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PROGRESS REPORT
TO
THE UNITED STATES ATOMIC ENERGY COMMISSION
CONTRACT AT(30-1)910
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:

It is desirable to have a system of mammalian cells in culture which would resemble certain types of tumors (e.g., large solid tumors) with respect to the presence of anoxic cells in poorly vascularized regions. With an in vitro system in which prolonged anoxia could be maintained without extensive loss of cell viability, the effect of radiations of various LET on anoxic cells could be investigated without interference from the many extraneous factors present in vivo. Finding conditions of irradiation under which cells would be least sensitized by oxygen is clearly of great importance for tumor radiotherapy. It was our objective to devise an improved system of anoxic or at least hypoxic HeLa cells for use in various radiobiological studies.

Other objectives of our research include the use of synchronized cultures of HeLa cells and metabolic inhibitors, as well as the use of pulsed electron and neutron irradiation in elucidating fundamental processes of radiation damage and repair.

Materials and Methods:

These have been described elsewhere (1,2) as well as in previous reports.

Main Research Accomplishments

A. Studies on Modification of Radiation Response in Synchronized HeLa Cells

Our studies with the radiosensitizer 5-bromodeoxyuridine (BUDR - analog of thymidine) have shown that although this compound alters the radiation response of HeLa cells only when incorporated into DNA, incorporation does not have to precede irradiation (3). Moreover, the usual fluctuation in radiation response as a function of cell age (as observed in synchronized cultures) of HeLa cells is absent in analog-containing cells. This may be partly due to the impairment of progression of analog-containing cells through the S phase which we observed (Figure 1), and points to the importance of DNA synthesis in the radiation response fluctuation phenomenon. In this context, it is not surprising that inhibition of DNA synthesis before or after irradiation in BUDR-containing cells

(with DNA synthesis already impaired) does not affect radiation response as much as in the case of control cells.

B. Studies with Density Inhibited Cells

Our principal objectives in studying cell systems whose growth is limited at high population densities are to determine the adequacy of such systems as experimental models of dormant tumor cell populations and to investigate their responses to radiation.

We have used two systems of density inhibited mammalian cells in culture: that of the naturally density controlled cell line 3T3 (whose maximum cell density is determined in part by serum concentration), and that of HeLa cells which are normally not density controlled but can be made so after incorporation of 5-bromodeoxyuridine; the latter observation is in agreement with those of Silagi and Bruce (4) using a different system.

An auxiliary problem in conjunction with 3T3 density controlled cells was their inability to incorporate significant amounts of ³H-thymidine under conditions in which such incorporation would be expected. Since incorporation of thymidine is a chief means of monitoring DNA synthesis and cell cycle parameters, we have given this problem considerable attention. It turned out, in spite of repeated negative laboratory reports, that the thymidine exclusion could be traced to mycoplasma contamination, which degrade exogenous thymidine, provided medium contains serum.

Although this phenomenon is sometimes observed in cases of mycoplasma contamination, repeated laboratory tests for the presence of this organism were negative. However, with the aid of a more sensitive detection procedure based on paper chromatography, we confirmed the presence of mycoplasma and determined some of its biochemical properties (including its ability to degrade exogenous thymidine and uridine).

We also carried out studies on HeLa cells containing BUdR which behaved in a density inhibited manner. It was found that neither the population density nor a post-irradiation incubation period (up to 48 hours) in the density inhibited state significantly affected the radiation response. Rather, the radiation sensitivity was governed almost entirely by the amount of BUdR incorporated.

C. Radiobiological Studies with Mammalian Cells Using High Intensity Pulsed Electrons (in collaboration with Dr. Edward R. Epp and Mr. Herbert Weiss)

The results of ultra high dose-rate experiments on bacterial cells, which demonstrate that sharp breaks in survival curves can be caused by radiation-induced depletion of intracellular oxygen, lead to the question of the response of mammalian cells exposed to such high intensity radiation. If the results obtained for bacterial cells could be validly extrapolated on physico-chemical grounds to mammalian cells, no break on an oxygen depletion basis would be expected in mammalian cell survival for a pulsed irradiation in air. This is because the measured break-point dose in air is about 55-60 kilorads for bacteria. Thus a break in mammalian cell survival would not be predicted unless the cells were equilibrated with an environment containing an oxygen concentration of only a few tenths of one percent. Evidence reported in the literature on this subject is completely conflicting. Some investigators (5,6) have reported evidence for a break in air at less than 1000 rads; others have reported no such evidence. Recently, results have been published indicating no break in air but a break at an oxygen concentration of about 0.35%, in agreement with what would be expected by extrapolating from bacteria.

A method was developed in this laboratory in 1970 for measuring the radiosensitivity of mammalian cells exposed to single high intensity pulses of radiation. The method involves a cell transfer technique which circumvents possible problems arising from migrant cells and from cells shielded from irradiation. Experiments indicated that the presence of such cells may be responsible for some of the disagreements reported in published data. Results obtained in this laboratory using this technique have not indicated the presence of a break in the radiosensitivity of HeLa cells irradiated in air with single short (3 nanoseconds) electron pulses. Further results obtained to date give every indication that a complete family of breaking survival curves for various initial oxygen concentrations will be obtained similar to those previously obtained at this Institute for bacteria. These data for mammalian cells are consistent with and can be predicted on physico-chemical grounds from bacterial data. They strongly imply that, at low oxygen concentrations, the initial part of a short electron pulse can serve to deplete oxygen within mammalian cells.

D. Studies on the Oxygen Enhancement Ratio (OER) of
Cyclotron Produced Neutrons

We have successfully developed a system of hypoxic HeLa cells in which viability was not greatly affected by as long as 24 hours in that state. Our system is especially suitable for O.E.R. studies using cyclotron-generated neutrons at low dose rates where longer exposure times are required.

The system of chronically hypoxic cells is prepared by mixing heavily irradiated "feeder" cells (in excess) with "assay" HeLa cells. The mixed cell suspension is then incubated at 37°C for 1.5 hours before irradiation to allow for oxygen depletion by the respiratory activity of the "feeder" cells. The (oxygenated) controls were prepared similarly, except that the "feeder" cells were killed by alcohol beforehand. In this system, plating efficiency was generally very good (over 60%) irrespective of whether or not feeders were metabolically active. With ^{60}Co gamma irradiation, an O.E.R. of 3.25 (taken as presumptive evidence of oxygen depletion) was consistently observed, while with neutron irradiation at a depth of 2.4 cm. in the phantom an O.E.R. of only 1.5-1.6 was obtained. This difference is highly significant and supports the use of neutron irradiation in radiotherapy of anoxic tumor cells.

E. Studies with Cf-252 Sources

Preliminary work on ^{252}Cf irradiation of HeLa cells is essentially completed. Arrangements for irradiation (including geometry of sources, dosimetry and holders for tubes containing cell suspensions) have been worked out. It is planned to utilize our system of chronically hypoxic cells in the proposed investigations. Californium sources deliver the mixed gamma and neutron irradiation at a slow rate and in ordinary systems, the complicating effects of prolonged anoxia could impair meaningful studies. Our system offers the advantage that cells do not proliferate during the irradiation period; this eliminates complications due to cellular multiplicity or of newborn cells not being exposed during the entire intended irradiation time.

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FIGURE 1. Incorporation of ³H-Thymidine Into Cultures Initiated from Mitotic HeLa Cells Which Incorporated Thymidine or BUdR During the Previous Cycle.

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