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DNA fibroblasts
Down's syndrome mutagenesis

Comparative Study of X-Ray and UV Induced Cytotoxicity, DNA Repair, and Mutagenesis in Down's Syndrome and Normal Fibroblasts

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Summary

Utilizing six age-matched human fibroblast cell strains (three normal and three Down's syndrome) cytotoxicity, DNA repair, and X-ray mutagenesis were measured. There was no significant difference in the colony-forming ability after ultraviolet (UV) or X-irradiation between normal and Down's fibroblasts. Similarly, UV-induced unscheduled DNA synthesis was not significantly different between normal and Down's cells. Finally, a comparison between the spontaneous and X-ray induced mutation frequency at the hypoxanthine-guanine phosphoribosyl transferase locus demonstrated no difference between the two cell types (normal and Down's).

Speculation

Down's syndrome has long been recognized as a cancer-prone human syndrome. Chromosomal radiosensitivity indicated the possibility of faulty DNA repair leading to the accumulation of somatic mutations. Because no biologic differences were detected in fibroblasts, the predisposition to cancer (especially leukemia) in Down's syndrome might be due to abnormal DNA repair in the white blood cells of these individuals.

It has been recognized now for many years that children with Down's syndrome are at an increased risk for neoplasia, especially leukemia (8, 9, 14, 15, 20, 22). In addition, chromosomes from individuals who are trisomic for chromosome 21 exhibit an increased aberration frequency (exchange type) after exposure to gamma rays (17). Chromosomes from Down's syndrome individuals have been demonstrated to be more susceptible to the chemical mutagen 7,12- dimethylbenz(a)-anthracene manifested as an increase in chromatid gaps or breaks (16). It has also been reported that fibroblasts from patients with Down's syndrome are more susceptible to SV 40 viral transformation than are normal controls (11, 25). Chromosome breakage after viral infection (chickenpox and measles) has also been reported to be more frequent in Down's syndrome patients (5, 6).

Implicit in any discussion of chromosomal sensitivity to mutagens and cellular susceptibility to oncogenic viruses is the role of DNA repair. After the demonstration that an initial stage of DNA repair may be defective in the cancer prone human syndrome xeroderma pigmentosum (2) and that fibroblasts of these XP patients had higher UV-induced mutation frequencies than normal fibroblasts (13), a number of other cancer-prone human syndromes (Fanconi's anemia, Bloom's syndrome, and ataxia telangiectasia) have been suspected as potential DNA repair mutants (3, 19); the authors include in this list Down's syndrome. Using a micronucleus assay for chromosomal damage, it was reported that the increased frequency of chromosomal aberrations produced by X-rays in cultured lymphocytes in Down's syndrome patients might be due to a defect in the rejoining system which repairs chromosomal breaks (4). It was also reported that leukocytes from Down's syndrome patients exhibited lower levels of UV-induced DNA repair synthesis (unscheduled DNA synthesis) than did leukocytes from normal controls (10).

In an attempt to further investigate the problem, the authors report here a series of experiments to measure: (1) colony-forming ability, *i.e.*, survival, of Down's and normal fibroblasts exposed to either UV or X-irradiation, (2) unscheduled DNA synthesis (excision repair) of Down's and normal fibroblasts after exposure to UV, and (3) spontaneous and X-ray induced mutation frequencies at the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) locus in Down's and normal fibroblasts.

MATERIALS AND METHODS

Primary fibroblast cultures of age- and sex-matched normal and Down's syndrome individuals were provided by Dr. D. Segal (Department of Pediatrics, University of Alberta, Edmonton, Alberta). These cell strains have previously been examined in terms of their *in vitro* cell cycle, origin, and growth (18). The Lesch-Nyhan cells (GM 1362) were obtained from the Human Genetic Mutant Cell Repository (Camden, NJ). The cells were grown in modified Eagle's medium (Earle's balanced salt solution with a 50% increase of essential amino acids and vitamins) supplemented with "nonessential" amino acids (100% increase), 1 mM sodium pyruvate, and 10% heat inactivated fetal calf serum. Under incubation conditions of 5% CO_2 in humidified air at 37°C the cells had a generation time of approximately 24 hr.

