PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 255–262 (1974)

Radiation-Enhanced Survival of a Human Virus in Normal and Malignant Rat Cells (37788)

KIKI B. HELLMAN, KAREN F. HAYNES, AND LARRY E. BOCKSTAHLER
(Introduced by H. G. Steinman)

Department of Health, Education, and Welfare, Public Health Service, Food and Drug Administration, Bureau of Radiological Health, Division of Biological Effects, Rockville, Maryland 20852

Certain repair processes known to occur in bacteria have been demonstrated in mammalian cells (1–3). Host-cell reactivation (HCR) of uv-irradiated SV40 virus was shown in normal human cells (1). Different mammalian cell lines expressed different levels of HCR, as measured by the survival of uv-irradiated herpes simplex virus in these cells (2). Enhanced survival of uv-irradiated herpes simplex virus (uv reactivation) occurred when host cells were exposed to uv radiation prior to infection (3). The amount of enhanced survival by uv reactivation (UVR) was approximately the same as that shown in bacteriophage–*E. coli* systems (4). Similarly, enhanced herpes simplex virus survival was observed in host cells exposed to X-radiation prior to infection with uv-irradiated virus: the effect termed X-ray reactivation (X-ray R) by analogy with UVR (5). The purpose of this study was to compare these repair phenomena in normal embryonic and malignant rodent cells and to investigate the relationship of HCR to UVR and X-ray R.

Materials and Methods. Cell cultures. To minimize species and strain differences, normal as well as malignant cells were derived from the Osborne-Mendel random bred rat. Normal rat embryo cells (OMRE) were obtained from primary cultures of trypsinized 10-day embryos and carried from 13 to 20 serial passages. Malignant cells were established in culture from an X-ray induced mammary adenocarcinoma arising in a 6-mo-old female rat. Rat mammary tumor (RMT) cells have undergone approximately 170 serial passages *in vitro* and have maintained their tumorigenicity for both X-irradiated and unirradiated female weanling rats. Stock cultures of OMRE cells were transferred weekly with ATV (6), while mechanical scraping with a rubber policeman was employed with RMT cells. For experimental cultures, both cell types were transferred with ATV. All cell cultures were propagated in Eagle's minimum essential medium (Microbiological Associates, Inc., Bethesda, MD) containing a twofold concentration of vitamins and amino acids, 4 m*M* glutamine, 100 units/ml each of penicillin and streptomycin, and supplemented with 10% fetal calf serum. Growth was at 37° in plastic 75 cm^2 flasks (Falcon Laboratories, Oxnard, CA) for routine stock culture maintenance and in plastic 25 cm^2 flasks and 50 mm petri dishes for experiments.

Virus assay. The macroplaque strain of Herpesvirus hominus-Type I (HV-1) was propagated in African green monkey kidney cells (CV-1) at 34° by modification of procedures previously described (2). Prior to infection of confluent monolayers, the medium was removed and the culture rinsed once with serum-free medium. One milliliter of the appropriate virus dilution was added to each of these cultures and adsorbed for 90 min at 37° with constant agitation. After virus adsorption, 3 ml of complete medium containing 0.25% immune serum globulin (Hyland, Inc., Los Angeles, CA) was added. Cultures were incubated at 37° for 4 days, fixed with ethanol, and stained with hematoxylin. The ability of HV-1 to cause plaques on OMRE and RMT cells was similar to that observed

on CV-1 cells.

Irradiation of virus and cells. Virus suspensions and cell monolayers were irradiated separately. For the purpose of uv exposure, virus suspensions (0.1–0.2 ml) were spread as thin layers in the centers of 50 mm plastic petri dishes. Cell monolayers were rinsed with serum-free medium, which was removed prior to uv or X-irradiation. The source of uv radiation was a germicidal lamp (General Electric G8T5 with radiation principally at 254 nm). Dose rates were measured with a uv dosimeter (Ultraviolet Products, Inc., San Gabriel, CA), by procedures described previously (2).

Incident dose rates to virus suspensions and cell monolayers were 29 and 5–6 erg/mm^2/sec, respectively. The X-radiation source consisted of a Westinghouse Coronado Therapeutic X-ray machine with no added filtration. The incident dose rate was 1480 rads/min. Both uv- and X-irradiated cell cultures were inoculated with appropriate dilutions of virus immediately (within 0.5 hr) after irradiation. Control experiments were conducted to show that multiplicity reactivation was not observed at the concentration of irradiated virus used in these experiments (2).

