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RESEARCH PROPOSAL

LOS ALAMOS MESON PHYSICS FACILITY

RADIATION REPAIR OF NORMAL MAMMALIAN TISSUES

Edward L. Gillette, Spokesman

PARTICIPANTS AND INSTITUTIONS

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John W. Watters, Colorado State University

Date: 2-25-75

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SUMMARY OF EXPERIMENT

The purpose of this investigation is to evaluate the response of capillary endothelial cells to negative pi mesons. A model has been developed at Colorado State University using the eyes of beagle dogs to examine effects of beta and x radiation on capillary endothelium at the limbus. This *in vivo* experimental system can be used to estimate the RBE of high LET radiation for endothelial cells. This has important clinical implications because effects on capillaries may determine the response of dependent tissues late after a course of radiation therapy. Additional stress to irradiated tissues may cause injury to fine vasculature leading to degeneration and necrosis. It is difficult to study radiation dose response and repair capability of nonproliferating or slowly proliferating mammalian tissues. However, knowledge about radiation repair by normal tissues becomes critical when considering use of radiation therapy techniques which may alter this capability.

During the past two years single- and split-dose techniques have been used to establish dose-effect curves and to quantitatively evaluate repair capability of endothelial cells. The proposed study at LAMPF will be only of single doses initially to determine the dose response of endothelium to negative pi mesons. This will be followed by a split-dose assay to determine effects on repair capability.

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PROPOSAL INFORMATION

Beam Area: A East

Beam Requirements:

These requirements are as previously established for human radiobiology experiments on the bio-med channel. This has been discussed with Jim Bradbury on February 25, 1975 via phone conversation. The peak region of the pion beam should be at the surface of the eye. These experiments should be done when a dose-rate of approximately 50 rads per minute in the peak is available.

Running Time Required:

Installation Time: 2 hours, Experiment 1; 4 hours, Experiment 2

Data Runs: 6 hours, Experiment 1; 9 hours, Experiment 2

Scheduling: 1 day in October, 1975 or as beam of required intensity is available for Experiment 1; 2 days approximately one month later for Experiment 2.

Space Required:

1. 10' x 10' area to perform surgical procedures. L. M. Holland, H-4, has been contacted concerning special equipment and anesthesiology support.
2. Housing facilities for 15 dogs for 1 day for Experiment 1; 4 days for Experiment 2.

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DETAILED STATEMENT OF THE EXPERIMENT

1. Purpose:

The long-term goals are to determine alterations in the response of capillary endothelial cells induced by high LET as compared to low LET radiation. Radiation effects on fine vasculature may be the determining factor in late response of organs composed mainly of slowly- or non-proliferating cells. Endothelial response to pion irradiation will be evaluated for exposures before and after stimulation of capillary proliferation. A comparison of these data with those for beta and x-ray exposure are of prime interest. Split-dose studies will be performed later to establish the repair capability of endothelial cells exposed to fractionated doses.

2. Background:

The literature on radiation pathology has been based largely on responses to either large single doses of radiation, protracted radiation within a narrow dose range or on clinical observations following radiotherapy. This material does not lend itself to critical assay of dose response or of capability of mammalian tissues to repair radiation damage. The latter point is extremely important in radiotherapy since the assumption of repair capability of normal tissues is the basis for fractionated dose regimens.

In recent years radiation response has been determined for rapidly-proliferating tissues in rodents such as bone

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marrow, skin, gut and spermatogonia (1,2,3,4,5). Skin and gut appear to have a large capability to repair radiation damage. Techniques for similar studies of nonproliferating or slowly proliferating tissues are much more difficult to develop.

Late complications from radiotherapy may be the result of either continual degeneration or regression of fine vasculature and/or continued proliferation and condensation of fibrous tissue in the irradiated area (6). Marked changes in dependent tissues can be caused by radiation induced changes in fine vasculature and interstitial tissue. This damage instituted by irradiation may ultimately lower resistance to additional injury. Added injury such as subsequent trauma or infection may cause tissues to undergo acute necrosis long after irradiation. Brain, heart, and kidney are critical organs commonly in radiation therapy fields and whose sensitivity may be determined by the sensitivity of capillary endothelial cells.

