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RADIATION RESEARCH

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RADIATION RESEARCH 52, 88–98 (1972)

Survival Curves and Age Response Functions for Chinese Hamster Cells Exposed to X-Rays or High LET Alpha-Particles[1]

E. J. HALL, W. GROSS, R. F. DVORAK, A. M. KELLERER, AND H. H. ROSSI

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HALL, E. J., GROSS, W., DVORAK, R. F., KELLERER, A. M., AND ROSSI, H. H. Survival Curves and Age Response Functions for Chinese Hamster Cells Exposed to X-rays or High LET Alpha Particles. *Radiat. Res.* **52,** 88–98 (1972).

Chinese hamster cells were synchronized with hydroxyurea, and exposed to either 800 rads of x-rays, or 250 rads of alpha particles at different points of the cell cycle. The pattern of response to x-rays is similar to that previously reported, namely, an increase in radioresistance to a maximum in late S, followed by a sensitive period in the late G_2 and M phases of the cycle. The pattern of response to alpha particles is qualitatively very similar; the variation between most sensitive and most resistant phases of the cell cycle is less than in the case of x-rays, but is still appreciable. The alpha particles have a LET of approximately 90 keV/μm, and it was thought possible that the age response function would be flat for such densely ionizing radiation; however, this was not found to be the case.

INTRODUCTION

It has been known for some years that survival curves for mammalian cells exposed to high LET radiation, approximate exponential functions of dose (*1*). This finding contrasts sharply to the situation for low LET radiations for which survival curves can have a large initial shoulder. For many cell lines, and in particular for the Chinese hamster cell which has been studied in great detail by Sinclair and others (*2*), the sensitivity to x-rays varies as a function of age in the cell cycle, and much

[1] Based on work performed under Contract AT-(30-1)-2740 for the United States Atomic Energy Commission.

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of this variation is due to a change in the shoulder of the survival curve. Consequently it might be expected that the response to high LET radiations might show significantly less change through the cell cycle than the response for low LET radiation, since there is little or no shoulder in the first place. Sinclair (*3*) exposed synchronized Chinese hamster cells to fission neutrons from the Janus reactor, and indeed found that the response as a function of cell age varied less than for x-rays. However, there was still an appreciable variation through the cell cycle. Hall (*4*) conducted similar experiments with hamster cells using 14-MeV neutrons and also found that the pattern of response through the cycle was preserved, though it was less pronounced than in the case of x-rays. The neutrons used in both studies referred to above can be considered to be of intermediate LET.

Recently Bird and Burki (*5*) exposed synchronized Chinese hamster cells to three different beams of high-LET charged particles; carbon ions (190 keV/μm), neon (650 keV/μm), and argon ions (2000 keV/μm). In all cases, the radiation response did not vary with cell age in the mitotic cycle. Elkind (*6*) has recently discussed unpublished data of Scarsgard in which synchronized hamster cells were irradiated with boron ions at a LET of 127 keV/μm. These data indicate that an age variation still exists, although it is much smaller than in the case of x-rays. The present report describes experiments in which synchronized hamster cells were exposed to monoenergetic alpha particles with a LET of about 90 keV/μm; a quantitative comparison with the previous experimental results for various radiation qualities will be given.

The question of age response functions with high LET radiations is of interest in two connections. First, it has important implications in an understanding of the mechanisms of cell killing by ionizing radiations. Second, it may have some relevance to recent trends in radiotherapy, since high LET modalities are being actively considered as an alternative or supplement to x- and γ-rays. The bulk of clinical experience has been accumulated with sparsely ionizing radiations, where the response to a dose of radiation varies widely with the cell age. The effect of a fractionated regimen almost certainly involves partial synchronization of the exposed cell populations, although the situation is very complicated and poorly understood at the present time. If there is no dependence of sensitivity on age for high LET radiations, this would be yet another altered variable when those radiations are used instead of x- or γ-rays, and possible changes in fractionation patterns may be desired.

MATERIALS AND METHODS

Culture of the Cells

V79 Chinese hamster cells were used, the original culture was supplied by Dr. M. M. Elkind of the Brookhaven National Laboratory. The cells were grown in nutrient medium F10 (*7*) supplemented with 10% fetal calf serum. The day before an

irradiation experiment, cells from a partly confluent actively growing stock culture were prepared into a single cell suspension by conventional methods (*8*), and plated out into Falcon plastic flasks for irradiation, or into specially constructed containers with a thin Mylar window for exposure to alpha particles. These special containers consist of a cylinder of stainless steel, with entry and exit ports for medium and gas; thin sheets of Mylar (0.82 mg/cm^2) were stretched over the end of this cylinder and held in tension by means of stainless-steel end-plates.

