## Title

Relative Radiosensitivities of Tetraploid and Diploid Chinese Hamster Cells in Culture Exposed to Ionizing Radiation



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# RELATIVE RADIOSENSITIVITIES OF TETRAPLOID AND DIPLOID CHINESE HAMSTER CELLS IN CULTURE EXPOSED TO IONIZING RADIATION

by

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## DISCLAIMER

# RELATIVE RADIOSENSITIVITIES OF TETRAPLOID AND DIPLOID CHINESE HAMSTER CELLS IN CULTURE EXPOSED TO IONIZING RADIATION

There exists a great deal of evidence that suggests that damage to DNA in mammalian cells is an important event in the sequence of events which lead to radiation induced reproductive death in mammalian cells[13]. In the Chinese hamster cells in culture much recent evidence points out that chromosome damage also plays perhaps the most important role in actual reproductive death in manifestations of visible chromosome aberrations[4,5,6]. Actually what mechanisms occur to transform DNA damage into chromosome damage are not yet clear.

If radiation induced reproductive death is induced by genetic or chromosomal damage, then one would expect that a change in ploidy would have a marked effect on the radiation response of the system in which it occurred.

The effects of ploidy on radiosensitivity of systems other than mammalian cells have been studied[10, 11, 17]. However the results appear to be contrary to each other. The results of ploidy experiments using mammalian cells appear also to be contrary to each other. In a variety of individual clones of aneuploid mouse cells having chromosome numbers from 51 to 109 Till[18] found no difference in radiosensitivity in terms of extrapolation number or $D_o$ dose. Sinclair[16] looked at a large number of cloned Chinese hamster cells with and without irradiation of 1500 rads and found no obvious relationship between isolated clones and the cell ploidy. Lockhard et al[9]

looked at HeLa cell clones and also found no obvious relationship between chromosome number and radiosensitivity. These results all suggested to Elkind and Whitmore[7] "that either the right measurements have not yet been made with mammalian cells..., or that in mammalian cells chromosome damage is not closely related to lethality."

The practical importance of the presence of higher ploidy cells in human tumours suggested to Slini and Hornsey[15] and to Berry[3] that the question of sensitivity to radiation and ploidy be studied using in vivo assay systems. Both experiments indicated a similar $D_o$ in hypertetraploid and hyperdiploid cells. However, the extrapolation number was increased in hypertetraploid over hyperdiploid cells. These results are consistent with recessive lethals being involved in cell killing such as chromatin loss and point mutations[7].

Bedford and Hall[1] were able to clearly demonstrate an increased extrapolation number in the survival curves of tetraploid Chinese hamster cells which arose spontaneously from a clone which was almost diploid (23 chromosomes). In addition they were able to demonstrate increased Elkind Sutton recovery in tetraploid cells compared to hyperdiploid controls. Also they observed a small decrease in $D_o$ for tetraploid cells which was statisitically significant from control values. In view of the importance of this type of experiment in the interpretation of damage leading to reproductive death we decided to repeat their experiment using colcemid induced tetraploids and a greater number of high and low dose survival points. This correspondence confirms and expands the results of Bedford and Hall[1] in that both the extrapolation number changes and the $D_o$ changes when two ploidies of hamster cells in culture are compared.

## Materials and Methods

The initial pseudodiploid line of Chinese hamster cells V79-S171 was kindly provided by a gift of W. Sinclair. This cell line has a modal chromosome number of 23 chromosomes. The culture medium is the same as described by Sinclair[16] and has a population doubling time of 10 hours in our laboratory conditions.

