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Fourth Annual

PROGRESS REPORT

September, 1961

A. E. C. Project No. 6

School of Veterinary Medicine

University of California

Davis, California

Operated for the Atomic Energy Commission

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PREFACE

Activities of this project have increased severalfold during the past year, and the pace set promises full operating capacity by early 1963. Building construction has continued, and a final preliminary plan for project construction was completed early this year. In September, the Veterinary Clinic building was finished, which provided space for academic personnel to locate on the project site. Construction of the Office and Laboratory started in January, and completion is scheduled for August. All experimental treatment facilities are scheduled for completion by early 1963; these facilities will accommodate approximately one-half of the specified number of dogs for this experiment to be dosed simultaneously.

While construction has continued and will continue over the next few months, a project staff has been organized. A threefold increase in personnel during the past year has been organized into sections: administration, radiobiology, veterinary, and pathology. Section leaders have assumed certain responsibilities toward the experimental design and project operation. The sections have been unified as a working team with the aid of weekly staff meetings.

In December, 1960, the continuous Sr-90 feeding program started with the 0.3 level, and higher levels were added later. Our present Sr-90 feeding facility for 60 dogs will be fully occupied at five dose levels by late summer. Experiences gained thus far give assurance that the large-scale treatment of dogs on all dose levels of both Sr-90 and Ra-226 will progress without undue delay. Experimental dams are now maturing to meet these demands for an enlarged scale of operation as facilities become available.

Since projects 4 and 6 will next year be placed under the SAN Financial Plan, a fiscal year (FY), which is in keeping with the University, is being established in addition to the calender (CY) that has been maintained. Thus, project administration is expected to become less cumbersome and more efficient.

The conscientious and cooperative efforts of the entire project staff to "get the job done" has been most gratifying. Furthermore, the reasonable

assurance that all building construction will be completed within the near future has greatly enhanced staff morale. Also, improved liaison with the staff of project No. 4 has greatly aided in reducing the problems inherent in the rapid growth of this program. These prerequisites, together with the material presented in this report, give promise that scientific achievements at this project should be worthwhile.

A. C. Andersen

PROJECT CONSTRUCTION AND FACILITIES

C. T. Potts and A. C. Andersen

Construction plans and completion stages to coincide with experimental requirements were partially described in past annual reports. A priority schedule was necessary to avoid undue delay in the experiment. Also, certain facilities required special designing and testing before they could be put into operation. Consequently, special equipment and structures have been constructed and have been in operation since 1958, while final plans for over-all project construction awaited allocations and engineering approval. This situation has proven to be both advantageous and disadvantageous. Disadvantages were a slight delay in the experiment and crowded working conditions. The advantages largely stem from a trial-and-error period for special facilities to be tested prior to completing the enlarged scale plan (master plan). For example, 60 specially designed metabolic-type cages were constructed in 1958. These units have been tested (1), and enough experience has been gained to proceed with a proven design for some 200 additional cage units. Conducting the actual experiment on a small scale has revealed several faults, which have been corrected in recent plans. Construction plans are now completed with all facilities scheduled for occupancy by early 1963. These plans and operation of project facilities will be discussed briefly.

I. Construction

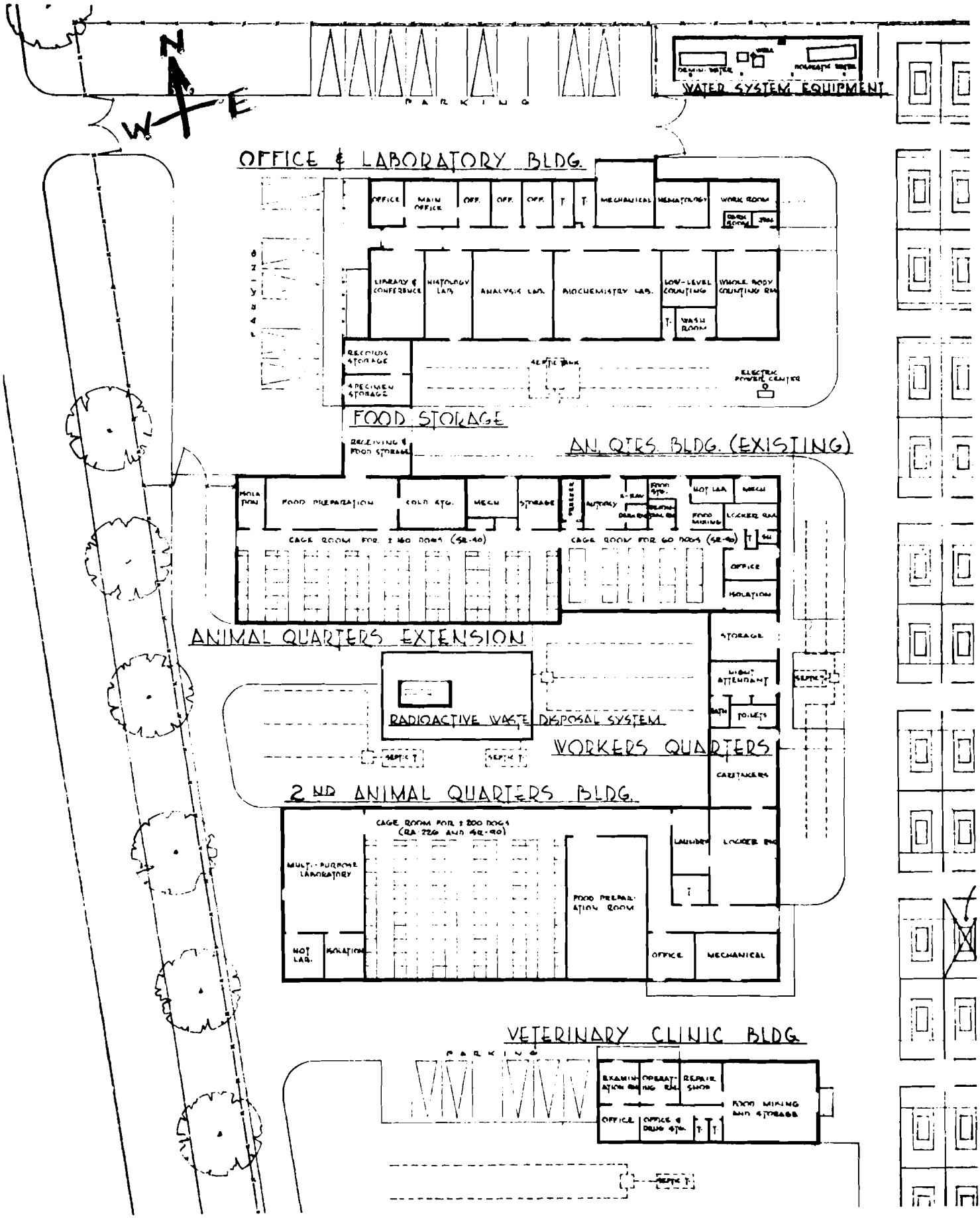
About three-fourths of the 5.7 acres assigned to this project will be occupied by outside holding pens, described in a previous report (2).

Roadways, parking areas, project lighting and a storm drainage system have been provided. A communication system will be installed to outside pens.

The over-all plan of project buildings is shown in Figure 1. The four main building units, from north to south, are: (a) Office and Laboratory--Radiobiology Laboratory, (b) Sr^{90} Ingestion Building--AH qtrs #1, (c) Sr^{90} and Ra^{226} Injection Building--AH qtrs #2, and (d) the Veterinary Clinic.

The 6,862-sq.-ft. Laboratory building, started in January, is to be completed in August, 1961. The building is designed to accommodate six academic and 10-12 nonacademic personnel.

Radioisotope administration will be conducted in two animal-holding-quarters buildings. AHQ#1 has been designed for continuous Sr^{90} feeding and the existing portion now provides satisfactory facilities for maintaining 60 dogs. Before dose levels 0.3, 2.0, 3.0, 4.0, and 5.0 could be fed, the following modifications were necessary: (a) air-conditioning units relocated and improved, (b) a quick-freeze room installed, (c) provision made for clothes storage, and (d) the isolation room converted into a chemical laboratory with a fume hood. Following these changes, the experiment was started in December 1960. AHQ#1 will be expanded to 180 dog capacity. Working drawings for AHQ#2 (radium injection) are now being completed. Both AHQ#1 and 2 are adjacent to and drain into the radioactive waste disposal unit. On the east side, both buildings will be connected by quarters for the animal caretakers and a night watchman. A receiving and storage area connects AHQ#1 with the Laboratory building. Thus, radioactive areas on the project are secured by either buildings or fences, yet these areas are accessible.


N
W
E
PARKING
WATER SYSTEM EQUIPMENT
OFFICE & LABORATORY BLDG.
OFFICE
MAIN OFFICE
OFF.
OFF.
OFF.
T
T
MECHANICAL
HEMATOLOGY
WORK ROOM
LIBRARY & CONFERENCE
HISTOLOGY LAB.
ANALYSIS LAB.
BIOCHEMISTRY LAB.
LOW-LEVEL COUNTING
WHOLE BODY COUNTING RM.
T
WASH ROOM
RECORDS STORAGE
SPECIMEN STORAGE
SEPTIC TANK
ELECTRIC POWER CENTER
FOOD STORAGE
RECEIVING & FOOD STORAGE
AN. QTRS. BLDG. (EXISTING)
ISOLATION
FOOD PREPARATION
COLD STG.
MECH
STORAGE
FREEZER
AUTOCLAVE
X-RAY
FOOD STG.
HOT LAB
MECH
FOOD MIXING
LOCKER RM.
CAGE ROOM FOR ± 160 DOGS (SR-40)
CAGE ROOM FOR 60 DOGS (SR-40)
T
SH
OFFICE
ISOLATION
ANIMAL QUARTERS EXTENSION
STORAGE
NIGHT ATTENDANT
SEPTIC T.
BATH
TOILETS
RADIOACTIVE WASTE DISPOSAL SYSTEM
WORKERS QUARTERS
SEPTIC T.
SEPTIC T.
CARETAKERS
2ND ANIMAL QUARTERS BLDG.
CAGE ROOM FOR ± 200 DOGS (RA-226 AND SR-90)
MULTI-PURPOSE LABORATORY
LAUNDRY
LOCKER RM.
T
FOOD PREPARATION ROOM
HOT LAB.
ISOLATION
OFFICE
MECHANICAL
VETERINARY CLINIC BLDG.
PARKING
EXAMINATION RM.
OPERATING RM.
REPAIR SHOP
FOOD MIXING AND STORAGE
OFFICE
OFFICE & DRUG STG.
T
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SEPTIC T.

Figure 1

The Veterinary Clinic building, south of the AH quarters, was completed in September 1960. Pending completion of the Laboratory, this building has been occupied by animal caretakers, professional staff, technicians, and a typist. The building was designed for veterinarians, animal caretakers, and food storage. An X-ray fluorescent spectrometer is located in the clinic shop. The veterinary portion (one-half) of this building includes an office, examination and surgical rooms, and drug storage. This building will be the examination center for dogs in outside pens.

The entire building area for this project totals 31,090 sq. ft., of which 13,000 sq. ft. will be completed by August 1961. Remaining construction (18,090 sq. ft.) is scheduled for completion by early 1963.

II. Project Facilities

New buildings, equipment, and facilities frequently require modifications for safe and efficient operation. Other modifications may include changes brought about by special experimental requirements and demands. These responsibilities as well as that of equipment maintenance are carried out by the Project Engineer. Complete plans of past and new construction, together with modifications or alterations, are kept on file. Major project facilities and recent alterations will be briefly described.

A. Project Water Supply and Drainage

Water is supplied through an 8-inch cased well (gravel packed) and a 5-hp turbine pump. The 2-inch discharge yields 60 g.p.m. at 60 p.s.i. Water is stored in a 1,000-gallon galvanized pressure tank that has a 2-inch distribution main to the demineralized water system, project buildings, and outside dog pens.

(1) Demineralized Water System:

From the well storage tank, a Barnstead F2 four-bed anion and cation exchange unit is installed. Ion-exchange resins are regenerated with concentrated NaOH and HCl. After each weekly regeneration, approximately 1,400 gallons of water are demineralized with a resistance in excess of 50,000 ohms. The demineralized water is stored in a 1,000 gallon galvanized tank that does not exceed 50 p.s.i., the optimum working pressure for the demineralizer. A 2-inch copper line supplies demineralized water to the laboratory building, animal holding quarters, and radioactive-disposal unit.

(2) Domestic Water System:

Domestic water is supplied to all buildings and outside dog pens. The main 2-inch water line is protected from pollution by a vacuum breaker in case of water shutdown or a break in the line. Buildings are supplied with a 2-inch main and valve from which water is divided into two systems--domestic and utility. These systems further provide added protection against possible water pollution. Each row of outside pens is supplied with shut-off and drainage valves.

(3) Recent Improvements in Water Supply:

(a) A shed-type building replaced a temporary weather protection one. This building also provides needed space for a small workshop and storage for volatiles (paint, solvents, etc.).

(b) An emergency pressure valve was installed on the domestic water tank.

(c) The demineralizer was modified to increase operating efficiency and water pressure necessary for cage cleaning.

(d) Main on-and-off valves to the dog pens were protected by commercial concrete-steel boxes.

(e) Water supply to each pen was modified to ensure weather protection of the self-watering devices.

(4) Future Water Demands:

Our present water supply is adequate for future buildings except for the fire-protection system. This system requires an emergency output of 1,300 g.p.m., which can be obtained only from the general campus water supply. Permission has been granted to install a 12-inch main from the deep campus well, approximately $1\frac{1}{4}$ miles from the project site. A fire-protection system is being installed in future project buildings. Water to supply this system is not expected until 1963.

In the meantime, it will be necessary to increase our existing water supply for the sprinkler system in the outside dog pens. Sprinklers are used to provide cooling by evaporation, for the dogs on excessively hot days. These sprinklers require water in excess of 100 g.p.m., so a 10-hp submersible water pump is being installed to double the present water supply.

(5) Drainage:

The project area (5.7 acres) requires adequate provision for storm drainage and as effluent from septic tanks. A large dry

well was dug, seven feet deep, ten feet wide, and sixty feet long. Large rock (2"-4") was placed in the bottom of this pit, small (1"-2") rock next, then building paper, and finally an 18-inch layer of soil. Underground tile pipe carries excess water from the various points of the project to this dry pit.

The sewage water from the office and laboratory building and X-ray room in AHQ#1 had originally been intended to drain into a leaching field. Since this area was needed as a paved area--three 30-ft. dry wells were dug and a pipeline installed to carry the septic effluent into dry wells. A small drain field was provided for the demineralizer effluent.

B. Quick-Freeze Room

An existing storage room was remodeled into a walk-in quick-freezer for packaged radioactive food. The five-ton water-cooled compressor selected is capable of holding the box to -20° C. with an additional load of 19,000 B.T.U. per hour. The inside of the freezer was designed to hold two carts with 17 trays each. By circulating the air over the food laden trays, maximum heat exchange is effected in a minimum of time.

At present the freezer is also used for storage of the frozen food. Upon completion of the extension of AHQ#1, a holding box will store the food. The freezer will then be used only for quick-freezing.

C. Heating and Ventilation

Air conditioning is accomplished in AHQ#1 building by: 2 one-ton, 1 three-ton, and 1 five-ton units mounted on the roof and air cooled. Efficiency of the three- and five-ton units was increased by moving them to

ground level and installing a water-cooled condenser. The five-ton air conditioner is operated by an inside thermostat, and the three-ton unit is activated when the outside temperature reaches 85 degrees. Two fans connected to the air conditioning and the two natural-gas heaters circulate air into the building.

The original filter system was not adequate for this building since frequent dust problems existed, so washable-type prefilters were installed to decrease dust entering the ventilation system. Washable dust filters have also been installed in the exhaust duct system.

A single unit, thermostatically controlled, heats and cools the Veterinary Clinic. This unit can recirculate air, or any amount of outside air can be introduced through a manually operated vent. Air is passed through washable filters. The compressor and condenser are air cooled. The heater is natural gas.

The Radioactive Disposal building is equipped with a large evaporative cooler (8 ft^3) that also serves to remove filtered off gases.

D. Radioactive Disposal Unit

To utilize the maximum volume of the underground settling tanks, a six-foot weir-type wall was installed in the influent section of the Imhoff tank. Sewage is lifted over the weir by a heavy-duty, non-clog submersible pump. The pump is equipped with an automatic float level which activates it at 140 gallon cycles.

For the remaining tanks, diaphragm-type transfer pumps with short strokes are designed to pump up to 60 p.s.i. Each of the 4 pumps operate at a different output, ranging from 85 to 300 g.p.m. A fifth pump is being

installed to transfer the sludge from the first chemical treatment tank back to the Imhoff tank. Thus, a sample can be taken when desired from any tank or during the pumping operation. A small laboratory sink has been added to facilitate chemical testing of the sewage.

III. Summary

Completed construction has basically been satisfactory for only minimum project operation. Frequent breakdowns and increasing demands made it necessary to review capacities and design of all project equipment and facilities. This was accomplished toward the later part of last year and several modifications have since enabled the project to operate at full capacity. Cages and associated facilities were made "fool-proof" enabling the 7-day Sr^{90} feeding experiment to proceed without interruption.

A master building plan for the project has been completed, and construction is proceeding as rapidly as possible. Every effort is being made to prevent undue delay in construction and plant operation. The unique requirements of this experiment have been solved, and full plant operation is anticipated by early 1963.

References

1. 3rd Annual Progress Report, AEC Project No. 6, 1960.
2. Andersen, A. C. and Goldman, M., JAVMA 137 (2): 129-135, 1960.

VETERINARY SECTION

May 1960 - May 1961

D. H. McKelvie

From 1956 to 1959 the veterinary aspects of this project were concerned primarily with designing and testing cage and pen facilities. By 1959, sixty-four outside pens (capacity 100 dogs) and fifteen cage units (capacity 60 dogs) had been constructed and tested as described earlier(1,2). Since then an additional sixty-four outside pens have been completed, and an equal number are now under construction. Breeding stock was obtained from Project 4 or on the open market. Additional experimental dams were purchased to meet the demands of the experiment proper. From this nucleus of dogs the following groups have been under veterinary supervision: (1) breeding colony; (2) maturing experimental dams; (3) Sr^{90} experimental dogs; and (4) surplus male pups or holding dogs. Other activities of the veterinary section pertain to caretaker organization, record keeping, and assistance in the various experiments.

The primary objectives of the veterinary section have been establishing suitable procedures and supplying an adequate number of experimental dams to fulfill the needs of the experimental design as construction is completed.

Through the combined efforts of all members of the project, the experiment proper began in December 1960, and the completion of all animal holding quarters is planned for early 1963. Thus, every effort is now being made to have 100 experimental dams 14 months old by that date.

This report summarizes the major activities of the veterinary section during the past year.

Imported Dogs-Quarantine*

The beagles obtained from Project 4--33 females and 10 males--were selected from breeding stock. These dogs were transferred directly to

1. 1959 Annual Progress Report, A.E.C. Project No. 6.
2. Andersen, A. C. and Goldman, M.: JAVMA 137, 2 (July 15, 1960).

* Conducted by Dr. R. Johnson and Mr. C. Hardesty, A. E. C. Project 4.

this colony after being dewormed, vaccinated, and tattooed for identification. They were placed in outside pens and assigned either to the breeding colony or to maturing experimental dams and formed the nucleus for the Project 6 colony.

In addition, purebred beagles were purchased on the open market and held in quarantine at Project 4. These dogs were selected for desirable qualities, given prophylactic treatment for parasites, tattooed, and vaccinated against distemper and hepatitis. The isolation period varied from 3 to 8 weeks, depending on the general health of the dogs. Four were culled * because of undesirable characteristics not detected at time of purchase.

Despite precautions before acquired beagles were introduced into the colony, both parasites and infectious diseases did become a problem. Moderate infestations of ascarids and fleas were introduced; fleas were controlled, but ascarids continue to be a problem in weaned pups.

Beagle Production and Assignment

The three sources of beagles (breeding colony, Project 4, and open market) have provided sufficient dogs to meet experimental demands and cull out undesirables. Undesirable phenotypic and genotypic traits were observed, and culling was enforced to prevent hindrance of the experiment. Unfortunately many of these traits were not recognized until several weeks or months had elapsed. This discussion summarizes the present status of the colony as well as explains the disposition of culled or surplus dogs.*

Since May 1959 the colony has whelped and weaned 97 litters, 62 of them during the past year. The net production from this colony since May 1959 is shown in Table I. This includes 5 litters whelped from experimental dams and designated as experimental dogs for Sr^{90} feeding. Pup mortality to the weaning age is shown in Table II. In large part,

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* Culled or surplus dogs have been either used for supportive (acute) studies or sent to other laboratories. (UCD Veterinary School; ANL; USNRDL; Lawrence; Hanford).

Table I

REPRODUCTION RECORD

(1959-1961)

Location or Conditions	No. Dams	No. Litters	Pups Whelped ♂	Pups Whelped ♀	Pups Weaned ♂	Pups Weaned ♀	Mortality (%)
I. Outside*							
A. Normal	44	66	196	166	141	121	27.7
B. Caesarean	7	7	19	22	5	13	56.1
II. Inside							
A. Normal	23	23	76	69	49	46	34.5
B. Caesarean	1	1	2	3	0	0	100.0
Total (AV)	68	97**	293	260	195	180	32.2

* Seven females whelped litters outside and inside.
** Sex Ratio (M/F) at weaning, 1.08.

Table II

AGE OF PUP LOSSES (PERCENT)*

	No. Pups	Neonatal Losses**	Nursing Losses	Total %
Outside Pens	362	61 (16.9%)	39 (10.8%)	27.7
Inside Cages	133	20 (15.0%)	18 (13.5%)	28.5
Total	495	83 (16.5%)	57 (11.5%)	28.3

* Very poor dams (1) or those requiring Caesarean section (8) are deleted. Dam production from 44 purchased and 24 proven stock. AEC Project No. 6.
** Neonatal losses include deaths from birth to 3-days of age.

pup losses to the weaning age were neonatal. These and other factors pertaining to reproduction are discussed more fully in a separate report*.

Of the 375 pups weaned, the assignment has been as follows:

8 ♀ - breeding colony.

4 ♂ - breeding colony studs.

33 ♀ - maturing experimental dams.

25 dogs are in AHQ#1 as experimental dogs.

4 dogs are as yet unassigned or being used in supportive studies.

29 ♀ were transferred to Project 4 for reproductive studies.

The remaining 192 beagles have been culled and released to other laboratories or used in acute studies.

The present status of the colony is as follows:

Breeding colony	51 (8 males; 43 females)
Maturing exp. dams	38
To be assigned**	94 (50 males; 44 females)
Total	183 Dogs

In addition to the 183 beagles kept in outside pens 39 dogs are currently maintained in the Sr^{90} ingestion building (AH#1): 4 experimental dams, 25 experimental dogs, and 10 growth-comparison dogs.

A growth-comparison study began while details for Sr^{90} treatment were being compared. Eight pregnant dams whelped their litters in cages according to the experimental design for this project. On being weaned, ten dogs were maintained as pairs (one of each sex from each litter) in cages. An equal number (sibs) were placed in outside pens. Weights have been recorded weekly, and roentgenographs taken monthly. An account of results for this study will be forthcoming.

During the past year, it has been necessary to cull 10 breeding colony dogs, 12 maturing experimental dams, and 107 weaned pups. The

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* Paper entitled, "Reproductive Fitness in Beagles" is in preparation.

** Pups (1-162 days of age) to be assigned as experimental dams or breeding colony dogs, depending upon experimental qualities and completion of future facilities.

reasons for culling were senility (above 5 years old), physical deformities (size, disease), or genetic defects (actual or potential). There were two unfortunate results from using stud M7. This stud showed no evidence of prognathism and was ranked excellent in conformation, disposition, and breeding ability. Nevertheless, approximately 40% of his 107 weaned pups showed slight to severe prognathism, and 2 developed hydrocephalus, making it necessary to cull both M7 and his progeny. In addition, stud dog M6 developed signs of epilepsy after being in the colony for several months. Increased recurrence of epileptiform seizures made it necessary to cull him from the colony.

