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# MORPHOLOGICAL CHARACTERIZATION OF THE RADIATION SENSITIVE CELL LINE, XRS-5

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## Abstract

Morphometric analysis was performed on the radiation sensitive Chinese hamster ovary (CHO) xrs-5 cell line, reverting xrs-5 cells and parental K1 cells. Several ultrastructural parameters (increased nuclear envelope membrane separation, cell and nuclear volume, nuclear to cytoplasmic ratio, and the nuclear surface area per unit volume of the cell) were measured and correlated with radiation sensitivity. A trend in increased cell size and radiosensitivity was observed. However, only the substantially increased nuclear envelope membrane separation in sensitive xrs-5 cells significantly correlated with radiation sensitivity. The maximal nuclear envelope membrane separation in sensitive xrs-5 cells was 270.8 nm. The maximal K1 cell nuclear envelope membrane separation was 134.8 nm, although, on average the K1 cell nuclear envelope membrane separation was 36.8 nm. The reverted xrs-5 cells had a smaller nuclear envelope membrane separation (maximal 83.6 nm), but the measured space did not completely revert to that for K1 cells. Therefore, we conclude that the nuclear envelope membrane separation is correlated with radiation sensitivity of xrs-5 cells, but it cannot be considered as the only defect correlatable with the radiation sensitivity.

**Key Words:** Nuclear ultrastructure, morphometry, radiation sensitive cells, CHO K1 cells, CHO xrs-5 cells.

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## Introduction

In the early 1980's, Jeggo *et al.* (1982) isolated various strains of mutants from the Chinese hamster ovary (CHO) cell line which were cross-sensitive to DNA damaging agents, such as ionizing radiation. The CHO cell lines have the benefit of being functionally hemizygous so the repair deficient mutants are expected to be mainly recessive. This factor makes the CHO mutants a desirable cell line for research in DNA damage and repair. Seven X-ray sensitive (xrs) strains were isolated, the most sensitive of which is the xrs-5 cell line, which is defective in the repair of DNA double strand breaks (Jeggo and Kemp, 1983).

Ultrastructural examination of xrs-5 cells show distinct morphological irregularities in the nuclear envelope (Yasui *et al.*, 1991). Other morphological differences noted (Yasui *et al.*, 1991) include cytoplasmic features: mitochondria in xrs-5 cells appear more dense and have non-parallel cristae (as compared to K1 cell mitochondria), there are several areas where the membrane of the endoplasmic reticulum is separated, creating cisternae, and there is an increase in the number and size of vesicles in xrs-5 cells. Also, cell size appears to be different between the two cell lines. Xrs-5 nuclei appear irregularly shaped (they have many invaginations and evaginations), and the cells appear larger.

Nuclear organization of K1 and xrs-5 cells also differ (Yasui *et al.*, 1991). Subtle differences in the distribution and staining density of heterochromatin along the nuclear periphery are found in xrs-5 cells. The heterochromatin in xrs-5 cells is more dispersed, although no differences in euchromatin or the nucleoli organization are apparent.

Several morphological parameters have been proposed to affect a cell's sensitivity to ionizing radiation, including cell size (Suciu, 1985, 1986) and the nuclear to cytoplasmic ratio (Arena, 1971). Suciu (1985, 1986) postulates that a larger cell size implies decreased radiation sensitivity and Arena (1971) postulates that a larger nuclear to cytoplasmic ratio implies increased sensitivity. Previously, we noted that xrs-5 cell radiosensitivity

reverted with increasing time in tissue culture and, concurrently, the irregularities in the perinuclear space disappeared in reverted xrs-5 cells (Yasui *et al.*, 1991). To determine if the increased nuclear envelope membrane separation correlated with radiation sensitivity, we analyzed several groups of xrs-5 cells that were in various stages of reversion. We also included several other ultrastructural parameters (cell and nuclear volume, nuclear to cytoplasmic ratio, and the surface area per unit volume of the cell) to measure and correlate with radiation sensitivity. Morphometric techniques of stereology (Weibel, 1979) were used to quantitatively describe our previous qualitative observations (Yasui *et al.*, 1991).

## Materials and Methods

### Cell maintenance

K1 and xrs-5 cells were maintained as sub-confluent cultures in McCoy's 5A medium, supplemented with 10% fetal bovine serum, at 37°C and 5% $CO_2$.

