ataxia cells $\phi \cong 0$.

Equation 4 is the track RMR survival equation. The repair ratio, μ, which is a constant in the general formula (Appendix 1, Equation 1), is here a function of m. The survival probability $S(m = 1)$ for the case when $m = 1$, when a single track crosses the cell nucleus, becomes of special significance. As shown below, the Ataxia cellular inactivation results allow us to evaluate $S(m = 1)$. The quantity $S(m = 1)$ may be regarded as a measure of the intratrack fidelity of repair. The initial slope of a survival curve is obtained by differentiating Equation (4):

$$\left[\frac{dS(m)}{dF}\right]_{F\to 0} = -\sigma(1-\phi) = -\sigma[1-S(m = 1)] \tag{5}$$

Interpretation of the Ataxia Data with the RMR Model

If we assume that all Ataxia survival equations are strictly exponential, the RMR model suggests various possibilities. One of these is that the U lesions are self-repaired very slowly, $\lambda \cong 0$. Another possibility is that the rate of self-repair is nearly normal, but that the fidelity ϕ is low: $\phi << 1$. A third possibility is that the rate of quadratic intratrack misrepair, k_1^*, is high. Any of these assumptions would lead to the conclusion that a single U lesion can kill the Ataxia cells. The slopes of the survival curves can then be used to calculate the yield of U lesions.

In the literature we find evidence that homozygous Ataxia cell lines are all highly radiosensitive. Anomalies in DnA synthesis have been observed in Ataxia cells, both in their failure to inhibit DNA synthesis immediately after exposure to ionizing radiation, and in their prolonged S phase (Murnane and Painter, 1982; Painter and Young, 1980; Houldsworth and Lavin, 1980; Edwards and Taylor, 1980). Some homozygous lines are reported to be deficient in repair replication and in the ability to remove γ-ray induced base defects, although others are not (Paterson et al., 1976, 1979a, 1979b, 1982). We do not know at present whether the deficiency in Ataxia repair lies in the DNA synthetic step or in some other phase of repair (e.g., steps involving ligation or gyration). It has also been shown that chromosomal aberrations involve ligation of chromatin and are more numerous than in normal cells, and that X-irradiated Ataxia cells are prone to produce abnormal quantities of chromosomal abnormalities (Zampetti-Bosseler and Scott, 1981; Scott et al., 1974; Taylor, 1978).

We assume for our calculation that for AT-2SF cells all repair is lethal misrepair; therefore, a single U lesion in the nucleus of a cell will inhibit the proliferative capacity of that cell. The inactivation coefficients, δ,

obtained from measured Ataxia survival data will therefore allow the calculation of the cross section, σ, for lesion production as a function of atomic number (Z) and LET (L) of a monoenergetic particle beam. The Ataxia cell survival becomes the simplified equation:

$$S = \exp(-\sigma F) = \exp(-\delta D) \qquad (6)$$

where F is particle fluence, D is dose in the appropriate units, and $\delta = \sigma/L$.

The yield of U lesions per cell can be calculated by multiplying the probability of a particle crossing the cell nucleus, with the probability that it can produce a U lesion in the nucleus. (We assume that lesions other than U lesions will not be indicated by the survival test.) We selected certain data points taken with 225, 425, and 670 MeV/amu neon beams and 330 and 570 MeV/amu argon beams to cover the LET range of interest. In analyzing the inactivation constants and cross sections for Ataxia cells, we find that:

1. There are at least two separate processes for the production of U lesions: the first one of these, the theta (θ) type, is proportional to LET, with the yield of $U_\theta = \theta L$ lesions. This is equivalent to the idea that at low LET, U lesions are proportional to dose: single delta rays are capable of causing U lesions. The presence of oxygen increases the yield of these lesions.

2. The second mechanism, the omega type (Ω) is due to higher order radical reactions in tracks. Usually one assumes a quadratic dependence on free radical density within the track; however, the actual interactions proceed presumably in very short time intervals of $< 10^{-9}$ seconds, while the track energy density is rapidly changing due to diffusion and competing radical reactions. We found it convenient to approximate the yield U_Ω as:

$$U_{\Omega} = \Omega L^2 (L/Z)^{0.2} \qquad (7)$$

The radical track structure at constant L is known to depend on the atomic number of the particles, and the last term is an attempt to account for this. If there is an oxygen effect in the omega process, the coefficient Ω would depend on the oxygen concentration at the time of the irradiation.

