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25 March 1960

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Dr. A. C. Andersen
School of Veterinary Medicine
University of California
Davis, California

Dear Bud:

As you requested in your letter of 11 March I have gone over the draft of the experimental design which you sent, and find that it is improving with every reissue.

The suggestions which I would now make are mainly in the nature of cleanup items, or are designed to improve the coverage of your document so that it can provide most of the answers to questions about the reasons for the design, without reference to associated files of correspondence.

Don't hesitate to call on me if you think I can be of further help in completing the record of your experimental design.

With every good wish.

Cordially yours,

ORIGINAL SIGNED BY
ROBLEY D. EVANS

Robley D. Evans
Professor of Physics

RDE:mms
enc.
cc: H.D.Bruner
A. M.Brues
W.H.Langham
H.A.Blair

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EXPERIMENTAL DESIGN FOR THE STRONTIUM^{90} AND RADIUM^{226} PROJECT

School of Veterinary Medicine
University of California at Davis
California

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HISTORY

In May, 1955, the USAEC, Division of Biology and Medicine, requested that the existing kennel facilities for Project No. 4 be considered as a possible site for a study on the effect of Sr^{90} in dogs. On July 16, 1955, the following proposals were suggested:

1. that dogs be treated elsewhere and returned to this kennel for life-span studies, or,

2. that the kennel be expanded to provide both treating and holding facilities to accommodate 70 more dogs.

It was requested that this proposal be revised to include an experiment involving five different dose levels of Sr^{90} in 125 dogs, or if possible, 250 dogs. For an undertaking of such magnitude, the cooperation of several laboratories was sought, and a second proposal was submitted, on November 11, 1956. The building should be large enough to accommodate 50 dogs (10/dose level) under treatment until a quota of 250 treated dogs would be reached. Of this number, 128 would be sacrificed at different ages and distributed to various laboratories for cooperative studies; the remaining 122 dogs would be kept at the Project 4 kennel for life-span studies. The following laboratories agreed to participate in this experiment: several departments in the University of California School of Veterinary Medicine at Davis and Donner Laboratory at Berkeley; Radiobiology Laboratory, University of Utah, Argonne National Laboratory, (Biology and Medicine), also Mass. Inst. of Tech. (on design, not on actual feeding study) and AEC Laboratory, Washington, D. C. After a series of meetings and discussions, it was unanimously agreed by those attending that a project site with laboratory facilities would be necessary to conduct the proposed experiment properly. A 4-acre site, Plot B-4, Armstrong Ranch, University of California, Davis, was selected, and the Dean of the Veterinary School assigned this area to the experiment. University approval was granted, and a third proposal for the enlarged program was submitted on February 24, 1956. This proposal was approved, and operating funds were received on July 1, 1956. The program was designated Project No. 6 and entitled "The Effects of Sr^{90} Administed during the Growth Period of the Dog." Personnel were recruited and preliminary construction plans were drawn up. Actual working drawings were started after January 1, 1957, when the University received construction funds. While plans for construction were in progress, the program was again reviewed, in June, 1957, and it was requested that

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both the size and scope of the experiment be expanded. It was requested that facilities be constructed for treating approximately 250 dogs and maintaining 700 to 800 dogs in outside holding pens. The number of treatments included: (1) six Sr^{90} ingestion levels, (2) one injected Sr^{90} level, and (3) five injected Ra^{226} levels. This program was agreed upon, and the project site was increased from 4.0 to 5.7 acres. Available construction funds were used to complete the first treatment building and additional facilities. A metabolic-type treatment building for 60 dogs was completed in December, 1958. During this period, pilot experiments were conducted on continuous Sr^{90} feeding of dogs and on selection of the highest designated dose level (1.83 μc Sr^{90}/gm Ca in feed). The results of this experience were reviewed in June, 1959. This meeting served to assimilate and integrate all previous suggestions and experience to formulate a long-term experiment involving the ingestion and injection of Sr^{90} and the injection of Ra^{226}.

The following persons participated in the development of this experimental design:

<u>Board of Consultants</u> - Dr. R. D. Evans, Dr. A. M. Brues, Dr. W. H. Langham, Dr. L. W. Tuttle, and Dr. H. A. Blair.

<u>U.S. Atomic Energy Commission</u> - Dr. H. E. Bruner, Mr. D. M. Gardiner, Dr. W. Claus, Dr. C. Dunham, and Dr. C. Dunham, and Dr. F. Pearson.

<u>Defense Atomic Support Agency</u> - Capt. J. Barton, Dr. C. L. Hansen, and Dr. J. Hilsken.

<u>U.S. National Laboratories</u> - Dr. M. Finkel, Dr. H. Foreman, Dr. J. H. Marshall, Dr. W. Norris, Dr. R. E. Rowlands, and Dr. M. A. VanDilla.

<u>Radiobiology Laboratory, University of Utah</u> - Dr. W. S. S. Jee, Dr. T. Dougherty, Dr. C. W. Mays, Dr. B. J. Stover, Dr. C. Rohfeld, and Dr. J. Arnold.