After overnight attachment for about 17 h, asynchronous populations of cells were irradiated with 210-kV x-rays or with alpha particles. At the time of irradiation, parallel cultures were fixed and stained and the mean number of cells per microcolony (the multiplicity) was determined under a low-power microscope.

To obtain a synchronized population cells were allowed to attach overnight, after which hydroxyurea (H.U.) was added to the growth medium to a final concentration of 1.25 m*M* and allowed to remain in contact with the cells for 3½ h. The medium containing the H.U. was then removed, the cells were washed, and fresh growth medium added. Hydroxyurea blocks DNA synthesis while allowing protein and RNA synthesis to continue in a normal fashion. In the concentration used, it is toxic to hamster cells synthesizing DNA (S cells) while allowing free passage of cells in the remaining phases of the cycle to the G_1/S interface where they are blocked. When the drug is removed, a wave of cells passes into S and constitutes a synchronized population (*9–11*). The cell cultures were then returned to the 37° C incubator and after various time intervals, as the cells moved through their mitotic cycle, samples were exposed to x-rays or alpha particles.

Methods of Irradiation

The source of x-rays was a 210-kV therapy machine, which at a treatment distance of 50 cm, produced a dose-rate of 70 rads/min. To expose cells to alpha particles of reasonably uniform LET, a special source was prepared which is illustrated in Fig. 1. The active deposit from ^{220}Em is plated on a pin in a device described in a previous report (*12*). This mixture of nuclides which has a half-life of 10.6 h, emits alpha particles at two energies, 8.78 and 6.07 MeV, but the path from the source to the cells is designed to stop completely alpha particles of the lower energy. This path (see Fig. 1) is 11.6 cm overall, a distance required in order to achieve acceptable uniformity of dose to the cells which are attached in a monolayer to a thin disc of Mylar 4.1 cm in diameter. This path consists of four parts. The first, inside the body of the irradiator, is through helium at atmospheric pressure and has a thickness of about 1.7 mg/cm^2. Next comes a Mylar window of 1.65 mg/cm^2, then 0.69 cm of air at atmospheric pressure (0.73 mg/cm^2), and finally 0.82 mg/cm^2 of Mylar, which constitutes the surface to which the cells are attached. The material between source and cells is equivalent to 4.5 mg/cm^2 of tissue. This is sufficient to absorb completely the 6.07-MeV alpha particle the range of which is 4.4 mg/cm^2.

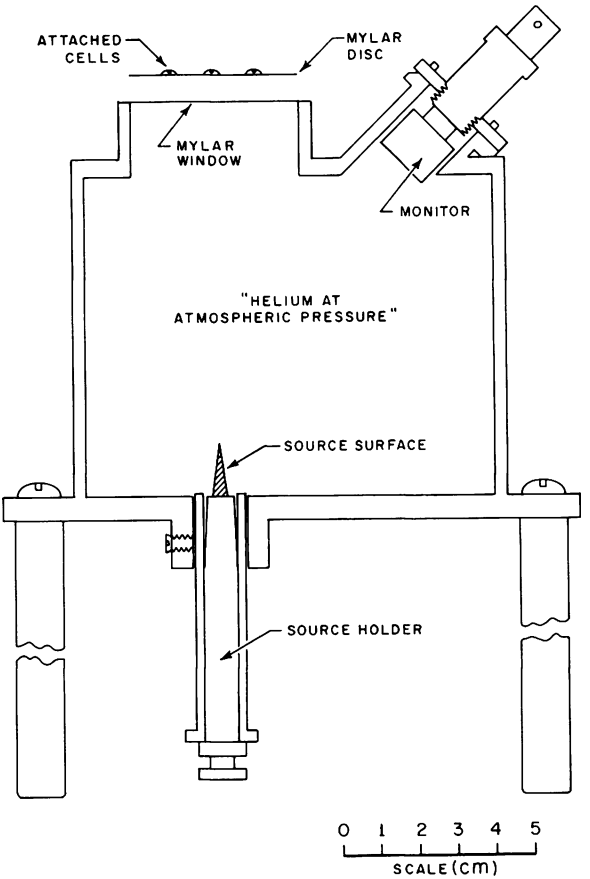

FIG. 1. Arrangement for irradiating cells with alpha particles. The radioactive isotope (^{220}Em) is plated on the conical pin. This nuclide emits alpha particles at two energies—8.78 and 6.07 MeV. The cells grow attached to a Mylar disc. The path between the source and the cells consists of helium, two thicknesses of Mylar, and a small air gap. This pathlength is sufficient to filter out the lower energy alpha particles before they reach the cells.

