Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 4752–4755, August 1983
Cell Biology

Intrinsic resistance to the lethal effects of x-irradiation in insect and arachnid cells

(cell survival/growth curves/colony formation/cell culture/cellular repair processes)

THOMAS M. KOVAL*†

Department of Radiation Therapy and Nuclear Medicine, Hahnemann Medical College, Philadelphia, Pennsylvania 19102

Communicated by Alexander Hollaender, May 6, 1983

ABSTRACT **Twelve cell lines representing 10 genera of three orders (Diptera, Lepidoptera, and Orthoptera) of the class Insecta and one cell line (Acarina) from the class Arachnida were examined to discern their sensitivity to the lethal effects of x-irradiation. Radiosensitivity was measured by a combination of colony formation and population growth curve techniques. Each of these arthropod cell lines is significantly more radioresistant than mammalian cells, though the degree of resistance varies greatly with order. Dipteran cells are 3 to 9 times and lepidopteran cells 52 to 104 times more radioresistant than mammalian cells. Orthopteran and acarine cells are intermediate in radiosensitivity between dipteran and lepidopteran cells. These cells, especially the lepidopteran, should be valuable in determining the molecular nature of repair mechanisms that result in resistance to ionizing radiation.**

The pronounced radioresistance of adult insects has been well documented (1, 2). This resistance has been attributed primarily to the lack of cell division in adult insects, in that the sensitivity of cells to irradiation is directly proportional to their reproductive activity (1–3), and because insects have minimal or no cell division in the adult state, they are extremely resistant to radiation (1, 2). Recent evidence has suggested that cells of insects have an intrinsic radioresistance (4). This cellular basis of resistance derives largely from studies with the TN-368 lepidopteran cell line. These mitotically active cells are approximately two orders of magnitude more radioresistant than mammalian cells (4–6). The unique nature and the ubiquity of radioresistance in insect cells have important implications concerning the innate ability of cells to tolerate large doses of radiation. Therefore, the present experiments examined 13 cell lines representing three orders of the class Insecta, along with 1 arachnid cell line (Table 1). All of the lepidopteran (moths and butterflies) cell lines exhibit a marked radioresistance similar to that observed in the TN-368 cells. The dipteran (flies and mosquitoes) lines are several times more resistant than mammalian cells but show greater sensitivity than the lepidopteran cultures. The orthopteran (cockroaches and grasshoppers) lines and the acarine (ticks and mites) line appear to be intermediate in radiosensitivity between the lepidopteran and the dipteran lines.

MATERIALS AND METHODS

Cell Cultures. All of the cell lines were cultured as monolayers in Costar 25-cm^2 tissue culture flasks containing 5 ml of medium. Flasks were maintained in a humidified incubator at 28°C. Further details concerning cell line designations, the organisms from which the lines were derived, growth media, population doubling times, passage numbers, and chromosome numbers are provided in Tables 1 and 2. TN-368, IPLB-SF-1254, IPLB-1075, and ATC-10 lines were obtained from W. F. Hink (Ohio State University); MRRL-CH1, clone GV1, and IAL-PID2 cells, from D. E. Lynn (U.S. Department of Agriculture); WR69-DM-1 and WR69-DM-2 cultures, from I. Schneider (Walter Reed Army Institute for Research); RU-TAE-14, RU-ASE-2A, and RAE-25, from T. J. Kurtti and U. G. Munderloh (Rutgers University); and UMN-BGE-2 and UMN-BGE-5β, from K. R. Tsang (University of Minnesota). V79 Chinese hamster lung fibroblasts were obtained as line GM-215 from the Institute for Medical Research in Camden, NJ. This line is among the more radioresistant mammalian cells and has been used extensively in radiobiological studies (6).

Population Doubling Times. Doubling times were derived from the logarithmic portion of growth curves obtained by seeding 10^6 cells in replicate 25-cm^2 flasks and counting at 24-hr intervals until the populations were well into stationary phase (each point on these growth curves represents at least two separate experiments having two duplicate flasks per experiment and four hemocytometer cell counts per flask).

X-Irradiations. A Picker Vanguard x-ray machine operated at 260 kV and 15 mA was used for all irradiations. X-rays passed through approximately 5 mm of glass but were otherwise unfiltered. Dosimetry, utilizing the same geometry as for experiments, was performed according to the method of Fricke and Hart (7).

