[CANCER RESEARCH 40, 926-932, March 1980]
0008-5472/80/0040-0000$02.00

Survey of Radiosensitivity in a Variety of Human Cell Strains[1]

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ABSTRACT

γ-Ray sensitivity for cell killing was assayed in 54 human cell strains, including some derived from individuals suffering from certain heritable diseases. The overall range of D_0 values in this study was 38 to 180 rads, indicating a considerable range of variability in humans. The normal sensitivity was described by a range of D_0 values of 97 to 180 rads. All ten ataxia telangiectasia cell strains tested proved radiosensitive and gave a mean D_0 value of 57 ± 15 (S.E.) rads, and these represent the most radiosensitive human skin fibroblasts currently available. Representative cell strains from familial retinoblastoma, Fanconi's anemia, and Hutchinson-Gilford progeria occupied positions of intermediate sensitivity, as did one of two ataxia telangiectasia heterozygotes.

Six xeroderma pigmentosum cell strains together with two Cockayne's syndrome cell strains (all known to be sensitive to ultraviolet light) fell into the normal range, indicating an absence of cross-sensitivity between ultraviolet light and γ-irradiation.

INTRODUCTION

The study of the response of human cells to UV (22) was stimulated by the discovery of an association between cellular sensitivity (4, 9, 22, 26), DNA repair (9, 22), and increased cancer proneness in exposed regions of the body in XP[3] patients (35). XP cells which are defective in excision repair are extremely sensitive to the lethal and mutagenic effects of UV (26); however, they do exhibit a range in response (2). Those XP cells which are competent for excision repair but defective in postreplicational repair show normal or near-normal levels of survival (4, 22) but are hypersensitive to the mutagenic effects of UV (26). Cell strains derived from patients with Cockayne's syndrome (37), Bloom's syndrome (17), and a sun-sensitive individual (strain 11961) from no defined syndrome (5) have all been shown to exhibit enhanced sensitivity to UV, although we have unpublished evidence indicating that not all Bloom's cells are sensitive. In none of these cases has any correlated defect in DNA repair been demonstrated which makes it necessary to invoke the existence of new, undiscovered repair processes to account for their sensitivity. It also implies that the repair defects in XP are important in carcinogenesis.

The heterogenous response of human cell strains to UV leads us to anticipate a similar variability for other DNA-damaging agents. With ionizing radiation, reports of enhanced cellular sensitivity have been made for AT cells (39) and for a retinoblastoma cell strain bearing a D deletion (40). For AT, defects in the repair of γ-ray-induced base damage have been reported for some but not all strains (32). A reduction in the ability of AT cell strains to increase the priming activity of γ-irradiated DNA in a DNA polymerase assay has been demonstrated (20). There is, as yet, no evidence for defects in the repair capacity for the sensitive retinoblastoma cells. The enhanced frequency of tumors in AT (18) and familial retinoblastoma patients (40) suggests that defects in repair might in these conditions, like XP, be correlated with carcinogenesis. By further extension of the UV model, we might expect to discover additional instances of sensitivity to ionizing radiation. Accordingly, in this and the accompanying contribution (41), we have attempted a survey of cellular sensitivity among cell strains from a variety of human conditions.

MATERIALS AND METHODS

Cell Culture. The source and description of the cell strains are provided in Table 1. Some were received as cell strains from other investigators; others were initiated from skin biopsies in our own laboratory using conventional techniques (9, 27).

The stock cultures were maintained in Eagle's minimal essential medium supplemented with 15% fetal calf serum (Flow Laboratories, Irvine, Scotland) in 75- or 175-sq cm plastic flasks (Corning, Scientific Supplies Ltd., London, England; or Nunc, Gibco Bio-cult, Paisley, Scotland). We attempt to maintain the stocks in such a way that they are normally subcultured only for an experiment and are not subjected to a loss of division potential by routine subculturing. We believe that actively growing cultures, *i.e.*, showing many cells in division, have a higher colony-forming potential than confluent static cultures. Thus, there is a tendency by many investigators to use stock cultures at approximately weekly intervals and to keep only sufficient cultures going to meet immediate requirements.

