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Fluorescent light-induced DNA crosslinkage and chromatid breaks in mouse cells in culture

(chromosomal aberrations *in situ*/chromosomal analysis/radiation damage)

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ABSTRACT **A single 20-hr exposure of mouse cells derived from embryonic or lung tissue to cool-white fluorescent light (4.6 W/m^2) causes both DNA damage and chromosome aberrations including chromatid breaks, exchanges, and minutes. In Kohn's alkaline elution technique, the DNA from exposed cells elutes more slowly than that from shielded cells. Because larger molecular weight DNA elutes slower than smaller, we interpret these results to mean that the DNA in cells exposed to light is crosslinked. The estimated frequency of crosslinks is sufficient to account for the number of chromatid breaks observed. The types of chromosome aberrations produced by light indicate that the primary lesion results in chromatid rather than chromosome breaks, and the results suggest an influence of cell density in that cells in densely populated cultures showed few or no chromatid breaks after irradiation. The present results, together with observations from the literature, suggest that the DNA crosslinkage and the chromosome aberrations produced by light may be related.**

In a previous study (1), exposure of mouse cells in culture to room fluorescent lights during routine handling produced a high frequency of chromosome aberrations. These aberrations appeared to result from chromatid breaks. Conceivably, fluorescent light might also damage cellular DNA, and such damage, in turn, could lead directly or indirectly to the chromatid breaks. In the present study we have examined, under controlled conditions and with the same cell line, the effect of fluorescent light on both DNA and chromosomes of mouse cells in culture. A technique for *in situ* examination of cells for chromosome aberrations (2) was modified and facilitated a more accurate determination of light-induced chromosome damage than obtainable with conventional methods.

MATERIALS AND METHODS

Source of Cells. Mouse cell lines NCTC 9038 and 9002 were originated from C3H$_f$/HeN 12-day embryo and newborn lung, respectively. NCTC line 8466 was from the lung of a 2-month-old C57BL/6N mouse, and line 9035 was from C3H$_f$/HeN whole embryo mince. All stock cultures were carried in Pyrex T flasks in Dulbecco–Vogt medium with 10% fetal bovine serum; medium was renewed three times weekly when cultures were gassed with a humidified mixture of 10% CO_2, 1% O_2, and 89% N_2. Cells were subcultured when confluent by a brief rinse with EDTA (1:5000, Microbiological Associates, Bethesda, MD) and dispersion with an EDTA/trypsin mixture (3). No antibiotics were used except for lines 9002 and 8466 from lung tissue, which received 0.1 mg of gentamicin (Schering Corp., Kenilworth, NJ) per ml. Stock and control cultures and culture medium were never exposed to light of wavelength below 500 nm; they were handled under gold or red fluorescent lights, and the flasks were wrapped in aluminum foil.

Conditions of Light Exposure. Cultures were exposed for 20 hr at 37° to a desk lamp containing two cool-white 15-W fluorescent Westinghouse bulbs (F15 T8-CW) at a distance of 40.6 cm and received 150 foot-candles (4.6 W/m^2; 1600 lux) at the growth surface as measured by a Weston model 614 light meter. Control cultures were handled identically but shielded from light.

Analysis for DNA Crosslinkage. DNA damage in cells exposed to light was determined by alkaline coelution through polyvinyl chloride filters (4–7) of DNA from a mixture of light-exposed and shielded cells. Cells (3×10^5 in 6 ml of medium) were planted in T-30 Pyrex flasks, prelabeled with either [2-^{14}C]thymidine (15 nCi/ml) or [*methyl*-^{3}H]thymidine (30 nCi/ml), and allowed to grow for 72 hr. The medium was then renewed to remove the isotopes and one set of cultures was light-exposed and the other set shielded. Both sets were then harvested, mixed together in 4 ml of medium, chilled to 0°, x-irradiated at 300 rads (3 Gy), and analyzed for DNA crosslinkage as described (6, 7). Briefly, cells were placed on a polyvinyl chloride membrane (Millipore, BSWP02500 2-μm pore) washed with phosphate-buffered saline, lysed with 20 mM EDTA, pH 10.2/0.2% Sarkosyl (wt/vol)/2 M NaCl, washed with 20 mM EDTA (pH 10.2), and eluted at a flow rate of 0.04 ml/min with 20 mM tetrapropylammonium EDTA (free acid form of EDTA titrated to pH 12.2 with tetrapropylammonium hydroxide). Fractions were collected every 90 min. After 18–20 hr, the elution was stopped, and the radioactivity remaining on the filter and in the funnel was determined (4). The cells and DNA were shielded from light after mixing and during filtration, washing, and elution.