Growth Curves. Exponentially growing cells were diluted in fresh growth medium to a concentration of 2×10^6 cells per ml. They were irradiated at room temperature in 2.0-ml aliquots as an aerated, stirred suspension at a dose rate of just under 0.83 Gy (83 cGy)/sec. Cells irradiated with a given dose were pooled and 1×10^6 cells were seeded into Costar 25-cm^2 flasks. Pooling was done to insure homogeneity among all replicates for each dose. Flasks were incubated at 28°C. Enough flasks were set up to enable frequent cell countings over a 2- to 3-wk period. Generally, four hemocytometer counts were performed on the cells of two replicate flasks per dose at each sampling. The flasks were then discarded.

Survival Curves. Cells in exponential growth were diluted in fresh growth medium to a concentration of 2×10^6 cells per ml. These cells were irradiated at room temperature as an aerated, stirred suspension at a dose rate of 83 cGy/sec. In addition, dipteran cells were also repeated at 15 cGy/sec to be

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

*To whom reprint requests should be addressed at: Natl. Council on Radiation Protection, 7910 Woodmont Ave., Suite 1016, Bethesda, MD 20814.

†Present address: Dept. of Radiology, The George Washington Univ. School of Medicine and Health Sciences, Washington, DC 20037; and Natl. Council on Radiation Protection and Measurements, Bethesda, MD 20814.

Table 1. Description of cell lines

Cell line	Order	Genus and species	Common name	Growth medium*	Passage number†
TN-368	Lepidoptera	*Trichoplusia ni*	Cabbage looper	Hink (8)	>1,000
IPLB-SF-1254	Lepidoptera	*Spodoptera frugiperda*	Fall armyworm	Hink (8)	>250
IPLB-1075	Lepidoptera	*Heliothis zea*	Corn earworm	Hink (8)	>250
MRRL-CH1, clone GV1	Lepidoptera	*Manduca sexta*	Tobacco hornworm	Hink (8)	202 parent; >50 clone
IAL-PID2	Lepidoptera	*Plodia interpunctella*	Indian mealmoth	Hink (8)	>50
WR69-DM-1	Diptera	*Drosophila melanogaster*	Fruit fly	Schneider (15)	>500
WR69-DM-2	Diptera	*Drosophila melanogaster*	Fruit fly	Schneider (10)	>500
ATC-10	Diptera	*Aedes aegypti*	Yellow fever mosquito	Mitsuhashi and Maramorosch (20)	>250
RU-TAE-14	Diptera	*Toxorhynchites amboinensis*	Mosquito	Leibovitz (20)	>50
RU-ASE-2A	Diptera	*Anopheles stephensi*	Mosquito	Leibovitz (20)	>10
UMN-BGE-2	Orthoptera	*Blatella germanica*	German cockroach	Mark (10)	>400
UMN-BGE-5β	Orthoptera	*Blatella germanica*	German cockroach	Mark (10)	>100
RAE-25	Acarina‡	*Rhipicephalus appendiculatus*	Brown ear tick	Leibovitz (20)	>50

* Number in parenthesis indicates the percent fetal bovine serum used as a medium supplement.
† Passage number indicates the minimal number of times the cells have been subcultured (ratio at subculture varying from about 1:3 for orthopteran lines to 1:10 or more for a few of the rapidly growing lines); the numbers primarily indicate that most of the lines are continuous.
‡ Class Arachnida.

more precise in administering low doses. Doubling or tripling the concentration of the cell suspension or slightly altering the temperature during the irradiation has no effect on the survival curve. Immediately after x-irradiation, aliquots of cells were plated into Falcon 60-mm polystyrene tissue culture dishes (five replicates per dose) containing 4 ml of medium and a previously x-irradiated (2,000 Gy for Lepidoptera and 500 Gy for Diptera) feeder layer (8) of the same cell line. To minimize any effect of the feeder layers and to keep plating efficiencies constant for all doses, the number of feeder cells per dish was varied to keep the total number of cells per plate constant. Plates were incubated in a humidified atmosphere at 28°C. Fourteen to 18 days later, the cells were stained with a neutral red solution and visible colonies having >64 cells were counted. Plating efficiencies were determined and surviving fractions were calculated (4).

