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Study of Ultraviolet-Induces Chromatid and Chromosome Aberrations as a Function of Dose in G1 Phase Vertebrate Tissue Cultures

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The Study of Ultraviolet-Induced Chromatid and Chromosome Aberrations as a Function of Dose in G_1 Phase Vertebrate Tissue Cultures*

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ABSTRACT

G_1 phase A8 *Xenopus laevis* (toad) and V79B *Cricetulus griseus* (hamster) tissue cultures were used to observe the frequency of ultraviolet-induced chromosomal aberrations as a function of dose. When cultures are irradiated with ultraviolet light, visible aberrations are virtually absent until a threshold of approximately 80 ergs mm^{-2} is reached. Aberrations then occur as a nonlinear function of dose. Chromatid aberrations are by far the most prevalent until doses in excess of 200 ergs mm^{-2} are administered, at which point chromosome aberrations become common.

INTRODUCTION

The study of ultraviolet light (UV)-induced chromosomal aberrations in eukaryotic cells has been limited in comparison with that of the induction of chromosomal aberrations by ionizing radiation. The frequency of chromosomal aberrations in G_1 vertebrate cells induced by ionizing radiation has been observed to be a logarithmic function of the dose administered. These aberrations are basically of the chromosome type, which can be subdivided into deletions, resulting from single breaks, and exchanges, resulting from multiple breaks and rejoining (Evans 1967). Humphrey et al. (1963) studied aberration production in hamster cells by a single dose of UV (100 ergs mm^{-2}, 254 nm) in the DNA-sensitive absorption range. Log phase cells in monolayers were irradiated and samples were collected for chromosome analysis when they reached the first mitosis after exposure. To determine whether the cells in a given collection sample were in the G_1, S, or G_2 phase of the cell cycle during exposure, the monolayers were flash labelled with tritiated thymidine before UV was administered. Somewhat surprisingly, Humphrey's group found that a high frequency of chromatid aberrations was produced in the G_1 cells, but the frequency of chromosome aberrations observed in these same G_1 cells was not significantly above the control level. Chu (1965) carried out a detailed study of aberration production in hamster cells, using 265 nm in the DNA absorption range and 280 nm in the protein absorption range. These studies with 265 nm were carried out in essentially the same manner as the work by Humphrey et al. (1963). Chu found, however, that doses such as 50, 100, and 200 ergs mm^{-2} produced a significant frequency of both chromatid and chromosome aberrations in G_1 cells. He also concluded that UV-induced aberration frequencies increased with increasing dose, as was the case with ionizing radiations. Both of these studies were complicated by the complex "mixing" of cells after UV exposure of log phase cultures, because of the fact that cells in different phases of the cell cycle during a given exposure are delayed by different time intervals in their progression toward mitosis. Griggs and Bender (1973), working with synchronous cultures of A8 *Xenopus* and V79B hamster cells, observed that a dose of 120 ergs mm^{-2} (254 nm) induced damage in G_1 cells which was expressed as chromatid aberrations in exposed cells that reached the first mitosis after exposure. A significant frequency of chromosome aberrations was not observed. By a rather detailed study of aberration frequencies as a function of UV dose (254 nm) over an extended dose range, using synchronous cultures of cells to circumvent the problem of cell mixing, the writers hoped to elucidate the mechanism of aberration production by UV in G_1 cells and to aid in the resolution of the apparent conflict mentioned above.

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MATERIALS AND METHODS

The A8 amphibian cell line is maintained in monolayer cultures at approximately 24C in glass tissue culture bottles and plastic tissue culture flasks in F12A medium. F12A medium consists of powdered F-12 medium (Grand Islands Biological Company) supplemented with fetal calf serum. Penicillin and streptomycin are added to aid in controlling contamination, and the pH is controlled with N-2-hydroxy ethylpiperazine-N'2' ethane sulfonic acid (HEPES) and sodium bicarbonate (Griggs and Bender 1972).

The V79B Chinese hamster cell line is maintained in monolayer cultures at 37C in F10 medium. This medium consists of F-10 powdered medium (Grand Islands Biological Company) supplemented with fetal calf serum. Penicillin and streptomycin are added to help control contamination, and the pH is controlled with sodium bicarbonate and carbon dioxide after titration with sodium hydroxide. Both types of media are sterilized by filtration.

Routine cultivation is achieved in the A8 cell line by vigorous shaking of the culture to produce a suspension of cells. The V79B cells, however, being more firmly attached to the surface of the container, are treated with a trypsin-versene solution to produce cell suspensions.

The UV source consisted of a single 15W germicidal lamp (Sylvania G15 T8). The lamp was suspended 60 cm above the floor of the radiation chamber, a glass enclosure with a plastic front containing entry ports (to permit the use of hands and for placing items within the chamber). Thin sheets of plastic were placed between the lamp and the radiation area to decrease the UV dose rate to the point at which relatively accurate dosages could be obtained by varying the exposure time. The exposure time was controlled by manually exposing the cells while recording the elapsed time. The modified dose rate, approximately 4 ergs mm^{-2} sec^{-1}, was checked periodically with a Westinghouse WL 755 phototube designed to read intensity at 254 nm.