Tetraploid cells were obtained by growing a cloned cell of the V79 cells containing 22 chromosomes in the presence of colcemid (0.037 micrograms/ml). Colcemid was added for 12-24 hours after which the cells were trypsinized and plated into fresh media in 30 ml falcon tissue culture flasks. After 7-8 days colonies were selected and removed and placed into a second falcon flask. Karyotypes were made for each of the clones selected in this fashion and for this study a line with 44 chromosomes and a population doubling time the same as the pseudodiploid cells was chosen. One week after clone selection the cells were grown in mass culture, and frozen slowly to liquid nitrogen in a solution 10% DMSO medium (vol/vol) and stored for future use. This storage may be necessary since there are known changes in karyotype which may occur when lines are cultured continuously. The tetraploid used in this study was quite stable with no changes observed in the eight weeks this clone was grown continuously.

An attempt was made to reiterate the procedure above and make several octoploid cell lines. The procedure was successful; however these octoploid cells were not stable for a period long enough for us to complete radiation studies. The octoploids were found to revert to tetraploids. Additional

studies were done using tetraploid cells which came about from an octoploid with the same population doubling times as the original diploid cells.

Tetraploid cells or hyperdiploid cells from overnight cultures were gently trypsinized using 0.03% trypsin solution for 5 minutes. Clumps of cells were removed by gently pipeting and corresponding dilutions for the survival curves made. When the cells had regained exponential growth as determined from growth curve experiments (2-3 hours in normal V79 and 4-6 hours in the tetraploid case) the cells were irradiated at 153 rads/min with 145 kvp X-rays in the presence of an air curtain incubator at 37 degrees centigrade. The X-rays were filtered with 1 mm Al and the half value thickness was 2 mm Al. Irradiation was at a distance of 39 cm. Dosimetry was performed using Fricke dosimetry solution.

After irradiation the cells were incubated for 7-10 days at 37 degrees centigrade in order to form visible colonies. It was found more reproducable to stain the colonies formed by the tetraploid cells with Bouins fixative than the usual methylene blue staining method. The plating efficiency of the unirradiated tetraploid cells was $48\pm13\%$ and the hyperdiploid controls $76\pm11\%$ (standard deviation is based on 12 experiments).

Data from these experiments werefitted to the single hit multi target model of mammalian cell survival by a linear least squares computer method.

## Results and Discussion

The number of metaphase chromosomes in each of 100 cells was determined for the two strains used in this study during the course of the experiments. The results for one of the tetraploid strains used are shown in Figure One

together with the corresponding hyperdiploid strain V79-S171. The modal chromosome numbers are 44 and 23 respectively. The second tetraploid strain also had a modal chromosome number of 44. The growth curves of these strains indicated a population doubling time of 10+1 hour in all of the strains used. The population doubling times were kept constant because a change in the population doubling time may affect the percent of cells in the various cell stages thus directly affecting the radiosensitivity of the cell population. When the tetraploid and the hyperdiploid cells were irradiated to determine the characteristic survival curves the results shown in Figure Two were obtained. These results are pooled data from six independent experiments. In four of the experiments one strain of tetraploid was used and in two of the experiments a different strain was used. The survival curve parameters determined from the least squares fit of all of the data are: tetraploid cells, N=19, $D_o$=127; hyperdiploids, N=4.3, $D_o$=154. These curves demonstrate a crossover at approximately 600 rads, i.e. at dose less than 600 rads the tetraploids are more resistant, while the opposite is the case at higher doses.

These results are in general agreement with the results of Bedford and Hall[1] who saw a slightly smaller increase in slope of the survival curves as the ploidy increased in cultured Chinese hamster overy cells. They also show the same direction of change in radiosensitivity between 2n and 4n cells as seen by Mortimer[10, 11] in the yeast Saccharomyces cerevisiae.

These results suggest that genetic damage is important in the events which lead to reproductive death in mammalian cells, since there is a variable relationship between radiosensitivity expressed in terms of

$(1/D_o)$[13] and the ploidy of cultured Chinese hamster cells. The degree of change in the slope of the survival curve is similar to that which is found in yeast after a change from diploid to tetraploid. The recent evidence, accumulated by Dewey et al [5,6], Grote[8] and Caranno[4] demonstrating a quite close similarity between visible chromosomal aberrations and radiation induced reproductive death in cultured mammalian cells, suggests that the reason for the increased radiosensitivity of tetraploid cells might be due to the increased efficiency of producing lethal chromosomal aberrations in tetraploid cells.