3. The number of U lesions increases as L increases; however, the amount of DNA in the cell nucleus is finite, and determines the maximum number of U lesions that a single particle track can cause. So the cross sections must "saturate" as L becomes large. In current experimental work we find that the maximum cross section occurs above L = 1,000 keV/μm.

The saturation cross section for both Ataxia and T-1 cells appears to be about 1.1 to 1.35 x 10^{-6} cm^2. This is smaller than the mean geometrical cross sections measured by microscopy in living cells attached to petri dishes. The actual structures that are critical to the radiation response are most likely chromatin fibers within the cell nucleus and may not include the entire nuclear volume.

We found a satisfactory formalism, a modification of one used much earlier by Todd (1964) and by Tobias (1971), for expressing the cross section for Ataxia cells:

$$\sigma = \sigma_{15,700} [1 - P \exp(-U_{\theta}) - (1-P) \exp(-U_{\Omega})] \qquad (8)$$

Here $\sigma_{15,700}$ comes from measurements by Blakely and Kraft at L≅15,700 keV/μm.[3]

[3] Ibid.

The parameter P is an adjustable coefficient that relates to the amount of lesions from low LET fragments and delta rays in a mixed field. The inactivation coefficient δ of Ataxia cells relates to the cross section by:

$$\sigma[cm^2] = \frac{\delta[Gy^{-1}] \cdot L[keV/\mu m]}{6 \times 10^8} \quad (9)$$

The continuous curves of Figure 7 represent an expression of the theoretical yield (δ) of U lesions produced by irradiation in aerobic and hypoxic environments; the actual data are also plotted. Based on these data, we can make several tentative conclusions:

1. Both aerobic and hypoxic lesion yield curves in Figure 7 are slightly higher for neon as compared to argon; this difference appears particularly at LET values greater than 100 keV/μm.

2. The linear term of Equation 8 accounts for most of the radiobiological oxygen effect; however, the data do not exclude the possibility of a small oxygen-dependent factor in the higher ordered Ω term.

3. Aerobic lesions peak at L = 120 keV/μm, whereas hypoxic lesions are maximum at L = 200 keV/μm.

4. The proportion of U lesions generated by the Ω process appears to be greater for neon ions than for argon, perhaps because neon ion tracks have lower velocities and smaller ionizing core diameters and higher local energy density than argon ions at the same LET.

The theoretical effect of specifying various values for coefficient P, the relative linear lesion yield, is shown on the right panel of Figure 8. Greater

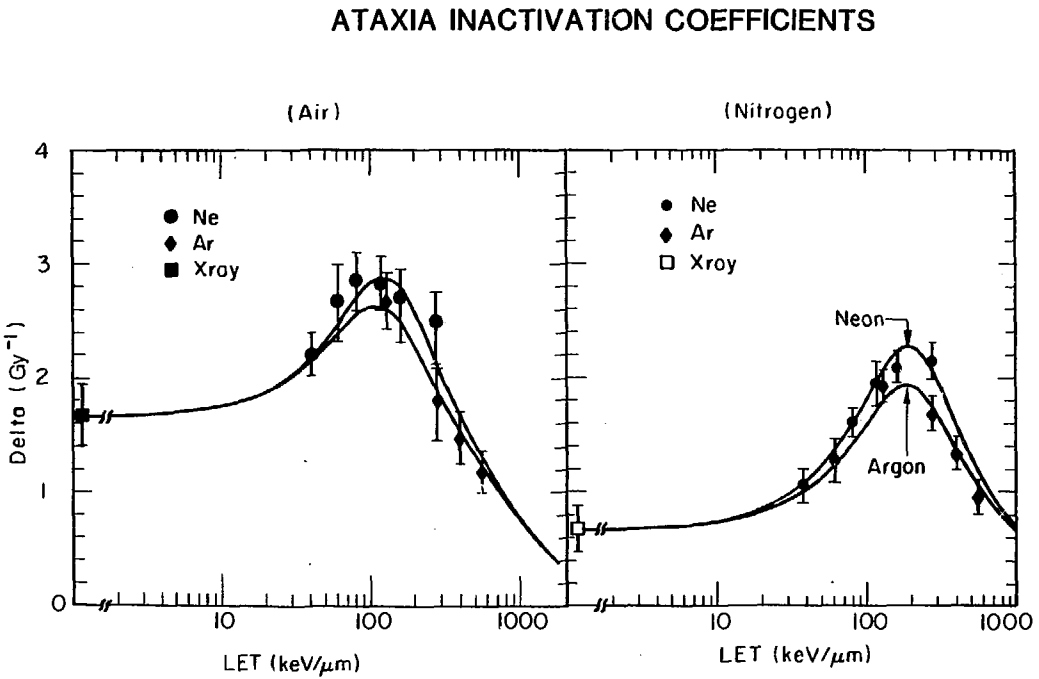

FIGURE 7. The LET dependence of the Ataxia cellular inactivation coefficient (δ) as used in the RMR model. The continuous curves correspond to calculated values of δ from Equations (8) and (9) with the appropriate correction from Equation (7) made for argon (lower curves) and neon (upper curves). (XBL 838-3973)