General Veterinary Aspects

A. Disease Immunity and Prophylaxis

Before being admitted to the colony each dog was given distemper and hepatitis vaccination. Nevertheless, distemper broke out among younger dogs in late spring of 1960. Antisera (20-30cc) and antibiotics (2cc Combiotic) were given to each dog in the colony, but therapeusis proved ineffective and the disease continued to spread. As a result, a "barrier" was set up whereby the colony was divided into groups of 20-24 dogs with vacant rows of pens between each group. This group isolation halted spread, but challenge to the colony resulted in ten (10) deaths among pups, and probably irreparable damage in others. Such disease challenge undoubtedly may produce active immunity, but losses from the disease necessitated an established immunological program plus quarantine procedures for imported dogs. Thus, the following immunization program will be enforced:

(1) Distemper and infectious canine hepatitis (Cyvac*) at weaning, at 6 months of age, and every 2 years thereafter.

(2) Leptospirosis (L. canicola and icterohemorrhagica) bacterin* at 4 months of age and annually thereafter.

(3) Rabies (modified live virus) vaccine* at 6 months of age and alternate years thereafter.

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* American Cyanimid Company, Pearl River, New York.

B. Parasite Control

Neither internal nor external parasites have been a problem in this colony. Nevertheless, as a prophylactic measure, pups have been given anthelmintics at 21, 30, and 40 days of age and repeated as needed. In general, pups are dewormed 5-6 times before one year old. Control of ectoparasites has been satisfactory from annual dipping of dogs in 1% chlordane solution.

C. Incidence of Disease and Other Syndromes

In addition to the distemper outbreak, forty-seven cases required treatment for the following conditions: enteritis (21), dermatitis(6), otitis externa (5), rickets (4), respiratory infection (7), metritis (2), and nephritis (2). These cases responded to routine therapeutic procedures.

Two of the aforementioned conditions warrant further comment, namely, enteritis and rickets. Nearly one-half of the treated cases showed symptoms of enteritis (diarrhea, vomition, constipation). The cause(s) of this condition remains one of concern. Routine X-rays consistently show varying amounts of crushed rock in the GI tract, and attempts have been made to decrease its ingestion. Several pens were surfaced 6 inches deep with either 1/4", 1/2", or 3/4" crushed rock, and X-rays were taken of each group after periods of 2 and 4 weeks. Pens surfaced with 3/4" crushed rock showed no ingestion by the dogs, but these pens proved difficult to clean properly. One-half-inch crushed rock proved far superior to 1/4", and pen cleaning was not difficult. As a result, all pens, both existing and new, are being surfaced with 1/2" crushed rock. In addition, two additional feet of asphalt pavement have been added to the pens. Fewer cases of enteritis have been observed since these additions.

Four pups, 2 1/2 to 3 months old, displayed marked clinical symptoms of rickets. Several slight cases were also apparent. The first concern was that the quality or quantity of calcium in the diet might prove subminimal. As previously discussed (3), the diet contains 1% calcium,

(3) 1960 Annual Progress Report.

which is well above the minimum calcium requirement (0.6%) for dogs (4,5). Investigation was made of other nutritional factors associated with calcium utilization. A possible vitamin deficiency was suspected, and the vitamin supplement was doubled. Within 2-3 weeks the symptoms of rickets decreased markedly, thus suggesting that the cause had been a loss of vitamin potency. Further possible incidence of rickets has been alleviated by ordering a fresh supply of vitamin supplement every four months and storing the vitamins in a cool area. The dietary regimen of the animals has otherwise been maintained unchanged from that reported in the 1960 Annual Progress Report.

Caretaker Organization and Training

Outside Crew

The outside animal caretakers were transfers from Project 4, where they had undergone a period of training under the principal animal caretaker (C. Hardesty). The requirement for animal caretakers has been one man for every 70-dog increase in the colony. This training period has minimized the veterinary work load on Project 6 and has assured experienced caretakers to meet the needs of the colony during its growth. Duties and procedures for this crew are similar to those described in a previous report (6).

Radionuclide Crew

Organization of the radionuclide crew began in 1956, when the high-level Sr^{90} pilot experiment was started. Additional pilot tests were conducted with Y^{90} when cage units became available, in 1959. The crew now consists of three animal technicians and a laboratory assistant, with supportive help from the staff.

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4. Jenkins, K. J., A Study of Some Mineral Requirements of the Dog. Thesis, University of Wisconsin (1958).
5. Campbell, J. R., Calcium and Phosphorus Imbalance in Growing Pups, Modern Vet. Practice, 41: 20 (Oct. 15, 1960).
6. 1958 Annual Progress Report, A.E.C. Project No. 6.

The duties of this crew differ markedly from those of the outside caretakers, and formal Sr^{90} procedures were enforced after special informative sessions. The technicians attended weekly staff orientation meetings on the fundamentals of radioisotopes and radiation. These meetings, conducted by the radiobiology section, consisted of demonstrations (movies), lectures, and informal discussions related to general radiological health physics. The success of this training for "respect" and not "fear" of radioisotopes was displayed in high-level Y^{90} feeding trials. Since these experiments, cooperation and enthusiasm by the caretakers has continued while the intricate details of organizing the feeding program have been put into effect. The duties of the crew include:

(1) Maintaining accurate records.

(2) Cleanliness of building and equipment.

(3) Feed preparation.

(4) Daily feeding and cleaning of the dogs, and

(5) Observation of the animals.

Neither an accident nor an error has been made by this crew in past months. This experienced crew should be capable of initiating the training of additional animal technicians to be employed as the Sr^{90} and Ra^{226} buildings are completed.

Record Keeping--General

Three general record-keeping systems have been in use during the past two years: (1) daily kennel log, (2) individual dog record, and (3) special records. Each animal caretaker crew maintains a daily log of kennel activities and observations; the senior animal technician and laboratory assistant are responsible for this record. At weekly intervals, the log is summarized for permanent recording, e.g., treatment of a dog is filed in the individual record and veterinary log, etc. The individual dog record is a complete account of each dog from birth to death. It includes the litter record, geneology, medical history, clinical and subclinical observations, and final disposition. Special records include those pertaining to dog assignment--to breeding colony, experimental dam,

experimental dog, or supportive studies. Each of these groups requires specified measurements or observations, which are recorded separately for evaluation. Thus, information accumulated in the daily log, weekly veterinary examination, and special records is tabulated under the related experiment.

The problem of maintaining accessible records has been discussed with the biometrician (F. Shultz) on several occasions. While organizing the program, the general scheme has been to keep individual records for future reference. This method is cumbersome, however, and will become obsolete as the colony increases. IBM punch cards are anticipated for all long-term recordings. This system is currently being studied as the experiment proper proceeds.

Discussion

The past year has brought forth several experiences emphasizing the importance of constant veterinary surveillance for successful conduct of the experiment. Facilities must necessarily be adequate and not overcrowded. Proper design of outside pens and inside cages merely assists in establishing a healthy environment for the dogs, but occupancy to maximum capacity is not practical. In general, it is felt that facilities should be kept filled to within 90% of their designed dog capacity, thus allowing space for control of infectious disease and other maladies common to a large colony of dogs.

The problems encountered in establishing a dog colony with ideal experimental qualities have been recently observed. The purchase of stock, even when selected and screened, frequently introduces disease and parasites. In addition, a long-term observation period is required before newly acquired dogs can be stated to have desirable qualities. After screening each dog for disease, parasites, poor disposition, etc., genetic constitution still remains to be tested. All of these factors relate directly to the future logistical success of this project. The possibility cannot be overlooked that one dog could require several times the amount of care of others, so culling has been instigated for elimination of animals that may prove a detriment to the experiment.

Recognized therapy has been given to assure maximum performance of every dog, yet, from a practical standpoint, any therapeutic and surgical procedures must be carefully scrutinized. The maintenance of a "weakling" that requires constant veterinary attention cannot be practical. An isolated example, that of M6 (exhibiting epilepsy), illustrates this point. Continued use of this animal as a breeding stud could introduce the syndrome through inheritance into a number of experimental dogs, increasing caretaker and veterinary work load as well as causing early death of an afflicted dog. A similar situation could arise from breeding dams that require Caesarean section. Past experience has indicated that some female dogs are prone to dystocia and other reproductive disorders. During the past year, eight Caesarean sections have been performed on breeding-colony dogs. Surgery was conducted for two reasons: (1) procurement of smaller (11"-12") beagles, and (2) increased puppy production. Progeny obtained by Caesarean section have been used discriminately as replacements in the breeding colony and for experimental dams. Only small males have been placed in the colony as studs, for the purpose of maintaining medium-size beagles. It is now felt that sufficient data has been accumulated on each dog to provide the experiment with dogs of ideal qualities for successful conduct of the experiment.

STRONTIUM-90 FEEDING

R. J. Della Rosa

The continuous feeding of Sr^{90} to 180 dogs (1) at different levels (6 levels plus controls) for an 18-month period requires an almost infallible system to minimize possible errors and accidents. A successful procedure of feed preparation (mixing and packaging) has been established over the past two years. The present procedure of feed preparation and feeding, the result of the joint efforts of the project staff, includes: (a) mixing of food containing the radionuclide, (b) semi-automatic packaging, weighing, and identification of food packages, (c) quick-freezing and storage of a 30-day food supply at each level, and (d) thawing, warming, and feeding of a daily ration to the experimental dams and their litters (the experimental dogs proper).*

The ingestion phase of the experiment was started on 23 December 1960. As far as possible, the various feeding levels are being expanded together, that is, the filling of cages with experimental dams is being completed at a nearly uniform rate. Described herein are the various steps in the preparation of the radioactive feed and the initiation of the continuous feeding program, studied in considerable detail during the past year.

I. Nutrition

A compounded dry ration (#613) of proper consistency, palatability, and nutrition has been in use for the past two years. The ingredients and analysis of this ration, as well as the composition of the diet, already described (1), are briefly summarized here. The diet consists of 60% by weight ration #613 (kibble form), 20% meat (ground beef-horse meat, "4-Paw"**), 20% tap water, blood meal, and vitamins (daily requirement per dog). The calcium dontent of the diet is approximately 1 percent, with a Ca/P ratio of 1.5/1. Three hundred grams of food per day is the average daily requirement for the adult beagle, with adjustments for pregnant and lactating dams and growing pups (A.E.C. Project No. 4).

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* Mr. William Kuster, Consulting Chemical Engineer, assisted in the design, testing and establishment of the feed preparation program.

** Wilson's Meat By-Products Co., Chicago, Illinois.

II. Feed Regimen

A. Radionuclide. A primary stock solution of radioactive strontium, Sr^{90}, in equilibrium with its daughter product, radioactive Yttrium, Y^{90}, in a carrier solution is stored in a lead-shielded steel box in an isotope preparation room equipped with a constant-exhaust hood.* The carrier solution consists of 15.2 mg/L $SrCl_2 \cdot 6H_2O$ and 19.5 mg/L $Y(NO_3)_3 \cdot 4H_2O$ (5 mg/L Sr^{++} and 5 mg/L Y^{+++}) in 1N HCl (to minimize adsorption on the surface of the glass container).

The primary stock solution was assayed using a National Bureau of Standards (Sr^{90}-Y^{90}) beta-ray standard. The standardization and assay were carried out in a beta well-type scintillation detector (stilbene) with an efficiency of about 5.3 percent for Sr^{90}-Y^{90}. Sub-dilutions of this primary stock are prepared for each dose level. The solutions are prepared with appropriate amounts of stock and carrier solutions so that a constant volume, irrespective of dose level, is used for each 20-kg batch of food. These working solutions are kept in polyethylene bottles and stored in the hood of the isotope preparation room. As required for each 20-kg mix, a 20-ml volume is pipetted into a 1-oz. polyethylene bottle and carried to the mixing room. To prevent possible errors, pipettes, bottles, and other equipment are segregated and identified. To minimize possible cross contamination, only one dose level of food is prepared at any one time.

B. Feed Mixing. The following ingredients are assembled prior to mixing:

Kibble (ration #613)	12 kg
Meat (ground beef - 4-Paw)	4 kg
Hot tap water	3.95 kg
Vitamin preparation	180 gm
Blood meal	90 gm
Solution of Sr^{90}	20 ml
Carrier solution	30 ml

A 1-HP Hobart food mixer (Fig. 1A) with stainless-steel accessory bowls, protecting hood, and beater is used for mixing. A separate bowl,

* The solution has been stored since 1956 and is Sr^{89}-free.

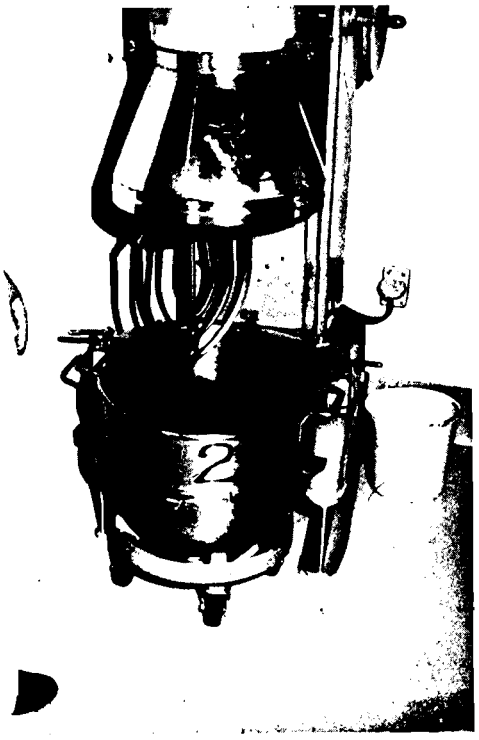

Figure 1A. HOBART MIXER - Showing bowl, beater and hood.

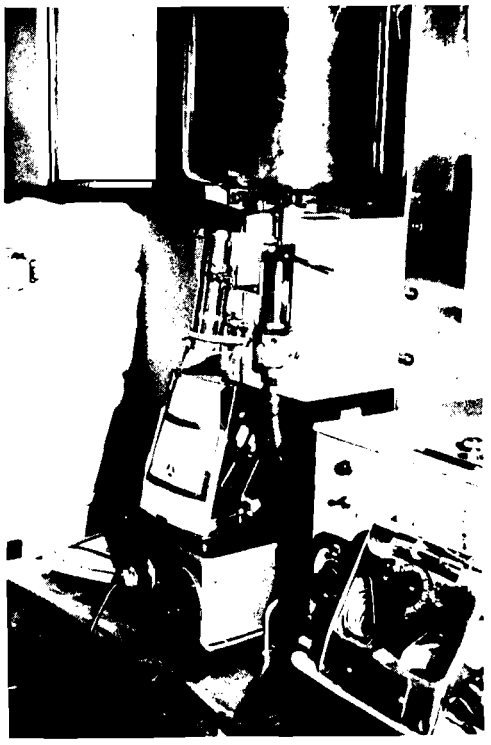

Figure 1B. GROSS WEIGHER AND SEALER - Showing hopper, pre-load tube pinch valve, food bag, and shadowgraph.

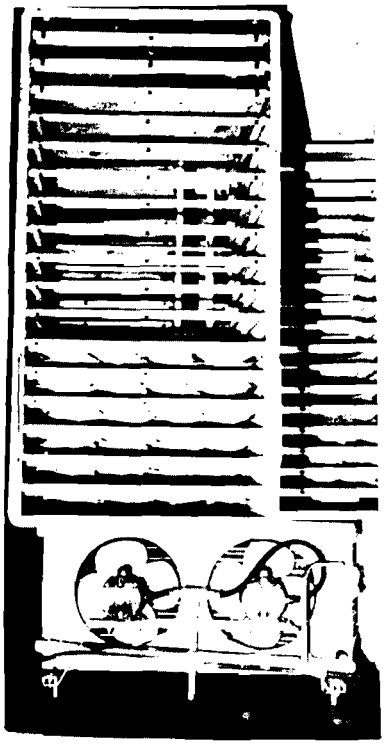

Figure 1C. FREEZER - Showing food cart and packages.

hood, and beater is reserved for each dose level. Each batch is prepared by mixing hot water, meat, and radionuclide (Sr^{90}-Y^{90}) for 1 minute at low speed in a mixing bowl. The bowl is lowered and the beater cleaned with a rubber spatula. The premixed kibble, vitamins, and blood meal are added, and the bowl is again raised into position and the entire contents mixed for an additional minute at low speed. The mixed food is then left in place for a "curing" period of 30-40 minutes to allow uniform absorption of the water and radionuclide into the dry kibble. The cured food is re-mixed (15 seconds) and transferred, by a large funnel, to a hopper of the automatic gross weighing machine.*

C. Food Packaging. The food is weighed and packaged with an automatic gross weighing machine shown in Figure 1B. The flat-bottom stainless-steel hopper has a capacity of approximately 2.4 cubic feet and is equipped with an eight-blade built-in involute rotor agitator. The food is dispensed by a feed rotor (powered by a 1/4-HP motor) into a food bag through a fixed-displacement preload tube located between the hopper and a gum-rubber pinch valve. The pinch-type valve is operated through an air cylincer and provides final closure of the material at the control point. The weighing mechanism utilizes a Shadowgraph scale** with control and photoelectric responders built into it. The container into which the food is being weighed is adjusted for tare, and the material is weighed directly. The start control is initiated through a foot switch, and the cutoff through photocell action. The hopper and attachments are designed for rapid assembly and disassembly for clean-up and decontamination. Four complete sets of hoppers and attachments are marked and reserved for control-level food, levels 0.3 and 1, levels 2 and 3, and levels 4 and 5.

A 300-gram ration is dispensed and weighed, with an accuracy of plus or minus 5 gm, into a serially numbered, labeled, and color-coded bag of

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* Designed and built by Don Turner, Associates, Santa Rosa, California.

** Exact Weight Scale Company, Columbus, Ohio.

laminated Kraft paper-polyethylene-aluminum* (1). The bag is then heat-sealed, recorded, and prepared for quick-freezing. For maximum freezing efficiency the packages are placed on a cart that is wheeled under the freezer coils of the quick-freeze room (Fig 1C). Freezing (-20°C) is complete within forty minutes. The frozen-food packages are then packed for storage in cartons (32 per carton) serially numbered, labeled, and color-coded to correspond with the food package identification. The color code and serial numbers for each dose level are presented in Table 1.

Table 1

IDENTIFICATION OF FOOD PACKAGES AND CARTONS

COLOR CODE AND SERIAL NUMBERS

Level	$\mu c\ Sr^{90}/gm\ Ca$	Color code	Serial numbers
Control	0.000005 **	black	000 000- 050 000
0.3	0.00154	violet	050 001- 075 000
1	0.00926	blue	075 001 100 000
2	0.0556	green	100 001 125 000
3	0.167	yellow	125 001 150 000
4	0.500	orange	150 001 175 000
5	1.500	red	175 001 200 000

* Weighing and packaging is accomplished by a team of four: a weighing machine operator, a sealer, a monitor-checker, and a package handler. The average packaging rate is about 3-4 per minute, with a yield of 67 packages per 20 kg of food mixed.

** See analytical section.

Present conditions of available freezing and storage space allow a supply of 20-30 days of each food level to be packaged, quick-frozen, and stored. This represents approximately 1500-2000 food packages for a full complement of 60 experimental dogs (capacity AHQ#1).

D. Daily Feeding Procedure. A successful feeding procedure, described in the 3rd Annual Progress Report, 1960, has been in operation for the past two years. This procedure is reviewed and summarized:

> The daily food requirement, designated by dose levels and bag numbers, is removed from cold storage 8 hours before use to allow adequate thawing. Twenty to twenty-five food packages are transferred to a domestic-type electric oven and heated 20 minutes at 200°F. After the oven is turned off, the packages are allowed to stand in the oven for an additional 20 minutes for uniform heating; the dogs prefer a temperature warm to the touch. The food packages (separated by dose levels) are then brought into the cage room and their serial numbers checked and recorded. While one caretaker prepares to serve the food, a second caretaker isolates both dogs to one side of the cage (sliding partition), and places the feed pans in the vacated cage. The food packages are opened and emptied into the feed pans. (The empty food bags are discarded into a polyethylene-lined disposal drum corresponding to the level being fed.) One food pan is withdrawn (to be served to the second dog), the sliding partition is opened and the first dog allowed acess to the food; the second food pan is then served to the second dog. One-half hour is allowed for feeding. Then the feed pans are collected, and the unconsumed food (if any) is weighed and the amount recorded. The unconsumed food is discarded into the cage and washed away with the excreta.

III. Radiological Health and Safety

The various aspects of the control and prevention of contamination during food preparation and daily feeding, including the environmental health and safety of personnel handling the radionuclides, are fully described in Section V. In brief, this is accomplished by careful monitoring at all steps involving possible contamination (e.g., survey meters, pocket dosimeters, and air sampling), the wearing of protective clothing, and the use of demineralized water for cage washing, and for cleaning and decontaminating the mixing utensils and feed pans. A dilute solution of HNO_3 (0.5N) is also used in the routine washing and decontamination of all stainless-steel utensils and feed pans.

IV. Analytical

As stated in the experimental design (1), a constant Sr^{90}/Ca^{40} ratio is to be maintained in each food level throughout gestation and lactation, and until the dogs reach 18 months of age (540 days).

To determine the uniformity of distribution of Sr^{90} in the prepared food, as well as the calcium and phosphorus content, three representative food packages from each 20-kg batch are reserved for analytical procedures. These are taken at the start, the middle, and the end of the packaging process, e.g., bag numbers 10, 40, and 60. Aliquots or the fractionated contents of each bag are used for analysis. The samples are dried and ashed in a muffle furnace (600°C), and the ash is dissolved in 6N HCl.

A. Methods.

1. Calcium: Total calcium is determined by one of two chemical methods currently in use: the chloranilic acid precipitation method of Ferro and Ham (2), and the ammonium hexanitratocerate titration method of Salomon et al (3). With the range of calcium-40 content in the food and skeletons, both methods are equally adaptable, but the chloranilic acid precipitation method is preferred because of its relative simplicity. The precipitated calcium chloranilate is dissolved in a solution of tetra-sodium ethylenediaminetetracetic acid (EDTA) and the optical density determined at 520 mμ.

2. Phosphorus: Inorganic phosphate is determined by the method of Fiske and SubbaRow (5).

3. Radiochemical Methods: Total beta activity is determined with a well-type scintillation detector. A 2-ml aliquot of the ash solution is sealed in a 3-ml polyethylene tube and is counted to the 95% limit of confidence. A like aliquot of control food ash is used as a blank (background) and for possible self-absorption corrections.

Since the present facilities do not permit low-level counting with any great accuracy, an independent analysis of the Sr^{90} content of the lowest dose level (0.3), as well as a determination of the fallout

content of the "control" food, has been carried out by two outside agencies*.

B. Results

1. Diet. The ingredients of the experimental diet (60% dry ration, 20% meat, 20% tap water) were analyzed for calcium and phosphorus. The results of one such analysis are presented in Table 2.

Table 2

ANALYSIS OF DIET

(Calcium and Phosphorus in dry ration, meat, and water)

Sample	Ca gms/100 gms	P gms/100 gms	Ca/P
dry ration (#613)	1.80	1.00	1.8
meat	0.08	0.20	0.4
tap water	0.02	0.004	

As is shown in Table 2, the relative contribution of the meat and water to the total calcium is very small and would represent a negligible fraction of the dietary calcium. It is of interest that 5 to 10% of the dietary phosphorus comes from meat.

Table 3 summarizes the analyses of calcium and phosphorus in the packaged food of the different dose levels.

Table 3

CALCIUM AND PHOSPHORUS IN PACKAGED FOOD

Level	Number of packages**	Ca gms/100 gms (average) (range)	P gms/100 gms (average) (range)	Ca/P (average) (range)
0.3	15	0.96 (0.79-1.09)	0.59 (0.44-0.86)	1.64 (1.35-2.48)
2.0	9	0.89 (0.72-1.09)	0.58 (0.43-0.73)	1.56 (1.24-2.08)
3.0	4	0.74 (0.52-0.92)	0.59 (0.51-0.88)	1.25 (1.02-1.30)

** Each food package was divided into three 100-gm fractions, and replicate analysis were performed on each fraction.

* Hazelton Nuclear Science Corporation, Palo Alto, California.
Robert A. Taft Sanitary Engineering Center, U.S.P.H.S., Cincinnati, Ohio.

As shown in Table 3, the present diet contains about 0.90 percent calcium and 0.60 percent phosphorus. These values (and ranges) compare favorably with previous analyses of the diet (1). The analysis of food continues on a routine basis not only to check on possible variation in incoming supplies but also to provide consistent preparation of radioactive feed over long periods.

2. Strontium-90 .

a. Control food: The lowest dose level (0.3) in the experimental design was based on the Maximum Permissible Body Burden of 1 μc Sr^{90}/70 Kg man[+] and is calculated to deliver about 1.5 times this body burden to a 10-kg beagle. It was therefore of interest to determine the "fallout" concentrations of Sr^{90} in foodstuffs used in compounding the ration, and its contribution to the lowest dose level.

