PROGRESS REPORT
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BIOLOGICAL EFFECTS OF RADIATION AND RELATED BIOCHEMICAL
AND PHYSICAL STUDIES

Proposal #2: Radiation Studies; Radiation
Mechanisms in Mammalian Systems
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RADIATION STUDIES

Radiation mechanisms in Mammalian Systems

Objectives:

One of our principal goals is to investigate the oxygen effect in mammalian cells exposed to radiation of different LET's. Since many solid tumors are believed to contain appreciable numbers of hypoxic cells which are relatively resistant to gamma radiation, the use of high LET radiation has the potential for reducing this protective effect of hypoxia.

We have been investigating the effects of hypoxia on the survival of HeLa cells using fast neutrons or californium-252, and have compared these with the results using x- and gamma radiation and radium. The experimental system we have employed to induce hypoxia is based on the physiological depletion of oxygen by dense cell cultures; we believe this system may mimic certain in vivo tumors in important respects.

Another objective of our studies is to investigate the effect of cell population density and proliferative state on the radiation sensitivity of different cell types. We also wish to determine whether one or more of these may, under appropriate conditions, serve as an experimental model of either normal non-dividing tissue, or of dormant or "G₀" tumor cells.

Materials and Methods:

These have been described elsewhere (1), as well as in previous reports.

Main Research Accomplishments:

A. Studies with chronically hypoxic cells

Hypoxia is induced in our HeLa cell cultures by incubating assay cells with an excess of heavily irradiated "feeder" cells. In this system, viability and hypoxia remain high over a period of 24 hours. An important objective was to allow enough metabolic activity to deplete the oxygen in the dense culture, yet prevent the accumulation of waste products which might diminish respiratory activity and allow oxygen to re-enter the system. We solved this problem by first incubating the cell suspension for two hours at 37°C to induce hypoxia, and then lowering the temperature to 30°C, at which hypoxia could be maintained without excessive accumulation of metabolic waste products. Incubation at 30°C also delayed cell progression through the division cycle, as determined using mitotically synchronized cells in conjunction with cytotoxic levels of tritiated thymidine. This inhibition of progression may prove useful for determining the responses of cells in different phases of the cycle to radiation at low dose rates.

With our system of hypoxic cells, we have determined the oxygen effect in synchronized HeLa cells irradiated with 280 kvp x-rays. High OER values were found in late G1 and S phase cells, while lower values were obtained in the M and early G1 phases, (Fig. 1).

B. Neutron Studies

The effect of hypoxia on HeLa cells irradiated with cyclotron-produced neutrons has been determined at two depths in a phantom. OER values of about 1.7 have been obtained at irradiation depths of both 2.4 and 16 cm. This result is relevant to clinical treatment, where one is concerned with the possibility of differential radiosensitivity of hypoxic cells in tumors situated at different depths within the body.

We have also conducted a series of experiments with synchronized HeLa cells made hypoxic in different parts of the division cycle, and irradiated with fast neutrons at 2.4 cm. depth. We found no significant differences in OER in cells irradiated in different phases of the cycle. To our knowledge, this is the first study of OER values using synchronized cells irradiated with neutrons, (Fig. 2).

C. Studies with californium-252 sources.

We have completed a series of experiments with californium and radium sources at a depth of 2.4 cm. within the phantom, using our system of chronically hypoxic HeLa cells (2). This system has the advantage over more classical methods for inducing hypoxia in that high viability is maintained for prolonged periods, making it suitable for low dose-rate irradiations. Employing californium sources, the overall OER was 1.72 ± 0.11 , (Fig. 3). including the contribution from the gamma component of the radiation. This compares with an OER of 2.53 ± 0.30 for radium. It is desirable to differentiate between the neutron and gamma ray components of californium-252 sources, as the contribution of each to the total dose varies with depth and the biological effect is dependent on dose and dose rate. Calculations were performed to determine OER and RBE values due to the neutron component alone at 2.2 cm. depth of irradiation. At this depth the OER for neutrons was 1.63 ± 0.12 . An RBE value of 2.74 ± 0.15 was obtained for neutrons emitted by californium-252, using data obtained with radium gamma-rays as a reference. While OER values agreed with data obtained by other workers, RBE values were substantially lower than commonly observed with this radiation modality. This may in part be accounted for by the smaller capacity of HeLa cells to accumulate and repair sublethal damage.

D. Studies with density inhibited cells.

The radiation sensitivity of cell lines whose proliferation is highly sensitive to cell population density and serum factors is being investigated. In order to determine whether their survival is dependent on the proliferative state of the cells, we

have obtained dose-survival curves following x-irradiation of mouse fibroblast 3T3 cells in both log and plateau phases. We found that the D_0 values were about 100 and 280 rads and the corresponding extrapolation numbers were 52 and 2.3 in log and plateau phases, respectively (3). Analogous studies with the HeLa cell line (whose proliferation is less affected by the above factors) yielded D_0 values of 144 and 123 rads, and extrapolation numbers of 1.6 and 3.4 for cells in log and plateau phases.

We are now carrying out similar studies in another "density dependent" cell line, the WI-38 line, derived from human embryonic tissue. This line probably constitutes an even better experimental model of normal dividing or resting human tissue than does the 3T3 line, since it is diploid as well as being of human origin.

Preliminary studies of the survival of these cells after irradiation in log or plateau phase indicated that when growth was limited by nutrient deficiency, survival of the plateau phase cells was similar to that of cells in log phase. When nutrient deficiency was not the principal factor limiting growth, the plateau phase cells appeared to become somewhat more resistant. These experiments, while provocative, have not yet been confirmed.

Biochemical investigations of this cell line have shown that when the cells were irradiated immediately following their release from confluency, the onset and rate of DNA synthesis were inhibited in a dose dependent manner. After a dose of 250 rads, the onset of DNA synthesis was delayed 6 hours, while at a dose of 1000 rads, DNA synthesis was almost completely suppressed. The rise in activity of thymidine kinase was markedly inhibited when the cells were irradiated under these same conditions. This sensitivity of thymidine kinase activity and DNA synthesis to x-irradiation is in contrast to the relative radioresistance of these processes in HeLa cells irradiated either at confluency or in the G_1 phase following mitotic selection.

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Fig. 1 Survival curves of mitotically synchronized HeLa cells exposed to 280 kVp X-rays, under oxygenated and hypoxic conditions. M: cells irradiated 1.5 hours after initiating the synchronized culture when 68% of them were still in mitosis. G1: cells irradiated 7.5 hours after initiation, when only 5% were labeled with ^3H -thymidine; and S: cells irradiated 13.5 hours after initiation of the culture, when 90% of the cells were labeled. Hypoxia did not delay progression.

Fig. 2 Survival curves of synchronized HeLa cells exposed to fast neutrons in different parts of the division cycle. Symbols M, G1, and S have the same meaning as in Fig. 1.

Fig. 3 Survival curves of HeLa cells (non-asynchronous) irradiated with californium sources. Empty circles: oxygenated cells; full circles: hypoxic cells.