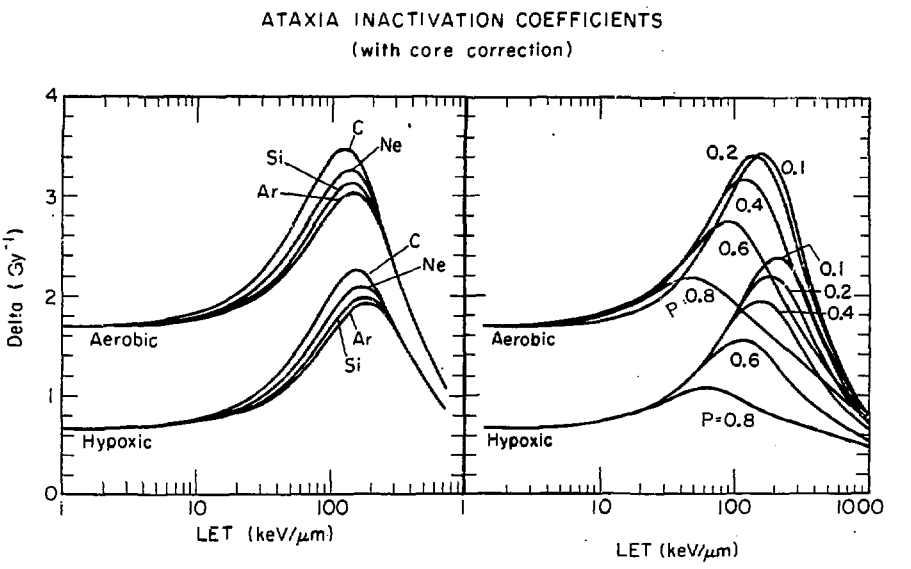

FIGURE 8. Samples of the theoretical LET dependence of the delta parameter for inactivation of Ataxia cells by heavy ions as these depend on particle track parameters.

Left. We believe that the cooperative U lesions are produced by interactions proportional to the square of radical density in the diffusing track core. Experimentally, however, we have obtained a better fit with $L^{-0.2}$. The correction term of $Z^{-0.2}$ has been applied here (normalizing to neon). Carbon, which has the smallest track core compared to other ions at the same LET, has the highest delta values, whereas argon has the lowest ones. The calculated argon and silicon inactivation coefficients are quite similar to each other; we find this also to be true experimentally.

Right. The linear dose dependence of lesion production, θ of Equation (8) is proportional to the track energy density of the particles. This portion, P, comes from the penumbra of tracks and also from low LET secondaries. A high linear proportion shifts the delta parameter down and to the left. (XBL 839-3992)

amounts of linear yield will decrease the height of the peak in yield curves and will move the peak to a lower LET. The P parameter may be helpful when we analyze data that have significant low-LET fragmentation doses.

In the left panel of Figure 8 we have plotted hypothetical yield curves for carbon, neon, silicon, and argon ions. The peak on the yield curve increases for ions of lower atomic numbers at the same LET where the track core diameter is smaller. Both of these correction methods become invalid for very high LET radiation (high atomic number and low velocities), where more complex phenomena take place. These corrections help to explain why the maximum RBE of low energy heavy ions is higher than that measured for high energy heavy ions and occurs at a lwer LET. Heavy ions of a few MeV/amu kinetic energy have a smaller low-LET penumbra and less theta-type lesion yield than high speed heavy ions. The yield curves in Figure 8 for P = 0.2 resemble the RBE curves obtained for low energy heavy ions by Todd (1964) and by Cox et al. (1977).

