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TO : Distribution

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712644

FROM : Stephany Wilson for N. M. Kligerman, M.D.

SUBJECT : Animal Control Studies to be Performed during Pion Beam Shutdown Period

SYMBOL : ADRT

The following is a summary of a meeting held on March 12, 1975, at the LAMPF conference room to discuss animal control studies to be performed with 250 Kvp x-rays during the current shutdown period. As Dr. Kligerman is out of town, I am sending these notes to you without his checking them, so that those of you who are responsible for the various experiments can complete your protocols. We will schedule a meeting in the next week or so, and at that time you can let me know if there are inaccuracies in these notes.

Attending the meeting were Dr. Kligerman; Drs. D. F. Petersen and Leo Gomez of the LASL Health Research Laboratory; Drs. R. E. Anderson, C. R. Key, Mario Kornfeld, W. C. Black, and W. E. Doughty of the UNM Pathology Department; and Stephany Wilson.

It was agreed that a series of x-ray control studies would be performed on the following sites: kidney, colon, brain, spinal cord, lung, heart, spleen, and skin. All control studies will be performed at the LAMPF Biomedical Facility, using the 300 Kvp unit. Dr. Gomez is to check with service representatives about modifying the unit to operate at the 250 Kvp level, so that comparisons can be made with studies already performed with 250 Kvp x-rays and so that back-up units can be used if necessary.

Dr. Gomez has also been assigned responsibility for scheduling the experiments, ordering animals, and making arrangements for short-term animal caging. Eddie Rivera has been assigned to the project full time by HRL as the animal technician. Individual caging after irradiation is desirable. Animals will be held at the Biomedical Facility for one week after irradiation and will then be transferred to Albuquerque for long-term holding at the UNM School of Medicine. The Pathology Department will make arrangements for animal caging in Albuquerque. Responsibility for arranging for transportation of the animals from Los Alamos to Albuquerque has not yet been assigned.

Each experiment will be conducted in two series, with half the animals irradiated at the various predetermined dose levels during the first series and the other half irradiated during the second series. Each group will be randomized separately to dose-level subgroups. Animals will be held in restrainers and and will not be anesthetized. Air supply through a tube should be sufficient in most cases. The possibility of giving the animals oxygen to avoid hypoxia during irradiation was discussed, but was rejected as long as a sufficient air supply can be maintained during the short period required for irradiation.

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For each organ site, a principal investigator was assigned to take responsibility for planning and executing the experiment, coordinating with the CBM and LASL persons who will be assisting him, ensuring that observation is carried out according to a predetermined schedule, involving visiting scientists who have expressed interest in participating in the experiment, and keeping the group informed of progress and results of his experiment. Each principal investigator is to develop a short protocol for his experiment and is to route it to the others for their review. A meeting will be scheduled in about a week to discuss the protocols and to make final arrangements. (Copies of the presentations made for the various organ sites at the time of the site visit for the preclinical grant are attached for your review, although in most cases they are no longer valid.)

All studies will be single-dose experiments. Fractionation studies will be performed later (at five fractions in five days), with doses to be determined based on the single-dose experiments. From the group of animals survi[redacted] at one week after irradiation, one will be randomly selected every two months for sacrifice and microscopic examination to detect early changes. In some experiments additional animals will be irradiated and subsequently sacrificed so that sufficient specimens can be obtained for both electron and light microscopic examination. If an animal dies during the two-months interval, he will be considered the sacrifice animal for that period. The remaining animals will be observed until death or until the end of two years when all surviving animals will be sacrificed for examination. The first day of irradiation will be considered Day 1 of the experiment, and schedules for observation and sacrifice will be calculated from that day.

Following is a list of persons assigned responsibility for each experiment:

Organ Site	Principal Investigator	Pathology	Radiotherapy	[redacted] Scientist
Kidney	Dr. Jordan	Dr. Jordan	Dr. Barnes	Dr. Phillips
Colon	Dr. Black	Dr. Black	Dr. Powell	
Brain	Dr. Kornfeld	Dr. Kornfeld	Dr. Kligerman	
Spinal Cord	Dr. Kligerman	Dr. Kornfeld	Dr. Kligerman	
Lung	Dr. Doughty	Dr. Doughty	Dr. Gomez	Dr. Phillips
Heart	Dr. Sternhagen	Dr. Key	Dr. Gomez	Dr. Stewart
Spleen	Dr. Andersen	Dr. Andersen	Dr. Kligerman	Dr. Carlson
Skin	Dr. Gomez	Dr. Gomez	Dr. Gomez	

A. Kidney (Jordan)

1. Treatment Schedule

Dose (rads)	2000	3000	4000	5000	6000
Number of animals	12	12	12	16	20

Animal System: Mice

Total Number Required: 72 (The BNM Pathology Department may have sufficient animals on hand for this experiment.)