### Radiation sensitivity

K1 and xrs-5 cells were both collected by trypsinization [0.25% trypsin + 0.27 mM ethylenediaminetetraacetic acid (EDTA) in phosphate-buffered saline without $Ca^{2+}$ or $Mg^{2+}$) and irradiated in suspension in McCoy's 5A medium with 10% FBS. A $^{137}Cs$ source was used, at a dose rate of 330 cGy/min. Three replicates were plated for each dose and the plates were maintained at 37°C and 5% $CO_2$. After a 7 day incubation, the cells were stained with 2% crystal violet in absolute methanol. Colonies containing 50 cells or more were considered a survivor.

While the radiation sensitivity of K1 cells remains constant over time, the radiation sensitivity of xrs-5 cells reverts back to the K1 cell sensitivity after a period of time in culture (Denekemp *et al.*, 1989; Nagasawa *et al.*, 1989; Yasui *et al.*, 1991). This reversion has been noted after 6 to 8 weeks in culture (Nagasawa *et al.*, 1989; Yasui *et al.*, 1991), although this time may vary (own observations). The rate of reversion seems to be dependant upon culture density, serum type and concentration and handling of cells (own data). If the xrs-5 cells reached a high density, their radiation sensitivity increased and their reversion process seemed to be inhibited (own observation).

### Transmission electron microscopy (TEM)

Both K1 and xrs-5 cells were collected by trypsinization (0.25% trypsin + 0.27 mM EDTA in phosphate-buffered saline without $Ca^{2+}$ or $Mg^{2+}$) from monolayer culture, centrifuged, and washed twice in a Sorensen's Na-K phosphate buffer. After the cells were washed, they were primarily processed for TEM with a 1.5% solution of glutaraldehyde in buffer (Sorensen's Na-K buffer was used throughout this procedure) for 1 to 2 hours, at 4°C. After this time, the cells were washed with buffer, resuspended in a 1% osmium tetroxide solution for 1 hour at 4°C. The cells were transferred to 70% acetone and left overnight. The cell samples were dehydrated using an acetone series and then the cells were infiltrated with Spurr epoxy resin, placed in BEEM capsules and the resin polymerized at 70°C for 16-18 hours.

Four separate fixations were performed. In 3 of the experiments both K1 and xrs-5 cells were processed for TEM. In the fourth experiment, only xrs-5 cells were processed for TEM. Both cell lines were processed using the same procedure, and were treated identically throughout the research. Four blocks were prepared for each cell line processed in every experiment.

Once the resin was polymerized, it was sectioned using glass knives in an LKB Huxley microtome. The sections were placed on 300 mesh copper grids. Sections for 2 to 3 grids were collected per block for a total of 8 to 9 grids per group. This procedure was used to help ensure the random sampling necessary for morphometric studies. The grids were stained in 2% uranyl acetate and Sato's lead citrate.

The samples were viewed in a Hitachi HS-9 TEM and approximately 30 cell profiles were collected for each group. All cell profiles were magnified 7000 times. Cell profiles were collected randomly. The cell profiles in the uppermost right hand corner of a grid hole were photographed, and only 5 to 8 micrographs were collected per grid. Two sets of prints (at no enlargement and at a magnification of 7x) were made from each micrograph.

A feature, noted in the xrs-5 cells, was the presence of viral particles, called C-particles or C-type viruses. These particles had a diameter of approximately 0.07 $\mu$m (Bozzola and Russell, 1982). The C-particles have been described as viral sequences which had previously been incorporated in cellular DNA. After irradiation and treatment with a mutagen, the viral sequences are activated to form C-particles (Aaronson *et al.*, 1971; Lieber *et al.*, 1973; Aaronson and Dunn, 1974). The C-particles were not infectious to other cell populations maintained in the laboratory as also noted by others (Lieber *et al.*, 1973), and did not appear to have an effect on the cells. Upon ultrastructural examination, it was noted that the C-particles were no longer present in older cell populations, nor were they ever present in the K1 cells.

