DNA REPAIR IN HUMAN PROGEROID CELLS*

James D. Regan and R. B. Setlow

Carcinogenesis Program, Biology Division, Oak Ridge National Laboratory,
and The University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences,
Oak Ridge, Tennessee 37830

Received June 14,1974

Summary — Human diploid fibroblast cells derived from normal subjects and from patients with progeria or progeroid syndromes were examined for the extent of their ability to repair γ-ray-induced single-strand breaks in their DNA. Cells irradiated and incubated as monolayers showed normal repair in all cell lines studied. Progeroid cells that were treated with trypsin, irradiated, and allowed to repair in suspension with shaking showed slightly less repair after long times than did normal cells. However, if the cells were not shaken, the progeroid cells remaining in suspension showed DNA degradation and an inability to repair DNA strand breaks in contrast to normal cells.

Progeria is a human genetic disease characterized by accelerated aging (1). Epstein et al. (2) used sedimentation in alkaline sucrose to study the rejoining of single-strand breaks in the DNA of γ-irradiated cells from a progeroid patient (K.H.). Their results indicated that the cells "failed to show evidence of normal DNA strand rejoining in vitro after exposure to cobalt-60 gamma irradiation," whereas control cells "showed essentially complete rejoining of radiation-induced strand breaks within 30 min after irradiation . . . ." Their results suggested "that an enzyme involved in DNA repair may be absent or greatly reduced in efficiency in cells from this patient with the progeria syndrome."

We have investigated the rejoining of DNA single-strand breaks in four human diploid fibroblast cell strains developed from patients with "progeroid" syndromes including K.H. cells (2). Although under certain experimental conditions we can repeat the observations of Epstein et al. (2), our results suggest that there is no inherent defect in DNA repair in progeroid cells.

The accompanying paper by Epstein et al. indicates that progeroid cells repair breaks less rapidly than do normal ones under experimental conditions similar but not identical to ours. We do not know why our results differ from theirs.

*Research jointly supported by the Carcinogenesis Program of the National Cancer Institute and by the U. S. Atomic Energy Commission under contract with Union Carbide Corporation.

MATERIALS AND METHODS

We used diploid fibroblasts derived from skin biopsies of normal subjects and from patients with progeria or progeroid syndromes (see Table I). Cell culture methods and the

TABLE I

Progeria or progeroid cells used*

L. D.	-	Classical progeria, passage 9
S. J.	-	Atypical progeria, passage 12
K. H.	-	Atypical progeria, passage 11, 12
H. M.	-	Werner's syndrome, passage 10

*These cells were generously supplied by Dr. Robert J. Hay, Department of Biological Sciences, Wright State University, Dayton, Ohio. Further details on these patients are available from Dr. Hay.

details of alkaline sucrose gradient centrifugation have been reported previously (3, 4). Cellular DNA was labeled by a 16-hr incubation in [^{3}H]dThd (2 μCi/ml) or $^{32}PO_4$ (15 μCi/ml). The labeling medium was then removed and replaced with regular growth medium for 2 hr prior to irradiation with 10 Krad of γ-rays from a ^{60}Co source. Cells were treated in one of 3 ways: (A) as monolayers on 50-mm plastic petri dishes (Lux Corp.) incubated at 37°C; (B) removed from dishes with 0.05% trypsin and irradiated and incubated at 37°C in suspension in glass centrifuge tubes, without shaking, as described by Epstein et al. (2); (C) removed with trypsin and irradiated and incubated in suspension in 25-ml plastic scintillation vials, with shaking on a rotary shake table (100 excursions/min) at 37°C. After irradiation the monolayer cells, (A), were removed from the dishes by scraping into a phosphate-buffered saline containing 0.6 mM EDTA (PBS-EDTA) solution (5) and resuspended at 2 X 10^5/ml cells. For cells incubated in suspension, (B) and (C), a 100-μl aliquot of cell suspension was diluted with 10 ml of PBS-EDTA, centrifuged for 3 min at ~1000 g to wash off the growth medium, and resuspended in PBS-EDTA at 2 X 10^5 cells/ml. Fifty μl of the cellular suspension was layered on top of an alkaline sucrose gradient containing 2 M NaCl as described previously (3, 4). Cells were permitted to lyse for 1 hr and the gradients were

centrifuged at 30,000 rpm for 180 min. Eight-drop fractions of the gradients were collected from a hole punched in the bottom of the tubes and counted as described (6). The data were analyzed by a computer to give weight-average molecular weights of the lower 0.8 of the gradients.

