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RESEARCH PROPOSAL

LOS ALAMOS MESON PHYSICS FACILITY

RADIOBIOLOGY OF NEGATIVE PI MESONS: A PRECLINICAL STUDY

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THE UNIVERSITY OF TEXAS (SOUTHWESTERN) MEDICAL SCHOOL
DALLAS, TEXAS 75235

Date: March 30, 1971

Recommendation of Program Advisory Committee:
Initial Exploration
To Run

Meeting held on:
7/5/71 - 7/11/71
date

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Proposal #44 - RADIOBIOLOGY OF NEGATIVE PI MESONS: A PRECLINICAL STUDY
Spokesman - D. E. Carlson, University of Texas (Southwestern) Medical School
Authors - D. E. Carlson, F. J. Bonte, and J. E. Dowdey.

Abstract - Experiments outlined in this proposal are designed to provide pretherapeutic radiobiologic information on essential aspects of stem cell inactivation by negative pion beams. Using an *in vitro* radiation procedure, spleen cell suspension will then be cultured *in vivo* according to two established assay procedures currently being used by the principal investigator, viz., hematopoietic colony formation, and secondary antibody formation. From the proposed experiments, stem cell inactivation as a function of depth will be determined, RBE values for both plateau and star regions will be determined, and also differential dose-rate-dependent cell inactivation will be investigated. Results from these experiments will provide a portion of the essential information that must be obtained before clinical trials for treating human neoplasms by pion beams are begun.

Beam Area, Channel - Area A and LEPC or biomedical area.

Particle - π^-

Energy - Variable but about 50 MeV.

Intensity - As much as possible.

Special Beam Requirements - Beam spot size and $\Delta p/p$ carefully controlled.

Targets - Lucite spleen cell container.

Time Requested - Install - 15 minutes
Tune - None
Data - 30 hours (30 min, 2X/day, for 30 days).

LAMPF Equipment Required - Standard dosimetric apparatus to be used for therapy.

Special Items or Effort - Environmental control may be a problem.

Date when Ready - When beam is available.

Previous Action by PAC - Committee suggested collaboration with LASL's H-Division to avoid animal transport problem.

Recommended Action by PAC - 30 hrs (7/71)

Approved Action by Director of LAMPF - Same

LAMPF Equipment -

Items Available:

Items to be Procured: Operations Cost -

Capital Equipment Cost -

Items to be Negotiated:

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SUMMARY OF EXPERIMENT

Experiments outlined in this proposal are designed to provide pre-therapeutic radiobiologic information on essential aspects of stem cell inactivation by negative pion beams. Using an *in vitro* radiation procedure, spleen cell suspension will then be cultured *in vivo* according to two established assay procedures currently being used by the principal investigator, viz., hematopoietic colony formation, and secondary antibody formation.

From the proposed experiments, stem cell inactivation as a function of depth will be determined, RBE values for both plateau and star regions will be determined, OER values will be determined, and also differential dose-rate-dependent cell inactivation will be investigated.

Results from these experiments will provide a portion of the essential information that must be obtained before clinical trials for treating human neoplasms by pion beams are begun.

Has this experimental system ever been employed by this investigator outside this laboratory?

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PROPOSAL INFORMATION

Beam Area: Area A or Radiobiology and Therapy Research Building.

Secondary Channel (if known): Unknown

Beam Requirements:

Type of Particle: negative pion

Momentum Range:
Momentum Bite:
Solid Angle:
Spot Size:
Emittance:
Intensity:
Beam Purity:
Target(s):

as defined by the pion therapy beam

Need definition of what ranges of these parameters will be studied —

Primary Beam Requirements: only those requirements imposed on the therapy beam.

Running Time Required:

Installation Time Required (no beam): 15 min. (includes time to remove apparatus at end of run)

Tune-up Beam (give intensity and hours needed): None (Incorrect if want to compare to therapy beam when running time-up will be necessary)

Data Runs: 30 hours Total Time (i.e., 30 min. occupancy, twice daily, total of 30 days. This estimate includes time for dosimetry)

Scheduling:

Realistic date when User will have the non-LAMPF apparatus ready: When therapy beam is on the air. — Expt Area A or on Board facility

Information that may affect scheduling: None

Major LAMPF Apparatus Required: None except standard dosimetric apparatus to be used for therapy.