Analysis of Chromosomes. The production of chromosomal aberrations by light was examined by a modification of Stanley's technique (2). A known number of cells was inoculated in 2 ml of culture medium into a Leighton tube containing a 9 × 50 mm coverslip (no. 1 thickness Bellco Glass Co., Vineland, NJ). Cultures were incubated in the dark for 24 hr. The experimental cells then received 20 hr of fluorescent light exposure as described above, whereas the control cells remained shielded. For mitotic arrest, 0.10 μg of Colcemid (GIBCO, Grand Island, NY, reconstituted in phosphate-buffered NaCl solution) per ml of culture medium was added for 2 hr. The culture medium then was decanted and replaced with hypotonic solution (75 m M KCl) for 15 min at 37°. Cells were fixed *in situ* with glacial acetic acid/methanol, 1:3 (vol/vol), for 30 min, air-dried, and stained for 5 min with 2% aqueous Giemsa stain (Harleco, Gibbstown, NJ). Coverslips were rinsed in tap water, air-dried, dipped in xylene, and mounted in Permount.

This technique was used for the following reasons. (*i*) The preexposure period after inoculation allowed time for repair

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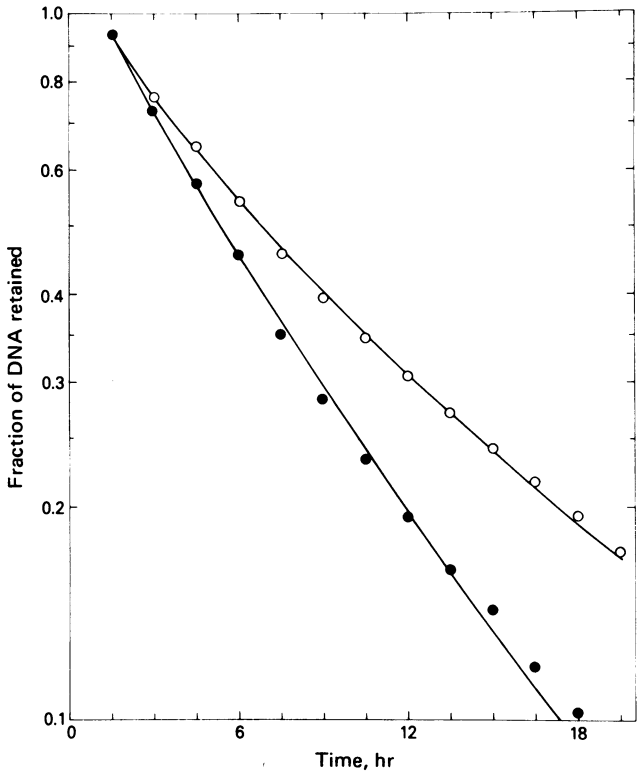

FIG. 1. Typical elution profile showing effect of light on DNA in mouse fibroblasts. These data are from Exp. 5 in Table 1, and the curve represents the least-squares fit of the equation described in the text. ○, ^{14}C from light-exposed cells; sum of all the radioactivity equals 30,600 dpm. $P_{(t)} = 1.301e^{-0.244t^{0.714}}$, in which $P_{(t)}$ is fraction of DNA retained at time t in hours. ●, ^{3}H from shielded cells; sum of all the radioactivity equals 162,800 dpm. $P_{(t)} = 1.346e^{-0.253t^{0.815}}$.

of trypsin damage to the cells, after which they entered logarithmic phase or maximal proliferative activity. (*ii*) The *in situ* fixation and examination of cells stained on the coverslips prevented cell damage and loss that would occur during conventional procedures of cell detachment from the flask and repeated centrifugations followed by spreading of the cells on the slide by air-dry or flame-dry methods. The present technique yielded well-spread metaphase cells with intact cytoplasm.

Table 1. Ratio of percentage of exposed DNA retained on filter to percentage of shielded DNA retained on filter at two points

Exp.	NCTC cell line	Passage no./ days *in vitro*	Ratio: exposed/shielded Shielded cells at 40% DNA retained	Shielded cells at 20% DNA retained
1*	9038	8/99	1.062	1.234
2*	9038	8/99	1.084	1.207
3	9038	11/127	1.030	1.056
4	9038	14/148	1.220	1.515
5	9038	16/162	1.240	1.558
6	9038	23/197	1.237	1.598
7	8466	16/225	1.182	1.535
		Mean ± SEM	1.151 ± 0.034 ($P = 0.004$)	1.386 ± 0.081 ($P = 0.003$)
8*	9035†	2/11	1.242	1.615
9*	9035†	2/11	1.158	1.505
10	9035	3/25	1.295	1.750
11*	9002†	2/17	1.265	1.720
12*	9002†	2/17	1.298	1.640
		Mean ± SEM	1.252 ± 0.026 ($P = 0.001$)	1.646 ± 0.043 ($P < 0.0001$)

* Reversed isotope. Experiments 1–7 were carried out in the Laboratory of RNA Tumor Viruses and experiments 8–12, in the Laboratory of Molecular Pharmacology.
† Light exposure was 24 hr; all other exposures were 20 hr.

