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X-Ray Sensitivity of Fifty-three Human Diploid Fibroblast Cell Strains from Patients with Characterized Genetic Disorders[1]

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ABSTRACT

The *in vitro* response of 53 human diploid fibroblast strains to X-irradiation was studied using a clonogenic survival assay. The strains, derived from patients with a variety of characterized clinical conditions, most with a genetic component, ranged in D_0 (a measure of the slope of the survival curve) from 43 to 168 rads. The mean D_0's of six strains from normal individuals was 140 to 152 rads, with an overall range, based on the extremes of their standard errors, of 128 to 164 rads. Three-quarters of the strains studied fell within this range. Strains identified as sensitive came from patients with ataxia telangiectasia, progeria, the two genetic forms of retinoblastoma, and partial trisomy of chromosome 13. No marked radiosensitivity was found among strains derived from patients with a number of other conditions associated with a predisposition to malignancy.

INTRODUCTION

Individuals with the rare autosomal recessive disease XP[3] are photosensitive and frequently develop malignant skin tumors as a result of exposure to sunlight. Their somatic cells have been shown to be highly sensitive *in vitro* to UV, and a defect in the repair of UV-induced damage at the molecular level has been demonstrated (3, 14). This observation, along with reports of *in vitro* sensitivity to UV in cell strains derived from patients with both Bloom's and Cockayne's syndromes (7, 17), has stimulated intense interest in a number of genetic diseases as models for the study of the role of DNA repair processes in mutagenesis and tumorigenesis.

More recently, it has been demonstrated that a second rare autosomal recessive disease, AT, may be an X-ray analog of XP. Patients with AT show an adverse reaction to radiotherapy, and experiments performed *in vitro* with fibroblasts derived from AT patients have shown that all cell strains examined to date show a marked sensitivity to killing by X-rays (14–16, 19, 22). Of additional interest is the observation that fibroblast strains and lymphocytes from AT heterozygotes show a degree of radiosensitivity intermediate between the homozygotes and normal controls (2, 16).

Fibroblasts from a patient with D deletion retinoblastoma have also been reported to be radiosensitive *in vitro*, although the magnitude of radiosensitivity was not as great as that reported for AT cell strains (21). This observation has recently been extended to a group of patients with hereditary or sporadic retinoblastoma (23). Fibroblasts from the former group, bearing the autosomal dominant form of the disease, were found to be more sensitive to X-irradiation than cell strains from patients with the sporadic form. The sporadic retinoblastoma cell strains, in turn, were indistinguishable in their radiosensitivity from normal controls. This observation is of particular interest since patients with hereditary retinoblastoma, as determined by bilateral involvement and/or a known family history of the disease, subsequently develop second tumors at sites distant from the orbit as well as within the irradiated field following radiotherapy (9, 11).

The interest in measuring the *in vitro* X-ray sensitivity of fibroblasts from patients with various genetic diseases as a model for investigating DNA repair has made the establishment of controlled base-line data on *in vitro* X-ray survival parameters of human diploid fibroblasts essential. The range of normal has been determined in many investigations for a large number of different types of animal cells both *in vivo* and *in vitro*. There are, however, relatively little reported data on the X-ray survival characteristics of human diploid cells. Recent improvements and standardizations in cell culture techniques have resulted in reasonable cloning efficiencies for most human diploid fibroblasts, along with the application of the colony formation assay for the determination of the radiosensitivity of a wide variety of cell strains. Establishment of base-line data on X-ray sensitivity is important in the identification of sensitive lines whether normal or malignant.

In this communication, we report on the *in vitro* radiobiological survival parameters of diploid fibroblast cell strains derived from patients with a variety of clinical syndromes associated with photosensitivity or X-ray sensitivity *in vivo*, a high incidence of spontaneous or radiation-induced neoplasia, precocious aging, chromosomal instability, or a characterized genetic anomaly. A parallel study done in a different laboratory by Arlett and Harcourt (1) accompanies this report. Included are strains from patients with the 3 forms of retinoblastoma, Fanconi's anemia, adenomatosis of the colon and rectum, progeria, several trisomies, and AT. Survival data from cell strains derived from 9 clinically normal individuals are also included in tabular or graphic form.