Samples of ashed control food were submitted for analysis to the U.S. Public Health Service and an A.E.C.-licensed commercial laboratory. Our basic (control) diet is presently estimated to contain about 5 $\mu\mu c$ Sr^{90}/gm Ca. This amount of Sr^{90} is not expected to contribute significantly to the 0.3 level since it represents less than 1 percent of the total Sr^{90} in the feed.

b. Sr^{90} in Experimental Dose Levels: Table 4 shows the Sr^{90} content in each of the different dose levels presently being fed.

Table 4

SUMMARY OF SR^{90} ANALYSES IN PACKAGED FOOD

Level	Number of packages*	μc Sr^{90}/gm Ca (calculated)	μc Sr^{90}/gm Ca Experimental (average)	(range)
0.3	6	0.00154	0.0014**	(0.0011-0.0018)
2.0	9	0.0556	0.0580	(0.0430-0.0837)
3.0	2	0.167	0.170	(0.1724-0.167)

* Each food package was divided into three 100-gm fractions, and replicate analyses were performed on each fraction.

** Compares favorably with values of Hazelton Nuclear Science Corp., (1390 ± 70 $\mu\mu c$ Sr^{90}/gm Ca).

+ National Bureau of Standards, Handbook 52.

V. Experimental Dogs

The introduction of pregnant experimental dams into the feeding program, and their subsequent production of experimental dogs, has proceeded at a fairly constant rate. According to the experimental design, the different dose levels (filling of cages) are being expanded at nearly the same rate. The number of dogs presently in the ingestion series is summarized in Table 5.

Table 5

DOGS ON CONTINUOUS SR^{90} INGESTION

(as of 15 May 1961)

Level	No. of dams	No. of puppies (♂,♀)	No. puppies surviving (♂,♀)	Deaths (♂,♀)	Sacrifice (♂,♀)
0.3	3	16 (7,9)	14 (7,7)	none	2 (0,2)
2	2	11 (5,6)	7 (4,3)	3 (1,2)	1 (0,1)
3	3	12 (3,9)	4 (1,3)	8 (2,6)	none
Total	8	39	25	11	3

The number of experimental puppies (25) represents nearly one-half the capacity of AHQ#1. It is anticipated that the 60-dog capacity of the present facility will be attained by late summer.

Puppies dying at birth, or shortly after, are retained for Sr^{90} analyses. Experimental dogs are also sacrificed at different ages to equalize numbers and distribution of sexes. The Sr^{90} content (body burdens) of these animals also determined, is presented in Section VII. Also, the various aspects of inside kennel management and the disposition of experimental dams are discussed in detail in the veterinary and phantom preparation sections.

VI. Summary

A successful semi-automatic procedure for the preparation, packaging, and storage of food at nearly constant Sr^{90}/Ca^{40} ratios has been established. A feeding regimen designed to minimize errors and radiation

hazards has been initiated at three different dose levels, involving a total of 39 experimental dogs. Analytical methods to support these studies have also been established.

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RADIOLOGICAL SAFETY

M. Goldman

With initiation of the radiostrontium feeding schedule, in the past year, a system of radiological health and safety procedures were established. These include the basic tenets of health physics: containment and confining of radionuclides under close supervision, plus strict adherence to hygienic methods. To date the radiation problems at this project are related primarily to the administration of Sr^{90}- Y^{90} in dog feed. In addition to the β-emitting nuclides there are two X-ray generating machines at the laboratory: a 100-KVP 200-ma diagnostic X-ray unit, and a 50-KVP 50-ma X-ray diffraction-fluorescent spectrometer. The latter is quite well shielded, and no stray radiation has been detected. The diagnostic unit, in a properly shielded room is so used that operating personnel have lead shielding.

The radiological involvement will be expanded shortly, with construction of additional Sr^{90}-Y^{90} feeding quarters plus a large Ra^{226} injection facility.

The laboratory is designed for low-level long-term feeding experiments, and in this feature assumes a possible uniqueness. Such an operation poses a peculiar approach to radiation safety. Very seldom will there be an opportunity for an acute type of incident. The metabolic nature of long-lived Sr^{90} and its short-lived daughter, Y^{90}, in the chemical forms used in these studies, would lead one to predict that the major concern would be to prevent accidental ingestion of the radionuclides by personnel. Superimposed on this consideration is the high probability that any hypothetical ingestion would be at a level below routine detection limits. In addition to such single incidents is the concern about any defect in experimental design that could permit such an event to become a chronic problem. The main defense against such episodes is a thorough analysis of experimental protocol, with emphasis on prevention of all possible contamination.

The organization of the health physics protocol centers on the two locations where radionuclides are present: the animal holding quarters

and the waste disposal plant. Access to these two areas is restricted to the permanent personnel who have specific duties therein. Entry to the animal holding quarters is through a single door in the outer lobby. In a locker room between the lobby and the radionuclide area all personnel don protective head covering, coveralls, and shoe covers, plus a dosimeter. Rubber gloves are also worn by personnel handling radioactive materials. Permanent and portable rate meters are located strategically throughout the building and at the exit from the radionuclide area.

To prevent the spread of contamination, a policy has been established that restricts the movement of personnel during operations and that includes frequent radiological monitoring between operations. For example, the mixing and packaging of radioactive feed is scheduled to avoid conflict with routine feeding and cleaning operations. To establish the flow of traffic desired, Dutch doors are placed between rooms were contamination might exist, and "cold areas" in the building are separated by locked doors. There are two emergency exits from the building, but these are sealed with tape to prevent unintended use.

The radionuclide areas are equipped with a strippable paint on the floors and the lower half of the walls. The drains in the floors, sinks, and cages are all connected to the waste treatment plant. The air in the animal rooms is used only once, and is exhausted through the cages and a set of filters in an effort to direct any airborne contamination away from personnel. Surface decontamination is effected with a filtered vacuum cleaner and the liberal use of demineralized water. The procedures instituted for potentially hazardous operations are as follows:

The daily feeding of the different dose levels requires an accounting of each feed package. The caretaker crew feeds only one level at a time, beginning with the lowest. The health physicist follows this operation and records the package fed each animal, as well as witnessing the operation, should his services be needed. (A monitor-checker is present at all feedings, thus assuring seven-day coverage.)

To date there have been no spills or accidents associated with the continuous Sr^{90} feeding program. Feed unconsumed after the one-hour

feeding period is removed from the building via the sewage system. Immediately after use the emptied feed pans are cleaned and decontaminated with demineralized water and a light rinse of dilute HNO_3, followed by a survey with a thin-window portable ratemeter. The pans are so identified as to preclude use for any but the designated dose level, although they are not separated within each level.

After each feeding period, and at several intervals during the day, the fiberglass cages are decontaminated by a demineralized water washing. This procedure has been satisfactory and is desirable since detergents and cleansers would lower the efficiency of the waste treatment plant. Excrement is washed away from the operator into the effluent sewage drain of each cage. The slight negative air pressure (the room air is exhausted through the rear of each cage) minimizes inhalation by personnel of aerosolized particles. The exit air stream passes through fiberglass filters before being exhausted. The filters have failed to show any detectable radioactivity to date. Frequent washing of cages limits the opportunity for particulates to become airborne and tends to keep all radioactivity in the liquid phase, which is most easily handled.

The feed mixing operation is fully described elsewhere in this report. The primary radionuclide stock solutions are all isolated in an isotope preparation room. A senior staff member dilutes stock solutions to the proper degree in an enclosed hood equipped with independent filters. At this time aliquots for standardization are also prepared. The procedure for feed mixing is designed to prevent errors in feed "labeling." Thus, in any one day of feed preparation only one dose bottle (sufficient for a 20-kg feed batch) is permitted in the feed mixing area at any one time. The actual handling of this amount of radioactivity in a small polyethylene bottle is minimal. The feed mixing machines are surveyed throughout the operation. In addition, air samples are taken during the packaging process to be analyzed for Sr^{90} content after the decay of the natural short-lived contaminants. The resultant feed packages are devoid of surface contamination and are stored in duly marked fiberboard containers in a large quick-freeze.

The amount of dilution of the radionuclides in the feed and the method of storage limit the possibilities of external radiation exposures. The external dose rate from these β-emitting nuclides is considered negligible at the levels and frequencies now in use.

The surface external dose from a package of food containing 0.5 μc Sr^{90} in equilibrium with its Y^{90} daughter has been measured at about 0.5 mr per hour (using a pocket dosimeter). If this is considered an average concentration and is multiplied by the daily period during which the bags are handled, one finds that the maximum dose to the hands would be about 0.5 mr per week. This dose is approximately 0.1% of the M.P.L. to skin surface as discussed in NBS Handbook 59. Pocket dosimeters worn by personnel have failed to show any increase in external radiation above background levels.

On two occasions short-term experiments were conducted with Ca^{47} and Sr^{85}. At the levels used, the maximum exposure recorded was about 50 mr per week for one person and less than 10 for all others. Since the physical decay was relatively rapid and the radionuclides were widely distributed (within experimental animals), these exposures were confined to two one-week intervals and were less than 10% of the M.P.L. of 600 mr/week for indefinite exposures.

When the laboratory building is complete, it is planned to use the whole-body dog counter to serve as an auxiliary means of determining personnel contamination. Until this equipment is calibrated, it is not known whether its detection capability can be extended to such a degree.

The operation of the waste treatment plant, described elsewhere in this report, presents several facets of concern to the health physicist. In this building, underground tanks of varying capacities receive and process the radioactive wastes. The sewage is initially pumped from a sump into a large Imhoff tank. This pump has occasionally been clogged by foreign matter and hair, some of which may have been carryovers from earlier construction phases. The pump was thus removed from the sump, hosed thoroughly with demineralized water, repaired, and replaced. The operation involved detectable amounts of Sr^{90}-Y^{90} but again at such low

levels ($\sim$ 1μc Sr^{90}/gal. waste) and for such short periods ($\sim$ 20 minutes) that no exposures were recorded on the personnel dosimeters. The pump has been replaced with a larger unit designed to eliminate maintenance. The liquid waste is transferred from one processing station to the next by pumps mounted on the decking above the tanks. Manifolds and drains at each point permit small quantities of waste to be removed without breaking the hatch seals. Off-gases from the tanks are passed through absolute filters. The entire deck surface is so sloped as to return to the Imhoff tank any spillage that might be expected following a pipe rupture or pump breakdown. Every aspect of the system is designed to be "failure-safe," and performance to date has been satisfactory.

The ion-exchange resins that concentrate the Sr^{90} from the processed wastes are packed into tight-mesh nylon cloth "socks" and then sealed in steel canisters. When the contaminated resin is expended, the nylon container simplifies its removal. The entire "sock" is concreted for offsite burial.

Im summary, the planning of the experiment and its facilities has borne fruit. To date, no defects in design and no accidents have been detected that violate established radiological health and safety practices.

DISPOSAL OF RADIOACTIVE WASTE

M. Goldman

Introduction

The experimental design for this program uses radionuclides, which require a waste disposal system. The system should safeguard public health and yet be simple in design and economical to operate. After several years of experiment and consultation, a system based on ion-exchange removal of cations was devised as a means of processing wastes and discharging effluents that do not exceed drinking-water M.P.L. requirements.

The raw waste consists of radioactive fecal matter, urine, and uneaten food, diluted by relatively large volumes of demineralized water and some domestic water. Since the quantity of radioactivity is easily determined (because of the nature of the experiment), one can readily calculate the potential daily load to be processed. The number of packages fed per day, times the radionuclide concentration per package for each level, is recorded daily; the rate of accumulation of waste is also known. If one then assumes that the amount retained by the dogs is negligible (<10%), the initial waste concentration can be estimated.

The experimental design was initiated in December, 1960, with introduction of the lowest dose level. Thereafter the higher levels were added so that each level would be represented by dogs of similar ages. Table 1 summarizes the rate of Sr^{90} feed consumption by experimental dogs. At present, the radionuclide utilization rate is doubling about every three weeks.

Table 1

SR^{90} FEED UTILIZATION

Month	μc Sr^{90} Fed	Cumulative Total
December 1960	0.08	0.08
January 1961	10.94	11.02
February "	17.94	28.96
March "	36.94	65.90
April "	90.28	156.18
May "	171.41	327.59

It is anticipated that about 190 μc Sr^{90} per day will be used when the 60-dog capacity of the present facilities is reached. When construction is complete, the total feeding schedule will amount to about 554 μc Sr^{90} per day. Since the age and number of dogs under treatment will vary continually, (some are to be sacrificed, some will be immature and eating smaller rations, and some may be finished before others start), a reasonable estimate is that, under full operation, the maximum daily feed consumption (ergo, waste production) will be between 400 and 500 μc Sr^{90} per day.

At this time, wastes are being produced at a rate of about 150 to 200 gallons per day. Under full capacity, this is expected to increase to some 400 gallons per day. This results in an estimated average concentration level of approximately 1 μc Sr^{90} per gallon (2-3 x 10^{-3} μc Sr^{90}/ml), a value of 3 x 10^4 times the present M.P.L. for off-site drinking water (10^{-7} μc/ml) (1). Thus, the waste treatment plant must achieve a radionuclide reduction factor of the order of 10^4.

The economy of operating a waste treatment facility must be considered in its planning. If all the raw wastes were collected, concreted, and buried offsite, the estimated cost would be about $2.00 to $3.00 per gallon (about $1,000 per day). During the course of the project the annual cost, then, could be over $200,000, and cost of waste disposal could easily exceed $1,000,000. Thus, the primary concern of this plant is to concentrate radioactive waste through the removal of liquids and yet assure adequate decontamination of the liquids to permit their discharge locally. Dr. Kaufman has expressed these two factors as follows:

$$\text{Volume Reduction Factor } (V_f) = \frac{\text{Initial Waste Volume}}{\text{Final Waste Volume}}$$

$$\text{Decontamination Factor } (D_f) = \frac{\text{Initial Radioactivity Concentration}}{\text{Final Radioactivity Concentration}}$$

The results of preliminary studies on ion-exchange resins as a means of arriving at the above factors are summarized below, largely a direct quote from a previous report (2).

Preliminary Studies

Tests of ion-exchange decontamination were performed with simulated liquid wastes of two types: aqueous solutions of calcium and strontium-90 in tap water; and a suspension prepared with distilled water, dog feces and urine, and strontium-90. For the first waste type an effort was made to select a range of calcium concentrations encompassing that expected in the effluent from the Davis Project dog cages. This simulated waste, being devoid of organic matter, was assumed to represent the effluent from a pretreatment system involving chemical coagulation, sedimentation, filtration, and chlorination. It typified a solution ideally compatible with the ion-exchange resin. The second waste type was a very approximate representation of the dog cage effluent after only simple sedimentation in an Imhoff tank. It was heavily laden with suspended and dissolved organic matter and perhaps characterized a liquid most unsuitable for direct application to an ion-exchange column.

The ion-exchange phases of the study with the simulated untreated Imhoff tank effluent made it amply clear that pretreatment would be necessary for proper performance of the ion-exchange portions of the treatment facility. The pretreatment studies included tests of aluminum and iron salts as chemical coagulants and an investigation of the influence of pH on clarification. Also examined were the possible advantages of employing chemical oxidation with chlorine and biochemical oxidation by prolonged aeration.

One of the most interesting observations was the passage of appreciable concentrations of yttrium-90 through 8 feet of cation-exchange column, preceding both calcium and strontium. Since yttrium is trivalent it was evident that the yttrium present in the simulated waste was either nonionic or complexed in a form having a low affinity for the cation resin. Fortunately, yttrium-90 has only a 61-hour half-life and can be removed from the plant effluent by simple retention.

On the basis of the laboratory studies reported herein, and with due consideration of the project's interest in simplicity and economy of operation, it is possible to make several concluding statements that will identify the major features of the required treatment facility. Figure 1 is a flow diagram of the proposed treatment process.

Treatment Prior to Ion Exchange

1. Maximum separation of solid organic material and wash water is desirable at the dog cages in the interests of

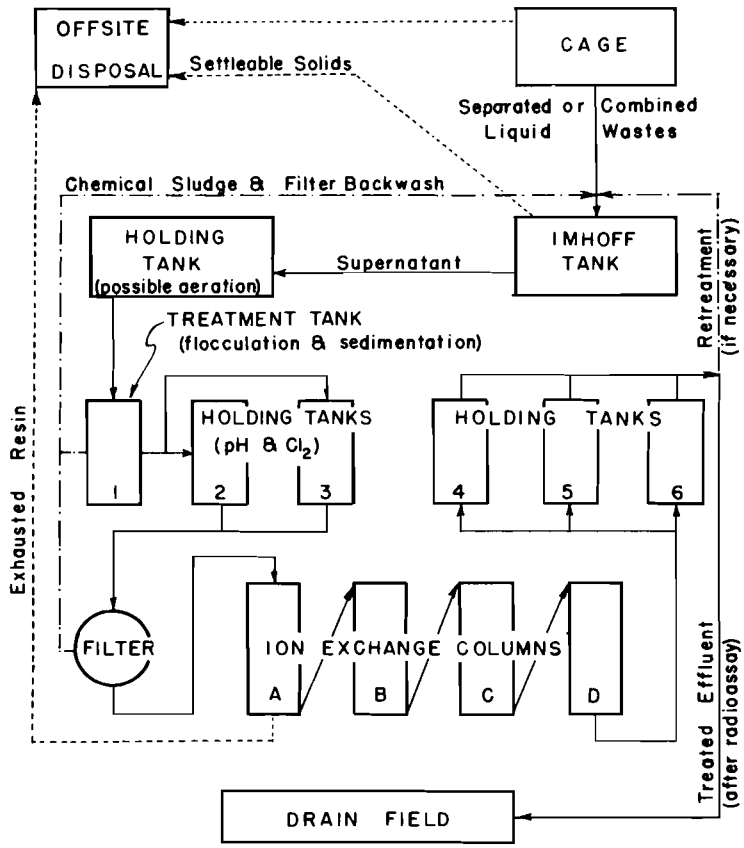
OFFSITE
DISPOSAL
Settleable Solids
CAGE
Separated or Liquid
Combined Wastes
Chemical Sludge & Filter Backwash
HOLDING TANK (possible aeration)
Supernatant
IMHOFF TANK
Retreatment (if necessary)
TREATMENT TANK (flocculation & sedimentation)
Exhausted Resin
HOLDING TANKS (pH & Cl_2)
HOLDING TANKS
1
2
3
4
5
6
FILTER
ION EXCHANGE COLUMNS
A
B
C
D
Treated Effluent (after radioassay)
DRAIN FIELD

Figure 1

economy, simplicity, and high strontium-90 decontamination in treating the waste. In this instance it is quite possible that personnel safety in animal care may require the water carriage of all wastes with the necessity of providing a high degree of decontamination of a complex organic waste. It has been assumed that this would be the case.

2. Ferric sulfate was found to be superior to aluminum sulfate as a coagulating agent. However, 95 per cent clarification of a simulated settled effluent containing wash water and all waste solids required over 500 mg/l of $Fe_2(SO_4)_3$. The resulting volumes of chemical sludge were excessive.

3. Prolonged aeration resulted in more satisfactory clarification, with an appreciable reduction in coagulant requirements. Approximately 250 mg/l of $Fe_2(SO_4)_3$ gave 95 per cent clarification, and the volume of chemical sludge was greatly reduced. Aeration promotes biochemical oxidation of the organic fecal solids, reducing coagulant demand. A similar, but probably greater, reduction could be achieved by careful waste segregation at the source, i.e., the utilization of a separate rather than a combined or water-carriage system.

4. The coagulant requirements during operation of the treatment facility must be determined by simple flocculation tests. The quantities of coagulant reported herein are based on tests of simulated wastes and are believed to be appreciably greater than those required in actual operation with a combined waste.*

5. Waste chemical sludges resulting from flocculation would normally be returned to the Imhoff tank influent and may aid in the initial clarification process.

6. At certain times it is quite possible that chemical coagulation will provide a decontamination sufficient that ion exchange will be unnecessary. This situation may prevail during periods where the dog population is being treated with the lower strontium-90 ingestion levels. A calcium phosphate precipitate would enhance the decontaminating

* For example, recent flocculation tests on actual settled combined waste from the treatment facility indicate that clarification can be adequate with applied alum of 100 mg/l at a pH of 4.0. The resulting sludge volume is only 2 per cent of the original waste volume under these conditions.

function of the coagulation operation and might be warranted at such times.

Ion-Exchange Treatment with a Sulfonated Polystyrene Resin

7. Ion-exchange columns will provide strontium-90 decontamination factors up to 10^4. However, to achieve this level of decontamination reliably, leakage must be minimized by employing long columns, on the order of eight feet, and the influent wastes must be free of suspended matter.

8. By employing segmented columns, operated in a countercurrent fashion, it is possible to use the ion-exchange resin to full capacity while achieving a decontamination factor of 10^4.

9. Although ion-exchange resins sorb strontium in preference to calcium, the latter reduces the unit capacity of an exchanger for strontium. The unit strontium-90 capacity of an exchanger is independent on the strontium-90 concentration of the waste and may be estimated from an equilibrium parameter and the calcium concentration of the waste. The strontium-calcium equilibrium parameter, K_{Sr-Ca}, was observed to vary between 1.5 and 2.0 for the resin under investigation. Minimizing calcium concentrations by employing demineralized water gives a greater capacity for strontium-90.

10. The achievement of high-order decontamination is dependent on minimizing leakage. This may be accomplished by employing long columns of relatively fine-grain resins and operating these at low flow rates.

The preliminary studies served to define many of the parameters of concern in the treatment of waste. It was initially decided not to separate the solid wastes at the source (the cages) but to permit all the solids to admix in forming the waste. (This includes the uneaten ration from each day's feeding as well).

Design of Waste Treatment Plant*

A. Waste Collection and Digestion. The basic design of the plant includes underground and above-deck facilities. The wastes drain from

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* The cooperation of Mr. Arnold Huff, Engineer, University of California, Davis, in the design of the waste disposal plant is appreciated. The efforts of Mr. Arliss Ray in the preliminary tests for this plant are likewise appreciated.

the building into a sump with a capacity of about 1000 gallons. A sealed sump pump then raises a 200-gallon quantity over a weir into the large Imhoff tank. This procedure in effect lowers the remaining tanks about 4 feet below the incoming drain inlet for gravity flow from the building. Raw waste is thus distributed along the length of the Imhoff tank, the first of 3 sections that compose the settling and digestion area. The capacity of the Imhoff tank is about 5000 gallons. At its effluent end is located an overflow weir that leads into the second settling volume, also about 5000 gallons. At the effluent end of the second settling tank is the remaining third of the digestion area, with a capacity of another 5000 gallons. These last two sections fill by overflow from the previous section. The final area is equipped with a pump to deliver the "digested" waste into the first treatment tank. Therefore, prior to treatment, each of the 5000-gallon areas is permitted to fill so that the Imhoff and settling tanks contain about 15,000 gallons of waste, the last 10,000 of which are the supernatant resulting from the Imhoff treatment. It is well to note that this large capacity and its relatively slow rate of fill (about 1% per day) are designed to afford maximum digestion of the raw wastes and agitation is minimum during the settling process. The degree of mixing of the wastes has not yet been established, but is assumed to be less than complete. Thus, if the radiostrontium concentration is doubling every three weeks and it takes about 10 weeks to fill the tank at present flow rates, one can assume that the effluent end, i.e., that pumped out first, will have a lower radionuclide concentration than is present at the influent end or sump. These values will be obtained by measuring the radiostrontium concentrations simultaneously at each end.

When the volume of waste in the settling tanks is at about 80% of capacity, a volume is transferred to the first chemical treatment tank. This volume is subsequently handled as an entity and is referred to as a batch with a nominal volume of 4000 to 5000 gallons.

B. Chemical Pretreatment. The batch that is pumped into the chemical treatment tank has already undergone considerable decomposition

and settling. The residual sludge contains the fraction of organically bound radionuclides that either floats or settles because of the design of the overflow weir in the Imhoff region. By using only the supernatant solution, a degree of decontamination is achieved.