RESULTS

DNA Damage. Fig. 1 shows a typical elution profile in which the DNA from cells exposed to light eluted more slowly than that from shielded cells. Because smaller molecular weight DNA elutes faster than large DNA (4, 5), we interpret these results to mean that the DNA in cells exposed to light is cross-linked in some way. Table 1 lists the results of 12 experiments from two laboratories, including reversed isotope experiments to eliminate artifacts due to radioactive thymidine. The statistical analysis was based on the ratio of the percentage of light-exposed DNA retained on the filter to that of shielded DNA retained. The elution curves were compared at two time points, when 40% and 20% of shielded DNA remained on the filter. For statistical evaluation of the results, elution curves were

Table 2. Effect of fluorescent light (20 hr) on chromosomal aberrations in cells of line NCTC 9038

Exp.	Passage no./ days *in vitro*	Treatment*	Inoculum, cells/cm² × 10^{-4}	Growth period, days Pre-exposure	Growth period, days Post-exposure	Cells studied, no.	Mean no. per cell Chromatid breaks	Mean no. per cell Minutes	Mean no. per cell Chromatid exchanges	Mean no. per cell Metacentrics
1	11/127	S	2.2	3	0	50	0	0.64	0	0.02
		E				84	0	1.14†	0.01	0.04
2	14/148	S	2.2	3	0	66	0	0.47	0	0
		E				50	0	0.68	0.02	0
3‡	15/155	S	1.0	3	3	150	0.01	0.60	0	0.01
		E				154	0.13§	1.16†	0.01	0.01
4	22/194	S	1.1	2	0	56	0.02	0.41	0.02	0
		E				50	0.50§	1.36§	0	0.06
5		S	1.1	2	1	108	0.02	0.46	0	0.07
		E				56	0.22§	1.88§	0.08†	0.07
6	23/200	S	1.7	2	0	78	0	0.47	0	0.03
		E				97	0.47§	0.92	0.21†	0.13

* S, shielded; E, exposed.
† $P < 0.05$.
‡ Cells exposed in T-30 flask. Immediately after exposure, control and exposed cells were trypsin-dispersed and inoculated at 1.1×10^4 cells/cm² on coverslips.
§ $P < 0.005$.

