ULTRAVIOLET LIGHT ENHANCED REACTIVATION OF A MAMMALIAN VIRUS

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Summary

When CV-1 mammalian cells are lightly UV-irradiated before infection with UV-irradiated herpes simplex virus, an increase in survival (UV-reactivation) of this virus is observed. The doses of UV to the cells necessary for maximizing UV-reactivation were a factor of about 10 lower than those previously reported for bacteriophage - bacteria systems.

Weigle (1) discovered that there is an increase in phage survival when UV-irradiated phage λ infected lightly UV-irradiated host cells E. coli K12S. This effect is called UV-reactivation (UVR). In addition to λ , UVR has been found with phages T1, T3, P22, P2, HP1, the Serratia-phage kappa, S13, and ØX174 (See review by Rupert and Harm (2)).

Many aspects of repair phenomena found in bacterial systems have been demonstrated in mammalian systems. The purpose of this paper is to show that UVR exists in UV-irradiated mammalian cells infected with a UV-irradiated animal virus. The herpes simplex virus-CV-1 cell system was selected for these studies, a system in which host cell reactivation is easily demonstrated (4).

MATERIALS AND METHODS

Cells and Culture Media

CV-1 cells, a permanent line of African green monkey kidney cells, obtained from American Type Culture Collection*, Rockville,

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Maryland, were used. NCTC-109 medium (Microbiological Associates, Inc., Bethesda, Maryland) was supplemented with 10% newborn calf serum (Colorado Serum Company Laboratories, Denver, Colorado), 4 mM L-glutamine and 100 units/ml each of penicillin and streptomycin. Cells were grown at 37°C in 50 mm plastic petri dishes.

Virus

The macro-plaque strain of herpes simplex virus (Herpesvirus hominus), a gift of S. Stein, Pennsylvania State University, was grown on confluent monolayers of CV-1 cells at 34°C.

Virus Assay

Freshly confluent monolayers of cells were inoculated with 1 ml of virus suspension (90 min. at 37°C with constant agitation). Subsequently 3 ml medium containing 0.25% immune serum globulin (Hyland, Division Travenol Laboratories, Inc., Los Angeles, Calif.) was added to the infected cell cultures, followed by incubation at 37°C. Cells were fixed with ethanol and stained with hematoxylin three days post infection. Virus inocula were diluted to give 20-250 plaques per assay dish.

Irradiation of Virus and Cells

Virus suspensions of 0.1 - 0.2 ml were spread into thin layers in the centers of petri dishes. A germicidal lamp (General Electric G8T5 with radiation principally at 254 nm) was used as the source of radiation. The incident dose rate used for virus irradiation was 34 erg/mm^2/sec. Dose rates were measured with a UV dosimeter (Ultraviolet Products, Inc., San Gabriel, Calif.).

Cell sheets were prepared for irradiation by rinsing with NCTC-109 serum-free medium. This medium was removed before irradiation. The incident dose rate used for irradiation of cells was 6 erg/mm^2/sec. The irradiated cultures were then immediately inoculated with appropriate dilutions of irradiated virus.

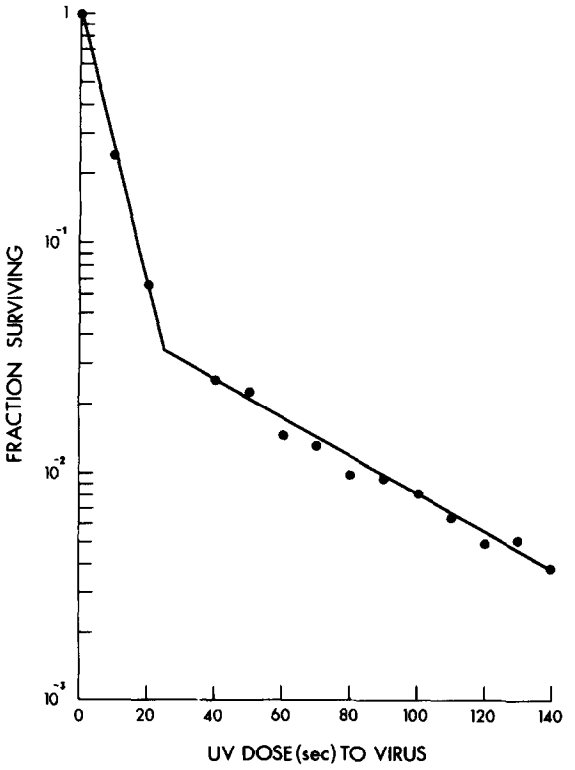

Figure 1. UV-survival of herpes simplex virus on unirradiated CV-1 cells.

RESULTS AND DISCUSSION

Figure 1 shows UV survival of herpes simplex virus in unirradiated CV-1 cells. Two components were found with an e^{-1} dose of about 210 erg/mm^2 for the first component and 1800 erg/mm^2 for the second. Control experiments showed that multiplicity reactivation was not observable at the concentrations of irradiated virus used for these experiments. Host cell reactivation of this virus in CV-1 and other mammalian cells will be described in greater detail elsewhere (4).