The spectrum of alpha particles which reach the position occupied by the mammalian cells is shown in Fig. 2. This was obtained with a lithium-drifted silicon solid-state detector and multichannel analyzer. It is of interest to note that, while there is a small spread due to straggling, the beam is still very nearly monoenergetic. In addition, the remaining energy (5.2 MeV) is sufficient to permit the alpha particles to traverse the thickness of the cells, which is not more than 10 microns. Over this distance the LET increases from 96 to 117 KeV/μm. This insures a reasonably uniform dose throughout the cells.

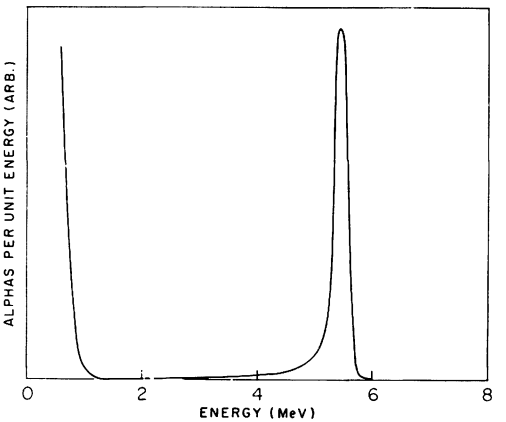

FIG. 2. Spectrum of alpha particles reaching the position occupied by the cultured mammalian cells.

The activity on the source pin has been found to be about 0.57 mCi after a plating period of 64 h. This activity results in a dose rate of 7.1 rads/min as measured with an extrapolation ionization chamber, and also computed from the fluence as measured with the solid-state detector. The contribution to the total dose by beta and gamma rays has also been determined with the ionization chamber and amounts to less than 3.5%. Another silicon solid-state detector, shown in Fig. 1, is incorporated into the body of the irradiator as a dose monitor. This detector was calibrated when the alpha dose was measured, and serves as a monitor for all cell irradiations. Its calibration is 3160 counts per rad and by appropriate calculation the dose to be delivered is transformed to total counts. These are displayed on a scaler and alleviate the need for any decay corrections.

RESULTS

Three experiments were performed in which asynchronous cells, after overnight attachment, were irradiated with a range of doses of either alpha particles or 210-kV x-rays. The data from the three experiments are shown in Fig. 3; the lines have been fitted by eye. For x-rays, the survival curve has an appreciable and unmistakable shoulder, followed on this semilogarithmic plot by an essentially straight portion. By contrast, the survival curve for alpha particles follows an exponential dependence on dose; there is no indication of an initial shoulder.

Figure 4 illustrates the results of a typical experiment in which cells which had been synchronized by treatment with H.U. were irradiated with either 800 rads of x-rays or 250 rads of alpha particles, at various times after removal of the H.U. For x-rays the pattern of survival is very similar to that previously reported (*9*, *10*). H.U. blocks cells at the G_1/S interface. When the drug is removed the cells

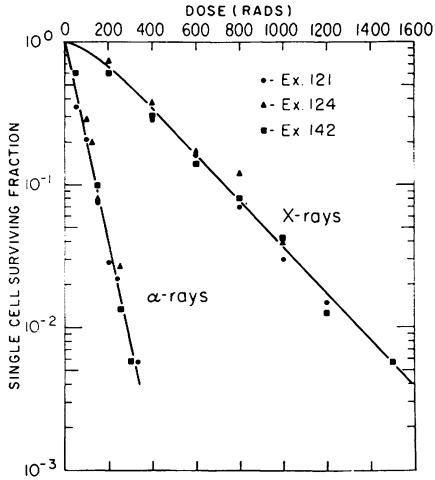

FIG. 3. Survival curves for asynchronous Chinese hamster cells exposed to 210-kV x-rays or alpha particles. The lines were fitted by eye through data from three repeat experiments.