Comparison between Ataxia and T-1 Cellular Radiobiology

We know that T-1 cells are aneuploid with variable chromosome numbers and that these cells have more DNA than AT-2SF cells. For the purposes of this paper, we assume that the magnitudes of the δ coefficients (see Equation 6)[4] are the same for Ataxia and T-1 cells. Using the approximations of Equation (8) to the Ataxia data, we then obtained a valu for δ at each L. The values of $\mu(L)$ and $\delta(L)$ could then be determined with a nonlinear least-squares routine for two variables using the appropriate T-1 cell experimental data. The results are shown in Table 1.

[4] Ibid.

Table 1. RMR Coefficients for Inactivation of T-1 Cells: 225 and 425 MeV/amu Neon Particles

Residual Range [cm water]	LET [keV/μm]	U Lesion Yield* [Gy^{-1}]	Repair Ratio [μ]	Fidelity [ϕ=S(1)]	Initial Slope $(dS/dD)_{D\to 0}=\delta(1-\phi)$ [Gy^{-1}]	Sigma [cm^2]
			Aerobic			
O 16.1	32	2.0	13	0.88	0.12	6.4×10^{-9}
A 6.8	41	2.2	16	0.90	0.22	1.5×10^{-8}
B 2.4	60	2.6	15	0.92	0.21	2.1×10^{-8}
C 1.2	80	2.7	26	0.76	0.62	8.3×10^{-8}
D 0.6	118	2.8	19	0.68	0.90	1.8×10^{-7}
E 0.35	164	2.7	37	0.64	0.99	2.7×10^{-7}
F 0.2	284	2.2	31	0.56	0.97	4.6×10^{-7}
			Hypoxic			
O 16.1	32	1.0	38	0.92	0.08	4.3×10^{-9}
A 6.8	41	1.05	41	0.91	0.095	6.5×10^{-9}
B 2.4	60	1.35	33	0.93	0.095	9.3×10^{-9}
C 1.2	80	1.6	34	0.90	0.16	2.1×10^{-8}
D 0.6	118	1.95	40	0.78	0.43	8.4×10^{-8}
E 0.35	164	2.2	35	0.76	0.53	1.4×10^{-7}
F 0.2	284	2.1	121	0.60	0.82	3.8×10^{-7}

*Assumed to be the same as theoretical expression obtained from Ataxia data.

We also calculated inactivation cross section values for the initial slopes of T-1 survival curves. These sigma values as well as continuous curves plotted using Equation 8 for Ataxia cells and Equation 5 for T-1 cells are shown in Figure 9. In this manner, one obtains a fairly consistent view of the similarities and differences between inactivation processes for Ataxia and T-1 cells.

At very high LET, well above 1,000 keV/μm, each particle that passes through an area of 110 to 135 μm^2 can kill either Ataxia or T-1 cells. At lower LET, the T-1 repair mechanims are operative. For example, at about L = 300 keV/μm, the probability that a single particle crossing the T-1 cell nucleus can actually kill the cell is only about $1 - \phi = 0.4$. The T-1 cells are so effective in eurepairing their U lesions that in air at low L values (L between 30 and 60 keV/μm) about fifteen eurepairs occur for each misrepair ($\mu \cong 15$). In spite of relatively large errors in determining the repair ratio, μ, it appears that U lesions produced in air are repaired with relatively greater probabilities for misrepair than U lesions produced in an hypoxic environment. Thus at a given L, $\mu_{(air)}$ is smaller than $\mu_{(nitrogen)}$. The repair ratios μ(L) seem to increase with L; however, the survival curves at high L become nearly exponential and the accuracy of determining L rapidly diminishes when L is large.

It would appear from this analysis that the magnitude of the oxygen effect in T-1 cells is greater at high dose than at low dose. This is what one would predict for mixed beams of low and high LET particles. The high energy heavy ion beams usually have a spectrum of LET values because of fragmentation, and the low LET portion of the track penumbra.