V79 cells were handled exactly the same as the insect cells. They were irradiated at a concentration of 2×10^6 cells per ml as an aerated, stirred suspension at 15 cGy/sec. Colonies were counted after 10 days of incubation at 37°C. The presence or absence of a feeder layer did not significantly alter surviving fractions. Irradiating suspensions of 1×10^6 or 4×10^6 cells per ml did not affect the results.

RESULTS

Population growth curves were used in this study to provide crude estimates of survival ability. Fig. 1 illustrates two representative examples of these growth curves. At least six doses of x-rays ranging from 10 to 400 Gy were examined along with control cultures for each cell line (200 Gy maximum for Orthoptera and Acarina). Irradiation was generally followed by a dose-dependent division delay (Fig. 1). The population was considered not to have recovered if it had not undergone a significant increase in number (approximately one doubling) over the initial seeding density within 2–3 wk. The maximal dose at which recovery was observed is given as the recovery dose in Table 2. The Lepidoptera, Orthoptera, and Acarina all recovered from the highest dose administered. These recovery doses are important in that they provide both crude comparative survival estimates for those lines for which no colony formation information was obtainable, and they provide as well a basis for selecting cell plating concentrations at various doses for the survival curve studies. In addition, the recovery doses noted in Table 2 correlate closely with *in vivo* sterilizing doses for the lepidopteran and dipteran insects (14).

Survival curves were possible for only 7 of the 13 lines studied and for only the lepidopteran and dipteran orders (Table 2 and Fig. 2) due to inherent difficulties entailed in the use of the colony formation technique to measure survival in insect cells. In general, the cells do not have very high plating efficiencies, and most insect cells grow loosely attached to growth vessels so that when formed, colonies disintegrate or float away upon removal of plates from incubation. Due to this loose attachment, the colonies are not easily counted, because fixing and staining contribute further to colony loss.

The TN-368 lepidopteran cell line exhibited an atypical multiphasic survival response composed of a steep slope region, followed by an inflection point, and finally a shallow slope region (Fig. 2) (12, 13). The D_0 for each slope and the width of the inflection point all increase proportionally, by approximately 1.7, for cultures irradiated under anoxic conditions (flushed with nitrogen prior to and during exposures) (12). Varying the dose rate from 202 to 9.1 Gy/min results in only a slight decrease in the slopes but does not alter the general shape of the curve (unpublished data). That two distinct cell populations are not represented has been demonstrated by culturing a population of cells previously irradiated with 60.6 Gy (from the lower portion of the resistant slope component) and repeating the survival assay with them. The same multiphasic response was obtained (unpublished data). The multiphasic response may be a consequence of cell cycle variations in radiosensitivity because it was decreased by partially synchronizing the cells (unpublished data).

Considering the D_0 of x-ray survival curves for most mammalian cells to be between 1.0 and 1.5 Gy, the dipteran cells demonstrate radioresistance 3 to 9 times that of mammalian cells, whereas the lepidopteran cells are 52 to 104 times more radioresistant. Survival curves for the two lepidopteran genera are nearly identical, and the five dipteran lines representing four genera (cultured in four different growth media) have curves very similar to each other in degree of radioresistance. For the remaining lepidopteran lines, along with the orthopteran and acarine lines, population growth curves are used to estimate radioresistance, because survival curves were not obtained. On