If this is the case how does one explain the results which we obtain at lower doses? One would expect that the chromosomal aberrations produced be the same per haploid set regardless of ploidy at lower doses of radiation and very low dose rates. Grote[8] has recently found that a tetraploid strain of Syrian hamster cells exhibited the same survival after 150 rads of X-rays in the G1 stage as their tetraploid counterparts while the number of total chromosomal aberrations increased twofold. Grote's[8] results tend to point out that tetraploid cells can most likely withstand more genetic loss from deletions than their diploid counterparts. Thus at low doses diploid cells might possess a greater probability of incurring lethal damage than the tetraploid cells.

The relative resistance of tetraploid cells compared to hyperdiploids might continue to be the same at higher doses if it were not for the increasing importance of exchange aberrations. Wolff and Bender[19] have reviewed much data that indicate that exchange aberrations increase approximately as the square of the dose up to a point of saturation, while deletions increase approximately as a linear function of radiation dose.

It is plausible then that the crossover observed in the survival curves might represent the differential saturation of asymmetrical exchange aberrations in diploid cells compared to their tetraploid counterparts. The point at which the tetraploid cells are equally likely to sustain a lethal chromosomal event as the diploid cells would be 600 rads on these experiments.

At doses beyond 600 rads, the total number of lethal chromosomal aberrations per cell (asymmetrical exchanges plus deletions) should increase less rapidly in diploid cells compared to tetraploid cells. The results of Norman and Sasaki[12] support this argument in that they seem to imply that saturation of asymmetrical exchanges is expected to occur at a higher exchange frequency with increasing chromosome number. The net result of this differential accumulation of damage should be to deplete the irradiated population of those cells without asymmetrical exchanges and deletions in the tetraploid cell population at a final greater rate than the final rate in the diploid population. Consequently the tetraploid cells would show a decreased survival when compared to hyperdiploid cells at larger doses if chromosome aberrations were important in reproductive death.