MATERIALS AND METHODS

Diploid fibroblast strains derived from foreskins, conjunctival biopsies, or punch biopsies of the skin were obtained from cell banks or other laboratories or were established in this laboratory. Sources of the cell strains are given in the tables. More than one-half of the strains used were karyotyped here or at the Institute for Medical Research, Camden, N. J., and their diploid chromosome number was confirmed.

Cultures were maintained at 37° in an atmosphere of 95%

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[3] The abbreviations used are: XP, xeroderma pigmentosum; AT, ataxia telangiectasia.

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air-5% CO_2. Growth medium consisted of Eagle's minimal essential medium with Earle's salts (Gibco F-15), supplemented with 15% fetal calf serum (Microbiological Associates, Walker, Md.), D-glucose (900 mg/liter), sodium pyruvate (0.66 mg/liter), and chlortetracycline (50 μg/ml; Lederle Laboratories, Pearl River, N. Y.). Three lots of serum were used in the course of these experiments. Serum was selected on the basis of reproducibility of growth rate, cloning efficiency, and response to radiation from lot to lot. Cells from each strain were studied at similar passage levels, usually the 4th to 25th mean population doubling, whenever possible.

For survival experiments, exponentially growing cells were detached with 0.25% trypsin in calcium-free and magnesium-free Earle's balanced salts solution and seeded at appropriate numbers into triplicate 10-cm-diameter Falcon tissue culture dishes containing 10 ml of culture medium. Feeder layers were not used, and the density of cells in these experiments never exceeded 4×10^4/dish. Dishes were irradiated 18 hr later (multiplicity = 1) at room temperature and ambient atmosphere with a 220 KVp General Electric Miximar unit operated at 15 ma and yielding a dose rate of 80 rads/min as determined by a Victoreen ionization chamber. The half-value layer of the beam was 0.5 mm of copper. At least 4 doses, ranging from 50 to 900 rads, were used per experiment. Following irradiation, the dishes were returned to the incubator, the medium was changed every 5 to 7 days, and the colonies were fixed and stained after 12 to 21 days. Satellite colony formation was not observed under these conditions. Experiments were scored under a dissecting microscope with colonies composed of 50 or more cells considered as survivors. Cloning efficiencies ranged from 0.4 to 44.0%. The calculated survival curve parameters were the D_0 in rads (inverse of the slope of the straight-line portion of the survival curve) and $\bar{n}$ (the y axis intercept, indicative of the magnitude of the shoulder region of the survival curve). They were derived from a least-squares linear regression analysis of points above 100 rads (except for the AT strains where the 50-rad survival data was included) from the number of experiments indicated ± S.E. Survival curves consistently showed a small but reproducible shoulder (n = 1.0 to 1.7); only D_0 values are therefore presented.

RESULTS

Tables 1 to 5 show the results of X-ray survival experiments performed on 53 human diploid fibroblast strains. The D_0's range from 43 (for a strain from a patient with AT) to 168 (for a strain from a patient with unilateral, sporadic retinoblastoma). In addition, a number of strains, including those from a member of a cancer-prone family with retinoblastoma, a patient bearing a partial trisomy of chromosome 13, and a progeroid patient, were intermediate in their radiosensitivity. Chart 1 shows the survival curves for these 5 strains.

