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Fluorescent light-induced chromosome damage and its prevention in mouse cells in culture

(cell culture/chromosomal aberrations/catalase/glutathione and ascorbic acid)

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ABSTRACT **Twenty-hour-exposure to fluorescent light produces chromatid breaks in a line of adult mouse lung cells grown in Dulbecco–Vogt medium supplemented with fetal bovine serum. The light-induced damage appears to be enhanced by increasing the concentration of oxygen in the gas phase of the culture. The effective wavelength(s) of light is in the visible range between 400 and 450 nm and is probably the mercury emission peak at 405 or 436 nm. Addition of catalase or glutathione with ascorbic acid to the culture medium reduced the number of chromatid breaks to a level not significantly different from that in the shielded cultures. It thus appears that the production of H_2O_2 in the culture medium or in the cell is responsible for the chromatid breaks. Most of the chromosomal abnormalities observed in long-term culture of mouse cells may result from exposure of cells or medium to fluorescent room lights in the presence of atmospheric oxygen. These genetic abnormalities can be minimized by shielding cells and medium from light, lowering the P_{O_2} of the medium, and including reducing agents such as glutathione and ascorbic acid in the medium formulation.**

Two features of rodent cells (particularly mouse cells) in culture are their karyotypic instability and their almost universal tendency to undergo malignant neoplastic transformation in the absence of deliberately added viruses or known carcinogens. In fact, normal mouse embryo cells initiated and maintained continuously in serum-free chemically defined medium usually transform in slow-growing primary culture even before the cells can be successfully passaged *in vitro* (1). Furthermore, changes in chromosomal constitution of mouse cells may occur as early as 10 days after explantation to culture (2). Any factors that prevent this karyotypic instability during routine handling of mouse cell cultures are important because this instability has hindered genetic, cell fusion, and oncogenic studies with cells from inbred mouse strains.

Three environmental factors have been found to influence the mouse karyotype: the type of serum used to supplement the medium (3), the concentration of oxygen in the gaseous phase (4), and, most recently, fluorescent light (5). Cultures shielded from room fluorescent light during routine handling by wrapping the flasks in aluminum foil showed fewer chromosomal abnormalities than did unwrapped controls. This observation led to the short-term experiments described here and to some long-term experiments, the results of which suggest that light and oxygen may play a role in the malignant transformation of mouse cells in culture (6). A parallel study (unpublished) with the same cell line and exposure conditions used here has shown that the DNA of the light-exposed cells is altered, an alteration interpreted as crosslinking.

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Fluorescent light produces photooxidative products that are toxic or mutagenic to mammalian cells in culture (7–10). Toxicity appears to result from photosensitization of riboflavin, with subsequent oxidation of tyrosine and tryptophan (7). In addition, irradiation of saturated oxygenated solutions of tryptophan by light gives rise to appreciable amounts of the mutagen H_2O_2 (11). In view of these observations, we explored the possibility that the light-induced chromatid breaks may result from a photodynamic oxidative process leading to the production of biologically active substances such as H_2O_2. Accordingly, we studied the influence of oxygen, preilluminated medium, wavelength of light, the reducing agents glutathione and ascorbic acid, and the enzymes catalase (hydrogen-peroxide:hydrogen-peroxide oxidoreductase, EC 1.11.1.6) and superoxide dismutase (superoxide:superoxide oxidoreductase, EC 1.15.1.1) on the production of chromosomal aberrations in a line of mouse lung cells in culture.

MATERIALS AND METHODS

Cell Culture. The origin and early history of NCTC line 8466 initiated from lung tissue of a 59-day-old C57BL/6N male mouse have been described (5). Cultures of this cell line had been maintained in serum-supplemented NCTC 135 medium with a gas phase of 1% O_2 and showed few chromosomal abnormalities compared with parallel lines grown with 18% O_2 (atmospheric) (5). The cells had been exposed to room lights [~42 footcandles (452 lm/m^2) in an unlighted laminar flow hood] for ~30 min three times weekly for medium renewals and subculturing. The cells of the present study were derived from cells frozen at the ninth subculture after 153 days *in vitro*. Soon after thawing, the cells were transferred from NCTC 135 medium to Dulbecco–Vogt medium with 10% fetal bovine serum and were carried in T-15 flasks with 3 ml of medium. Medium was renewed three times weekly and cultures were gassed with a humidified mixture of 1% or 0% O_2/10% CO_2/N_2. When confluent, cells were subcultured by a brief rinse with EDTA (1:5000, Microbiological Associates, Bethesda, MD) and dispersion with an EDTA/trypsin mixture ATV (12). Glutathione (reduced), ascorbic acid, catalase, and superoxide dismutase were obtained from Calbiochem, Merck, and Sigma, respectively.