The chemical treatment effects further decontamination. In this step, the waste treatment consists of pH adjustment and coagulation to obtain a solution of maximum clarity. Coagulation requirements are determined for each batch by means of simple flocculation tests. Aliquots of the tank are pumped to a special testing outlet (which drains back into the Imhoff tank), and clarity and pH are measured. At present, empiric criteria are used. Several jar (flocculation) tests are performed with various amounts of pH adjustment and added coagulant (alum). The test consists of constant mixing of the two additions of 200-ml aliquots of waste, a standard settling time, and then measurement of clarity in a turbidometer. The values thus obtained are used as guides for lowering the pH of the entire batch to about pH 4, with 30 to 40 liters of 12N HCl. In addition, a batch of 4-5000 gallons requires 7 to 10 lbs of alum, to achieve optimum flocculation. Mixing in the tank is accomplished by a system of submerged air jets operated by a compressor on the deck. Before further treatment (see figure 2) the chemical sludge is allowed to settle for 24 hours to prevent carry-over into the next tank. The supernatant liquor is pumped out through a swivel pipe suspended from a float whose inlet is maintained several inches below the liquid surface. A fixed pipe inlet is also located at the bottom of the tank to permit the transfer of accumulated chemical sludge to the Imhoff tank when necessary. It is presently estimated that 5 to 10 batches can be chemically processed before the sludge level builds up to the point where it interferes with the clarification step. Transfer of the chemical sludge to the Imhoff tank also improves initial clarification. Ultimately the total sludge level will necessitate cleaning the Imhoff tank completely, but present estimates place this at about a biannual frequency.

Figure 2

Interior view of chemical treatment tank with capacity of 4600 gallons. Note aerations jets (center pipe above floor), sludge pumpout pipe (at base of center post), and swiveled supernatant inlet pipe (attached to square polystyrene float at right). With minor variations in plumbing, each of the six tanks is constructed in a similar manner.

C. Chlorination. The clarified supernatant of the chemical treatment step is transferred to a holding tank, where it is further treated with 5% sodium hypochlorite to minimize bacterial growth in subsequent steps. Each new batch treated by chemicals in the previous step is mixed with some 15% of the sludge residual volume derived from earlier treatments. If the radioactive concentration were always constant, sludge samples might indicate the degree of decontamination due to flocculation. But the current case is one in which each subsequent batch is at a higher radionuclide level than its predecessor, and therefore the sludge serves to dilute the batch being treated.

D. Filtration. Following chlorination, the batch is pumped through a rechargable sand filter before passage through the ion-exchange resin columns. The sand filter can be cleaned by back-flushing it with demineralized water that drains into the Imhoff tank.

E. Ion-Exchange Treatment. The resin is contained in a series of 5 cannisters, each containing one cubic foot of resin. The total resin bed length is 15 feet, with a 9-inch diameter. Each cannister has influent and effluent valves and pressure gauges, permitting rearrangement of the cannisters as they become exhausted, and simple replacement of the resin. The resin is Dowex 50 x 8, 20-50 mesh, sodium form. Within each cannister the resin is encapsulated in a nylon cloth "sock", which is discarded with the resin. The "sock" rests on a perforated stainless-steel plate at the base of the cannister to provide uniform liquid flow through the column. The rate of flow through the resin is currently at about 120 gallons per hour (4.5 gal/min./ft.^{2}).

F. Decontaminated Wastes. The final effluent is stored in a series of holding tanks with a total capacity of 14,000 gallons. These tanks each contain one batch and provide a time safety factor for the Sr^{90} to come into equilibrium with its Y^{90} daughter as well as excess Y^{90} decaying. Each batch is sampled at the final stage prior to its release. The radionuclide concentration of the effluent after ion-exchange treatment is below the present counting capabilities of this laboratory. However, accurate estimates of radioactivity are being

obtained by commercial analyses following the AEC-HASL procedures. The results are expected shortly and should be considerably below the 10^{-7} μc Sr^{90}/ml MPL.

Results of Treatment

Table 2 summarizes the first six months of operation of the waste treatment plant. It should be noted that most of Batch No. 1 was retained and stored with Batch No. 2. Furthermore, there is a large residual volume representing the sludge in each pretreatment tank, thus preventing a quantitative transfer of each batch. However, by sampling the batches before and after each step, a reasonable accountability of decontamination efficiency is possible. It was necessary to accumulate a large volume of waste before such determinations could be meaningful. Should any batch exceed the limits placed upon it, i.e., the average annual M.P.L., it can be transferred back to the Imhoff tank for further reprocessing or diluted to the proper level. The former procedure is presently part of the policy for waste disposal.

Table 2

BATCH TREATMENT OF RADIOSTRONTIUM CONTAINING WASTES

				Pretreatment					Ion Exchange		
Batch No.	Initial Volume (gals)	Total μc Sr^{90} (est)	Date	HCL (1)	Alum (lbs)	pH Final	Clarity (0-100)	Settling Time (days)	Gal/hr	Gal.	Storage (Tank #)
1	2225	5	4/18/61	12.5	8.5	5.1		3		875	leach field
2	4560	23	5/5/61	40.0	7.0	3.7	25	6	128	4600	4
3	4570	34	5/22/61	34.0	8.75	4.6	25	2	121	3525	5

Acknowledgment

Much of the information in this section was obtained from the report of two of our consultants resultant from a cooperative agreement between the project and the Sanitary Engineering Research Laboratory, College of Engineering, and the School of Public Health, University of California at Berkeley, under the direction of Prof. Warren J. Kaufman and Mr. Arliss D. Ray. Their efforts made possible the development of the present disposal plant.

REFERENCES

(1) USAEC Title 10, Part 20. Standards for Protection Against Radiation, Federal Register, Nov. 17, 1960.

(2) Arliss D. Ray and Warren J. Kaufman, An Investigation of Ion Exchange Treatment of Strontium 90 Contaminated Organic Wastes. Preliminary Report (49 pps.). Sanitary Engineering Research Laboratory, College of Engineering and School of Public Health, University of California, Berkeley, January 1, 1961.

BIOPHYSICAL MEASUREMENTS

M. Goldman

A major effort of the past year has been directed toward implementing the experimental design. One of the facets of the experiment is concerned with accurate appraisal of the dose delivered to beagles during and following a diet containing Sr^{90}. Several basic approaches can be integrated to give meaningful information on the Sr^{90} body burdens in these dogs. The classic approach is direct: the animal is sacrificed and his radionuclide content assayed by radiochemical means. This procedure permits infinite choice of depth of study, but the experimental design requires that the large majority of animals be maintained for their natural life span. Removing a limited number of dogs from the experiment at different periods of development, however, gives an accurate insight into the remaining animals in the group.

When this approach is coupled with *in vivo* measurements, appraisal of the variability and distribution of radionuclide burdens is reasonably complete. The method of whole-body counting of individuals with radionuclide burdens is relatively recent. However, the analytical technics that are available offer the investigator considerable latitude. In the absence of a laboratory and counter, progress in establishing this technic has been somewhat limited. However, a whole-body counting facility has been designed and assembled, and very shortly will be available for use.

In measuring the body burden of dogs containing Sr^{90}, suitable standards under controlled geometric configuration are necessary to quantitate body burdens in the living dog. Progress in this direction is described in this section. In addition, certain aspects of dose calculations are discussed. Finally, a series of studies on the retention of orally administered radiostrontium in beagles are reported.

Part A

PHANTOM PREPARATION

R. D. Phemister and A. C. Andersen

Introduction

Whole-body counting procedures are greatly facilitated by accurate standard preparations, or phantoms. Accuracy in this case depends on not only the amount but also the distribution of radioactivity in the phantom. This is particularly true when bone-seeking isotopes are considered. With this in mind, efforts were directed toward establishing a technique whereby dogs can be preserved indefinitely for use as phantoms. Such phantoms, with any desired amount of radioactivity (physiologically distributed), will provide a means of standardizing whole-body counting procedures and of comparing conditions with those in other laboratories.

During the earliest phases of this project, Professor R. D. Evans (MIT) suggested a cooperative effort to produce calibrated standards for whole-body counting. This project was to prepare phantom specimens containing Sr^{90}. Three such preparations, containing different burdens, were to be sent to M.I.T. for whole-body counting and direct beta assay. The results and an intact phantom were then to be returned to the project to standardize whole-body counting procedures here.

A number of specimens were prepared in 1956-57 by conventional embalming techniques and dipping or spraying. After several months had passed without evidence of decomposition, three phantoms were sent to M.I.T., but they began decomposing soon after their arrival there. Several attempts failed to correct the fault, and the program was interrupted until late in 1960. Following is a report of the latest procedure and the results obtained with it.

Materials and Methods

Nine dogs 120 to 670 days old were obtained over a 5-month period as culls from the kennel. The following procedure evolved for preparing phantoms:

1. The animal is fasted for 24 hours and given one ounce of castor oil the evening before it is sacrificed.

2. Following a preanesthetic dose of atropine, the animal is anesthetized with pentobarbital sodium. This is followed by intravenous injection of 10,000 units of heparin.

3. The entire body is then clipped and the dog is placed on its back.

4. The right common carotid artery and the left external jugular vein are isolated via incisions on each side of the trachea.

5. Catheters (8F Bard) are inserted, caudad and cephalad in the jugular, and caudad in the carotid. Each catheter, once the flow of blood is good, is secured in place. Those in the jugular are used to exhaust the blood, and that in the carotid is connected to a gravity-flow source of warm physiological saline solution.

6. The blood is slowly replaced by saline. This usually requires 30 to 45 minutes or longer. Throughout this time the heart continues to beat, greatly facilitating the flushing process.

7. After the heart stops, the infusion is changed to a 2.5% formaldehyde and 2.5% glycerin solution with enough Furaspor* added to make a 3% solution. This continued for an hour.

8. Then the solution is changed to 7% formaldehyde and 7% glycerin plus Furaspor for another hour.

9. This is followed by a 14% formaldehyde and 14% glycerin solution plus Furaspor for another hour or until the dog becomes edematous, whichever is longer.

10. While the final solution is being infused, some of the same concentration (14%) is injected into the thoracic and abdominal cavities, into the catheterized bladder, and into the cisterna magna (following aspiration of cerebrospinal fluid).

11. The infusion apparatus is then disconnected, and the catheters are left in place overnight to allow additional drainage.

12. The following morning the cervical incisions are closed. Then follows an indefinite period during which the cadaver is allowed to

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* Eaton Laboratories, Norwich, New York.

dehydrate until it approaches its original (live) weight.

13. It is then prepared for coating by suturing all body orifices and soaking in acetone.

14. When dry it is ready for the succession of dips in vinyl plastic** that produce a multi-layered, clear, permanent coating. This coating, free of heavy metals, closely approximates the dog's hair coat.

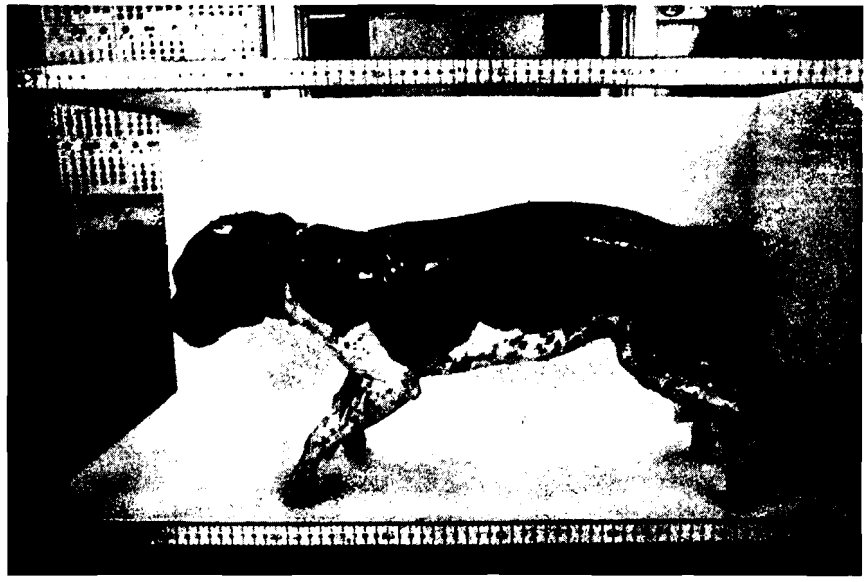

Figure 1. A phantom
(photograph taken 7 months after sacrificing animal)

Results

To date 8 dogs have been successfully preserved for periods up to 7 months (Table 1). One other dog dehydrated badly and began to putrefy in the inguinal region 2½ months after preparation (but before being dipped in plastic).

** Ply-On, Coat B (Ply-On, San Francisco, California).

Table 1

WEIGHT LOSS OF PHANTOMS

Day Sacrificed			Phantom Data to 7-12-61				
Dog	Age (Days)	Live Weight (Kg)	Time Preserved (Months)	Weight (Kg) Day 1	Present Weight (Kg)	Kg. Loss/ Month	% Wt. Loss/ Month
1	124	5.170	7	5.890	4.950	.134	2.2
2	130	4.950	6.5	8.000	6.080	.295	3.6
3	172	5.720	5	6.930	6.160	.154	2.2
4	174	8.150	5	10.200	8.380	.364	3.5
4A	635	7.700	3	8.910	7.520	.397	4.4
11A	600	6.900	3	7.750	6.760	.330	4.2
4C	668	9.360	2.5	10.200	8.900	.520	5.0
21A	664	5.800	2.5	8.160	6.550	.644	7.8

Discussion

Several facts became apparent upon examination of the data. First, the dogs become heavier during the original perserving (infusing) process. This takes the form of a generalized edema and is related to the breakdown of the vascular bed.

Second, the weight loss per month is greater in the more recently prepared specimens. In other words, weight loss is greatest shortly after infusion. There are at least two reasons for this: (1) the lost weight is primarily the "surplus" edematous portion, and (2) the phantoms are not yet coated with plastic.

It is probably significant that while the last 4 preparations were waiting to be coated (until they reached live-weight values) the temperature rose well over 100°F for a few days. During this time the uncoated preparations lost considerable weight while the coated ones were not particularly affected.

It is anticipated that the phantoms will lose some weight, but at a very much reduced rate. To further minimize weight loss, the phantoms will be kept in a temperature-controlled environment.

Summary

At present, 8 phantoms have been prepared by a method that involves embalming followed by plastic coating. They are being studied to determine their suitability as permanent specimens.

DOSIMETRY

Part B

M. Goldman

Whole-Body Counting. A temporary "whole-body counter" has been used during the last year to provide preliminary information, the details of which have been described (1). The 2 x 2 NaI (Tl) crystal detector feeds the signal to a single-channel pulse-height analyzer. The detector, surrounded by lead, faces the wide end of an open lead cone whose solid angle encloses the counting area. The limited sensitivity of this configuration restricts its usefulness in measuring body burdens much below 4 μc Sr^{90}. The background count rate is about 200 per minute, and one microcurie of Sr^{90} in a beagle would increase this number by only 12 counts per minute with the geometry now in use. One survivor of a pilot study (5S2) contains about 200 μc Sr^{90} and has been counted several times. Her litter mate succumbed to an osteogenic sarcoma last year, and a portion of his skeleton (about 17%) was imbedded in a cylinder of paraffin to provide a phantom of uniformly labeled bone containing about 43 μc Sr^{90}. This "standard" was compared with those available at the Radiobiology Laboratory of the University of Utah as well as with results of radiochemical analyses of portions of the skeleton.

The whole-body counting of animals containing Sr^{90} is based on measurement of the bremsstrahlung produced by the interaction with the surrounding matrix of the β rays from Sr^{90} and its Y^{90} daughter. The intensity of this external bremsstrahlung is proportional to the Z number of the absorber with maximum photon energies that can equal that of the β ray (2). In the case of bone-seeking radionuclides, the bremsstrahlung production is greatly influenced by the mineral content of the bone. Bremsstrahlung measurements are difficult to calibrate in growing animals since the effective thickness of the absorber (i.e., bone) is changing as the skeleton mineralizes. Furthermore, as the skeleton becomes more "dense," the efficiency of bremsstrahlung production increases. These two factors may be self-canceling. In older animals, the self-absorption due to increased weight may decrease the efficiency of bremsstrahlung detection.

Thus it is necessary to use several calibrating sources in estimating Sr^{90} burdens in dogs fed the radionuclide over a major portion of their skeletal development period.

Sr^{90} Dosimetry. The important consideration in estimating dose to skeletons is the fact that a sizable fraction of the β energy from skeletally deposited Sr^{90}+Y^{90} is expended outside the skeleton, Mays (3) has calculated. From his findings for different mammalian species, one can calculate that this fraction can vary by a factor of 2 when men and mice are compared. In the case of an animal continually fed Sr^{90} from fetal age to adulthood, the fraction of β energy absorbed from the deposited radionuclides is continually increasing. Therefore the dose rate to the skeleton as well as the bremsstrahlung production can be expected to change with time. Based on information for the beagle skeletal growth curve, figure 1 shows how β energy might be absorbed over the life of a dog on a continuous Sr^{90} diet. In the fetus, it is estimated that 30 to 50% of the β energy of skeletally deposited Sr^{90}+Y^{90} is absorbed by the skeleton. While the pup is nursing, this figure increases to about 55 to 60%, and then as the dog matures the energy expended within the skeleton can reach 80-85%. The greater the percentage of β energy absorbed within the skeleton, the greater the bremsstrahlung production per μc Sr^{90} in bone. Over the range of skeletal weights encountered during the life span of a dog one could empirically estimate that each time the skeletal weight doubles, 5 percent more of the β energy from Sr^{90} + Y^{90} is absorbed by the skeleton (covering the approximate range from 10 gms to 1 kg of fresh skeleton). Parmley et al (4) have shown that this exponential relationship holds reasonably well over the skeletal weights included in the average dog growth curve, but that at higher or lower values the relationships are less variable per unit change in skeletal mass.

If additional factors are known, such as the rate of mineralization of the skeleton and the accretion and retention of the radionuclides during and after the feeding period, the average skeletal dose in rads can be estimated over the lifetime of the animal. In this particular experiment,

it is calculated that the dose-rate curve in dogs on a Sr^{90} diet from the onset of fetal ossification to adulthood (18 months old) will assume a steep positive sigmoid shape, with less change in dose rate at very early (fetal, nursing) and adult (post-puberty) ages. After the feeding period the dose rate will fall more slowly than an acutely labeled skeleton, following a power function whose main contributors are the rate of bone turnover, Sr reutilization, and the physical decay rate of the radionuclide. If all of the energy from one microcurie of Sr^{90}+ Y^{90} is delivered to a gram of bone, one can calculate that the daily dose rate in rads would be:

$$\text{rads/da} = \frac{\text{d/da/}\mu\text{c}}{\text{Mev/gm/rad}} \times \frac{\mu\text{c}}{\text{gm bone}} \times \text{Mev}_{\text{eff/d}}$$

$$\text{rads/da} = \frac{3.1068\text{x}10^9}{6.24 \times 10^7} \times 1.0 \times 1.12$$

$$\text{rads/da} = 57.38$$

However, since only a portion of the β energy is absorbed by the skeleton, it can be seen from Fig. 1 that on this basis alone, an adult dog would receive about 45 rads/day/μc Sr^{90} + Y^{90}, whereas a weanling pup would receive only about 20. These assumptions were combined with data on the rate of mineralization of beagle skeletons, realizing that immature skeletons do not have as much mineral per gram of bone as adults. Figure 2 therefore shows that, on a basis of μc Sr^{90}/gm Ca in bone, the calculated dose is less than in the preceding example. Note that a gram of bone may have only about 0.2 grams of calcium at maturity whereas at younger ages, when much of the skeleton is not mineralized, the percent calcium in bone is much less than at maturity, and the fraction of β energy absorbed is also lower. After the feeding period, the dose rate will decrease because of biological elimination of Sr^{90} and physical decay.

The foregoing approach has been used in calculating the skeletal dose received by dogs in the pilot experiment discussed in the Radiation Pathology Section. For discussion we have divided the lifespan of

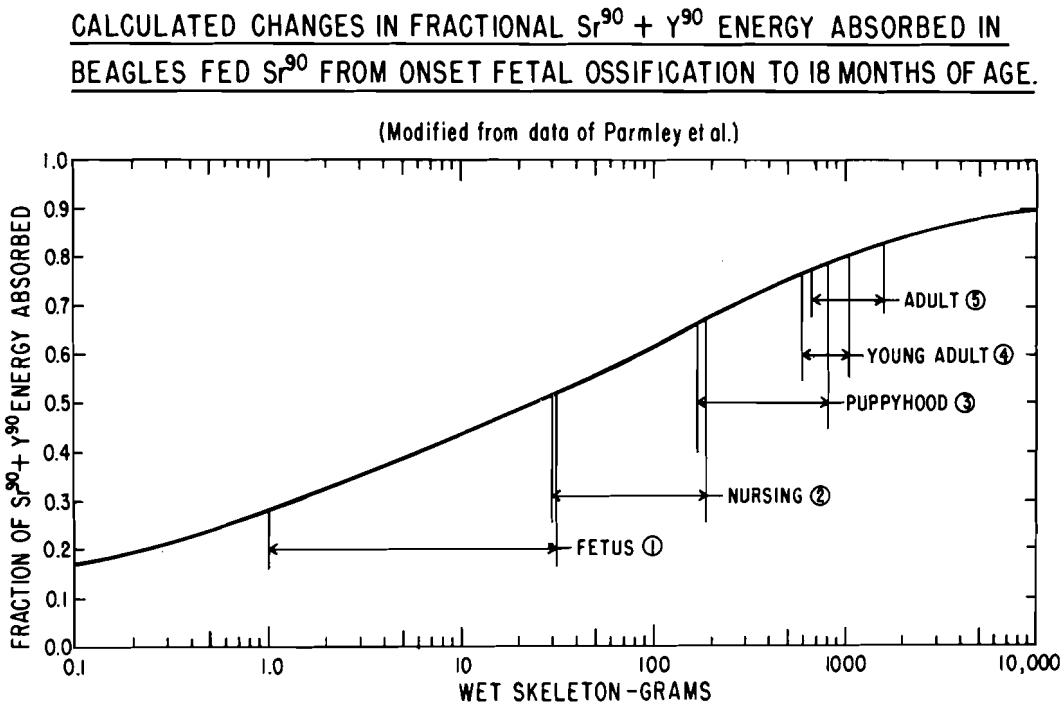

Figure 1

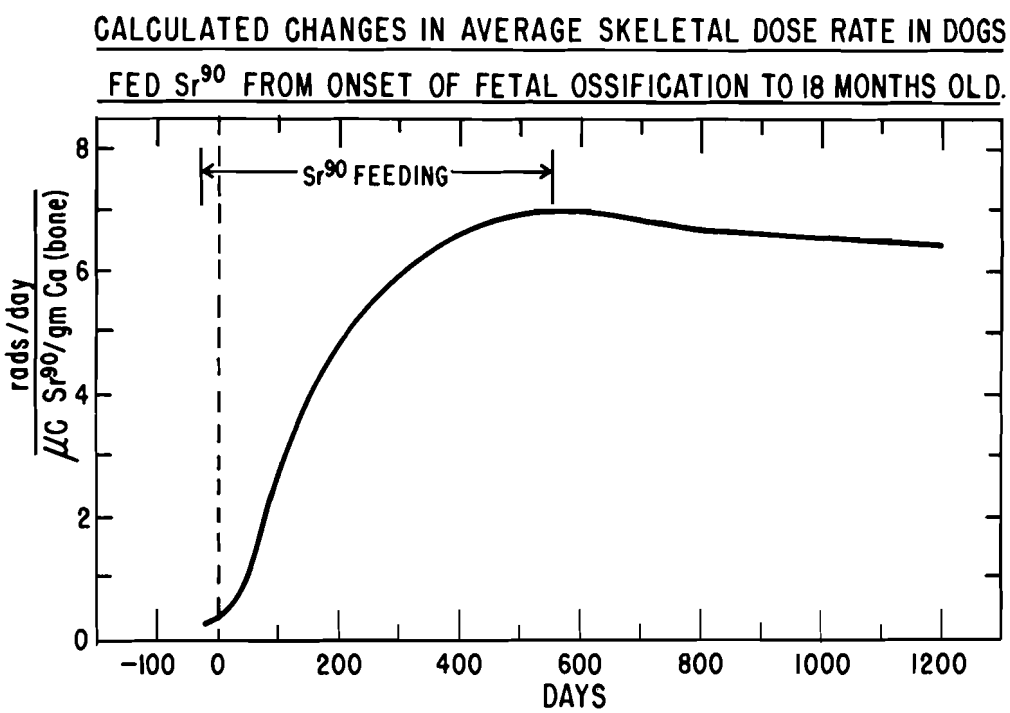

Figure 2

beagles in such a feeding experiment into 5 basic periods as summarized in Table 1.

