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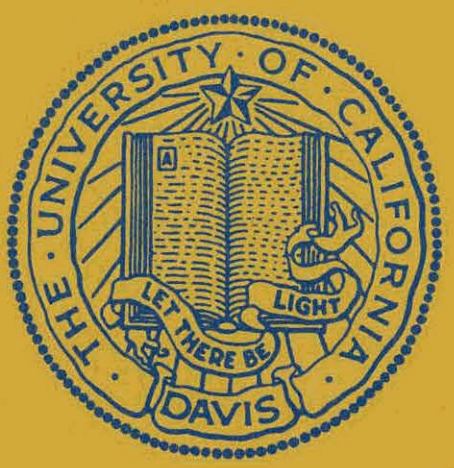
1968 Annual Report



Radiobiology Laboratory

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RADIOBIOLOGY LABORATORY
School of Veterinary Medicine
University of California, Davis

ANNUAL REPORT - Fiscal Year 1968

Issued June, 1968

by

The Staff

of the Radiobiology Laboratory

Leo K. Bustad, Director

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FOREWORD

This is the third annual report since the formal unification of two radiation projects to create the US Atomic Energy Commission-sponsored Radiobiology Laboratory. The first project began in 1951 and was designed to define the late effects of acute and fractionated doses of X-irradiation on female Beagles; only 25 Beagles from this study remain. Fractionation of the dose had no noticeable effect when the total dose was 100R; however, with a total dose of 300R, there was an eight-day increase in survival for every day elapsing between the first and last exposure. Modest effort is being expended to determine the ovarian effect of low-level fractionated exposure in pups prior to weaning.

The principal emphasis continues to be on the second--and larger--project, which is devoted to the effects of bone-seeking radionuclides. Efforts in cell biology and biochemistry are continuing. Blood cells from Ra-226-treated dogs appear to manifest radioresistance. The response of lymphocytes to *in vitro* X-irradiation was less marked, and surviving cells showed greater transformation; marrow cells incorporated twice as much DNA precursor per cell as did control cells at 24 hours.

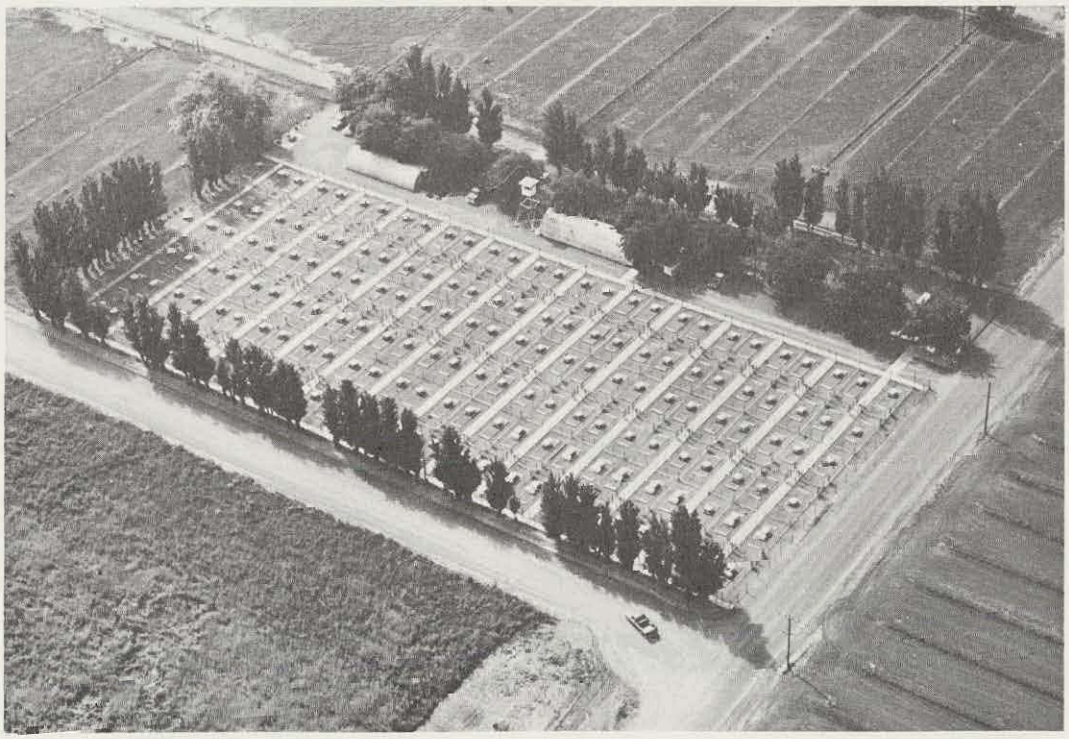
Bones from older Beagles fed high levels of Sr-90 develop a condition that we refer to as "pachyostosis" due to the unexpected increase in density, thicker cortex, and decreased remodeling that is observed. The tentative conclusions are that the catabolic activity in bone is affected to a greater extent than is anabolic activity during remodeling and development. Another unexpected finding was a decrease in the mucopolysaccharide content of costal and articular cartilage in the skeletons of dogs treated with Ra-226 at the highest levels.