Each culture for irradiation was prepared and irradiated as follows. The stock culture bottle was shaken lightly and the medium changed to remove dead cells and start the synchronization of the culture for G_1 cells. Cells were collected from the stock culture by similar "shake-offs" every 30 minutes, to remove only those cells that had just come through mitosis. Then 2 ml of each resulting cell suspension was deposited carefully in the center of a 10-cm petri plate so as to form a large drop. Within approximately two hours at room temperature the cells had settled to the bottom and attached to the surface, with no cells more than 2 cm from the center of the plate and with essentially no touching or overlap; thus exposure was uniform without interference from the shadow cast by the sides of the petri plate. Once the cells had attached to the plate, the medium was removed, the cells were irradiated for a specific length of time according to the desired dose, and fresh medium was gently reapplied.

All A8 irradiations were done under red light to avoid photoreactivation, and the irradiated cultures were transferred immediately to a light-proof container. Irradiated cultures were incubated at 24C in these covered glass containers, which kept the humidity near 100%, until the cultures were ready for fixing. Colcemid, to a final concentration of 10^{-6}M, was added after the appropriate incubation period, and 24 hours later the cells were fixed on glass slides with 3:1 methanol-acetic acid and stained with crystal violet. Chromosome spreads were scored by conventional methods (Wolff 1961). Spreads containing a normal complement of chromosome (36 for A8 cells and 22 for V79B cells) were examined for chromatid terminal deletions, isochromatid breaks, chromatid exchanges, chromosome terminal deletions, interstitial deletions, and chromosome exchanges (rings and dicentrics).

The monolayers of V79B G_1 cells were treated throughout the experiments in essentially the same manner as the A8 cells with the following exception. All cultures were maintained at 37C in glass containers and irradiated cultures were fixed approximately 15 hours after treatment with colcemid to a final concentration of 4×10^{-6}M.

RESULTS AND DISCUSSION

Extensive mitotic index determinations not only provided a guideline for appropriate incubation periods, but also yielded informative data for doses between 0 and 120 ergs mm^{-2} (Fig. 1). A marked increase in the time required for the cells to reach mitosis after irradiation was observed through the 60 erg point, beyond which the required time became more consistent. A similar pattern was noted in the effect of the radiation dose on the optimum mitotic index (Fig. 2); there was a relatively sudden decrease in the fraction of cells reaching mitosis, again through the 60 erg point. These determinations have an important bearing on the validity of data concerning chromatid aberrations at doses below 200 ergs mm^{-2} (Griggs and Bender 1973). Figures 1,2, and 4 and Table I show only data for the A8 cell line to maintain clarity, but similar data were observed for the V79B mammalian line. Griggs and Bender (1972) reported that the surviving fraction for A8 cells varied logarithmically with dose, which also seems true of the writers' mitotic index data between 0 and 60 ergs mm^{-2}.

When aberration frequencies are plotted as a function of dose, no clear increase in aberration frequency is observed until doses exceed 60 ergs mm^{-2}, beyond which the curves sharply rise (Fig. 3). This rise is first noticed in the V79B cells at the 75 erg point, and appears in the A8 cells at about the 90 erg point (Fig. 3, and Table I). This event occurs after the aforementioned observed effect of UV radiation on mitotic index. These data suggest that some threshold radiation value must be exceeded before aberrations appear, and thus imply that an "accumulation of UV-induced events" leads to aberrations. This effect is not observed in aberration production with ionizing radiation, and has not been observed before in studies reported on UV-induced aberration production, probably because no one has studied doses below 50 ergs mm^{-2} for damage induced by wavelengths in the DNA-sensitive absorption range.

Separate compilation of the chromatid and chromosome aberration frequencies showed that chromosome aberrations became noticeably more prevalent at 200 ergs mm^{-2} than at any lower dose administered (Fig. 4). The fact that relatively low frequency of chromosome aberrations was noticed in the controls and at low radiation levels suggests a threshold radiation value for chromosome aberrations at a higher dose level than the threshold for chromatid aberrations.

The data are somewhat similar to Chu's (1965) findings at higher doses, but are more in accord with the data presented by Humphrey et al. (1963) for doses of about 100 ergs mm^{-2}. Consideration of the long and complicated division delays (more than 70 hours in some cases) reported by Chu suggests that he may have scored many cells at the second mitosis after exposure instead of the first. For example, irradiation of a given G_1 cell might result in damage which would appear in a cell scored at the first division as a chromatid terminal deletion. However, if this cell were capable of moving through a second cell cycle, the damage would appear at the second mitosis as a terminal chromosome deletion. Such an effect could significantly bias results in such a manner as to overestimate chromosome

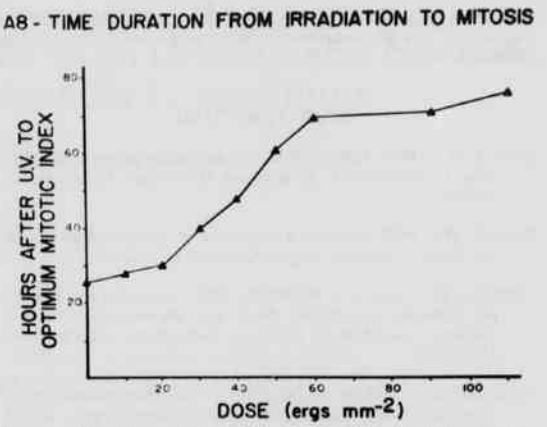

Figure 1. Time interval of the A8 cell line from irradiation to the maximum mitotic index as a function of dose.