The right kidney will be removed surgically, and the left kidney will be irradiated four weeks after surgery. Additional animals are to be irradiated in the 5000 and 6000 rad groups mainly to provide enough for a comparison of electron versus light microscopy. An attempt will be made with these animals to determine if changes can be picked up earlier with EM and, if so, how much earlier. The resulting data may provide supporting evidence of the need for an electron microscope for pathology studies associated with the pion clinical program.

The treatment cone will be approximately 1.5 cm., large enough to encompass the entire diameter of the kidney. An attempt will be made to displace as much of the intestinal tract as possible with the treatment cone, so the radiation field can be limited as closely as possible to the kidney alone. It was suggested that a few mice be fed barium and studies done to see how adequately the cone displaces the intestine.

2. Observation Schedule

To be determined.

3. Endpoints

a. Length of survival

b. Quality of survival

c. Histologic changes

d. Chemical changes (Dr. Kligerman is to check with Dr. Joseph Concannon to determine the most profitable chemical tests in his irradiations of dogs.)

B. Colon (Black)

1. Treatment Schedule

Dose (rads)	2000	3000	4000	5000	6000
Number of animals	12	12	12	16	20

Animal System: Rats

Total Number Required: 72

The animals will be irradiated using lateral ports, with the dose confined to the rectum. Dr. Kligerman suggests using barium on a few animals to determine the precise field. Dosimetry can be done with TLD dosimeters available through [redacted] (Dr. Robert Thomas has this equipment). Microscopic examination of serially sacrificed animals will be directed mainly toward detecting early changes in the muscularis and submucosa.

2. Observation Schedule

To be determined.

3. End Points

a. Perforation

b. Stricture

c. Ulceration

d. Diverticular formation

e. Any other complication leading to death

C. Brain (Kornfeld)

1. Treatment Schedule

Dose (rads)	1000	2000	3000	4000	5000	6000
Number of animals	20	20	24	28	10	10

Animal system: Rats

Total Number Required: 112

Whole brain irradiation will be delivered through lateral ports. A larger number of animals will be irradiated for this experiment because the particular techniques required for EM microscopy results in the brain tissue specimens unsuitable for light microscopy.

2. Observation Schedule

Animals will be observed every two hours for the first 24 hours. The observation schedule after that time is still to be determined.

3. End Points

a. Early acute death (expected for rats in 5000 and 6000 rad groups)

b. Ataxia with recovery

c. Diarrhea

d. Decreased food intake and/or weight loss

e. Motor alterations

D. Spinal Cord (Kligerman)

1. Treatment Schedule

Dose (rads)	1000	2000	3000	4000	5000	6000
Number of animals	20	20	24	28	10	10

Animal system: Rats

Total Number Required: 112

Irradiation will be delivered through a portal no less than 1.5 cm in diameter to the lower thoracic spinal cord (approximately T-12), excluding the esophagus from the treatment field. The treatment area will be depilitated, and the animal will be tattooed to mark the upper and lower boundaries of the treatment field.

2. Observation Schedule

To be determined. When an animal becomes paralyzed to the extent that he is unable to eat or becomes severely debilitated, he will be selected for serial sacrifice.

3. End Point

Paralysis.

E. Lung (Doughty)

1. Treatment Schedule

Dose (rads)	1000	2000	3000	4000	5000
Number of Animals	12	12	12	12	12

Animal System: Mice

Total Number Required: 60

The right lung will be irradiated, with the left lung serving as a control.

2. Observation Schedule

To be determined.

3. End Points

To be determined.

F. Heart (Sternhagen)

1. Treatment Schedule

Dose (rads)	2000	3000	4000	5000	6000
Number of Animals	12	12	12	16	20

Animal System: Rabbits

Total Number Required: 72

This experiment will be similar to the kidney experiment, but the doses will be delivered from the side. Extra animals at the high dose levels are for comparison of early changes detected by electron versus light microscopy. Rabbits are chosen as the test system for comparison with Stewart's earlier work. Dr. Stewart has expressed interest in working with us on this experiment.

2. Observation Schedule

To be determined.

3. End Points

a. EKG abnormalities

b. Interstitial fibrosis

c. Enzyme Studies

d. Others as appropriate

C. Spleen (Anderson)

This experiment will parallel earlier in vitro spleen studies by Anderson et al. for comparison of results using the machine at the Biomedical Facility with those obtained with the one at UNM. However, in vivo experiments will also be performed using mice. The number of mice required was not specified. Dr. Anderson will need to notify Dr. Comez soon of his animal requirements.

H. Skin (Comez)

Considerable work has already been done on skin by Dr. Raju. Dr. Comez will do a few additional mouse feet using the machine at the Biomedical Facility for comparison with Dr. Raju's results using the machine at HRL. Dr. Comez is to outline all specifications for this experiment.

DISTRIBUTION:

R. E. Anderson
J. E. Barnes
W. C. Black
W. E. Doughty
L. Comez ✓
S. Jordan
C. R. Key
N. M. Kligerman
M. Kornfeld
D. F. Petersen
C. J. Sternhagen