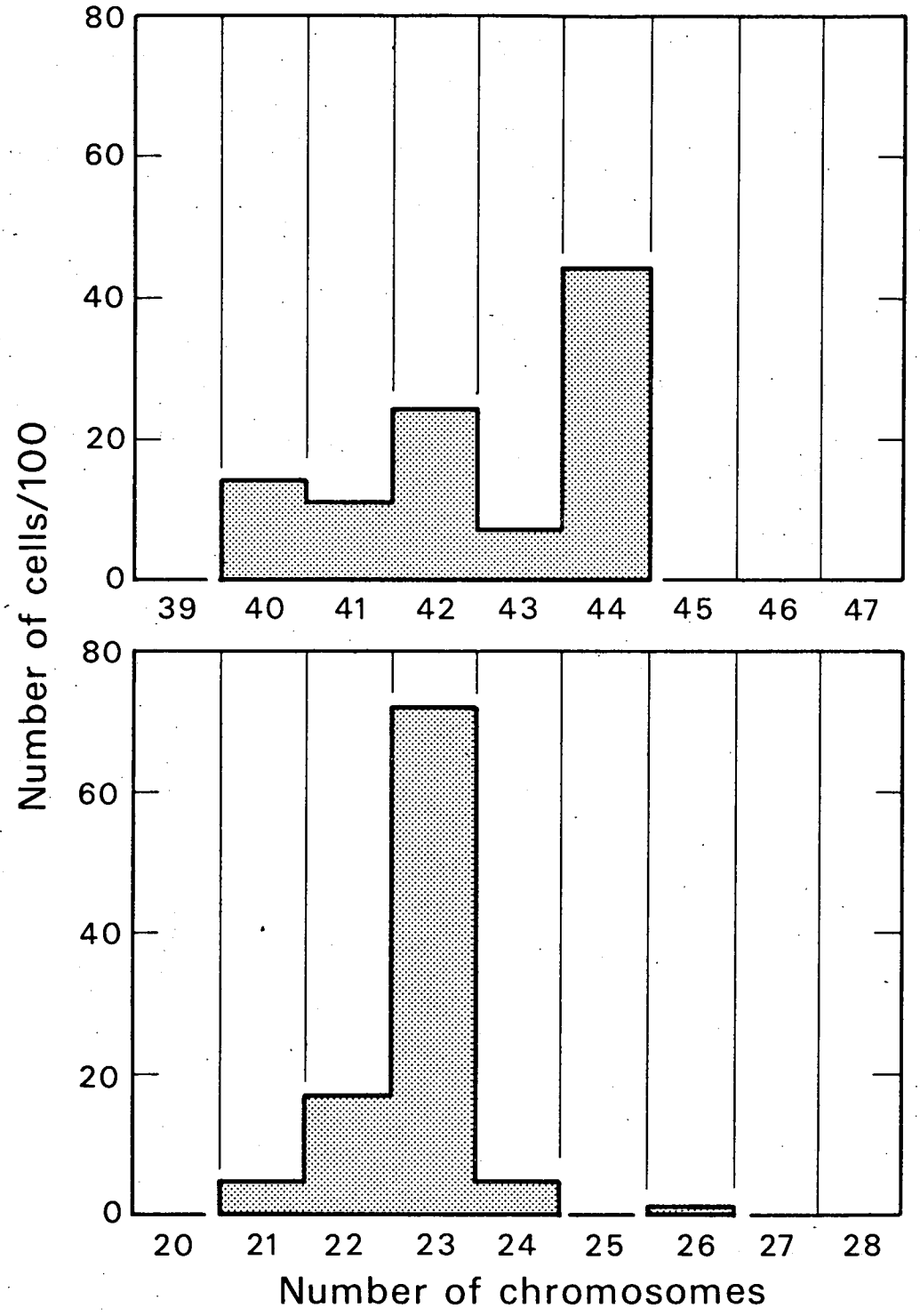
Another interesting aspect of the survival of tetraploid cells compared to hyperdiploid cells, which might help to explain the results of this experiment, is the possible relative importance of dose rate or dose fractionation on the survival curves. Bedford and Hall[1] showed data which indicated an increased capacity for Elkind-Sutton recovery in tetraploid Chinese hamster cells over hyperdiploid ovary cells. This type of recovery is reflected in hamster cell survival data by an increased extraplation number which we also obtained in our experiments. It is well known that as the dose

rate of ionizing radiation administered to mammalian cells is greatly lowered the survival curves become single hit with increased $D_o$ and much reduced extrapolation numbers. The slopes of the survival curves approach those obtained in the shoulder region of the survival curves. We would expect that if the survival curves of tetraploid and diploid cells were compared at dose rates of around 1.5 rads per minute or less that recovery mechanisms would make the tetraploids more resistant than the hyperdiploids similar to our acute low dose results, thus giving data similar to that found in plant studies by Sparrow et al[17]. In these chronic irradiation studies the tetraploids would be expected therefore to be more resistant than their hyperdiploid counterparts.

## FIGURE LEGENDS

FIG. I. A. Histogram of chromosome number of 100 cells from one induced tetraploid strain of V79-S171 cells. B. Histogram of chromosome numbers of 100 cells in V79-S171 hyperdiploid cell line.