Shielding and Enclosures Required: None

Special Services Required: Laboratory equipment with Coulter counter or microscope and temporary space for mouse cages. (digital, graphic display of [illegible] spectrum, or what?) #, size

Space Required: insignificant. (1 x 1 cm?)

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DETAILED STATEMENT OF THE EXPERIMENT

AIM

Before clinical work is undertaken, the distribution of π^- meson dose with depth in tissue, or a tissue-equivalent absorber, must be determined. Techniques must be used which are sensitive enough to distinguish between absorbed dose along the entrance path of the pion beam, and the anticipated higher absorbed dose in the star region. It is the latter region which must be regulated and expanded to encompass any neoplasm which is to be treated with this modality. Further, the therapist will need to know how the Relative Biological Effectiveness (RBE) and Oxygen Enhancement Ratio (OER) change as a function of depth dose within a patient, particularly near the junction between star production and the low LET region.
depth dose in depth

We are proposing experiments designed to provide information for depth dose distribution, to determine RBE values for plateau and star regions, and to determine OER values, for negative pions at the Los Alamos facility. Two established methods will be used to measure radiosensitivity in terms of reproductive capacity of spleen cells irradiated in vitro but cultured in vivo. Further, since the dose rate will change with field size and may also change in proportion to beam utilization upstream, we propose to investigate the differential effects of dose rate in both the plateau and star regions.

Two-dimensional [illegible] for the [illegible] of [illegible] measure dose-distribution [illegible] been [illegible]

METHOD OF STUDY

The classical method of Till and McCulloch (5) will be used to assay cell killing of splenic colony-forming cells; Makinodan's technique (6) of spleen cell transfer will be used to determine cell killing of secondary antibody-producing cells. Although these procedures will involve in vitro irradiation of cells, we feel that our method will yield quantitative dose-distribution measurements considerably more precise than could be obtained by irradiating spleens in situ.

Materials and Methods

Mice: Specific-pathogen-free CD_2F_1 (Balb/cXDBA$_2$)F_1 male mice, purchased from Cumberland View Farms, Clinton, Tennessee, will be used in all experiments. This strain is chosen because of its low level of natural hemolysins to sheep erythrocyte antigens (7). Mice will be kept in cages equipped with filter tops both before and after experimental manipulation. Only mice between 12 and 16 weeks of age will be used.

Antigen: Sheep erythrocytes from whole blood, obtained by venapuncture, will be available from a sheep maintained by our Animal Care Facility.

Colony-forming cells: The procedure of Till and McCulloch will be used (5). Nucleated spleen cells, irradiated in vitro in a manner described below, will be injected intravenously, at concentrations to be determined, into heavily irradiated syngeneic recipient mice. After 9 or 10 days recipient spleens will be removed, stained with Bouins' solution and gross spleen colonies will

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be enumerated either visually or from serial subsections examined microscopically according to Jenkins, et al. (8). Classifications of colonies into cell type, e.g. erythropoietic, granulocytic, etc., may be done but is not central to objectives of this work.

Antibody-forming cells: The spleen cell transfer technique of Makinodan (6) will be used for assaying radiosensitivity of antibody-forming cell precursors. Spleens from mice that received a presensitizing dose of sheep erythrocyte antigen four weeks earlier will be removed, cell suspensions will be made in medium-199, counted, adjusted to appropriate concentrations, and irradiated as described below. Appropriate concentrations of cells will be intravenously injected into heavily irradiated mice followed by injection of ½ ml of 2 percent erythrocyte suspension. After 6 days mice will be killed by exsanguination, individual blood samples will be collected, sera will be removed and stored at -20°C until antibody assay. Antibody activity will be determined by making two-fold dilutions of serum in saline using microtiter diluters; a standard concentration of sheep erythrocytes will be added, samples will be incubated for 4 hours at room temperature and read by macroscopic agglutination. The highest dilution of serum that exhibits detectable agglutination for each sample will be taken as the end point, defined as the antibody titer.

Irradiation

Cobalt-60 gamma: As a reference irradiation for RBE determinations and for irradiation of recipient mice, a 3,000-Curie Theratron-80 Cobalt-60 source will be used. Recipients will receive 1000 rads whole-body irradiation at 80 rads per minute. Spleen cells will be irradiated as described later.

Negative pions: Normal donor mice and heavily irradiated recipients will be transported to the LAMPF site; spleen cells will be exposed to pion beams. On the basis of recent estimates (3) we are assuming a pion dose rate of ~25 rads per minute.