We are following up our observation that the myelogenous disorders induced by radiostrontium are, in fact, progressive stages in a leukemic response. This is being done by techniques directed toward defining the pathogenesis in animals having a high probability of manifesting the disease. In addition to the Beagle colony, a small *Marmosa mitis* colony has been established; developmental studies on marmosa are described in this report. Three strains of mice are also being utilized to determine the age at which Sr-89 exposure is most critical for leukemia induction.

The increase in contributions to this year's progress report reflects our increased scientific effort.

L. K. Bustad, Director

RADIOBIOLOGY LABORATORY



RADIOBIOLOGY LABORATORY ANNEX

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IN MEMORIAM

In appreciation of their fine contributions
and cooperative spirit,
we dedicate this issue to three of our associates
who died during the past year

JUDY M. BOYDEN
IRVING I. HERTZENDORF
ROBERT P. STARBUCK

A special fund has been established at the University in honor and memory of Dr. Irving I. Hertzendorf, an outstanding postdoctoral fellow who was tragically killed.

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BEAGLE X-IRRADIATION STUDIES

In order to obtain data that could later be extrapolated to assess possible late effects of irradiation of humans, Beagle dogs were exposed to single or fractionated whole-body X-irradiation as outlined in Table 1.

Table 1. EXPERIMENTAL DESIGN FOR WHOLE-BODY X-IRRADIATION STUDY

Subgroup	Exposure*	Total R	No. Dogs
1	Control (sham-irradiated)	0	57
2	25R at 28-day interval	100	22
3	25R at 14-day interval		25
4	25R at 7-day interval		20
5	50R at 28-day interval		21
6	50R at 14-day interval		21
7	50R at 7-day interval		20
8	100R single		23
		Subtotal	152
9	75R at 28-day interval	300	22
10	75R at 14-day interval		23
11	75R at 7-day interval		26
12	150R at 28-day interval		25
13	150R at 14-day interval		21
14	150R at 7-day interval		23
15	300R single		11
		Subtotal	151
		Total	360

* Radiation factors: 250 kvp, 30 ma, Thoraeus II filter (HVL 2.65 mm Cu). Dose rate, 8.5R/min, bilateral midline air dose at 140-cm distance. Female Beagles, 8 to 12 months old when exposed.

The Beagle dog was chosen as an experimental subject because it has a relatively long lifespan, a low incidence of spontaneous tumors of bone and blood-forming organs, and is readily available; its short hair and medium size were also advantageous.

One of the objectives of the study was to evaluate any decrement in work capacity. Reproduction was utilized as a measure of work potential since it is a natural process of radiobiological interest which requires energy expenditure and produces quantitative data. Results of the experiments are reported in the succeeding papers.

In a limited study the effects on the ovary of 10 or 30R exposure on alternate days to pups 2 to 42 days of age will be determined; the ovaries will be examined at 6 months of age for the presence of follicles.

SURVIVAL OF CONTROL AND X-IRRADIATED BEAGLES

L. S. Rosenblatt
A. C. Andersen

The study of the effect of X-irradiation on survival of female Beagles was updated. Survival during the 3 yr immediately after irradiation was inversely related to total X-ray dose. All irradiated subgroups evidenced lifespan shortening relative to controls. Lifespan shortening was independent of total elapsed time between first and last exposure for 100R subgroups but a negative correlation was observed among 300R subgroups. Gompertz equations of mortality rates fit the data of only the last 6 yr of each irradiation group; during these periods the increase in mortality rates was identical for the three groups.

As of April 1968 there were 27 survivors of the X-ray study, including 8 control dogs. The present report updates the results of the survival study to January 31, 1968. Over a 6-yr period beginning in late 1952 a total of 360 Beagles were entered into the experiment; of these, 57 were sham-irradiated controls, 152 received a total of 100R, and 151 a total of 300R. Data relative to numbers of dogs and exposure regimens are given on p 1 of this report.