The range of mean D_0's for normal strains was 140 to 152, a range much narrower than that reported by Arlett and Harcourt (1). Shown as the shaded area in Chart 1 is the overall range of normal as determined by the extremes of the standard errors of the strains in Table 1 (D_0 = 128 to 164 rads). Possible sources of variation are examined in the discussion. Survival data from 4 additional strains, all derived from the foreskins of clinically normal 3-day-old infants at approximately the same time and tested in parallel in a single experiment using only 3 doses, are plotted in Chart 2. The mean D_0 was 150 rads. Cloning efficiencies ranged from 8.3 to 44.0%. Of interest is the observation that there is very little variation in radiosensitivity among these strains or between these strains and the other normal strains shown in Table 1.

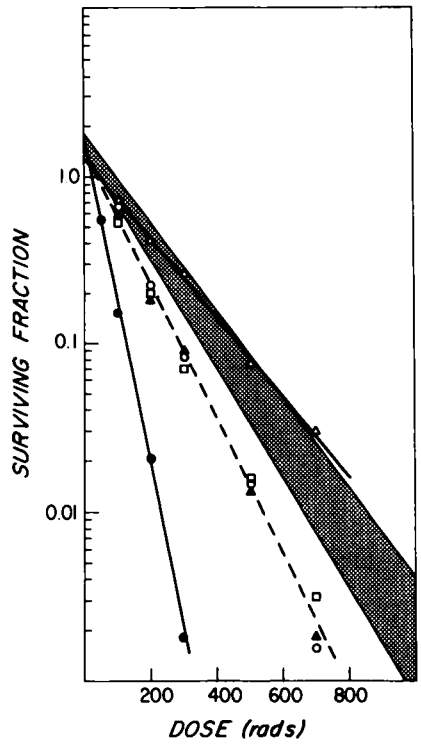

Chart 1. *In vitro* X-ray survival curves for: HL21, a sporadic unilateral retinoblastoma strain (△); CRL1343, an AT strain (●); AG1880, a familial cancer syndrome-retinoblastoma strain (▲); GM1663, a partial trisomy 13 strain (○); and KH2, a progeroid strain (□). *Shaded area* is delineated by the extremes of the standard errors of the 5 normal strains shown in Table 1.

Strains Showing Normal Radiosensitivity from Patients with Various Clinical Disorders. Table 2 shows cell strains derived from individuals with a variety of clinical syndromes in which the mean D_0 of the X-ray survival curves ranged from 126 to 159. Excluded from this table are several progeria and retinoblastoma strains which have been grouped together by disease in Tables 4 and 5. Included are strains derived from individuals with conditions associated with a predisposition to malignancy, such as Fanconi's anemia, basal cell nevus syndrome, adenomatosis of the colon and rectum, and XP, as well as strains bearing a characterized genetic defect at the chromosomal level (several trisomies, a partial trisomy, Lesch-Nyhan syndrome, ring 13, D deletion syndrome, and bilateral ocular melanoma). None of these strains was significantly more radiosensitive than the normal controls shown in Table 1.

X-Ray-sensitive Strains. Cell strains classified as sensitive are tabulated in Table 3. Again, several progeria and retinoblastoma strains are excluded from this table and grouped by disease in Tables 4 and 5. The X-ray-sensitive strains in Table 3 fall into 2 distinct groups: those from individuals with AT and those with retinoblastoma and/or a defect of chromosome 13. Strains from the rare autosomal recessive disease AT have been extensively described in the literature, and a sizable number of them are included in the accompanying report. Their D_0 values tend to cluster in the 40- to 50-rad range, making them the most sensitive mammalian cell type *in vitro* examined

Table 1

Diploid cell strains from normal individuals

Strain	Sex	Age	Source	No. of experiments	Cloning efficiency (%)	D_0 (rads)
Li106	M	46 yr	HSPH[a]	8	1.0-6.3	149 ± 7[b]
EX25	M	27 yr	HSPH	3	3.2-12.7	140 ± 4
BZWF	M	3 days	HSPH	3	17.8-28.5	142 ± 14
GM1381	F	9 mos.	IMR	3	0.6-1.7	148 ± 9
AG1518	M	3 days	IMR	4	11.6-25.9	152 ± 12
AG1522	M	3 days	IMR	4	11.9-44.0	145 ± 3

[a] HSPH, Laboratory of Radiobiology, Harvard School of Publich Health, Boston, Mass.; IMR, Institute for Medical Research, Camden, N. J.
[b] Mean ± S.E.