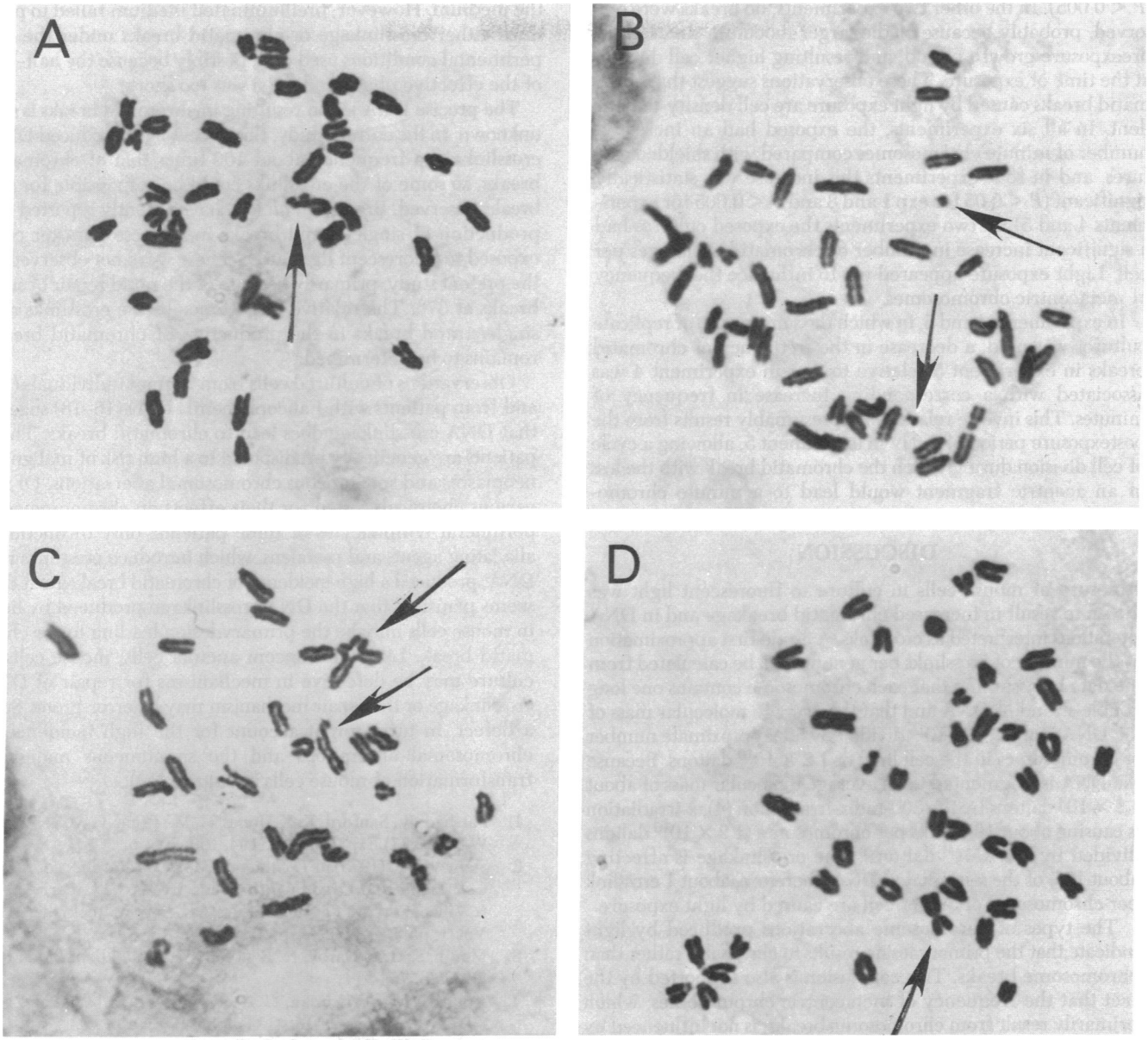

FIG. 2. Metaphase plates of mouse cell line NCTC 9038 showing chromosomal aberrations produced by 20 hr of exposure to fluorescent light. Arrows indicate: (*A*) chromatid break; (*B*) two minute chromosomes; (*C*) chromatid break (lower) and chromatid exchange (upper); and (*D*) metacentric. (×2700.)

obtained by fitting the equation:

$$P_{(t)} = ae^{-bt^c}$$

to the experimental data by the method of least squares. In this expression, $P_{(t)}$ is the fraction of DNA retained at time t and the parameters a, b, and c are estimated from the elution data. In all cases there was an excellent fit of this equation to the data (Fig. 1). At both time points, there was a significantly higher percentage of DNA retained from cells exposed to light ($P < 0.005$ for each point). However, the DNA of cells treated with 20-hr preilluminated culture medium eluted at the same rate as that from untreated cells.

In this assay, the cell mixture is x-irradiated to fragment the DNA prior to elution. An alternative interpretation of the slower elution of the light-exposed DNA is that the exposed cells can repair the x-ray breaks more rapidly, yielding higher molecular weight DNA during the interval from x-irradiation in one building to lysis on the filter in another. To test this hypothesis, cultures were lysed immediately after x-irradiation and mixed with cultures lysed later in the usual way. The elution curves were virtually identical. Thus, the slower elution of the light-exposed DNA appears to result from crosslinkage rather than from a more rapid repair of x-ray-produced breaks.

Chromosomal Aberrations. Table 2 summarizes the effect of a single exposure (20 hr) of fluorescent light on the frequency of chromosomal aberrations in cell line NCTC 9038 at various passage levels and days in culture. The following four types of chromosomal aberrations were observed: chromatid breaks, minutes, chromatid exchanges, and metacentrics (Fig. 2), and their frequency per cell was recorded. Abnormalities scored as chromatid breaks showed clear discontinuity and chromatid nonalignment (Fig. 2 *A* and *C*). Minutes were chromosomes less than half the length of the smallest chromosome in the mouse karyotype (Fig. 2*B*). Chromatid exchanges were interchromosomal (Fig. 2*C*).

