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SECOND QUARTERLY PROGRESS REPORT

June 1958

A. E. C. Project No. 6

Contract (11-1) GEN 10

THE EFFECTS OF SR-90 ADMINISTERED DURING THE GROWTH PERIOD OF THE DOG

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Davis, California

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II. INTRODUCTION

Since submitting our last quarterly report (March 1958), construction activity of this project has gained momentum. The current status of construction is as follows: 1) a perimeter security fence (1900 lin. ft.) has been installed; 2) 68 outside pens have been completed with the exception of dog houses and surfacing with 1/4" crushed rock; 3) a well, pump, and pressure system is ready for final testing (bacteriological); 4) plans for the animal-holding quarters are out-to-bid, the closing date for bids being June 12; 5) one cage unit has been delivered to the X-ray Project and is now under trial. Details concerning the outside runs will be described in the September 1958 report when these units will have been completed and occupied.

Water Supply. A well was established at a depth of 270 feet, and the logs read as follows:

0 - 5'	Sandy soil	71 - 84'	Clay	241 - 252'	Clay
5 - 58'	Sandy clay	84 - 128'	Gravel	252 - 265'	Gravel
58 - 62'	Fine gravel	128 - 223'	Clay	265 - 270'	Clay
62 - 71'	Sandy clay	223 - 241'	Sand		

Since an adequate water supply was attained in the lower strata of gravel, perforated 8" casing was sunk into the lower clay strata (265'). An 18" gravel filled bore encased the upper 165 feet of the well to prevent any possible surface contamination. Only a minimum amount of sand was removed from the well on testing, and the well withstood a pumping capacity of 150 gallons per minute. The water table rose to within 25 feet of the surface. Thus, we are assured that this well will accommodate any future water demands by this project. A 5 HP, 3 phase motor drives a turbine set at a depth of 100 feet. From this pump, a 1,000 gallon storage tank and pressure system supply a 2" main pipe to the buildings and outside runs.

Animal-holding Quarters. We are most pleased with the final plans for the animal quarters. These plans represent the combined efforts of architects from the University, a private architectural concern, Department of Sanitation, Division of Radiation Safety, our own group, and also, an array of consultants regarding specific issues. This building is considered as an initial unit for future expansion. To illustrate the position of this building in relation to the overall program, a general plot plan (Fig. 1) is shown on the following page. The plot plan also depicts the capacity of this area for future expansion. The project site is now designed for a maximum capacity of 700 dogs in outside runs and cage space for 250 dogs. Under the present schedule, occupancy of these quarters is expected by October 15, 1958.

Disposal. We have been cooperating with Radiation Safety (University of California, Berkeley) to establish a controlled campus-wide disposal area. The south 50-60' of the project site will be designated for this purpose. Radiation Safety has provided appropriations for building a 10' roadway along the west perimeter fence for access to the disposal site. They will maintain the burial ditches and provide any special facilities needed for disposal of "hot" materials. Any person requiring access to the disposal area will report to the project secretary to gain permission for entry.

Were they buried on site but where

Personnel. The arrival of laboratory equipment has greatly stimulated the enthusiasm of the group. Various isotope analyses have been underway and will be described in more detail in this report. The caretakers are showing increased interest regarding the care of the Sr^{90} treated dogs, and some caretakers from the X-ray Project have voluntarily expressed their desire to be trained for this type of work; thus, no difficulty is anticipated in obtaining lay help for the proper conduct of this experiment.

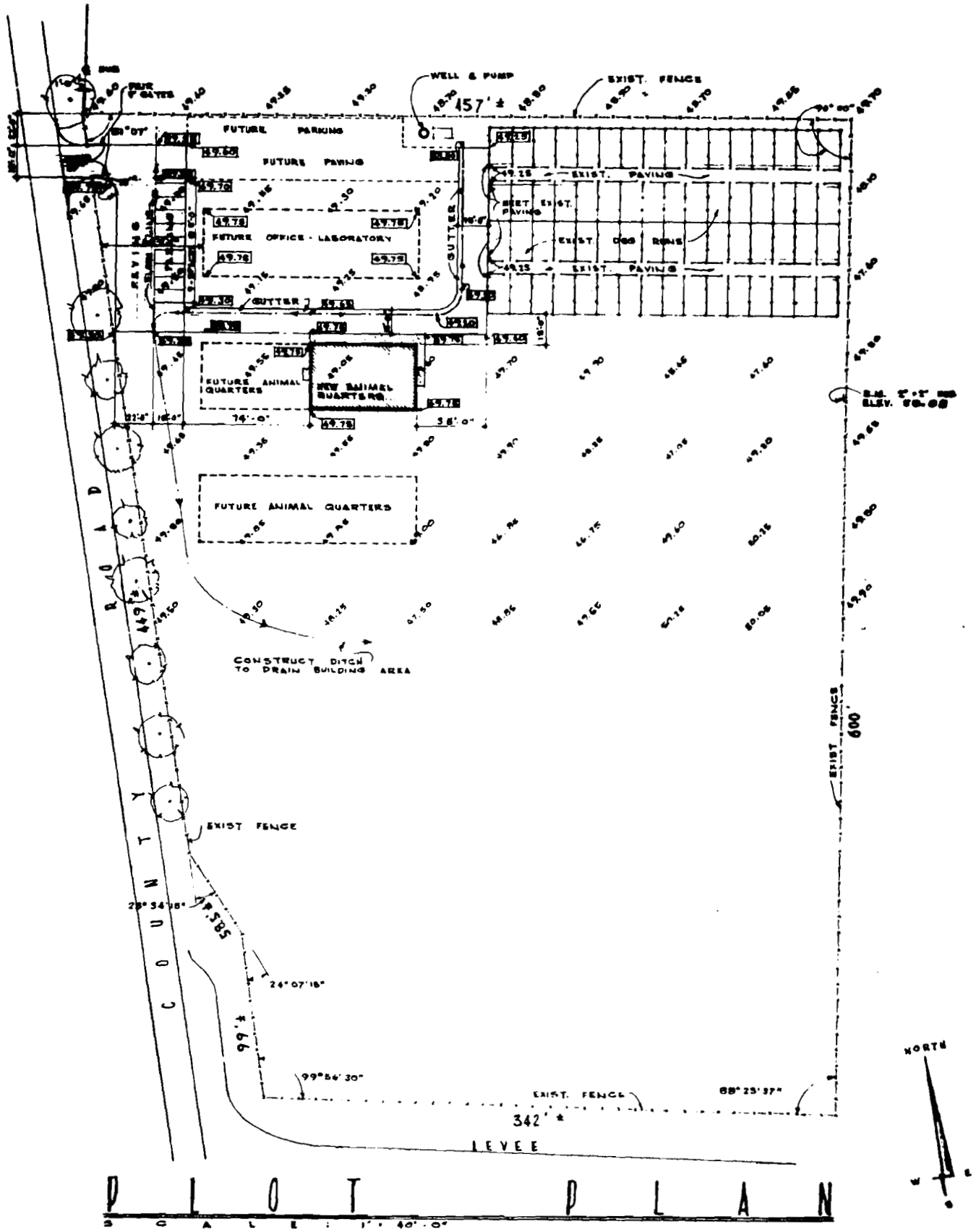

Fig. 1. Plot plan of Strontium-90 Project site

III. SELECTION OF BREEDING STOCK

Many casual observers remark about the uniform size of our beagles maintained on the X-ray Project. Such observations are somewhat ambiguous, because actual measurements of the dogs reveal considerable variation in both size and body weight. Size and body weight may exceed a 50% variation between individuals. These factors have recently been brought to our attention since much of the equipment to be used in this project will be designed for a particular size of dog. Past experience has suggested that the following size for experimental dogs is optimum: females weighing between 300 and 360 ounces (8.5 - 10.2 Kg) and measuring 13" in height and 16" in length; males weighing between 320 and 380 ounces (9.1 - 10.8 Kg) and measuring 14" in height and 17" in length. Nine dogs with the above qualifications have been selected from the breeding colony (X-ray Project). These dogs, varying in color markings from that of light yellow to dark tricolor, will supply progeny for this experiment. From a genetic standpoint, line breeding has been followed exclusively; this feature is important since spontaneous mutation rates have not been established for the dog.

IV. DOG CAGES

Maintaining a large colony of dogs indoors from birth to 2 years of age presents many problems. The animals must be permitted companionship but cannot be so crowded as to drastically limit space for exercise. Adequate lighting must be provided for the dogs; yet, the cages must be sealed well enough to prevent a radiation hazard. With these prerequisites in mind, we now feel that an appropriate cage design has been perfected.

After one year of study and experimentation, we have developed a cage unit which is versatile and appears to solve the problems mentioned above. The unit,

as shown in Figure 2, is divided into 4 cages mounted on tubular legs with four, 5" rubber-tired wheels - the front two being equipped with brakes. The wire-reinforced glass doors in front swing up and in, a feature similar to garage door installations. We feel that this type of door opening presents less hazards to radiation exposure than that which swings to the outside subjecting the animal caretakers to possible accidental contamination. When the door is up, the inner surface can be easily washed and all wash water is confined within the cage. It will be noted, also, that air passage into the cage is provided for with a 3" space above the door and a space between the floor and collecting tray. The units have rear windows to avert a darkened cage interior.