Spleen cell irradiation: A lucite container with inside dimensions ~3 cm wide by ~2 cm deep by 30 cm long will be constructed with partitions of millipore material every 2 mm perpendicular to the long axis of the container. The container will be equipped with fittings so that either air, oxygen , or nitrogen can be bubbled through each chamber. A known concentration of spleen cells in medium-199 will be placed in each chamber immediately before irradiation; the long axis of the container will be placed parallel with the radiation. Immediately after irradiation spleen cells from each compartment will be withdrawn with a syringe and injected into the previously irradiated recipients. Small aliquots of cells will be taken to determine the fraction of viable cells by Eosin exclusion.

By partitioning the chamber, it will be possible to determine responses of cells as a function of beam depth (to within 2 mm) from the point of entrance throughout the plateau, star region, and beyond. Further, this will enable us to measure the OER as a function of depth, and to provide values for determining RBE also as a function of depth.

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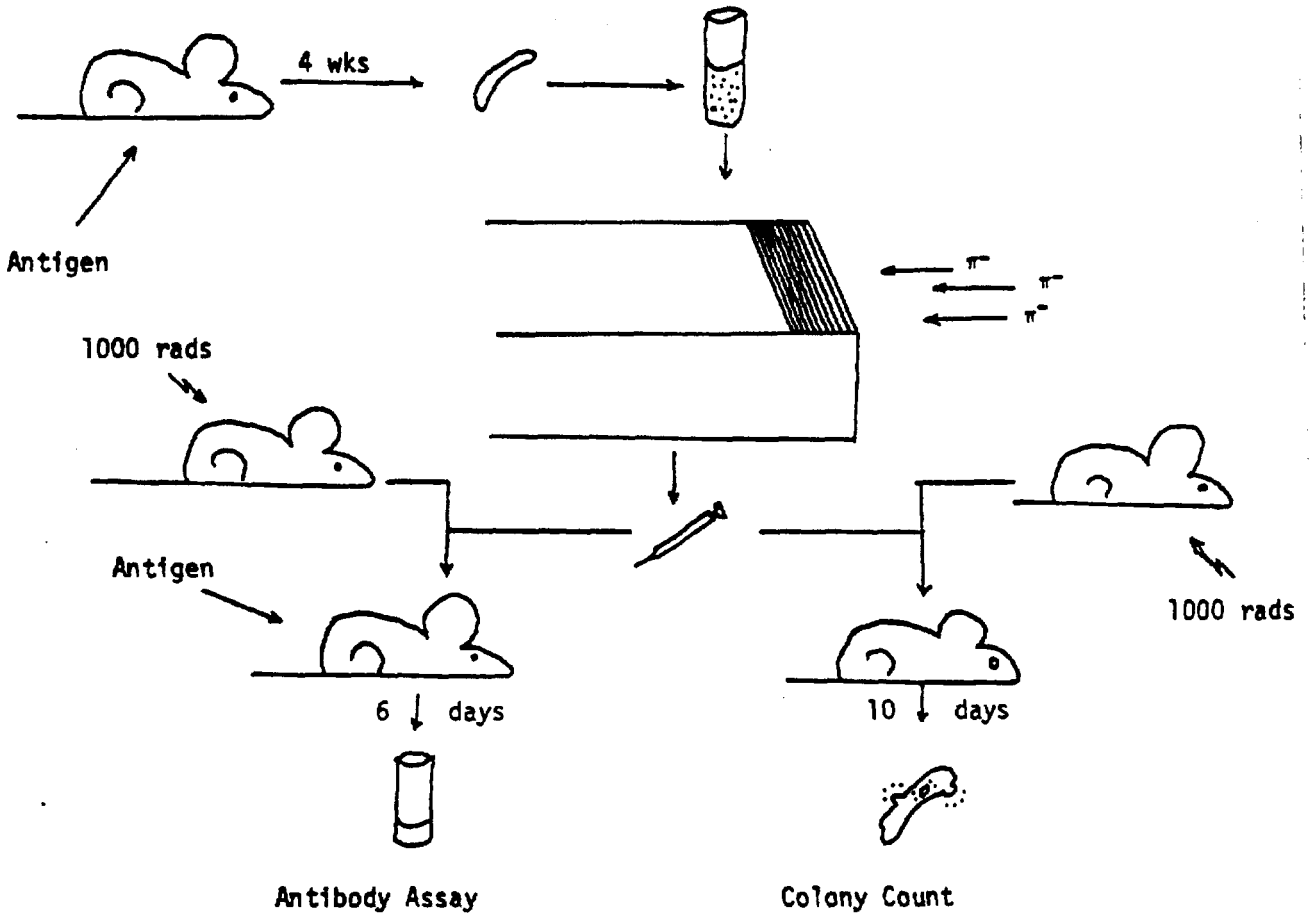