RESULTS

(A) Cells in monolayer. Figures 1 and 2 show the sedimentation profiles of the

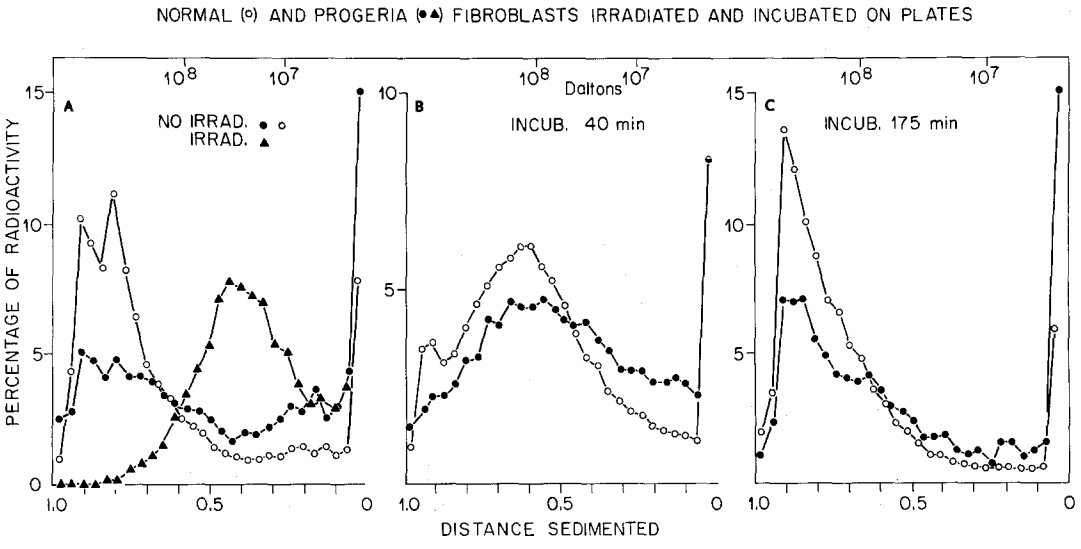
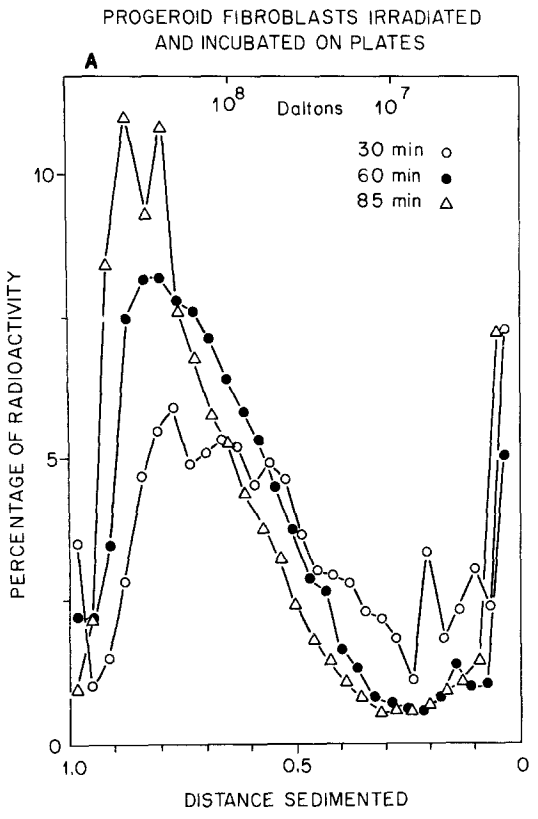


FIG. 1. Alkaline gradient profiles of [^{3}H]dThd-labeled normal (HSBP) and progeroid (L.D.) cells irradiated and incubated for various times on petri plates before collection for centrifugation in the SW-56 rotor of a Beckman L3-50 centrifuge for 180 min at 30,000 rev/min.

DNA of normal diploid and two progeroid fibroblast cell lines irradiated and incubated for various times on petri dishes. There is no suggestion from these data that a defect in DNA repair is present in progeroid cells. The table in Figure 2 summarizes the gradient data for one normal and four progeroid cell types in terms of the weight-average molecular weight. There are no impressive differences in the rate of repair of strand breaks between the various progeroid cells and normal cells.