The cell survival experiments were conducted similarly for UV and X-irradiation. To determine the percent survival fraction of cells treated with UV, the cells were seeded in 100 mm tissue culture dishes (4 plates per UV dose) 6 hr before medium removal and UV irradiation. After attachment, the medium was removed, and the cells were exposed to a germicidal lamp (GE 15T8-15W) which delivered a flux of 10 ergs/mm^2/sec(1 J/M^2/sec); fresh medium was then added to the cells. Growth was terminated at approximately 2 wk; at which time the colonies were fixed, stained, and counted.

In order to determine colony forming ability after exposure to X-rays, known dilutions of cells in suspension were irradiated with a General Electric Maxitron 300 X-ray machine. The exposure rate was 184 R/min (250 kV, 20 mA, with 3 mm of Aluminum filtration). The cells were immediately seeded in 100 mm tissue culture dishes (4 dishes per X-ray dose) and were permitted to grow for approximately 2 wk. The colonies were then fixed, stained, and scored.

Unscheduled DNA synthesis was measured after UV irradiation after the deficient medium-hydroxyurea protocol (21).

Measurement of forward mutations to 6-thioguanine resistance (HGPRT) was conducted according to the protocol in Table 1. As can be seen in Table 1, a replating technique utilizing multiple expression times was followed which allowed us to characterize

Table 1. *Frequency of X-ray induced 6-thioguanine resistant mutants in normal and Down's syndrome cells as a function of expression time*

		Expression times			
		4, 8, 11 or 10, 14, 21 days		7 hr	21 days
Irradiation of cell in suspension (300R X-ray)		Deliver to bottles, for attachment and growth	Pool cells and replate for mutation analysis	Add 6-TG (5 μg/ml)	Score
Experiment	Cell line	Expression time (days)	No. colonies/plates scored	% Plating[1] efficiency	Induced mutation frequency/ 10^6 survivors
1	N.F.3	4	7/96	8.2	22.1
1	N.F.3	8	47/83	12.1	117.2
1	N.F.3	11	50/84	12.6	118.2
1	D.S.2	4	2/96	5.9	8.8
1	D.S.2	8	49/72	15.1	112.5
1	D.S.2	11	83/84	22.0	112.2
2	N.F.3	10	35/75	11.8	99.3
2	N.F.3	14	13/73	9.7	46.0
2	N.F.3	21	1/75	19.0	1.8
2	D.S.2	10	24/55	14.8	73.8
2	D.S.2	14	13/75	9.2	47.3

[1] Average of four plates.

Table 2. *Dose response for X-ray induced 6-thioguanine resistant mutagenicity in normal and Down's syndrome fibroblasts*

		4 days	4 days	7 hr	21 days
Irradiation of cells in suspension	Deliver to bottles for attachment and growth		Subculture	Pool cells and replate for mutation analysis	Add 6-TG (5 μ/ml) Score
Cell line	X-ray (rads)	No. colonies/plates scored	% Plating[1] efficiency	Mutation frequency/10^6 survivors	Corrected mutation frequency[2]
N.F.3	0	5/46	37.25	7.3	7.4
N.F.3	150	13/47	35.38	19.5	20.1
N.F.3	300	21/46	30.80	37.1	39.3
D.S.2	0	4/46	54.88	4.0	4.1
D.S.2	150	18/48	45.63	20.5	20.3
D.S.2	300	22/48	29.10	39.4	38.7

[1] Average of four plates.
[2] Corrected for recovery of Lesch-Nyhan cells as described in Table 3.

the HGPRT mutation system for our cell lines. In addition, a reconstruction experiment (Table 3) designed to measure the recovery of 6-thioguanine resistant Lesch-Nyhan cells seeded in the presence of normal and Down's syndrome fibroblasts was performed after the protocol of Albertini and DeMars (1) and Jacobs and DeMars (7). Table 2 depicts the protocol utilized for the X-ray, dose response experiment.

RESULTS

Figure 1 illustrates the survival of fibroblasts from normal and Down's syndrome individuals upon exposure to varying doses of UV irradiation. A total of four cell strains was employed (two normal and two Down's), and as can be seen in Figure 1 (a and b) there seems to be no difference among any of the cell strains tested.

Figure 2 represents the survival of six different cell strains (three normal and three Down's) exposed to varying doses of X-irradiation. As was the case with UV irradiation, there seems to be no significant difference among the cell strains tested.