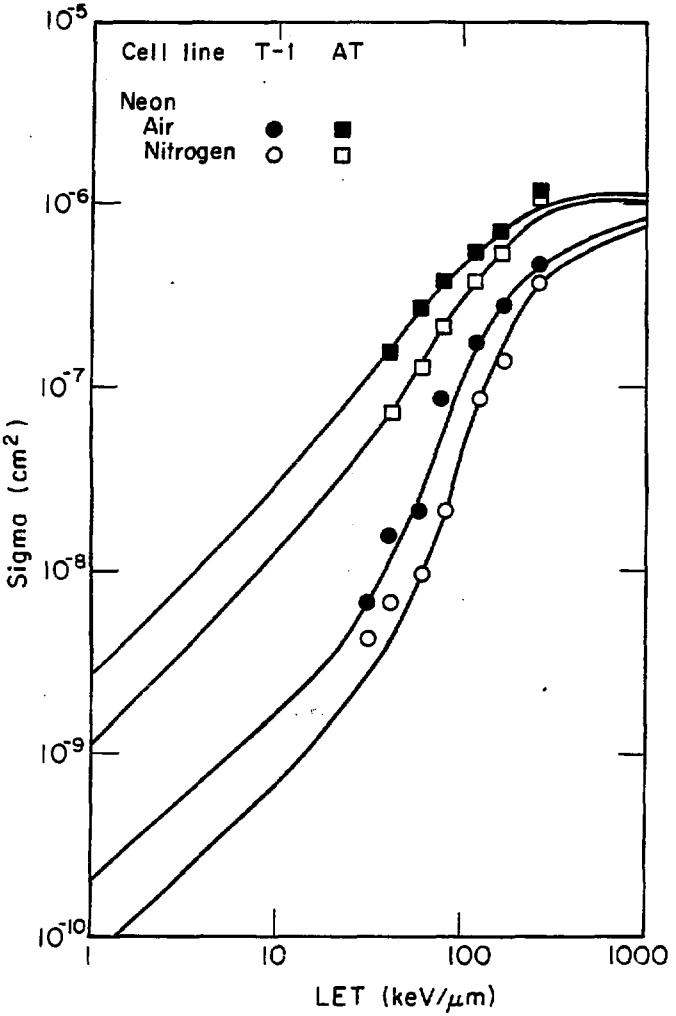

FIGURE 9. We plotted the cross sections for producing lesions in Ataxia and T-1 cells by neon ions (upper two curves), based on Equation (8). The lower curves correspond to the cross sections for T-1 cells, after repair has occurred. These curves are based on Equations (4) and (5); the data are also shown. (XBL 838-3971)

DISCUSSION

The purpose of this investigation is to compare the responses of a genetically radiosensitive human cell line (Ataxia telangiectasia) and the relatively radioresistant human T-1 cells to accelerated heavy ions. Our work is in general agreement with Lucke-Huhle et al. (1982), who found that homozygous Ataxia cells exposed to ^{241}Am alpha particles have an RBE_{10} of 1.9, and also with Cox (1982), who measured the responses of plateau phase Ataxia cells to accelerated helium ions and other radiations up to L = 140 keV/μm.

We have obtained Ataxia inactivation data with two particles in a broad range of LET values. There are some similarities and also some differences between the Ataxia and T-1 cell results. The differences relate to the magnitude of the effects seen. The similarities include: (1) the general trends of the response of both cells is the same at the LET values studied, and (2) we find that when L is greater than 100 keV/μm, the Ataxia cells are more sensitive to neon ions than to argon ions at the same LET. This effect could be attributed either to the different radial structure of neon and of argon tracks, or to different contributions to the effects by the low LET fragments; however, the dose contributions from fragmentation were relatively low.

There has been considerable discussion concerning the magnitude of the oxygen effect in Ataxia cells. Paterson found that the gamma ray OER values for Ataxia heterozygotes was lower than the OER for Ataxia homozygotes or normal cells. This led Paterson to the hypothesis that Ataxia cells can be categorized by their differential ability to repair damage produced under oxic and hypoxic conditions even though the effect of posttreatment at low temperature on the oxygen effect in cellular survival is ignored (Alper and Bryant, 1974; Powers, 1962).

Ritter et al. (1979) could not find a statistically significant difference between the OER values of four normal fibroblast lines and a homogyzous Ataxia cell line. Kinsella et al. (1982) found a variety of OER values for normal and for Ataxia cells, depending on their method of calculation. We found that AT-2SF cells had consistently lower OER_{10} values than T-1 cells in the range of LET studied below 200 keV/μm; however, the differences at individual points are not significant. To date, all published Ataxia OER results are based on work with asynchronized cells; however, there are several reports of lower OER values in mitotic and G1 cells compared to S phase cells (Kruuv and Sinclair, 1968; Legrys and Hall, 1969; Pettersen et al., 1977; Sapozink, 1977; Sapozink et al., 1974). It is possible that some of the variability reported for Ataxia OER values may relate to variable population distributions across the cell cycle.