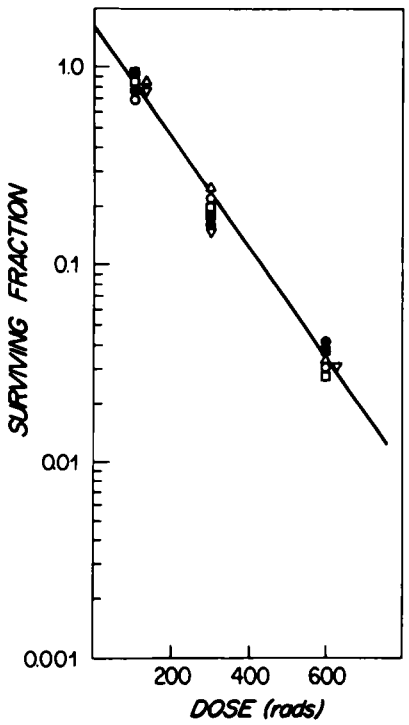

Chart 2. Composite *in vitro* X-ray survival curve for a single experiment involving 5 normal foreskin-derived diploid strains (AG1518 to AG1522) established in parallel. The mean D_0 for the group is 150 ± 4 rads.

to date. The second group of radiosensitive cell strains are from patients who bear either of the genetic forms of retinoblastoma (the D deletion type or the hereditary type), or whose cells bear a defect on the long arm of chromosome 13, a chromosome closely related to the etiology of retinoblastoma (13). Several other sensitive strains from patients with hereditary retinoblastoma are included in Table 5. Of interest is the fact that the D_0's for the retinoblastoma-chromosome 13-related strains cluster around the 90 to 100 range, suggesting an analogy to clustering of the AT strains. Sensitive strains from 3 individuals with progeria, a disease associated with clinical signs of premature aging or progeroid symptoms, are included in Table 4 and fall in the same range.

Strains from Progeric or Progeroid Individuals. The Hutchinson-Guilford progeria syndrome is a disease associated with clinical signs of premature aging (5). Some patients have progeric symptoms, but not all the manifestations of the classical Hutchinson-Guilford progeria syndrome. As shown in Table 4, strains from progeria patients formed a heterogeneous group ranging from normal [D_0 = 139 ± 9 (S.E.)] to sensitive (D_0 = 96 ± 6) in their radiosensitivity. KH2 and KH3 are strains derived from biopsies of the same individual with atypical progeria taken 4 years apart. Cells from an earlier biopsy of this same individual had a very abbreviated *in vitro* life span, did not form colonies, and were found to be defective in single-strand break rejoining as determined by an alkaline sucrose gradient technique (6). This defect was not detected in cells from subsequent biopsies in this or in other laboratories. Two other strains in this group, PRO1PV [also studied by Arlett and Harcourt (1)] and AG991, seem to be sensitive to killing by X-irradiation as well. On the basis of the clinical information available to us, there appears to be no clear relationship between the clinical syndrome (atypical or classical progeria) and radiosensitivity.

Hereditary and Sporadic Retinoblastoma Patients. Table 5 shows the results of survival experiments performed on 8 sporadic and 7 hereditary retinoblastoma patient-derived cell strains. Patients with the hereditary forms of the disease have an elevated incidence of malignant second tumors, both spontaneous and radiogenic. This is not observed in patients with sporadic retinoblastoma. As reported previously (19) and extended here with 3 additional strains, those from the hereditary group, considered together, are significantly more sensitive (p = 0.05) than those from the sporadic group. It is of interest that the D_0's for the hereditary group range from 132 rads for AG1131, just within the range of normal, down to 92 rads for FRBW. The latter and AG1880, with a D_0 of 98 rads, are similar in their radiosensitivity to AG1142, the strain derived from a retinoblastoma patient with a detectable deletion in chromosome 13. The heterogeneity in response among hereditary retinoblastoma strains is similar to that found among the progeric strains.