(B) Cells in suspension — no shaking. Immediately after irradiation at 0°C, the tube containing the cells in suspension was placed in a 37°C water bath. After 15 min incubation, the DNA of normal cells shows partial repair of strand breaks but the



B

The weight-average molecular weight (x 10^6) after 10 Krad of γ irradiation, and subsequent incubation on petri plates

(For unirradiated cells, M_w = 140–160)

Cells	Time after irradiation					
	0	15	30	40	60	85
L.D.	42	—	84	104	—	145 130
K.H.	42	—	105	—	138	157
S.J.	—	81	83	—	115 106	123
H.M.	48	92 84	116	—	—	—
HSBP	53	87	96	—	125	128

FIG. 2. (A) Alkaline gradient profiles of progeroid (K.H.) cells after γ-irradiation (10 krads). (B) Summary of DNA repair results with normal and progeroid cells.

progeroid cells show much less (Figure 3). With continued incubation, both normal and progeroid cells exhibit much the same pattern. The slower moving DNA decreases in molecular weight, and its relative amount increases. The amount of low molecular weight DNA is much greater for the progeroid cells. A complication in these experiments was the attachment of cells to the surface of the tube during incubation and the consequent decrease in the number of cells put on the gradients. There was a 75% decrease for progeroid and a 90% decrease for normal cells. Thus, in both the normal and the progeroid cells, there were two populations—one attaching rather readily to the tube substrate and the other attaching slowly. In the slowly attaching normal cells there was some rejoining of strand breaks, but in the progeroid cells remaining in the suspension most of the DNA decreased in size.

(C) Cells in suspension—with shaking. The experiments were designed to insure that all cells would remain in suspension during the postirradiation period. The results

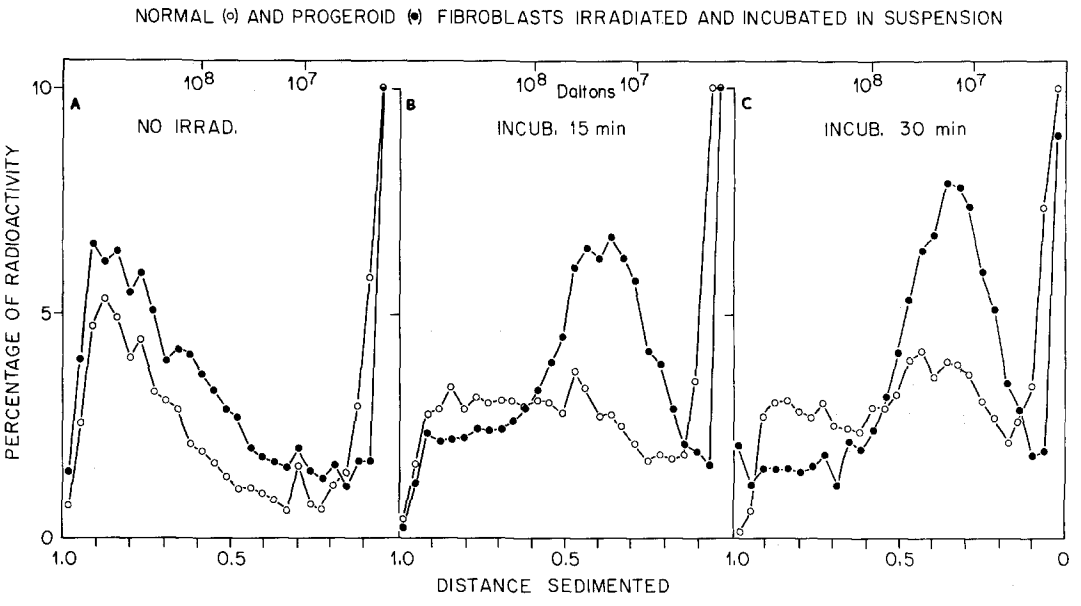


FIG. 3. Alkaline gradient profiles of ^{32}P-labeled normal (HSBP) and [^{3}H]dThd-labeled progeroid cells (K.H.) after trypsinization. The two types of cells were mixed, irradiated, and incubated without shaking at 37°C in suspension. The heights of the ^{32}P profiles are less than those of the ^{3}H profiles because an appreciable part of the label (~30%) is at the top of the gradient in non-DNA components.

are shown in Figure 4. After 15 and 30 min incubation, the DNA's of normal and progeroid cells are returning to control molecular weights. Continued incubation with shaking results in the appearance of some DNA of molecular weight less than 10^8.

DISCUSSION

Both K.H. and normal cells in monolayer cultures and while held in suspension, are able to repair γ-ray-induced single-strand DNA breaks under the experimental conditions reported here. On the other hand, cells remaining in suspension in nonagitated cultures do not repair, but degrade their DNA. This degradation by a small fraction of the initial population could account for the changes observed between 30 and 60 min in the irradiated, shaken cultures (Figure 4). Preliminary experiments on normal and progeroid cells that were treated with trypsin, irradiated in suspension, and replanted on petri dishes yielded inconclusive results.