Conditions for Light Exposure. To examine the effect of fluorescent light, cells were inoculated at 5–7 × 10^4 cells per 2 ml of culture medium into replicate Leighton tubes each containing a 9 × 50 mm coverslip (no. 1 thickness, Bellco Glass Co., Vineland, NJ). After a growth period of approximately 24 hr with a gas phase containing 18% O_2 and 10% CO_2, the experimental cultures were exposed to light for 20 hr at 37°. Control cultures, the stock line, and culture medium were never exposed to light of wavelength <500 nm (they were routinely

handled under gold or red light). The experimental cultures were placed at a distance of 40.6 cm from two cool-white Westinghouse bulbs (F15 T8-CW) or, in one experiment, two green bulbs (F15 T8/G) yielding approximately 150 or 220 footcandles, respectively, at the level of the growth surface as measured by a Weston model 614 light meter. The two Plexiglas filters UF-3 and G [0.060 inch (1.5 mm) thick] were obtained from Read Plastics, Inc. (Rockville, MD) while the P-3 filter was obtained from Eastman Kodak (Rochester, NY).

Chromosome Analyses. Immediately after the exposure to light, 0.1 μg of Colcemid (GIBCO, Grand Island, NY, reconstituted in phosphate-buffered saline) per ml of culture medium was added to control and experimental cultures. After 2 hr of incubation to produce mitotic arrest, the culture medium was decanted and replaced by hypotonic solution (75 mM KCl) for 15 minutes 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 (Harleco, Gibbstown, NJ). After staining, the coverslips were rinsed in tap water, air-dried, dipped in xylene, and mounted in Permount. In each experiment, approximately 200 metaphase plates were randomly selected from four experimental and four control cultures for analyses. Coverslips were coded with respect to precise experimental treatment and were examined without knowledge of their history. Except as indicated, the χ^2 test of heterogeneity was applied for statistical evaluation of the data.

RESULTS

Four types of chromosomal aberrations were observed: chromatid breaks, minutes, chromatid exchanges, and metacentrics. The chromatid break probably is the primary lesion from which the other three types evolve.

In all seven experiments of this study, 20-hr exposure of cells at 1 day after inoculation produced a significant increase in chromatid breaks ($P < 10^{-4}$). In four of the experiments there was a significant increase in the frequency of chromatid exchanges after exposure to light ($P < 10^{-4}$–10^{-2}), and in one experiment, a significant increase in frequency of minutes and metacentrics was also seen ($P = 0.003$ and 0.02, respectively). The average number of metacentrics per cell showed a cumulative increase with period of culture *in vitro* as indicated by the results in Tables 3, 4, and 5 on cells carried for 273, 301, and 329 days *in vitro* (22, 26, and 29 passages), respectively.

Effect of Oxygen and Preilluminated Medium. Cells were exposed to light with 1% or 18% (atmospheric) O_2 in the gaseous phase of the culture [culture medium P_{O_2} approximately 40–60 mm Hg at 1% and 125–130 mm Hg at 18% O_2 (13)] (Table 1). Because very few cells had more than one chromosome abnormality, a three-way classification of data (exposed or shielded, 1% or 18% O_2, percentage of cells with one or more aberrations) leading to a $2 \times 2 \times 2$ contingency table was analyzed by exact statistical tests (14, 15). There was no significant oxygen effect or interaction between the percentage of O_2 and light exposure for any of the four categories of chromosomal aberrations. Although the oxygen–light interaction was not statistically significant, the effect of light exposure on incidence of minutes and metacentrics was enhanced at 18% O_2. (For minutes, the relative odds for exposed cells compared to shielded was 1.65 at 1% and 4.11 at 18% O_2; for metacentrics, the relative odds for exposed cells compared to shielded was 2.17 at 1% O_2 and 7.64 at 18% O_2.)