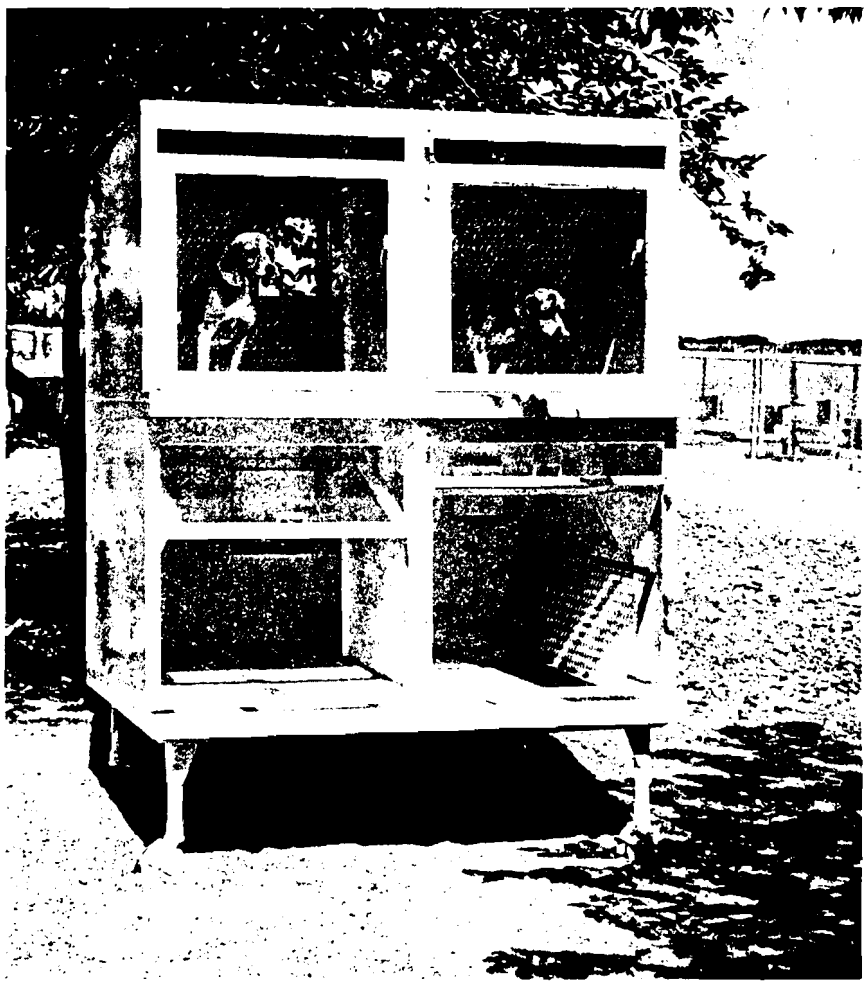

Fig. 2. Front view of cage unit showing floor and door construction, and air space provision.

As shown in Figure 3, the 2 cages at either level can double as one, merely by opening the sliding glass door divider, and thus allow the dogs an area of two cages (24.5 sq. ft.) for exercise and companionship. The dividing glass door will be left open except at feeding or cleaning time. By pairing the dogs to one cage, the unoccupied cage can be cleaned easily with minimum handling of the dogs. The cage floor (figs. 2 and 3) is made of expanded metal welded to angle iron frames which is hot-dip galvanized after fabrication. The floor is in 2 interchangeable sections below which the collecting tray extends 4" in front of the cage and slopes toward the rear.

Fig. 3. Close-up view of cage interior showing inner sliding glass door divider.

The drain at the rear of each collecting tray empties into a 3" flexible plastic hose connecting each cage to the sewer. Since each cage has a separate drainage system, individual excreta can be collected by placing the plastic hose into a separate container whenever desired. Preliminary trials have shown that the cage can be satisfactorily cleaned with as little as 1 gallon of water per dog. Periodic flushing of the collecting trays during the day greatly reduces urine odors.

Air entering the front of the cage is exhausted through a 6" vent in the rear of the cage, as seen in Figure 4. This vent will connect to a central exhaust system which will filter any air-borne radioactivity and discharge the

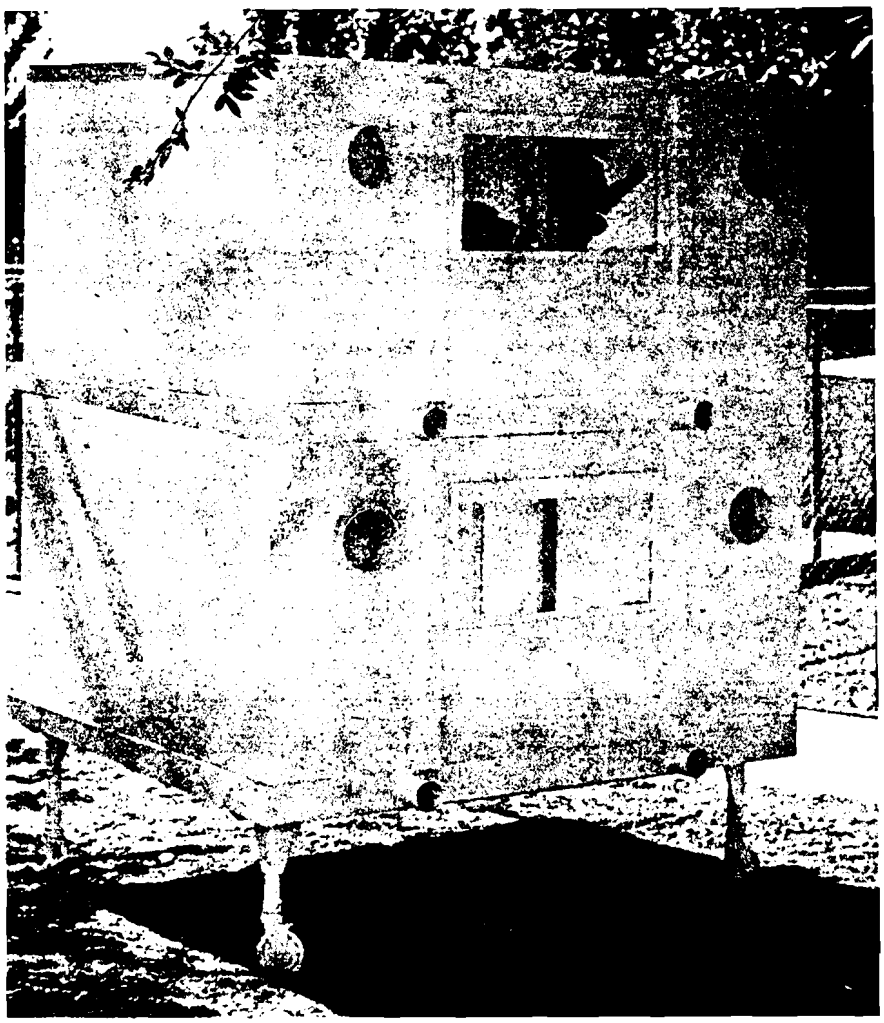

Fig. 4. Rear view showing air exhaust outlets and excreta drainage outlets.

filtered air through a duct located on the roof of the building. This feature will be of special concern in dealing with radium treated dogs since radon can be properly controlled.

The 4 units constructed to date have been built on a contract basis at a cost of $960.00 per unit. The units are constructed of 18 gage galvanized steel and painted with white baked enamel. The cages are painted white to facilitate the detection of soiled surface. Our objection to a painted surface concerns chipping during cleaning, etc. This objection may require resorting to stainless steel construction in part or all of the cage unit. Since stainless steel construction would greatly increase the net cost of these units, we are presently conducting a number of trials to try to overcome our objections to the materials used in building the first 4 units. We are confident that a practical and efficient unit will be soon forthcoming, and that such units can be constructed on a production basis.

V. HISTOPATHOLOGY OF ACUTE SR^{90} TOXICITY BY THE ORAL ROUTE*

INTRODUCTION

The First Annual Progress Report (July 1956 - September 1957) described the clinical and autopsy findings in 2½-month-old dogs given various levels of SrY^{90} orally. In this experiment, dose levels varied from 50 to 9,000 μc SrY^{90}. Based on survival, the dogs could be placed into three groups: for example, doses of 1,000 to 9,000 μc resulted in death within 6 to 7 days (peracute lethality), doses of 500 to 1,000 μc resulted in death within 30 to 50 days, and doses of 200 μc or less was not found to cause death within 60 days after dosage. The last group was sacrificed for pathological study 106 days following treatment.

*Terminology to denote various syndromes in this experiment is based on clinical signs in respect to dosage given, i.e., levels above 9,000 uc SrY^{90} would not be expected to appreciably decrease the 6-7 days elapsing before resulting in death.

MATERIALS AND METHODS

Tissues representing each organ system were placed in alcohol-formol or Bouin's fixative, dehydrated, and infiltrated with nitrocellulose. Sections, 5 to 10 microns in thickness, were affixed to slides directly from the knife. The following stains were used in this study: H and E, Pollak's trichrome, and Periodic Acid - Schiff's Reagent. One section was cleared for phase microscopy study.

One dog from each group typifying lesions observed among the various clinical syndromes will be discussed.

A. PERACUTE SYNDROME

Dog S-11 (4D7) weighing 2.01 Kg was given 4,500 μc Sr^{90} orally. Death occurred 7 days later, and necropsy revealed massive hemorrhage in the bone marrow cavity and severe necrosis of the lower bowel.

Histopathology. Severe lesions were observed in the gastro-intestinal tract, lymph nodes, and bone marrow. The extent of damage within these organs appeared to be sufficient to explain the cause of death although other organs did reveal varying degrees of pathology.

Gastro-intestinal damage began in the mucosa of the stomach and increased progressively toward the lower bowel. Within the stomach slight necrosis of the lining epithelium with subsequent pseudomembranous formation was noted. The submucosal blood vessels were congested and contained numerous polysaccharide globules. These globules stained similarly to those previously identified and described in the acute X-irradiation syndrome.* In the upper duodenum, mucosal

*Andersen, A. C. A Substance Observed Within the Vascular System of Dogs Receiving Lethal Exposures of Whole-Body X-irradiation. Rad. Res. 6 (3), March 1957.

damage was limited to necrosis of the tips of the villi; erythrocytes were not found within the lumen. In contrast, the lower region of the duodenum showed extensive damage of villi and presence of erythrocytes within the lumen. Villi in this area of the gut were representative in shape, but their contents consisted of a necrotic mass. Necrosis did not extend below the neck of the mucosal crypts. Lymphocytic nodules in the duodenal region appeared normal. From the jejunum posteriorly, the entire mucosa was denuded. Necrosis in the lower gut extended only to the muscularis mucosa below which congested blood vessels demarcated the submucosa. The lumen of the gastro-intestinal tract from the jejunum posteriorly was filled with erythrocytes and debris. Numerous polysaccharide globules of varying size (1 to 20 microns in diameter) were seen within blood vessels (afferent and efferent) to this gut region.

Other lesions consisted of varying amounts of congestion and/or hemorrhage especially obvious in bone marrow, lymph nodes (medulla), heart and thyroids. Considerable numbers of polysaccharide globules were identified within these organs; the presence of this substance appeared to be related to the extent of vascular damage observed. For instance, few petechial lesions occurred in the thyroid where follicles and interfollicular tissue adjacent to hemorrhagic foci did not reveal radiation damage, but, on the contrary, these parenchymal elements appeared normal. This observation would suggest an embolic action of the polysaccharide globules resulting in endothelial rupture (hemorrhage).