Experimental Protocol

Preliminary: For the antibody-forming-cell experiments a linear relationship between the spleen cell dose and antibody titers will be established for unirradiated cells. From this reference line a value for the effective number of viable cells in any irradiated cell population can be determined.

Likewise, a linear relationship between the number of spleen colonies and inoculum size for unirradiated colony-forming cells will be determined. This will become the reference function for determining fractional survival in each subsequent irradiation experiment.

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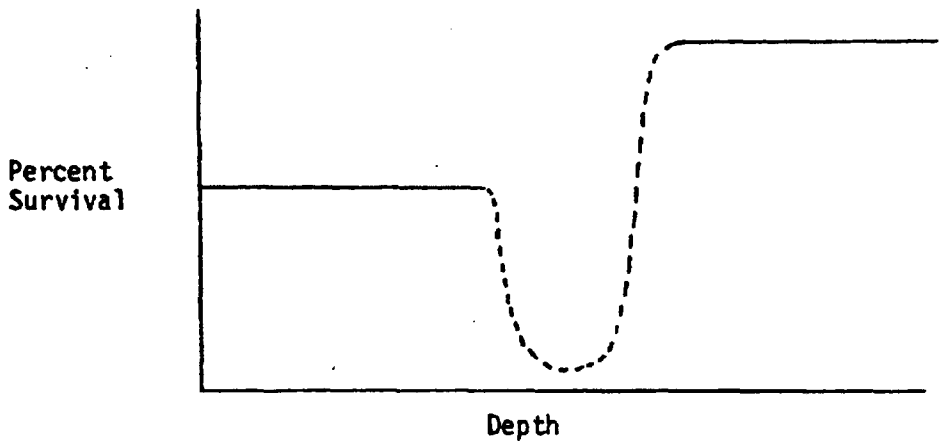
Experimental:

Gamma rays: Survival curves, where $\log_{10}$ of the surviving fraction of cells (S) is plotted against radiation dose, will be determined using gamma rays, for both antibody-forming cells and colony-forming cells irradiated in either 20 percent oxygen-80 percent nitrogen, 100 percent oxygen or 100 percent nitrogen environments. These curves will provide reference parameters for comparison with negative pion irradiation.

Negative pions: For π^- meson irradiation an RBE of ~2.5 will initially be assumed for the star region, with respect to Co-60 gamma rays. Spleen cells will be placed in the irradiation chamber, positioned with the long axis parallel to the pion beam, and exposed to doses expected to fall on the exponential portion of the survival curve. Appropriate concentrations of cells from each chamber will be injected into recipients as described before, and assayed as described. Cells will be irradiated in either oxygen, nitrogen, or air-equivalent environments as above. Thus, it will be possible to determine (1) dose distribution as a function of depth, (2) RBE for the plateau and star regions, (3) OER for both regions, and (4) dose-rate dependence as follows:

(1) Dose Distribution. By assaying cells from each compartment, i.e., each 2 mm increment of depth, fractional cell survival as a function of distance will be determined using reference functions previously constructed. With ~50 MeV pions, S values can be determined for cell compartments both at the proximal and distal edges of the star region. A typical experiment is expected to yield results such as are sketched below.



(2) RBE: From S values on the plateau and star regions for a particular pion rad dose, RBE will be determined as:

$$\frac{S(\text{gamma rays @ e.g. 200 rads})}{S(\text{pions @ e.g. 200 rads})} - \text{RBE}$$

Not an RBE but a ratio of survivals for same dose. RBE is ratio of std/test units for same biological effect.

Obviously, accurate RBE values must be derived from the exponential portion of the gamma-ray survival curve.

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(3) OER: Since dose distribution will not change as a function of oxygenation or anoxia, only chambers corresponding to the mid-plateau, mid-star regions and the plateau-star-region border will be filled with culture medium. Comparison of highly ozygenated and anoxic environments will reveal the maximum OER, thus, the OER will be calculated for the three zones as:

$$\frac{S(100\%\ N_2)}{S(100\%\ O_2} = OER$$

OK

? interface effects - may be present due to use of [illegible] filters & get unpredictable dose gradients invalidating results.