Evidence is accumulating that Ataxia cells have no PLD repair of low LET radiation damage (Weichselbann et al., 1978; Cox et al., 1981; Arlett and Priestly, 1981). Because SLD repair is associated by definition with the shoulder of the radiation survival curve, Ataxia cells are assumed to have no SLD repair either; however, from cytogenetic data Taylor et al. (1975) suggested that gamma irradiated Ataxia cells did rejoin DNA strand breaks. Harrihan et al. (1981) and Fornace and Little (1980) used an alkaline elution technique that is highly sensitive in the detection of single strand breaks. They found that the molecular rate of DNA repair was normal in x-irradiated Ataxia cells, and stated that the techniques used in molecular rejoining studies offer no insight into the rate at which structural defects are generated during the course of end-to-end reunion of broken strands. The molecular and cellular studies are then in apparent contradiction unless we assume that a certain amount of the repair that is progressing at the molecular

level is misrepair. If we assume, however, that Ataxia cells have a defective proofreading-repair mechanism operating in the late stages of DNA synthesis, then we obtain an explanation for the low plating efficiency and high radiation sensitivity of this cell line.

Painter and Young (1980) demonstrated that Ataxia cells lack the usual delay that occurs in cell progression in normal cells after a dose of X-rays. The role of the delay may be to allow repair synthesis to take place before cell division occurs. In studies of heavy ion effects on V79 cells these cells did not suffer major delays in S phase after exposure to heavy ions (Lücke Huhle et al., 1979) or alpha particles (Hieber et al., 1981). It appears then that normal cells recognize X-ray injury and, in response, slow down S phase to repair the lesions, but this may happen to a lesser degree after heavy ions. Ataxia cells seem to have lost this ability to recognize X-ray induced lesions. Painter (1983) has shown that the chain elongation phase of DNA synthesis in Ataxia cells fails to be inhibited by a dose of X rays, and Zampetti-Bosseler and Scott (1981) have shown that Ataxia cells have less mitotic delay than normal cells.

Our findings are consistent with the idea that the essential radiolesions responsible for lethal effects are qualitatively similar or identical for Ataxia cells at low or at high LET. At the moment we have no evidence that the lesions produced in Ataxia and T-1 cells are qualitatively different at any LET. There have been recent alkaline sucrose gradient studies that indicate no differences between Ataxia and normal diploid fibroblasts in the yield of X-ray or γ-ray induced single strand breaks (Vincent et al., 1975; Sheridan and Hueng, 1979; Fornace and Little, 1980; Harrihan et al., 1981; Painter, 1983) or double strand breaks (Lehmann and Stevens, 1977).

For analysis by the RMR model, we assumed that the lesions were qualitatively and quantitatively similar to those in T-1 cells; however, Ataxia cells had a faulty repair mechanism in the sense that most lesions were misrepaired, whereas in normal cells most lesions were eurepaired. Viewed in this light, the cross section for inactivation of Ataxia cells could be equated with the production of U lesions. We obtained a mathematical representation of these cross sections, and calculated the coefficients representing eurepair and misrepair in T-1 cells. The conclusions, discussed below, depend on the accuracy of our assumptions. Furcinitti (1983) reports that caffeine potentiates the effects of X rays on Ataxia cells. If this work is confirmed, it may be adviseable to reexamine the conclusions we have drawn based on the RMR model.

When we compared the Ataxia and T-1 survival curves with the RMR-track model, it became evident that T-1 cells can eurepair at least 14/15th of the U lesions produced by X rays. For heavy ions, it appears likely that the increase of Ataxia inactivation cross sections with increasing LET relates to the production of multiple U lesions along each track and the lack of ability to repair all of these lesions. Roots et al. (1979) have shown that T-1 cells repair strand breaks produced by heavy ions. From our survival data, we conclude that heavy-ion induced lesions are also effectively repaired in T-1 cells; however, when dealing with multiple lesions it becomes more probable to find that at least some of these lesions become misrepaired and thus kill the cells. We were able to calculate the single track survival probability for T-1 cells and found that at L = 300 keV/μm there is a 60% probability that one particle passing through the cell nucleus will kill a T-1 cell.

Other interesting suggestive results of the RMR model are the findings that U lesions produced in the presence of oxygen appear to be repaired less

efficiently in T-1 cells than U lesions produced under hypoxic conditions. This statement holds only if the oxygen effect for lesion production in Ataxia cells is lower than in T-1 cells. Part of the oxygen effect in T-1 cells could be expalined if the lesions produced in an hypoxic environment are repaired more efficiently than lesions produced in air. Palcic et al. (1982) demonstrated in Chinese hamster ovary cells that the oxygen effect is a function of X-ray dose. There is clearly need for additional research on the oxygen effect in T-1 cells and Ataxia cells.