### Morphometry

Point counting was used to obtain the volume fraction of the cells (Weibel *et al.*, 1966; Suciu, 1986) and

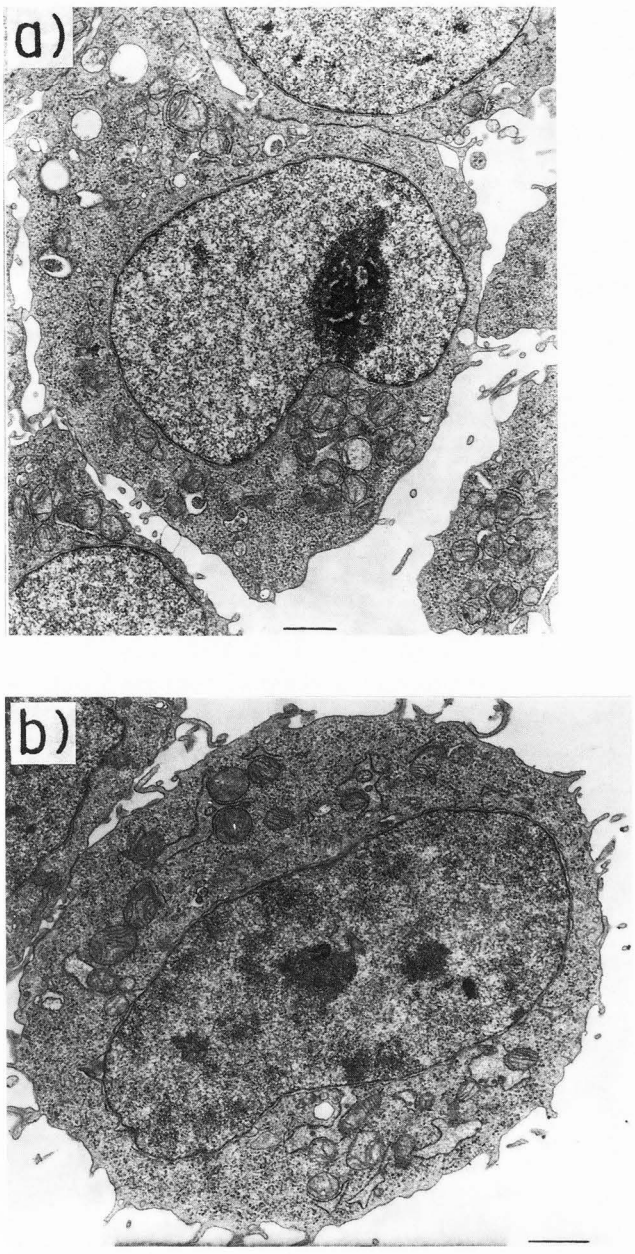


**Figure 1.** Electron micrographs of K1 (**a**) and sensitive xrs-5 cells (**b**). The xrs-5 cell had been maintained in tissue culture for 1 week. Bars = 1 $\mu$m.

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the nuclear to cytoplasmic ratios. A 0.7 cm grid was placed over the unenlarged micrographs, and the number of intersections which lay within the total cell and the nucleus were counted. This grid size was found to be sufficient by calculating the relative standard error (RSE) (Williams, 1977; Toth, 1982), where an RSE of 5% was well exceeded. While point counting is not a direct measure of cell size, it can be used as an estimation of cell size for a comparison between the cell lines.

Morphometric techniques can be used with either light microscopy (LM) or TEM. When using LM, tissue shrinkage in the resin and compression of the sections need not be considered. They are, however, major factors which must be accounted for in TEM (Toth, 1982). When samples are compared among themselves, as in this study, these factors are equal to one another and thus need not be corrected for. If the results are to be compared to other studies, however, correction factors must be used to account for shrinkage and compression. The TEM was calibrated using a calibration grid with 2160 lines/mm.

Biological variation was taken into account by using a cumulative average curve, as described by Williams (1977). A micrograph was analyzed, averaged with a second micrograph, then those 2 micrographs were averaged with a third, etc. This cumulative average was then plotted against the number of micrographs. The number of micrographs at the point where the curve plateaus is the number needed to account for the biological variation. A cumulative average versus number of cell profiles graph was plotted for each cell line and for each variable tested to determine the number of micrographs to score.

To ensure proper sampling of cellular components which are not evenly distributed throughout the cell such as the nucleus (as opposed to mitochondria, for example), micrographs were taken of any cell which was located in the upper right hand corner of a grid hole, so all sections of the cells were represented: some with no nucleus, some with a glancing view of the nucleus, and most with the section through the center of the nucleus. When data from all of these micrographs were averaged, an estimate of the actual cellular values was obtained.

## Results

### Ultrastructure of K1 and xrs-5 cells

Electron micrographs of K1 and sensitive xrs-5 cells are shown in Figure 1. All quantitative data were collected from such micrographs.