Table 1

SIGNIFICANT PERIODS IN SR^{90} FED BEAGLES

Period	Age (days)	Comment
1 Fetus	-30 to 0	Onset of fetal ossification to birth, mineral accretion determined by placenta.
2 Nursing	0 to 35	Skeleton begins significant mineralization, rate determined by milk supply.
3 Puppyhood	35 to 250	Dog on radioactive feed, rapid accretion, greater Sr^{90} retention.
4 Young Adult	250 to 540	Epiphyseal closure, skeleton assuming adult size and mineral content.
5 Adult	540 to death	Sr^{90} feeding stops, Sr^{90} body burden decreases slowly.

At the end of Period 4, the skeleton is uniformly labeled with Sr^{90}. Radiochemical analysis* of bones from an animal sacrificed at the end of the feeding regimen indicated that the radionuclide was very evenly distributed both among bones and within the humerus (Fig. 3). It was observed that the percent variation from the average for the entire bone does not differ between trabecular and compact regions of the humerus. If compared to bones labeled by a single injection of radio-nuclide during adulthood, trabecular bone may have 10 times the activity of compact bone (5). During Period 5 it has been observed that following the cessation of Sr^{90} feeding, the loss of Sr^{90} from the uniformly labeled skeleton appears to be primarily from sites of trabecular bone. Figure 4 shows that the compact bone appears to have a much slower remodeling rate, and, in one instance studied (5S1), it did not lose more than a few percent of the average values at the end of feeding, although the total skeletal loss of Sr^{90} was about 20% in two years.

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* Performed by B. J. Stover, Radiobiology Division, Department of Anatomy, University of Utah.

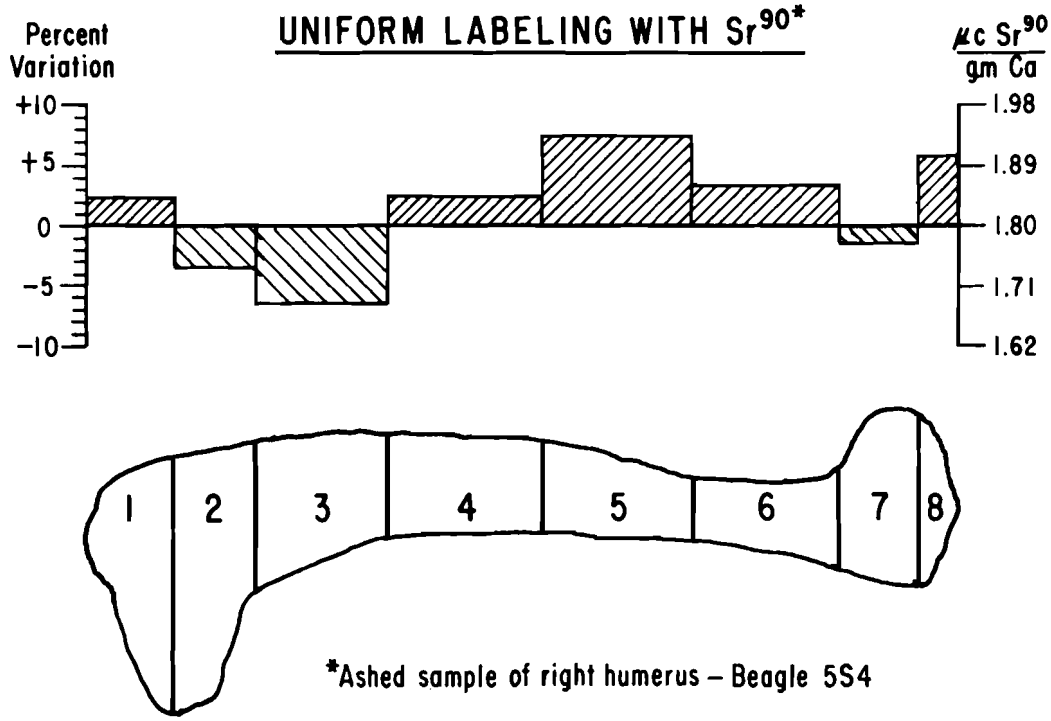

Figure 3

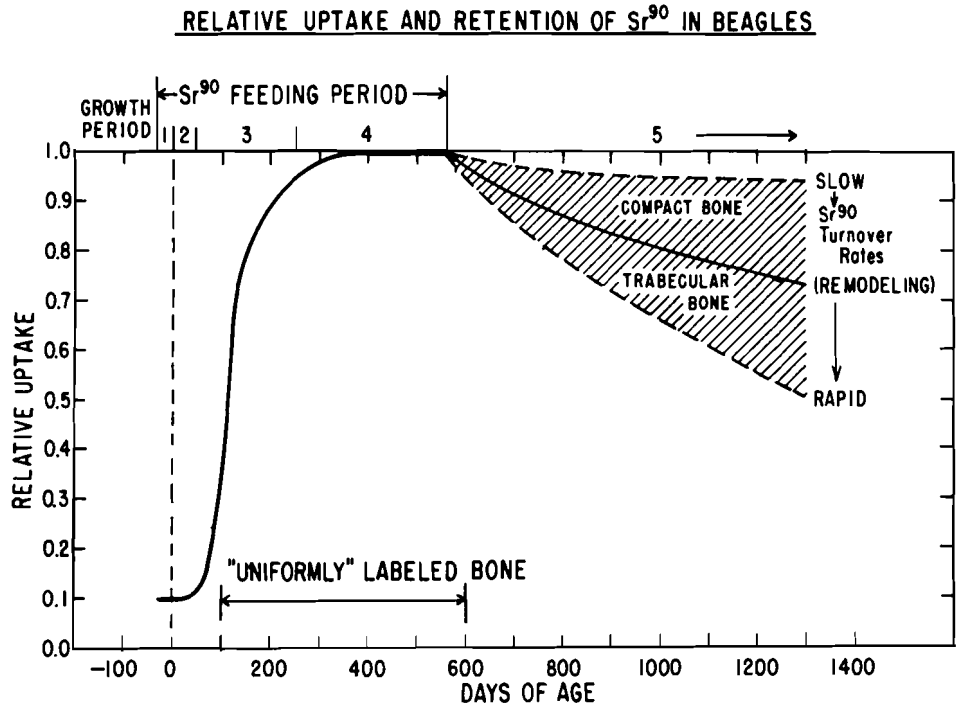

Figure 4

This observation suggests that, on the average, the uniformly labeled skeleton loses its radionuclides at a slower rate than the same burden deposited via a single injection at adulthood. Such might be the case if the adult type of acute deposition (injection) primarily involved trabecular areas of the skeleton with more rapid remodeling and diffusion constants, whereas in the uniformly labeled skeleton a comparatively larger fraction of the burden was deposited in compact bone.

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BIOPHYSICAL MEASUREMENTS

Part C

THE RETENTION OF INGESTED STRONTIUM IN BEAGLES

(Oral Discrimination)

R. J. Della Rosa and M. Goldman

Introduction

Strontium and calcium have been shown to be remarkably similar in behavior in biological systems (1). Studies of the metabolism of strontium and calcium in various species (cow, goat, sheep, rat, and man) have shown that the two elements, though qualitatively similar, have distinct quantitative differences (2). This differential behavior of strontium and calcium occurs mainly in such processes as (a) absorption from the gastrointestional tract, (b) reabsorption in the tubules of the kidney, (c) transfer across the placenta, and (d) secretion into milk. As a measure of this over-all behavior of the two elements, Comar et al (3) proposed the term Observed Ratio (OR) to represent the over-all behavior of the two elements between a given sample (e.g. skeleton) and its precursor (e.g. diet). The Observed Ratio is given by:

$$OR_{\text{sample-precursor}} = \frac{\text{Sr/Ca sample}}{\text{Sr/Ca precursor}}$$

The term Discrimination Factor (DF) applied to the particular physiological process that brings about the differential behavior indicates the contribution that it makes to the Observed Ratio. Table 1 presents Observed Ratios for some species. This differential behavior of ion pairs is currently thought to occur during physiological and biochemical processes that

Table 1

STRONTIUM-CALCIUM OBSERVED RATIOS (DIET TO BONE)*

Species	Diet	Method	Remark	OR Value
Cow	Normal	Double tracer	8 animals	0.21
Goat	Normal	Double tracer daily dose	2 animals	0.23
Sheep	Grass	Stable Sr/Ca ratios	6 animals	0.24 (0.15-0.31)
Sheep	Grass	Sr^{90}/Ca ratios	6 animals	0.23 (0.09-0.42)
Rat	Milk	Double tracer		0.57
Rat	Non-milk	Double tracer		0.27
Rat	Non-milk	Lifetime feeding of Sr^{90}/Ca		0.28
Rat	Non-milk	Stable Sr/Ca ratios		0.27
Man	Milk	Double tracer with each meal	4 patients (9 to 73 yrs.)	0.54 (0.50-0.62)
Man	Non-milk	Double tracer with each meal	4 patients	0.44 (0.37-0.51)
Man	Normal	Stable Sr/Ca in diet and bone	1 normal	0.24

* Taken from United Nations Report, 1958. Ref. 2.

involve ion transport across biological membranes. The preferential absorption of calcium over strontium may thus be attributed to differences in mass and size of ionic radii, differential binding by complexing agents, or to preferential movement by active transport mechanisms (4).

The absorption and retention of ingested radiostrontium in rats have been shown to be affected by age, and by the calcium and phosphate content of the diet (5, 6, 7). An increase in dietary calcium will proportionately decrease the retention of strontium in the growing rat. This effect occurs, however, only if the phosphate is elevated simultaneously with the calcium, that is, maintaining the normal calcium to phosphorus ratio (6, 7).

In the present experimental design, an estimate of OR would find practical use for predicting the Sr^{90} body burden in beagles continually ingesting the radionuclide at known Sr^{90}/Ca ratios. During the past year a series of experiments have been carried out to determine the absorption and biological retention of strontium, exploring such factors as age, single versus chronic ingestion, and the effect of varying the dietary calcium levels. The OR has been determined in:

A. beagles ingesting Sr^{90} at a constant Sr^{90}/Ca^{40} ratio from before birth (onset of fetal ossification) to 100 days of age;

B. beagles of different ages,

   1. 48-1550 days, maintained on a 1% diet calcium level and a Ca/P ratio of 1.5/1, following a single radioactive meal utilizing Sr^{85} and Ca^{47} as tracers;

   2. 83-2057 days, maintained on a 2.2% dietary calcium level and a Ca/P ratio of 1.6/1, following a single radioactive meal utilizing Sr^{85} and Ca^{47} as tracers;

3. weaned puppies that had received Sr^{90} *in utero* and during lactation from a dam with a previously acquired body burden of Sr^{90} (approximately 200 μc).

Methods and Results

A. Continuous Feeding.

(1) Newborn Content. Table 2 shows the Sr^{90} content of puppies born to dams continuously ingesting Sr^{90} (at a constant Sr^{90}/Ca^{40} ratio, 1% calcium level, and Ca/P ratio of 1.5/1) during pregnancy as outlined in the experimental design (8). The skeletons of the newborn (neonatal deaths) were removed from the carcasses and ashed at 600° C, and the ash was dissolved in dilute HCl. The radionuclide content was determined as outlined in Section VII.

Table 2

RETENTION OF SR^{90} IN NEWBORN BEAGLES (NEONATAL DEATHS)

Dog (level)	Wgt (gms)	Ash Wgt (gms)	Total Ca (gms)	Total P (gms)	Ca/P	μc Sr^{90} / gm Ca	OR**
D2F02*	210	---	1.67	1.02	1.64	0.004	0.07
D2F03	290	---	2.08	1.29	1.61	0.005	0.09
D2M05	260	---	1.67	1.05	1.59	0.004	0.07
D3F05	200	4.2	0.74	----	----	0.020	0.12
D3F06	240	4.1	1.59	0.61	2.61	0.008	0.05
D3F08	170	3.4	1.05	0.90	1.17	0.010	0.06
D3F09	190	4.4	1.27	0.73	1.74	0.011	0.07
D3M03	180	3.5	1.12	----	----	0.009	0.06

* Identification of dogs and levels (8).

** $OR = \frac{Sr/Ca \text{ fetus}}{Sr/Ca \text{ dams' diet}}$

The Sr^{90} body burden of the newborn puppies was derived from dams on the D2 level (0.0556 μc Sr^{90}/gm Ca) and the D3 level (0.167 μc Sr^{90}/gm Ca). The Observed Ratios (newborn skeleton to dams' diet) are seen to average 0.08 (range 0.05 to 0.12) and to be independent of the radionuclide content of the dams' diet.

(2) Retention at various ages. The retention of Sr^{90} in beagles ingesting the radionuclide continuously has been determined at 47 and 76 days for level D03, and at 53 and 100 days for level D2. The animals were sacrificed by electrocution and exsanguinated 12 hours after a final radioactive meal, and their skeletons grossly dissected. The skeletons were ashed at 600° C and the ash dissolved in dilute HCl. The skeleton of D2F04 was analyzed in sections (Table 3) because of the volume of material and the desire to compare the various sections.

Tables 3 and 4 show the retention of Sr^{90} (skeletal content) by growing beagles continuously ingesting the radionuclide. It is evident (Table 3) that the Observed Ratios (OR = Sr/Ca bone/Sr/Ca diet) of dogs at levels D03 and D2 (0.45 and 0.33, and 0.37 respectively) are not significantly different, and that the Observed Ratio is not affected by the difference in age, that is, the degree of skeletal development.

The retention of Sr^{90}, skeletal content, in beagle D2F04 is presented in Table 4. The range of Observed Ratios for the different fractions is not interpreted as a lack of uniform labeling. The values obtained are consistent with the variability in sampling techniques and biological materials.

Percent retention of the ingested radionuclide can be estimated from the daily consumption record of each animal and the total Sr^{90} content of

Table 3

RETENTION OF SR^{90} IN GROWING BEAGLES

Dog (level)	Age (days)	Wt (kg)	Ash Wt (gms)	Total Ca (gms)	Total P (gms)	Ca/P	Total µc Sr^{90} Fed*	Total µc Sr^{90} in bones	% reten-tion	µc Sr^{90} / gm Ca	OR**
D03F07	47	0.69		7.82	5.78	1.35	0.013	0.004	30.8	0.00069	0.45
D2F05	53	1.56	40	12.71	7.52	1.69	1.50	0.26	17.3	0.02033	0.37
D03F01	76	2.56	65	24.28	11.32	2.14	0.118	0.011	9.3	0.00051	0.33

* Estimated from total no. of packages fed, percent consumed, and µc Sr^{90}/gm Ca measured at each dose level.

** $OR = \frac{Sr/Ca \text{ bones}}{Sr/Ca \text{ diet}}$

Table 4

RETENTION OF SR^{90} IN A BEAGLE AT 100 DAYS OF AGE*

(Distribution within Skeleton; Dose 0.0556 µc Sr^{90}/gm Ca)

Fraction	Wt. Ash (gms)	Sr^{90} (µc)	Total Ca (gms)	Total P (gms)	Ca/P	µc Sr^{90} / gm Ca	OR	Total Sr^{90} Fed (µc)	% retained
Vertebrae	15.1	0.133	4.48	----	----	0.0293	0.53		
Skull and Jaw	33.0	0.268	10.81	5.84	1.85	0.0247	0.45		
Long bones	35.0	0.320	7.66	6.67	1.15	0.0396	0.71		
Ribs	14.2	0.123	4.62	3.04	1.52	0.0267	0.48		
Pelvis and Scapulae	9.0	0.090	2.81	1.70	1.65	0.0316	0.57		
Total	106.3	0.934	30.38			0.1519		8.9	10.5
Average						0.0306	0.55		

* Wt. = 4.45 Kg.

the skeleton. Thus, beagle D2F04 ingested 51 packages of food in 49 days (8.9 μc Sr^{90}). The skeleton contained 0.934 μc, 10.5% of the amount ingested. In like manner, beagle D2F05 retained ≈ 17.3%, D03F01 retained ≈ 9.3%, and D03F07 retained ≈ 30.8%.

B. Single Feeding* (Double Tracer Studies).

(1) Retention of Sr^{85} and Ca^{47} at a 1% Dietary Calcium Level. The absorption and retention of strontium and calcium by beagles of different ages were determined following ingestion of a single meal containing known amounts of Sr^{85} (carrier-free, 0.5-mev gamma ray) and Ca^{47} (1.3-mev gamma ray). The energies of the gamma rays are sufficiently different to permit good electronic differentiation.

Standards of each radionuclide were prepared at the time of feeding by placing 200 μc Sr^{85} or 500 μc Ca^{47} in a 2-liter plastic bottle the size of the smallest test animal. Annular cylinders of paraffin were prepared to approximate medium- and larger-sized animals.

A total of eleven purebred beagles (9 females and 2 males) were used ranging from 48 to 1550 days of age. Before the experiment the animals had been maintained on a 1% calcium diet (Ca/P ratio of 1.5/1) for several months, in most cases since birth.

The radioactive meals were prepared by adding equivalent aliquots of the radionuclides as used in the standards to a mixture of a dry kibble ration (60%), meat (20%), and water (20%). To ensure complete consumption, the food was offered after a short fast--with very young animals, in two

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* The authors acknowledge the cooperation of Dr. C. W. Mays in the planning and execution of this experiment. Dr. Mays is with the Radiobiology Division, Department of Anatomy, University of Utah, Salt Lake City, Utah.

divided portions a few hours apart.

Within one hour of ingestion, the total radioactivity of the radionuclides, in the dogs as well as standards, was measured by whole-body counting of the gamma rays (8). The dogs were restrained in a counting box in a manner that maintained a constant geometry between the animal and the detector. The dogs did not require anesthesia or medication of any kind during the 1- to 2-minute daily counting time. Counting the standards and dogs alternately eliminated any need for corrections for physical decay.

Figure 1 shows the biological retention curves of each radionuclide expressed as the percent of administered dose versus age. The retention curves indicate that Sr and Ca are absorbed differently. The retained fraction of strontium and calcium decreases rapidly with increasing age of the animal. After 250 days there is little effect of age on the percent retention of the two radionuclides, averaging about 5% for calcium and 2% for strontium between 3 and 9 days after administration. The highest retention in the youngest dog was about 43% for calcium and 19% for strontium.

Figure 2 shows Observed Ratios expressed as the ratio of Sr^{85}/Ca^{47} in the body to Sr^{85}/Ca^{47} in diet. The line was drawn through OR = 0.44, the average OR for all ages. Thus, under the conditions of this experiment, no effect of age was evident.

(2) <u>Retention of Sr^{85} and Ca^{47} at a 2% Dietary Calcium Level</u>. The effect of increased dietary calcium on the retention of strontium and calcium was studied in five beagles maintained on a compounded biological diet containing approximately 2% calcium with a Ca/P ratio of 1.6:1. The dogs were kept on this diet for 30 days prior to the experiment to allow

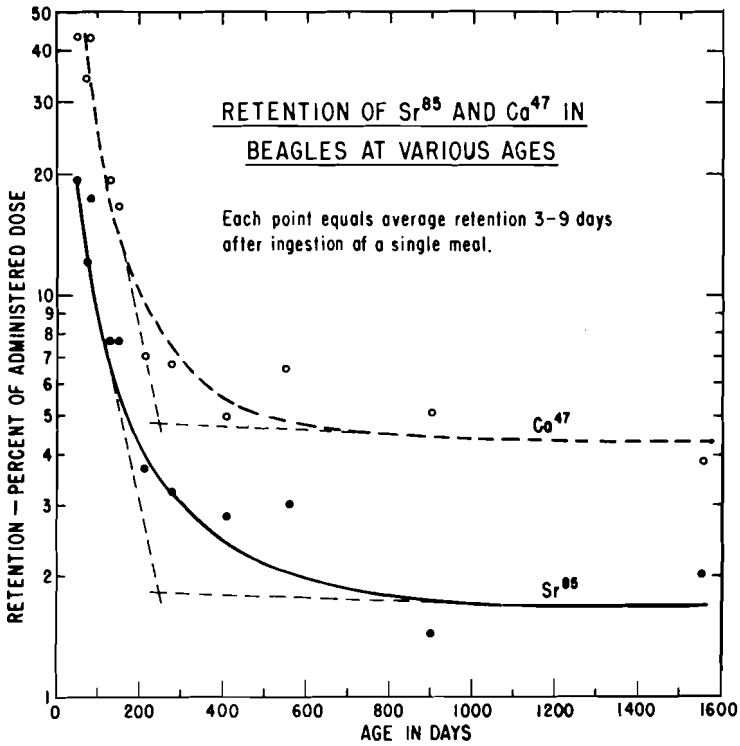

Figure 1

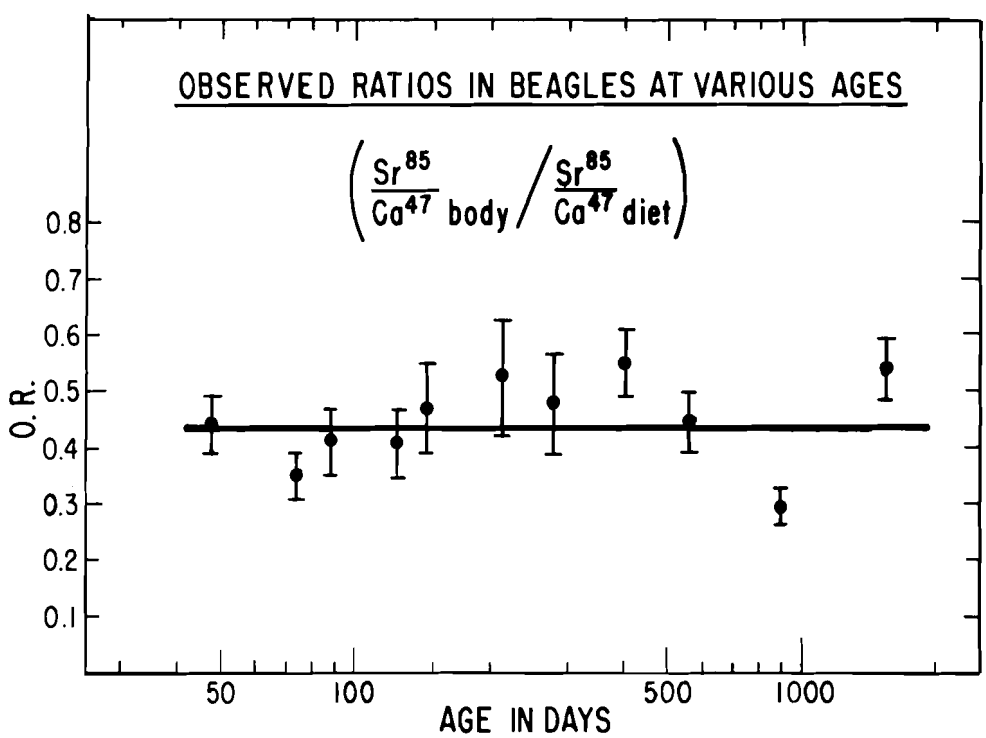

Figure 2

adaptation and skeletal equilibration to the increased calcium intake. All other conditions were the same as described above.

The data (Table 5) are compared with that obtained from the 1% calcium diet. The percent retention (3-9 days after ingestion) for both Sr^{85} and Ca^{47} is significantly reduced in the beagles on the 2% calcium diet. A twofold increase in calcium ingestion resulted in about a twofold decrease in the retention of Sr^{85} and Ca^{47}. The OR (body-diet) was essentially the same at both levels of calcium intake.

C. Retention of Sr^{90} in Weaned Puppies.

The biological retention of Sr^{90} in a litter of 8 puppies receiving the radionuclide *in utero* and during lactation was determined by whole-body counting* (bremsstrahlung) of the puppies. The maternal Sr^{90} body burden had been acquired (one year) prior to breeding, and the dam had been maintained on a non-radioactive diet containing 1% calcium and a Ca/P ratio of 1.5/1. Therefore, the body burdens of the puppies would represent newly mobilized skeletal radiostrontium. The OR was estimated from the body burdens of the puppies and a radiochemical analysis of the milk** obtained during lactation.

The average body burden of the weaned puppies was 0.66 μc Sr^{90} per puppy. At an average body weight of 2 kg or approximately 12 gm of skeletal calcium (8), this would be equivalent to 0.055 μc Sr^{90}/gm Ca.

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* Performed by Dr. C. W. Mays.

** Performed by Dr. Betsy Stover, University of Utah, Radiobiology Division, Department of Anatomy, Salt Lake City, Utah.