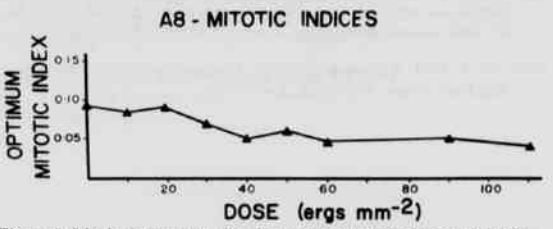

Figure 2. Maximum fraction of A8 cells reaching mitosis as a function of dose.

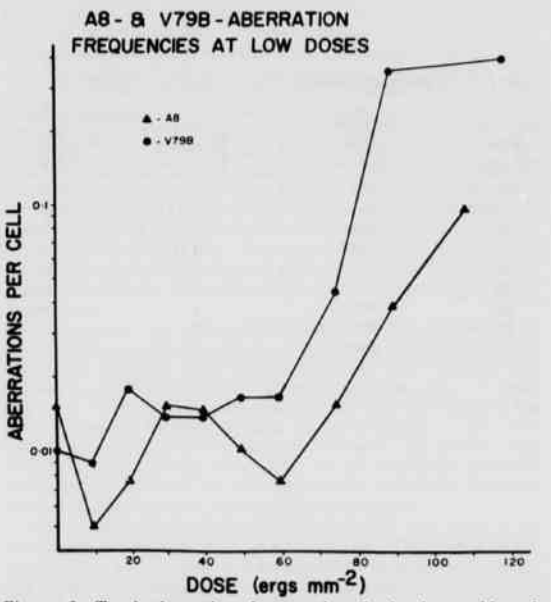

Figure 3. Total aberration frequencies (both chromatid and chromosome types) of the A8 and V79B cells lines as a function of dose between 0 and 120 ergs mm^{-2}. Average number of cells scored was 365 for the A8 and 780 for the V79B.

aberration frequencies and underestimate chromatid aberration frequencies. Use of synchronous cultures minimized this problem in the writers' study.

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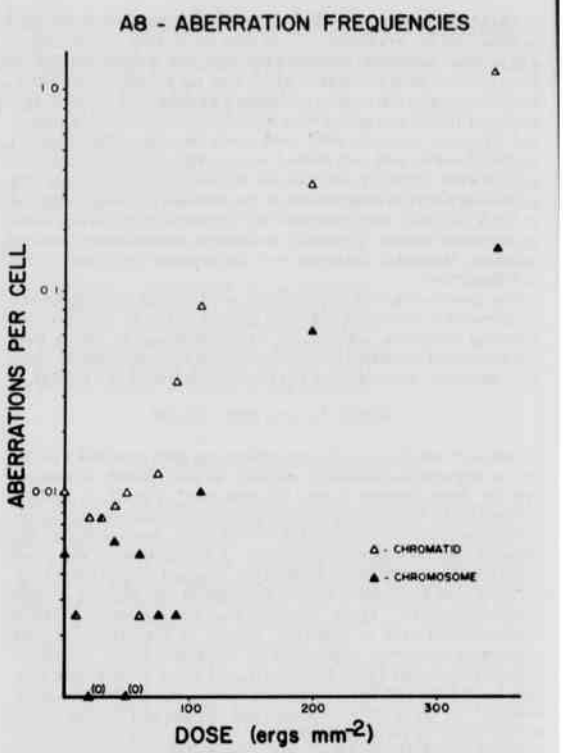

Figure 4. Aberration frequencies of the A8 cell line over an extended dose range, with aberrations separated into chromatid and chromosome types. Average number of cells scored was 425.

Table I. Aberration Distribution for A8 Cells at Low Doses

Dose ergs mm^{-2}	Number cells scored	Chromatid aberrations			Chromosome aberrations		
		Terminal deletions	Isochromatid breaks	Exchanges	Terminal deletions	Interstitial breaks	Exchanges
0	200	2	0	0	1	0	0
10	400	1	0	0	1	0	0
20	300	2	0	0	0	0	0
30	310	2	0	0	1	1	0
40	500	3	0	1	3	0	0
50	400	4	0	0	0	0	0
60	400	1	0	0	2	0	0
75	400	6	0	0	1	0	0
90	400	11	2	1	0	0	1