### Reversion of xrs-5 cells

The cell survival data detailing the reversion of the xrs-5 cells with time in tissue culture for this experiment are shown in Figure 2. These survival data agreed with the previously published data: the $D_0$ for the sensitive xrs-5 cells was 45 cGy while that for K1 was 200 cGy (Denekamp *et al.*, 1989; Nagasawa *et al.*, 1989; Yasui *et al.*, 1991). As the survival of the xrs-5 cells is determined over time, the tail end of the survival curve approaches the curve for the K1 cells (also see Denekamp *et al.*, 1989).

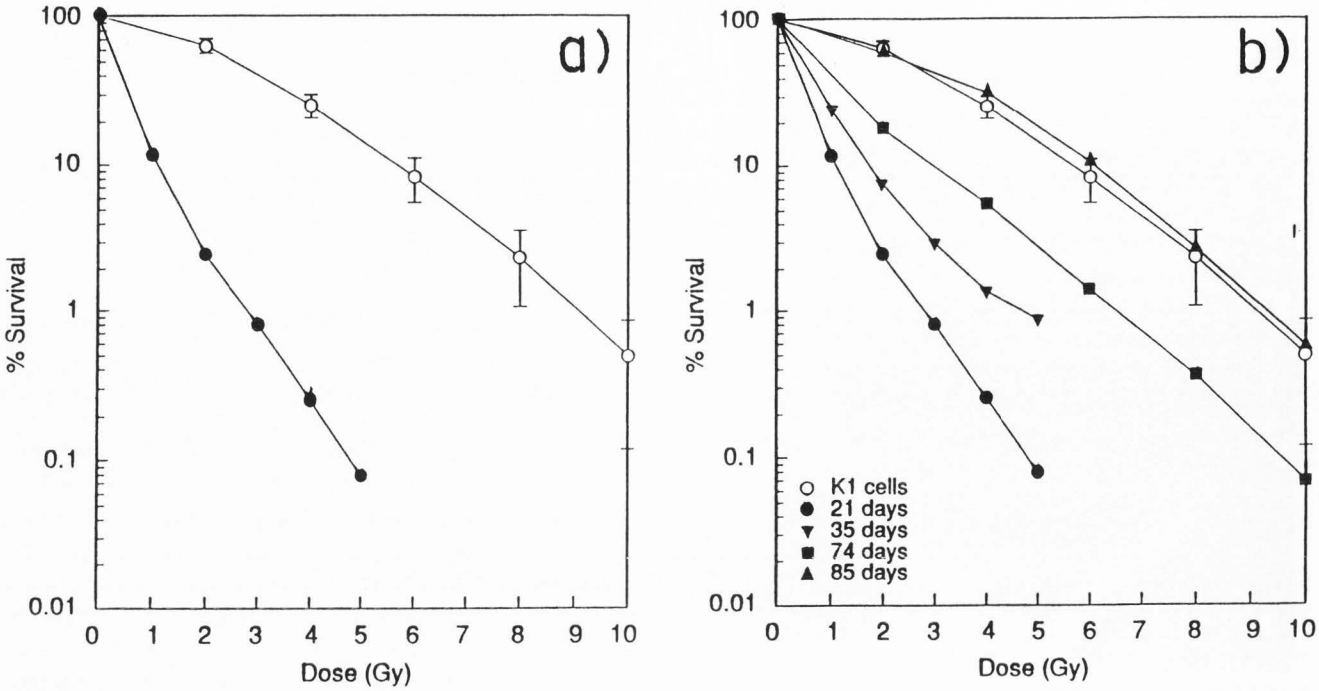


**Figure 2.** Representative survival curves where the per cent survival is plotted against the dose. (a) xrs-5 cells (●) at one week of age, and (b) xrs-5 cells throughout the reversion process. The K1 (○) curve in both graphs represents an average of 18 experiments where the error bars represent one standard deviation of the mean.