B. ACUTE SYNDROME

Dog S-8 (4A8), 206 Kg in weight, received 970 μc SrY^{90} orally. Death occurred 43 days following administration. On necropsy, ulcers of the lips and feet, and atrophy of the tonsils were noted.

Histopathology. No morphological evidence of lesions were observed in the following: cerebrum, cerebellum, thyroids, esophagus, stomach (fundic region), duodenum, ileum, jejunum, liver, gallbladder, heart, testes, pancreas, kidneys, and adrenals. By far the most severe damage was present in the lungs. Diffuse capillary hemorrhage with moderate amounts of atelectasis and emphysema were readily apparent. Of considerable interest was the finding of large quantities of polysaccharide globules similar to those described in the acute X-irradiation syndrome. In the lung, such globules ranged from 1 to 30 microns in diameter appearing as if they were filtered within capillaries of this organ. This material was also found in arterial capillaries terminating within lymph nodes, splenic red-pulp, heart, gastro-intestinal tract, and kidneys; with few exceptions, the intravascular globules were small in diameter (1 to 5 microns) in these organs. Only a few larger globules were found in the kidneys and subgross lesions were limited to a few ischemic glomeruli. Whether the presence of intravascular globules could be related to the state of decreased blood flow in such glomeruli was not determined.

Congestion and hemorrhage were found in the subepithelial capillaries of the colon. The presence of these lesions did coincide with an abundance of large-size (10 to 25 microns) polysaccharide globules; a finding that will be correlated with the over-all study of this substance found within the vascular system during the post-radiation syndrome.*

Moderate hypoplasia appeared in all lymph nodes examined. This was made evident by the near lack of cellularity within the medulla and sinusoids. Lymphocytic follicles were apparently regenerating as evidenced by numerous mitotic and juvenile cells.

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*Paper to be presented: International Congress of Radiation Research Meeting, Burlington, Vermont, August 17, 1958.

C. SUBACUTE SYNDROME

Dog S-3 (4A5), a female weighing 1.90 Kg, was given an oral dose of 87 μc Sr^{90}. She was sacrificed 106 days following treatment, and necropsy showed only pale coloring of the lymph nodes.

Histopathology. No morphological lesions were found in the lung, thyroids, adrenals, pancreas, ovaries, tonsil, kidney, lung, liver, gallbladder, and gastro-intestinal tract. However, the stomach mucosa did show a limited amount of mucosal damage followed by connective tissue proliferation. This damage was not found in the stomach of other dogs in this series.

The spleen showed evidence of lymphocytic degeneration followed by follicular regeneration as proliferating young lymphocytes were numerous. Apparently, the red pulp suffered a moderate amount of damage as small hemorrhagic areas and an accumulation of hemosiderin was present. However, these lesions surrounded foci of active hemopoiesis and repair. In general, examination of sections of the spleen gave the impression that considerable damage may have occurred some 1 to 3 weeks following treatment and followed by repair.

CONCLUSION

Single oral doses of 50 to 200 μc Sr^{90} caused moderate but apparently reparable damage to hemopoietic tissue, whereas 500 to 600 μc Sr^{90} approximated an LD 50/30 dose in the dog. Larger doses (1.0 mc or over) resulted in death within 6 to 7 days after administration. Such cases revealed massive hemorrhage of hemopoietic tissue and severe mucosal damage in the lower gastro-intestinal tract.

Mucosal and associated damage were apparently the effect of beta irradiation whereas hemorrhagic lesions could have resulted from the indirect effects of

ionizing radiation. The latter sequella is presently under intensive investigation in studies being conducted in relation to acute X-radiation damage.

SUMMARY

Massive denudation of the mucosa from the jejunum posteriorly characterized severe radiation damage resulting from extremely large Sr^{90} oral doses. Erosion of the mucosa and consequent removal of the intestinal lining provided an easy access for bacteria. Thus, large doses of Sr^{90} conceivably caused death by dehydration and septicemia.

Widespread pulmonary hemorrhage, congestion of subepithelial capillaries within the colon and lymph node hypoplasia were the most characteristic lesions observed in the acute Sr^{90} syndrome. The presence of these lesions seem correlated with the finding of intravascular PAS positive globules. It seems rather unlikely that thrombocytopenia could have existed to the degree of causing hemorrhage since numerous hemopoietic foci containing relatively normal numbers of megakaryocytes were observed in the liver and other tissues.

VI. PATHOLOGY OF CHRONIC SR^{90} ADMINISTRATION - DOG 5S3

INTRODUCTION

Four dogs have been on a daily diet in which Sr^{90} has been incorporated in their feed since January 1957. These dogs will continue on this diet until July 1958 (18 months of age) when 2 of the 4 dogs will be sacrificed to determine the amount of retained Sr^{90} in the skeleton.

Unfortunately, one of the dogs (male - 5S3), scheduled to be sacrificed in July was found dead quite unexpectedly by the animal caretaker on April 26, 1958. During the last 30 days of life, this dog showed periodic evidence of minor gastro-intestinal disturbance. Once or twice a week he vomitted after eating and frequently voided a gray, pasty, soft or semi-solid stool. Between these

episodes, this dog showed evidence of normal appetite and stool. As a matter of fact, his weight was normal prior to the time of death even though he displayed marked listlessness and anorexia.

A complete set of X-rays were taken of this dog (5S3), and a careful autopsy was performed. Tissues of all organs were placed in fixative, and the skeleton was weighed, measured, and placed in absolute alcohol or acetone. Organ weights were also recorded.

Periodic blood samples have been taken from the 4 dogs on this experiment. None of these samples showed deviations from the normal until about 2 weeks before dog 5S3 died when his blood picture was as follows: PCV - 35 mm, RBC - 5.41×10^6, Hgb - 11.5 gms/100 ml, and corrected sedimentation rate of 25 mm/hr. The leukocyte count on this dog was 14,700 per mm^3 with relative normal differential values (70 + 2 hetero; 1 - mono; 26 + 1 lympho). This blood sample suggests embarrassment of erythropoiesis. It should be mentioned that blood examination of the remaining 3 dogs in this group are normal.

A. GROSS PATHOLOGY

Dog 5S3 weighed 11.8 Kgs and was 471 days old at the time of death. Necropsy revealed multiple subcutaneous hemorrhage, lesions along the dorsal aspect of the neck, withers and loin, as well as in the ventral abdomen region. There were also deep intramuscular hemorrhages in the area of the thigh and the deep muscles of the neck and foreleg. Mucous membranes were pale with mucosal hemorrhages in the buccal cavity. The tonsils were studded with hemorrhagic lesions.* The lungs showed edema, hemorrhage and emphysema, with marked edema especially noticeable in the mediastinum. The heart was flabby and enlarged and the liver

*It should be mentioned that this group of 4 dogs have shown tonsilar atrophy since puppyhood but dog 5S3, at necropsy, showed well-developed tonsils.

was swollen and bulged on cut section. The spleen was greatly enlarged, weighing some 5 times (257.1 gms) that of a normal dog at this age. The gastro-intestinal tract showed echymotic hemorrhages from the stomach to the cecum which increased in severity in the lower portions of the tract. The rectum was filled with black, tarry feces as further evidence of severe internal hemorrhage. The mesenteric lymph nodes were swollen and hemorrhagic. Likewise, kidney showed areas of hemorrhage, and the cortex appeared to be somewhat thinner in relation to the medulla than found among normal dogs. Hemorrhage was also found in the epididymus. Subdural hemorrhages of the brain (cerebrum) were noticeable over the cerebral cortices. The parathyroid glands were large and readily visible to the naked eye.

Careful study of the skeleton has failed to reveal any indication of impending tumor formation. Preliminary study of the long bones of the skeleton suggest that the linear measurements correspond to those found in normal dogs, but these bones weighed some 20 percent more.(wet weight) when calculated as a percent of body weight and compared to that of normal dogs of comparable weight and age.

All sections were affixed to slides directly from the microtome knife. Five sections from each of 23 blocks of tissue were stained as follows: hemotoxylin and eosin, Pollak's trichrome, Periodic acid - Schiff's reagent plus a control. One section was left unstained for phase microscopy.

B. HISTOPATHOLOGY

Tonsils and anterior cervical lymph nodes. The entire node showed diffusely arranged lymphocytic-type cells. Few lymphocytic follicles could be distinguished in a normal architectural arrangement, and these cells were so numerous as to obscure the sinusoids. Limited numbers of erythrocytes were present within

arterial capillaries and moderate to heavy accumulations of hemosiderin and hematoidin appeared in the medullary portion of the node. Close examination of the lymphocytic-type cells revealed considerable variation in the size, shape, and phases of activity. Numerous mitotic figures and young cells as well as pyknotic nuclei took on a uniformly dispersed appearance throughout the tissue. In general, these cells showed large eccentrically located vesicular-type nuclei with condensation of paranuclear chromatin. A single, large false nucleolus (PAS positive) was present within most nuclei. The cytoplasm was scant in amount with irregular clumps of amorphous granular substance. In some areas, these cells were compactly arranged and infiltrating the adjacent connective tissue of the parotid and parathyroid glands.

Thyroid. No visible lesions; However, subcapsular follicles were compressed and interspersed with islets of diffuse parathyroid tissue.

Parathyroid. No visible lesions. Some evidence of medullary edema in one portion of the gland.

Lungs. Considerable emphysema and edema covering a wide portion of the tissue examined. Moderate amounts of hemosiderin within macrophages. The lumen of all blood vessels were virtually filled with lymphocytic-type cells.

Heart. No visible lesion. Capillaries contained relatively small numbers of erythrocytes, but numerous lymphocytic-type cells. Minute acidophilic droplets (1 to 3 microns in diam.) were observed within capillaries.

Liver. Sinusoids enlarged and laden with lymphocytic-type cells similar to those found in the cervical region. Kuppfer cells contained an abundance of hemosiderin. Surprisingly few erythrocytes were noted within the sinusoids. Hepatic cells were relatively free of lipids. The triad and in particular the peritriad space (Space of Mall) contained numerous lymphocytes.

Stomach. No visible lesions. Blood vessels filled with lymphocytic-type cells.