The analyses reported on here consist principally of life-table studies (cumulative survival rates) and of studies that involved the fitting of Gompertz equations to observed mortality rates. The cumulative survival rates of the individual subgroups, combinations of subgroups, and groups (0R, 100R, and 300R) did not change appreciably from those observed last year (UCD 472-114, 1967, p 7). The latest cumulative survival rate curves for groups are shown in Fig. 1. The 300R group has lower survival rates than the controls from the beginning, while those of the 100R dogs are slightly below control values in the beginning but do not really begin to differ from controls until about 10 yr post-irradiation. It was of interest to determine whether the early mortality observed among the irradiated dogs was dose-related. The two major causes of death in the younger dogs were classified as acute diseases--infectious and non-infectious--and reproductive disorders, primarily dystocia and gangrenous mastitis. A compilation of the basic data was presented last year (UCD 472-114, 1967, p 10). For deaths occurring during the first 2 yr post-irradiation, mortality was definitely related to dose (Fig. 2). Hence, the reduced survival rate of the irradiated dogs (in particular, the 300R group) relative to controls could not be said to be independent of irradiation. In fact, the data appear to indicate the existence of synergisms between irradiation treatments and environmental stresses. Retrospective studies strengthen this idea, in that deaths attributable to distemper occurred only among irradiated dogs and gangrenous mastitis was observed only among dams given 300R.

The cumulative survival rate curves were recomputed utilizing data only for dogs that had survived to 3 yr post-irradiation, thus ruling out early mortality. The results (Fig. 3) do not appear to differ appreciably from those shown earlier (Fig. 1) when the data for the higher ages are compared. When all dogs were utilized (Fig. 1), the median survival times (MST) in years post-irradiation were 11.6, 10.5, and 9.2 yr for the 0R, 100R, and 300R groups, respectively. For the populations of dogs surviving to 3 yr post-irradiation, the MSTs are shifted upward by 0.1 to 0.2 yr. Lifespan shortenings, however, were unaltered; they averaged 9% for the 100R and 19% for the 300R groups relative to controls.

The assessment of lifespan shortening by the use of averages for groups may be somewhat misleading. The experimental design (p 1) shows that there were six fractionated exposure subgroups per group. The interval between exposures was either 7, 14, or 28 days and either 2 or 4 exposures were given. The total protraction of the dose (total elapsed time) varied from 7 days (2 exposures, 7 days apart) to 84 days (4 exposures, 28 days apart). When lifespan shortenings are plotted against total elapsed time the results shown in Fig. 4 are obtained. It is clear that all subgroups have suffered some shortening of lifespan. Among the 100R subgroups there is no correlation of lifespan shortening with total elapsed time. Among the 300R subgroups such a correlation is strikingly evident. The observed regression coefficient ($b = -0.17$) indicates that lifespan shortening is reduced by 0.17% by the addition of one day between first and last exposures, within the range studied (7 to 84 days). Thus, an overall difference of 77 x 0.17% or 13% was noted. It is also of interest to note that the two 100R subgroups that exhibited the greatest lifespan shortening were each exposed at 14-day intervals (subgroups 3 and 6). The only 300R subgroups found to be above the regression line were those also exposed at 14-day intervals (subgroups 10 and 13). Although this observation does not justify the drawing of a conclusion, it may indicate that cell synchrony at the time of irradiation had a prolonged effect on survival.

A number of attempts were made to fit Gompertz equations to observed mortality rates. The form of the Gompertz used was $Y = ae^{kt}$, where k is the rate constant or, on a semi-log scale, the regression coefficient, representing the rate of change of mortality rates relative to changes in age. The mortality rate at the x^{th} age (q_x) was defined as the number dying in the x^{th} interval relative to the number of dogs at risk during that interval, and expressed in percent. The first attempts involved fitting either all of the data or all but that for the first 3 yr post-irradiation by the method of least squares.

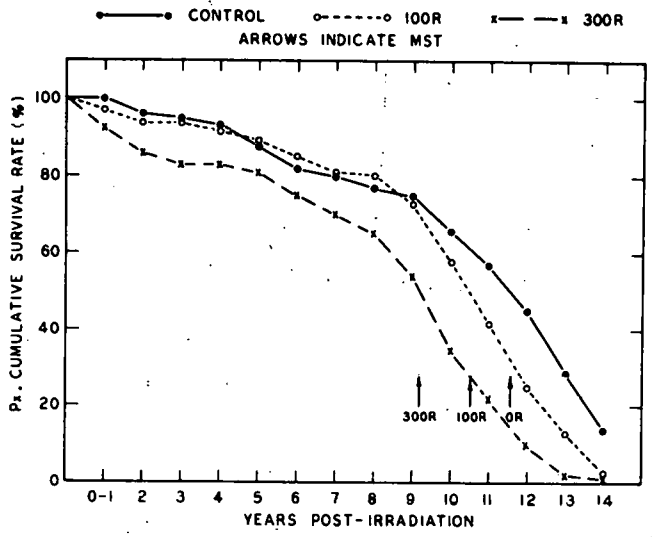

Fig. 1. Cumulative survival rates for control and X-irradiated Beagles. All dogs surviving 90 days post-first-irradiation are included. Arrows indicate median survival time for each group.