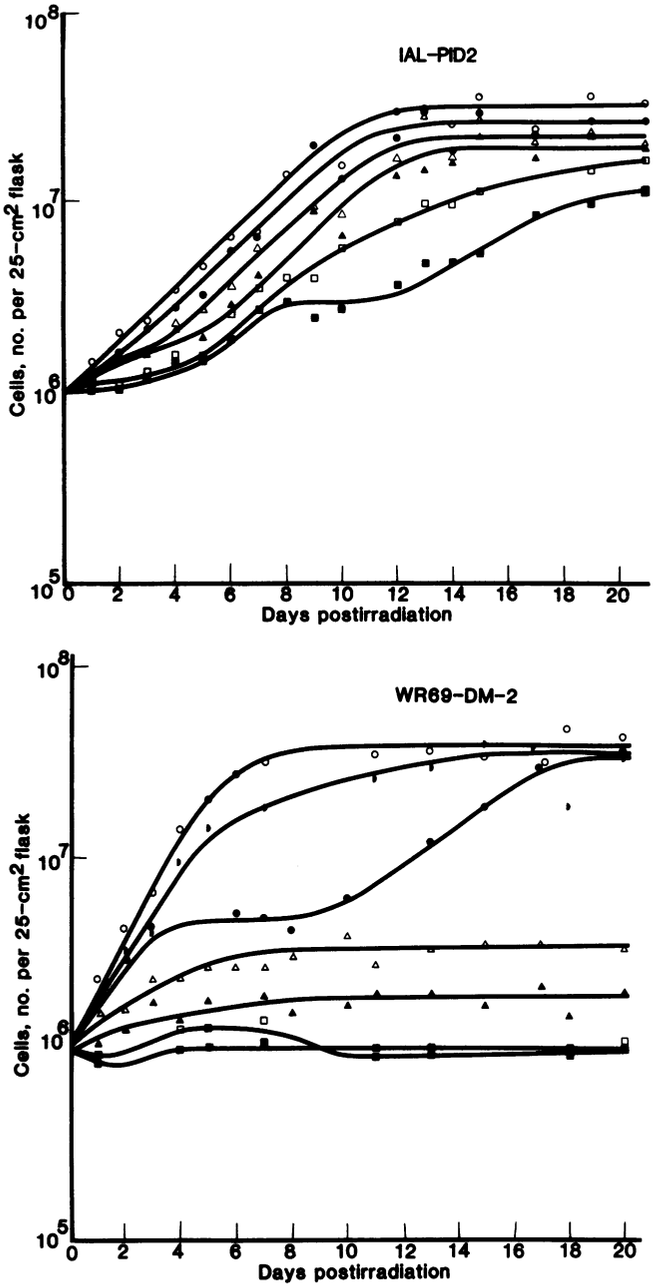

FIG. 1. Representative population growth curves of IAL-PID2 (lepidopteran; *Upper*) and WR69-DM-2 (dipteran; *Lower*) cells x-irradiated with 0 Gy (○); 10 Gy (◗); 25 Gy (●); 50 Gy (△); 100 Gy (▲); 200 Gy (□); and 400 Gy (■). Each point represents the mean of at least two separate experiments having two duplicate flasks per experiment and four hemocytometer counts per flask.

this basis it appears that the orthopteran and acarine lines show intermediate radioresistance between the dipteran and the lepidopteran cell lines.

DISCUSSION

This study demonstrates unequivocally that the cells of several higher eukaryotic organisms, mainly insects, have the inherent ability to survive exceptionally large radiation doses. The degree of radioresistance varies greatly with order, the lepidopteran cells being considerably more resistant than the dipteran cells. The basis for this unique finding may provide valuable insights into heretofore unrevealed cellular and molecular mechanisms of dealing with radiation damage.

Table 2. Comparative cell characteristics and radiosensitivities

Cell line	Population doubling time, hr	Approximate chromosome number*	Recovery dose, Gy†	D_0, Gy‡
TN-368	19	88	400	65.7; 130.2§
IPLB-SF-1254	24	>100	400	63.9
IPLB-1075	29	>100	400	—
MRRL-CH1, clone GV1	48	130	400	—
IAL-PID2	52	120	400	—
WR69-DM-1	30	8	100	5.1
WR69-DM-2	24	8	100	6.5
ATC-10	16	6	100	3.6
RU-TAE-14	48	6	100	6.2
RU-ASE-2A	26	6	100	10.2¶
UMN-BGE-2	100–150	23	200	—
UMN-BGE-5β	100–150	46	200	—
RAE-25	60	23	200	—

* All lepidopteran lines are heteroploid, with a near-tetraploid number of chromosomes in the majority of cells (9, 10). Polyploidy is common for lepidopteran tissues *in vivo* (11), making it difficult to arrive at a definite diploid chromosome number. At least 50% of the cells of the lines representing the remaining orders have a diploid chromosome number as shown, except for the UMN-BGE-5β line, which has a majority of tetraploid cells (as listed).

† Recovery doses were determined as described in the text.

‡ D_0 values (D_0, dose at which survival = $1/e$ on the exponential portion of a survival curve) were obtained from the slopes of the survival curves presented in Fig. 2.

§ Two D_0 values were given for the TN-368 line because a multiphasic survival response having two logarithmic portions was observed (12, 13).