To determine whether the light effect was mediated through the culture medium, 10 ml of medium in a T-60 flask gassed with 18% O_2 was illuminated for 20 hr before use. Cells exposed to the preilluminated medium for an additional 20 hr showed a slight increase in the number of chromatid breaks and minute chromosomes but neither increase was statistically significant.

Table 1. Effect of oxygen and preilluminated culture medium on frequency of chromosomal aberrations induced by fluorescent light

Passage/ days in culture	Treatment	Average no. per cell: Chromatid breaks	Minutes	Chromatid exchanges	Metacentrics
10/167	1. Shielded, 1% O_2	0.017	0.035	0.004	0.009
	2. Light-exposed, 1% O_2	0.202*	0.056†	0.086*	0.026‡
	3. Shielded, 18% O_2	0.044	0.027	0.011	0.006
	4. Light-exposed, 18% O_2	0.279*	0.105†	0.105*	0.041‡
12/189	5. Shielded, 18% O_2	0.025	0.015	0	0.020
	6. Light-exposed, 18% O_2	0.285*	0.045	0.050§	0.065
	7. Preilluminated medium, 18% O_2	0.045	0.020	0.005	0.025

* $P < 10^{-4}$ between treatments 1 and 2, 3 and 4, and 5 and 6.
† $P = 0.003$ between treatments 1 and 2, and 3 and 4.
‡ $P = 0.021$ between treatments 1 and 2, and 3 and 4.
§ $P < 0.010$ between treatments 5 and 6.

Effective Range of Wavelengths. To determine whether the effective wavelength(s) lies in the UV (<400 nm) or visible range, three types of filters were used: Plexiglas G and UF-3 with cool-white and P-3 with green fluorescent light (green light was used to furnish more distinct mercury emission peaks at 365, 405, and 436 nm). The transmittance properties of these filters are illustrated in Fig. 1. Both the Plexiglas G and UF-3 filters did not eliminate the light-induced chromatid damage in that the frequency of chromatid breaks was significantly higher than in the shielded controls ($P < 0.0002$). However, with both filters, and especially the UF-3 which has a cutoff near 400 nm, the frequency of chromatid breaks was reduced from that in the light-exposed cultures, presumably because of the 10% reduction in overall transmittance and the partial cutoff of the 405-nm peak with the UF-3. On the other hand, the P-3 filter, which has a cutoff at ~450 nm, completely eliminated the light-induced chromatid breaks (Table 2). These results indicate that the effective wavelength is in the visible range between 400 and 450 nm and is probably the mercury emission peak of 405 or 436 nm.

Addition of Catalase and/or Superoxide Dismutase. To test whether a photodynamic oxidative process leading to the production of singlet O_2 or H_2O_2 might cause the chromatid breaks, the enzymes catalase and superoxide dismutase were added to the culture medium during the light exposure. Catalase decomposes H_2O_2, and superoxide dismutase scavenges superoxide radical. The frequency of light-induced chromatid breaks and exchanges was significantly reduced by the addition of catalase 0.05 or 0.1 μg/ml and was not significantly higher than in shielded cultures (Table 3). Addition of superoxide dismutase alone did not diminish the light-induced chromatid damage; however, the addition of catalase and superoxide dismutase did significantly reduce the number of chromatid breaks, although not to the level in the shielded cultures (Table 4). These results suggest that the light-induced chromatid breaks