(4) Dose Rate: The question of differential dose-rate-dependent inactivation will be investigated by exposing cell suspensions to a broad range of dose rates as defined by the pion therapy beam. Using depth-dose information previously established we will determine dose-rate dependent inactivation for the same three depth regions as proposed for the OER determinations. We expect that dose rate dependence will be significant in the plateau region, but somewhat less differentially demonstrable at depths corresponding to star production.

Finally, we will determine the D_0 of colony formation and antibody response by constructing survival curves. Spleen cells in culture will be irradiated at dose increments from ~25 rads to an appropriate maximum dose. As previously described, responses will be expressed as $\log_{10} S$ as a function of rad dose for both oxygenated and anoxic cells.

SIGNIFICANCE OF RESEARCH

Work outlined in this proposal will yield important radiobiologic information on the effects of negative pion irradiation on two different cell populations within the same organ. Our data will permit evaluation of dose distribution, RBE and OER for pion beams in material approximating soft tissue. The assay systems have been established as valid measures of radiosensitivity, and are analogous to clinical therapy in that the end points measure the reproductive capacity of cells. Data from studies such as these must be available for necessary evaluation before clinical trials for human treatment are begun.

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RELEVENT WORK DONE BY OTHERS

Negative pi mesons (pions or π^- mesons) are negatively charged elementary particles, with a mass 273 times that of an electron, and a mean life of about 2×10^{-8} seconds. Originally predicted theoretically, charged mesons were first observed in 1947 in cosmic irradiation (1). Although negative pions can be produced in any nuclear interaction (providing the energy of the primary particle is sufficient), they are produced abundantly when an accelerated proton beam strikes a beryllium target.

Supported by calculations relating to the characteristics of energy distribution in tissue-equivalent material, Fowler and Perkins proposed the possibility of using negative pions for human cancer therapy (2). There is now general agreement that negative pions may provide an important method for treating human tumors, based on their physical properties. In tissue-equivalent material, π^- mesons with energies of $\sim$25 MeV or greater have a long mean-free path for nuclear collisions and almost always come to rest before undergoing nuclear interaction. Thus, as π^- mesons pass through tissue-equivalent medium, the dose increases slowly with depth, producing a Bragg peak near the end of the range. The π^- mesons at rest are then captured in an outer orbit of a heavy atom, e.g., carbon, nitrogen or oxygen in tissue, replacing an electron in that atom. Within 10^{-10} seconds the π^- meson cascades down from one orbit to the next, causing emission of low-energy X-rays, and finally comes to rest in the lowest orbit of the capturing atom. The wave functions of the π^- meson and nucleus overlap, and the negative pion is thus captured by the nucleus, which then disintegrates into smaller fragments consisting of short-range heavily ionizing fragments and neutrons. The burst of particulate energy formed by the exploding nucleus is termed star formation. Fowler has calculated that for a π^- meson (rest mass $\sim$ 140 MeV) captured by oxygen, about 40 MeV is expended in overcoming the binding energy of the nucleus, 70 MeV is carried off by neutrons and about 30 MeV appears in the form of protons, alpha particles and heavier ions (3). Particles with charge greater than 1 are mainly short-range, High-LET particles, and produce local high energy deposition in the immediate vicinity of capture. By adjusting the energy of the pions, this region of high LET, high RBE local radiation can be deposited at varying depths, and unique deposition of radiation within a tumor mass can be achieved, therefore, with concomitant sparing of surrounding tissue. Calculations by Fowler (3) and measurements by Richman, et al. (4) and Curtis and Raju (5) show that at the end of the π^- meson path length (the region of the star production about three times as much energy per unit path length is deposited as along the initial plateau.

In soft tissue, i.e., free of bone, the frequency of π^- meson capture by elements present is expected to be 73 percent in oxygen, 20 percent in carbon, and 3 percent in nitrogen, leaving about 4 percent captured in heavier elements (6). This distribution follows closely the relative abundance of these atoms in tissue.

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Negative pions may also be captured by hydrogen, forming neutral pi mesons which diffuse through the medium and either decay to two gamma rays (1) or become transferred to nuclei causing nuclear disintegration.