On the first day of an experiment, cultures were dispersed using 0.25% trypsin (Difco Laboratories Ltd., West Molesey, Surrey, England) in Dulbecco's Buffer A for 20 min at 37°. The culture vessel was then shaken to loosen any attached cells, and any clumps of cells were broken up by vigorous pipetting. The suspension of cells was then spun down in a MSE minor centrifuge (Measuring and Scientific Equipment, Crawley, Sussex, England) at approximately 800 × *g* for 3 min, the supernatant was discarded, and the pellet was broken up by flicking the base of the centrifuge tube (Sterilin, Ltd., Teddington, Middlesex, England) with the index finger. The cells were then resuspended in complete medium and counted on a Hawksley hemocytometer slide (Arnold Horwell Ltd., London, England). The original and any new stock culture vessels were then reinoculated at a density appropriate to the surface area (~5 × 10^5 for 75 sq cm and ~1 × 10^6 for 175 sq cm), and any new vessels were gassed with a 5% CO_2 in air mixture. A new 75-sq cm vessel was inoculated with 5 to 10 × 10^5 cells in 15 ml medium for use in the experiment the next day.

[1] This work was supported in part by Euratom Contract 111 953 72E.

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

Received December 7, 1978; accepted November 28, 1979.

The plating technique was that of Cox and Masson (11), which uses a feeder layer of homologous cells. Thus, part of the cell suspension was diluted to 6×10^4 cells/ml in complete medium and sterilized with a dose of 5 krads γ-irradiation. This suspension was then diluted 10-fold with Eagle's medium supplemented with 15% newborn calf serum (Gibco-Biocult), and 10 ml were dispensed into the appropriate number of 9-cm-diameter disposable vented dishes (Nunc). We have elected to use calf serum for the experimental but not the maintenance phase of these studies on the grounds of economy; it is usually necessary to test a number of samples before a batch with good growth-promoting properties is identified. These dishes are then incubated overnight with a 5% CO_2 in air atmosphere in a LEEC injection model incubator or sealed up in plastic cake boxes in an anhydric incubator with the gas atmosphere provided by interacting a mixture of tartaric acid and sodium bicarbonate (3).

On the second day, the 75-sq cm culture was trypsinized as described and spun down with 5 ml of complete medium, which aids in the formation of the pellet. A dilution series was then made up in complete medium, with the concentration of cells at each dilution depending upon the intrinsic cloning efficiency and the sensitivity of the particular cell strain. The aim is to achieve a maximum of 100 viable units/ml of suspension after each treatment level. The suspensions were then irradiated, and 1-ml aliquots were added to the feeder layer plates, usually 6 per dose point. The cells were spread evenly by rocking the plates, which were then reincubated for 15 to 16 days using the box system (2). Where more than one cell strain was used, the plates were mixed in the boxes to achieve some degree of randomization. At the end of the growth period, 2 to 3 ml of 1% methylene blue (East Anglia Chemicals, Hadleigh, Suffolk, England) was added to each plate for a minimum of 1 hr. The medium, plus stain, was then poured off, and the plates were drained dry, rinsed with tap water, and scored either wet or dry at a stereomicroscope. We use the criterion of a clone containing 50 or more cells being a survivor. Since these experiments covered a period of some 40 months, there has been some evolution to the above method; originally, feeder layers and irradiated cells were plated at the same time, and a change of medium was made after 7 days. These practices have been discontinued without any change in the quality of the data.

Irradiation. All irradiations were performed in air with radiation from a ^{60}Co source at ambient temperature. Doses were given as seconds of treatment, and, since over the time course of these experiments the dose rate decayed from 3.19 to 1.80 krads/min, it was appropriate to convert these timed doses to the dose rate in rads on a monthly basis when computing the results. The accuracy of these timed doses was checked by irradiating lithium fluoride crystals in the place of cells and measuring the absorbed doses with respect to a standard calibrated source. The standard error was ±13% for all treatment times. Fortunately, experiments with the radiosensitive AT cell strains took place at lower dose rates. A limited number of irradiations were performed using a Betratron radiotherapy cobalt source at the Royal Sussex Hospital, Brighton, England. We are indebted to Peter Cross for making this facility available to us and for providing information on dosimetry.

Data Processing. After clone counting, survival was calculated using the counts on unirradiated plates as 100% survival. The data were processed by computer to fit a linear regression (we are indebted to Lynne Mayne for assistance with this facility). With the possible exception of cell strain 1BR (N = 1.3), all other cell strains gave an extrapolation number (N) equal to unity.