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**Table 1.** Total number of points counted over K1 and xrs-5 cells. The time in culture is shown in days. ***Two separate fixations for day 8 are indicated by (1) and (2) in all tables.** The average number of points ± 1 standard deviation of the mean is shown. Also indicated is the range (maximum and minimum) of points for the samples. The number of micrographs analyzed is indicated by n. The average number of points over the K1 cells was not statistically significantly different from the average number of points over xrs-5 cells ($p = 0.05$).

| Days | | K1 cells | xrs-5 cells |
|---|---|---|---|
| 8 (1)* | Average | 160.05 ± 49.95 | 177.49 ± 67.01 |
| | Range | 264 - 52 | 340 - 52 |
| | n | 38 | 35 |
| 8 (2)* | Average | 124.42 ± 49.76 | 148.32 ± 56.22 |
| | Range | 226 - 26 | 232 - 25 |
| | n | 33 | 31 |
| 59 | Average | --- | 192.89 ± 85.17 |
| | Range | --- | 412 - 36 |
| | n | --- | 27 |
| 85 | Average | 205.29 ± 76.44 | 206.19 ± 74.3 |
| | Range | 340 - 60 | 344 - 71 |
| | n | 28 | 31 |

**Table 2.** Volume fraction ($V_v$) in percent of nucleus and average nuclear to cytoplasmic ratios (Nuc/Cyto) of K1 and xrs-5 cells. The time in culture of these cells is shown in days. The nuclear volume fraction and the average nuclear to cytoplasmic ratios of K1 cells was not statistically significantly different from those values for xrs-5 cells ($p = 0.05$).

| Days | | K1 cells | xrs-5 cells |
|---|---|---|---|
| 8 (1) | $V_v$ | 39.43 | 36.91 |
| | Nuc/Cyto | 0.3715 | 0.3562 |
| 8 (2) | $V_v$ | 29.76 | 33.01 |
| | Nuc/Cyto | 0.2711 | 0.2803 |
| 59 | $V_v$ | --- | 34.33 |
| | Nuc/Cyto | --- | 0.3108 |
| 85 | $V_v$ | 38.27 | 35.86 |
| | Nuc/Cyto | 0.3326 | 0.3362 |

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In these series of survival studies, xrs-5 cells required 85 days to revert to a "K1-like" cell survival. Parallel cell samples were collected at the times indicated on the survival curves for the morphometric analysis of K1 and xrs-5 cells.

**Table 3.** Cell volume determination of K1 and xrs-5 cells. The average value ± 1 standard deviation of the mean is indicated. The ranges (maximum and minimum values) are also indicated. The number of micrographs analyzed is indicated by n. The time in culture of these cells is shown in days, and the data are represented in $\mu m^3$. Except for day 8, no statistically significant difference in cell volume was detected between K1 and xrs-5 cells ($p = 0.05$).

| Days | | K1 cells | xrs-5 cells |
|---|---|---|---|
| 8 (1) | Average | 447.93 ± 58.72 | 633.15 ± 91.50 |
| | Range | 546.01 - 364.16 | 749.16 - 513.79 |
| | n | 6 | 6 |
| 8 (2) | Average | 345.38 ± 65.12 | 400.78 ± 43.94 |
| | Range | 427.93 - 27.01 | 456.91 - 356.51 |
| | n | 6 | 6 |
| 59 | Average | --- | 631.26 ± 201.31 |
| | Range | --- | 927.39 - 403.71 |
| | n | --- | 6 |
| 85 | Average | 581.07 ± 106.19 | 670.56 ± 95.37 |
| | Range | 746.78 - 470.52 | 769.90 - 533.67 |
| | n | 6 | 6 |

**Table 4.** The average nuclear volume (calculated by multiplying the volume fraction from Table 2 with the determined cell volume of K1 and xrs-5 cells. The time in culture of these cells is shown in days and the data are represented in $\mu m^3$. No statistically significant difference in average nuclear volume between K1 and xrs-5 cells was detected ($p = 0.05$).

| | K1 cells | xrs-5 cells |
|---|---|---|
| 8 Days (1) | 176.62 | 233.70 |
| 8 Days (2) | 102.79 | 132.30 |
| 59 Days | --- | 216.71 |
| 85 Days | 222.38 | 240.46 |

## Point counting

The total number of points over K1 cells, reverting xrs-5 cells and sensitive xrs-5 cells was determined (Table 1) to obtain the volume fraction of the cells. The average number of points and the range (maximum and minimum) of points was determined. The average number of points indicated that xrs-5 cells were consistently larger than the K1 cells, although the values were not statistically different at the 0.05 level (compared using a student's t-test). The average nuclear volume fraction of the cells (Table 2) was determined by dividing the number of points in the nucleus by the total number of points over the whole cell. The nuclear volume fractions were not statistically different at the 0.05 level for the xrs-5 and K1 cells (Table 2).