Experiments were repeated at least three times, with data averaged in each case, unless otherwise indicated.

Results. Representative survival curves for uv-irradiated virus in control (unirradiated) cells are demonstrated in Fig. 1. The multicomponent survival curves were similar and could be resolved into two components (2, 7). The dose rquired for an average of one lethal hit, e^{-1} dose, for the first component (initial slope) was 189 erg/mm^2 for RMT cells and 169 erg/mm^2 for OMRE cells. The e^{-1} doses for the resistant components (final slopes) for RMT and OMRE cells were 652 and 630 erg/mm^2, respectively. The ranges of e^{-1} doses for this component, as observed from several experiments, were 616–774 erg/mm^2 for RMT cells and 620–780 erg/mm^2 for OMRE cells. The fraction of total infected cells represented by the resistant component was determined by extrapolation of this component to the zero dose and

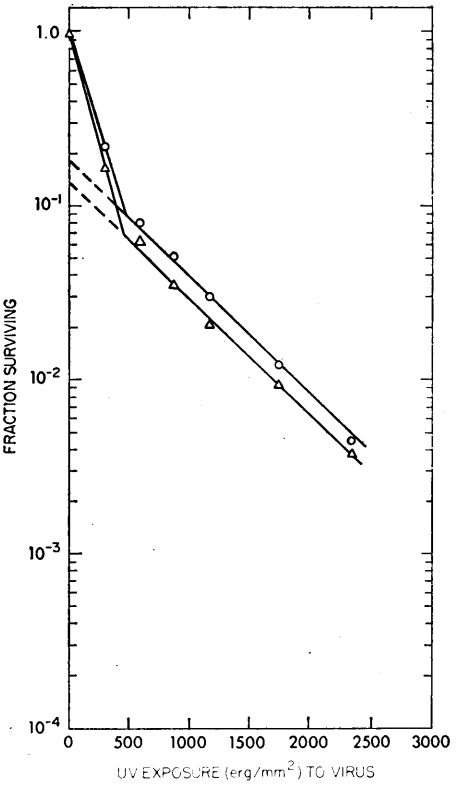

FIG. 1. Survival of uv-irradiated herpes simplex virus in unirradiated rat tumor (RMT) and rat embryo (OMRE) cells. The dose-rate of the uv source was 29 erg/mm^2/sec. (○) RMT; (△) OMRE.

found to be similar: 18 and 15% for RMT and OMRE cells, respectively. Therefore, using the repair of uv-irradiated virus as an indicator, both cell types exhibited similar levels of HCR. These paralleled those found for other mammalian cells of rodent origin and for certain human cell lines (2).

The capacity of uv-irradiated RMT cells to support plaque formation of control virus decreased with uv exposure, as indicated in Fig. 2A. Enhanced survival of uv-irradiated virus (UVR) was observed for virus uv doses falling within the resistant component of the survival curve. Since capacity and UVR are reflected in the curves for irradiated virus, quantitation of UVR by first order elimination of the capacity effect was achieved by determining the ratio of the virus survival fraction on irradiated cells to the virus survival fraction on unirradiated cells, hereafter referred to as the "uv reactivation factor"