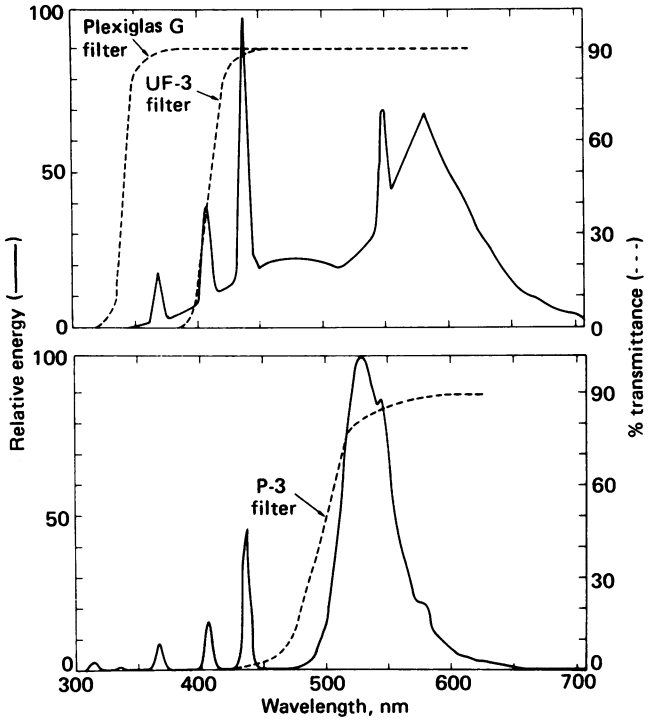

FIG. 1. Transmittance properties of Plexiglas G, UF-3, and P-3 filters. Spectral distributions of cool-white fluorescent lamp (*Upper*) and of green lamp (*Lower*) were kindly supplied by Peter Baumbusch, Westinghouse Electric Corporation, Bloomfield, NJ.

result primarily from H_2O_2 produced in the culture medium or in the cells.

Addition of Reducing Agents. To determine whether reducing agents that promote destruction of H_2O_2 would influence the light-induced chromatid damage, glutathione alone or in combination with ascorbic acid was added to the culture medium during the exposure to light. The addition of glutathione significantly reduced the number of light-induced chromatid breaks but not to the level in the shielded control cultures (Table 5). However, the combination of glutathione and ascorbic acid with either 0% or 18% O_2 reduced the number of light-induced chromatid breaks to a level not significantly different from that in shielded cultures. Neither glutathione nor catalase at the low concentrations used were absorbing light of wavelengths 300–450 nm to any appreciable degree.

Table 2. Effect of filters on frequency of chromosomal aberrations induced by fluorescent light

Passage/ days in culture	Treatment	Average no. per cell: Chromatid breaks	Minutes	Chromatid exchanges	Metacentrics
16/231	1. Shielded	0.025	0.015	0	0.125
	2. Light-exposed	0.405*	0.025	0.040†	0.095
	3. Light-exposed and UF-3 filter	0.175‡	0.040	0.005§	0.090
	4. Light-exposed and Plexi-G filter	0.215¶	0.040	0.005§	0.100
23/280	5. Shielded	0	0.030	0	0.775
	6. Light-exposed (green light)	0.170*	0.040	0.005	0.795
	7. Green light + P-3 filter	0.010	0.025	0	0.750

* $P < 10^{-6}$ between treatments 1 and 2, and 5 and 6.
† $P = 0.022$ between treatments 1 and 2.
‡ $P = 2 \times 10^{-4}$ between treatments 2 and 3, and 1 and 3.
§ $P = 0.074$ between treatments 2 and 3, and 2 and 4.
¶ $P = 0.001$ between treatments 2 and 4; $P = 1 \times 10^{-4}$ between treatments 1 and 4.

Table 3. Prevention of light-induced chromatid breaks by catalase

Treatment	Average no. per cell: Chromatid breaks	Minutes	Chromatid exchanges	Metacentrics
1. Shielded	0.030	0.030	0	0.600
2. Shielded + catalase 0.05 μg/ml	0.015	0.030	0	0.610
3. Shielded + catalase 0.1 μg/ml	0.025	0.025	0	0.655
4. Light-exposed	0.285*	0.010	0.040†	0.550
5. Light-exposed + catalase 0.05 μg/ml	0.045‡	0.014	0	0.577
6. Light-exposed + catalase 0.1 μg/ml	0.070§	0.020	0.005	0.575

* $P < 10^{-6}$ between treatments 1 and 4.
† $P = 0.020$ between treatments 1 and 4.
‡ $P = 0.143$ between treatments 2 and 5.
§ $P = 0.060$ between treatments 3 and 6.