Although no important difference was detected between normal and Down's syndrome fibroblasts in terms of their survival to irradiation, because it had been reported that there was a DNA repair deficiency in Down's syndrome (3), it was decided that UV induced "unscheduled" DNA synthesis might be an important parameter to measure. Figure 3 clearly demonstrates no consistent difference between the two cell types (normal and Down's) at two doses of UV and two periods of ^{3}H-TdR incorporation. The authors are quite confident that no differences exist between normal and Down's syndrome fibroblasts in their response to ultraviolet radiation as measured by cytotoxicity and unscheduled DNA synthesis. However, as reported previously in this paper, Down's syndrome cells do exhibit increased sensitivity to X-irradiation as measured by chromosomal breakage. In light of this observation, the authors conducted a series of experiments to measure the spontaneous and X-ray induced mutation frequencies in normal and Down's syndrome fibroblasts. The rationale was that X-ray sensitivity as measured by chromosomal aberrations might indicate a defect in the chromosomal rejoining system which could potentially result in the fixation of mutations.

In a preliminary series of experiments (Table 1), it was determined that the maximum recovery of X-ray induced 6-thioguanine resistant mutants occurred at 8–10 days after irradiation, followed by a subsequent decline in mutation frequency for both normal and Down's syndrome cell strains. Consequently, an 8-day expression time was chosen as the time of maximum mutant recovery. Figure 4 clearly indicates that at two doses of X-irradiation (150R and 300R), in addition to a OR point (spontaneous mutation to 6-thioguanine resistance), there is no difference between the mutation frequencies of normal and Down's syndrome fibroblasts.