Three classes of data are provided. In the first, a number of replicate experiments were performed, each based upon the response to 4 doses of radiation spanning a range of survival from 40 to 0.01% survival, depending upon sensitivity; such data are indicated in Table 1 by a mean D_0 value ± S.E. The second class of data was computed from a single dose-response curve; here the D_0 value is indicated without an estimate of standard error. A third class of data was obtained from a screening test using single doses (380 to 400 rads); the D_0 value obtained by extrapolation from zero dose through the observed point is indicated by *brackets*. This class represents instances where it was believed that the result from the single-dose assay of survival did not justify further replication. The difference between mean D_0 values was tested by Student's t test against the values for cell strains 1BR and 2BI and was regarded as significant when p is less than 0.05 (Table 1).

RESULTS

The results are summarized in Table 1, and some representative curves are illustrated in Chart 1. A number of technical points need to be made before reviewing the results. The first concerns the effect of serum batch; our observations covered a period of 40 months, and during this time 8 batches of serum were used. Cell strains 2BI and 1BR are regarded as the reference normal material (see below), since most of the experiments on the other cell strains involved pairing with one or the other of them. It was thus possible to test for the influence of serum batch with these 2 cell strains; no effect was detected. The second concerns cloning efficiency. Cell strains 2BI and 1BR grow and clone well, but variation in the efficiency of cloning was observed during the period of these studies; indeed, cloning efficiencies as low as 3 and 14%, respectively, were recorded. A small but nonsignificant negative correlation between cloning efficiency and sensitivity was detected. This suggests that poorer cloning efficiency might lead to a higher survival and that we were thus unlikely to overestimate sensitivity in those cell strains which were only available to us at late passage and which did not clone well under our culturing conditions. Finally, for 4 cell strains, 1BR, 2BI, 19BR, and 11961, independently derived biopsies were taken, and thus some check was possible for differences between distinct cell strains obtained from the same individual. The mean D_0 and the standard errors of these cell strains are shown in Table 2 and indicate that there is no effect of the biopsy.

The reference cell strains 1BR and 2BI were established from individuals with no specific clinical defects, who should be described as normal but who are significantly different in γ-ray sensitivity in this series of experiments. It was decided, therefore, to assign sensitivity to those cell strains which were significantly more sensitive than 2BI. In this way, a minimal assignment of sensitivity was achieved since, clearly, more cell strains would prove sensitive to 1BR than to 2BI. In those cases where a statistical evaluation was possible, 11 cell strains were not more sensitive than 2BI, although they exhibited a lower D_0 value. In 10 instances, greater D_0 values were not significantly