<u>University of California at Davis</u> - Dr. A. C. Anderson, Dr. M. Goldman, Dr. F. Shults, Dr. J. R. Luick, Dr. H. R. Parker, and Dr. D. K. Jasper.

1. <u>PROJECT NO 6 TITLE</u>

The former title, "The Effects of Sr^{90} Administered During the Growth Period of the Dog," will be changed to:

"THE EFFECTS OF CONTINUAL SR^{90} INGESTION DURING THE GROWTH PERIOD OF THE BEAGLE, AND ITS RELATION TO RA^{226} TOXICITY."

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2. FUNDAMENTAL CONCEPT OF THE EXPERIMENT

The purpose of the experiment is evaluation of the relative toxicity of Sr^{90} and Ra^{226} in beagles. The Sr^{90} aspect of the program is based on continuous ingestion of Sr^{90} from before the onset of fetal ossification (21 days after mating) until early adulthood (18 months of age). The relative toxicity of Sr^{90} ingestion in humans may then be estimated by the following approximate relationship:

$$\frac{\text{Human } Sr^{90} \text{ and continuous exposure}}{\text{Human } Ra^{\ }\text{, brief exposure}^{*}} \approx \frac{\text{Beagle } Sr^{90} \text{ and continuous exposure}}{\text{Beagle } Ra^{\ }\text{, brief exposure}^{*}}$$

$$\frac{X}{A} \approx \frac{B}{C}$$

$$X \approx A\left(\frac{B}{C}\right) \qquad X \simeq A\,(B/C)$$

The Davis Project will provide estimates for B and C. Estimates of A will be derived from the results of other investigators.

3. SELECTION OF BEAGLES AS THE EXPERIMENTAL ANIMAL

Practical experience in the care and management of a large beagle colony has been obtained over the past nine years. The physical characteristics of the beagle provide a reasonable basis for estimating radiation effects. Their body size, bone structure, metabolism, pathology, and life span are sufficient from an irradiation standpoint to permit a more accurate extrapolation to humans than would be possible from smaller experimental animals.

4. NUMBER AND SEX OF BEAGLES PER DOSE LEVEL

this is a definitional "label" not group"

A. Definition of terms:

1. "Dose Level" - amount of a designated treatment.

2. "Group" - the number of dogs per dose level. Thirty dogs will constitute one group receiving a designated treatment, and the major control groups will consist of sixty dogs. In addition, each group will contain dogs for sacrifice at approximately 2, 6, 12, and 18 months of age with an additional sacrifice age yet to be determined. Thus, each experimental group will consist of 35 dogs or more.

see redefinition on attached page 3a.

on p.4 is called "consecutive order" by birthdate also "dog number" within a dose level

*Human cases of internally deposited radium are due mostly to brief exposures on the order of 3 years, with administration either by repeated intravenous injection or by roughly continuous oral ingestion. To imitate such exposures, the "brief exposure" in dogs is to be a series of 8 injections over a period of 4 months (approx. 3 human years) from age 14 months to 18 months. The radium dogs are to be caged from birth in the same manner as the Sr^{90} dogs.

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insert for page 3

2. "Group" connotes a litter of pups entering the experimental colony simultaneously. At SLC a "group" or litter is divided among a series of dose levels, thus M03S2, M03S3, and M03S4 constitute a "group" and were injected on the same day but at different dose levels (S2, S3, and S4). At Davis all pups of a given litter are necessarily at the <u>same</u> dose level. Thus a "group" of Davis pups might be S4F03, S4F04, S4M05, S4M06. A "group" at SLC has the same "consecutive order" or "dog number" (03 in the example used here), but a "group" of pups entering the Davis experimental colony has consecutive "dog numbers" (03, 04, 05, 06 in the example). "Group" has an explicit technical meaning in the SLC design, but in the Davis design "group" is synonymous with "litter".

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3. "Series" - refers to experimental ~~procedure~~ treatment; i.e., isotope and route of administration.

B. Sacrifice dogs will be selected from a given dose level in such a way as to adjust the sex ratio of the remaining experimental dogs as close to unity as possible.

C. Genetic differences between dose levels will be minimized, but differences within dose levels should be maximized. This will be accomplished as follows:

1. Purchase of healthy purebred beagles representing different family lines.

2. A male will breed only one (1) dam per dose level.

3. Parental sisters will be distributed between different dose levels.

4. A dam will whelp her first and only litter of experimental dogs after reaching 14 months of age.

D. None of the experimental dogs will be bred; control and treated dogs are to be an F_1 generation and remain virgins throughout life.