DISCUSSION

These results show that 20-hr exposure to fluorescent light produces chromatid breaks in adult mouse cells grown in Dulbecco–Vogt medium supplemented with fetal bovine serum. The light-induced damage appears to be slightly enhanced by using 18% compared with 1% O_2 in the gas phase of the culture. However, complete elimination of the damage by sparging the cultures with 0% oxygen was not attained, presumably because the culture medium P_{O_2} was still relatively high (13). The effective wavelength of light is in the visible range, between 400 and 450 nm, and is probably one of the mercury emission peaks at 405 or 436 nm.

In the present study, catalase and glutathione with ascorbic acid, agents known to promote the destruction of H_2O_2 (16), appeared to prevent the light-induced chromatid breaks when

Table 4. Effect of superoxide dismutase and catalase on frequency of chromosomal aberrations induced by fluorescent light

Treatment	Average no. per cell: Chromatid breaks	Minutes	Chromatid exchanges	Metacentrics
1. Shielded	0.015	0.010	0	1.055
2. Shielded + SOD	0.005	0.015	0	1.065
3. Shielded + SOD + C	0.005	0.030	0	0.985
4. Exposed	0.310*	0.010	0.025	1.015
5. Exposed + SOD	0.315*	0.035	0.060	1.045
6. Exposed + SOD + C	0.055*†	0.010	0.020	1.085

SOD = superoxide dismutase, 100 units/ml; C = catalase 0.1 μg/ml.
* $P < 10^{-6}$ between treatments 1 and 4, 2 and 5, and 5 and 6.
† $P = 0.008$ between treatments 3 and 6.

Table 5. Effect of glutathione and ascorbic acid on frequency of chromosomal aberrations induced by fluorescent light

Treatment	Average no. per cell			
	Chromatid breaks	Minutes	Chromatid exchanges	Metacentrics
1. Shielded + GSH	0.005	0.025	0	0.935
2. Shielded + GSH + Asc	0.005	0.020	0.005	0.845
3. Exposed	0.240*	0.035	0.025	0.825
4. Exposed + GSH	0.065†‡	0.010	0.005	0.965
5. Exposed + GSH + Asc	0.030*§	0.030	0.015	0.975
6. Exposed + GSH + Asc	0.020¶	0.020	0.005	0.975

GSH = glutathione (reduced), 0.02 mg/ml; Asc = ascorbic acid, 0.1 mg/ml. Gaseous phase contained 0% O_2 in 1–5, 18% in 6.
* $P < 10^{-6}$ between treatments 1 and 3, and 3 and 5.
† $P = 0.003$ between treatments 1 and 4.
‡ $P < 10^{-4}$ between treatments 3 and 4.
§ $P = 0.127$ between treatments 2 and 5.
¶ $P > 0.250$ between treatments 2 and 6.

added to the culture medium. It therefore seems likely that the chromatid damage induced by light is mediated through the production of H_2O_2 in the culture medium or in the cells. The lack of a significant effect of preilluminated medium on chromatid damage does not preclude the possibility that H_2O_2 is produced in the medium because H_2O_2 decomposes rapidly in solution. H_2O_2 has been suggested as the agent responsible for cell injury including chromosome damage by ionizing radiation (17, 18) and single- or double-strand breaks or crosslinks in DNA (19–21).

It thus appears that most of the chromosomal abnormalities observed in long-term cultures of mouse cells result from the production of H_2O_2 during exposure of cells or medium to fluorescent light and atmospheric oxygen during routine handling. Such damage can be minimized by the addition of reducing agents to the medium, shielding the cultures and medium from light of wavelength below 450 nm, and lowering the P_{O_2} of the medium. No abnormalities were observed in mouse cells shielded from light and grown in a medium containing glutathione and ascorbic acid, NCTC 135, with a gaseous phase of 1% O_2. On the other hand, cells of the same line under the same culture conditions but grown in Dulbecco–Vogt medium lacking these reducing agents showed many chromosomal abnormalities by the end of 2 months in culture (6).

Although the role of light as a causative factor in the spontaneous malignant transformation of mouse cells in culture is yet to be firmly established, limited studies to date suggest that repeated exposure of cells to fluorescent light in medium with a high P_{O_2} and lacking glutathione and ascorbic acid enhances malignant transformation (6). Under these conditions, light-exposed cells also show a higher frequency of chromosomal abnormalities than do the shielded controls.

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