Ileum. The extent of autolysis made histopathological study difficult, since since about one-half of mucosal surface was absent.

Spleen. The capsule outlined a uniform distribution of lymphocytic-type cells. A large number of these cells obscured the sinusoids as well as other architectural features of this organ. Only scant numbers of lymphocytic follicles could be recognized. A few petechial lesions and macrophages containing hemosiderin could be detected between large numbers of diffusely arranged lymphocytic-type cells. Evidence of hemopoiesis was lacking although mitotic as well as necrobiotic cellular changes were apparent. Lymphocytic-type cells forming the largest portion of this organ were similar in type to those described in the tonsils and cervical lymph node.

Pancreas. Connective tissue invasion by lymphocytic-type cells.

Kidney. Connective tissue invasion by lymphocytic-type cells.

Pathological Anatomical Diagnosis (Dog 5S3)

The histological finding of numerous lymphocytic-type cells infiltrating many organs and tissues suggests a type of highly malignant lymphoma. Some confusion exists among pathologists in regard to the classification of specific types of lymphomas. The description and classification offered by Ogilvie[1] indicate that the findings in this dog (5S3) characterize a lymphosarcoma. Not only does our histological evidence typify this type of neoplasm, but the clinical and necropsy report are also characteristic, i.e., rapid growth of neoplastic cells with whitish appearance of some organs and patches of necrosis and hemorrhage as well as anemia developing late in the terminal stages. Thus, it appears that death of Dog 5S3 was attributed to lymphosarcoma. However, it cannot be

[1] Ogilvie, R.F.: Pathological Histology, 2nd ed., The Williams and Wilkins Company, Baltimore, 1945.

stated with certainty whether the pathogenesis of this tumor was due to Sr^{90}. At this time, we can only suggest internal radiation as the cause since such tumors are known to occur in non-radiated cases of seemingly spontaneous origin. On the other hand, we have examined a large number of beagles in the X-ray Project during past years without evidencing this type of tumor as the cause of death. The coincidence of Sr^{90} and this type of malignancy will be carefully studied in other experimental dogs.

C. RADIOCHEMISTRY

Dog 5S3 had been fed daily ad libidum an amount of strontium equal to 1.83 μc Sr^{90}/gm Ca in the feed. At termination the dog weighed 11.8 Kg. If we estimate the skeletal weight to be 10 percent of the body weight, Dog 5S3 would have had 1180 gms of skeleton. If we had assumed that there was no discrimination in favor of the retention of Ca or Sr^{90}, the estimated body burden of the dog was 303 μc Sr^{90} or 26 μc Sr^{90}/Kg body weight. Evidence to the effect that there was no discrimination between Ca and Sr is given in Section VII.

We have estimated the radiation dose rate in rad/day from the formula: $D = 51 \times C \times \bar{E}$, where C is the concentration of Sr^{90} expressed as microcuries Sr^{90}/gm skeleton and $\bar{E}$, the average energy per disintegration which is equal to 1 mev. If we assume that the Sr^{90} was distributed evenly throughout the skeleton, the daily dose rate is 13 rads/day. The accumulated dose from the date of whelping to termination - a total of 471 days - would be approximately 6100 rads. The total accumulated dose, assuming that the skeleton is formed from the day upon which dosing was begun, is estimated to be 6500 rads.

VII. BIOCHEMISTRY

A. STRONTIUM AND CALCIUM IN MEAT, FEED, BONE

Preliminary radiochemical analyses are now available on the four dogs which have died or have been sacrificed during the course of the preliminary experiments. These data are listed on Table 1. The dogs and their dams have been fed daily 1.83 μc Sr^{90}/gm food Ca ad libidum. This ratio is close to the highest proposed level, D_5, which is 1.93 μc Sr^{90}/gm Ca.

The discrimination ratio (Sr^{90}/Ca in the feed ÷ Sr^{90}/Ca in bone) in the weaned puppy (5S5) and in the adult dog (5S3) was approximately unity. This indicates that the growing dog does not discriminate against Sr in favor of Ca in the over-all transfer from the gut to bone. Nor does there appear any discrimination against Sr^{90} in the transfer from blood to milk to bone. In this case, at least, the beagle resembles more closely the chicken than the mouse[2]. The reasons for this difference are not readily apparent. Certainly the condition of our experiment differs significantly from all others; whereas we are studying the continuous uptake of Ca and Sr^{90} in dog, most of the earlier work have been done with small laboratory animals, for example, the mouse and the rat following a single intravenous injection of labeled Ca and labeled Sr.

The Sr to Ca ratio in the bones of the 2 stillborn puppies was only 1/3 that of the diet (Table 1); thus it appears that there is a mechanism which discriminates against the transfer of Sr^{90} across placental membranes in the dog.

The naturally occurring strontium content of the dry dog ration, hamburger, and bone, has been determined by activation analysis at ORNL. These results are given in Table 2. Since more than 90 percent of the dog's dietary calcium is supplied by the dry ration, we may also use these data to estimate the Ca/Sr

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[2] Comar, C.L., and R. H. Wasserman. Progress in Nuclear Energy. Series VI, Volume 1, Permagon Press Ltd, London (1956).

TABLE 1. CALCIUM AND STRONTIUM-90 CONTENT OF DOG BONES

Dog No.	Disposition	Bone	Ca Content*	Sr^{90} Content**	Sr^{90}/Ca
5S3	Died at 471 days	Scapula	0.188	0.475	2.5
		Ulna	0.153	0.290	1.9
		Rib	0.207	0.389	1.9
		Atlas	0.174	0.291	1.7
		Vertebra	0.162	0.388	2.4
		Femur	0.190	0.378	2.0
				$\bar{M}$ =	2.1
5S5	Sacrificed at Weaning (2nd generation on Sr^{90})	Vertebra	0.124	0.296	2.4
		Pelvis	0.145	0.333	2.3
		Femur	0.153	0.353	2.3
		Rib	0.179	0.348	1.9
				$\bar{M}$ =	2.2
5S6	Stillborn (2nd generation on Sr^{90})	Scapula	0.136	0.0713	0.52
		Femur	0.131	0.0868	0.66
		Rib	0.183	0.0957	0.34
		Vertebra	0.140	0.0720	0.51
				$\bar{M}$ =	0.51
5S8	Stillborn (2nd generation on Sr^{90})	Scapula	0.153	0.113	0.74
		Femur	0.137	0.094	0.68
		Rib	0.157	0.084	0.53
		Vertebra	0.155	0.079	0.51
				$\bar{M}$ =	0.61

*Grams Ca per gm dry defatted bone

**Microcuries Sr^{90} per gm dry defatted bone

*****

TABLE 2. CALCIUM AND STRONTIUM CONTENT OF DIET AND BONE

Sample	Ca Content gm Ca/gm	Sr Content µg Sr/gm	Ratio Sr/Ca
Dry ration	0.0327	0.77	23.5
Hamburger, #1	0.00129	0.105	82.
Hamburger, #2	0.000316	0.0081	25.6
Dog bone	0.234	3.34	14.3

discrimination in favor of calcium retention which is in the order of 1.6/1. These analyses are to be repeated in the near future using a sample of the entire skeleton of the dog rather than of a single bone.

B. CARBON-14 METABOLISM STUDIES

The C^{14} metabolism studies which were reported in the First Quarterly Progress Report (March 1958) have been continued and extended to include glucose 1 C-14, glucose 2 C-14, and glucose 6 C-14. Trials with X-irradiated dogs using glucose U-C^{14} are now scheduled for July 1958. The scope of these experiments have been widened to include the study of the biochemical composition of dog milk. In addition, we shall attempt to determine the chemical precursors of the peculiar mucopolysaccharide which Dr. Andersen[3] has reported to be present in the vascular bed of the lethally X-irradiated dog.

C. HEALTH PHYSICS

Personnel who have worked on the Sr^{90} dogs have worn CWS protective mask filters. These filters were changed after having been worn 4 months. The filters worn by Mr. Mori and Dr. Parker, and a Control filter were analyzed for radioactivity. The filters were ashed and planchetted on 1" stainless steel cups. The results indicate that Mr. Mori's filter had trapped 2.2×10^{-4} μc and Dr. Parker's, 3.8×10^{-5} μc of radioactivity.

From these data we may estimate the maximum hazard of working under these rather primitive conditions. If we assume that a person wore no mask while handling the food and excreta of these dogs and if we further assume that 100% of the inhaled activity would be retained, then we may calculate that it would require 1500 years to accumulate the maximum permissable level of Sr^{90} in the body of 1 μc.

---

[3] Andersen, A.C.: A Morphological Substance Within the Vascular System of Dogs after Whole-body X-irradiation. Rad. Res. 5 (4), 446 (1956).

VIII. PUBLICATIONS AND REPORTS

A. C. Andersen*

April 24, 1958 — Members of the Forestry Service, Redding, California. Effects of Radiation.

May 20, 1958 — Sacramento Society for Medical Improvement, Sacramento, California. Radiation Effects in Relation to the Atomic Era - Contributions being made by the School of Veterinary Medicine.

J. R. Luick

April 24, 1958 — Senior Veterinary Students in Public Health, Davis Campus. Aspects of Fallout of Concern to Veterinarians.

Publication: J.R. Luick, D. Torrel, and W. Siri. A method for the determination of water intake in grazing sheep. (using $H^3{}_2O$) Int. J. Applied Rad. & Isotopes (in press)

Max Kleiber, A.L. Black, J.O. Gutte, and J.R. Luick. Specific oxidation and utilization of acetate and glucose by cow, sow, dog and chicken. 2nd Conf. Peaceful Uses of Atomic Energy, Geneva, Switzerland. 1958.

J.R. Luick, R. A. Garber, N. Akesson, and L.D. Leach. A method for the study of fungicide distribution by farm machinery using Rb^{86}. (submitted to Int. J. App. Rad. & Isotopes)

H. R. Parker

May 26, 1958 — Rotary Club, Oroville, California. The Establishment and Maintenance of a 500-dog Beagle Colony.

*See Progress Report (Proj. 4) for additional talks and publications.