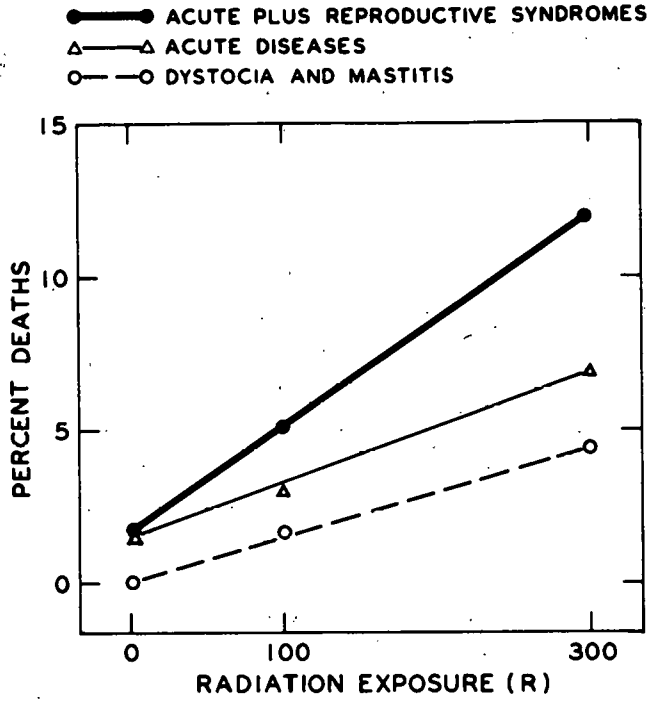

Fig. 2. Percentage of mortality from acute syndromes in control and X-irradiated Beagles during the first 2 yr post-irradiation.

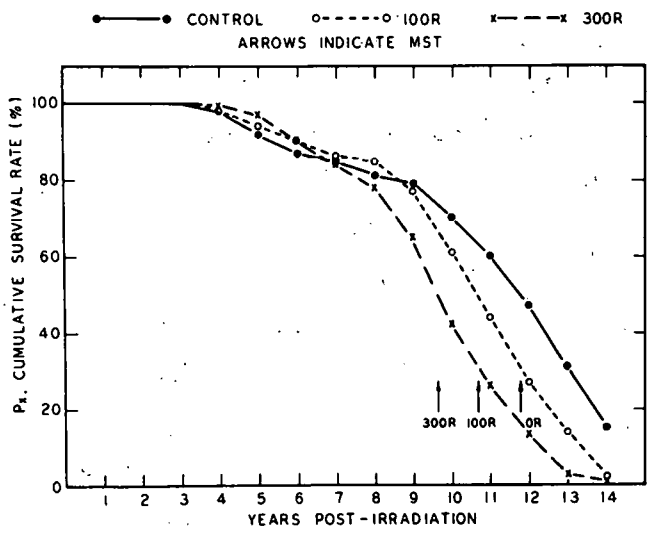

Fig. 3. Cumulative survival rates for control and X-irradiated Beagles surviving 3 yr post-irradiation. Arrows indicate median survival time for each group.

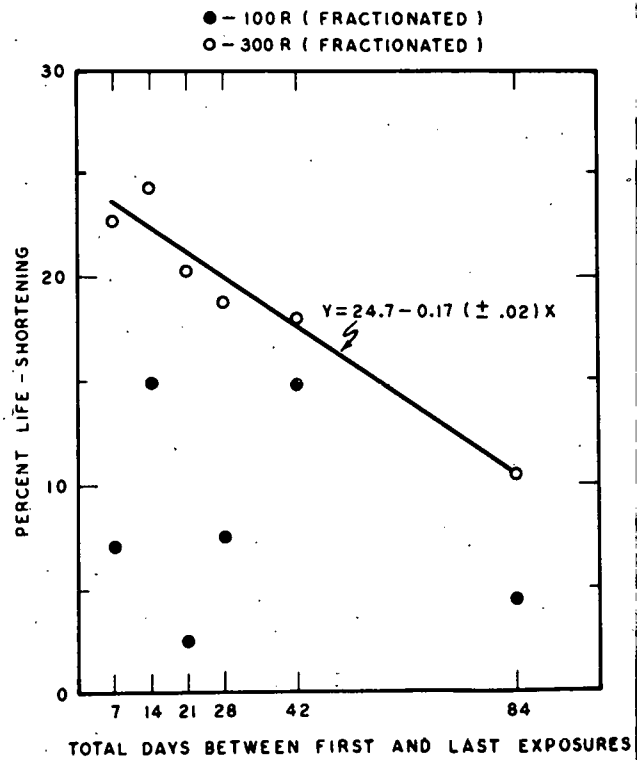

Fig. 4. Lifespan shortening in relation to the interval between first and last exposures.

