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SHORT COMMUNICATION

APPLICABILITY OF A MICROBIOLOGICAL TECHNIQUE TO SELECTION OF HCR⁻ MUTANTS OF MAMMALIAN CELLS[1]

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There have been many studies on mechanisms of repair of damage induced by radiation in mammalian cells since the phenomenon of cellular recovery was discovered by Elkind and Sutton (Elkind and Sutton, 1959). But, because of the complexity of mammalian cells and the lack of appropriate repair deficient mutants of mammalian cells, the repair mechanisms in mammalian cells are not yet as clear as in bacteria. In the bacterial system, UV-irradiated viruses are reactivated by repair enzymes of the host cell and as a result kill the cell possessing this repair system. This property, known as host cell reactivation (HCR), was used for selection of repair deficient mutants of bacteria (Howard-Flanders and Theriot, 1962). Recently, cells from a patient with xeroderma pigmentosum were discovered to be defective in repair replication (Cleaver, 1968). These cells were later reported to be HCR deficient for growth of UV-irradiated herpes virus and for transformation by UV-irradiated SV40 virus (Rabson, Tyrrel and Legallais, 1969; Aaronson and Lytle, 1970). Previous studies have also suggested the existence of an HCR mechanism in avian cells (Závadová and Závada, 1968). To establish the first step for selection of radiation sensitive mutants of mammalian cells using the method of Howard-Flanders and Theriot, we studied the existence of an HCR mechanism in a cell system with herpes simplex virus. This virus has recently been used for the same purpose by another author (Lytle, 1971).

The herpes simplex virus used in this work was a clone of the +GC Miyama strain, which has been maintained for many years in this laboratory. All the cell lines used for assay of the virus were grown in square bottles in Eagle's Minimum Essential Medium (MEM) supplemented with 10% calf serum. The virus was easily inactivated at 37 C but serum had a protective effect against this heat inactivation (Fig. 1). Therefore, phosphate buffered saline with 5% calf serum was used for dilution of the virus. The virus was irradiated under a germicidal lamp (15 w; Toshiba Electric Co.) at a distance of 41 cm from the sample and 0.2 ml of irradiated diluted virus preparation was added to a monolayer of host cells. After 2 hr adsorption at 37 C, the monolayer was overlaid with MEM with 5% calf serum and 1% Difco agar (agar medium). Plaques were

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counted on the third day after virus inoculation.

The curve for survival of virus after UV-irradiation suggested the existence of multiple component inactivation kinetics, but there were insufficient points on the survival curve to calculate the sizes of these different components. Irradiated viruses showed different UV-sensitivities when assayed on different host cells (Fig. 2 and 3). Herpes simplex virus was more UV-resistant when assayed on BSCl cells (monkey origin) than when assayed on RK13 cells (rabbit origin) or on FL cells (human origin).

These differences probably represent differences in the HCR capacities of the different cell lines, and the following data support this possibility. Caffeine is known to have an inhibitory effect on the HCR mechanism of bacterial and avian cells (Sauerbier, 1964; Závadová and Závada, 1968). When 2 mM caffeine was added to the assay system of BCSl cells, the plating efficiency of the virus was not affected, but its UV-sensitivity increased markedly (Fig. 4).

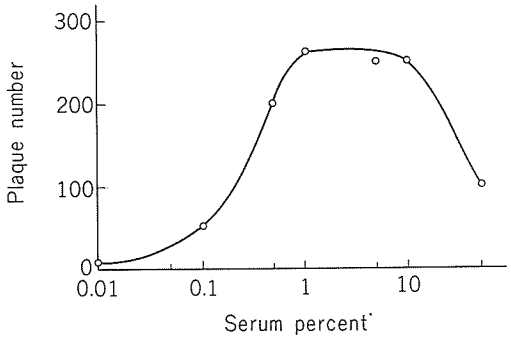

FIGURE 1. *Protective effect of calf serum against heat inactivation of herpes simplex virus. The virus was diluted with phosphate buffered saline with varying amounts of serum and was incubated for 2 hr at 37 C. Plaque forming activity was tested in BSC1 cells.*