FIG. II. Survival of tetraploid and hyperdiploid Chinese hamster cells as a function of radiation dose. Closed circles are hyperdiploid cells; open circles tetraploid cells. Means $\pm$ standard errors for six experiments.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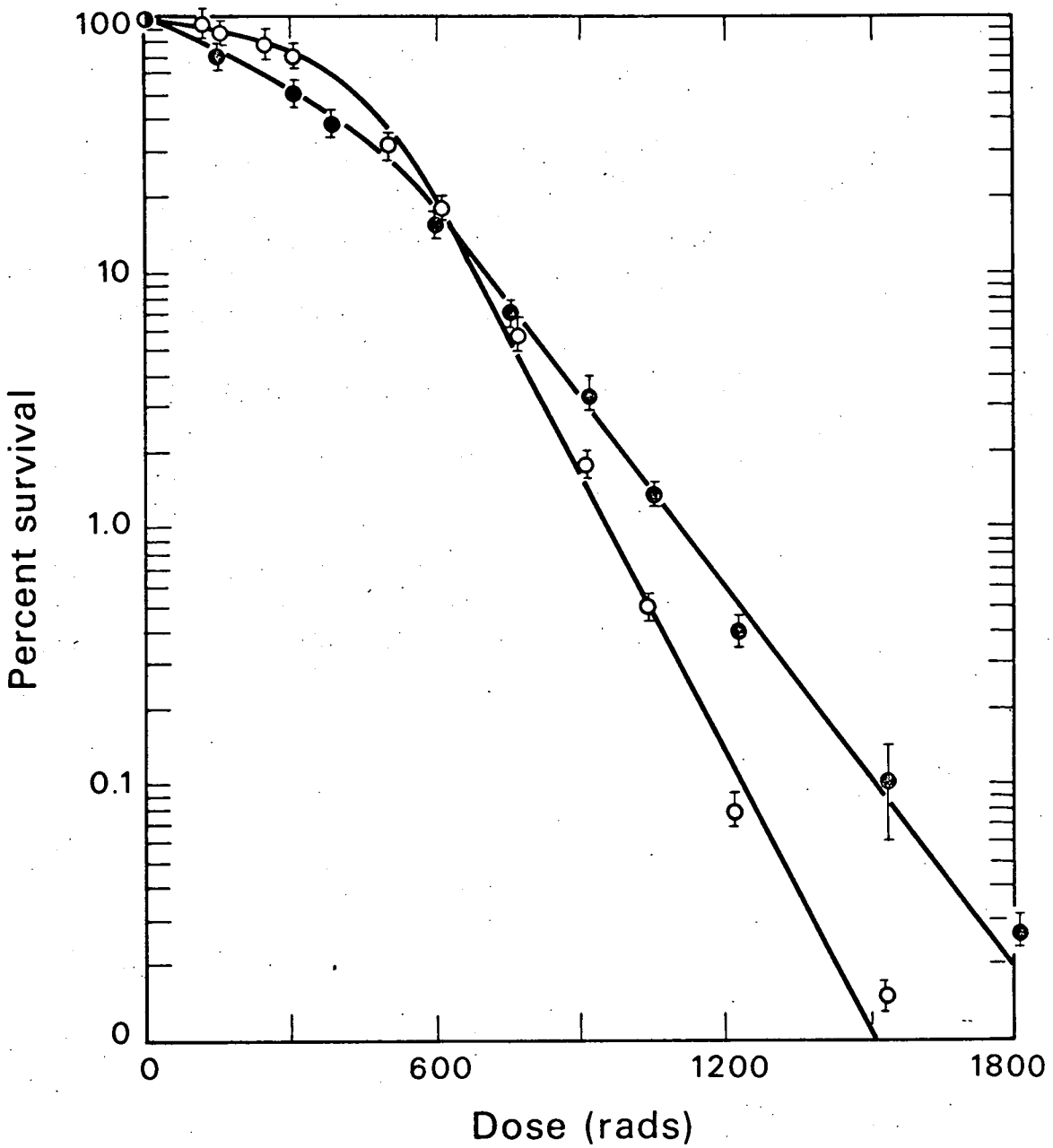


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