¶ These cells grow initially as a firmly attached population, but as they spread out, the cells form spherical vesicles as well as a confluent monolayer. These vesicles may remain attached to the growth vessel or may float freely in the medium with the possibility of reattaching later. Therefore, even though the cultures are trypsinized to form a single cell suspension prior to irradiating and plating, vesicle formation and migration could occur during the survival assay, leading to the calculation of a slightly inflated radioresistance.

Degree of mitotic activity does not appear to play a significant role in determining degree of radiosensitivity in the cell lines studied here. The dipteran and lepidopteran cells examined have the same range of population doubling times (approximately 16–19 hr to 48–52 hr) in each order (Table 2), yet the difference in radioresistance between the orders remains well identified and quite distinct (Fig. 2). Chromosome number does not appear to correlate with radiosensitivity in insects either (14). For example, chromosome number between the Diptera and Lepidoptera (Table 2) differs greatly, the Lepidoptera having a large number of very small holokinetic chromosomes (which preclude accurate chromosome studies) and the Diptera having a small number of relatively large monokinetic chromosomes (15, 16). Holokinetic implies that kinetochores or centromeres are spread out or "diffused" along the chromosome. Because chromosomal breakage due to radiation damage could be expected to result in the loss of chromosome parts and subsequent cell death in monokinetic species and because chromosome fragments could be retained in chromosomally holokinetic species, thereby decreasing the amount of cell killing, an attractive explanation for the extreme radioresistance of the Lepidoptera might be hypothesized on this basis. However, the Hemiptera (true bugs) (17) and the Homoptera (aphids and leafhoppers) (18) also have holokinetic chromosomes and do not require the extremely large x-ray doses needed for sterilization in the Lepidoptera (19, 20). Indeed, hemipteran cells have been reported to have nearly twice the holo-

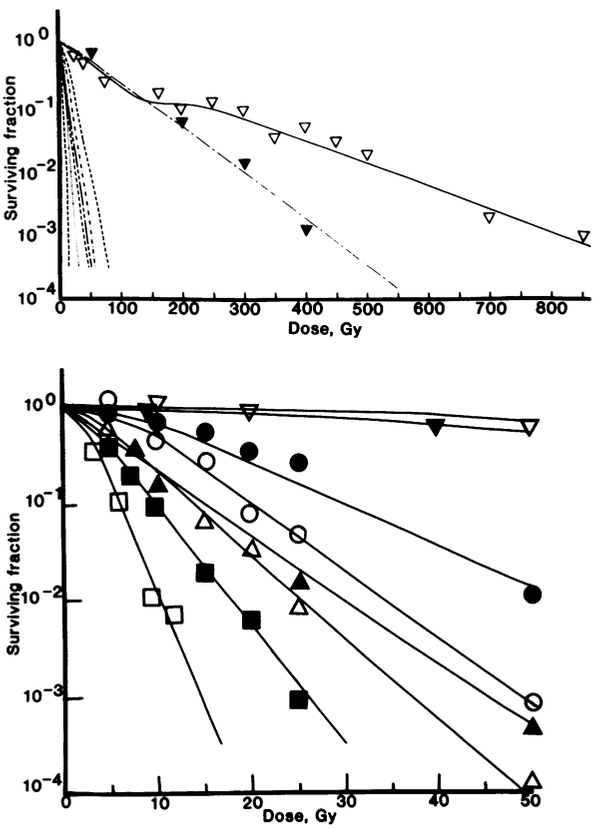

FIG. 2. Lepidopteran, dipteran, and mammalian cell survival comparisons: TN-368 (▽); IPLB-SF-1254 (▼); RU-ASE-2A (●); RU-TAE-14 (○); WR69-DM-2 (▲); WR69-DM-1 (△); ATC-10 (■); and V79 (□). (*Lower*) Blowup of the low-dose portion of *Upper*. Each point represents the mean ± SEM of at least five separate experiments with five replicates per experiment. Survival curve shoulders were fitted by eye and straight line portions by using linear regressions.

centric activity of lepidopteran cells yet demonstrate far greater radiosensitivity (19). DNA content of the TN-368 cells is approximately 7 pg per cell, whereas that of the V79 cells is 12–14 pg per cell (21). This is a relatively slight difference and there is no reason to believe it is responsible for the large difference in radiosensitivity. Perhaps chromosome size or DNA content per chromosome is more important in determining insect cell radiosensitivity.