A more direct method, utilizing the method of moments technique, indicated that the Gompertz equation fit only the data of the last 6 yr of each group. The curves were then projected back to the origin. The results of the last named analyses are shown in Fig. 4.

The mortality rates clarify the results of the survival analyses (Figs. 1-3). There is a graded response to dose for early mortality (1-3 yr post-irradiation), with the 300R group on the high side, immediately followed by a similar graded response to mortality (4-6 yr post-irradiation), with the controls on the high side. All groups show relatively low mortalities 7-8 yr post-irradiation. These results may be explained on consideration of a genetically homogeneous population exposed to stress over a period of time: less vigorous animals would die early; somewhat more resistant ones, later on. The picture is completed with the imposition of the stress due to X-irradiation and the attendant synergisms. The fitted Gompertz curves indicate that the mortality rates during the first 8 yr post-irradiation exhibit components which are not Gompertzian, and it is postulated that these components are those discussed immediately above.

The growth parameters estimated by the method of moments technique for each of the three groups were almost identical, indicating that, when the death rate in each of the groups began to increase, it increased at the same rate for each group. This may be seen in the lower right-hand plot of Fig. 5, where the parallel nature of the three curves for the later period is evident. The times at which these curves lifted off the basal level differed by 1 yr for the 300R-100R and 100R-0R comparisons. The data indicate that the 300R group became "extinct" at 13 yr post-irradiation and the 100R at 14 yr, while the controls were not yet extinct at 15 yr post-irradiation.

The results of this study tend to confirm those of similar studies performed on mice and rats. However, the Beagle data are believed to serve as a better model for evaluation of irradiation effects on human survival, in that genetically homogeneous populations were exposed and maintained under conditions more natural for the species involved.