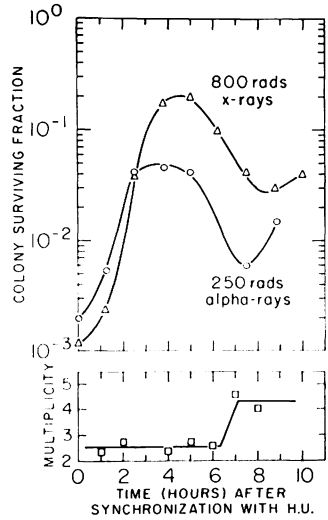

FIG. 4. Top panel: Response of cells to a dose of either 800 rads of x-rays or 250 rads of alpha particles, as a function of the time after synchronization with hydroxyurea. Lower panel: The multiplicity, or average number of cells per microcolony, as a function of time after synchronization.

move into S and become progressively more resistant; maximum resistance is observed in late S, this is followed by an increase in sensitivity as the cells progress through G_2 into mitosis. For alpha particles the change of radiosensitivity as the cells move through the cell cycle is qualitatively similar to that for x-rays, but the extent of the variation is less.

COMPARISON WITH PREVIOUS EXPERIMENTAL FINDINGS

A comparison of experimental data on the variation of sensitivity throughout the cell cycle has been given by Bird and Burki (*5*), who plotted "survival variation in life cycle compared with x-rays" as a function of LET. Although no explicit exposition of the meaning of this quantity is given we infer from the discussion that this parameter is defined as

$$V' = \frac{R(D)/S(D)}{R_x(D_x)/S_x(D_x)},$$

where $R(D)/S(D)$ is the ratio of the surviving fractions of the most resistant to the most sensitive subpopulations at dose D of the test radiation and $R_x(D_x)/S_x(D_x)$ is the corresponding ratio for a dose D_x of x-rays. In particular, Bird and Burki chose doses of the two types of radiation to produce equal survival level for the most sensitive cells so that $S(D) = S_x(D_x)$. Then one has

$$V' = \frac{R(D)}{R_x(D_x)}.$$

The disadvantage of this parameter is that it is dose dependent even for high LET where the survival curves are approximately exponential. It is, therefore, practical to choose a modified parameter which will be designated by V:

$$V = 1 - \frac{\ln R(D)}{\ln S(D)}.$$

If the survival curves for the sensitive and the resistant subpopulations are exponential viz:

$$S(D) = e^{-\alpha_s D}$$

$$R(D) = e^{-\alpha_r D}$$

Then:

$$V = 1 - \frac{\alpha_r}{\alpha_S}.$$

The parameter V can assume values between 1 and 0, and in this special case in which resistant and sensitive subpopulations exhibit exponential survival curves it is independent of dose.

In the general case where survival curves have a shoulder, V is dose dependent. If one assumes that, as commonly observed, the resistant subpopulation has a larger shoulder than the sensitive fraction, one must expect that the values of V are largest for smallest doses or highest survival. It is, therefore, necessary to specify the dose, or the survival level, for any value of V. Table I gives a compilation of V, together with the survival rates in the G_1-phase. In addition, the values of LET are given. This poses no difficulty in those cases in which the experiments were performed with monoenergetic charged particle beams. For other radiations, one must give an average value of LET. It has been shown (*13*) that the relevant average is the dose mean LET ($\bar{L}_D$); accordingly this quantity is also given in Table I, for both x-rays and neutrons. For a comparison with the plot given by Bird and Burki (*5*), one must note that these authors have used track average LET ($\bar{L}_T$) for 14-MeV neutrons; this value is smaller than $\bar{L}_D$ by a factor of 7.

From a consideration of Table I it would appear that the relative variation of sensitivity throughout the cell cycle is not clearly dependent on LET in any systematic way. Indeed a consistent functional dependence on LET is not to be expected, even in the case of the track segment method, because particles of different

TABLE I

COMPARISON OF SYNCHRONY DATA FROM VARIOUS AUTHORS

Author and reference	*Type of radiation*	*Dose (rads)*	S_s	$\frac{ln\,R(D)}{ln\,S(D)}$	$V = 1 - \frac{ln\,R(D)}{ln\,S(D)}$	$\rho = \frac{V}{V_x}$	$\frac{LET^a}{(keV/\mu m)}$
Sinclair (*3*)	250-kV x-rays	710	0.018	.437	.563		
	Janus fission	125	.2	.715	.285	.506	
	neutrons	225	.03	.63	.37	.655	~85
		325	.007	.712	.288	.512	
Hall (*4*)	250-kV x-rays	800	0.056	0.50	0.50		
	14-MeV neutrons	500	0.042	0.67	0.33	0.66	94
Skarsgard cit. Elkind (*6*)	Boron ions	380	.012	0.72	0.28		127
Bird and Burki (*5*)	145-kV x-rays	950	0.025	0.625	0.375		
	carbon ions	250	.032	1.0	0	0	190
	neon ions			1.0	0	0	650
	argon ions			1.0	0	0	2000
Present authors	210 kV x-rays	800	.0013	.23	0.77		
	α-rays	250	.0015	.49	0.52	0.67	105

[a] For monoenergetic particles the quantity used is L_∞ ; in the case of the neutrons the dose average LET is quoted.

charge and different velocity can produce the same value of $\bar{L}_D$. Such particles can have different radial profiles of energy deposition around their tracks. Microdosimetric considerations show that, the use of $\bar{L}_D$ as an indicator of radiation quality is even more seriously limited for a mixed radiation field.