Respectfully submitted,

Harold R. Parker, DVM
A.E.C. Project No. 6

Approved by:

A. C. Andersen, VMD, PhD.
Principal Investigator
A.E.C. Projects 4 and 6

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FIRST QUARTERLY PROGRESS REPORT

March, 1958

A. E. C. Project No. 6

Contract (11-1) GEN 10

THE EFFECTS OF SR-90 ADMINISTERED DURING THE GROWTH PERIOD OF THE DOG

School of Veterinary Medicine

University of California

Davis, California

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VII. PUBLICATIONS AND REPORTS

I. SUMMARY

During the period covered by this report, January - March, 1958, there has been an increase in the activity of all aspects of the project. The final plans for the Animal Holding Quarters are essentially complete and we hope construction will begin in the very near future. Also during this period, nearly all of the laboratory equipment has arrived. Most of this equipment will remain crated until facilities for its operation are available. Considerable attention has been given to the radiological health and safety aspects of the project - particularly in regard to protective clothing, safe handling of all phases of isotope handling, monitoring systems, and the waste disposal plant.

The preliminary studies on the effects of continuously ingested Sr^{90} at the higher dose level (D_5) have continued in spite of several major obstacles. The dogs which have been dosed daily at 1.83 uc Sr^{90} per gram food calcium for 15 months weigh 20 percent more than non-irradiated dogs which are maintained on the X-ray project grounds. The cause of this greater growth has not yet been determined. The laboratory research program includes: a biochemical and morphological study of the growth and development of the beagle skeleton; establishing procedures of analysis of biological material containing radioisotopes including: ashing methods, calcium, phosphorus, citrate, strontium and strontium-90; intermediary metabolism of C^{14} labeled metabolites in lactating beagle dogs; and preparation of plasticized beagle dogs to be used as phantoms for a whole body counter.

II. PERSONNEL

Staff

Principal Investigator ..... A. C. Andersen, VMD, PhD

Co-director ..... D. E. Jasper, DVM, PhD 2-0155
Dean, Veterinary School

Radiobiologist ..... Jack R. Luick, PhD

Veterinarian ..... Harold R. Parker, DVM 2-0164 & 23599

Technical Assistance

Senior Lab. Tech. ..... A. M. Gau

Animal Caretaker ..... Ray Mori

Typist-clerk ..... Ida Yamaguchi

Student Help

Thomas Banks

Carlo Besio

Richard Chance

III. INTRODUCTION

Since the last Semi-annual Progress Report was submitted (December, 1957), the Atomic Energy Commission Operational Offices in San Francisco expressed the opinion that Quarterly Progress Reports would be preferred to either Annual or Semi-annual Reports. The opinion was expressed that frequent reports would keep those interested in this project currently advised on its progress. Furthermore, informal reports would reduce the work load of the personnel involved in the project proper.

Several people have visited us during the past 3 months. We were particularly pleased that Dr. Robley Evans could visit with us on his recent trip to the west coast. We were pleased to learn that the proposed expansion of this project is under serious consideration. He requested that we submit a letter in answer to Dr. Henry Blair's criticism of the experimental design of the expanded project. This letter was submitted and we have received a reply from Dr. Bruner favoring the acceptance of the original proposal. Apparently the only major issue still in question pertains to the inclusion of reproductive studies into the experimental groups. In regard to this question, the original proposal included reproductive studies with definite objectives in mind. These objectives will be brought to the attention of the Steering Committee for further discussion (June, 1958) in relation to the future research activities of this project.

Captain J. Barton visited with us for one day on his tour of the West and seemed pleased with a first hand description of the progress being made. Mention should be made of Dr. Collery from the School of Veterinary Medicine in Ireland. Dr. Collery visited with the objective of gaining knowledge on the general aspects of radiobiology, and in particular, the

effects of whole-body X-irradiation and the future studies involving internal emitters. He expressed his gratitude to the group here for their cooperation in making his 3-week visit with us an informative and profitable stay.

In November, 1957, authority was given to put the construction and equipment funds into effect. Final plans for the animal-holding quarters are now nearing completion and are expected to receive the Regents approval during the current month. We have not been able to proceed with the fencing and well drilling due to the heavy rains in this area. To date, more than 10 inches of precipitation above the normal has kept the project site inaccessible for construction. In the meantime, some 20 tons of fencing material have arrived and a contract has been let for the drilling of the well and installation of a pressure water system. Contractors have assured us that they will begin construction at the first possible opportunity. During the past month, considerable amount of laboratory equipment has arrived - counters, microscopes, balances - and all are now being put into use. The X-ray machine has arrived and is ready for installation upon the completion of the animal-holding quarters. Receipt of the histological equipment will enable us to proceed with sectioning and the study of tissues that accumulated during the past year.

IV. HEALTH PHYSICS ASPECTS

A. University of California Radiation Safety Program.

One function of the Division of Radiation Safety, University Physician's Office, University of California, Berkeley, is to monitor all of the locations on the many university campuses where radioactive isotopes are used.

To date, monitoring of the Davis campus has been performed by a representative from the Berkeley campus - usually on a twice monthly basis. The Strontium-90 Project has necessited a significant increase in the man hours spent monitoring on the Davis campus. The enlarged Strontium and Radium study should justify a resident monitor on the Davis campus. To this end, Dr. John Heslep, Chief of the Division of Radiation Safety, has proposed that a full-time monitor be assigned to the Davis campus, July 1, 1958.

B. Proposed Monitoring System.

Considerable time has been given to the design of a total-body survey instrument ("hand-foot" monitor type) which would be suitable for our situation. Commercial instruments are in general not well suited since they all contain one or more of the following limitations:

1. Monitor only hands and feet
2. Electronically integrated into an elaborate alarm system
3. Unduly expensive
4. Relatively insensitive to beta particles.

Dr. Luick and Mr. Robert Magill of the Division of Radiation Safety have proposed to fabricate a personnel safety monitor out of transistorized beta G. M. Survey Meters. The thin window probes of these meters are to be mounted in the casement of the door which leads into the locker room, and each meter is to give an audio-visual signal in the presence of radioactivity. In practice, a person wishing to monitor himself will stand as close as possible to the detecting tubes and slowly turn 360 degrees in place. This instrument is to be used only as a "quick and dirty" means for detecting contamination; it is not to exclude the use of a more thorough survey using a portable meter.

C. Cesium-137 Contamination.

A package containing a small vial of Strontium-90 solution was sent to Dr. Charles Mays, Radiological Laboratory at Salt Lake for radiochemical assay. This package was found to contain 2 microcuries Cs^{137} as a radiochemical contaminant. Further investigations indicated that the contamination was in the packing material. Since Cs^{137} has not been used on the Davis campus and since the packing material came originally from Oak Ridge, it seems probable that the radiochemical contamination also originated at Oak Ridge. No Cs^{137} was found in the Sr^{90} solution.

D. X-ray Facility.

The primary requirement of the X-ray unit on the Strontium-90 Project is to produce accurate radiograms to reveal pathological changes such as tumor formation. We have purchases a General Electric Model TX-21 generating unit, General Electric Model HRT-111 X-ray tube, Bucky diaphragm, and electronic timer. The machine and equipment will be mounted in an X-ray room especially designed for safety and demands for this project. The X-ray room is connected with the darkroom by a leaded pass box to increase the efficiency of the installation. The darkroom will be equipped with a 10 gallon tank and a high air-flow film dryer. As shown in previously submitted plans (December, 1957), the X-ray equipment is located adjacent to the autopsy and examining rooms to minimize transport of the anesthetized dog. The door in the X-ray room will have safety switches so designed that the X-ray unit cannot be operated unless the door is closed - the control panel is outside the room. Dr. John Heslep, Radiation Safety Division, Berkeley, has specified the amount of shielding necessary and

this has been included in the plans. This installation, which was designed by Dr. Paul Stivender, General Electric Engineer, has been accepted by the Architects and Engineers and by the University Physician.

V. RESEARCH (PRELIMINARY STUDIES)

A. Plasticizing of Buildings.

For the past year, we have been investigating various types of plastics to obtain a suitable strippable coating for the new animal-holding quarters. Most of the plastics put to trial have been too brittle, insufficient in tensile strength, or not resistant to the chemical action of the cleansing detergents. One plastic which we now feel will be suitable as a protective coating for the interior finish of the animal-holding quarters is called "Ply-On*." This product is pliable, resistant to acid and alkali, is relatively inexpensive and strips easily without tearing. Furthermore, "Ply-On" can be applied by our own personnel with spray equipment.

Preliminary experiments with "Ply-On" have been satisfactory. The plastic is usually applied in three coats. The A coat is applied as a primer and sealer. The B coat constitutes the main body of the plastic and may be applied up to 32 microns in thickness. The C or final coat is approximately 4-6 microns thickness and contains the color pigment. The total thickness of the 3 coats can vary from 10 microns to 40 microns. A 40 micron thick coat is suitable as a floor covering. If it is necessary to remove a contaminated area, the area involved can be cut with a sharp instrument, stripped, and repaired with satisfaction.

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*Ply-On Coatings, Inc., San Francisco, California.

B. Phantom Dogs.

A year ago, preliminary studies were begun on the preparation of plasticized phantom dogs. These phantoms are to be sent to M.I.T. for isodose standardization curves for Bremsstrahlung analyses. Various types of plastics were tried and Vinyl Plastic B* compound appeared promising; however, this material became hardened and cracked at room temperature, and the embalmed plasticized dog lost weight. Therefore we have continued the search for a more suitable plasticizing material.

Several embalmed dogs were sprayed with "Ply-On." The embalmed specimens sprayed with this material have been withstanding room temperature, and the plastic has remained elastic and flexible. An example of specimens prepared with this material is shown in Figure 1.