Table 5

RETENTION OF Sr^{85} AND Ca^{47} IN BEAGLES

(Percent of Dose: Average 3-9 Days after Ingestion of Single Meal)

Age (days)	1% Ca Diet			2% Ca Diet		
	Sr^{85}	Ca^{47}	OR*	Sr^{85}	Ca^{47}	OR
48	19.2	43.7	0.44			
74	12.0	34.1	0.35			
79	17.3	43.2	0.41			
83				8.7	16.9	0.52
83				10.0	17.1	0.59
94				12.9	23.6	0.55
125	7.8	19.2	0.41			
145	7.8	16.7	0.47			
215	3.7	7.0	0.53			
279	3.2	6.8	0.48			
405	2.8	5.1	0.55			
560	3.0	6.6	0.45			
589				1.2	2.4	0.49
900	1.4	5.0	0.29			
1550	2.0	3.8	0.54			
2057				1.1	2.1	0.52
Average			0.44			0.53

* $OR = \frac{Sr/Ca\ body}{Sr/Ca\ diet}$

The milk assayed at 1.11×10^{-4} $\mu c\ Sr^{90}/mg\ Ca$ or $0.11\ \mu c\ Sr^{90}/gm\ Ca$. Thus, the $OR_{puppy\text{-}milk} = \frac{\mu c\ Sr^{90}/gm\ Ca\ puppy}{\mu c\ Sr^{90}/gm\ Ca\ milk} = \frac{0.055}{0.11} = 0.5$.

Discussion and Summary

Evidence of the preferential absorption and retention of calcium over strontium in beagles was obtained from animals on a continuous Sr^{90} ingestion regimen and from animals receiving a single tracer dose of Sr^{85} and Ca^{47}. The Observed Ratio (OR) was used to represent the over-all differences in the metabolic behavior between the two elements. The Observed Ratios obtained in beagles (0-2057 days of age) under various experimental conditions are summarized in Table 6. Note that the values for the beagle appear to be of the same magnitude as those quoted in Table 1 for man. It is possible that the metabolic (pathways) processes, or the dietary qualities of the two species, are more similar than in the other species studied. One might interpret these observations as further justification for extrapolating to humans the data obtained in beagles.

Data obtained thus far indicate that:

1. the biological retention of strontium and calcium in growing beagles decreases with increasing age;
2. the OR values do not change with age, being nearly the same for all ages studied;
3. the OR values obtained for single versus chronic ingestion are nearly the same, respectively averaging 0.44 and 0.42;
4. an increase in the dietary calcium intake (maintaining a constant Ca/P ratio) decreases the retention of both strontium and calcium without greatly affecting the OR.

Table 6

STRONTIUM-CALCIUM OBSERVED RATIOS IN BEAGLES*

No. of Dogs	Age (days)	Diet	Method	OR*	Remarks
8	0	1% Ca (Ca/P 1.5/1)	Continuous Sr^{90} ingestion (pregnancy)	0.08 (0.05-0.12)	Neonatal Deaths
4	47-100	"	"	0.42 (0.33-0.55)	
11	48-1550	"	Double Tracer (Sr^{85} and Ca^{47}) Single Meal	0.44 (0.29-0.55)	
5	83-2057	2% Ca (Ca/P 1.6/1)	"	0.53 (0.49-0.59)	
8	40	Milk (dam on 1% Ca Ca/P 1.5/1)	Continuous Sr^{90} ingestion	0.5	Single litter of weaned puppies

* Body (or bone) - diet.

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THE STANDARD BEAGLE

SKELETAL GROWTH AND DEVELOPMENT*

M. Goldman

Analyses completed on 92 beagles of varying ages can be applied to predicting skeletal growth rates from external measurements. The scope of the study, and the need for such information, have been discussed (1, 2). Since this project concerns lifetime measurements of the effects of bone-seeking radionuclides, it is of considerable interest to know as much as possible about skeletal growth and size in this relatively stable colony. Such data have practical applications in dosimetry calculations, studies on mineral metabolism, identification of direct and indirect radiation effects upon the skeleton and its growth, and pathological-anatomical analysis of long-term effects. In addition, very little information on the growth and development of the beagle skeleton, or of dogs in general for that matter, includes sufficient quantitation for venturing beyond the realm of qualitative descriptions.

It is recognized that 92 animals of both sexes and varying ages do not, by any means, fulfill the requirements for such quantitation, but the information is hopefully offered as a step in that direction. The reader must be further cautioned about the author's use of the word "standard." Our definition is limited to animals reared under the special environmental, nutritional, and genetic conditions of this colony. It is not suggested

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* The contributions of H. R. Parker, J. R. Luick, R. Chance, and A. M. Gau in collecting the material used in this study are appreciated.

that these parameters apply to all beagles or localities. Many of the criteria used in maintaining this colony may be quite unacceptable to other investigators. What we do suggest is that under our experimental conditions, it is possible to draw some general conclusions concerning the manner and rate of growth in the beagle.*

This report describes the derivation of predictive equations for estimating various "internal" (i.e., physical) measurements. The first question raised in this analysis was how one could best estimate the total skeletal weight of a living dog. One estimate that has assumed wide acceptance is that, in many mammals, the skeleton is 10% of the body weight. This figure was used in the initial experimental design for this project. However, it is known that as animals reach old age, obesity may occur and cause estimates of skeletal weight to exceed actual weight. In addition, during the early, rapid-maturing phase of an animal's life span there is no assurance that the ratio of skeletal to body weight remains constant. The immature skeleton is less mineralized than its adult counterpart. The further this line of thinking is pursued, the greater the probability that for only one brief period in life does the skeleton account for 10% of the body weight. Preliminary analyses of our data support this contention (2).

These data indicate that the ratio of skeletal to body weight is constantly changing throughout the life of the dog. It is about 0.12 at birth, decreases to 0.08 during maturity, and in senescence decreases further to 0.06-0.07. Thus it is suggested that such estimates are usually not very accurate, and although they are convenient their shortcomings

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* The beagles used in this study were surplus to the experimental needs of AEC Project 4, where they were raised. They were carefully selected on this basis of good health and as being representative of standards set for the beagle breed.

should be recognized.

Our data suggested possible analysis along the classical approach advocated by D'Arcy W. Thompson, P. B. Medawar, J. S. Huxley, and others, whose researches into growth and form over the last 70 years have been partially summarized (3).

We discussed the development of the fetal dog in a similar manner (2). The fetus appears to develop in an allometric fashion due to its multicative growth. By measuring its length or weight or knowing its age, the remaining fetal parameters can be predicted quite well. Weight of fetus was exponentially related to its age, and classically followed a power function relation to the length. The data also suggested an exponential relation between fetal age and mineralization rates.

After birth, however, these simple relations no longer hold. The rate of skeletal growth follows a different curve from that which could describe the entire animal. Simply stated, during early puppyhood the skeleton grows at a faster rate, on a mass basis, than the entire dog. This rate is continually decreasing, but never assumes a constant value. The "plateau" reached at puberty continues until senescence, whereupon it again assumes an increasing negative slope. It is not the purpose of this report to discuss the many physiological interactions that govern the rate processes whose sum is known as growth. A complete understanding of their integration would provide a firm basis for predicting absolute growth patterns of any organ system.

The information now available permits estimates of relative growth rates. For example, how does the femur develop in relation to the entire

skeleton or the hind limb? Are there valid correlations between height and appendicular skeletal mass and between length and axial skeleton size? Does the time of epiphyseal plate closure mark a significant change in the growth pattern of that bone? How important are age and body weight in skeletal size estimates? Are some parameters in predicting skeletal information of greater importance at certain times during the life span than at other times? Such are the types of questions we attempted to answer within the limits of the experimental material available.

As a first approach to the data, it was decided to attempt a multiple regression analysis in which up to 27 independent variables were used in predicting total skeletal weight.* These are summarized in Table 1. The first six variables are easily obtainable from the living animal. Considerable past effort has been devoted to the appendicular long bones. Consequently, they were analyzed in detail. Since the skull composes one-fifth of the skeleton of the dog and the mandible and pelvis are also of considerable interest, these were also included. The skeleton was divided into 21 parts in collection of the data. Nine of these 21 parts (Table 1) were included in this analysis.

A statistical analysis of the data was devised and programed in a manner compatible with a computer treatment of the data. The data were first coded on I.B.M. punch cards. To maintain a more linear relation between values, all the variables were transformed into their $\log_{10}$ values. The multiple regression analysis was then performed on an I.B.M. 650 computer.

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* Statistical analysis performed by Leon Rosenblatt, Animal Breeding Consultants, Livermore, California.

Table 1

VARIABLES USED IN ADDITION TO SEX IN MULTIPLE REGRESSION ANALYSIS

No.	Variable	No.	Variable
1.	Height*	15.	Skull length
2.	Length	16.	Skull zygomatic factor
3.	Width (at 10th rib)	17.	Skull cranial factor
4.	Depth (at 10th rib)	18.	Radius length
5.	Body weight (gms)	19.	Radius diameter x 10
6.	Age (days)	20.	Radius factor = $Lx\ (\frac{10D}{2})^2$
7.	Femur length	21.	Ulna length
8.	Femur diameter	22.	Ulna diameter x 10
9.	Femur factor = $Lx\ (\frac{D}{2})^2$	23.	Ulna factor = $Lx\ (\frac{10D}{2})^2$
10.	Humerus length	24.	Tibia length
11.	Humerus diameter	25.	Mandible-coronoid length
12.	Humerus factor = $Lx\ (\frac{D}{2})^2$	26.	Mandible-condyle length
13.	Skull zygomatic width	27.	Pelvis length
14.	Skull cranial width	28.	Total skeleton (gms)

* All measurements are in cm unless otherwise noted.

The computer program used was a "stepwise regression" in which each variable that entered the regression analysis was evaluated by F test to determine whether it would make a significant contribution to the regression. An F value of 1.8 was chosen since it approximated the 0.20 level of significance for the number of degrees of freedom available. In this manner, the variable was tested against an F value that was neither too stringent nor too lenient from a practical statistical viewpoint. Thus, for each variable tested, the higher its F value the greater its contribution to the resultant predictive equation.

The problems were solved in several fashions, each solution resulting in a predictive equation that used one to seven of the twenty-seven variables available. Each of the 18 problems analyzed by the computer was independent of the other problems. The results of the analyses indicate that, with these data, total dry skeletal weights can be predicted with errors ranging from 1.6% to 8.4%.

Dry skeletal weights were used in the computations rather than fresh (wet) weights, to eliminate one of the larger variables, i.e., the inability to dissect-out bones repeatedly without errors in moisture content. Preliminary examination of the data indicate that there may be a relatively more constant relation between mineral content of bones and their dry weight. Further analyses are under way to relate the variables used in these equations to chemical composition of bones. The dry-weight estimate is therefore an intermediate figure between a true chemical determination and a biologically important figure (live skeleton weight). The former is necessary in evaluating whole-body counts in a live animal (i.e., <u>in vivo</u>

estimates of $\mu c\ Sr^{90}$/gm Ca), and the latter is necessary in obtaining dose-rate figures (calculated on $\mu c\ Sr^{90}$/gm bone).

The following analyses are based on some 15,000 measurements of the animals used in these studies. The data, therefore, were quite adaptable to analysis by computer methods. Once the programs had been designed and the information properly coded, the actual computer analysis for each problem was completed in 10 to 40 minutes.

The problems were divided into several basic categories of increasing complexity. The results indicate that the more variables available to the analysis of a problem, the smaller the resultant predictive error.

The first breakdown of the data was to compute the data on a sex basis. Here the data were analyzed separately for males, for females, and for all dogs regardless of sex. The analysis was done without regard to age of the dog, and then repeated with age as a variable. When only the "external" (i.e., first 5 in Table 1) variables were considered, the resulting solutions were as reliable as those obtained when the dog's age was also available to the computer. In the latter case, age was not considered a significant contributor to the analyses.

The next approach was based on the appearance of the raw data. There was a suggestion that the dogs might be divided into two groups: those 35 to 250 days old ("immature") and those 250 to 550 days old ("mature"). Arrangement of the animals in this manner resulted in a set of solutions (i.e., males, females, and combined) that were again based on the first 6 variables.

The final set of analyses included all 27 variables, divided the dogs again at the 250-day age bracket, and considered males, females, and

all dogs. These equations provided estimates whose error was about 1.5 to 3.5%, whereas all the previous solutions indicated errors of 5 to 9%.

The results of these analyses indicated that in most cases the body weight was a major factor in predicting skeletal weight. It was used fifteen times in solving the eighteen problems. Table 2 ranks the physical factors (variables) in decreasing order of frequency, i.e., number of times used in the eighteen equations.

Table 2

Variable No.	Frequency	%
5	15/18	83
1	7/18	39
2	6/18	33
6	5/18	28
4	4/18	22
9	3/18	17
20	3/18	17
22	3/18	17
27	3/18	17
14	2/18	11
15	2/18	11
17	2/18	11
3	1/18	6
7	1/18	6
13	1/18	6
19	1/18	6
21	1/18	6
24	1/18	6
25	1/18	6

Many of the variables barely passed the F test and contributed very little to the equation, although the contribution was statistically significant at the level chosen. The rather random manner in which most of the variables appeared in each equation with rather low significance levels leads one to conclude that the data are not sufficient to make population predictions of any magnitude in most cases.

The conclusions of merit brought out by the analyses were that not only is age an important factor, but the stature of the dog can be of considerable aid in predicting skeletal mass. In addition, dividing the dogs roughly into two populations on the basis of age significantly improved accuracy of the prediction of skeletal mass. Table 3 summarizes the results of the problems and is divided into the sections described above. (10 of the 92 dogs were not included since they revealed omissions in measurements.)

Table 3-1

Using variables 1-5 to estimate dry skeletal weight (Y).

Problem 1 - all males	N = 32 ;	height, body weight
2 - all females	N = 46 ;	height, length, depth
3 - males plus females	N = 78 ;	height, width, body weight

Predictive Equations*

Problem 1 $Y = -1.69233 + 0.28487X_1 + 1.00519X_5$

2 $Y = -1.70600 + 0.34161X_1 + 0.49533X_2 + 0.33242X_4 + 0.67813X_5$

3 $Y = -1.74735 + 0.39480X_1 + 0.32463X_3 + 0.84302X_5$

*Note that in these and subsequent tables all values are the logarithm to base 10 so that the cm or gm values should be corrected to their respective log values to predict Y, which is the log 10 of the dry skeletal weight. The X subscripts refer to the variables in Table 1. The number of dogs and the variables used precede each set of predictive equations in these tables.

Table 3-2

Using variables 1-6 to estimate dry skeletal weight (Y).

Problem 4	- all males	N = 34	; height, body weight
5	- all females	N = 48	; length, body weight
6	- males and females	N = 82	; height, length, body weight

Predictive Equations

Problem 4 $Y = -1.71964 + 0.41295X_1 + 0.96320X_5$

5 $Y = -1.88991 + 0.74681X_2 + 0.85644X_5$

6 $Y = -1.76330 + 0.41271X_1 + 0.39487X_2 + 0.81165X_5$

Table 3-3

Using variables 1-6 for dogs between 35 and 250 days of age to estimate dry skeletal weight (Y).

Problem 7	- males	N = 17	; depth, body weight, age
8	- females	N = 26	; body weight, age
9	- males and females	N = 43	; body weight, age

Predictive Equations

Problem 7 $Y = -1.04623 - 0.36548X_4 + 0.78674X_5 + 0.48726X_6$

8 $Y = -1.29816 + 0.83433X_5 + 0.30664X_6$

9 $Y = -1.12509 + 0.74441X_5 + 0.38992X_6$

Table 3-4

Using variables 1-6 for dogs between 250 and 550 days of age to estimate dry skeletal weight (Y).

Problem 10	- males	N = 6	; body weight
11	- females	N = 15	; height, length, depth
12	- males and females	N = 21	; height, body weight

Predictive Equations

Problem 10 $Y = -1.37359 + 1.03054X_6$

11 $Y = -1.11129 + 1.01984X_1 + 0.56860X_2 + 1.08830X_4$

12 $Y = -1.36596 + 1.04784X_1 + 0.62623X_5$

Table 3-5

Using variables 1-27 for dogs between 35 and 250 days of age to estimate dry skeletal weight (Y).

Problem 13	- males	N = 17	; variables used from table 1 = 2, 4, 5, 6, 7, 22, 27
14	- females	N = 26	; variables used from table 1 = 9, 15, 17, 20, 21, 24, 27
15	- males and females	N = 43	; variables used from table 1 = 3, 5, 6, 14, 20, 22

Predictive Equations

Problem 13 $Y = -1.13401 + 0.48641X_2 - 0.49360X_4 + 0.53564X_5 + 0.67329X_6 - 0.69836X_7 + 0.35055X_{22} + 0.41742X_{27}$.

14 $Y = +0.18133 + 0.35265X_9 + 3.04753X_{15} + 1.79547X_{17} + 0.52574X_{20} + 0.53881X_{21} - 0.42787X_{24} - 0.48173X_{27}$

15 $Y = -0.63764 - 0.19368X_3 + 0.24878X_5 + 0.32289X_6 + 0.96648X_{14} + 0.20287X_{20} + 0.45020X_{22}$

Table 3-6

Using variables 1-27 for dogs between 250 and 550 days of age to estimate dry skeletal weight (Y).

Problem 16 - males	N = 6 ;	variables used from table 1 = 13
17 - females	N = 15 ;	variables used from table 1 = 5, 9, 14, 17, 19, 22
18 - males and females	N = 21 ;	variables used from table 1 = 5, 9, 15, 20, 25, 27

Predictive Equations

Problem 16 $Y = -0.48524 + 2.71998X_{13}$

17 $Y = -0.45065 + 0.33423X_5 + 0.22331X_9 - 1.36280X_{14} + 0.74053X_{17} + 0.84245X_{19} + 0.50307X_{22}$

18 $Y = +0.24249 \quad 0.16620X_5 + 0.34817X_9 - 0.41670X_{15} + 0.13454X_{20} + 0.52567X_{25} + 0.59622X_{27}$

Table 3-7

Percent error in estimating total dry skeletal weight for the predictive equations, including the errors in estimating either sex from equations in which sex was not a variable in the solution.

Problem		Average Error %	Range %
1 Males		8.4	0.5 to 19.5
2 Females		6.8	0.2 to 16.0
3 All dogs	Males	7.7	1.1 to 23.8
	Females	7.4	0.3 to 18.7
	Total	7.5	0.3 to 23.8
4 Males		8.4	0.1 to 24.9
5 Females		7.4	1.3 to 18.7
6 All dogs	Males	7.3	0.2 to 24.2
	Females	7.6	0.5 to 18.8
	Total	7.5	0.2 to 24.2
7 Males (35-250 days)		2.0	0.3 to 10.4
8 Females		6.1	1.1 to 15.0
9 All dogs	Males	4.3	0.6 to 17.8
	Females	6.9	0.2 to 18.2
	Total	5.9	0.2 to 18.2
10 Males (250-550 days)		5.5	2.1 to 12.0
11 Females		4.9	0.6 to 13.0
12 All dogs	Males	6.5	1.8 to 16.3
	Females	6.8	0.4 to 14.9
	Total	6.7	0.4 to 16.3
13 Males (25-250 days)		1.5	0 to 3.8
14 Females		2.0	.2 to 5.5
15 All dogs	Males	3.4	.1 to 7.0
	Females	3.3	0 to 9.8
	Total	3.3	0 to 9.8

Table 3-7 (Cont'd.)

Problem		Average Error %	Range %
16 Males (250-550 days)		3.2	.8 to 7.6
17 Females		1.6	.1 to 4.3
18 All dogs	Males	1.9	.6 to 4.1
	Females	1.9	.2 to 5.8
	Total	1.9	.2 to 5.8

By comparing the errors obtained with these equations, it appears that the most practical equations are those that predict well with a minimum number of variables. Of the 27 variables listed in Table 1, only the first six are easily obtained for any given dog. In addition, the best representation of the dog population is that which includes the greatest number of dogs. Therefore, the equations for problems 9 and 12, which include both males and females, are offered as the most practical approach to estimating the dry skeletal weight in the beagle.

As an example of how these equations can be used in a practical way, the following dog was randomly picked from the group and the skeletal weight estimated.

Dog No. 50 was a male, 201 days old, weighing 8510 grams. The equation to use would be number 9 (35-250 days; males or females). This equation is:

$$Y = -1.12509 + 0.7441X_5 + 0.38992X_6.$$

Therefore, the information necessary is X_5 (bodyweight) and X_6 (age), using the units as described in Table 1 for this dog:

X_5 = body weight in grams = 8510 gm; $\log_{10}$ value = 3.92993

X_6 = age in days = 201 days; $\log_{10}$ value = 2.30320

$Y = -1.12509 + 0.74441(3.92993) + 0.38992(2.30320)$

$Y = -1.12509 + 2.92548 + 0.89806$

$Y = 2.69845 = \log_{10}$ of dry skeletal weight = 499.4 grams.

Therefore, the equation would predict a skeletal weight of 499.4 grams (although these calculations were carried out to the fifth place, a slide rule would give a reasonably close value for Y with a minimum of effort). The resultant estimate is 6% too low, since the actual dry skeletal weight was 531.1 grams.

If a more accurate estimate is necessary, of a living dog, the equation in problem 15, which employs more information, could be used. Such information might in fact be obtainable from the X-rays of the dog, but this is not suggested as a routine procedure.

The limited scope of this study perhaps explains the inclusion of certain variables that were used very infrequently by the computer. Were a greater number of skeletons available, distributed over the normal life span of the dog, a general pattern would probably have evolved in which a minimum number of parameters were repeatedly used, each contributing to the equation a comparable degree of significance. Such appears to be the case with age, body weight, and height.

In summary, the results of these analyses indicate that a single ratio of skeletal weight to body weight is not a reliable estimate, since these two variables change at different rates during the life span of a beagle. The suggestion is offered that the best present estimate of dry skeletal weight in a living dog requires two general equations.

These equations (nos. 9 and 12) use body weight, age, and/or height to give average estimates to within 6% of actual values. If greater accuracy is required, equations 15 and 18, using a more complicated approach (including measurements from skeletal roentgenograms), can provide estimates of dry skeletal weight within an average error of 2 to 3%. It is suggested that the preceding approach can be of considerable practical use in dosimetry calculations involving chronic irradiation of the skeleton of long-lived species with bone-seeking radioisotopes.

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PATHOLOGIC SEQUELLAE IN BEAGLES FOLLOWING CONTINUOUS FEEDING OF SR^{90} AT A TOXIC LEVEL

A. C. Andersen and M. Goldman

In 1956, a long-range Sr^{90} feeding program was established with a preliminary experiment to determine the toxicity of body burdens calculated to reach values of about 27 μc Sr^{90}/Kg body weight at 19 months of age (one month after completing the feeding schedule). This report discusses clinical, sub-clinical, and post-clinical syndromes manifested by the dogs throughout the experiment.

Late in 1956, a female beagle of normal size and conformation was isolated in a metabolism cage after being bred. Throughout gestation and lactation she was fed ad libitum a specially prepared radioactive diet: by weight, 60% dry ration containing 3.27% calcium and 1.91% phosphorus, 20% water (including the Sr^{90}), and 20% hamburger. Sr^{90} was maintained at a constant Sr^{90} to Ca ratio, and the amount ingested per day ranged between 10-20 μc Sr^{90}. In January 1957, the beagle whelped 4 pups (2 males; 2 females, which were maintained on the same diet until 18 months old. While on the radioactive ration, one female (5S2) was bred and whelped a litter of six pups, likewise maintained on the Sr^{90} diet. Ten dogs were kept on the Sr^{90} diet, and except for one survivor (5S2) were studied after sacrifice or death. The information obtained is sufficient to illustrate the toxic effects to be expected in dogs fed continuously on a high level of Sr^{90}. It is estimated that the dogs ingested an average of 13 μc Sr^{90} per day.

A dam and stud of lineage comparable to that of the dogs fed Sr^{90} pro-

duced two sham-treated controls (male and female). Control data represent these dogs and those from our beagle colony.

The estimated body burden of the dogs reached 20-30 c Sr^{90}/Kg. Rate of deposition of the isotope during feeding and retention curves were estimated from radiochemical analyses of bones and whole-body gamma ray (bremsstrahlung) counting of survivors. Calculation of the resultant rad dose rate is complicated by the marked skeletal changes during the periods of interest. Figure 1 illustrates the cumulative rad dose of one dog and shows how the dose rate changes during the five periods of interest in this experiment.

