714509

Copied From Los Alamos
National Laboratory Archives

Log no. 242
Date 11/11/75
Book 3 Pg 49
By M. Rigga

Survival of Synchronized Cultured Human Kidney T-1 Cells Exposed to Stopping Pions and X-Rays at Various Times after Mitosis

REPOSITORY LANL/ARC
COLLECTION MP-DO
BOX No. A-91-011
FOLDER 105-9

Paul Todd
618 Life Sciences Bldg.
University Park, Pennsylvania 16802
814-865-0242

Spokesman: Paul Todd

Beam Line: Biomedical Pion Channel

Proposal for submission to:

Los Alamos Meson Physics Facility
Program Advisory Committee
Biomedical Subcommittee Members
L. Rosen, Director

COPIED FOR
RECORD

FILE BARCODE
00133508

00133508.001

1092451

Requirements for

Survival of Synchronized Cultured Human

Kidney T-1 Cells Exposed to Stopping

Pions and X Rays at Various Times after Mitosis

Primary Beam: protons

Secondary Beam: negative pions

Beam line: biomedical pion channel

Momentum: 170 MeV/c

Momentum bite: $\Delta p / p = 2\%$

Special beam characteristics:

Intensity = 40 rad/min stopping pions

Area = 4 x 4 - 4 x 8 cm x cm

Stopping medium = water

Special requirements:

Dosimetry (integrated normalized current) with in-house instrumentation at LAMPF

Water tank with stopping region localized

LAMPF cell culture lab

Time requirements:	Experiment 1	2 shifts
	Experiment 2	2 shifts
	Experiment 3	4 shifts

COPIED FOR
HSPT

1092452

00133508.002

A. Background

It is ironic that one of the "standard" test cell lines (human kidney T-1) for high LET radiations is extremely difficult to synchronize by mitotic selection. In 1974 we devised a highly inefficient method for obtaining populations of T-1 cells with approximately 94% of the cells in late metaphase or anaphase. The response of cells in the first half of the cycle has been studied following irradiation with 250 kVp x rays and ^{239}Pu alpha particles. The high LET response as a function of cycle phase is clearly different.

Although the radiation responses of synchronized Chinese hamster cells have been studied following a variety of low and high LET radiations, the information provided may be only partially applicable to the human situation. Cultured human cells have two radiation-resistant periods during their cycle, whereas Chinese hamster cells have only one, unless the G1 phase is artificially prolonged by conditions of deprivation. It is therefore important to study synchronized human cells in addition to Chinese hamster cells with respect to their responses to stopping pions.

B. Methods of Procedure

Human kidney (T-1) cells will be selected in mitosis from a population enriched in mitotic cells by prior accumulation of late G1 cells in 2.5 mM thymidine medium. 9 hr after thymidine removal the cell monolayer will be vigorously washed with low-calcium medium and incubated 2 additional hr in 3 ml/flask of the same medium. The monolayer will be washed gently in this medium which, when it is poured off, will contain late metaphase and anaphase cells. These cells will be seeded into T-30 plastic flasks for underwater irradiation with stopping pions, in three separate experiments.

COPIED FOR HSPT

In the first experiment the first 14 hr of the cell cycle (which is 22 hr long) will be studied by irradiating cultures every 1.5 hr with

1092453

2 doses of x rays and 3 doses of stopping pions. More doses will be used at 4 hr and 11 hr, the points of maximum and minimum resistance, respectively, in order to obtain complete survival curves.

In the second experiment the mitotically selected cells will be allowed to progress for 8 hr, after which they will be "resynchronized" by 4 hr incubation in 2 mM hydroxyurea, which inhibits DNA synthesis but does not kill established cultured human cells. Cultures will then be irradiated every 1.5 hr to discern the time-dependence of survival of cells progressing through the second half of the cycle—S, G2, M. Two doses of x rays and 3 doses of stopping pions will be used at each point. More doses will be used to establish survival curves at 8 hr and 12 hr, which are expected to correspond to late S and M, respectively, in the "resynchronized" populations.

The third experiment will be designed around the results of the first two and will consist of two survival-vs-time curves, each using one pion dose chosen on the basis of the first two experiments. Survival will be determined every 1.5 hr over a 28 hr period, and cells to be irradiated more than 12 hr after exposure will be resynchronized as in the second experiment.

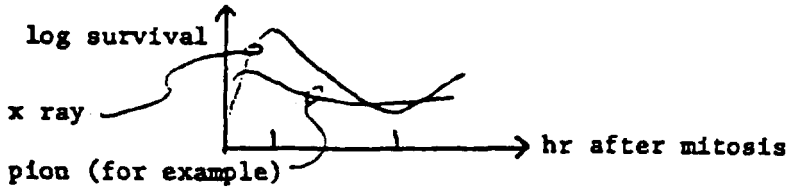
A logical fourth experiment is the determination of survival curves at selected phases of the cell cycle. This will be proposed in a future application, the contents of which will also depend upon the outcome of the above experiments.

COPIED FOR
HSPT

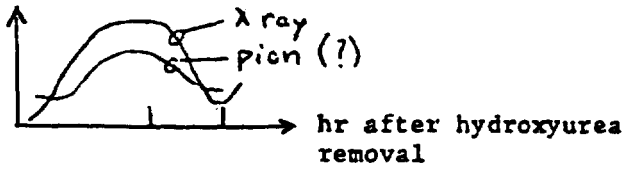
C. Results

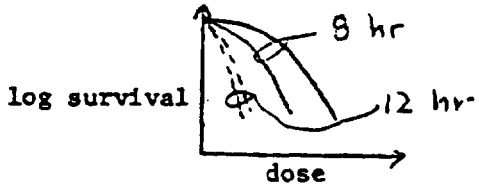
The data sets will be plotted on the following axes:

Experiment 1: log survival

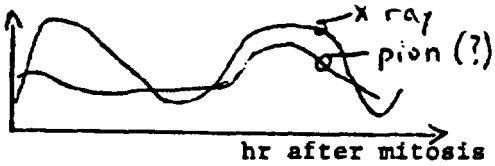


Experiment 2: log survival





Experiment 3: log survival



Cells from all populations used will be monitored for their ability to incorporate ^{3}HTdR to establish the position of the cells in the cycle at the time of each irradiation. Both continuous and pulse labeling will be used.

COPIED FOR
HSF

1092455

00133508.005

D. Significance

Until recently it was thought that the only advantage of high LET radiation might be the reduced OER. But J. F. Fowler pointed out that if high LET radiation has a therapeutic gain factor it should be exploited whatever the cause. Upon analyzing carefully-executed experiments in which synchronized Chinese hamster cells were irradiated with X rays and alpha particles, Raju pointed out that the RBE changes throughout the cell cycle and, given that normal and neoplastic cells are in different parts of the cycle, significant therapeutic gain factors might be realized under a properly timed high LET therapy schedule. The proposed experiment aims to determine to what extent this situation might hold for human cells irradiated with stopping pions.

Financial support for this study is available through a grant to the principal investigator from the National Cancer Institute. An attempt will be made to coordinate the timing and execution of the experiments in this proposal with those to be performed by the principal investigator in "Experiment #143" using the same biological test system in fractionation and recovery experiments.

COPIED FOR
REPORT

1092456

00133508.006