In recent years techniques have been devised to study the effects of radiation on endothelial cells. Van den Brenk et al. (7) used the Seyle pouch technique to stimulate angiogenesis and growth of the repair blastema in rats. They measured radiosensitivity of capillary blood vessels in the subcutis. They obtained a D_0 of 240 rads based on colony counts and a recovery capability of 180 rads based on split-dose radiation studies. There was no significant difference in response to single doses of radiation given

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either shortly before or after raising the pouches, however, a delay of two or three weeks before raising the pouches caused an increase in the dose required to produce a given effect by 550-625 rads for single doses in the range of 1500-1800 rads given to intact skin. This amount of "repair" could have been influenced by a number of factors including the possibility of endothelial proliferation induced by radiation.

Reinhold and Buisman (8) studied vascularization in subcutaneous air pouches in rats. They obtained a quantitative indication of the vascular index related to the local amount of an intravenously injected tracer. A "survival curve" based on the amount of tracer as a function of radiation dose gave a D_o value of about 170 rads and a D_q of 540 rads. Irradiation was done immediately following stimulation of capillary proliferation. Split-dose experiments with an initial dose of 500 rads followed by a variable test dose 24 hours later gave a $D_2 - D_1$ of about 290 rads. When they irradiated prior to stimulation of proliferation they obtained a D_o of 237 rads (9).

At Colorado State University a method has been developed to assay response of proliferating and nonproliferating endothelial cells. A lamellar keratectomy is performed in the center of the cornea to induce superficial neovascularization. Neovascularization is detected by a redness of the limbus produced by the presence of blood in the newly formed vessels. Irradiation of the limbus was done either before or after induction of proliferation. The percentage of eyes

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with neovascularization within one week following surgery was plotted against dose. Split-dose studies were carried out to determine the radiation repair capability of endothelial cells. Split-dose studies done either before or after induction of capillary endothelial proliferation indicated that the radiation repair capability was approximately 540 rads in either case.

Methods have now been developed to utilize a modified Chalkley point-hit type evaluation to determine survival curves following radiation (10). Histologic sections are made from superficial layers of the cornea near the limbus. The newly formed capillaries are present in this tissue in less than one week following the partial keratectomy. An estimate is made of the percentage surface area occupied by capillaries. This has been found to be inversely proportional to radiation dose and is probably related to the number of endothelial cells surviving irradiation. Experience at Colorado State University was obtained in this type of analysis on a study of the microvasculature of tumors at variable intervals following irradiation (11,12).

3. Preliminary Data:

Split-dose studies for beta radiation have been completed. For irradiation immediately following surgery the dose required to prevent neovascularization in 50% of the irradiated eyes (NVD_{50}) is 934 rads (Table I). The NVD_{50} 6 hours after induction of capillary proliferation was 1000 rads and at 24 hours after induction was 864 rads. The