Cell size and nuclear volume fraction did not correlate with different radiation sensitivity for these cells. The data also showed that the nuclear to cytoplasmic ratios were equal for the two cell lines, so it can be concluded that this was also not correlated with radiation sensitivity. No significant trends were observed in the volume fractions or nuclear to cytoplasmic ratios of the xrs-5 cells over time. There was a trend in the data, however, that indicated that the total cell size (as estimated by the total number of points counted) increased as the xrs-5 cells became more radiation resistant. This trend was further studied using direct measurements to calculate volume.

## Volume measurements

It is proposed that nuclear volume is associated with the degree of radiosensitivity a cell exhibits (Suciu, 1986). According to this hypothesis, the xrs-5 cells should have a smaller nuclear volume than the K1 cells because xrs-5 cells are more sensitive than K1 cells.

To determine the volume of the cells, a procedure described by Suciu (1986) was used where the longest diameter (length, l) and the line perpendicular and at the midpoint of the length (width, w) of the cell were measured in millimeters. The cell volume (V, Table 3) was calculated according to the following formula, which was reported as being applicable to both spherical and non-spherical objects (Suciu, 1986):

$$V = (4\pi/3)\ \{(l \cdot w) / 4\}^{3/2} \qquad (1)$$

The nuclear volume was calculated by multiplying the nuclear volume fraction determined from point counting by the volume of the cell (Table 4).

Since the cells were sectioned at random, the plane of the cell shown in the micrograph may not necessarily represent the midpoint of the cell. Therefore, to ensure that the actual cell volume was being measured (and not the volume at either end of the cell), only the 6 largest cell volumes were used. These results cannot be compared to results outside of this study, however, since correction factors for the shrinkage of the tissue in the resin and the compression of the sections due to sectioning have not been applied. The data in Table 3 (represented in $\mu m^3$) shows that the xrs-5 cells were consistently larger than the K1 cells, although the difference for 8 days (1) was the only group for which this difference was significant at $p = 0.05$ level. Interestingly,

**Table 5.** Surface area of nuclear membrane per units volume of cell of K1 and xrs-5 cells. The average value ± 1 standard deviation of the mean is shown. The range (maximum and minimum) nuclear surface areas are also shown. The number of micrographs analyzed is indicated by n. The time in culture of these cells is shown in days, and the values are in $mm^2/mm^3$. No statistically significant difference in surface area of nuclear membrane between K1 and xrs-5 cells was found ($p = 0.05$).

| Days | | K1 cells | xrs-5 cells |
|---|---|---|---|
| 8 (1) | Average | 0.03332 ± 0.0113 | 0.0322 ± 0.0143 |
| | Range | 0.0494 - 0.0000 | 0.0763 - 0.0000 |
| | n | 36 | 35 |
| 8 (2) | Average | 0.0310 ± 0.0169 | 0.0274 ± 0.0148 |
| | Range | 0.0648 - 0.0000 | 0.0480 - 0.0000 |
| | n | 33 | 31 |
| 59 | Average | --- | 0.0281 ± 0.0119 |
| | Range | --- | 0.0452 - 0.0000 |
| | n | --- | 27 |
| 85 | Average | 0.0247 ± 0.0127 | 0.0273 ± 0.0113 |
| | Range | 0.0472 - 0.0000 | 0.0461 - 0.0000 |
| | n | 28 | 31 |

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the cells which completely reverted in their radiation sensitivity (85 days) showed the largest nuclear volume, which was statistically equivalent to that of the K1 cells.

These results are consistent with the point counting results, which showed that the xrs-5 cells were consistently slightly larger than the K1 cells. However, the differences between the K1 and xrs-5 cells were generally not statistically significant. The conclusion drawn from these data was that there was no appreciable size difference between the two cell lines, although the xrs-5 cells appeared to become larger as their radiation sensitivity decreased.

### Surface area

As previously mentioned, the nucleus in each xrs-5 cell is very irregularly shaped and appears to have many invaginations and evaginations compared to the K1 cells (Yasui *et al.*, 1991). To express this feature quantitatively, the surface area of the nucleus per unit volume of cell was determined.

A series of lines, 1 cm apart, were placed over a micrograph and the line length was measured across the cell and totaled for the micrograph. The number of intersections of the nuclear envelope with the test lines was also counted, and the surface area of the nuclear envelope per unit cell volume was calculated.

