MOLECULAR RADIOBIOLOGY OF HUMAN CELL LINES. I. COMPARATIVE SENSITIVITY TO X-RAYS AND ULTRAVIOLET LIGHT OF CELLS CONTAINING HALOGEN-SUBSTITUTED DNA*.

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The incorporation of 5-halogenated thymidine analogs, 5-bromo- or 5-iododeoxyuridine (BUdR, IUdR), into deoxyribonucleic acid (DNA) of mammalian cells increases their sensitivity to ultraviolet light (UV) and also to X-rays (Djordjevic and Szybalski, 1960). The present study extends these observations to a somewhat more exhaustive evaluation of the effect on X-ray sensitivity. In addition to BUdR and IUdR, a third thymidine analog, 5-chlorodeoxyuridine (CUdR) was examined as a possible radiosensitizer.

EXPERIMENTAL. - The general tissue culture techniques were described previously (Djordjevic and Szybalski, 1960). The cell line, D98/AG (Szybalski and Smith, 1959), was selected for this study because of its favorable cultural characteristics. Prior to irradiation, 25×10^4 cells were inoculated into 2-oz. prescription bottles containing 5 ml of E_{90} medium alone or supplemented with various concentrations of CUdR, BUdR or IUdR together with a specific inhibitor of thymidylate synthetase (Heidelberger et al., 1957), 5-fluorodeoxyuridine (FUdR) at a concentration of 0.004 µg/ml. The cells were grown on the glass surface for four days (with a medium change on the second day), removed by trypsinization, centrifuged, and the pellet resuspended in a balanced salt solution with phenol red omitted (BSS). The resulting suspension was filtered through cheese cloth and the cell concentration determined by microscopic count. To determine UV survival, a sample of this filtered cell suspension, diluted in BSS to obtain 10^5 cells/ml, was added (10 ml) to a 9 cm open dish, and irradiated at a distance of 60 cm with a 15 watt Westinghouse Sterilamp, with agitation. Samples (0.1 ml) were transferred at specified times to 60 mm dishes containing 5 ml E_{90} medium. These were incubated 7 days in 5% CO_2 atmosphere, with a medium

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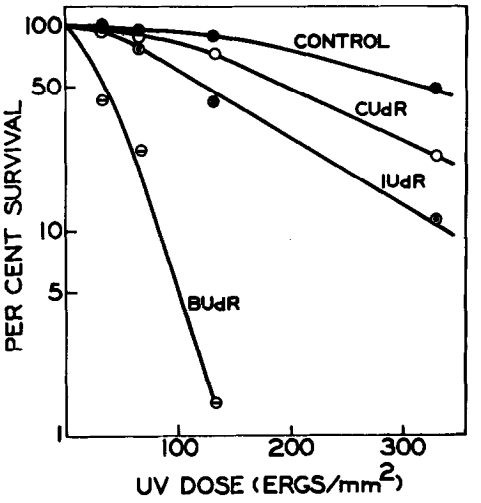
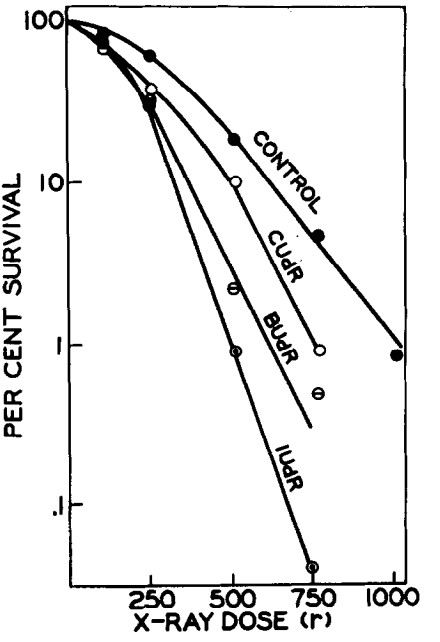
change every two days, stained, and the colonies counted. For X-irradiation*, another sample of the filtered cell suspension was diluted in E_{90} medium with or without 10 per cent glycerol to obtain $5x10^4$ cells/ml. One ml aliquots were irradiated in stoppered polypropylene tubes. After irradiation 0.2 ml samples from each tube were plated in duplicate. To permit more uniform colony development throughout the entire experiment, the cells were stained and counted either on the 7th day (for doses up to 500 r) or on the 10th day (for doses higher than 500r). One of the parameters of the survival curve, the mean lethal dose (MLD), as defined by Elkind and Sutton (1960), is used here to represent the radiosensitivities of normal and halogen-labeled cells. The MLD is the radiation dose necessary to reduce the cell survival to 37 per cent of the non-irradiated controls, calculated from the slope of the exponential portion of the survival curve.