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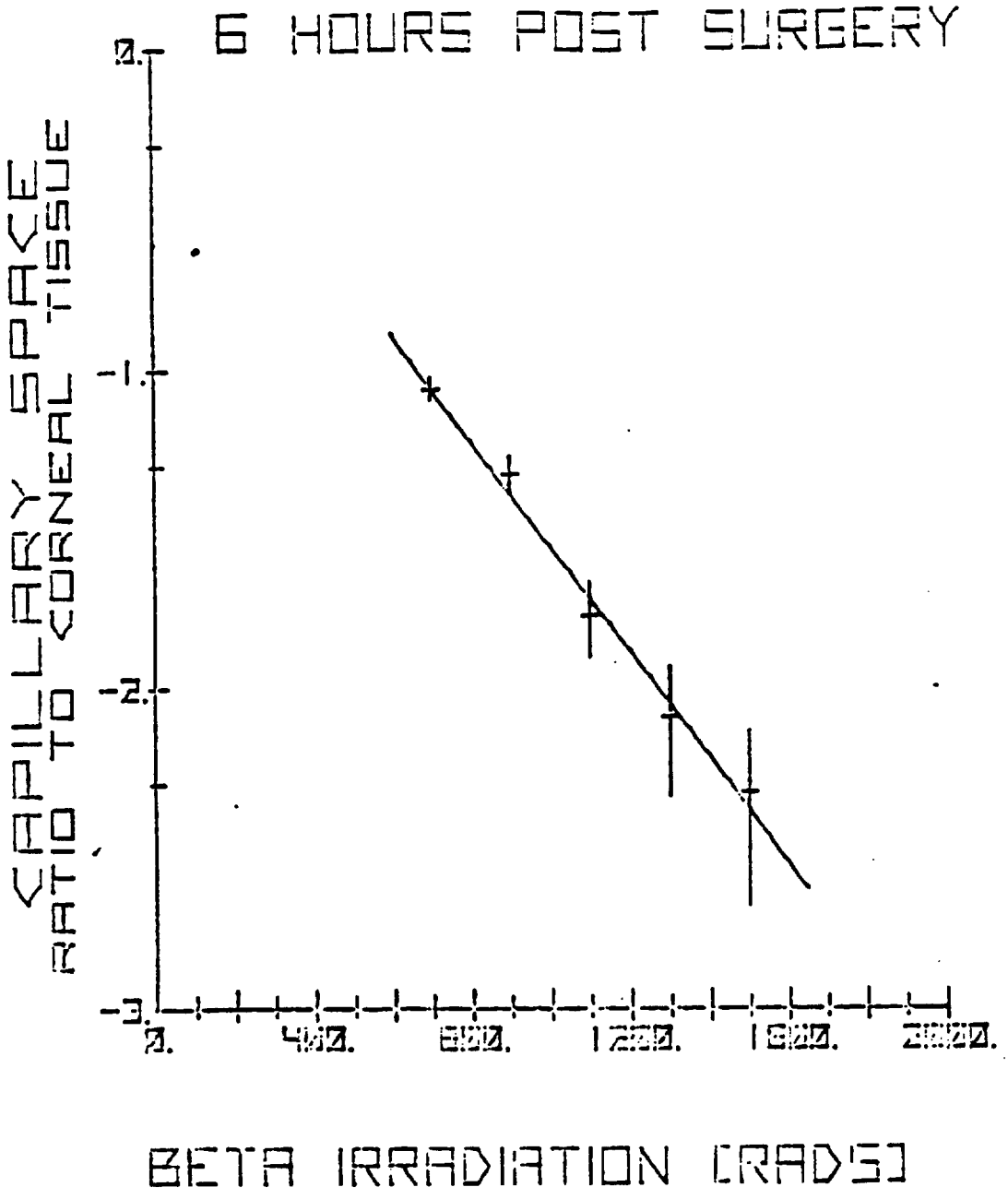
Figure I



Beta radiation dose-effect curve which is related to endothelial cell survival. Capillary space represents the log of the mean of four estimates of the fraction of total tissue volume occupied by capillaries. Corneal sections were taken near the limbus 10 days following induction of capillary proliferation. The eyes were irradiated 6 hours after induction. The "D_o" is 260 rads.