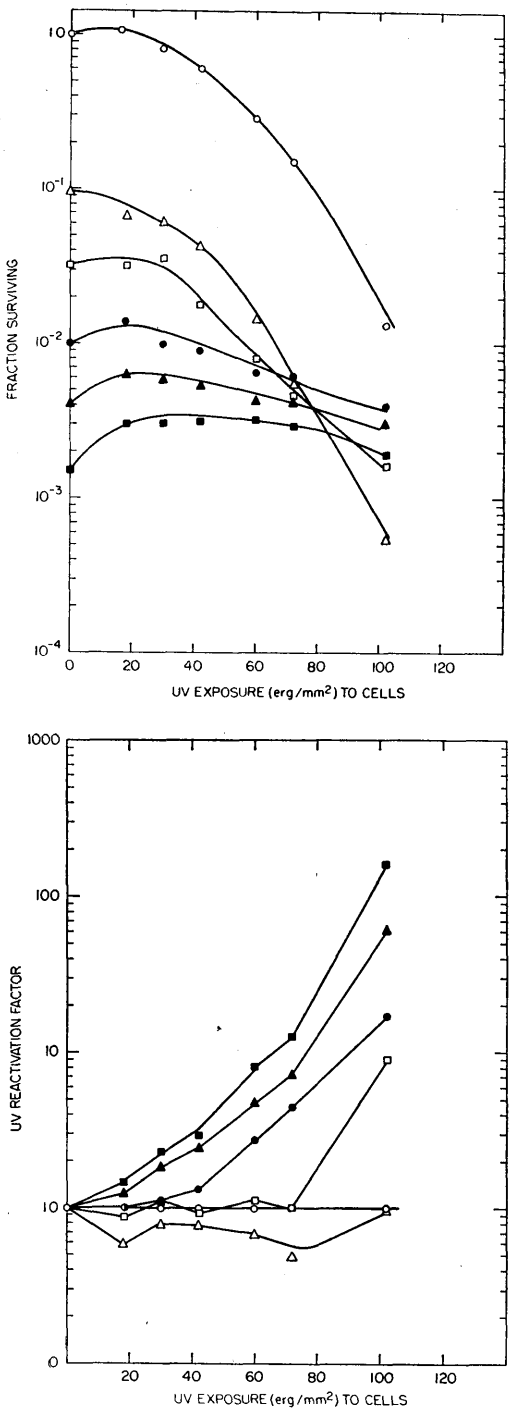

FIG. 2A. (top) Survival of uv-irradiated herpes simplex virus in uv-irradiated rat tumor (RMT) cells expressed as function of uv exposure to cells. The dose-rate of the uv source was 6 erg/mm²/sec for cell monolayers and 29 erg/mm sec for virus samples. Cells and virus were irradiated separately. Ultraviolet exposure to virus: (○) 0 erg/mm²; (△) 435 erg/mm²; (□) 870 erg/mm²; (●) 1740 erg/mm²; (▲) 2610 erg/mm²; (■) 3480 erg/mm². (B) (bottom) Ultraviolet reactivation of herpes simplex virus as function of uv exposure to rat tumor (RMT) cells. The dose-rate of the uv source was 6 erg/mm²/sec for cell monolayers and 29 erg/mm²/sec for virus samples. Ultraviolet exposure to virus: (○) 0 erg/mm²; (△) 435 erg/mm²; (□) 870 erg/mm²; (●) 1740 erg/mm²; (▲) 2610 erg/mm²; (■) 3480 erg/mm². "uv reactivation factor" (UVR factor) is the ratio (virus survival fraction on cells irradiated with the dose indicated on the abscissa)/(virus survival fraction on unirradiated cells). Values of UVR factor greater than 1.0 indicate UVR.

(UVR factor) (8). As demonstrated in Fig. 2B, the increase in UVR factor clearly indicates UVR in irradiated RMT cells for all virus doses examined above 435 erg/mm². The amount of UVR increased with increasing virus dose and increasing cell exposure. The maximum UVR factor achieved was approximately 160. Although UVR factors of similar magnitude have been reported for phage–bacterial systems (8), the results obtained with RMT cells constitute an initial observation of such a pronounced enhancement effect in mammalian cells. Previous studies with CV-1 cells and HV-1 have shown a two- to threefold enhancement (3).

The UVR effect was similarly investigated in OMRE cells (Fig. 3A). It was observed that the ability of uv-irradiated OMRE cells to support plaque formation of control and virus irradiated with different uv doses decreased at approximately the same rate as a function of cell uv exposure. From results expressed in terms of the UVR factor (Fig. 3B), it is clearly evident that UVR of irradiated virus is absent in OMRE cells.

