Radiation Resistance in Lipovirus-Altered Human Cells

Abstract. *Human cells altered by infection with the "lipovirus," a transmissible cytopathic agent, are highly resistant to ionizing radiation. Whereas about 500 roentgens will inhibit cell multiplication in normal cells, more than 500,000 roentgens are required to produce a similar effect in the altered cells. This gross change in cellular radiosensitivity may provide a useful system for studying in vitro the mechanisms of inhibition of cell division by radiation.*

The "lipovirus" is a transmissible cytopathic agent presumably originating from the blood of a patient with infectious hepatitis (*1*). When cultured human cells are inoculated with this agent they undergo unique changes. These include shrinkage of the cell nucleus to about one-third its normal size, the appearance of marked DNA and thymine catabolism, an increase in cellular lipids, and the presence of spongiform bodies (*2, 3*). Such lipovirus-altered cells, despite an 80- to 90-percent reduction in average DNA content of the cell, are capable of continuous multiplication in a culture medium enriched with casein hydrolyzate and yeast extract (*3*). As a result, they maintain their characteristic abnormalities over many generations of replication. The uninfected parent cell line we have used is an established strain of epithelial-like cells (Lich-1) which has been derived from an explant of human liver (*4*). To facilitate description, the lipovirus-altered cells shall be referred to hereafter as ILEM cells (Infected Lich-1 in Enriched Medium) (*3*).

In our investigation, we have studied the response to ionizing radiation of both uninfected Lich-1 cells and ILEM cells. The radiosensitivity of Lich-1 cells was similar to that of other strains of human cells grown in vitro, but the ILEM cells were about 1000 times more resistant to single doses of radiation. To our knowledge, this extreme degree of resistance to irradiation is without parallel in cultured mammalian cells, and it seems unlikely that the change in radiosensitivity can be explained simply on the basis of the quantitative reduction in DNA content of these cells, or on the possible presence of an intracellular radioprotective compound.

The parent cells (Lich-1) were grown on glass as monolayers in a medium consisting of 5 to 10 percent inactivated horse serum in a modified Eagle's medium (*5*). In each experiment, a series of replicate cultures was prepared, each containing about 50,000 cells in 1 ml of medium. The radiation source used for Lich-1 cell cultures was a 250-kv (peak) x-ray generator operating at 15 ma with 1-mm aluminum added filtration, yielding a dose-rate within a culture tube of 68.5 roentgen/min at 60 cm. The dose-rate was checked before and after each experiment with a Victoreen condenser ionization chamber. Immediately after irradiation the medium in all cultures was changed, and the number of cells in four unirradiated controls was counted. The remaining control and irradiated cultures were placed on a roller drum (12 rev/hr) at 37°C; nutrients were renewed on alternate days, and cell numbers were again counted on the 3rd and 6th days.

In experiments in which uninfected Lich-1 cells were irradiated (Fig. 1) a minimum exposure dose of 500 r was required to inhibit cell multiplication after 3 days, though doses as low as 100 r caused a definite decline in cell multiplication. These findings are similar to those reported for other human cell lines (*6*).

Two strains of lipovirus-altered (ILEM) cells which had been kept in continuous cultivation in enriched medium for 1½ to 2 years were irradiated as suspensions of about 3.5×10^5 cells in 5 ml of enriched medium in sealed glass ampules. In order to achieve a sufficiently high dose-rate, irradiation was carried out with a cobalt-60 source (*7*). This source contained slightly less than 10^6 curies of cobalt-60 arranged in two parallel planes. The absorbed radiation dose-rate to the midline, 12.55 cm equidistant from each plane, was 7.5×10^4 rad/min when the source was in a fully raised position. Additional exposure occurred during raising and lowering of the source into a deep water tank, and the total absorbed dose to each culture was determined by means of ferrous-copper dosimeters. Cultures were irradiated in a ventilated carrier within which the temperature was maintained at about 24°C. After irradiation, the cells were diluted 1 : 20 in 2 ml of fresh enriched medium and incubated at 33°C, a temperature optimum for the propagation of the lipovirus (*8*). Samples from these suspensions were then taken for counting at 1 or 2 day intervals.

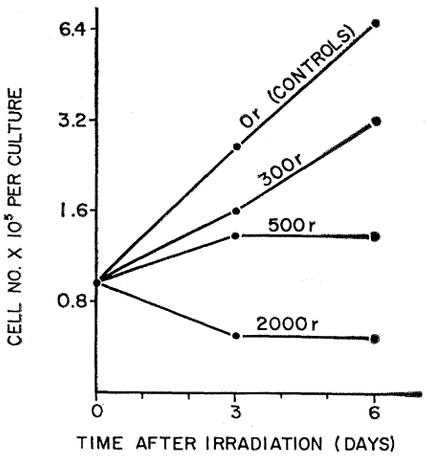

Fig. 1. Effect of x-irradiation on multiplication of human Lich-1 (liver) cells in vitro. Semilog graph. Each point represents average of four cultures in single experiment. Doses shown are exposure doses in roentgens.