Table 1

Cell survival after γ-irradiation of various cell strains

Strain	Description	Sex	Age at biopsy (yr)	No. of experiments	Range of cloning efficiency	D_0	*t* test vs. 2BI	*t* test vs. 1BR
1. 1BR	Normal	M	23	15	14–100	160 ± 7[a]	Sig.[b]	
2. 2BI[c]	Normal	M	26	14	3–100	124 ± 6		Sig.
3. C62TO[d]	Normal	M	35	2	5, 14	101 ± 6		Sig.
4. 1BI[c]	Normal	M	35	2	20, 44	130 ± 4		
5. 4BR[e]	Lesch-Nyhan hemazygote (28)	M	3	2	10, 14	125 ± 11		Sig.
6. 19BR[f]	Normal, light-sensitive skin	M	75	7	8–32	116 ± 7		Sig.
7. 21BR[f]	Normal, light-sensitive dermatitis	M	72	2	3, 7	118 ± 12		
8. 22BR[f]	Normal, multiple rodent ulcers	F	72	3	7–23	151 ± 23		
9. 25BR[f]	Solar keratosis	M	64	1	50	[120]		
10. 30BR[g]	Darier's disease (29)	F	34	1	75	[121]		
11. 32BR[g]	Darier's disease	M	20	1	53	[123]		
12. HG885[h]	Meiotic defect	M		2	13, 17	113 ± 6		Sig.
13. 75RD24[i]	Multiple tumors			3	10–36	163 ± 10	Sig.	
14. XP1BR[j]	XP (9), Complementation Group D	F	41	1	23	126		
15. XP4BR[j]	XP, unassigned	M	13	1	13	129		
16. XP4LO[j]	XP, Complementation Group A	M	10	2	53, 64	128 ± 2		
17. XP4RO[k]	XP, Complementation Group C	F	16	2	14, 41	129 ± 13		
18. XP7TA[k]	XP, variant (23)	F	20	1	16	162		
19. XP30RO[k]	XP, variant	M	30	3	12–34	147 ± 16		
20. XP33RO[k]	Sun-sensitive hyperkeratosis, no UV cellular sensitivity	M	33	1	35	[120]		
21. XP34RO[k]	Spinocellular tumor on cheek	M	13	1	44	[110]		
22. HG916[h]	Bloom's syndrome	F	1	2	1, 5	108 ± 4		Sig.
23. GM1492[l]	Bloom's syndrome (16)	M		2	3, 25	180 ± 27	Sig.	
24. CS7SE[m]	Cockayne's syndrome (25)	F	9	1	2	[131]		
25. CS5HO[m]	Cockayne's syndrome	M	21	1	4	[131]		
26. BCNS[c] 1BI	Gorlin's syndrome (34)	M	38	6	6–70	144 ± 10		
27. BCNS[c] 2BI	Gorlin's syndrome	M	74	5	14–39	119 ± 12		
28. BCNS[c] 3BI	Gorlin's syndrome	M	14	6	16–50	134 ± 8		
29. 11961[n]	Light-sensitive, UV cellular sensitive (5)	M	1.5	4	7–33	143 ± 3		
30. 04516[o]	Multiple self-healing squamous epithelioma (14)	F	32	1	30	121		
31. AT1BI[c]	AT (6)	M	7	2	1–31	79 ± 19	Sig.	
32. AT3BI[c]	AT	M	4	6	2–41	60 ± 10	Sig.	
33. AT4BI[c]	AT	M	6	8	34–90	50 ± 3	Sig.	
34. AT5BI[c]	AT	M	18	3	40–54	43 ± 1	Sig.	
35. AT6BI[c]	AT	F		1	6	48		
36. AT7BI[c]	AT	F	16	1	1	[38]		
37. ATH7BI[c]	AT heterozygote, mother of AT7BI	F	39	2	41, 44	106 ± 2	Sig.	
38. AT1LO[p]	Atypical ataxia	M	11	2	10, 28	88 ± 4	Sig.	
39. AT181TO[d]	AT	F	21	1	1	56		
40. AT95TO[d]	AT	F	10	3	3–8	63 ± 10	Sig.	
41. AT97TO[d]	AT	M	16	3	4–10	41 ± 2	Sig.	
42. ATH96TO[d]	AT heterozygote, mother of AT95 and 97TO	F		4	8–37	96 ± 3	Sig.	
43. PRO1PV[q]	Hutchinson-Gilford progeria (12)			3	11–35	96 ± 4	Sig.	Sig.
44. 525LAD[i]	Werner's syndrome (13)	M	28	2	16–24	97 ± 7		Sig.
45. 531LAD[i]	Werner's syndrome	M	31	1	7	135		
46. PRO3RO[i]	Hallerman-Streif progeria (36)			3	5–10	109 ± 9		Sig.
47. PRO1RO[i]	Originally thought to be Werner's syndrome; probably not	M	37	2	46, 56	108 ± 5		Sig.
48. FA1BI[c]	Fanconi's anemia (29)	M	22	4	1–18	69 ± 5	Sig.	
49. Rb1BI[c]	Sporadic bilateral retinoblastoma (1), normal chromosome constitution	M	5	4	1–9	72 ± 4	Sig.	
50. RbH1BI[c]	Mother of Rb1BI	F	23	3	25–31	92 ± 2	Sig.	
51. RbH2BI[c]	Father of Rb1BI	M	27	2	12–22	98 ± 9		Sig.

Table 1—*Continued*

Strain	Description	Sex	Age at biopsy (yr)	No. of experiments	Range of cloning efficiency	D_0	*t* test vs. 2BI	*t* test vs. 1BR
52. A5570[r]	Familial bilateral retinoblastoma, normal chromosome constitution	M	3	3	5–23	84 ± 12	Sig.	
53. A7983[r]	Sporadic retinoblastoma	F	2	3	11–33	116		
54. GM1142[s]	Retinoblastoma, D deletion type	F	2	2	8	89 ± 4	Sig.	