These results indicate that expression of UVR depends on the cell type. Additionally, it depends on the survival level of irradiated virus (Fig. 2B). This latter possibility was further investigated (Fig. 4). It was observed that irradiation of RMT cells resulted in a change in the slope of the resistant component of the survival curve (e^{-1} dose of 774 erg/mm² in control cells; e^{-1} dose of 1294 erg/mm² in cells exposed to 72 erg/mm²),

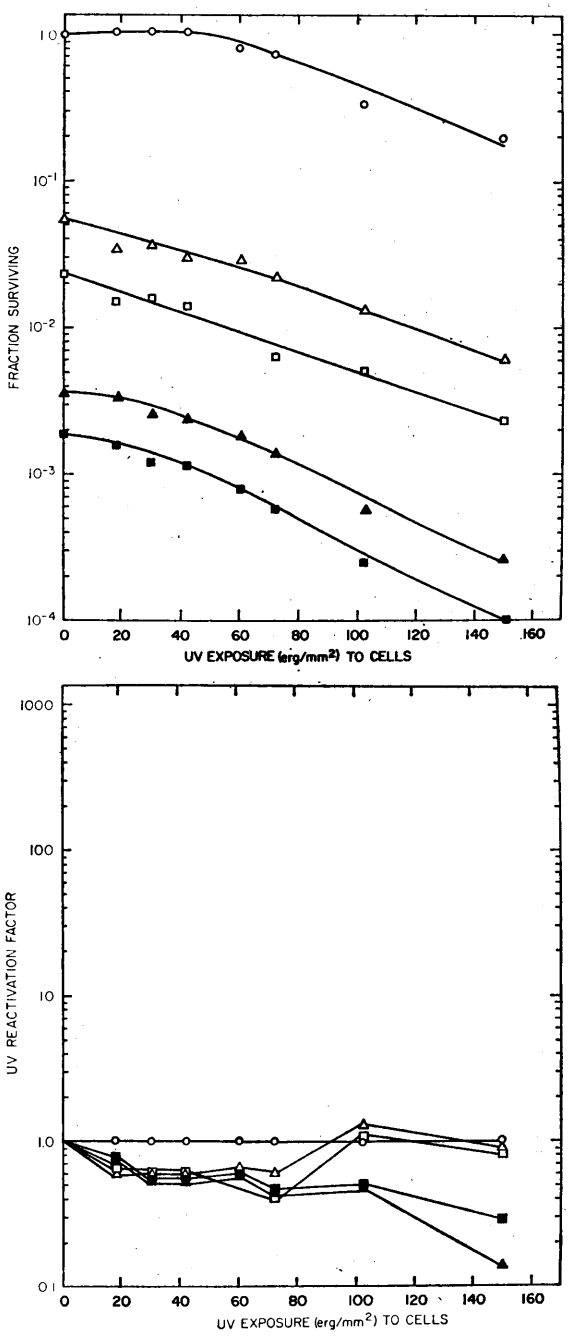

FIG. 3A. (top) Survival of uv-irradiated herpes simplex virus in uv-irradiated rat embryo (OMRE) cells. The dose-rate of the uv source was 6 erg/mm²/sec for cell monolayers and 29 erg/mm²/sec for virus samples. Cells and virus were irradiated separately. Ultraviolet exposure to virus: (○) 0 erg/mm²; (△) 435 erg/mm²; (□) 870 erg/mm²; (▲) 2610 erg/mm²; (■) 3480 erg/mm². (B) (bottom) Ultraviolet reactivation of herpes simplex virus as function of uv exposure to rat embryo (OMRE) cells. The dose-rate of the uv source was 6 erg/mm²/sec for cell monolayers and 29 erg/mm²/sec for virus samples. Ultraviolet exposure to virus: (○) 0 erg/mm²; (△) 435 erg/mm²; (□) 870 erg/mm²; (▲) 2610 erg/mm²; (■) 3480 erg/mm². "uv reactivation factor" (UVR factor) is the ratio (virus survival fraction on cells irradiated with the dose indicated on the abscissa)/(virus survival fraction on unirradiated cells). Values of UVR factor greater than 1.0 indicate UVR.