Our results also suggest that there are two mechanisms for the production of U lesions: the first is proportional to the transferred energy density and is subject to the usual oxygen effect; the second at high L values is due to a higher order radical reaction and is proportional to about $L^{2.2}$ and also depends on the atomic number of the beam particles. Usually it is assumed that this damage component is independent of the oxygen effect; however, our results suggest that there might be a small oxygen effect in this second component. More accurate experimental work with Ataxia heterozygotes may further clarify some of these findings.

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GLOSSARY

U = uncommitted lesions

U_0 = initial U lesions at time t = 0

S, $S(m)$ = survival

D = dose

eurepair = perfect repair

misrepair = repair that has mistakes and an altered DNA structure

μ, $\mu(m)$ = repair ratio (rate of linear eurepair/rate of quadratic misrepair)

ϕ = fidelity of linear eurepair

λ = time rate of linear repair

L = LET (linear energy transfer)

m = mean number of initially uncommitted U lesions

k = time rate of quadratic (mis)repair

k_i, k_i^* = intratrack repair coefficients

k_e, k_e^* = intertrack repair coefficients

α = linear inactivation coefficient (linear-quadratic model)

β = quadratic inactivation coefficient (linear-quadratic model)

σ = LET dependent cross section for production of U lesions

n, n_0 = number of U lesions produced by a track of LET = L

F = fluence of heavy particles

Z = atomic number

P = an adjustable coefficient that relates to the amount of lesions from low LET fragments and delta rays in a mixed field

δ = σ/L = yield of U lesions per unit dose

θ = linear yield of U lesions in a track of LET = L

Ω = higher order yield of U lesions in a track of LET = L

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APPENDIX I:

RMR Model for Low LET

In developing the RMR model it was our intent to provide a flexible framework for the quantitative description of the processes that lead to the production of radiolesions, for the recognition of the presence of these lesions by cells, and for the enzymatic repair processes that act on them. A key to the development of this model was the realization that most of the expressed cellular biological effects appear after a delay, and that an external observer is not able to recognize the individual radiolesions in a single living cell without, in the process of observation, causing other lesions that might also be deleterious. These unobservable biochemical lesions are called uncommitted (U) lesions. Because ionization processes are so rapid, it is assumed that the U lesions originate in a time frame too short for recognition by living cells. Mammalian cells may take a millisecond or more to recognize these lesions and the elementary actions of repair that follow usually take 10^{-4} to 10^{-2} seconds for each step. There are probably several kinds of U lesions and also an assortment of different environmental agents can cause the same type of U lesions. For explaining the data in this paper, however, a single type of U lesion has been assumed.

Central to the RMR model is a description of the kinetics of the repair processes. For X-rays, we proposed two types of repair kinetics. The rate of the first one, "self repair," is proportional to the number of lesions present. The second type of kinetics assumes cooperative interaction between different lesions; in its simplest form it is proportional to the square of the lesions present. This repair, sometimes called quadratic repair, was

introduced because of the obvious analogy between the repair of DNA strand breaks and of chromosome breaks.

Both linear and quadratic repair might be imperfect repair. We introduced the terminology "eurepair" for repair that restores DNA to the true, original or perfect structure and coding; "misrepair" corresponds to an altered physical or chemical structure of the repaired DNA. The fidelity of self-repair, (ϕ), occurs explicitly in the survival equation. To derive survival probabilities one must also choose appropriate statistics. With the use of Poisson statistics, in the simplest case of a single type of U lesion, the probability of survival S is given by:

$$S = e^{-U_o} \cdot \left[1 + \frac{U \cdot \phi \cdot (1-e^{-\lambda t})}{\mu}\right]^{\mu} \qquad (1)$$