[a] Mean ± S.E.
[b] Sig., significant.
[c] Supplied by Dr. A. M. R. Taylor, Birmingham, England.
[d] Supplied by Dr. David Hoar, Toronto, Ontario, Cananda.
[e] Supplied by Dr. R. O. McKeran, London, England.
[f] Supplied by Dr. Patrick Hall-Smith, Brighton, England.
[g] Supplied by Dr. P. V. Harrison, Newcastle, England.
[h] Supplied by Dr. J. German III, New York, N. Y.
[i] Supplied by Dr. M. F. Niermeijer, Rotterdam, The Netherlands.
[j] Supplied by Dr. D. A. Burns, London, England.
[k] Supplied by Dr. B. Bootsma, Rotterdam, The Netherlands.
[l] Supplied by The Human Genetic Mutant Cell Repository, Camden, N. J.
[m] Supplied by Dr. R. D. Schmickel, Mich. CS75E is also known as 7447 and GM1428; CS5HO is also known as GM1098.
[n] Supplied by Dr. F. Giannelli, London, England.
[o] Supplied by Dr. M. A. Ferguson-Smith, Glasgow, Scotland.
[p] Supplied by Dr. R. Cox, Harwell, England.
[q] Supplied by Dr. A. Falaschi, Pavia, Italy.
[r] Supplied by Professor D. G. Harnden, Birmingham, England.
[s] Supplied by Dr. J. B. Little, Boston, Mass.

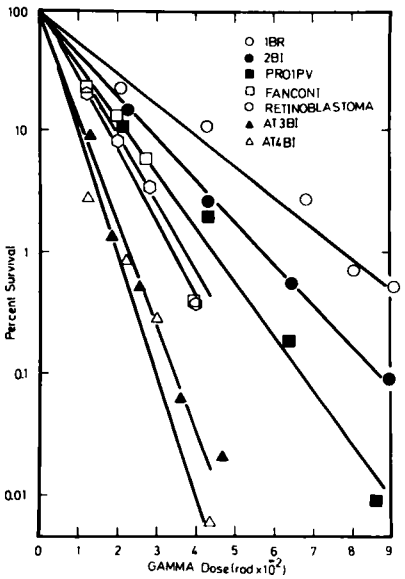

Chart 1. Cell survival of various cell strains after γ-ray irradiation. The data are taken from the full survival curves used to compute D_0 values and are given in Table 1. The following strain numbers refer to Table 1. ○, 1BR (strain 1); ●, 2BI (strain 2); ■, PRO1PV (strain 43); □, FA1BI (strain 48); ⬡, Rb1BI (strain 49); △, AT3BI (strain 32); ▲, AT4BI (strain 33). *Curves* fitted by eye.

different, while 1BR, 75RD24, and GM1492 were significantly more resistant. The range of D_0 values which were not significantly different from that of 2BI was 97 to 151 rads, which can be extended to 180 if the significantly more resistant set are included. We thus regard the D_0 range of 97 to 180 rads as being a summary of the range for 24 cell strains which did not differ from the 2 strains from the normal individuals. A further 13 cell strains, where no statistical evaluation is possible, fall within the range. The mean of the 37 cell strains was 126 ± 17 (S.E.) rads, which is remarkably close to the value for 2BI and gives some justification for the use of this strain as a reference. Many of these cell strains were from individuals who

Table 2

Cell survival after γ-irradiation measured in cell strains from independently derived biopsies from the same individual

Strain	Biopsy	D_0
1BR	1	169 ± 7[a]
	2	139 ± 13
2BI	1	124 ± 22
	2	131
19BR	1	120 ± 9
	2	114 ± 14
11961	1	158
	2	139 ± 9

[a] Mean ± S.E.

suffered from a variety of clinical disorders of greater or lesser severity (Table 1) and thus could not be described as normal in the clinical sense. However, we wish to define them as having normal radiosensitivity.

The 10 AT cell strains tested were more sensitive than was 2BI, although in 2 cases (AT7BI and AT181TO) no statistical comparison was possible. The mean D_0 value was 57 ± 15 rads. The ataxia data were heterogeneous when tested statistically but became homogeneous when AT1LO was excluded. All of these cell strains with the exception of AT1LO[4] were established from patients where there was no doubt about the clinical definition. Two AT heterozygotes were tested. One of them (ATH96TO) was more sensitive than 2BI: the second (ATH7BI) was not, although it was significantly more sensitive than 2BR.