Relationship between *in Vitro* Radiosensitivity and Cloning Efficiency. We observed no consistent relationship between cloning efficiency and D_0 despite up to a 10-fold variation in cloning efficiency within several strains. In AG 1142, a D deletion retinoblastoma strain, the cloning efficiency over 6 experiments ranged from 0.7 to 6.5%, a difference of approximately 9-fold, and yet little variation was observed in the D_0 (94 ± 5 rads). With respect to absolute D_0, 2 strains, AT 3BI (AT) and CCL 122 (Fanconi's anemia) had similar (low) cloning efficiencies (0.8 to 1.1% and 0.8 to 1.0%, respectively), but the first was X-ray sensitive (D_0 = 43 ± 1 rads) and the second was normal (D_0 = 148 ± 16 rads) in its response. AT5BI, another AT strain, had a D_0 similar to AT 3BI (54 ± 4 rads) but a 20-fold higher cloning efficiency (14.0 to 21.3%). Strains from normal individuals varied by as much as 90-fold from one another in cloning efficiency (0.6 to 44.0%) and yet the D_0's of

Table 2

Diploid cell strains showing normal radiosensitivity

Strain	Clinical class	Sex	Age	Source	No. of experiments	Cloning efficiency (%)	D_0 (rads)
CCL54	Trisomy 21	M	2 mos.	ATCC[a]	2	9.8–13.0	143 ± 5[b]
CRL1175	Basal cell nevus syndrome	M	48 yr	ATCC	2	5.4–10.9	130 ± 6
CCL1113	Lesch-Nyhan disease	M	12 yr	ATCC	2	6.3–16.4	139 ± 6
CCL122	Fanconi's anemia	M	6 yr	ATCC	2	0.8–1.1	148 ± 16
GM1492	Bloom's syndrome	M		IMR	3	3.1–3.3	159 ± 4
CCL1223 (XP12BE)	XP(A)	F	7 yr	ATCC	3	7.9–23.0	159 ± 11
Ocmel	Bilateral ocular melanoma	F		HSPH	4	2.4–5.7	140 ± 13
VF#5	Adenomatosis of the colon and rectum	F	31 yr	L. Kopelovich[c]	2	4.0–12.7	152 ± 5
GM729	Ring 13 syndrome	M	14 yr	IMR	3	1.3–4.0	136 ± 5
GM84	Trisomy 22	M	1 mo.	IMR	3	5.2–9.0	135 ± 21
GM2719	Coloboma of the retina	M	28 yr	IMR	4	9.0–22.2	149 ± 11
GM250	D deletion syndrome	F	15 mos.	IMR	3	0.3–1.0	147 ± 6
AG1615	Wilm's tumor	M	9 yr	IMR	2	9.6–12.3	150 ± 6
GM1555	Partial trisomy 13	M	13 yr	IMR	3	3.4–4.9	126 ± 9
AG2718	D deletion retinoblastoma	F	11 yr	IMR	5	21.8–44.0	139 ± 8
FA1BI	Fanconi's anemia	M		C. Arlett[d]	3	1.5–3.0	160 ± 16

[a] ATCC, American Type Culture Collection, Rockville, Md.; IMR, Institute for Medical Research, Camden, N. J.; HSPH, Laboratory of Radiobiology, Harvard School of Public Health, Boston, Mass.
[b] Mean ± S.E.
[c] Memorial Sloan Kettering Cancer Center, New York, N. Y.
[d] MRC Cell Mutation Unit, University of Sussex, Brighton, England.