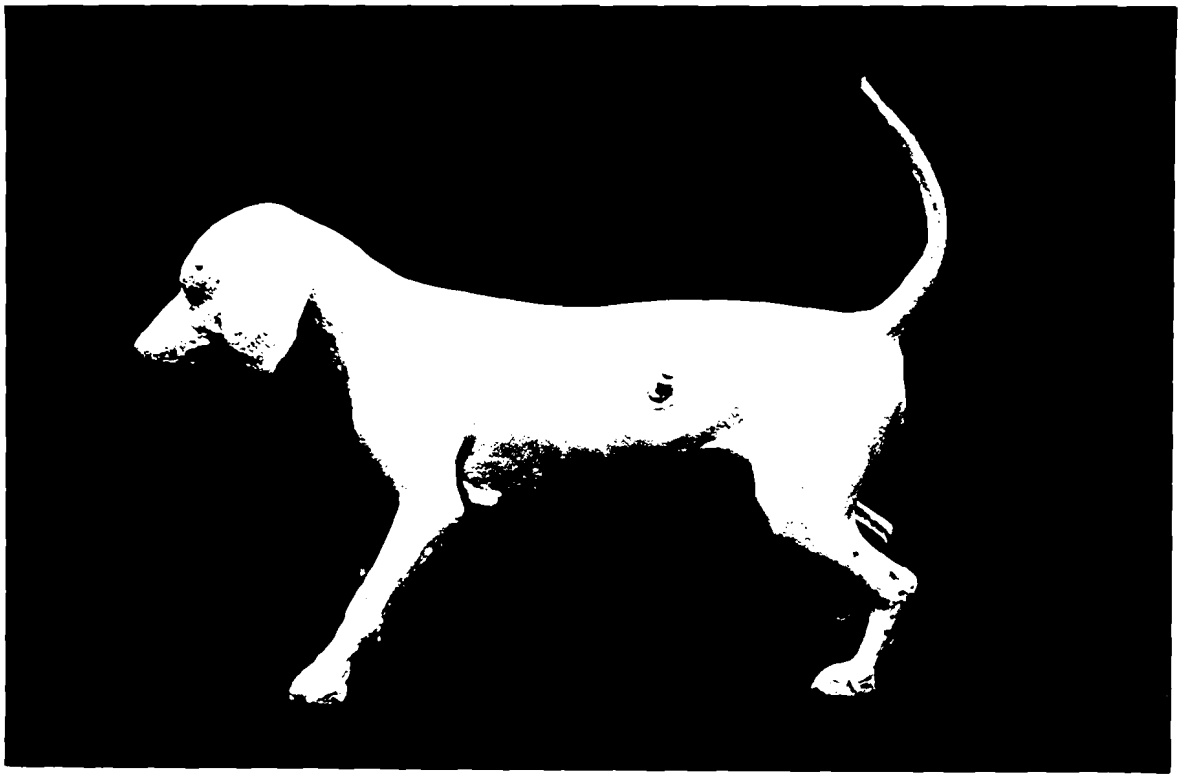

Fig. 1. Plasticized Dog No. 5B8. This specimen has been kept at room temperature for 60 days. The tube, as seen, is connected to a balloon inserted in the stomach for research purposes.

*Bakelite Company, Texas City, Texas.

A "cold" phantom and one containing Strontium-90 are now being prepared for Dr. Robley D. Evans.

Chemical analysis of "Ply-On" revealed an undesirable high level of heavy metals, especially titanium (See Chemical Analysis of Plastics, Part C). Heavy metals are undesirable since they frequently are contaminated with trace amounts of radioactive isotopes. Furthermore, our objective is to use a "tissue-equivalent" plastic; thus, further trials will be conducted using non-pigment material.

C. Chemical Analysis of "Ply-On."

The chemical analysis of the white plastic "Ply-On" film which was used to plasticize 2 beagle dogs is as follows:

Chemicals	Percent of the White Plastic Film
Carbon	39.8
Hydrogen	5.5
Chlorine	24.6
Oxygen	6.6
Ash	20.0
titanium	8.0
calcium	0.8
phosphorus	0.5
lead	0.2
silicone	less than 0.5
iron	0.01
magnesium	less than 0.01
antimony	less than 0.01
copper	less than 0.01

D. Airport Site.

The Animal Husbandry Annex in which we have kept our Strontium-fed dogs for the past 18 months is to be torn down before the buildings of the new Strontium-90 Project will be completed. For this reason, it has become necessary to find new quarters to maintain the chronically dosed Sr^{90} dogs.

Mr. Cecil Norris, Business Manager of the Davis Campus, has made available a small hut located several miles west of the campus proper near the university airport. This building is 10'x15' in size, and although small, has proved adequate for our purposes. This building has been modified to meet the requirements of the Radiation Safety Division. Four large dog cages have been placed in the building. All cleaning of dog cages and disposition of waste material is accomplished with a minimum of water. Since there are no plumbing facilities or means of washing down the floor, it is necessary to avoid spillage outside of the cages. Using such a semi-dry system has presented a severe challenge since any spill would cause major contamination which must be cleaned by mopping and sponging. As a result, we have gained valuable information on some of the factors involved in completely containing isotopes that are being used.

The interior of this building was sprayed with a plastic material called "Cocoon*." This material has proved unsatisfactory and we have had to seal the inside of the building with polyvinyl sheeting. This liner will be removed and disposed of after the animals have been transferred to the new building site. Adjacent to this shed is an old housing unit which has kitchen and bathroom facilities. These have been modified to our requirements for use as a dressingroom and a place to store our protective clothing.

E. Summary Report on Sr^{90} Dogs.

To date 24 dogs have been dosed with Strontium-90 in the preliminary studies conducted in preparation for the new facility to be completed this summer. Twelve of these dogs were given high levels of Sr^{90} orally, and

*R.M. Hollingshead Corp., Camden, New Jersey.

the acute effects of the isotope were reported in the First Annual Progress Report (July - September, 1957).

The other 12 dogs have been given daily oral doses of Sr^{90}. Six of these dogs (4 adults and 2 pups), are still receiving isotope daily in their feed. The 4 adult dogs have been receiving high levels of Sr^{90} daily for 15 months, and to date, there are no apparent signs of skeletal pathology. Periodic blood samples have remained essentially normal.

An interesting observation on these treated dogs has been their growth. As shown on Figures 2 and 3, there are one or two periods of 3-4 weeks duration when growth slows appreciably. After 40 weeks of age, the dogs have reached a growth plateau similar to dogs kept on the X-ray Project. However, at this age, Sr^{90} treated dogs were approximately 20 percent heavier than expected. We cannot at this time state whether this increase in body weight (growth) is an isotope or environmental effect. Control puppies (OS0 and OS1) are now being raised under similar environmental and nutritional conditions as the Sr^{90} dosed dogs to evaluate basic reasons for this increased growth rate.

Depigmentation of the iris in one male treated with Sr^{90} has been observed. This condition is somewhat similar to that described by Dr. Carl Rehfeld at the June meeting at Davis in 1957. However, we have not observed fundic changes as described by Rehfeld in radium treated dogs.

The other two chronically dosed pups, 5S7 and 5S10, are progeny from the 15 month Sr^{90} treated female (5S4). This bitch had a litter of 5 puppies, two of which were stillborn and one was sacrificed at weaning. Two puppies are being raised as an F-2 generation on Sr^{90}. One of these pups will be sacrificed at 12 months of age, and the last pup will be sacrificed at 18 months of age. From these studies, information will be

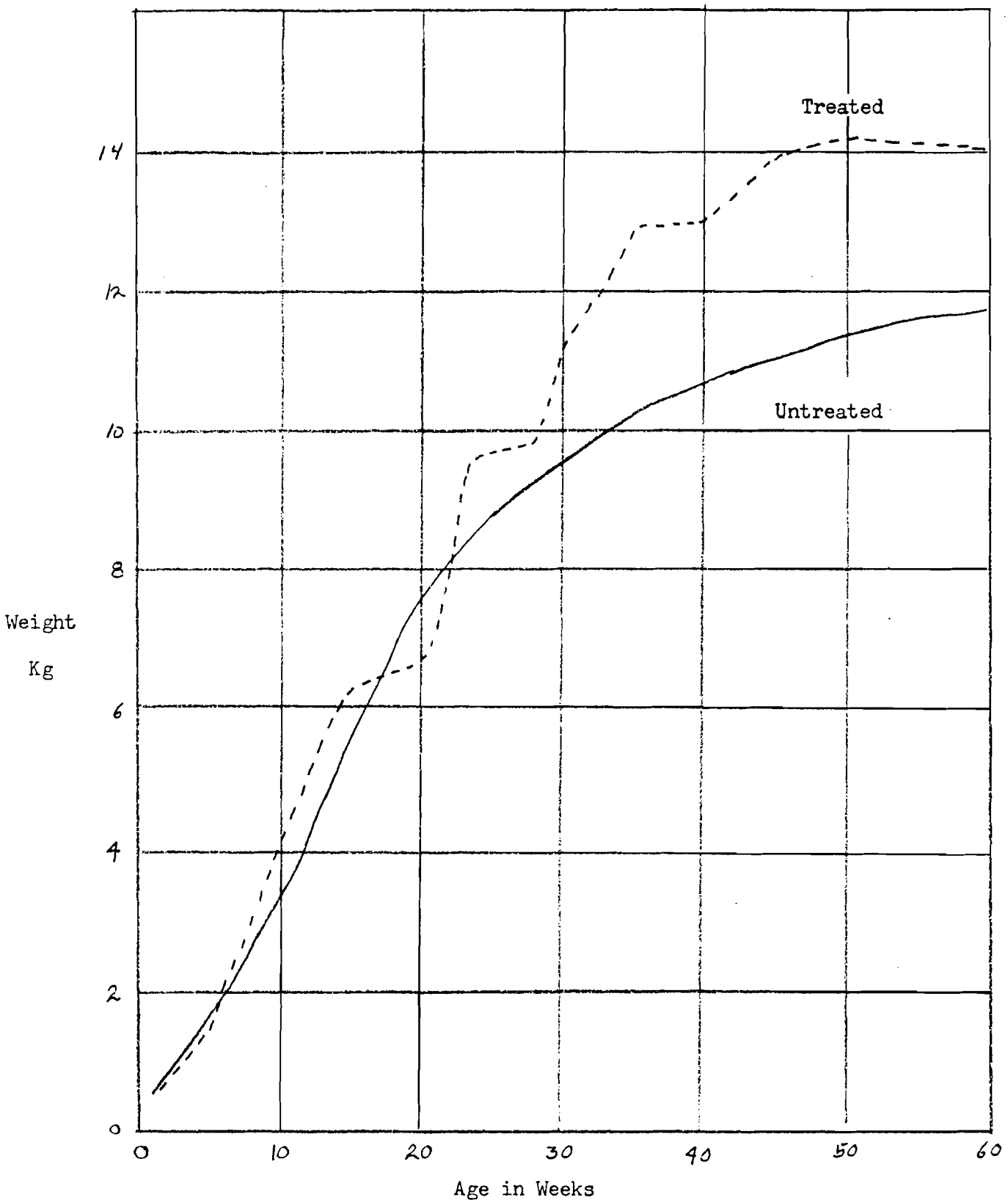

Fig. 2. Comparison of Sr^{90} treated to untreated dogs. MALES.