Among the other sensitive cell strains was the D-deletion retinoblastoma (GM1142; D_0 = 89 ± 4 rads) which was studied by Weichselbaum *et al.* (40). One other retinoblastoma cell strain (Rb1BI; D_0 = 72 ± 4), from a sporadic bilateral case with a normal chromosome constitution was more sensitive than 2BI. Of considerable interest is the sensitivity of one of

[4] R. Cox, personal communication.

the heterozygotes (RbH1BI), the mother of Rb1BI. A cell strain (A5570) established from a familial bilateral case of retinoblastoma proved sensitive (D_0 = 84 ± 12), while material from a unilateral sporadic case (A7983) was not sensitive. Neither of these cases bears any chromosome abnormalities.[5]

We have examined a number of progeric cell strains, one of which (PRO1PV; D_0 = 96 ± 4) was more sensitive than 2BI. A single Fanconi's anemia cell strain (FA1BI) was also found to be γ-ray sensitive (D_0 = 69 ± 5).

Among those which could not be distinguished from 2BI in terms of γ-ray sensitivity were 6 XP cell strains, single representatives of Complementation Groups A (XP4LO), C (XP4RO), D (XP1BR), and unassigned (XP4BR), all with defects in excision repair, and 2 XP variant cell strains (XP7TA and XP30RO) defective in postreplication repair (22). The 2 strains designated XP33RO and XP34RO were not considered to be genuine XP's because of the limited clinical descriptions and since the cellular responses for survival and repair[6] were not characteristic of XP cells. Two Bloom's cell strains (HG916 and GM1492) were not sensitive, and neither were the 2 UV-sensitive Cockayne's syndrome cell strains (CS7SE and CS5HO).

DISCUSSION

Considerable variation in the response to γ-irradiation for cell killing was observed, with the overall range in mean D_0 values being 38 to 180 rads. Our experimental design used cell strains from 2 clinically normal individuals (1BR and 2BI), who were selected at random, as references, and most of the other cell strains were checked for radiosensitivity in tests when paired with one or the other of them. The considerable volume of data generated for these 2 cell strains made it possible to rule out, for them at least, any influence of serum batch, cloning efficiency, or particular biopsy on radiosensitivity. We are not able to exclude the possibility of any cell cycle effects confounding the results, but an analysis of cell cycle effects must await the development of techniques to synchronize cells which can be applied with efficiency to human material.

Some 27 cell strains did not differ in radiosensitivity from 1BR and 2BI, and this provides us with a range of D_0 values from 97 to 180 rads which we regard as being representative of the normal range, although the cells were, in many cases, taken from individuals who were not normal clinically. This range is in agreement with that obtained for normal individuals using essentially similar experimental techniques (10, 11) but with X-ray not γ-ray, irradiation. Cox[4] has noted a skewed response with more representatives at the lower than at the upper end of the range, a result which is confirmed by our observations. Weichelsbaum *et al.* (41) provide a range of D_0 values of 128 to 164 rads based upon a set of cell strains from 6 normal individuals exposed to X-rays. The range, while being very similar to ours, has a higher mean which is surprising in the light of the difference in the relative biological effectiveness between X-rays and γ-rays. It is not known if some of the more sensitive cell strains among those defined as showing normal sensitivity might be heterozygous for radiosensitive genes. There is a requirement to increase the size of the sample of the normal population to resolve this difference.

Normal cellular sensitivity was confirmed in Gorlin's syndrome (34), which has given some indications of radiosensitivity (19). Six XP cell strains which, with the exception of the variants, show marked sensitivity to UV (22)[7] been tested, and thus far none has proved sensitive to γ-irradiation. The identification of X-ray-sensitive XP's remains a possibility since their existence has been reported (42). Other UV-sensitive cell strains from Cockayne's syndrome (37) and a sun-sensitive individual with cellular sensitivity to UV (5) are also not sensitive to γ-irradiation. Thus, in no case was UV sensitivity associated with cross-sensitivity to ionizing radiation, in marked contrast to the situation in bacteria (7). This implies that the repair pathways which handle UV damage are distinguishable in at least one step from those which are concerned with γ-ray damage. Bloom's syndrome cells which we have not been able to confirm as UV sensitive (17) have also proved not to be sensitive to γ-irradiation.