In an effort to determine important radiobiological parameters such as RBE and OER for π^- mesons in the plateau and star regions, several workers have used the Berkeley 184 inch syncyclotron. This facility is, to date, the most intense source of low energy π^- mesons; at peak operation 10^6 pions per second are produced, which, depending on the beam size, is from 5 to 30 rads per hour. Although the Berkeley pion beam is contaminated with 25 percent electrons and 10 percent negative mu mesons (expressed as percent of total particles), and the dose rate is quite low, work summarized below constitutes the bulk of our present knowledge of the effects of π^- mesons in biological systems.

Feola, et al. (7,8) measured the proliferative capacity of either two-day-old or five-day-old ascites tumor cells irradiated in vivo in mice. Tumor-bearing donor mice were killed after irradiation and tumor cells were injected into normal syngeneic recipients; mice were evaluated at 8 weeks for presence of tumors. In these experiments, using Co-60 gamma rays as a reference irradiation, an RBE value of 5.4 ± 8 was calculated for π^- mesons in the star region. They suggested that the high RBE was probably due to repair at the low gamma ray dose rate of 5 rad/hour. Throughout the plateau region, however, the RBE appeared to be very close to 1.

Loughman, et al. (9) examined tumor cells for polyploidy that had received from 50 to 351 rads, from mice of the preceding experimental series. For the plateau region an RBE of 1 was reported; in the star region the RBE was 2.15. The RBE for stars only, in the Bragg peak region was estimated to be 3.64, a suggested upper limit for polyploidy induction by π^- mesons.

Richman, et al., (10) reported a peak-to-plateau ratio of about 2.4 for production of abnormal anaphases in roots of the bean Vicia faba. Further, they found an oxygen enhancement ratio (OER) of 1.35 in the star region, which can be compared to a value approximating 2 for radiations such as Co-60 gamma rays at corresponding dose rates. Since the OER depends upon dose rate at room temperature, they repeated the experiments at 4°C, found an OER of 1.5, and suggested that this value may be closer to that expected from an acute π^- meson dose rate.

Raju and Richman (6) reported preliminary results using cultured human kidney T-1 cells. The RBE appeared to be near 2 with cells irradiated while frozen in liquid nitrogen. Burki, et al. have shown that frozen cells exhibit no dose-rate dependence (11).

Preliminary results of experiments measuring reverse mutations in Saccharomyces cerevisiae Z-34 strain of yeast were also reported by Raju and Richman (6). These studies indicated an RBE of 1.18, an OER of 1.5 to 1.9 for the system. Cobalt-60 gamma rays yielded an OER of 2.5.

These studies, while not definitively applicable to high dose-rate π^- meson work, do present guidelines for designing experiments for the Los Alamos

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meson Physics Facility. We expect to find RBE values approximating 2 in the star region, compared to Cobalt-60 gamma rays, and approximately 1 in the plateau. The effect of oxygen is expected to be no greater than 2, probably nearer to 1.5. These values are in keeping with theoretical calculations of Curtis (12).

Techniques to be used in the work proposed are straightforward and established. The technique for measuring the viability of irradiated hemopoietic stem cells was introduced by Till and McCulloch (13), who showed that the number of spleen colonies observed after 9 or 10 days in heavily irradiated mice is linear with the number of nucleated bone marrow cells injected. Thus, by assuming that the inverse relationship holds, i.e., that the number of colonies observed from a population of irradiated cells is a linear function of the number of viable colony-forming cells, they constructed x-ray survival curves that resembled typical cell culture curves. The D_0 for colony forming cells irradiated in vivo was 115 ± 8 rads using Co-60 gamma rays. Subsequently they reported a slightly lower D_0 value of 95 ± 9 rads for the colony forming ability of marrow cells irradiated in vitro (14). Histologic examination of spleen nodules revealed that they consisted of rapidly proliferating hematopoietic tissue (13).

Direct evidence that spleen colonies are clones, i.e., that each arises by proliferation from a single cell, was presented by Becker, et al. (15). They irradiated mice to a dose of 250 rads, transplanted 10^7 nucleated bone marrow cells, then exposed recipient mice to an additional 650 rads in two fractions. Based on the observations that hemopoietic repopulation in irradiated mice could be accomplished by few cells, and that cell clones originating from these few cells often had abnormal karyotypes, they reasoned that if a spleen colony develops from a single cell with abnormal karyotype then all cells of the colony would carry the same abnormality. If two or more cells produced a colony, the karyotypes of cells present were expected to be different since the probability of inducing identical chromosomal abnormalities in two or more cells is very small. By examining abnormal karyotypes of 70 or 100 cells each from 4 different colonies, they showed that 95 to 99 percent of the cells contained at least one marker characteristic of each colony. Subsequent confirmation of these results has been reported (16, 17).