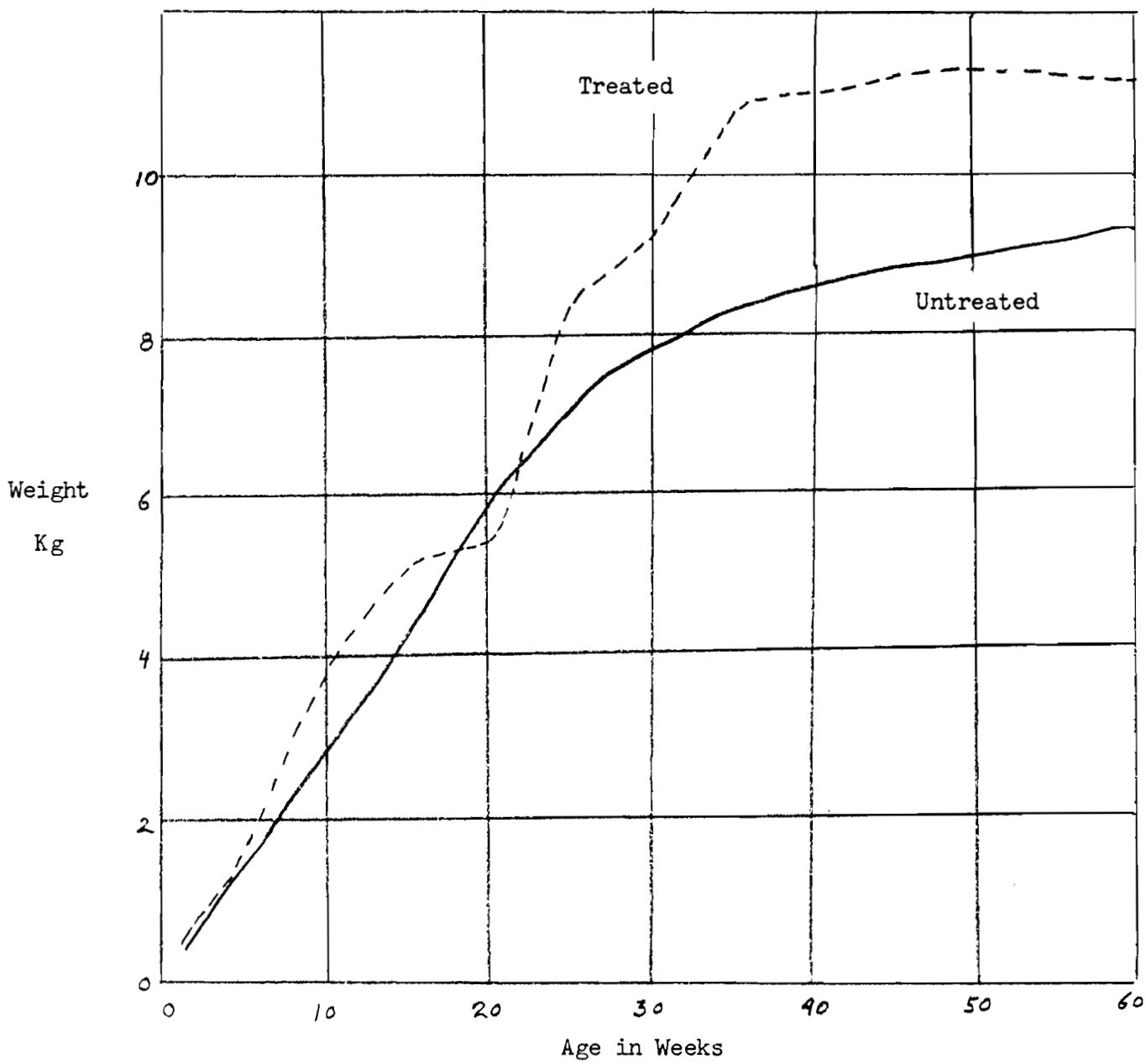

Fig. 3. Comparison of Sr^{90} treated to untreated dogs. FEMALES.

ACUTE ORAL TOXICITY STUDIES

Trial No.	Litter No.	Dose Level	Frequency	Disposition	Remarks
XS1	4C5	23 μc Sr^{90}/Kg Body Wgt.	Single oral	Sacrificed 106 days after dosing	Minor pathology
XS2	4D5	23 "	"	" "	"
XS3	4A5	46 "	"	" "	"
XS4	4B5	46 "	"	" "	"
XS5	4A6	90 "	"	" "	"
XS6	4B6	90 "	"	" "	"
XS8	4A8	470 "	"	Died 43 days after dosing	Major pathology
XS9	4F7	470 "	"	" 32 "	"
XS10	4B8	23 "	"	" 7 "	"
XS11	4D7	23 "	"	" 7 "	"
XS12	4C8	4.6 "	"	" 6 "	"
XS13	4E7	4.6 "	"	" 7 "	"

PRELIMINARY STUDIES DAILY INGESTION Sr^{90}

Trial No.	Litter No.	Dose Level	Frequency	Disposition	Remarks
5S0	C76	1.83 µc Sr^{90} per gm Ca	daily	Sacrificed at weaning pups	
5S1	1C	"	"	Longevity	First generation on Sr^{90}
5S2	1A	"	"	Longevity	"
5S3	1D	"	"	To sacrifice at 18 mos. (7-9-58)	"
5S4	1B	"	"	To sacrifice at 18 mos. (7-9-58)	", whelped second generation
5S5		"	"	Sacrificed at weaning (1-14-58)	Second generation on Sr^{90}
5S6		"	"	Stillborn (12-7-57)	"
5S7		"	"	To sacrifice at 12 mos. (12-7-58)	"
5S8		"	"	Stillborn (12-7-57)	"
5S10		"	"	To sacrifice at 18 mos. (6-7-59)	"
5S12		"	"	To sacrifice when puppies wean	Whelped 2, both stillborn
5S13		"	"	Stillborn (4-1-58)	
0S0	37D	0	0	Control	
0S1	37E	0	0	Control	

available on the effects of Sr^{90} on successive generations of dogs.

Another female, 5S12, was started on the D_5 level of Strontium-90 at 30 days gestation. Her offspring were to supply the F-1 generation to compare to the above mentioned F-2 puppies. Unfortunately, this bitch whelped only 2 puppies, both stillborn. This bitch will be sacrificed and embalmed and prepared as a "hot" phantom.

F. Skeletal Studies.

The studies on the beagle skeleton were started in October 1956, and are still in progress. Since the September 1957 Progress Report, 25 additional skeletons have been completed. This study offers a great deal of information on the growth and development of the normal beagle skeleton. Most of the dogs involved in the study are between 0 and 18 months of age. A few aged animals are also included. Body measurements of the intact dog include: heighth, length, width, and depth of chest. Each dog is X-rayed before autopsy to obtain permanent record of the skeleton. Also, measurements are obtained from these X-rays and compared to actual dimensions taken at the time of death. After autopsy, the skeleton is separated into the following groups:

1. Skull
2. Mandible
3. Cervical vertebrae
4. Thoracic vertebrae
5. Lumbar vertebrae
6. Sacrum and Caudal vertebrae
7. Ribs
8. Sternum
9. Pelvis
10. Femur
11. Tibia
12. Fibula
13. Rest of Hind limb
14. Scapula
15. Humerus
16. Ulna
17. Radius
18. Rest of Front leg
19. Teeth
20. Costal Cartilage
21. 10th Rib
22. Os Penis

Wet, dry, and dry defatted weights of the above bones as well as linear and volumetric measurements are obtained.

G. Chemical Analyses

1. Skeleton. The chemical analysis of the skeletons of these beagle dogs is proceeding rapidly. Thus far, 22 skeletons have been defatted and volumes of the various bones in the groups listed in Part F (Skeletal Studies) have been determined. Twelve skeletons are being analyzed for calcium, phosphorus, nitrogen, and ash content. These determinations are being completed on a contract basis with Morse Laobratory, Sacramento, California. In addition, we are studying the advisability of determining citrate in these bones since recent investigations indicated an important role for citrate in bone matabolism.

In our laboratory, Mr. A. M. Gau, Senior laboratory technician, is perfecting a wet ashing technique which we plan to use in preference to muffle ashing of bone. This will decrease considerably the hazards which arise in handling dried radioactive ash. These studies involve time - course studies with nitric, hydrochloric, and perchloric acids and with combinations of these acids.

Investigations have continued on methods for the determination of calcium in biological meterials. A method by Solomon et al. (Arch. Biochem., Vol. 11, p. 433, 1946), as modified by the radiochemistry group at M.I.T. (Evans) has proved excellent for materials containing micro amounts of calcium. The well known Clark-Collip oxalate-permanganate titration still seems the most satisfactory for macro amounts of calcium. A new method recently appeared in Anal. Chem., 29, 258 (1957) which uses EDTA and the indicator, Calcon is being investigated. According to the authors, this method may be favorably applied to the determination of micro quantities of calcium. Large number of samples may be handled with ease and magnesium ion does not interfere.

2. Food. This project calls for the feeding of a diet which contains an unchanging calcium to strontium-90 ratio. This diet is to be fed *ad libidum*. Several analyses have been made by us and by independent laboratories of the calcium and phosphorus content of the dry ration. The mean values, on a dry weight basis, are as follows: 3.23 percent calcium, and 1.91 percent phosphorus. We also deemed it wise to establish a bank of sealed strontium-90 standard solutions and to submit aliquots of this solution to various laboratories for radiochemical assay. These assays have now been completed to our satisfaction.

3. Strontium. Arrangements have been made with Oak Ridge National Laboratory to determine the Strontium content of our more important biological materials. Samples of dry defatted dog bone, defatted hamburger, and of the dry ration, are to be sent in the near future for activation analysis.