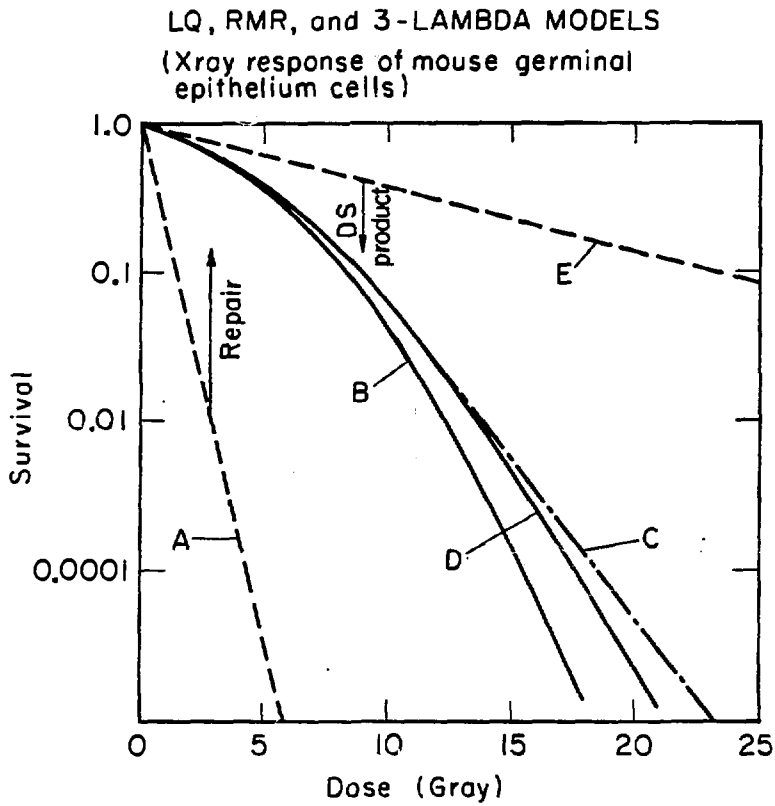
where U_o is the quantity of initial U lesions, which are produced as a function of dose and radiation quality; ϕ is the fidelity of linear self-repair; λ is the time rate; and μ is the repair ratio, the ratio of the rate of linear eurepair to the rate of quadratic misrepair. There is also a time factor: if the lethal effect must express itself prematurely, then survival can be modified with the time factor.

It is important to gain a qualitative understanding of the RMR survival equation. The first term on the right side of equation (1) is the lesion term: it is a simple negative exponential relationship representing the survival probability of cells that either do not repair at all, or cells in which all repair is lethal misrepair. The second term is the repair term: if there is repair, this term is greater than unity; if the rate of linear eurepair is much greater than the rate of quadratic misrepair then a shoulder appears on the dose-survival function.

Relationship to Other Models

There are well-defined relationships between the RMR model and several other known models of cellular inactivation by radiation (Tobias et al., 1983a), some of which are illustrated in Appendix Figure 1. The initial low-dose part of the "linear quadratic" survival equation can be exactly translated into the RMR model; at higher doses the RMR survival is higher than would be predicted from the usual linear quadratic equation. When a third order term is added to the linear-quadratic model, its survival predictions run above the RMR curve. Conceptually, however, there are important differences between the two models. The initial slope of the linear quadratic model is often regarded as the result of irreversible radiation injury, whereas in the RMR model the initial injury might be much greater, and the initial slope as well as the curvature of survival equations are due in great measure to repair and its fidelity.

A typical RMR survival curve will exactly superimpose for several log cycles over the survival predicted by the 3-λ model of Douglas and Fowler (1976), though they have very different conceptual origins. The RMR model also relates to the target theory: in the target theory a J hit survival curve usually means that cells survive 0,1,2 ... or J-1 hits, but die if they receive J or more hits. The RMR theory assigns a finite probability of survival for each class of 1,2 ... J hits. The recent LPL model of Curtis (1983) uses the process introduced by Tobias et al. (1980b), but adds a second class of irreparable U lesions as in the model of Pohlit and Heyder (1981). Albright has also approached the RMR model as a chain of Markov processes (1980).



APPENDIX FIGURE 1. Survival curves calculated by the linear quadratic (Chadwick and Leenhouts, 1973), RMR (Tobias et al., 1980b), and 3-λ (Douglas and Fowler, 1976) models. The RMR and 3-λ curves are essentially identical over 4 log cycles (Tobias et al., 1983a). The "lesion" term in the RMR survival equation appears as a dashed exponential line on the left, and repair increases the survival and alters the shape of the survival curve. There is a basic difference in the interpretation of survival curves by the linear quadratic and RMR models. In the linear-quadratic model of Chadwick and Leenhouts (1973) the upper dashed exponential curve is the initial lesion curve, and in the RMR model a reduction in survival is assumed to occur as a result of repair. (XBL 838-3970)

Tobias and Ngo (Tobias et al., 1980a; Ngo et al., 1981) derived a number of rules for interaction between modalities which are used by the RMR model for split dose experiments and for administration of two kinds of deleterious agents (which might make the same kind of U lesions) or different kinds and for different treatments (which influence the various repair mechanisms).