RESULTS AND DISCUSSION. - UV and X-ray survivals for the analog-free control and for the cells after incorporation of each of the three analogs are represented in figures 1A (UV) and 1B (X-ray). In agreement with the unpublished results of Dr. G. Ragni of this laboratory, CUdR was found to be the best thymidine substitute, as judged by its ability to revert FUdR-inhibition. As seen in figures 1A and 1B, however, it also had the least pronounced radiosensitizing effect. When employed at equimolar concentrations; BUdR was by far the best radiosensitizer toward UV, while IUdR incorporation resulted in the highest sensitivity toward X-rays. The MLD's for control, $2x10^{-6}$M CUdR, BUdR, or IUdR were 180, 110, 100, and 75 r (X-rays), and 280, 175, 27, and 140 ergs/mm^2 (UV) respectively. It has been found (unpublished results) that growth prior to X-irradiation at increasing CUdR concentrations did not appreciably increase radiosensitivity any further than represented in Fig. 1B. However, increasing BUdR concentrations did increase sensitivity, as shown in Fig. 2. The concentrations used and their respective MLD's were: $2x10^{-7}$M, 150 r; $2x10^{-5}$M, 90 r; and $8x10^{-5}$M, 55 r. Difficulty was encountered in studying the survival at increasing levels of IUdR because of the high toxicity of this analog. At the ratio of cells to FUdR concentration used in these experiments, there is an incomplete block of thymidylate synthetase, and some thymidine is

*The cells were irradiated with a General Electric machine operated at 5 ma and 140 kV with a 1 mm Al filter. The HVL was 3 mm of Al, and the samples were 16.0 cm from the target. The dose delivered was measured with a Victoreen dosimeter placed inside a polypropylene tube at the same level as the cells. We are indebted to Dr. K. H. Clifton and Dr. H. Vermund of the Department of Radiology for the help and hospitality extended to us in conjunction with the use of the X-ray equipment.





Figures 1A and 1B. Comparative UV (1A) and X-ray (1B) survival of D98/AG cells grown for 4 days prior to irradiation in the absence (control) or in the presence of 0.004 μg FUDR/ml and 2 x 10^{-6}M CUdR, BUdR or IUdR.

synthesized. The radiosensitivity at any given level of BUdR may be increased with higher FUdR concentrations, but unfortunately also with augmentation of the toxicity. The supplementation of the post-irradiation plating medium with thymidine is presently under evaluation.

The lethal effects of X-rays were reduced when the cells were suspended, at the time of irradiation, in medium in which 10 per cent of the water had been replaced by glycerol. This has been observed previously in yeast and bacteria (Marcovich, 1957; Wood, 1959; Dewey, 1960). The relative protection of normal cells and cells after BUdR incorporation was practically the same, as shown in Fig. 2. The MLD's for normal cells with and without glycerol were 370 and 180 r respectively. The corresponding MLD's for cells sensitized with BUdR were 200 and 90 r. Since the relative protection by glycerol for each of the two conditions was nearly the same, it appears that BUdR causes sensitivity to both the glycerol-independent ("direct") and glycerol-dependent ("indirect") effects of X-rays.

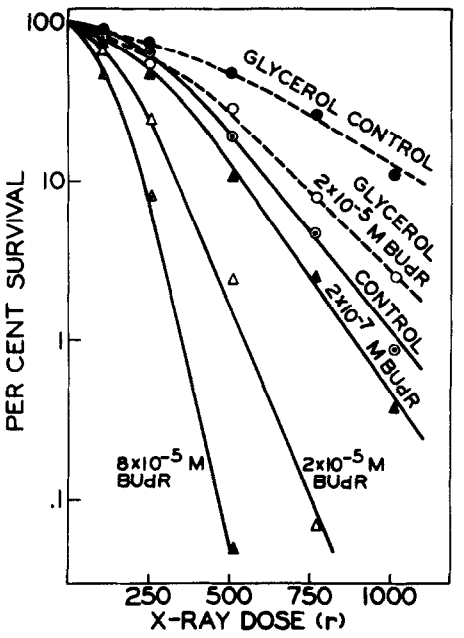


Figure 2. X-ray survival of D98/AG cells grown at various BUdR concentrations and irradiated in glycerol-free (solid lines) or 10% glycerol-supplemented (broken lines) medium.

In summary, the results presented here demonstrate the increased sensitivity of mammalian cells to X-irradiation after incorporation of CUdR, BUdR, or IUdR. It was found that CUdR produced the least and IUdR the greatest sensitivity to X-irradiation. Ten per cent glycerol in the medium during irradiation reduced the lethal effects of X-rays by nearly the same factor, in both normal cells and cells with BUdR-labeled DNA. The molecular mechanism of the radiosensitizing effects of halogenated thymidine analogs was discussed earlier (Szybalski, 1960) and is at present under active study.

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