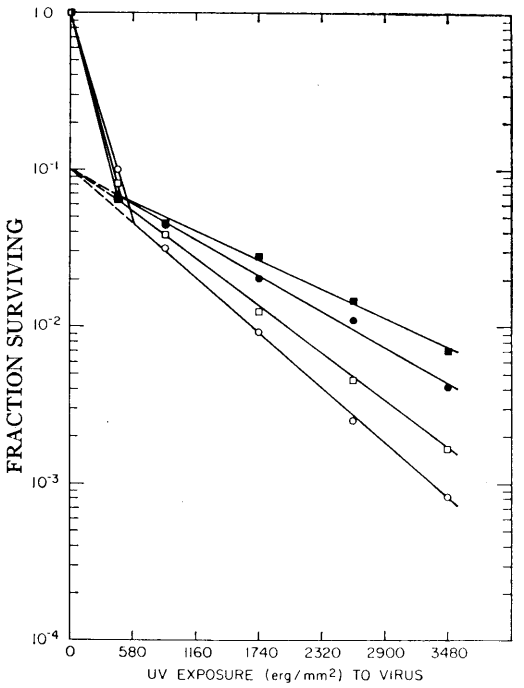

FIG. 4. Survival of uv-irradiated herpes simplex virus in uv-irradiated rat tumor (RMT) cells expressed as function of uv exposure to virus. Ultraviolet exposure to cells: (○) 0 erg/mm²; (□) 30 erg/mm²; (●) 60 erg/mm²; (■) 72 erg/mm².

but not in the percentage of infected cells representing that component (10%). This indicates that uv irradiation enhanced the capacity of individual cells in the resistant population to reactivate the irradiated virus.

When survival curves for uv-irradiated virus on control and irradiated OMRE cells were obtained, it was observed (Fig. 5) that the slope of the resistant component remained the same (e^{-1} dose of 765 erg/mm² in control cells; e^{-1} dose of 857 erg/mm² in cells exposed to 72 erg/mm²) and the percentage of infected cells representing that component decreased (8% in control cells; 2.5% in irradiated cells). This result indicates that enhanced capacity for reactivation of irradiated virus did not occur with irradiated OMRE cells. In fact, a decrease in the resistant cell population was observed with increasing cell uv exposure.

A recent report has shown that X-irradiation enhancement (X-ray R) of HV-1 survival occurs in a mammalian cell (5). In view of the contrasting UVR effect observed in the two types of rat cells, it was of interest to investigate enhanced reactivation after X-irradiation of host cells.

Figure 6 demonstrates plaque formation by uv-irradiated virus on X-irradiated RMT cells. The doses to the virus were such that virus survivals fell within the resistant component of the survival curve where UVR was readily demonstrated. The ability of irradiated cells to support growth of control virus was not affected over the range of X-ray exposures investigated up to 9 krads. The plaque-forming ability of irradiated virus increased with increasing X-ray dose to the cells and plateaued at approximately 1 krad. Survival enhancement of two to five times, depending on virus dose, was observed.

Examination of OMRE cells showed that X-ray R was absent (Fig. 7). In fact, a decrease in irradiated virus survival was observed, as a function of X-ray exposure up to 1 krad.

Discussion. Reactivation of uv-irradiated

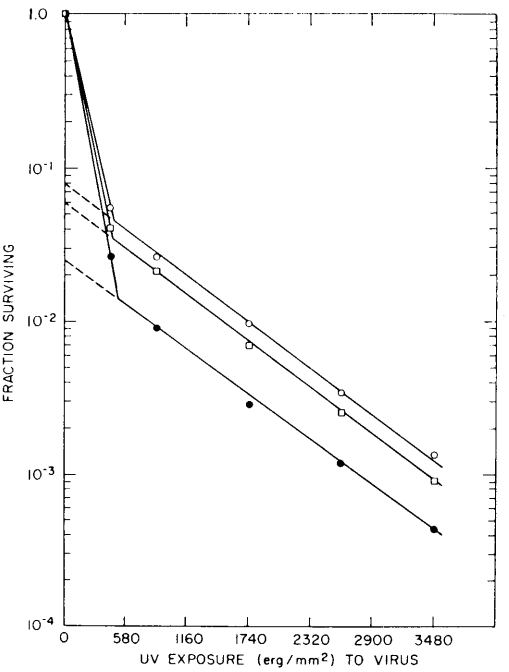

FIG. 5. Survival of uv-irradiated herpes simplex virus in uv-irradiated rat embryo (OMRE) cells expressed as function of uv exposure to virus. Ultraviolet exposure to cells: (○) 0 erg/mm²; (□) 30 erg/mm²; (●) 72 erg/mm².