On the basis of these results we will assume that spleen colonies observed result from proliferation of single transplanted cells that lodge in recipient spleens.

Siminovitch et al. (18) suggested a method for determining the fraction of injected colony-forming cells that lodge in individual spleens (f). They transplanted known numbers of nucleated marrow cells to heavily irradiated primary recipients, then removed spleens from a group of recipients after 2 hours and transplanted nucleated cells to a second group of irradiated mice. After 9 days the number of spleen colonies was determined in the secondary recipients, which provided an estimate for the number of colony forming cells present in the spleens of primary recipients. Since the number of cells initially injected was known, the fraction of colony forming cells residing in the primary recipients' spleens could be calculated. They reported a mean value of 0.17 for this fraction.

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Recently, Kretchmar and Conover (19) presented convincing evidence that the method of Siminovitch, et al. for estimating f is invalid since this value changes as much as three-fold depending upon the time after initial transplantation of marrow that the secondary transfer occurs. They suggested that fluctuations in f may be due to circulating of colony forming cells, i.e. that constant immigration and emigration of cells through the spleen occurs, thus, a particular f value reflects not only cells that may be lodged in the spleen but also those in transit at the time of spleen removal and subsequent transplantation. Thus, there appears to be no precise method for measuring values for f. We feel, however, that this difficulty should not eliminate our choide of method for determining effects of cell killing by π^- mesons. Values to be determined for S, the surviving fraction, will be expressed in terms of colonies observed as a function of the nucleated cell inoculum. We will assume a constant value for f since the maximum number of cells will be relatively small with respect to the normal nucleated cell content of the spleen (20). Thus, our values for D_0 may be inaccurate by a constant, f, which cannot be precisely determined; but values for RBE and OER will be accurate since these numbers are ratios, i.e., the effect of a constant will be divided out. Furthermore, the dose-depth distribution profile will be inaccurate only in vertical magnitude; the peak-to-plateau ratios will not be affected, nor will the delineation of the star region.

The technique for assaying antibody formation was introduced by Makinodan, et al. (21). For both primary and secondary responses, they demonstrated a linear relationship between the number of nucleated spleen cells injected into heavily irradiated recipients and the $\log_2$ antibody titer observed 5 or 6 days later. Further, this relationship was used to determine the radiosensitivity of both primary and secondary antibody formation from irradiated, transplanted spleen cells (22). They observed D_0 values of 70 R for both primary and secondary responses, an extrapolation number of 2.72, with 250 kVp x-rays. One of us has previously used this technique in assaying the effects of radiation exposure rate on antibody-forming spleen cells (23).

We propose to utilize spleen cells from presensitized donors for the following reasons. Recent work by Mitchell and Miller (24) and others (25,26) indicates that the primary antibody response involves activity of at least two different cell types, viz. thymus-dependent antigen-reactive cells, and antibody-forming-cell precursors derived from bone marrow. The relative proportions of these cells in the spleen are not equal (27), nor is the apparent radiosensitivity of each population equal (28). The secondary or anamnestic response, however, is triggered by interaction of antigen with long-lived memory cells, already differentiated or "primed" to begin rapid proliferation and further differentiation into mature antibody-forming cells. Secondary antibody responses for the antigen of choice are much stronger than for primary responses, i.e., antibody titers are greater by 60- to 100-fold (22). In addition, the variability of responses from presensitized spleen cells is significantly lower than that observed from primary responses (20).

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Although the localized hemolysin in gel technique of Jerne (29) with modifications of Wortis and Dresser (30) permits actual enumeration of splenic antibody-forming cells in irradiated spleen-cell recipients, we feel that the immense amount of time required to assay 200 or more mice at one time (the result of one typical π^- meson experiment) the additional equipment and manpower that would be required are not justified. Furthermore, by assaying humoral antibody activity the responses of all sites of immune cell localization and antibody production can be accounted for.