Another complication in the attempt to find a functional dependence of sensitivity variation on LET, results from the fact that different experiments are not always strictly comparable, because the experimentally observed values of V are usually less than maximal because of imperfect synchronization of the cells. This accounts in part for the differences in the various observed values of V for x-rays given in Table I. For this reason it is practical to normalize the parameters V relative to the value for x-rays obtained in the same experiment. Owing to the limitation of the experimental data, one must be content with an approximate comparison, and for this reason the parameter

$$\rho = \frac{V}{V_x}$$

is also listed in Table I. Because V_x is expected to be largest at highest survival levels, ρ must be smallest at highest survival levels. For each value of ρ, the survival level for G_1 cells exposed to x-rays is given (S_s).

It should be noted that the limitations of the comparison in Table I are due mainly to the fact that the change of the survival curve with cell age cannot be described realistically by the variation of one parameter. However, in most cases an analysis in terms of several parameters cannot be performed, because the survival throughout the cell cycle has been determined at only a limited number of dose levels.

The survival curve for an asynchronous cell population exposed to α-particles is observed to be an exponential function of dose within experimental uncertainty (see Fig. 3). This result might be considered to be surprising since the survival curves for synchronized cells in various phases of the cycle each appear to be exponential (*5*). The curve for asynchronous cells is a composite of those for the various phases of the cycle. Superposition of exponential curves of different slope results in a curve which is concave from above. Accordingly the survival curve for asynchronous cells would have to be nonexponential.

A quantitative evaluation shows, however, that the deviation from the exponential survival cannot be expected to be observed experimentally. Figure 5 shows an estimate of the cellular sensitivity throughout the cell cycle which would result if the curve in Fig. 4 were corrected for increase in cellular multiplicity and the loss of synchronization with cell age. If one uses this curve and assumes that at any given cell age survival is exponential, one can calculate the resulting curve for a nonsynchronized population in logarithmic growth. The result is shown in Fig. 6 as a solid curve. One notes that the curve indeed deviates from the exponential shape. Despite the fact that the slope of the curve decreases by about a factor of 2 with increasing

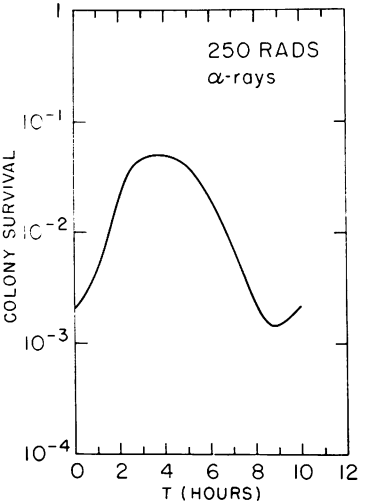

FIG. 5. Survival of synchronized Chinese hamster cells exposed to 250 rads of α-particles at different times after synchronization. The curve represents an estimate of the data in Fig. 4 corrected for multiplicity increase and loss of synchrony with time.

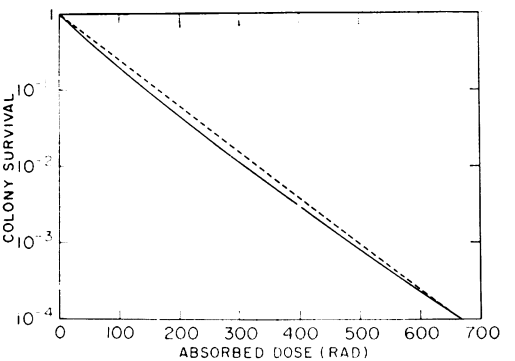

FIG. 6. Survival curve (solid line) for a nonsynchronized population in logarithmic growth under the assumption that the survival curves for all individual cell ages are exponential. The broken line is inserted to indicate the deviations from an exponential function.

dose, the deviation from the exponential shape is certainly within the limits of experimental accuracy. This is indicated by a comparison with the exponential function represented by the broken line. This consideration also implies that there may be cumulative damage which is masked by the limited experimental accuracy and the sensitivity variations.

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