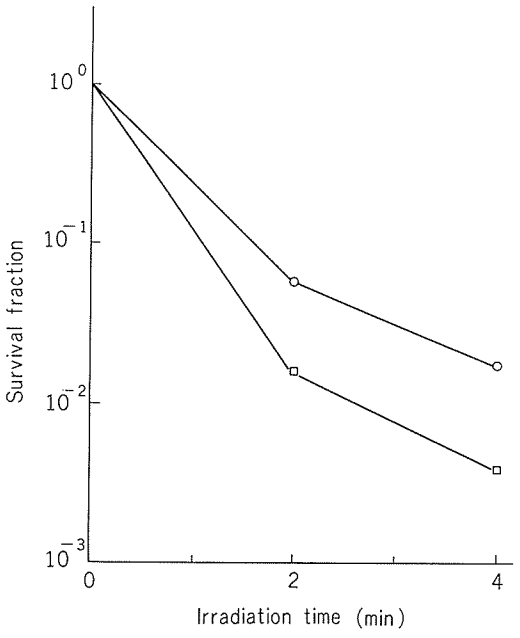

FIGURE 2. *UV-inactivation curve of herpes simplex virus tested with BSCl cells and FL cells. Symbols: (○) virus titer assayed on BSC1 cells and (□) that on FL cells.*

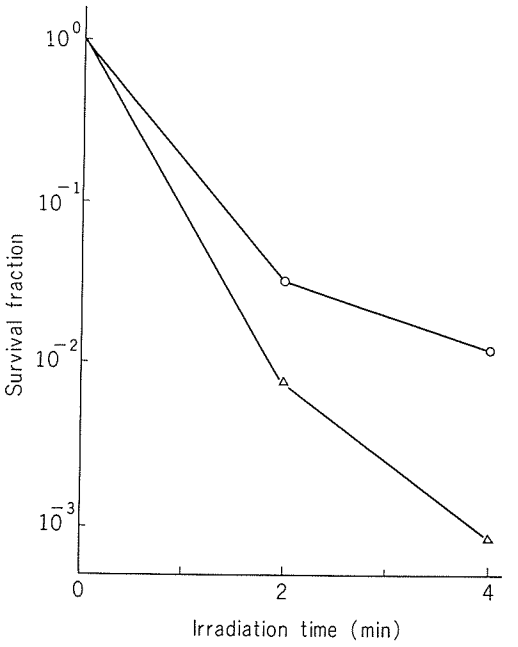

FIGURE 3. *UV-inactivation curve of herpes simplex virus tested with BSC1 cells and RK13 cells. Symbols: (○) virus titer assayed on BSC1 cells and (△) that on RK13 cells.*

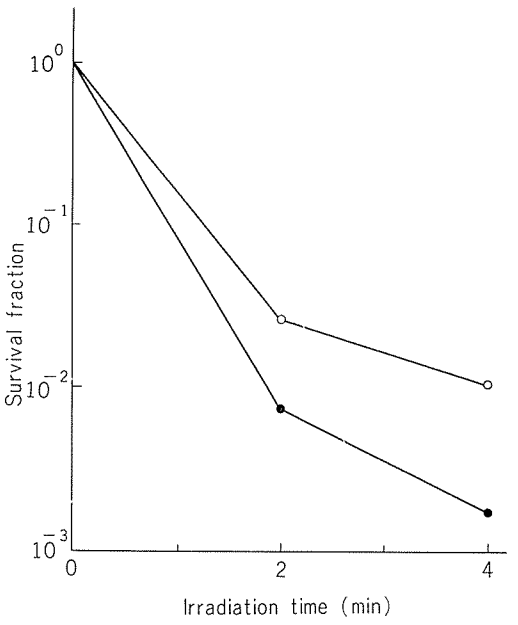

FIGURE 4. *Effect of caffeine on survial of UV-irradiated herpes simplex virus. After irradiation, 2 mM caffeine was added to the virus suspension during the adsorption period. Caffein was also added to the agar overlay medium. Symbols:* (●) *caffeine, 2 mM;* (○) *control.*

Our results suggest that an HCR mechanism which is sensitive to caffeine exists in BSCl cells. The HCR capacity in BSCl cells, which is suppressed by caffeine, is not so large as that of bacteria or human cells (Aaronson and Lytle, 1970). However, the method of Howard-Flanders and Theriot may be applicable for selection of mutants of mammalian cells lacking the HCR mechanism.

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