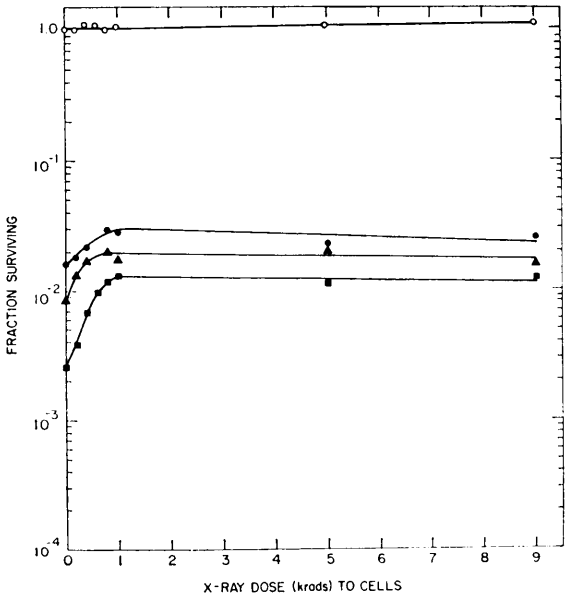

FIG. 6. Survival of uv-irradiated herpes simplex virus in X-irradiated rat tumor (RMT) cells. The dose-rate of the X-ray source was 1480 rads/min for cell monolayers; dose-rate of uv source was 29 erg/mm²/sec for virus samples. Ultraviolet exposure to virus: (○) 0 erg/mm²; (●) 1740 erg/mm²; (▲) 2610 erg/mm²; (■) 3480 erg/mm².

HV-1 was examined and compared in normal embryonic (OMRE) and malignant (RMT) rat cells of the same strain. Both cell types expressed similar levels of HCR of irradiated virus. However, marked differences were observed between the two cell types in radia-

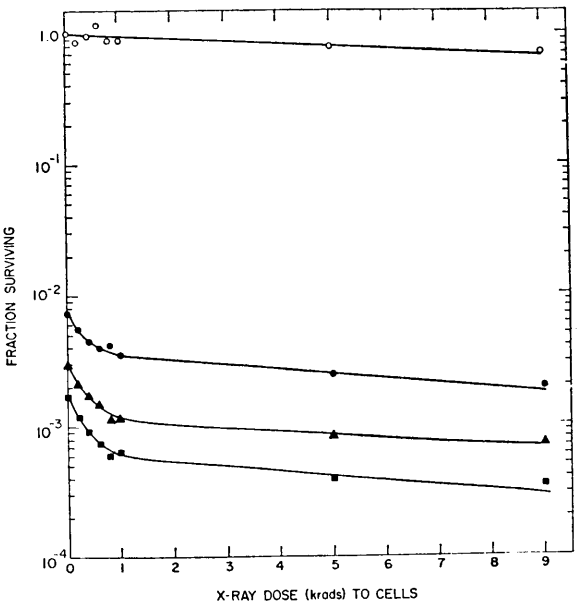

FIG. 7. Survival of uv-irradiated herpes simplex virus in X-irradiated rat embryo (OMRE) cells. The dose-rate of the X-ray source was 1480 rads/min for cell monolayers; dose-rate of the uv source was 29 erg/mm²/sec for virus samples. Ultraviolet exposure to virus: (○) 0 erg/mm²; (●) 1740 erg/mm²; (▲) 2610 erg/mm²; (■) 3480 erg/mm².

tion-enhanced reactivation: UVR and X-ray R of HV-1 occurred in RMT cells but were absent in OMRE cells. Similar relationships have been observed between normal (human embryonic lung) and malignant (HeLa) human cells (9). Thus, HCR and radiation-enhanced reactivation (UVR and X-ray R) of HV-1 appear to operate independently in mammalian cells. A similar conclusion was reached through biochemical studies on HCR and UVR with the bacteriophage λ–*E. coli* system (10). HCR of λ was shown to involve host cell enzyme excision of pyrimidine dimers, whereas excision was not involved in UVR of λ.

The magnitude of the UVR response in RMT cells is noteworthy. The amount of UVR of HV-1 was considerably greater in these cells than in either CV-1 or HeLa cells (3, 9). It was clearly demonstrated with RMT cells that the amount of UVR increased with virus uv dose and cell uv exposure. Survival enhancement of HV-1 by X-irradiation of RMT cells was similar in magnitude and radiation response to that observed previously with CV-1 cells (5).