Table 3

Diploid cell strains showing sensitivity to X-rays

Strain	Clinical class	Sex	Age	Source	No. of experiments	Cloning efficiency (%)	D_0 (rads)
CRL1343	AT	F	7 yr	ATCC[a]	6	0.7–3.5	46 ± 3[b]
AT3BI	AT	M	4 yr	C. Arlett[c]	2	0.8–1.0	43 ± 1
AT5BI	AT	M	18 yr	C. Arlett	2	14.0–21.3	52 ± 4
AG1880	hereditary retinoblastoma, cancer-prone family	F	37 yr	IMR	6	4.3–20.5	98 ± 6
AG1142	D deletion retinoblastoma	F	3 yr	IMR	6	0.7–6.5	94 ± 5
GM85	Partial trisomy 13	M	14 days	IMR	4	1.8–3.8	95 ± 4
GM1663	Partial trisomy 13	M	14 days	IMR	3	0.8–4.7	75 ± 8
RbH1BI	Mother of bilateral retinoblastoma patient	F	23 yr	C. Arlett	5	10.8–20.0	95 ± 11

[a] ATCC, American Type Culture Collection, Rockville, Md.; IMR, Institute for Medical Research, Camden, N. J.
[b] Mean ± S.E.
[c] MRC Cell Mutation Unit, University of Sussex, Brighton, England.

Table 4

Diploid cell strains from patients with progeria

Strain	Sex	Age	Source	No. of experiments	Cloning efficiency (%)	D_0 (rads)
KH2	M	16 yr	HSPH[a]	3	1.5–2.3	114 ± 6[b]
KH3/AG1710	M	19 yr	HSPH/IMR	2	4.2–7.6	96 ± 6
EX442			MGH	2	8.7–19.3	136 ± 9
AG991	M	4 yr	IMR	2	0.4–1.8	117 ± 6
AG1972	M	14 yr	IMR	2	1.0–2.0	134 ± 16
A77	M	14 yr	HSPH	2	2.3–11.4	139 ± 9
PRO1PV			C. Arlett[c]	2	0.6–5.8	118 ± 11

[a] HSPH, Laboratory of Radiobiology, Harvard School of Public Health, Boston, Mass.; IMR, Institute for Medical Research, Camden, N. J.; MGH, Clinical Genetics Unit, Massachusetts General Hospital, Boston, Mass.
[b] Mean ± S.E.
[c] MRC Cell Mutation Unit, University of Sussex, Brighton, England.

the strains with the highest (AG 1522) and the lowest (GM 1381) cloning efficiencies had similar D_0's (145 ± 3 and 148 ± 9 rads, respectively). Indeed, an analysis of the results of all experiments included in Tables 1 to 5 showed no systematic relationship between cloning efficiency and X-ray sensitivity.

DISCUSSION

Before relevant comparisons can be made among strains derived from patients with a variety of clinical conditions, it is important to determine the range of radiosensitivity for normal diploid fibroblast strains. In the accompanying communication (1), Arlett and Harcourt consider the range of "normal" to be delineated by the mean D_0's of those cell strains, regardless of clinical diagnosis, not significantly different from either of the 2 reference strains IBR and 2BI. Their overall range of normal, expressed as the extremes of the standard errors of the most radioresistant and radiosensitive normal cell strains, is 90 to 207 rads (97 ± 7 rads for 525LAD to 180 ± 27 rads for GM1492). Our range of normal determined through multiple experiments with 6 cell strains derived from clinically normal individuals is 128 to 164 rads, a far narrower range than that

Table 5
Diploid cell strains from patients with sporadic or hereditary retinoblastoma