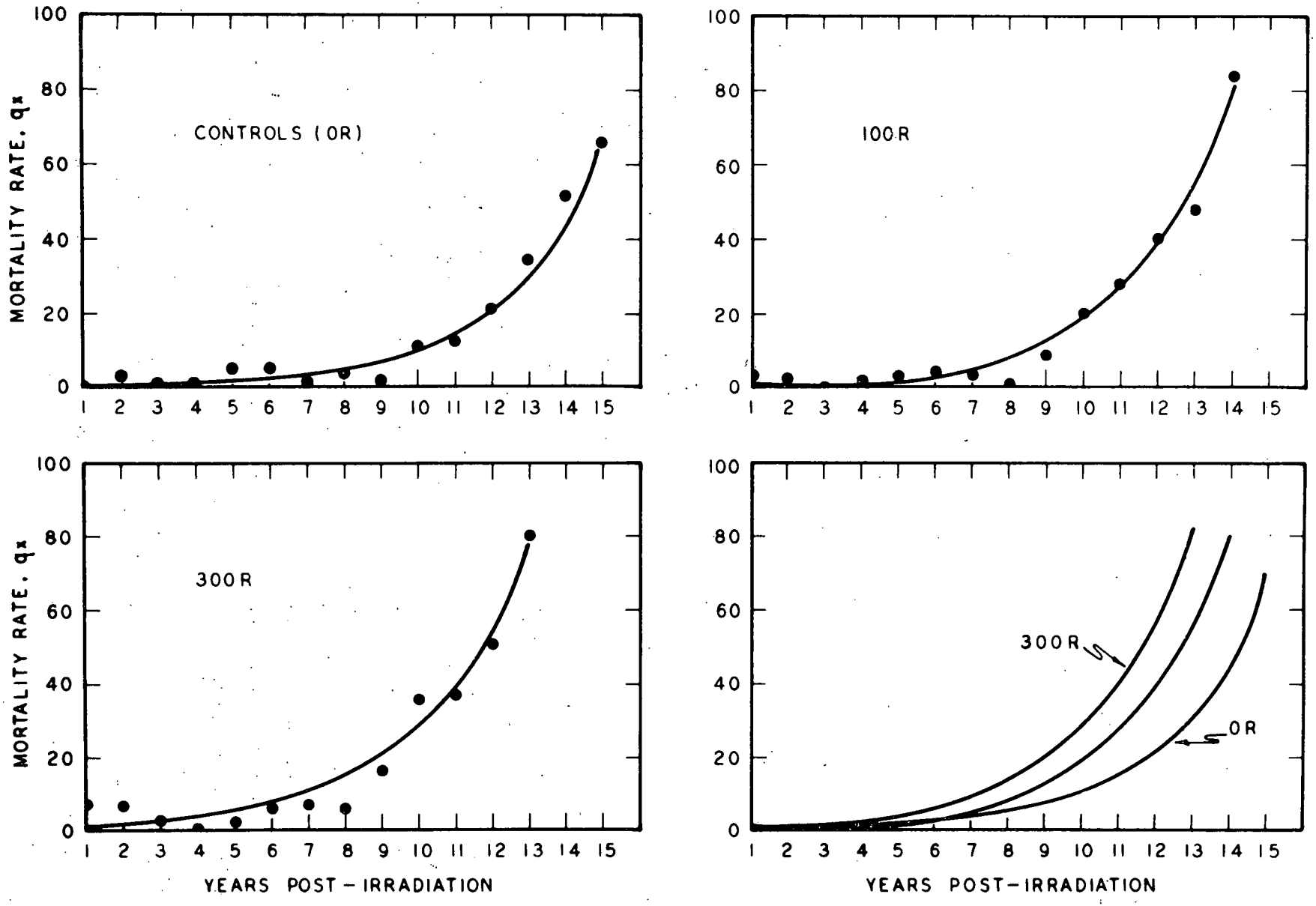

Fig. 5. Observed mortality rates for each group, with Gompertzian equation fitted. The final figure combines the individual group curves.

EFFECTS OF WHOLE-BODY X-IRRADIATION ON THE ABILITY OF FEMALE BEAGLES TO REPRODUCE

A. C. Andersen

The reproductive ability of female Beagles exposed as young pups to a single midlethal dose of X-rays was impaired but not eliminated. These Beagles, up to 6.9 yr of age, weaned an average of 17 pups, 33% fewer than control dams. Fractionated irradiation was utilized to sterilize female Beagles; 26 estrous cycles were exhibited by 7 females, all were infertile. The ovaries at sacrifice revealed no evidence of corpora lutea or corpora albicans.

Studies at this laboratory give evidence that the female dog (Beagle) cannot be sterilized by a single whole-body X-ray exposure (UCD 472-114, 1967, p 3-5). Midlethal dose (290R) survivors, X-irradiated as pups (group B) or prior to puberty (group C) do show a reduction in their pup production when bred on each successive estrous period. The reproductive ability of this colony between 0.8 and 6.9 years of age is shown in Table 1.

Table 1. REPRODUCTION OF FEMALE BEAGLES* 0.8-6.9 YR OF AGE X-IRRADIATED AS PUPS OR PRIOR TO PUBERTY

Group (No. Beagles)	No. Litters	Failure to Conceive (%)	No. Pups Whelped	No. Pups Weaned	Pups Weaned per Dam
A 20 controls	135	25	728	500	25
B 15 pups exposed	94	22	383	246	17
C 22 prepuberty exposed	148	20	687	453	21

* All were survivors of midlethal dose (290R) of X-irradiation.

Thus, relative to controls, a single X-ray exposure of pups from birth to 90 days of age lowered the reproductive ability 33%. A comparable X-ray exposure prior to puberty (7-9 months) showed less effect (17%). These results agree with well established findings in mice, in which radiosensitivity of the ovary varies greatly depending upon age at exposure. However, sterility was not manifested in irradiated female Beagles. Individual records revealed fertile estrous periods in all dogs, and the decrease in reproduction by irradiated dams was attributable primarily to smaller litter size and higher pup mortality to the weaning age, as well as to lowered conception rates.

Since reproduction in female Beagles could not be drastically impaired by single X-ray exposures at various ages, fractionated exposures were used. Seven out of 16 Beagles survived 50R weekly from birth to 14 weeks of age (750R total), and estrus occurred as shown in Table 2.

Table 2. AGE AT ESTRUS OF FEMALE BEAGLES GIVEN FRACTIONATED X-IRRADIATION (50R FOR 14 WKS = 750R)

Beagle No.	Age (days) at Mating						
13C	614	784	967	1071	1321	1446	
14A	1221						
16B	624	764	974	1067	1317	1451	
18B	-						
18C	1503						
21B	328	573	720	1359	1527		
21C	335	402	566	840	1162	1302	1428

Estrus was determined by the date on which females would accept mating; all matings were infertile. A genetic difference in response is indicated by the fact that sibs 18B and 18C continued in anestrus while sibs 21B and 21C had the usual estrus cycles. When the dogs were sacrificed at 4.5 yr of age, the ovaries were about one-third the usual size, almost devoid of follicles, and corpora lutea or corpora albicans were not observed. Therefore, infertility could be explained by atresia of developing follicles. Studies are currently under way to test the effects of lower X-ray exposures on the ovary. Pups from 2 to 42 days of age are being exposed on alternate days (10R or 30R) and the ovaries will be examined at 6 months of age for the presence or absence of follicles.

PRENATAL DEVELOPMENT OF THE OVARY IN THE BEAGLE

A. C. Andersen
Miriam E. Simpson

The following is a resume of a portion of a monograph in preparation: "Development of the Ovary in the Dog (Beagle)." Other parts of the work include: Introduction, Post-natal Development, Estrous Cycle, Aging, and Pathology.