The mechanisms of mammalian cell UVR and X-ray R are unknown. The fact that both of these phenomena occur in RMT cells and both are absent in OMRE cells suggests that the two mechanisms may be similar. Observations from bacterial and mammalian cell systems suggest the following possible mechanisms for radiation-enhanced reactivation: (a) absolute increase in the number of certain repair enzymes induced by irradiation; (b) a shift in the balance between destructive (nucleolytic) and repair enzymes (7); and (c) recombination between viral and host genes (11). Any of these mechanisms acting either alone or in combination could account for the enhanced reactivation of virus in this study.

In view of the contrasting results obtained between the normal embryonic and malignant cells, it is of interest to speculate on the role of cell origin in relation to UVR and X-ray R phenomena. It has been demonstrated that embryonic cells, especially at critical stages in development, are highly sensitive to X-irradiation (12). Furthermore, certain enzyme systems concerned with intermediary metabolism are deficient or not fully expressed in embryonic cells (13–15). Such may also be the case for possible repair enzyme systems involved in UVR and X-ray R.

Results shown in this study have demonstrated the relative radiation senstitivity of normal embryonic and malignant cells with respect to enhanced reactivation of virus.

Summary. The repair phenomena of host-cell reactivation (HCR), uv reactivation (UVR) and X-ray reactivation (X-ray R) were studied in normal embryonic (OMRE) and malignant (RMT) rat cells of the same strain by examining the survival of uv-irradiated herpes simplex virus (HV-1). Experiments indicated that HCR was present to the same extent in both OMRE and RMT cells as evidenced by similar e^{-1} values for both components of the virus survival curve as well as similar extrapolation numbers for the resistant component. UVR and X-ray R were demonstrated in RMT cells as indicated by enhanced survival of irradiated virus; however, both of these repair processes were absent in OMRE cells. The amount of UVR in RMT cells increased with virus uv dose and cell uv exposure. It was evident that HCR and radiation enhanced reactivation (UVR and X-ray R) may operate independently in these mammalian virus–host cell systems.

We thank Lynda Kramer and Wah Lee for assistance in X-irradiation of cells and C. David Lytle, F. Alan Andersen, and Thomas J. Withrow for helpful discussions and critical review of the manuscript.

1. Aaronson, S. A., and Lytle, C. D., Nature (London) **228**, 359 (1970).

2. Lytle, C. D., Int. J. Radiat. Biol. **19**, 329 (1971).

3. Bockstahler, L. E., and Lytle, C. D., Biochem. Biophys. Res. Commun. **41**, 184 (1970).

4. Weigle, J. J., Proc. Nat. Acad. Sci. USA **39**, 628 (1953).

5. Bockstahler, L. E., and Lytle, C. D., J. Virol. **8**, 601 (1971).

6. Madin, S. H., and Darby, N. B., Jr., Proc. Soc. Exp. Biol. Med. **98**, 574 (1958).

7. Rupert, C., and Harm, W., *in* "Advances in

Radiation Biology" (L. G. Augenstein, R. Mason and M. Zella, eds.), Vol. 2, p. 1. Academic Press, New York (1966).

8. Fauquet, P., and Defais, M., Mutat. Res. **15**, 353 (1972).

9. Hellman, K. B., Haynes, K. F., Lytle, C. D., and Bockstahler, L. E., Abstr. Annu. Meet. Amer. Soc. Photobiol., 1st, Sarasota, FL 10–14, June (1973).

10. Boyle, J. M., and Setlow, R. B., J. Mol. Biol. **51**, 131 (1970).

11. Hart, M. G. R., and Ellison, J., J. Gen. Virol. **8**, 197 (1970).

12. Rugh, R., Radiology **99**, 433 (1971).

13. Nemeth, A. M., Ann. N. Y. Acad. Sci. **3**, 199 (1963).

14. Greengard, O., and Dewey, H. K., J. Biol. Chem. **242**, 2986 (1967).

15. Feuer, G., and Liscio, A., Nature (London) **223**, 68 (1969).

Received Sept. 14, 1973. P.S.E.B.M., 1974, Vol. 145.