This procedure was performed on all of the micrographs, and an average surface area per unit test volume was obtained. Since there was variability in the position of the cell on the micrograph, and to ensure that the sampling was not biased, the micrographs were measured, rotated 90°, and the measurements were repeated.

These data in Table 5 (represented in $mm^2/mm^3$) show no significant difference in nuclear surface area to volume ratio. There were also no apparent trends in the data.

To summarize the data thus far, no appreciable differences have been found between the xrs-5 and K1 cells which could account for the differences in radiation sensitivity.

### Evaluation of nuclear envelope membrane separation

A direct measurement method was employed to evaluate the nuclear envelope membrane separation (Table 6). Micrographs which contained the nuclear envelope were photographically enlarged 7 times. While in many cases the entire nucleus did not fit on an 8x10 print, the portion of the nucleus in the right-hand side of the field was positioned so as to fit as much of the nucleus as possible on the print. Two-centimeter intervals were marked off around the membrane, and electronic calipers (with an accuracy of ± 0.025 mm) were used to measure the separation at those points. An average of these measurements for each cell was calculated. Additionally, the maximum and minimum separation (the position of which was estimated by eye) was measured.

The maximum and minimum values in Table 6 represent the measurements described above, and not the maximum and minimum measurements of the 2 cm interval data (the standard deviation was calculated for all micrographs). The data (represented in nm) show that the nuclear envelope membrane separation of xrs-5 cells was consistently larger than the K1 cells. These differences were significant at the 0.05 level. When the older xrs-5 cells were compared to the younger cells, there was a slight difference (although not statistically significant) in the separation: the cells which had reverted in the radiation sensitivity had a slightly smaller average membrane separation.

The minimum separations, as would be expected, were all statistically equivalent. The most apparent difference between the cells was in the maximum separations. The sensitive xrs-5 cells showed a very large maximum separation, while the xrs-5 cells which had reverted in the radiation sensitivity showed a much smaller separation (this difference was statistically significant). It should be noted, however, that the membrane separation of the reverted xrs-5 cells did not equal that of the K1 cells, although they approached one another. The conclusion, which must be drawn from these data, is

**Table 6.** Membrane separation of the inner and outer nuclear membranes as measured by an electronic caliper. All values are given in nm with 1 standard deviation of their mean. The number of micrographs analyzed is indicated by n. Nuclear envelope membrane separation of young xrs-5 cells was statistically significantly larger than for K1 cells ($p = 0.05$). The t statistic used to determine the p value is indicated for each data set.

| | | K1 cells | xrs-5 cells | t | p < |
|---|---|---|---|---|---|
| 8 Days (1) | Average | 35.46 ± 3.03 | 46.11 ± 10.94 | 4.76 | 0.002 |
| | Maximum | 55.04 ± 8.43 | 163.99 ± 124.03 | 4.77 | 0.001 |
| | Minimum | 19.21 ± 4.56 | 20.96 ± 6.77 | 0.89 | 0.5 |
| | n | 9 | 30 | | |
| 8 Days (2) | Average | 36.31 ± 3.97 | 60.55 ± 23.46 | 4.55 | 0.002 |
| | Maximum | 77.56 ± 57.24 | 70.79 ± 224.34 | 3.72 | 0.005 |
| | Minimum | 22.25 ± 2.84 | 22.55 ± 3.36 | 0.31 | 0.5 |
| | n | 19 | 20 | | |
| 59 Days | Average | --- | 54.15 ± 19.8 | | |
| | Maximum | --- | 199.73 ± 202.55 | | |
| | Minimum | --- | 26.06 ± 5.81 | | |
| | n | --- | 19 | | |
| 85 Days | Average | 38.92 ± 5.47 | 44.68 ± 5.66 | 3.27 | 0.01 |
| | Maximum | 57.34 ± 14.79 | 83.58 ± 28.28 | 3.67 | 0.01 |
| | Minimum | 26.06 ± 5.66 | 24.56 ± 6.21 | 0.80 | 0.5 |
| | n | 20 | 20 | | |

that the membrane separation did not appear to have a cause and effect relationship with radiation sensitivity, although there did seem to be a correlation between the two.