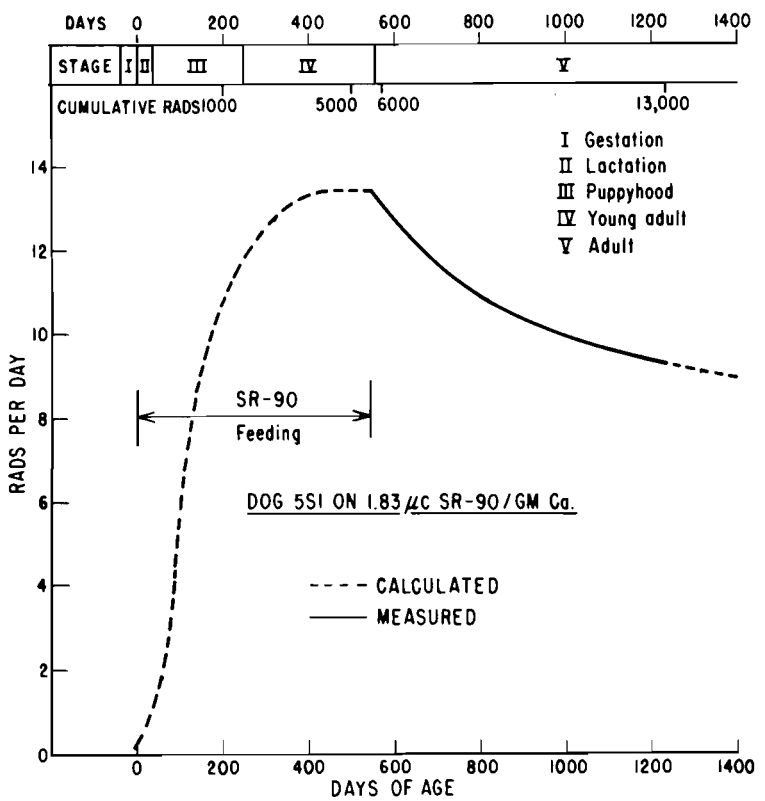


Figure 1: Skeletal dosage in beagles fed Sr^{90} during growth period.

The curve shows how the percent dose absorbed by the skeleton during the life of the dog is affected by growth, mineralization, and excretion. The early growth period is further corrected for discrimination during gestation and lactation, and for the fact that as the skeleton increases in size, more of the β energy from the radionuclide is absorbed (1). Young, rapidly growing dogs may differ from adult dogs in radiation sensitivity, especially to the skeleton and bone marrow, and this could influence the significance of the dose and dose rate at subsequent ages.

Under the conditions of this experiment the effects of high levels of Sr^{90} in the diet are noticeable quite soon. Histologic examination of the femur from a pup sacrificed at birth gave evidence of disturbed ossification although the skeleton appeared normal upon gross examination. Sections of the femur revealed alternate foci of necrosis and acellularity between active areas of osteogenesis. Despite histological evidence of defective ossification at birth, dogs maintained on the Sr^{90} diet showed normal development of the skeleton throughout growth, except for a sternal bulge. The xiphoid region protruded ventrally 2-3 cm until treated dogs were about one year old; then the defect subsided. Throughout growth, palpable lymph nodes and tonsils were estimated to be about one-half the size normal for the beagle. Peripheral blood examinations at monthly intervals showed the normal trend in values to maturity, but leukocytic values did not exceed 60-70% of the normal mean values for the beagle(2). Leukocyte counts ranged from 5,000 to 7,000/mm^3, but thrombocyte counts were normal, ranging from 110,000 to 430,000/mm^3. At 4 years old, $2\frac{1}{2}$ years after Sr^{90}-treatment ceased, the surviving dog has not yet attained normal blood values.

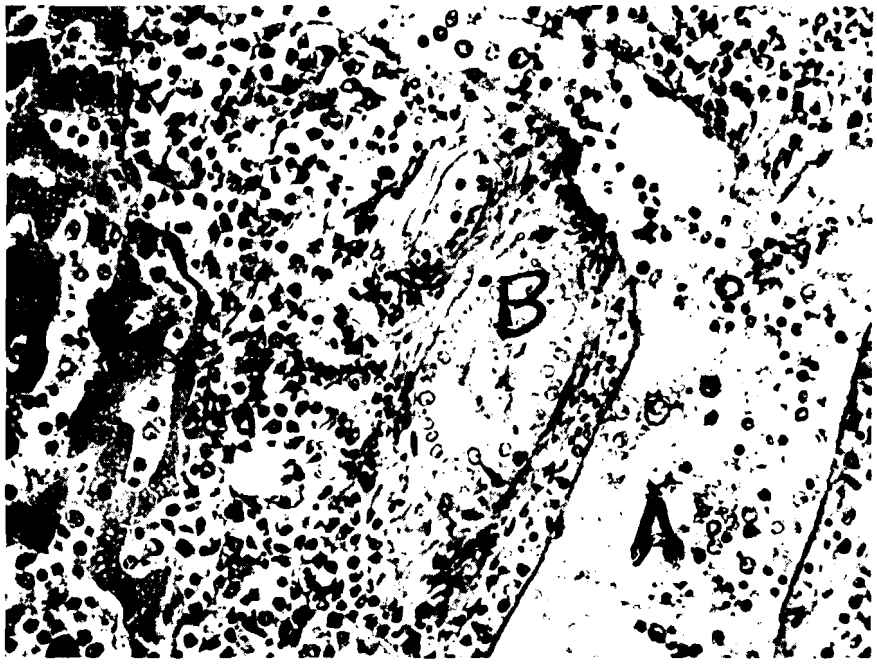
Two highly malignant neoplasms were observed during Sr^{90} administration. At $6\frac{1}{2}$ months old, one dog (5S5) showed signs of anorexia, bloody diarrhea, and rapid loss of body weight; death occurred 5 days later. Readily apparent at necropsy were marked icterus, widespread hemorrhage, and multiple neoplastic growths. Histological examination revealed the neoplasm to be a primitive type of lymphosarcoma (Figs. 2a, 2b).* As shown in Figure 2a, the lymphoblastic sarcoma had the characteristic feature of penetrating interstitial tissue; its metastatic ability was evidenced by lymphoblastic cells in peripheral blood during terminal illness and metastatic cells within the lumen of blood vessels after death.

A similar case occurred in one dog (5S3) at 15 months of age. Palpation of the retropharyngeal lymph nodes revealed progressive enlargement as terminal illness progressed. Upon necropsy, at 471 days of age, widespread hemorrhage and metastatic neoplasms resembled the other case. Blood examinations before death demonstrated a total leukocyte count of 9,250 per mm^3, of which 83% were lymphoblastic. Features of this neoplasm are unlike leukemia in that the vascular system merely acts as a vehicle for metastases.

Two dogs were maintained for longevity studies. At 38 months old, one dog (5S1) exhibited a posterior paresis that progressed to posterior paralysis. As osseous neoplasm in the 5th lumbar vertebrae was seen roentgenographically. The animal had to be sacrificed 7 weeks later. Roentgenograms (Fig. 3) at weekly intervals during the dog's illness gave evidence that the diameter of the neoplasm doubled in size every 16 days. At death, the tumor had spread to the 4th, 5th, and 6th lumbar vertebrae with extensions into neighboring muscle. The neoplasm was identified histologically as an osteogenic

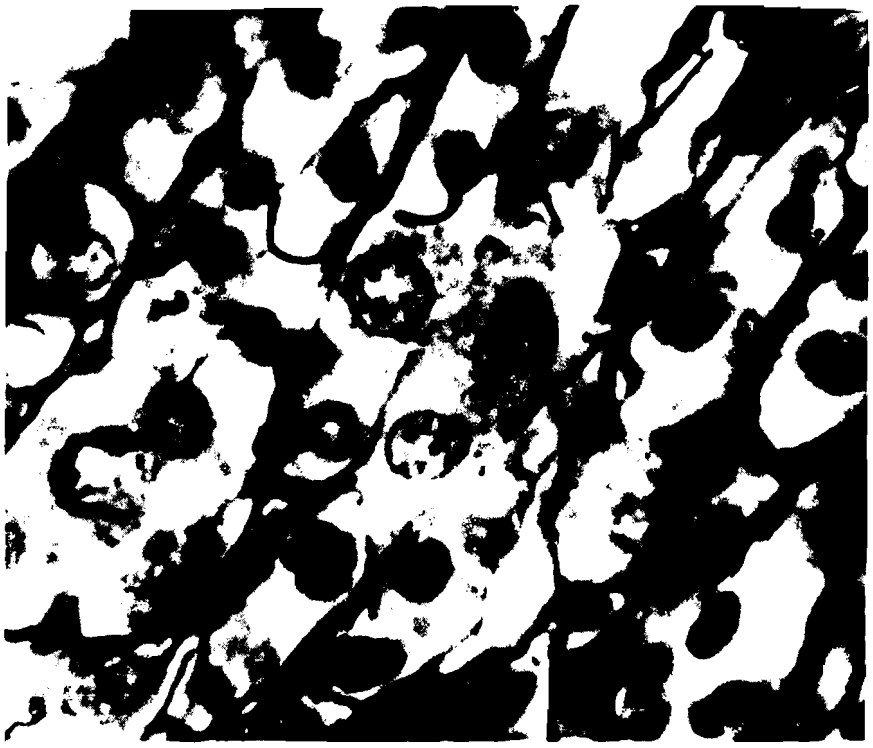
*Synonyms: malignant lymphoma, reticulosarcoma, reticulum cell sarcoma, large round cell sarcoma.

Figure 2a



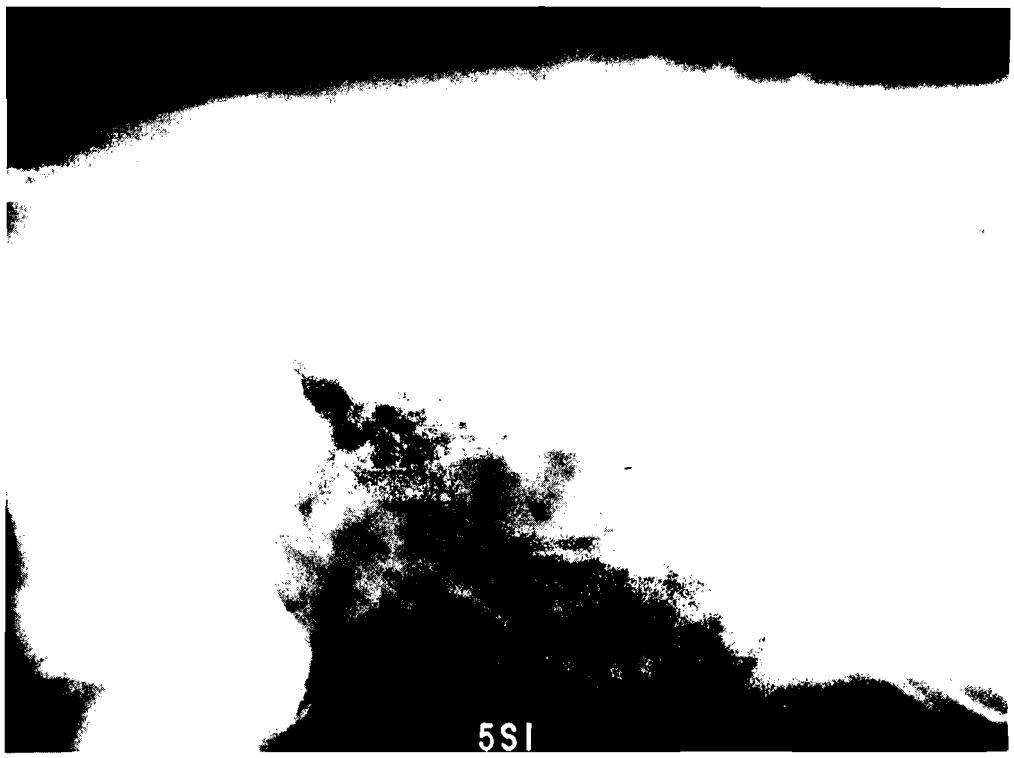
Sr^{90} induced lymphoblastic sarcoma. Invasion of portal triad and hepatic parenchyma. A, blood vessel containing metastatic cells, B, bile duct, C, hepatic laminae. Bouin's nitrocellulcse, hematoxylin and eosin, 100X.

Figure 2b



Higher magnification of lymphoblastic sarcoma, shown in Figure 2a, depicting various size cells and reticulum stroma. Bouin's, nitrocellulose, Foot and Menard method for reticulum, 1200X.

Figure 3



Roentgenogram of male beagle 5S1 showing neoplasm in the 4th, 5th, and 6th lumbar vertebrae and surrounding soft tissues. Posterior paresis followed by paralysis and death at 1226 days of age.

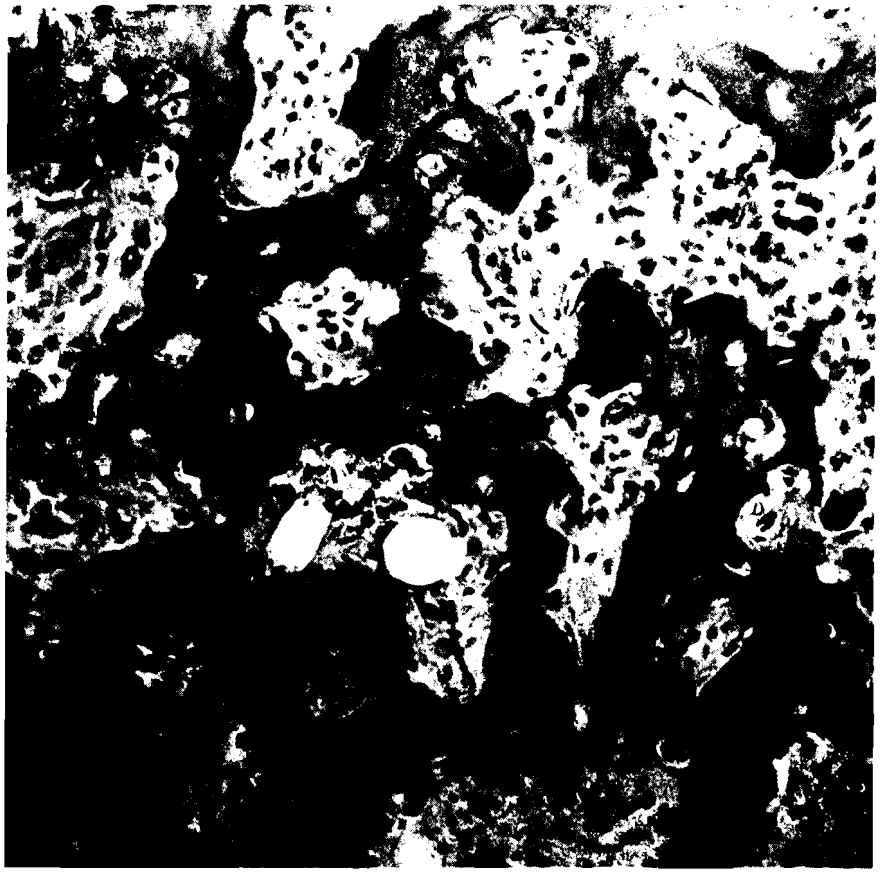
sarcoma (Fig. 3a) with multiple small metastases to the lung (Fig. 3b). Upon gross and microscopic study, the organ systems, including parathyroids and bulbus oculi, appeared normal. However, other skeletal damage was apparent both radiographically and histologically.

A 5-mm slice of the femur revealed two pathognomic lesions from radioisotope damage (Fig. 4). Autoradiograph (Fig. 4,A) demonstrated a circular non-radioactive area about 1.5 cm in diameter in the proximal metaphysis. This semi-solid noncalcified mass contained uniformly distributed pyknotic nuclei as evidence of an aseptic osseous necrosis; the necrotic area was avascular and encapsulated by dense bone. Figure 4, A and B, also demonstrated cortical areas of decreased bone density and radioactivity. Such areas have been studied histologically and roentgenographically in the last survivor (5S2) in this experiment.

Histological study of cortical bone revealed alternate areas of osseous necrosis and repair. Figure 5a, A, shows an area of cortical bone erosion containing necrotic osseous tissue and mesenchymal elements between spicules of compact bone. Histological study of surrounding areas gave evidence that osseous necrosis precedes fibrous tissue replacement (Fig. 5b, C). Thus, this lesion may be described as a circumscribed osteolytic fibrosis that is apparently progressive with advancing age. A similar lesion following radium deposition has been described in humans (3).

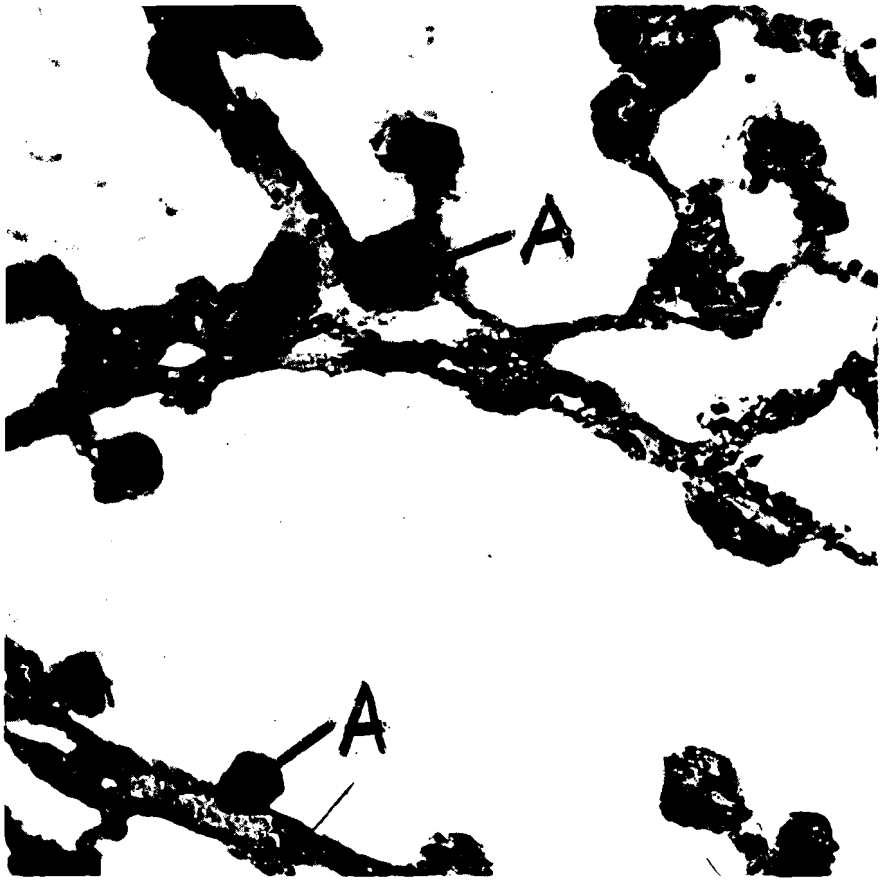
During the past year, we have been able to observe the rate of radiation-induced osteolytic fibrosis in the dog. As shown in Figures 6a and 6b, the lesion is not confined to wither endosteal or periosteal surfaces, and almost doubles in severity in about one year. Dogs surviving to maturity on this Sr^{90} level showed an increased radio-opacity in the metaphyseal

Figure 3a



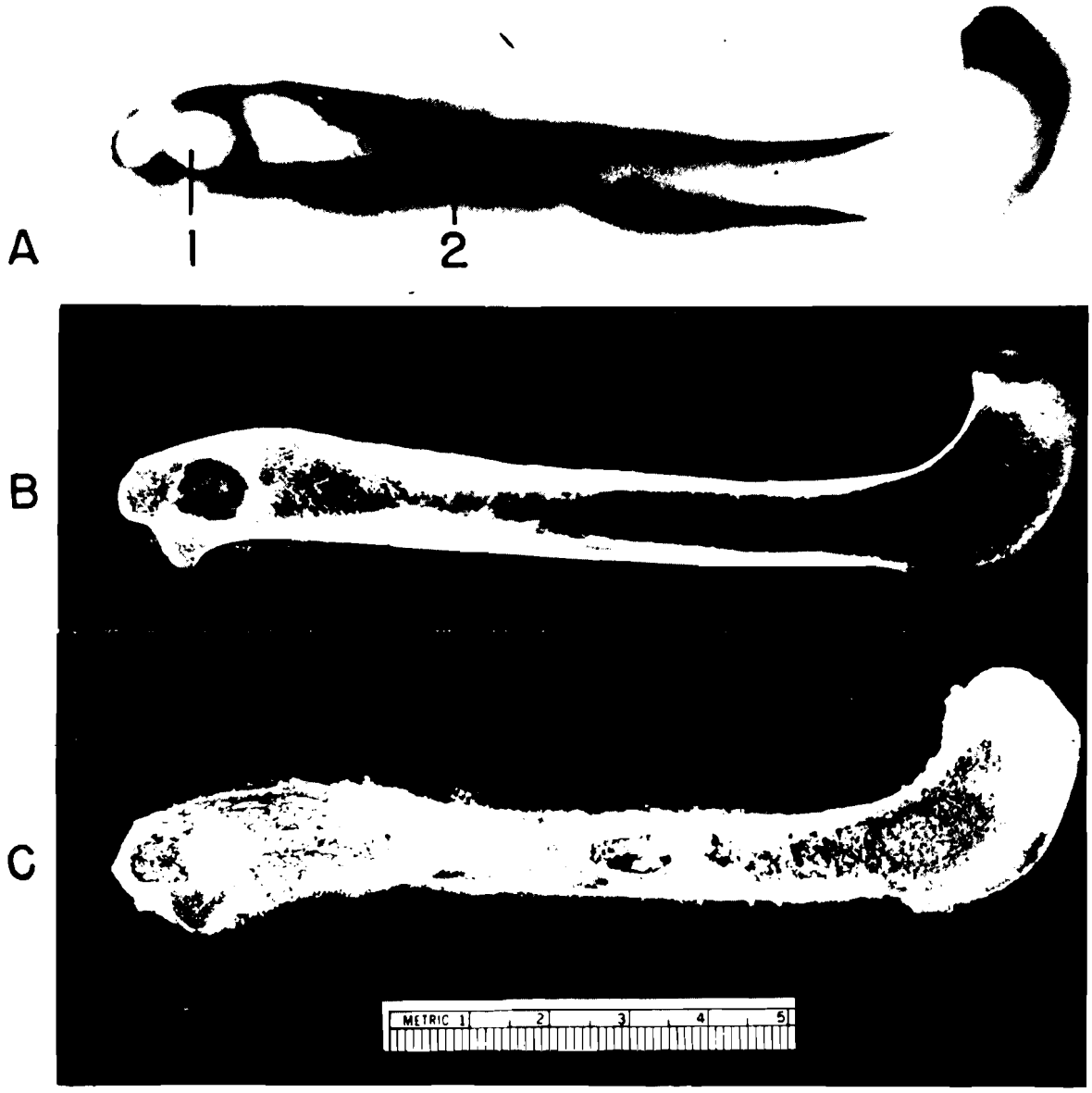
Photomicrograph of osteogenic sarcoma shown in Figure 3. The primary neoplasm consisted of rather uniformly distributed osseous and osteoid tissue. Bouin's, decalcified (formic acid method), nitrocellulose, hematoxylin and eosin, 100X.

Figure 3b



Islands of meta stases in lung parenchyma (A) from primary osteogenic sarcoma of the lumbar vertebrae (Dog 5S1). Nitrocellulose, Bouin's, H and E, 200X.

Figure 4



Right Femur – Beagle 5S1 (male).