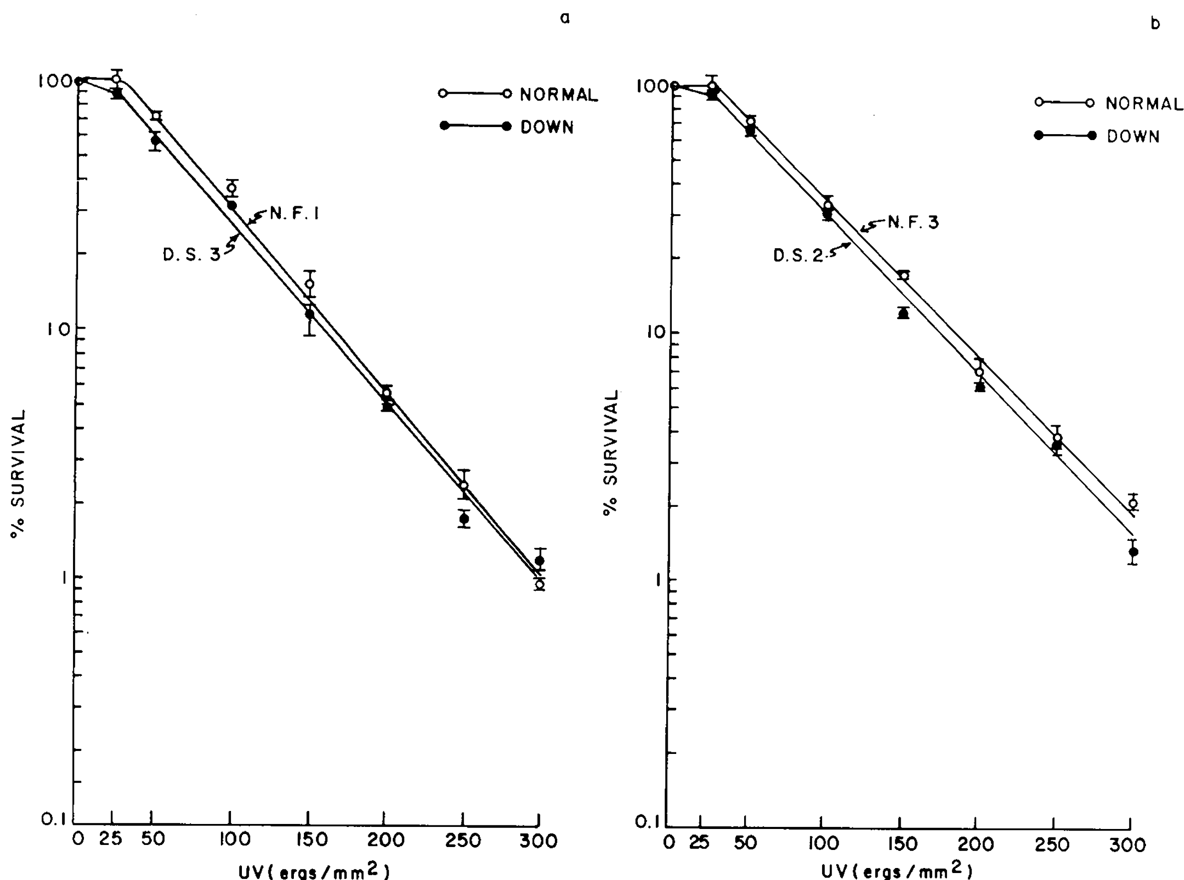


Fig. 1. (*a* and *b*) Survival of normal and Down's syndrome fibroblasts after UV exposure. *Bars* represent SEM.

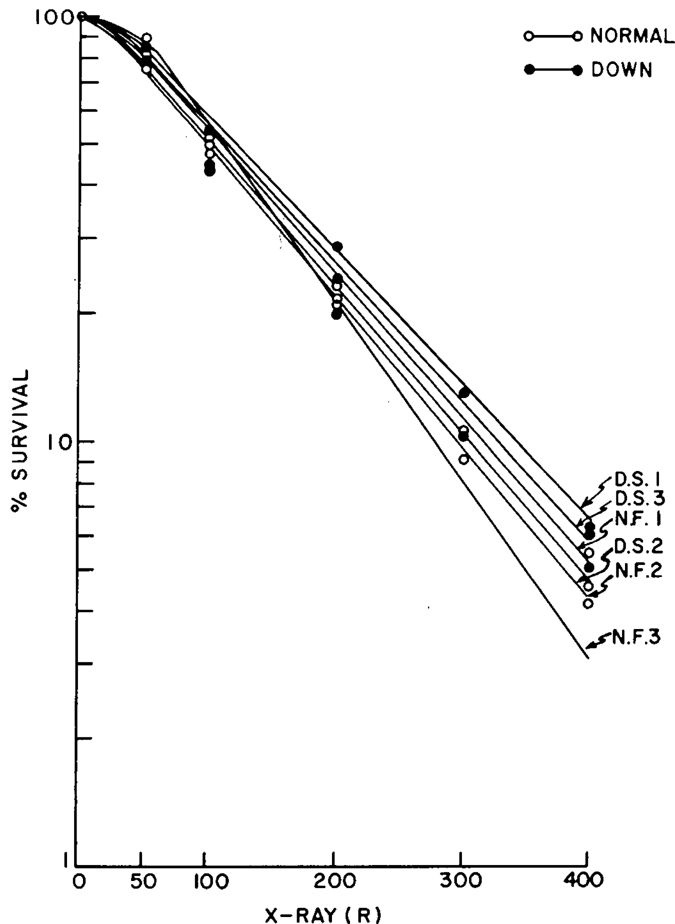
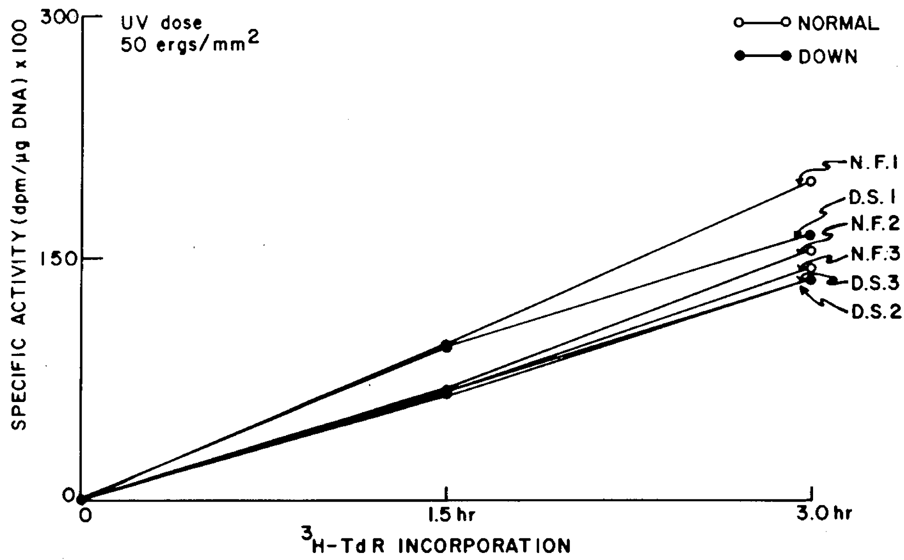