## Discussion

The radiation sensitive CHO mutants isolated by Jeggo *et al.* (1982) have been widely used by radiation biologists to study the effects of double strand breaks and the repair processes of these lesions. The defect in the repair process in xrs-5 cells, however, remains a mystery. While many researchers are looking for a defective enzyme, or a specific mutation which causes the increased sensitivity of xrs-5 cells, this study takes a different approach by looking at the distinctive morphological features noted (for example see Fig. 1) in the xrs-5 cells (Yasui *et al.*, 1991). If the morphological changes are shown to be correlated with radiation sensitivity, then the specific cause and effect of these morphological changes can be more specifically addressed.

We find xrs-5 cells revert to K1 radiosensitivity (Fig. 2) and nuclear morphology (Yasui *et al.*, 1991). The concurrent radiosensitivity and morphological reversion permits us to quantitate various morphological differences to determine if they correlate with differences in radiation sensitivity between the two cell lines. Experiments were performed to quantify these differences and to correlate them with the changes in radiation sensitivity.

Upon examination, xrs-5 and K1 cells appeared to differ in size but no significant differences between the two cell lines in volume fraction of the nuclei and nuclear to cytoplasmic ratio were noted for either of these factors. Therefore, we concluded that these factors are not correlated with radiation sensitivity and could not be used to provide an explanation for the difference in sensitivity between the two cell lines. To further quantify the cell and nuclear volumes, volume was calculated using direct measurements of the cells. These results again showed no significant differences between the two cell lines, although there was a trend in the data where the xrs-5 cell volumes increased as their radiation sensitivity decreased. This trend was not significant, however, at the 0.05 level (as tested by an ANOVA). These data provided further evidence to support the fact that cell size is not a major factor in the increased radiation sensitivity of the xrs-5 cells, as compared to that of the K1 cells. No significant difference between the two cell lines in the invaginations and evaginations of the nuclear envelope as measured by the surface area per unit volume of cell were detected. Again, the conclusion was reached that these irregularities are not correlated with radiation sensitivity (in this instance) and do not play a major role in the change in sensitivity of the xrs-5 cells.

The nuclear envelope membrane separation was quantified by using direct measurements from micrographs. The average separation of the xrs-5 cells was consistently (and significantly) larger than that of the K1 cells. The separation of the nuclear membranes in the xrs-5 decreased as the cells reverted in their radiation sensitivity. As the cells reached the K1 level of radiation sensitivity, however, the separation of the nuclear membranes did not equal the separation measured in K1 cells. From these results, it was concluded that the irregular membrane separation may play a role in the radiation sensitivity. Since the nuclear membrane separation of xrs-5 cells did not completely revert to that of K1 cells, the relationship cannot be considered a cause and effect relationship. These data, however, provided evidence of a correlation between radiation sensitivity and the changed nuclear envelope membrane separation.

The nuclear envelope is the membrane system that forms the boundary between the cell nucleus and the cytoplasm in eukaryotes. It is composed of a double membrane, pore complexes, and the nuclear lamina. The nuclear lamina provides an anchoring site for interphase chromosomes. Chromatin appears closely associated with the lamina in most cell types (Gerace, 1986). The nuclear lamina is also thought to provide an architectural framework for the nuclear envelope (Gerace, 1986). The close association of the lamina with the inner nuclear membrane could be important for physically coupling the dynamics of the lamina to nuclear membrane structure and function (DNA replication, DNA damage repair, gene expression, etc.) (Gerace, 1986). The increased nuclear envelope membrane separation quantitated here may represent an altered template on the lamina for DNA damage repair so that the cells do not repair lethal damage, thereby accounting for the radiation sensitivity. Others have found the nuclear envelope area to be an extremely sensitive radiation target (Munro, 1970; Alper, 1979).

## Conclusions

These quantitative EM data suggest that cell size, nuclear volume, nuclear to cytoplasmic ratios and nuclear surface irregularities do not correlate with radiation sensitivity in xrs-5 cells. Increased nuclear envelope membrane separation did correlate with radiation sensitivity but the separation did not completely revert in size to K1 nuclear membrane separation. Therefore, the increased nuclear envelope membrane separation in sensitive xrs-5 cells may be a factor in the radiosensitivity of the xrs-5 cells but it can not be the sole defect responsible for the radiation sensitivity of xrs-5 cells.

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**Editor's Note:** All of the reviewer's concerns were appropriately addressed by text changes, hence there is no **Discussion with Reviewers.**