Ultraviolet-irradiated virus was assayed on cells that were lightly UV-irradiated at various doses (Fig. 2a). Virus irradiated with doses which fall in the region of the second component of the

survival curve shown in Fig. 1 (exposures greater than 25 sec.) exhibited UVR. UVR was not apparent for virus irradiated with doses falling within the region of the first component. In order to better characterize the UVR effect the experiment was repeated for virus irradiated for 60 and 120 sec. and more data were taken for low doses to the cells (Fig. 2b). These two curves clearly demonstrate UVR. The reason the shapes of these curves are not quantitatively the same as in Fig. 2a has not yet been determined and may be due to a parameter of the cell system such as differences in cell ages at time of assay between the two experiments.

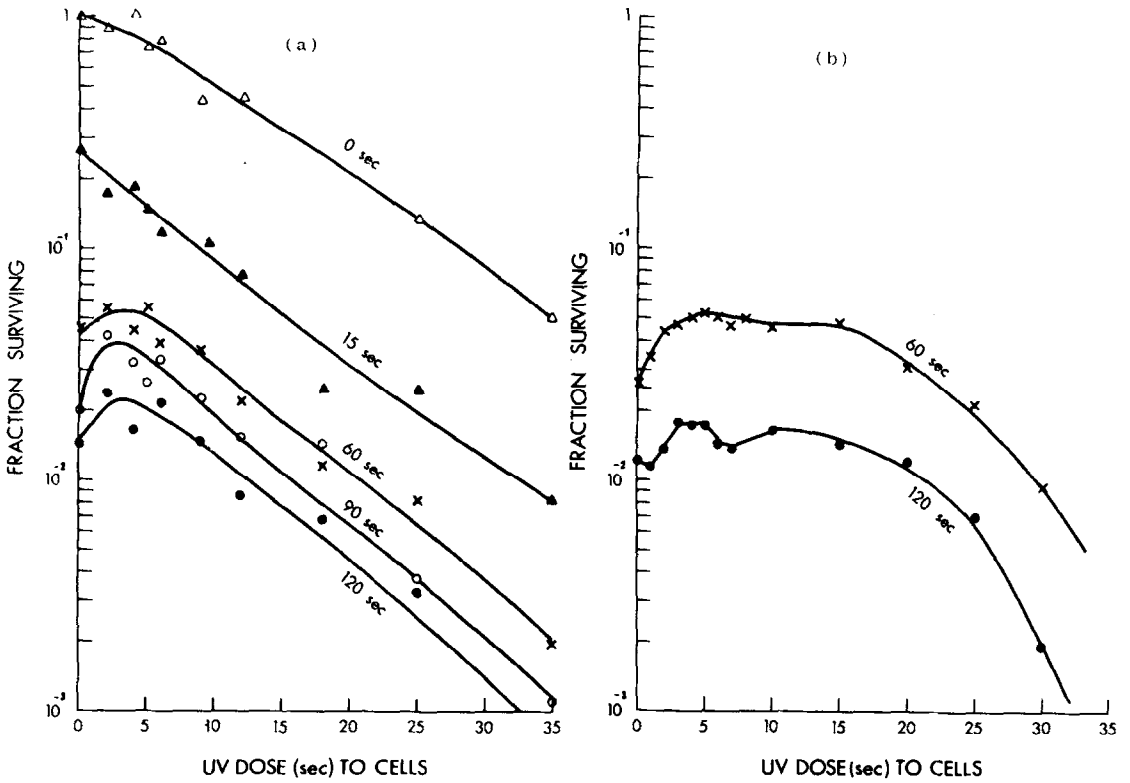

Figure 2. (a) UV-reactivation of herpes simplex virus on CV-1 cells. Virus and cells were irradiated separately. The virus exposure (in sec.) is indicated for each curve (see Materials and Methods for dose rates).

(b) Same experiment repeated in triplicate for virus exposures of 60 and 120 sec.

These results show that UVR apparently exists in a mammalian virus - cell system and it is suggested that UVR may be a general phenomenon. Doses to the cells necessary for demonstrating UVR were an order of magnitude lower than those for phage - E. coli systems (3). The amount of enhancement of survival by UVR was approximately the same in both systems.

It may be of interest that UVR was clearly demonstrated only for heavily irradiated virus, i.e. for the second component of the virus survival curve. It has recently been found that the host cell reactivation (HCR) present in this second component has properties (such as caffeine sensitivity) which are very similar to the HCR in bacteria (4). In bacteria UVR is manifested only in HCR positive strains (5). Thus the HCR-UVR relation in this mammalian system may be similar to that of bacteria. The quantitative differences in UVR for these two types of cells is probably a result of different physical target sizes and of radiation repair capacities which differ in efficiency.

The mechanism of UVR is unknown (see review by Rupert and Harm (2)). One possibility is UV irradiation of a cell might induce an absolute increase in the number of repair enzymes. However, UV irradiation of cells creates additional substrate for both destructive and repair enzymes. Therefore, another possible mechanism is that the balance between these enzymatic functions could also be shifted towards a higher relative efficiency of the repair process by reducing the amount of available destructive enzymes (2). Studies are in progress in this laboratory which may help select among these possible mechanisms.

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