Fig. 2. Survival of normal and Down's syndrome fibroblasts after X-irradiation.

Table 2 presents the data, used for Figure 4 fully corrected for mutant recovery as measured using prototype Lesch-Nyhan cells in a reconstruction protocol.

DISCUSSION

The observations dealing with increased sensitivity of Down's syndrome cells to radiation, chemical carcinogens, and both oncogenic and nononcogenic viruses led us to hypothesize that perhaps the susceptibility of Down's syndrome patients to malignancy reflects errors of DNA repair systems leading to the accumulation of mutations. The authors now know that increased frequencies of somatic mutations are associated with cancer-proneness, *i.e.*, xeroderma pigmentosum (13); therefore, a demonstration of increased frequencies of mutations in Down's cells would not be unexpected. The authors were not able to detect any increases in UV or X-ray cytotoxicity, UV-induced unscheduled DNA synthesis, or X-ray-induced mutations in Down's syndrome fibroblasts. However, because the most prevalent malignancy in Down's syndrome appears to be leukemia, perhaps an examination of white blood cells would be more fruitful. There is evidence which documents defects in lymphoid tissue, specifically T-cell function in Down's syndrome (12). There also is evidence that DNA repair capacities may vary depending upon the cell line one examines. B-cells are known to have a more efficient system of UV repair and, consequently, are much less radiosensitive than T-cells (24). More importantly, it has recently been demonstrated (Biedemann, unpublished results) that Down's syndrome lymphocytes exhibit significantly higher levels of sister chromatid exchanges in response to mitomycin C and gamma radiation. Sister chromatid exchanges are thought to be quite sensitive indicators of unrepaired lesions in damaged DNA (23). Perhaps, during the course of cellular differentiation, tissue-specific differences are generated which predispose white blood cells to the accumulation of genetic mutations and ultimately malignancy. Additional studies on the comparative cytotoxicity, DNA repair, and induced mutagenesis in Down's and normal white blood cells would be warranted.



b.

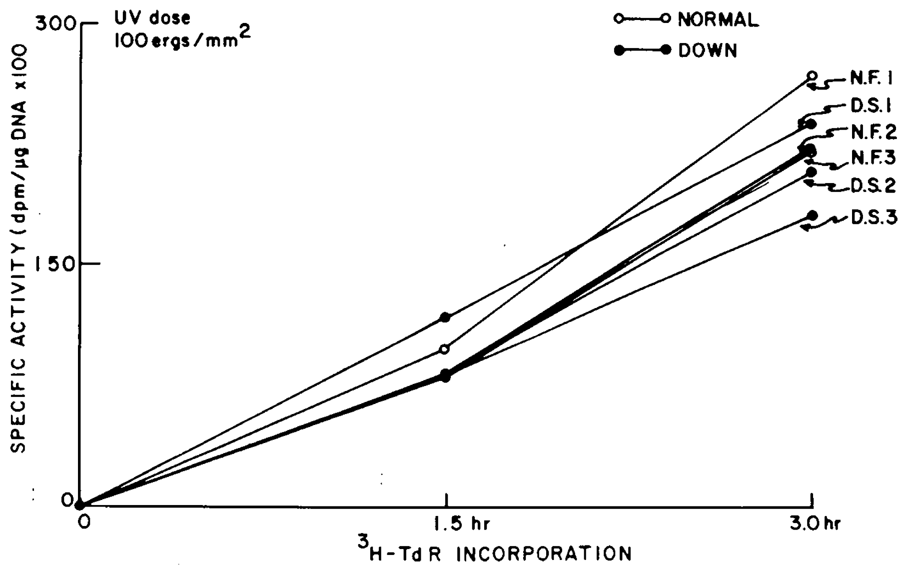


Fig. 3. (*a* and *b*) UV induced unscheduled DNA synthesis of normal and Down's syndrome fibroblasts.