In four experiments (Fig. 2 is an example), the lowest dose with a consistent inhibitory effect on cell multiplication was 290,000 rad. With 120,000 rad, slight inhibition of growth was noted in only one of three experiments in which this dose was used. In none of the experiments were doses up to 580,-

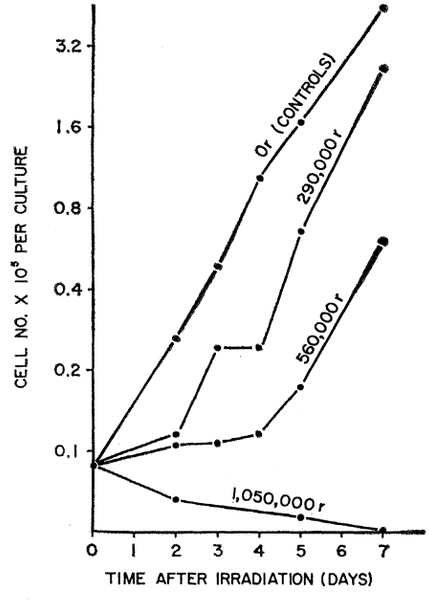

Fig. 2. Effect of cobalt-60 irradiation on multiplication of lipovirus-altered (ILEM) cells in vitro. Semilog graph. Each point average of duplicate cultures in single experiment. Each curve represents response to single radiation exposure with maximum exposure time 14 minutes. Doses shown are actually absorbed doses in rads.

000 rad sufficient for complete inhibition of cell multiplication. Doses in the range of 10^6 rad resulted in complete cell death in five of eight cultures; recovery of cell multiplication in the remaining three cultures occurred about 2 to 3 weeks after irradiation. After recovery from irradiation, all cultures were serially transferred at intervals of 1 to 3 weeks for a total of at least six transfers. At each transfer, 0.1 ml of the ILEM cell suspension was transferred to 1.9 ml of fresh, enriched medium. In all instances, cell density of about 200,000 cells per milliliter was attained at the termination of the experiment.

In parallel experiments, ILEM cells irradiated with similar doses while suspended in isotonic saline showed the same response to radiation as those suspended in enriched medium. On the other hand, unirradiated ILEM cells, suspended for 3 hours in enriched medium previously irradiated with 10^6 rad, propagated at the same rate as those suspended in unirradiated medium. Enriched medium showed no protective effect on normal Lich-1 cells. In order to determine whether this phenomenon of extreme resistance to irradiation was in any way related to the source of radiation (cobalt-60 γ-rays compared to 250-kv (peak) x-rays), ILEM cells were irradiated by the same x-ray generator, and the factors used were those employed for normal Lich-1 cells (with the exception of a target-culture distance of 20 cm, above 10,000 r). No significant effects on growth characteristics were demonstrated with graded x-ray exposures ranging from 1,000 to 130,000 r.

The mechanism for the development of this unusual radioresistance in ILEM cells is not known. It is improbable that the radioresistance is due merely to the quantitative decrease in DNA content of these cells; on the basis of general relationships between radiation lethality and cellular DNA content reported for various cells and viruses (*9*), a 90-percent reduction in cellular DNA alone should result in only a 10- to 20-fold increase in the radiation dose required to kill cells. Chemical radioprotective agents in vitro, on the other hand, have thus far been shown to cause only a two- to fourfold increase in radioresistance (*10*). Radioresistant mutant strains of cultured cells and bacteria have been isolated, but these show a maximum increase of tenfold in radioresistance, and there is nothing to suggest that the mechanism of radioresistance in these instances is related to that observed in ILEM cells (*11*). The fact that infection with the lipovirus has produced a line of cells whose radioresistance is increased 1000-fold suggests, rather, that some basic alteration in a radiosensitive "target" in the cell has taken place as a result of changes induced by this cytopathic agent.

It appears that in ILEM cells doses up to 100,000 rad do not significantly affect the basic metabolic functions governing cellular synthesis and growth. However, the primary effect of ionizing radiation in most rapidly dividing cultured cells is the inhibition of mitosis, and the mitotic process itself is normally very sensitive to ionizing radiation. Certain differences have been observed in the mechanism of cell division in ILEM cells. First, preliminary studies indicate that the multiplication of ILEM cells is not delayed by colchicine at a concentration of $10^{-4}M$. (Division of Lich-1 cells is inhibited at the concentration of $10^{-6}M$.) Secondly, it has been shown that the division time of the ILEM cell is only about 6 minutes, as compared to 15 to 20 minutes for Lich-1 cells, though their cell-doubling times are roughly similar (*3*). Possibly, infection with the lipovirus has sufficiently altered the process of cell division such that it is no longer sensitive to ionizing radiation, and cell death results from a different mechanism requiring considerably higher radiation doses.

Further work is needed to elucidate the metabolic changes which give rise to radioresistance in lipovirus-altered cells. A system in which such gross alterations in cellular radiosensitivity can be selectively produced, however, offers another means of studying the mechanisms of radiation damage in human cells.

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12. Supported in part by a Rockefeller Foundation grant, by PHS Bureau of State Services grants 5R01-OH-00103-03 and 4T1-RH27-03 (64), and by grants 5-K3-GM15,131 and AI04,998 from NIH.

1 April 1965

Trematode Parasitism and Polymorphism in a Marine Snail

Abstract. Velacumantus australis *is a common Australian snail which harbors several larval trematodes. Many populations have a small proportion of banded shells. Analysis of three samples from a coastal lake shows that banded snails are less likely to harbor larval trematodes than unbanded snails.*

The Australian mud whelk *Velacumantus australis* occurs in large numbers on sandy mud flats of many coastal lakes, estuaries, and sheltered bays on the eastern and southern coasts of Australia. It commonly harbors several species of larval trematodes, and the incidence of infection in adult snails of some populations is as high as 50 percent (*1*).

Many populations have a small proportion of individuals with banded shells, in which a white band about 1 to 2 mm wide is present in the lower part of each whorl. Banding is found in snails of all ages, including very young juveniles which do not carry larval trematodes. Banded snails are scattered randomly among the other snails.

Three samples of *V. australis* were collected from different areas of Lake Burril, 145 miles (233 km) south of Sydney, during December 1964 and January 1965. A total of 25,155 snails were collected, of which 3.92 percent had banded shells. The frequency of banded snails tended to be highest in the youngest juveniles and lowest in old adults.

Random samples of 815 banded and