The genital ridge was not found in embryos less than 7.0 mm in crown-to-rump (C-R) length. Nineteen embryos ranging from 4.0 to 6.4 mm in C-R length (21 to 26 days post-coitum (pc)) did not reveal gonocytes; hence the migratory aspects of these cells from the wall of the gut and mesentery could not be studied in the Beagle. First evidence of the genital ridge and enlarged spherical cells (gonocytes) was found in embryos ranging from 7.0 to 10.5 mm in C-R length (28 to 30 days pc). The genital ridge began immediately posterior to the developing adrenal gland and continued caudally along the dorso-lateral aspect of the coelomic cavity for 1.0 to 2.2 mm. In cross section, the dense cellularity of the ridge contrasts with the loose connective tissue of the adjoining mesonephros (Fig. 1a). Histologically, the genital ridge consists of a covering epithelium (mesothelium) and the underlying concentration of mesenchymatous tissue. The surface epithelium is pseudostratified and becomes thinner medially and laterally, continuing as a single, flattened layer of mesothelium lining the coelomic cavity. Enlarged cells with sharply defined, light-staining cytoplasm (gonocytes) were not identified consistently in the genital ridge.

In the 10-mm Beagle embryo the genital ridge protrudes further into the coelomic cavity. The central portion increases in both height and width to form the undifferentiated gonad. The distinct gonadal mass rapidly enlarges in embryos between 12 and 20 mm in C-R length. The gland consists of mesenchymal and epithelioid cells with interspersed capillaries (Fig. 1b). Evidence of a "first ingrowth" was not definite; there is an indication that gonadal enlargement occurs by proliferation of both epithelial and mesenchymal cells. The undifferentiated gonad is somewhat elongated and projects anteriorly and dorsally from the mesonephros, to which it is attached by a broad stalk (Fig. 1b). [Wilhelm Bischoff (Entwicklungsgeschichte des hundeeies, F. Vieweg und Suhn, 1845) described a pair of egg-shaped bodies (or gonads) without definite internal structure in a 27-day-old dog embryo. By actual measurement of the illustration in Bischoff's treatise, this embryo was 15 mm in C-R length, which compares both in age and size to embryos observed in this study.] Gonocytes were not found in undifferentiated gonads of 66 Beagle embryos examined.