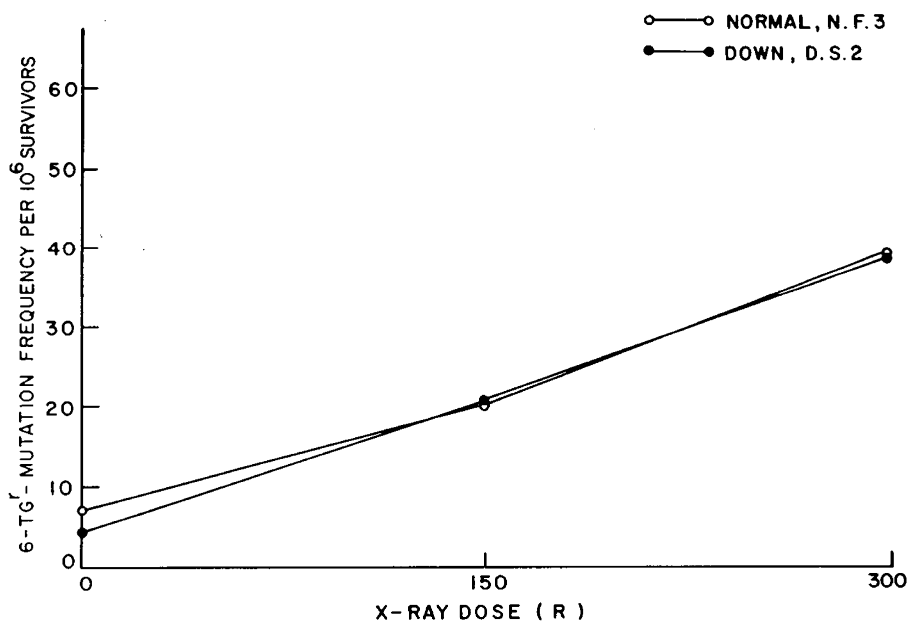


Fig. 4. Spontaneous and X-ray induced mutation frequencies of normal and Down's syndrome fibroblasts.

Table 3. *Recovery of prototype 6-thioguanine resistant cells (Lesch-Nyhan) in the presence of treated normal and Down's syndrome cells*

7 hr			21 days	
At time of replating for mutation analysis, pool Lesch-Nyhan and normal or Down's syndrome fibroblasts and replate		Add 6-TG (5 μg/ml)		Score
No. of cells plated				
Normal	Down's syndrome	Lesch-Nyhan[4]	No. of colonies formed (average of four plates)	Recovery (%)[5]
		200	114.5	100.0
4×10^4 [1]		200	113.5	99.1
4×10^4 [2]		200	111.0	96.9
4×10^4 [3]		200	108.0	94.3
	4×10^4 [1]	200	110.8	96.7
	4×10^4 [2]	200	115.5	100.8
	4×10^4 [3]	200	116.5	101.7

[1] Cells from mutagenesis experiment, 0 rads X-ray.
[2] Cells from mutagenesis experiment, 150 rads X-ray.
[3] Cells from mutagenesis experiment, 300 rads X-ray.
[4] Cells used were GM 1362 Lesch-Nyhan Syndrome fibroblasts.
[5] Corrected from plating efficiency of Lesch-Nyhan cells.