In four of six experiments, compared with shielded controls, the cultures exposed to light had a significant increase in percentage of cells with breaks and a significantly higher number of chromatid breaks per cell based on the χ^2 test of homogeneity

($P < 0.005$). In the other two experiments, no breaks were observed, probably because of the larger inoculum size, longer preexposure growth period, and resulting higher cell density at the time of exposure. These observations suggest that chromatid breaks caused by light exposure are cell density-dependent. In all six experiments, the exposed had an increased number of minute chromosomes compared with shielded cultures, and in four experiments this increase was statistically significant ($P < 0.05$ for exp 1 and 3 and $P < 0.005$ for experiments 4 and 5). In two experiments the exposed cultures had a significant increase in number of chromatid exchanges per cell. Light exposure appeared not to influence the frequency of metacentric chromosomes.

In experiments 4 and 5, in which the same group of replicate cultures was used, a decrease in the frequency of chromatid breaks in experiment 5 relative to that in experiment 4 was associated with a corresponding increase in frequency of minutes. This inverse relationship presumably results from the postexposure period of 1 day in experiment 5, allowing a cycle of cell division during which the chromatid break with the loss of an acentric fragment would lead to a minute chromosome.

DISCUSSION

Exposure of mouse cells in culture to fluorescent light was shown to result in increased chromatid breakage and in DNA alterations interpreted as crosslinks. A crude first approximation of the number of crosslinks per genome can be calculated from the data by assuming that each chromosome contains one long double-strand of DNA and that the average molecular mass of the DNA is about 6×10^{11} divided by 50 (approximate number of chromosomes in the cell line) or 1.2×10^{10} daltons. Because the DNA is fragmented to an average molecular mass of about 1.2×10^9 daltons by the 300-rad x-irradiation (4), x-irradiation is causing about 10 breaks per chromosome (1.2×10^{10} daltons divided by 1.2×10^9 daltons). The crosslinkage is affecting about 10% of the x-irradiated DNA; therefore, about 1 crosslink per chromosome or 50 per cell are caused by light exposure.

The types of chromosome aberrations produced by light indicate that the primary lesion results in chromatid rather than chromosome breaks. This conclusion is also supported by the fact that the frequency of metacentric chromosomes, which primarily result from chromosome breaks, is not influenced by light exposure. The present results suggest an influence of cell density in that cells in densely populated cultures showed few or no chromatid breaks after irradiation. A similar effect of cell density on the cytotoxic effect of fluorescent and black light has been reported by Wang and associates (8, 9). They attributed the toxicity of visible light for mammalian cells to photoproducts produced in the culture medium and suggested that the cell-killing effect is density dependent because, when more cells were present, the amount of photoproducts absorbed by each cell was correspondingly less. From their experimental results, they concluded that the photochemical reaction involves the riboflavin-sensitized oxidation of tryptophan and tyrosine in the medium. Bradley and Sharkey, using different conditions of exposure, observed mutagenicity and some toxicity of fluorescent light for V-79 Chinese hamster cells that appeared to be direct and not mediated through the culture medium (10). In contrast to these toxic effects, a proliferative response of human diploid fibroblasts has been observed after short exposure of cells or culture medium to fluorescent light, whereas long exposures were cytotoxic (11, 12). In experiments comparable to those used for human cells, mouse cells failed to show a proliferative or cytotoxic response (13). DNA crosslinkage and chromatid breaks could result from photoproducts formed in the medium. However, preilluminated medium failed to produce either crosslinkage or chromatid breaks under the experimental conditions used (13), possibly because the half-life of the effective photoproduct(s) was too short.

The precise DNA lesion resulting in chromatid breaks is still unknown. In the current study, fluorescent light produced DNA crosslinks at a frequency about 100 times that of chromatid breaks, so some of the crosslinks could be responsible for the breaks observed. Bradley *et al.* (14) have recently reported the production of single-strand breaks in Chinese hamster cells exposed to fluorescent light at 1°. These were not observed in the present study, primarily because of the rapid repair of such breaks at 37°. The relative importance of the crosslinks and single-strand breaks in the production of chromatid breaks remains to be determined.

Observations of cultured cells from normal individuals (15) and from patients with Fanconi anemia (FA) (16–18) suggest that DNA crosslinkage does lead to chromatid breaks. These patients are genetically predisposed to a high risk of malignant neoplasms and spontaneous chromosomal aberrations. Of the various chemicals tested for their effects on chromosomes in peripheral lymphocytes of these patients, only difunctional alkylating agents and psoralens, which introduce crosslinks into DNA, produced a high incidence of chromatid breakage. It thus seems plausible that the DNA crosslinkage produced by light in mouse cells may be the primary lesion leading to the chromatid break. Like the Fanconi anemia cells, mouse cells in culture may be defective in mechanisms for repair of DNA crosslinkage or the repair mechanism may be error-prone. Such a defect, in turn, might account for the high incidence of chromosomal aberrations and the spontaneous malignant transformation of mouse cells in culture (19).

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