5. IDENTIFICATION OF EXPERIMENTAL DOGS

A. At birth, each experimental dog, dead or alive, will be assigned a five-digit number. Nursing pups will be identified by hair slip until weaning. At weaning, the identification number will be tattooed in the ears as follows:

1. Left ear (treatment series and dose level):

1st digit - series - letter D = ingested strontium
S = injected strontium
R = injected radium
X = other

2nd digit - dose level 0 = control level
0.3 = level 0.3
1 = level 1
2 = level 2
3 = level 3
4 = level 4
5 = level 5

ie "control" is a "level" not a group

2. Right ear (sex and consecutive order by birthdate):

3rd digit - M = male
F = female

4th and 5th digit - dog number (consecutive order) within a dose level

these "consecutive order" numbers are the "groups" at SLC. The SLC concept of "group" is not needed at Davis.

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NOTE: the code sequence S4F0 also shows that this is a female dog. The animal at Vaitian's City which received the same treatment has the code sequence F0354.

3. **Example:**

not: "female number 3" but "dog number 3"

insert

Left ear - S4 (treatment; dose level)
Right ear - F03 (sex; dog no), or,
S4F03 = injected four-level strontium, female, dog number 3

comma

B. Each row of cages will be assigned a specified dose level. Dogs within a cage row will be rotated to minimize inter-cage environmental variables.

C. Experimental series and levels groups will be randomized in outdoor pens; pen location will not identify a given treatment.

"Group" was not used as defined in page 3, changed to "series and levels"

6. FACTORS RELATED TO TREATMENT

A. Sr^{90} and Ra^{226} effects, and their ratio (B/C of section 2 above), are anticipated to be dependent on both total dose and dose rate.

B. Retention will be estimated by whole-body counting with a bremsstrahlung detector calibrated with standards and phantoms. Whole-body counts will be conducted at periodic intervals throughout the life of each dog. Retention will be further quantified by β-ray assay of sacrificed dogs.

insert

C. A constant Sr^{90}/Ca^{40} ratio will be maintained in each level throughout gestation and lactation until the dogs are 18 months old. Factors related to calcium metabolism (quantity and quality) will be maintained reasonably constant throughout the experiment, and all dogs will receive the same stable Sr^{88}/Ca^{40} diet.

D. The experiment will proceed with the ingestion series, followed by the injection series as facilities become available. The existing animal holding quarters, containing five rows of metabolism cages, will be initially used for dose levels 0.3, 2, 3, 4, and 5. The expanded area of this building will handle levels 0 through 5 simultaneously, with controls placed at two different locations.

E. Methods to keep errors to a minimum will include use of trained personnel properly supervised; identification of all utensils, facilities, data sheets by code (marker and color) and maintenance of an error log as well as a record of all experimental procedures.

F. The guiding philosophy of the program will regard each dog as an essential link in the study of radiation-induced sequellae. Prophylactic, therapeutic, and ancillary studies will be

Retention in the 0.3-level Sr^{90} dogs will probably be too small to permit bremsstrahlung measurements, and will be estimated by β-ray counting on specimens from an enlarged number of 10 serial sacrifices.

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performed to ensure the clearest possible understanding of treatment effects. The recognition of pathological syndromes includes appropriate clinical, enzoclinical, and autopsy procedures. In the event of terminal illness, a dog will be sacrificed shortly before death.

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7. **EXPERIMENTAL DOSE LEVELS**

I. **INGESTION OF Sr^{90} SERIES** (Dose selected to give ~~same~~ cumulative rad dose at age 2.5 years which equals that in Salt Lake City dogs 2.5 years post injection; eg 8100 rad for 5-level).

Levels	No. Dogs ±	Sacrifice	μc Sr^{90}/gm Ca in Diet	Estimated Body Burden* (18 mos; μc Sr^{90}/kg, 2 days after last dose)
5	30	5	1.500	18
4	30	5	0.500	6
3	30	5	0.167	2
2	30	5	0.0556	0.67
1	30	5	0.00926	0.11
0.3	30	10	0.00154	0.02
0	60	5	0.00000	0.00
	240	40		Subtotal 280

II. **INJECTION OF Sr^{90} SERIES** (Single injection at 18 mos. of age to correlate with Salt Lake City 4-level single-injection Sr^{90} dogs)

4 ~~5~~ 20 5 33 mc/kg 9.0 mc/kg (80 days post inj.) 4

Subtotal 25

III. **INJECTION OF Ra^{226} SERIES** (8 semi-monthly injections starting after 14 months of age and finishing at 18 months of age)

(Dose selected to give same total Ra/kg as for a single injection at same level at Salt Lake City; eg. total of 10 μg/kg for 5-level)

Levels	No. Dogs**	Sacrifice	Dose/Injection μc Ra^{226}/kg	Estimated Body Burden (μc Ra^{226}/kg, 30 days after last injection)
5	30	5	1.25	2.5
4	30	5	0.42	0.8
3	30	5	0.14	0.28
2	30	5	0.047	0.09
1	30	5	0.008	0.015
0	60	5	0.000	0.00
	210	30		Subtotal 240
				Total 545

*Based on Sr-Ca discrimination factor of 1.0, 10 kg dog, 12 gm skeletal Ca/kg.

**If at all possible.

± Number alive at say 18 months, ie not including stillbirths.

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