The data discussed clearly demonstrate the innate ability of cells from a variety of insects to withstand large doses of x-irradiation. The fundamental process(es) responsible for this ability are yet to be determined. Studies have shown that lepidopteran insect cells have superior x-ray-induced unscheduled DNA synthesis and DNA single-strand break repair mechanisms compared to mammalian cells (21, 22) and that ultraviolet-type repair mechanisms function in both dipteran (23, 24) and lepidopteran (25) cells, though at levels similar to mammalian cells. It is possible that superior x-ray repair confers a certain amount of radioresistance in all insect cells and that this resistance is magnified in the Lepidoptera through unique chromosomal features. These insect cells should be extremely valuable in determining the molecular characteristics of repair systems leading to radiation resistance.

I thank W. F. Hink, D. E. Lynn, I. Schneider, T. J. Kurtti, U. G. Munderloh, and K. R. Tsang for providing me with cell cultures and H. Walton for technical assistance. This project was supported by U.S. Public Health Service Grants R01-CA30648 and R01-CA34158 awarded by the National Cancer Institute.

1. Ducoff, H. S. (1972) *Biol. Rev.* **47**, 211–240.
2. O'Brien, R. D. & Wolfe, L. S. (1964) *Radiation, Radioactivity and Insects* (Academic, New York).
3. Bergonie, J. & Tribondeau, L. (1906) *C.R. Fr. Seances Acad. Sci.* **143**, 983–985.
4. Koval, T. M. (1980) in *Radiation Biology in Cancer Research*, eds. Meyn, R. E. & Withers, H. R. (Raven, New York), pp. 169–184.
5. Hall, E. J. (1978) *Radiobiology for the Radiologist* (Harper & Row, New York).
6. Elkind, M. M. & Whitmore, G. F. (1967) *The Radiobiology of Cultured Mammalian Cells* (Gordon & Breach, New York).
7. Fricke, H. & Hart, E. J. (1966) in *Radiation Dosimetry*, eds. Attix, F. H. & Roesch, W. C. (Academic, New York), Vol. 2, pp. 167–239.
8. Puck, T. T. & Marcus, P. I. (1955) *Proc. Natl. Acad. Sci. USA* **41**, 432–437.
9. Hink, W. F. (1976) in *Invertebrate Tissue Culture, Research Applications*, ed. Maramorosch, K. (Academic, New York), pp. 319–369.
10. Ennis, J. T. & Sohi, S. S. (1976) *Can. J. Genet. Cytol.* **18**, 471–477.
11. White, M. J. D. (1973) *Animal Cytology and Evolution* (William Clowes & Sons, London), 3rd Ed.
12. Koval, T. M. (1981) *Radiat. Res.* **87**, 500 (abstr.).
13. Koval, T. M. (1983) *Radiat. Res.* **95**, in press.
14. LaChance, L. E., Schmidt, C. H. & Bushland, R. C. (1967) in *Pest Control: Biological, Physical, and Selected Chemical Methods*, eds. Kilgore, W. W. & Doutt, R. L. (Academic, New York), pp. 147–195.
15. Bauer, H. (1967) *Chromosoma* **22**, 101–125.
16. Murakami, A. & Imai, H. T. (1974) *Chromosoma* **47**, 167–177.
17. Hughes-Schrader, S. & Schrader, F. (1961) *Chromosoma* **12**, 327–350.
18. Schrader, F. (1947) *Evolution* 1, 134–142.
19. Gassner, G. & Klemetsan, D. J. (1974) *Can. J. Genet. Cytol.* **16**, 457–464.
20. Berg, G. J. & LaChance, L. E. (1976) *Ann. Entomol. Soc. Am.* **69**, 971–976.
21. Koval, T. M., Myser, W. C., Hart, R. W. & Hink, W. F. (1978) *Mutat. Res.* **49**, 431–435.
22. Koval, T. M., Hart, R. W., Myser, W. C. & Hink, W. F. (1979) *Int. J. Radiat. Biol.* **35**, 183–188.
23. Trosko, J. E. & Wilder, K. (1977) *Genetics* **73**, 297–302.
24. Boyd, J. B. & Setlow, R. B. (1976) *Genetics* **84**, 507–526.
25. Koval, T. M., Hart, R. W., Myser, W. C. & Hink, W. F. (1977) *Genetics* **87**, 513–518.