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PREVIOUS WORK ACCOMPLISHED RELEVANT TO THIS PROPOSAL

Work of the principal investigator has mainly been involved with effects of ionizing radiations on immune responses. The homograft response following bone marrow transplantation has been studied from two points of view. Lethally irradiated rats were injected with either syngeneic or allogeneic bone marrow from donors of different C/D antigenic phenotype. Since these antigens are agglutinable in saline, differential agglutination of circulating erythrocytes after Fe-59 uptake, and spectrophotometric hemoglobin determination enabled us to determine erythropoietic rates of both donor and host components in radiation chimeras (1).

Exposure-rate dependence of homograft suppression was studied in mice exposed to lethal gamma irradiation at rates between 4 R/min and 53 R/min. Irradiated mice that received either rat or foreign mouse bone marrow transfusions, were assayed according to 30-day mortality, presence or absence of donor-type erythrocytes in peripheral circulation, and presence or absence of foreign (rat) granulocytes in marrow and spleen by alkaline phosphatase staining. By these three measures of transplantability, we showed that marrow grafting was more successful after lethal irradiation at a high rate, i.e., 53 R/min, than the same total exposure administered at 4 R/min or 20 R/min (2,3). These data demonstrated that radiation at the high rate depressed the homograft response to a greater extent than did radiation at lower rates, therefore facilitating successful marrow transplantation in the high-rate experiments leading to an increased proportion of donor-type cells in circulation in hematopoietic tissues, and greater 30-day survival. Since differential immune suppression occurred at lethal radiation levels, we suggested that radiation mortality per se is not a measure of immune incompetence (4,5).

In view of the demonstrated exposure-rate dependence of homograft immunity, we studied the effects of exposure rate on humoral antibody formation to sheep erythrocyte antigens (6). Mice were exposed to gamma rays from 200 R to the 25 percent lethal exposures using rates from 8 R/min to 72 R/min. Exposure-rate-dependent antibody activity, observed over a 35-day test period, varied with the total radiation exposure, antigen dose, and the time differential between antigen injection and radiation exposure. Maximum exposure-rate-dependent antibody formation occurred when 700 R was given 12 hours after antigen administration; responses of mice exposed at 8 R/min were 64 times greater than those exposed at 40 R/min or 70 R/min (7).

Further, we compared the role of exposure rate for antibody depression to its role in differential 30-day mortality for the same mouse strain. Thirty-day mortality was interpreted as reflecting principally hemopoietic death since mice were free of Salmonella and Pseudomonas spp. and deaths occurred on or after the ninth post-irradiation day. Exposure rates between 4 R/min and 40 R/min were highly differentially suppressive to antibody formation, however, this same range of rates only slightly altered mortality. Mortality increased to a greater extent after exposure to rates above 40 R/min, while antibody formation was depressed only slightly more by exposure rates between 40 R/min and 100 R/min. On the basis of these data we suggested that cells responsible for post-irradiation recovery and survival were sensitive to different exposurerates from those cells involved in antibody formation (8).

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The possibility that interacting cells involved in the primary immune response to sheep erythrocytes may possess different radiation-rate sensitivites is currently under investigation. Preliminary results indicate that the antibody-forming-cell precursor compartment derived from bone marrow is highly exposure-rate dependent. This observation has led us to question whether different populations of cells from the bone marrow are affected differently by radiation rate. Preliminary studies have been designed to compare the exposure-rate sensitivity of bone-marrow-derived antibody-forming -cell precursors with bone-marrow=derived colony-forming cells. Our results to date suggest that these two activities are not equally exposure-rate dependent.

While previous and current work has not involved RBE and OER determinations, it has, however, involved a rather thorough understanding of techniques to be employed in achieving the objectives of research outlined in this proposal.

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PUBLICATIONS, PRINCIPAL INVESTIGATOR

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EXTENT OF PARTICIPATION

Experimental work per se will be carried out by D.E. Carslon, principal investigator, with his technical personnel. F.J. Bonte will be consulted regarding explicit experimental design and the subsequent evaluation of data. J.E. Dowdey will act as physical consultant to the project, will assist in design and fabrication of apparatus, and will be available to perform any necessary physical measurements.

FINANCIAL SUPPORT

Funds have been requested for this project from the American Cancer Society.

Backup funds are available.

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SPECIAL APPARATUS TO BE FABRICATED BY LAMPF

None

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IDENTIFICATION OF HAZARDS ASSOCIATED WITH THE EXPERIMENTAL APPARATUS AND PRECAUTIONS TO BE TAKEN:

None

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