H. Carbon-14 Studies.

A pilot experiment in which carbon-14 labeled acetate was injected into a lactating beagle dog was described in the Second Semi-annual Progress Report of December, 1957. Since then, we have modified an existing sheep respiration apparatus for the use with dogs. We have substituted a small pressure chamber for the mask which was used on the dog in the previous trial. This chamber is large enough to accommodate the dog but not so large that it creates a serious dead air space problem. One side of the chamber has a glass window through which it is possible to determine the chamber temperature, the respiration rate, activity, and signs of distress on the part of the dog. Dr. Parker has suggested that we might fabricate a small plastic glove box with an airlock entrance to use in place of this steel chamber. Thus, we would be able to obtain blood samples from the dog and to perform acts within the chamber without interrupting the trial.

A series of trials were completed on a lactating bitch during the month of March. These trials involved the use of acetate 1 C-14, acetate 2 C-14, and uniformly C-14 labeled glucose. The trials were of 18-hour duration during which time samples of respired CO_2 were collected and the dog was completely milked out 4 times. These milk samples are now being separated into their major carbon containing constituents, namely: casein, albumin, lactose, and butterfat. By studying the amount of radioactivity in each sample and the pattern of labeling, we should be able to establish the normal pathways of carbon metabolism in the lactating beagle dog. Future trials call for a similar study into the pathways of metabolism of the lethally X-irradiated beagle dog.

VI. COOPERATIVE STUDIES

A. Spontaneous Rickets in Lambs

An outbreak of spontaneous ovine rickets in the Davis area this spring has renewed the interest of Dr. Luick and several other workers in the School of Veterinary Medicine in this disease - the etiology of which is unknown. The disease is characterized by multiple fractures of the long bones and ribs which lead to complete disability, starvation, and death. Drs. Blaine McGowan and Glen Spurlock are the principal investigators; Dr. Luick is assisting by analyzing certain bones of these sheep for calcium, phosphorus, citrate, nitrogen and percent ash content.

B. The Chemistry of Milk

A group of workers in the Department of Dairy Industry are investigating the chemistry of copper and iron in milk. Dr. Luick is assisting this group by actively supervising those phases of the investigation which require the use of radioisotopes, mainly Fe^{59} and Cu^{64}.

C. Water Content of Range Grasses.

In response to the request from Mr. Donald Torrell, Department of Animal Husbandry, Dr. Luick proposed a method by which the water content of pasture grasses which are actually eaten by range sheep would be calculated. This method involves the labeling of body water, particularly saliva, with tritium, and subsequently analyzing saliva and the egesta which is collected from an esophageal fistula for their tritium content. This method has recently been put to the test in a series of trials at the Hopland Field Station by feeding sheep lawn clippings. The reliability of Dr. Luick's method is indicated by the fact that there was no significant difference between the direct determination of water content of the lawn clippings by dessication from that calculated by the indirect method using tritium labeled saliva. Mr. William Siri of the Donner Laboratory, University of California, cooperated by performing the tritium analysis.

D. Anatomy of the Beagle.

For the past year, Dr. Howard Evans from the Department of Anatomy, School of Veterinary Medicine, Cornell University, has been studying during his sabbatical year with Dr. L. M. Julian of the Davis Anatomy Department. Dr. Evans has been studying the anatomy of the beagle and has found some abnormalities in the muscles of the hyoid area in the beagles from the Cornell Research Kennel. We have made available to Dr. Evans many of the dogs undergoing routine autopsy in our beagle kennel. To date, he has not found the muscular abnormality in dogs examined from our beagle colony. This suggests the existence of a genetic mutation in the Cornell strain of beagles.

E. Musculo-skeletal Relationship in the Beagle

Dr. Logan M. Julian, Chairman of the Department of Anatomy, School of Veterinary Medicine, Davis, has been conducting a study on muscle mass-length relationships, and muscle-bone length relationships. We have made available to Dr. Julian many of the dogs which are included in our skeletal studies. As a result of this cooperative study, data will be made available to compare the muscle-skeletal relationships of the beagle to that of other breeds of dogs.

VII. PUBLICATIONS AND REPORTS

A. Publications*

1. The Metabolic Fecal Phosphorus Excretion of the Young Calf.

   G.P. Lofgreen, Max Kleiber and Jack R. Luick.
   J. Nutrition, 47, No. 4, pp. 571-582 (August 1952).

2. Distribution of Intravenously Injected Radioactive Phosphorus (P^{32}) Among Sheep Tissues.

   A.H. Smith, Max Kleiber, A.L. Black, J.R. Luick, R.F. Larson and W.C. Weir.
   J. Animal Sci., 11, No. 4, pp. 638-645 (November 1952).

3. Butyrate as a Precursor of Milk Constituents in the Intact Dairy Cow.

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4. Glucose as a Precursor of Milk Constituents in the Intact Dairy Cow.

   Max Kleiber, A.L. Black, M.A. Brown, C.F. Baxter, J.R. Luick, F.H. Stadtman.
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*Jack R. Luick. Not listed previously and brought up to date.

7. Calcium and Phosphorus Transfer in Intact Dairy Cows.

Max Kleiber and J.R. Luick.
An. N.Y. Acad. Sci., 64, pp. 299-313 (August 1956).

8. An Improved Method for the Determination of Metabolic Fecal P.

Jack R. Luick and G. P. Lofgreen.
J. Animal Sci., 16, No. 1, pp. 201-206 (February 1957).

9. Partition of Calcium Metabolism in Dairy Cows.

Jack R. Luick, J.M. Boda and Max Kleiber.
J. Nutr., 61, No. 4, pp. 597-609 (April 1957).

10. Some Biokinetic Aspects of Calcium Metabolism inDairy Cows.

Jack R. Luick, J.M. Boda and Max Kleiber.
Am. J. Physiol., 189, No. 3, pp. 483-488 (June 1957).

11. Fructose Metabolism in Dairy Cows.

Jack R. Luick, Max Kleiber, J.M. Lucas and T.A. Rogers.
Am. J. Physiol., 191, No. 1, pp. 90-94 (October 1957).

12. Phosphate Exchange in Marrow-Filled and Pneumatized Bones of Birds.

A.H. Smith, D.G. Reck and J.R. Luick.
Am. J. Physiol., 190, No. 3, pp. 455-456 (September 1957).

13. Circulation Cleaning.

W.G. Jennings, A.A. McKillop and J.R. Luick.
J. Dai. Sci., 15, No. 11, pp. 1471-1479 (November 1957).

14. Acetate Metabolism in Ketosis of Dairy Cows.

G.K. Macleod and J.R. Luick.
Proc. W. Div. Am. Dai. Sci. Assn., pp. 73-85 (1955).

Further Studies on Fructose Metabolism in Dairy Cows using U-C^{14} Fructose. (in preparation)

A Method for the Determination of Water Intake of Grazing Sheep using H^3-Labeled Water. (in manuscript)

A Respiration Apparatus for Studies on the Intermediary Metabolism of Small Animals, Especially Dogs. (in preparation)

Acetate Metabolism in the Lactating Dog using 1 C^{14} and 2 C^{14} Acetate. (in preparation)

Glucose Metabolism in the Lactating Dog using U-C^{14} Glucose. (in preparation)

B. Lectures and Seminars

A. C. Andersen*

Jan. 9, 1958	La Perita Garden Club, Courtland, California. Radiation Effects in Relation to Animal Production.
Feb. 14, 1958	California Young Farmers of America Group, Fresno, Calif. Panel Discussion, "Atoms in Agriculture." Pathological Aspects of Sr^{90} and X-rays in Animals.
Feb. 27, 1958	Joint Meeting of Calif. Cattlemen's Assn., Farm Advisors, and Related Groups. Radiation Effects in Relation to Animal Production.

H. R. Parker

Jan. 29, 1957	A.C. Andersen, H.R. Parker. Informal discussion and demonstration on the biological effects of ionizing radiation followed by a conducted tour of the X-ray Project. CSVMA mid-winter Conference, Davis Campus.
Apr. 13, 1957	Picnic Day Public, Davis Campus. Construction and Management of AEC Project No. 4.
May 6, 1957	Sophomore Class, Veterinary School, Davis Campus. Kennel Activities, AEC Project No. 4.
June 14, 1957	High School and College Science Teachers - Civil Defense, Radiological Safety Services, Sacramento, California. Biological Effects of Sr^{90} and X-ray.
Nov. 8, 1957	A.C. Andersen, H.R. Parker. The Production and Use of Beagles in Radiobiological Research. Animal Care Panel, San Francisco, California.
Mar. 12, 1958	North Sacramento Rotary Club, Sacramento, California. The Establishment and Maintenance of a 500-dog Beagle Colony.

J. R. Luick

June 22, 1957	Assn. Operative Millers (General Mills, Inc.), San Luis Obispo, California. Use of Radioactive Isotopes in Farm Animals.
Spring, 1957	University personnel, Davis Campus. Lecture, laboratory and field exercises. Radiological Mobile Laboratory Operators Course (69 hours).

*Additional publications and lectures listed in Project 4 Progress Reports.

Aug. 25, 1957	Pacific Coast Section for the Am. Assn. Adv. Sci., Stanford, California. Further Studies on the Fructose Metabolism in Ruminants.
Dec. 6, 1957	Seminar: Graduate Group in Physiology, Davis Campus. The Role of Milk and Dairy Products in the Atomic Age.
Jan. 27, 1957	Pacific Gas and Electric Employees (Civil Defense, Radiological Safety Service), Davis, California. Radiation Detection Instruments and their use in the Field.
Feb. 14, 1958	California Young Farmers of America Group, Fresno, Calif. Panel Discussion, "Atoms in Agriculture." Radioactive Isotopes for Research with Farm Animals.
Feb. 26, 1958	Seminar: Graduate Group in Plant Science, Davis Campus. Radiation Detection Instruments and Methods of Radioassay.
Mar. 11, 1958	Soroptomist Club, Davis, California. California's Program for Radiological Defense.
Mar. 13, 1958	Seminar: Graduate Group in Education, Davis Campus. Background of Radiological Defense for the Needs of our Community.
Spring, 1958	Interested Public, Esparto, California. Civil Defense (Calif. State Div. Rad. Safety Serv.). Radiological Meter Operator's Course (15 hours).
Spring, 1958	Interested Public, Woodland, California. Civil Defense (Calif. State Div. Rad. Safety Serv.) Radiological Monitor Course (41 hours).

Respectfully submitted,

Jack R. Luick, PhD.
Radiobiologist
A.E.C. Project No. 6

Approved by:

A. C. Andersen, VMD, PhD.
Principal Investigator
A.E.C. Projects 4 & 6