Strain	Sex	Age	Source	No. of experiments	Cloning efficiency (%)	D_0 (rads)
Sporadic						
HL21	M	1 yr	MEEI[a]	3	13.6–14.7	168 ± 11[b]
RbME	M	9 mos.	HSPH	3	12.8–34.2	167 ± 7
RbCH	F	6 mos.	HSPH	3	2.8–9.2	164 ± 7
HL23B	M	1 yr	MEEI	3	11.3–17.0	144 ± 7
AG1947	M	2 yr	IMR	3	11.5–22.8	142 ± 6
AG1946	M	18 mos.	IMR	3	5.5–21.0	141 ± 7
AG1979	M	2 yr	IMR	3	0.93–2.5	135 ± 8
Hereditary						
AG1131	F	9 mos.	IMR	3	3.0–16.5	132 ± 4
AG1980/1408[c]	F	1 yr	IMR	6	2.0–12.9	128 ± 11
AG1879	F	11 yr	IMR	5	1.4–8.0	128 ± 9
AG1123	F	9 mos.	IMR	3	1.2–12.4	121 ± 4
AG1880	F	37 yr	IMR	6	4.3–20.5	98 ± 6
FRBW	M	9 mos.	HSPH	3	11.6–15.4	92 ± 5

[a] MEEI, Massachusetts Eye and Ear Infirmary, Boston, Mass.; HSPH, Laboratory of Radiobiology, Harvard School of Public Health, Boston, Mass.; IMR, Institute for Medical Research, Camden, N. J.
[b] Mean ± S.E.
[c] Since first publishing these data (19), we have learned that AG1980 and AG1408 were derived from biopsies of the same patient taken about 1 year apart. Shown here are the pooled data for both strains.

of Arlett and Harcourt (1). The discrepancy results in part from their use of 2BI as a reference strain. This strain, with a mean D_0 of 124 rads, is more sensitive than all our normal strains and is significantly more sensitive than their second reference strain 1BR (mean D_0 = 160 rads) and results in the inclusion of many cell strains as normal which we would have considered to be "sensitive." We suggest that this strain and normal strain C62TO (mean D_0 = 101 rads) may possibly be from individuals heterozygous for a disease associated with an increased X-ray sensitivity. Recent estimates for the number of AT and Fanconi's anemia heterozygotes in the population at large have ranged as high as 2% (18).

In an earlier study, Weichselbaum *et al.* (20) reported on the *in vitro* radiosensitivity of several strains of diploid fibroblasts derived from patients who demonstrated unusually sensitive clinical responses to ionizing radiation during radiotherapy. The D_0 for 2 of these strains was 101 and 108 rads. Because the D_0 for one of the normal strains included in the study was 112 rads, it was concluded that the cells from these 2 radiosensitive patients were not abnormally sensitive to the lethal effects of X-irradiation *in vitro*. Based on the results of the current investigation, this conclusion may have been incorrect. Unfortunately, these strains are no longer available for testing under present conditions. There was significant variability in the experimental results for each cell strain in the first study, as well as an extrapolation number of less than unity for several strains. Retrospectively, this variability probably resulted because 3 different investigators performed these experiments at different times under variable culture conditions. The results in the present investigation were obtained by one individual under constant conditions with large, carefully selected lots of serum.

The *in vitro* radiosensitivity of cell strains from patients with AT and hereditary retinoblastoma is of considerable interest since these patients as a group have a higher than expected incidence of solid tumors, leukemia, and lymphoma. In an earlier communication (23), we discussed the appropriateness of retinoblastoma over AT as a model for *in vivo* mutagenesis, tumorigenesis, and the issue of genetic susceptibility to cancer. Among hereditary retinoblastoma patients, tumors are mesenchymal in origin rather than primarily lymphoreticular as is the case with AT, with no observed abnormalities of the immune system. However, malignancy in AT may not be solely due to immune perturbations, and the lymphoreticular system may simply allow the expression of phenotypic neoplasia (8, 10).

The differences observed previously in this laboratory between hereditary and sporadic retinoblastoma patients, as well as the radiosensitivity of a cell strain from a patient with the D deletion form of the disease, are confirmed by Arlett and Harcourt (1).