All AT cell strains were more sensitive than 2BI. Some variation was observed, but, if we include a further set of 7 strains (10), then it becomes difficult to avoid the conclusion that cellular radiosensitivity might be taken as a diagnostic for AT. Cellular sensitivity is at present, however, not feasible as a prenatal test for AT because of the time taken to establish cultures and to perform the survival experiments. A rapid cellular test of sensitivity such as dye exclusion (21) is required urgently for this purpose. Sensitivity at the chromosomal level (19, 33) might permit an early diagnosis.

There is a considerable temptation to regard AT as an ionizing radiation analog of XP, particularly with respect to the excision of base damage. There are at least 2 complementation groups with defects in repair and a third class represented by AT4BI, AT5BI, and AT7BI with no detectable defect (31). However, this third class is not equivalent to the XP variant, since the cells are as sensitive to cell killing (this report) as those carrying the repair defect, while XP variants show no or only slightly enhanced sensitivity to UV for cell killing (4, 26).

One of the 2 AT heterozygotes showed intermediate sensitivity between its homozygote and 2BI. It has also been shown that, under anoxic radiation conditions, AT heterozygotes also fall into 2 classes for sensitivity (30). The intermediate sensitivity of some heterozygotes was correlated with defects in repair. Lavin *et al.* (21) have produced evidence that indicates that AT heterozygotes can be shown to be intermediate in radiosensitivity between controls and homozygotes. These observations are of some significance in carcinogenesis, since Swift (38) has shown that cancer may be more frequent in AT heterozygotes than normals. With a gene frequency approaching 1% of the population, detection of AT heterozygotes is of value in identifying and possibly monitoring individuals at risk, and it might also be of importance with respect to genetic counselling.

While AT cells, with a reasonable sample size, are all considerably more sensitive than normals, the situation with retinoblastoma is more complex. Cell strains from the hereditary form of the disease are more sensitive than those from its sporadic form which could not be distinguished from a series of normals (24). Three of the 4 retinoblastoma cell strains tested by us proved to have sensitivity intermediate between the normal and AT ranges; one of these was the strain with the D deletion tested by Weichselbaum *et al.* (40). Both the other sensitive strains had a normal chromosome constitution and were established from bilateral forms of the disease, one with a familial

[5] J. Moreten, personal communication.

[6] C. F. Arlett, S. A. Harcourt, and A. R. Lehmann, unpublished results.

[7] C. F. Arlett and S. A. Harcourt, unpublished observations.

history (A5570) and the other described as sporadic (Rb1BI). The fourth nonsensitive strain was again of normal chromosome constitution and was sporadic and unilateral. These observations based upon independently obtained material are in agreement with those of Little (24).

No information is available, to date, on any repair defects in this condition, but sensitivity for cell killing, by analogy with AT and XP, may be taken as a strong indication of the existence of a defect in repair. The sensitivity of some members of this group is relevant to carcinogenesis, since there is an increased frequency of cancer in individuals with the hereditary form of the disease (24). The sensitivity to ionizing radiation and possible repair defect may be more significant than in AT because there is no evidence of any immune deficiency and thus defects in repair may, like XP, be responsible for the tumor increase.

Two other cell strains proved to be more sensitive than 2BI and occupy positions intermediate between the normal range and the AT range; these were from a Fanconi's anemia patient and a Hutchinson-Gilford progeria patient, respectively. The accompanying paper shows heterogeneity in the progeric cell strains, and thus our observation might be fortuitous. It would, nevertheless, be of considerable interest if the aging syndromes could be identified on the basis of their radiosensitivity. The Fanconi's anemia case is to be contrasted with 2 other published results for cells from this syndrome (15, 40), although they do represent material from different patients. The results with FA1BI provide the only instance of a direct disagreement between ourselves and Weichselbaum *et al.* (41). There is, at present, no explanation for this discrepancy; possible reasons are being tested experimentally. These results may suggest heterogeneity for γ-ray sensitivity in this syndrome, but they also show, clearly, how important it is to investigate a representative selection of cell strains in any condition. Because of this, the present report should not be considered a definitive survey, with the possible exception of AT. With AT and using the model of XP, the discovery of a true "variant" form with little or no radiosensitivity may be expected. We have already pointed out that with XP radiosensitive cell strains might also be anticipated.

ACKNOWLEDGMENTS

We are grateful to Drs. B. A. Bridges and A. R. Lehmann for much helpful criticism of the manuscript. We are also indebted to the colleagues who sent us the cell strains and skin biopsies.

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