We suggest that progeroid cells do not have an inherent reduced ability to rejoin radiation-induced DNA single-strand breaks, but that they may have a reduced and

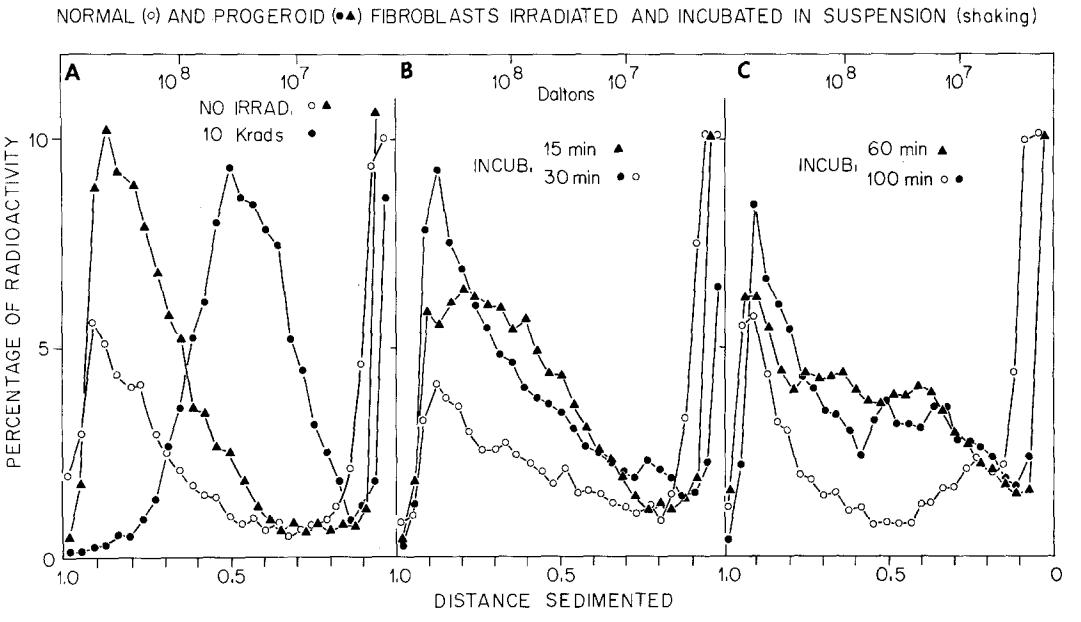


FIG. 4. Alkaline gradient profiles of ^{32}P-labeled normal (HSBP) and [^{3}H]dThd-labeled progeroid cells (K.H.) after trypsinization, irradiation, and incubation with shaking at 37°C for various times.

variable ability to recover from trypsin treatment. The cells remaining in suspension in nonagitated cultures after irradiation may represent either cells with a reduced ability to recover from trypsin treatment or cells that have sustained greater trypsin damage than others. The cloning efficiencies and X-ray survival of HeLa and Chinese hamster cells are greatly affected by the time allowed for recovery from trypsin damage (7, 8). Conceivably, progeroid cells are very sensitive to this damage and slow to recover. This sensitivity could be reflected in several ways, one of which could be slow DNA repair.

Acknowledgments — We are very grateful to Dr. Robert J. Hay for the gift of the progeria cells and to Drs. J. Epstein, J. R. Williams, and J. B. Little for helpful discussions.

REFERENCES

1. DeBusk, F. L. (1972) Pediatrics 80 (Part 2), 697-724.
2. Epstein, J., Williams, J. R., and Little, J. B. (1973) Proc. Nat. Acad. Sci. USA 70, 977-981.

3. Regan, J. D., Setlow, R. B., and Ley, R. D. (1971) Proc. Nat. Acad. Sci. USA 68, 708-712.
4. Regan, J. D., Setlow, R. B., Kaback, M. M., Howell, R. R., Klein, E., and Burgess, G. (1971) Science 174, 150-153.
5. Setlow, R. B., Regan, J. D., German, and Carrier, W. L. (1969) Proc. Nat. Acad. Sci. USA 64, 1035-1041.
6. Carrier, W. L., and Setlow, R. B. (1971) Anal. Biochem. 43, 427-432.
7. Berry, R. J., Evans, H. J., and Robinson, D. M. (1966) Exp. Cell Res. 42, 512-522.
8. Barendsen, G. W., and Walter, H. M. D. (1964) Radiat. Res. 21, 314-329.