Our data suggest no marked X-ray sensitivity among cell strains representing a variety of diseases in which chromosomal instability, increased incidence of neoplasia, or precancerous lesions are observed. This observation is for the most part supported by Arlett and Harcourt (1). There are, however, several cases of heterogeneity of radiosensitivity seen in both laboratories among patients with a particular disease. Among patients with progeria, for example, we have observed a range of D_0's from 96 to 140. It is possible that in a number of the other diseases represented by single patients, the normal observation was fortuitous. At the cell survival level, this heterogeneity is analogous to the reported response of cell strains derived from XP patients to UV (3).

In only one case, that of Fanconi's anemia strain FA1BI, do our results differ significantly from those of Arlett and Harcourt (1). Possible reasons for this difference are currently under investigation. Shown in Table 6 are the 7 strains which were examined by both laboratories. With the exception of FA1BI, a remarkable degree of agreement exists between our laboratories in the determination of *in vitro* radiosensitivity, despite significantly different methodologies. The differences in cloning efficiencies between laboratories (significantly higher with the feeder layer technique of Arlett and Harcourt), coupled with an agreement with respect to the D_0's of the strains involved, further point out the lack of correlation between cloning efficiency and D_0 demonstrated by each of our laboratories.

We agree with Arlett and Harcourt (1) that no cell strain studied to date seems defective in both UV and X-ray repair. Deficiencies in either the UV or X-ray repair pathway seem, in some cases, each to lead to the phenotypic expression of malignancy. The determination of clonogenic survival *in vitro*

Table 6
Comparison of radiosensitivities and cloning efficiencies for 7 strains examined in parallel by 2 laboratories

	Weichselbaum *et al.*		Arlett and Harcourt (1)	
Strain	D_0 (rads)	Cloning efficiency (%)	D_0 (rads)	Cloning efficiency (%)
RbH1Bl	95 ± 11[a]	10.8–20.0	92 ± 2	25–31
AG1142	94 ± 5	0.7–6.5	89 ± 4	8
PRO1PV	118 ± 11	0.6–5.8	96 ± 4	11–35
AT3Bl	43 ± 1	0.8–1.0	60 ± 10	2–41
AT5Bl	52 ± 4	14.0–21.3	43 ± 1	40–54
GM1492	159 ± 4	3.1–3.3	180 ± 27	3–25
FA1Bl	160 ± 16	1.5–3.0	69 ± 5	1–18

[a] Mean ± S.E.

seems to be a useful method for screening syndromes which may involve a deficiency in DNA repair. Physicochemical investigations, extensively used in the case of XP, have fallen short when applied to X-ray-sensitive strains. Paterson (15) has described 2 complementation groups among AT strains, one of which is deficient in the repair of X-ray-induced lesions sensitive to attack by an endonuclease purified from *Micrococcus luteus*. Survival, however, does not appear to be dependent on the involved molecular repair process, since strains from both groups show the same hypersensitivity to the lethal effects of X-rays.

It is of interest that one group has used the X-ray survival curve method for confirming the diagnosis of patients with AT (4). In another case, cell strains from clinically normal parents of a patient with bilateral (and therefore hereditary) retinoblastoma with no family history of retinoblastoma were shown by Arlett and Harcourt (1) to be X-ray sensitive. In light of Knudson's (12) hypothesis, this observation may represent the introduction of a heritable mutation leading to a predisposition to retinoblastoma and possibly to the second tumors associated with this disease into a family lineage. Analysis of similar families as well as "cancer-prone" families may lead to a better understanding of the genetics of susceptibility to cancer. *In vitro* investigation of the spontaneous and induced mutation rates in cells derived from such individuals, along with biochemical investigations on the nature of the repair deficiency associated with radiosensitivity, may provide information relevant to the mechanisms for tumorigenesis *in vivo*.

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