REFERENCES AND NOTES

1. Albertini, R. J., and DeMars, R.: Detection and quantification of X-ray induced mutation in cultured, diploid human fibroblasts, *Mut. Res., 18:* 199 (1973).
2. Cleaver, J. E.: Xeroderma pigmentosum: a human disease in which an initial stage of DNA repair is defective. *Proc. Nat. Acad. Sci., 63:* 428 (1969).
3. Cleaver, J. E.: Human inherited diseases with altered mechanisms for DNA repair and mutagenesis. In: J. W. Littlefield, J. D. Grouchi, and F. J. G. Ebling: Birth Defects (Oxford, 1978).
4. Countryman, P. I., Heddle, J. A., and Crawford, E.: The repair of X-ray-induced chromosomal damage in trisomy 21 and normal diploid lymphocytes. *Can. Res., 37:* 52 (1977).
5. Higurashi, M., Tamura, T., and Nakatake, T.: Cytogenetic observations in cultured lymphocytes from patients with Down's syndrome and measles. *Pediatr. Res., 7:* 582 (1973).
6. Higurashi, M., Tada, A., Miyahara, S., Hirayama, M., Hoshina, H., and Tamura, T.: Chromosome damage in Down's syndrome induced by chickenpox infection. *Pediatr. Res., 10:* 189 (1976).
7. Jacobs, L., and DeMars, R.: Quantification of chemical mutagenesis in diploid human fibroblasts: induction of azaguanine-resistant mutants by N-methyl-N-nitro-N-Nitrosoguanidine, *Mut. Res., 53:* 29 (1978).
8. Krivit, W., and Good, R. A.: The simultaneous occurrence of leukemia and mongolism. *J. Dis. Child., 91:* 218 (1956).
9. Krivit, W., and Good, R. A. Simultaneous occurrence of mongolism and leukemia. Report of a nationwide survey. *J. Dis. Child., 94:* 289 (1957).
10. Lambert, B., Hansson, K., Bui, T. H., Funes-Cravioto, F., Lindsten, J., and Holmberg, M.: DNA repair and frequency of X-ray and UV-light induced chromosome aberrations in leucocytes from patients with Down's syndrome. *Ann. Hum. Gen., 39:* 293 (1976).
11. Levin, S., and Hahn, T.: Transformation of cells in culture from children with chromosomal and immune deficiency disorders. *Israel J. Med. Sci., 8:* 133 (1972).
12. Levin, S., Erga, Nir, and Mogilner, B. M.: T-system immune-deficiency in Down's syndrome. *Pediatrics, 56:* 123 (1975).
13. Maher, V. M., and McCormick, J. J.: Effect of DNA repair on the cytotoxicity and mutagenicity of UV irradiation and of chemical carcinogens in normal and xeroderma pigmentosum cells. In: J. M. Yuhas, R. W. Tennant, and J. D. Regan: Biology of Radiation Carcinogenesis, pp. 129–145 (Raven Press, N.Y., 1976).
14. Merritt, D. H., and Harris, J. S. Mongolism and acute leukemia. *Arch. Dis. Child., 92:* 41 (1956).
15. Miller, R. W.: Persons with exceptionally high risk of leukemia. *Can. Res., 27:* 2420 (1967).
16. O'Brien, R. L., Poon, P., Kline, E., and Parker, J. W. Susceptibility of chromosomes from patients with Down's syndrome to 7,12-dimethylbenz(a)-anthracene induced aberrations *in vitro*. *Int. J. Cancer, 8:* 202 (1971).
17. Sasaki, M. S., and Tonomura, A. Chromosomal radiosensitivity in Down's syndrome. *Jap. J. Human Gen., 14:* 81 (1969).
18. Segal, D. J., and McCoy, E. E. Studies on Down's syndrome in tissue culture. I. Growth rates and protein contents of fibroblast cultures. *J. Cell Physiol., 83:* 85 (1974).
19. Setlow, R. B.: Repair deficient human disorders and cancer. *Nature, 271:* 713 (1978).
20. Stewart, A., Webb, J., and Hewitt, D.: Survey of childhood malignancies. *Brit. Med. J., 1:* 1495 (1958).
21. Trosko, J. E., and Yager, J. D.: A sensitive method to measure physical and chemical carcinogen-induced "unscheduled DNA synthesis" in rapidly dividing eukaryotic cells. *Exp. Cell Res., 88:* 47 (1974).
22. Wald, N., Borges, W. H., Li, C. C., Turner, J. H., and Harnois, M. C.: Leukemia associated with mongolism. *Lancet, 1:* 1228 (1961).
23. Wolff, S.: Sister chromatid exchange. *Ann. Rev. Genet., 11:* 183 (1977).
24. Yew, F. H., and Johnson, R. T.: Human B and T lymphocytes differ in UV-induced repair capacity. *Exp. Cell Res., 113:* 227 (1978).
25. Young, D.: The susceptibility to SV 40 virus transformation of fibroblasts obtained from patients with Down's syndrome. *Eur. J. Cancer, 7:* 337 (1971).

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