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TABLE I

NVD_{50} Values for Single Dose Beta Irradiation
After Induction of Capillary Endothelial Proliferation

Time After Induction (hours)	NVD_{50} (rads)	95% Confidence Limits
0	954	753-1152
6	1000	789-1209
24	864	660- 939
48	1397	1179-1685

TABLE II

NVD_{50} Values for Single Dose Beta Irradiation
Before Induction of Capillary Endothelial Proliferation

Time Before Induction (hours)	NVD_{50} (rads)	95% Confidence Limits
24	1475	1236-1679
48	1391	1136-1629

TABLE III

Split-Dose NVD_{50} Values for Beta Irradiation Before
and After Induction of Capillary Endothelial Proliferation

Time of D_1 (hours)	Time of D_2 (hours)	NVD_{50} (rads) ($D_1 + D_2$)	95% Confidence Limits
0 postinduction	6 postinduction	1355	1136-1560
48 preinduction	24 preinduction	1746	1545-1912

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NVD_{50} 48 hours after induction was 1397 rads. All these postinduction NVD_{50}s are within approximately the same range except the 48-hour NVD_{50}. The higher 48-hour postinduction NVD_{50} may have been due to cellular proliferation and a greater number of cells.

Irradiation was done prior to surgery to determine the response of slowly-proliferating cells. The NVD_{50} 24 hours preinduction was 1475 rads (Table II). The NVD_{50} 48 hours preinduction was 1391 rads. The NVD_{50}s are significantly higher than those following surgery except for the 48-hour postinduction NVD_{50}. These single dose experiments indicate either some capability for radiation repair or a decreased sensitivity of slowly-proliferating endothelial cells possibly due to an increased D_o.

A split-dose study was done in which a first dose of 500 rads was given immediately post surgically and variable second doses were given 6 hours after surgery. The NVD_{50} was 1355 rads (Table III). This was approximately 350 rads greater than either the immediate post surgical or 6-hour post surgical single-dose NVD_{50}s. A split-dose experiment before induction of endothelial pr-liferation was done. The first dose of 500 rads was given 48 hours before induction and the variable second doses given 24 hours preinduction. This split-dose NVD_{50} was 1746 rads. This was 271 and 355 rads greater than the 24-hour and 48-hour single-dose NVD_{50}s. Therefore, the repair capability appears to be about 350 rads as determined either before or after induction of endothelial proliferation. Single-dose NVD_{50}s for cells prior to induction

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of capillary proliferation may indicate an increased resistance of the slowly-proliferating cells.

Recently, a modification of the Chalkley point-hit method for determining the percentage surface area occupied by capillaries near the limbus of the eye has been used to obtain data for survival curves. The method is to remove a tissue layer from the cornea and make a cross section parallel to the limbus of the eye. The percentage capillary surface area is inversely proportional to dose. The sections are evaluated using a light microscope. The eyes were irradiated with beta particles from a ^{90}Sr source. The survival curve determined 6 hours after induction of proliferation has a D_o of 260 rads (Fig. 1). Data has been obtained for survival curves following x irradiation, and are presently being analyzed.

4. Methods To Be Used:

Male and female beagle dogs between 8 and 14 months of age will be randomly selected to examine the response of conjunctival endothelial cells to negative pi meson irradiation. It is proposed to use five levels of radiation with exposure of four to six eyes at each dose. The range will be from 250 to 1000 rads.

The first experimental protocol will be to irradiate the eyes 48 hours prior to lamellar keratectomies. An 8 mm diameter central lamellar keratectomy with a corneal trephine set to a depth of 0.5 mm produces superficial neovascularization at the limbus of the eye. A 4 mm distance between the circular incision and the limbus permits evaluation

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The corneal epithelium will be scraped from the lesion each day to insure capillary infiltration. Corneal tissue will be collected 10 days post-induction and sections prepared for microscopic examination of capillary invasion. Data analysis will include: (1) Dose-response curves, (2) Dose required to reduce the capillary ratio by 50% (CD_{50}), (3) Comparison of these data with those from beta and x-irradiation studies.

For Experiment 2, a constant dose of 500 rads will be given to each eye 48 hours prior to induction of capillary proliferation. Variable second doses will be given 24 hours later in the range of 200 to 900 rads to determine repair capability.

4. Facilities and Equipment Required:

Space will be required for a surgery table, gas anesthesia machine and an operation microscope. All of the equipment will be furnished by Colorado State University. The working area need not be more than about 10 feet square. Temporary facilities will be required for housing 15 beagle dogs.

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