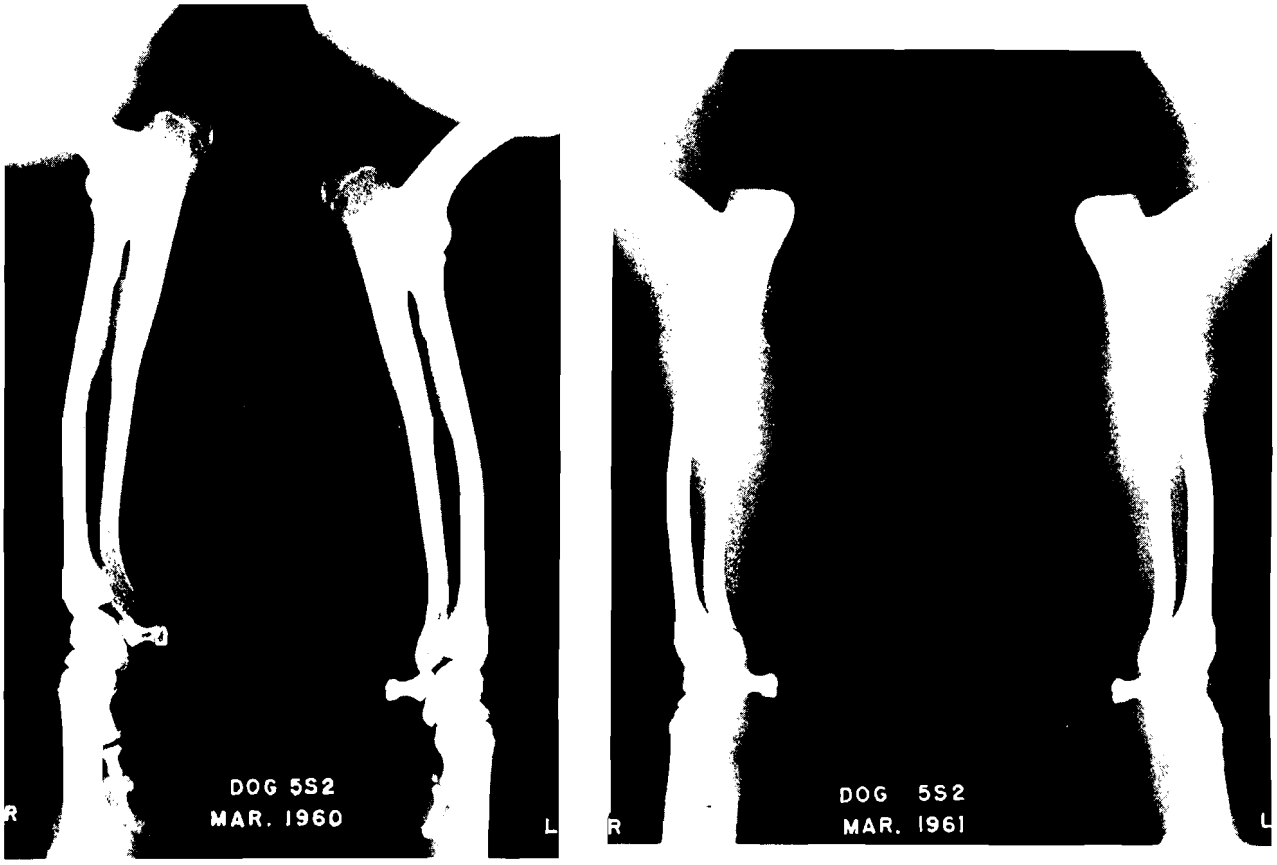
Femur slice, beagle 5S1. A-1 autoradiogranh showing absence of Sr^{90} in aseptic necrotic area, and A-2 decreased Sr^{90} content of circumscribed osteolytic area in diaphysis. B, roentgenogram: note decreased radio-opaqueness of diaphysis corresponding to A-2. C, photograph. 2/3 normal size.

Figure 5a

Erosion of compact bone in diaphysis of femur (Fig. 4, A-2). A, necrotic osseous mass surrounded by mesenchymal elements--osteoclasts, osteoblasts, and fibroblasts. B, normal compact bone. Such lesions were repaired by fibrosis. Bouin's, decalcified (formic acid method), nitrocellulose, 100X.

Figure 5b

Photomicrograph of apparently dormant osteolytic fibrotic area C shown in Figure 4, A and B. Vascular fibrous tissue has become organized. Bouin's nitrocellulose, 200X.



Figures 6a and 6b: Anterior limbs of beagle 5S2. Roentgenograms were taken at 3 and 4 years of age ($1\frac{1}{2}$ and 3 years after Sr^{90} treatment). Comparison of the diaphysis of humerus, radius and ulna reveal the slow progression of circumscribed areas of osteolytic fibrosis. The intense radio-opacity of the metaphyseal region in the distal radii persists. Same radiographic and photographic procedures were used in both figures.

region of certain long bones. This non-specific lesion, easily recognized in roentgenograms of the distal radius, is apparently irreparable.

In summary, this experiment demonstrated the sequence of pathological events in dogs fed high levels of Sr^{90} to early maturity (18 months of age). Malignant soft tissue neoplasms (lymphoblastic or reticulum cell sarcomas) preceded bone neoplasia. Similar results have been reported in mice, and the opinion is held that soft tissue neoplasia occur through indirect mechanisms rather than direct irradiation (4, 5). Two years after Sr^{90} administration, one dog succumbed to osteogenic sarcoma in lumbar vetebrae. Spontaneous osteogenic sarcomas are not uncommon in the dog, but their occurrence is largely restricted to long bones of large breeds (6, 7). As discussed by Upton (8), the mechanism of radiation-induced neoplasms appears to be a complex multistage process. The authors are in agreement with the opinions of others that osseous necrosis followed by early fibrosis constitutes the pre-cancerous lesion for osteogenic sarcoma (9, 10). The long-term effect of Sr^{90}, besides neoplasms, is a slow process in bone (osteolytic fibrosis) that forms non-osseous low-density tissue. Lecroix (11) has observed that bone remodeling is a continuous process throughout life with individual bone responses to radiostrontium being influenced by age dependent variables. Consequently, an adequate understanding of the effects of a constant Sr^{90} environment in osseous tissue must include consideration of the complex variables which contribute to the net effect.

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FURTHER STUDIES ON THE POST-IRRADIATION INTRAVASCULAR SUBSTANCE

A. C. Andersen and H. S. Winchell*

Introduction

The finding of an intravascular substance (IVS) after lethal total-body X-irradiation was discussed in previous reports (1, 2, 3). In brief, lethality from 300-r exposures consistently revealed large intravascular globules of an acidophilic substance that was PAS-positive, and erythrocyte-negative. Attempts to isolate the substance from tissue homogenates yielded complex material, but a mucopolysaccharide substance (Fraction C) was obtained in limited quantity by formamide extraction (4). This precipitate was likewise complex and would require considerable analytical procedures to remove contaminants. Although IVS has not been isolated, it is certainly present in animals that succumb to acute radiation exposures; it is easily recognized in histologic examination if procedures are proper. Alcohol-formol fixation and Pollak's rapid trichrome stain reveal intravascular globules larger than erythrocytes but of various sizes. These are frequently observed occluding blood vessels and associated with congestive and hemorrhagic lesions. The substance has been found in dogs succumbing to roentgen rays, neutrons (fast), and beta rays (oral Sr^{90}). A limited number of species

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* Cooperative study. Dr. H. S. Winchell, Lawrence Radiation Laboratory, Berkeley, California, has been conducting research studies on Y^{90} given systemically.

have been examined, and the contention remains that hemorrhage-prone species (swine; guinea pig) do contain large quantities of the substance following acute radiation exposure. Species less hemorrhage-prone (hamster, rat, mouse, rabbit) may show the substance; however, its identity is complicated by IVS droplets smaller than erythrocytes.

This study was undertaken to examine the role of some target organ or organs in the formation of IVS. Posteriorly exposed dogs (1,100 to 1,700 rad posterior to the liver) developed the substance, whereas those given comparable exposures anteriorly did not. Furthermore, over 1,100 rads to the exteriorized small intestine produced IVS that was identified in petecchial lesions of the kidney--an abscopal effect. The present study was made to evaluate the role of the integrity of the GI mucosa in the production of IVS. It was hoped that this could be done by comparing the finding of IVS following systemically and orally administered Yttrium-90. Y^{90} was selected for intestinal mucosal irradiation on the basis of its remaining within the lumen of the intestinal tract and its energetic ($\bar{E}_{AV}$ = 0.9 Mev) beta radiation. Thus, orally administered Y^{90} Cl_3 would irradiate the gastrointestinal mucosa almost exclusively. The comparison of the effects of such local irradiation with the effects of systemic beta radiation from the same isotope should give insight into the role of the GI mucosa in the production of IVS.

Materials and Methods

Forty-two purebred beagles between 8 months and 2 years old were divided into systemically and orally treated groups. Systemically treated Y^{90} Cl_3 as a chelate (DTPA) via jugular vein and bladder-catheter return technic for 18-24 hours as described by Winchell et al (5). Y^{90} Cl_3 + DTPA was injected

intravenously and re-circulated to obtain whole-body beta irradiation. The distribution of the Y^{90} DTPA chelate in various body tissues is complicated, and the estimated dosimetry to individual organs is given elsewhere (5). All systemically radiated dogs were observed daily until death. Autopsies were performed soon after death of the animal (one-half to 18 hours in most cases). Oral administration of $Y^{90}Cl_3$ (carrier-free) was given in various doses (2-90 mc Y^{90}) after a 24-hour fast. Twenty beagles were divided into pairs, and doses of 4, 8, 16, 30, 60, and 90 mc $Y^{90}Cl_3$ were added to the feed. The relative amount of "spiked" ration ingested by each dog was determined within 4 hours of feeding. In addition, six beagles, after fast, were given doses of 2-30 mc $Y^{90}Cl_3$ in 50 gms $BaSO_4$ via stomach tube. The latter series was examined radiographically at hourly intervals to obtain "passage-time"; to prevent constipation, the dogs were given two ounces of castor oil about twelve hours after $BaSO_4$ administration. The "passage-time" of $BaSO_4$ through major portions of the gastrointestinal tract was: stomach, 2.2 hours; small intestine, 2.5 hours; and large intestine, 12.5 hours.

The dose to the GI tract was estimated with the following assumptions: (1) the 250 gms of food given orally remained as a bolus of constant volume, (2) density of the bolus was approximately equal to the density of water, (3) $Y^{90}Cl_3$ was equally dispersed throughout the bolus, (4) the radionuclide was not absorbed or adsorbed for any appreciable time by the intestinal mucosa, and (5) radiation flux at the surface of the bolus was one-half of the equilibrium flux within the bolus. The approximate energy given up by the bolus to the GI mucosa was estimated as equal to one-half the energy associated with the equilibrium flux within the bolus. Thus, the expression

(vol. sample/vol. bolus)(dpm/mc)(Mev/d)(ergs/Mev)(rad/erg x 0.5) would give an estimate of the rad dose to the mucosa, or, $(1/250)(2.22 \times 10^{9})(0.9)(1.6 \times 10^{-6})(10^{-2})(0.5) = .06395$ rad/min/mc administered. On a rad-mc basis, various portions of the GI tract received:

Stomach = 132 (2.2 hrs.) x 0.064 = 8.4 rad/mc
Small intestine = 150 (2.5 hrs.) x 0.064 = 9.6 rad/mc
Large intestine = 750 (12.5 hrs.) x 0.064 = 48.0 rad/mc

The above is admittedly a "barn-door" type of estimate, since many variables exist in oral dosimetry; however, these values are quite helpful in evaluating the level of Y^{90} required to produce a demonstrable effect, either clinically or histologically.

The dogs dosed orally were housed in 12 ft^2 metabolism cages throughout the experiment in Animal Holding Quarters No. 1 (Project 6). Those given systemic irradiation were treated and maintained at the Lawrence Radiation Laboratory. All animals were examined several times daily for clinical signs of illness (radiation effects). Sacrifice was by electrocution (110 V for 30 seconds). Routine blood examinations were made throughout the experiment.

Upon death or sacrifice, necropsy was performed and representative tissue samples were placed in Bouin's concentrated fixative. Tissues selected for study were: dorsal cerebrum, liver, lung, heart, kidneys, ileum, and colon. These were embedded in nitrocellulose or tissuemat, sectioned at 8 microns, and stained in hematoxylin and eosin, Pollak's rapid trichrome, and toluidine blue 0. Each organ was examined for parenchymal damage, congestion or hemorrhage, and the presence of IVS. Globules of IVS within the vascular system or hemorrhagic lesion were graded 0-4 plus, depending on their size and abundance; globules less than twice the diameter of erythrocytes were excluded.

Results

Systemic irradiation (over 8 mc Y^{90}/lb. body wgt.) caused death within 4 to 26 days (av. = 12.5 days). Four dogs died at a later date without showing characteristic sign of radiation sickness. Two of these had been given autologous bone marrow, which saved them from acute radiation deaths. One was given sublethal radiation but succumbed after two months of distemper. A general course of radiation sickness (emesis, diarrhea, etc.) was apparent a few days prior to death. Blood values showed a marked depression in leukocytes, but thrombocyte levels were maintained above 100,000 per mm^3 in most of the animals. Massive hemorrhage was especially noticeable grossly in thoracic and abdominal organs. Histologic examinations revealed bone marrow and lymph-node hypoplasia, lamina propria hemorrhage of gastrointestinal tract, and widespread damage to the reticuloendothelium tissue. Damage to the RE tissue varied between different dogs and organs. For instance, six cases showed marked multiple areas of telangiectasis of the liver, with consequent parenchymal damage (necrosis).

Oral administration of 30 mc Y^{90} or more resulted in depression, anorexia, and diarrhea on the 2nd to 3rd day. Such symptoms progressed, and death was supervened by sacrifice on the 7th day post-treatment. Blood values remained within normal range throughout the experiment. Gross examination at sacrifice revealed no lesions in dogs receiving less than a single 30 mc Y^{90} dose, but those receiving greater doses showed pulmonary congestion, hyperemia of the liver, and mucosal denudation of the colon. These lesions were confirmed histologically.

A survey of tissues (brain, lung, heart, liver, and kidney) revealed IVS only in systemically treated dogs (Table I). Hence, it can be stated that more general irradiation damage than that resulting from colonic irradiation alone is required to produce IVS. Of considerable importance was the finding of massive hemorrhage in systemically treated dogs in the absence of thrombocytopenia but in the presence of IVS. Likewise, fibrin was noted intravascularly in cases where tissue fixation was delayed. Consequently, radiation-induced hemorrhage in this experiment could be related to endothelial damage either directly from irradiation, or possibly indirectly from partial to complete IVS occlusion of blood vessels. The consistent finding of large IVS globules occluding blood vessels (emboli), resulting in an infarct from loss of vascular integrity, is not incomprehensible. Study of numerous sections containing IVS indicate that the substance is at least somewhat plastic and molds to the shape of the vessel similarly to a drop of oil in aqueous solution. Photomicrographs on succeeding pages illustrate these phenomena (Figures 1, 2, 3, and 4).

TABLE I: Y^{90}--SYSTEMIC AND GASTROINTESTINAL--IVS SURVEY

Dog No.	Treatment[1]	Death or Sacrifice			IVS (0-4+)*
82	S 330	Died	7	Days	++
M8(x)	S 346	"	4	"	++
234655	S 536	"	4	"	++
Brutus	S 338.6	"	12	"	++
4G	S 389.5	"	12	"	+++
M1	S -----		Dead		++++
Chain	S 450	"	14	"	+++
W7	S 578	"	26	"	+
David	S 315.5	"	19	"	++++
2A	S 458.8	"	14	"	++++
R14	S 517	"	12	"	++
F19	------	"	5	" (distemper)	±
Joe	S 306	"	2	Mos.	-
Fatty***	S 356	"	1	"	-
W4***	S 339	"	2	" (ulcer)	-
5F	G 2.0	Sac. "	7	Days	-
5G	G 2.0	"	7	"	-
33A	G 3.7	"	7	"	-
22E	G 3.7	"	7	"	-
5H	G 4.0	"	7	"	-
5E	G 4.0	"	7	"	-
D24	G 7.3	"	7	"	-
8C3	G 7.3	"	7	"	-
F1	G 14.6**	"	7	"	-
22F	G 14.6**	"	7	"	-
97B	G 21.0**	"	7	"	-
1D	G 21.0**	"	7	"	-
C24	G 29.3**	"	7	"	-
B24	G 29.3**	"	7	"	±
C24	G 29.3**	"	7	"	-
1C	G 37.0**	"	7	"	-
20B	G 58.5**	"	7	" (intestinal necrosis)	-
M8	G 58.5**	"	7	"	+
12G	G 87.8**	"	7	"	-
4H	Control	"	7	"	-

(1) S = systemic; G = ingestion; mc Y^{90}.

* Estimated by 100 x survey of 1.5 x 2.0-cm sections of heart, lung, and kidneys. Tissue fixed in Bouin's, nitrocellulose or paraffin embedding, stained with Pollak's trichrome.

** Symptoms--diarrhea progressively with increasing dose. Dose above 30 mc was followed by anorexia, listlessness, and bloody diarrhea (intestinal syndrome) 2-3 days prior to sacrifice. Histopathology--severe necrosis of colon mucosa.

*** Given autologous bone-marrow transplant post-irradiation.

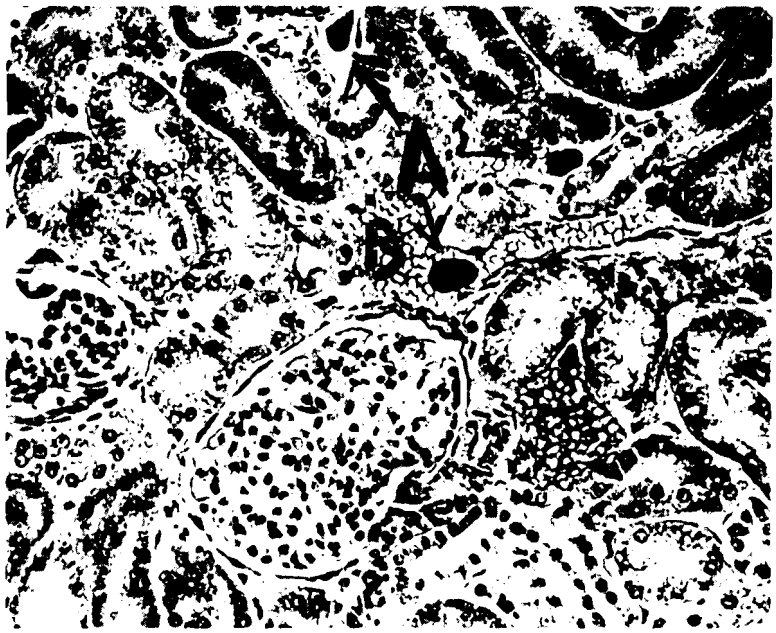


Figure 1: Large globules of IVS (A) in congested blood vessel (B) of kidney from dog 2A dying two weeks after receiving 459 mc Y^{90} intravenously. Note degenerating glomerulus (C). Bouin's, paraffin, Pollak's trichrome, 200x.

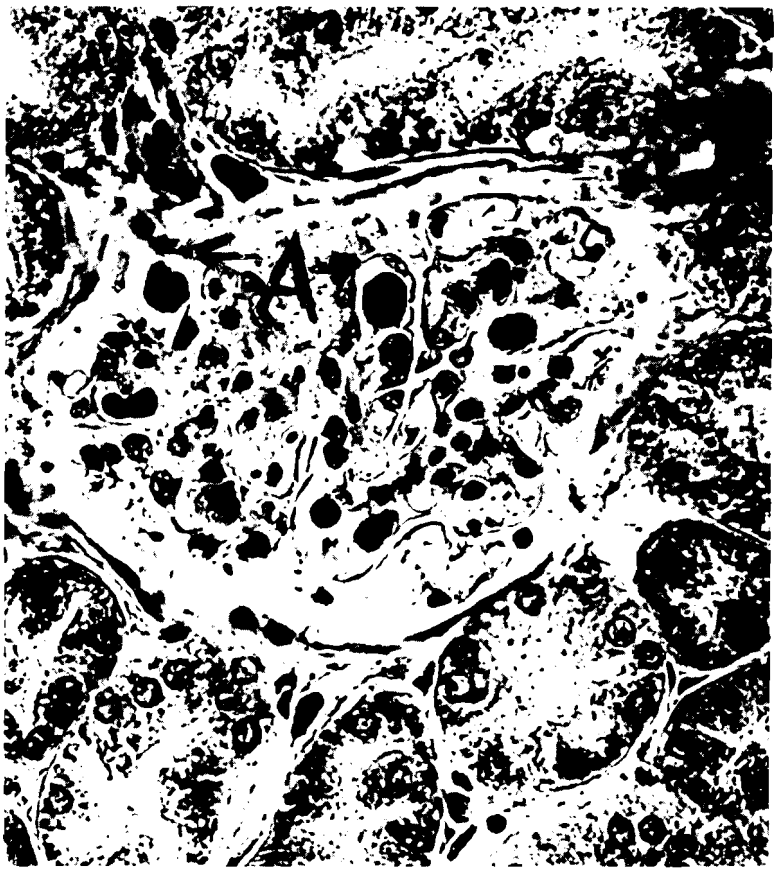
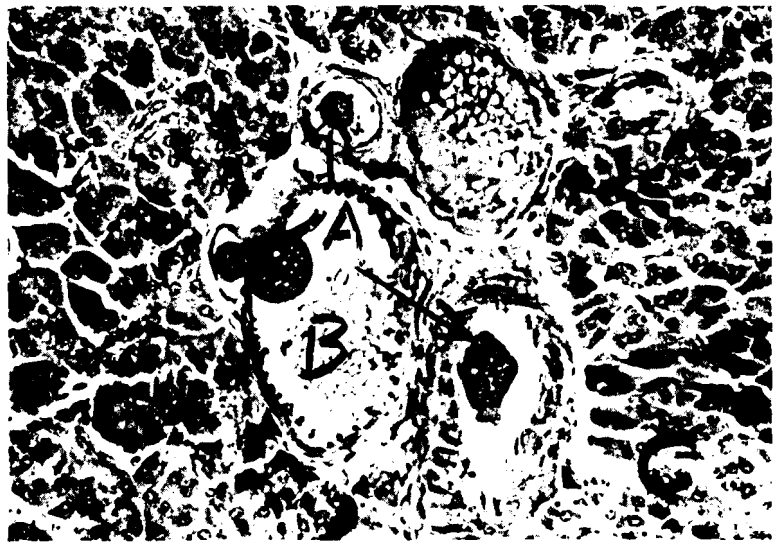
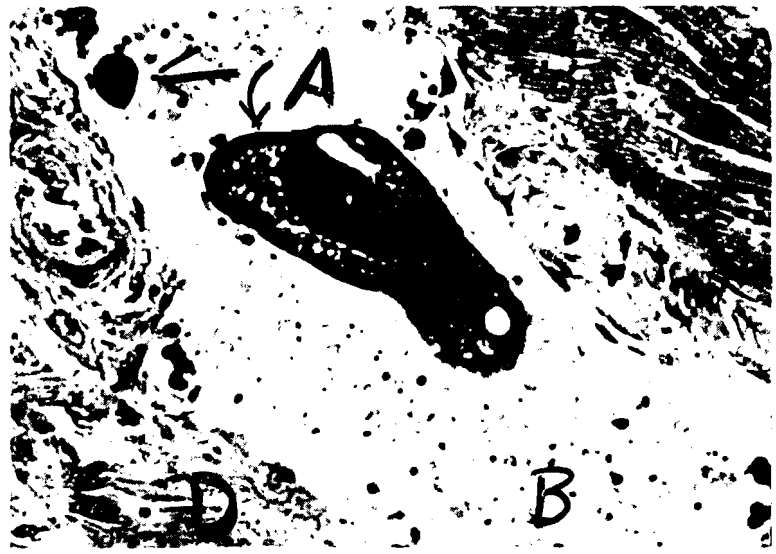


Figure 2: Globules of IVS within glomerulus tuft (A); note enlargement of capillaries. Dog 2A (Figure 1) 600x.





Figures 3 and 4: Photomicrographs of pancreas (Figure 3) and heart (Figure 4) blood vessels containing globules of IVS (A). Note position and shape of globules: (B) erythrocytes, (C) pancreas, (D) cardiac muscle. Beagle 234655 died on 3rd day following 539 mc Y^{90} given intravenously. Bouin's, paraffin, Pollak's trichrome. 200 and 400x.

CONCLUSION

1. Total-body beta irradiation produced IVS.

2. Severe beta-ray damage to the terminal ileum and entire colon failed to produce IVS.

3. Systemic beta irradiation caused a massive hemorrhage that was apparently not due to thrombocytopenia.

4. The study of numerous sections gave the impression that large IVS globules could induce loss of endothelial integrity by occluding blood vessels partially to completely.

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SUMMARY

The completion of adequate facilities for the daily feeding of Sr^{90} to beagles from zero ossification (in utero) to 18 months of age has permitted initiation of the experimental design. With a capacity for 60 dogs, several dose levels at a constant Sr^{90}/Ca^{40} ratio have been included to date. Facilities and procedures that provide for adequate environmental and personnel health and safety as well as that of the experimental animals have been integrated and include feeding of Sr^{90}, decontamination, and disposal of radioactive wastes. Predictive equations that estimate the skeletal mass in living dogs are presented. The determination in the beagle of the Observed Ratio (O.R. dog-diet) using orally administered radiostrontium is discussed, as are criteria in estimating bone doses from Sr^{90} in growing dogs. Pathological sequellae from an oral, toxic level of Sr^{90} are discussed in terms of resultant lymphoid and osseous malignancies as well as progressive osteolytic fibrosis. Also reported is the design of some of the facilities necessary to the program, and the veterinary aspects of beagle maintenance are discussed. Plans summarized for additional expansion include treatment of 350 beagles with Sr^{90} and Ra^{226} by 1963.