

X-Ray-Enhanced Reactivation of Ultraviolet-Irradiated Human Virus

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Received for publication 2 July 1971

When CV-1 mammalian cells were X-irradiated before infection with ultraviolet (UV)-irradiated herpes simplex virus, an increase in survival of this virus was observed. X-ray reactivation is proposed as the name of this phenomenon by analogy with UV reactivation. The amount of survival enhancement was about the same as that found for UV reactivation in the same virus-host system. The enhancement reached a plateau at approximately 1000 rads and appeared to be radiation-insensitive at high doses.

The survival of ultraviolet (UV)-irradiated viruses depends on many factors which affect the host cell. An enhancement in survival of UV-irradiated virus results when the host cells are lightly UV-irradiated. This phenomenon, called UV reactivation (UVR) and known to occur in phage-bacterial systems, was recently reported for a mammalian virus-host cell system (1). The mechanism of UVR is unknown [see review by Rupert and Harm (6)]. The question arises as to whether the UV damage in the cell induces an increase in the number of repair enzymes which can act upon the damaged virus. X rays produce a different quality of lesions than UV light. The existence of X-ray enhancement of UV-irradiated virus survival could give information regarding the mechanism accomplishing radiation enhancement of virus survival. This preliminary report demonstrates that X-ray survival enhancement of UV-irradiated virus does occur for a mammalian virus-host cell system. To our knowledge, data concerning this effect have not been published for any virus-host cell system.

The macroplaque strain of the human deoxyribonucleic acid virus herpes simplex virus (*Herpesvirus hominus*) and CV-1 cells (African green monkey kidney) were used. Culture and virus assay methods have been described (1). Virus and cells were irradiated separately. Virus was irradiated with a germicidal lamp (General Electric G8T5 with radiation principally at 254 nm). The incident dose rate was 34 ergs per mm² per sec. Confluent monolayers of cells were irradiated with 250 kv (constant potential) X rays. Cell sheets were prepared for irradiation by rinsing with NCTC-109 serum-free medium. The dose rate was 1,252 rads per min from a Westinghouse

Coronado Therapeutic X-ray machine with no added filtration. The irradiated cell culture was inoculated with virus suspensions as soon as possible (within 0.5 hr) after irradiation.

Figure 1 shows the survival of UV-irradiated virus on X-irradiated cells normalized to that for unirradiated virus and unirradiated cells. Virus samples exposed to two different UV doses were compared with unirradiated virus. The UV doses were sufficiently high that virus survivals fell within the second component of the normal two-component survival curve for this system (1). At these doses to the virus, UVR is easily demonstrated (1).

The top curve in Fig. 1 shows that, in these cells (confluent monolayers), doses up to 12 krad did not affect the ability of the cells to support unirradiated virus growth during the 3-day infectivity assay. In separate experiments, decreases in capacities for growth of irradiated and unirradiated virus were obtained with doses of 20 krad or higher (*unpublished data*). The high radiation resistance in growth capacity reported here contrasts with results found by Powell in L cells (5). The reason for this difference is not known; the difference in virus assay and cell condition may affect the capacity property.

Both curves for UV-irradiated viruses show absolute increases in plaque-forming ability with nearly linear dependence on X-ray dose to about 1,000 rads. Above that dose, plaque-forming ability of the virus does not change (up to at least 12 krad). Since X rays at the doses used here do not affect capacity for virus growth, it may be concluded from the lower curves that irradiation of the host cells produces a maximum

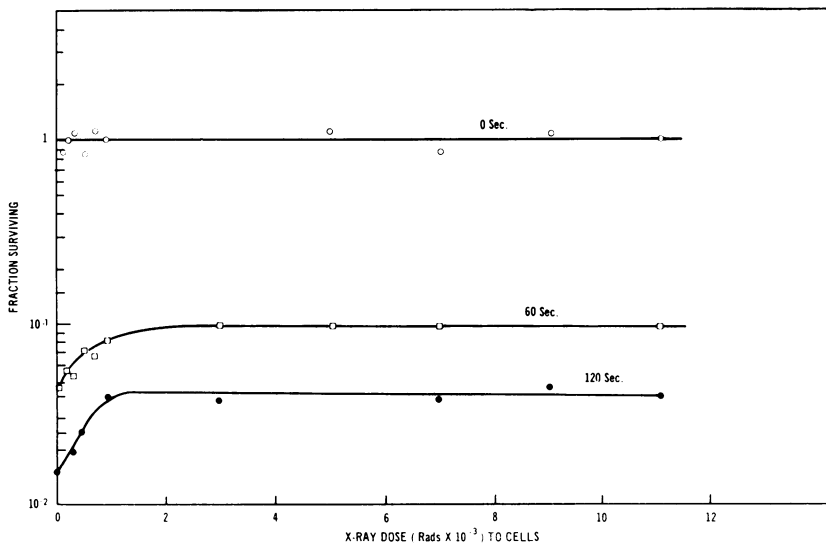


FIG. 1. Survival of UV-irradiated herpes simplex virus on X-irradiated CV-1 cells. UV doses to the viruses were: 0 sec, ○; 60 sec, □; 120 sec, ●.

survival enhancement of two to three times. This is similar to that found for UVR (1). As a control for further investigation, the experiment in Fig. 1 has been repeated a number of times in this laboratory with essentially the same results. To the best of our knowledge, this is the first time X-ray data of this type have been presented for any biological system. Brief mention has been made that this may occur in a bacterial system; however, no data were presented (3, 6). We propose by analogy with UVR to designate this phenomenon X-ray reactivation.

One hypothesis to explain UVR employs induction of an increase in number of repair enzymes by UV damage, i.e., pyrimidine dimers. Since X rays do not cause appreciable amounts of pyrimidine dimers (2), it can be concluded that other mechanisms are probably present and that radiation enhancement of virus survival is not UV-specific. That reactivation of UV-irradiated virus can occur by treatment of host cells with an agent other than radiation, at least in the case of a phage-bacterial system, was shown by Otsuji and Okubo (4), who demonstrated enhancement of λ phage UV survival by pretreating host bacteria with mitomycin C. Experiments are in progress to elucidate mechanisms of UVR and X-ray reactivation.

The excellent technical assistance of Karen Haynes and Juliet Tanada is gratefully acknowledged. The authors thank Lynda Kramer and Wah Lee for assistance in X irradiation of cells and Walter Harm, William Leach, and Kiki Hellman for critical review of the manuscript.

A preliminary report of this work was given at the 15th Annual Meeting of the Biophysical Society, New Orleans, La., 17 February 1971.

ADDENDUM IN PROOF

After this paper was accepted for publication, it was brought to our attention that Ono Shimazu (Virology 29:295-302, 1966) demonstrated an X-ray reactivation-like effect for the single-stranded bacteriophage ϕ R.

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