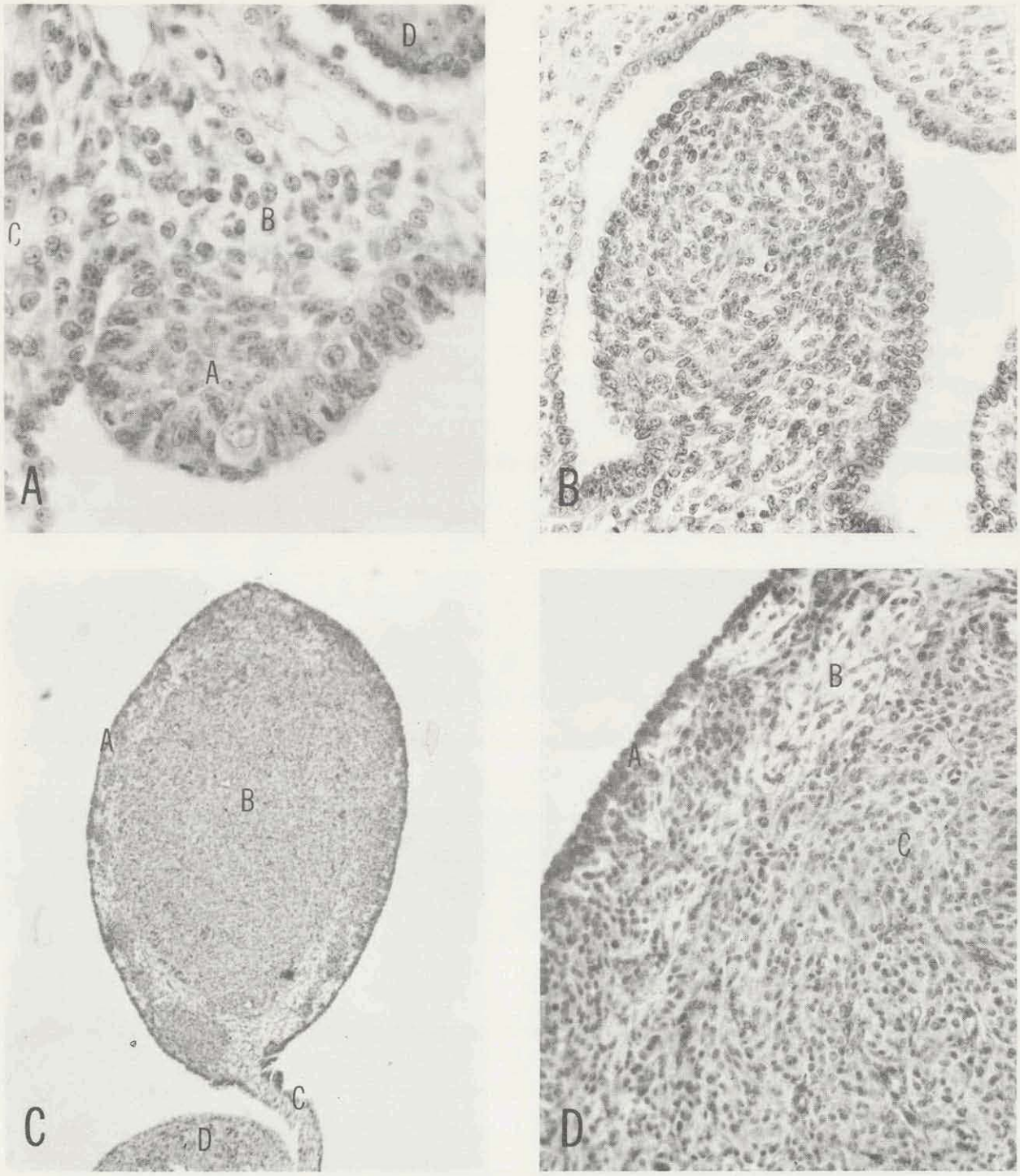

Fig. 1. Early development of the gonad

a. Cross section of (A) genital ridge. Note gonocyte in surface epithelium. (B) underlying mesenchyme. (C) mesentery. (D) mesonephros. Embryo - C-R 8.2 mm; 30 days pc; H and E; 400X.

b. Undifferentiated gonad. Note poorly defined surface epithelium, broad stalk and variation in cell types. Embryo - C-R 15mm; 27 days pc; body wt. 0.47 g; H and E; 125X.

c. Ovary. (A) cortical rim, (B) core, (C) mesovarium, (D) mesonephros. Embryo - C-R 36 mm; 36 days pc; body wt. 3.3 g; H and E; 30X.

d. Higher (100X) magnification of (A) cortical rim, (B) primitive tunica albuginea and (C) core of ovary shown in Fig.

Recognition of the testis precedes that of the ovary in several species including man (Witshi, Development of Vertebrates, W. B. Saunders Co., 1956; van Wagenen and Simpson, Embryology of the Ovary and Testis, Homo sapiens and Macaca mulatta, Yale University Press, 1965). Testes were first recognized in the 19.0-mm Beagle embryo (29 days pc) as a few cord-like structures within the gland. Such cords were readily apparent by the lightly staining center and peripheral concentration of nuclei. The ovary could not be recognized in embryos smaller than 29.0 mm in C-R length (38 days pc). Three distinct zones, characteristic of the ovary, were noted: namely, an irregular thickening of the surface epithelium, which contrasted to the underlying loose connective tissue (primitive tunica albuginea), and a dense cellular core. The core represents the mass of the original ovary. The superficial zone is the definitive cortex, which is commonly referred to as the "second ingrowth". The superficial zone of the ovary in the dog does not have a definite pattern in early stages of differentiation; rather, the cells are in close apposition to the surface epithelium and extend into the gland as irregular clusters or strands of cells (Fig. 1c, 1d).

Jonckheere (Arch. Biol. (Fr). 40: 357, 1930) described and illustrated the "second ingrowth", or proliferation of the surface epithelium, in the ovaries of the prenatal dog. His observations were made on a limited number of specimens whose ages were estimated, and no other measurements were stated. The present account utilizes extensive, accurately dated material to describe the manner in which the ovary develops in the prenatal Beagle. As fetal growth progresses, the ovary likewise increases in size. In the 60-mm fetus (42 days pc), the superficial zone of the ovary ("second ingrowth") appears as irregular clusters of cells forming a cortical rim (Fig. 2a). At this time an increasing number of enlarged, spherical shaped cells (oögonia) become discernible (Fig. 2b). The rete ovarii and associated structures of the ovary are quite conspicuous and assume definitive characteristics (Fig. 2a). The rete ovarii appears as irregular cords and tubules within the core and hilium. In serial sections, cords of the rete ovarii are seen in apposition to the mesonephros, from which it is generally considered to be derived. The hilum also contains blood vessels which continue in the core and branch into numerous capillaries within the primitive tunica albuginea. In the 62-mm fetus,* the Wolffian and Müllerian ducts are distinguishable (Fig. 2a).

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* External features are recognizable in pups of approximately 50 mm; hence, the designation of fetus instead of embryo.

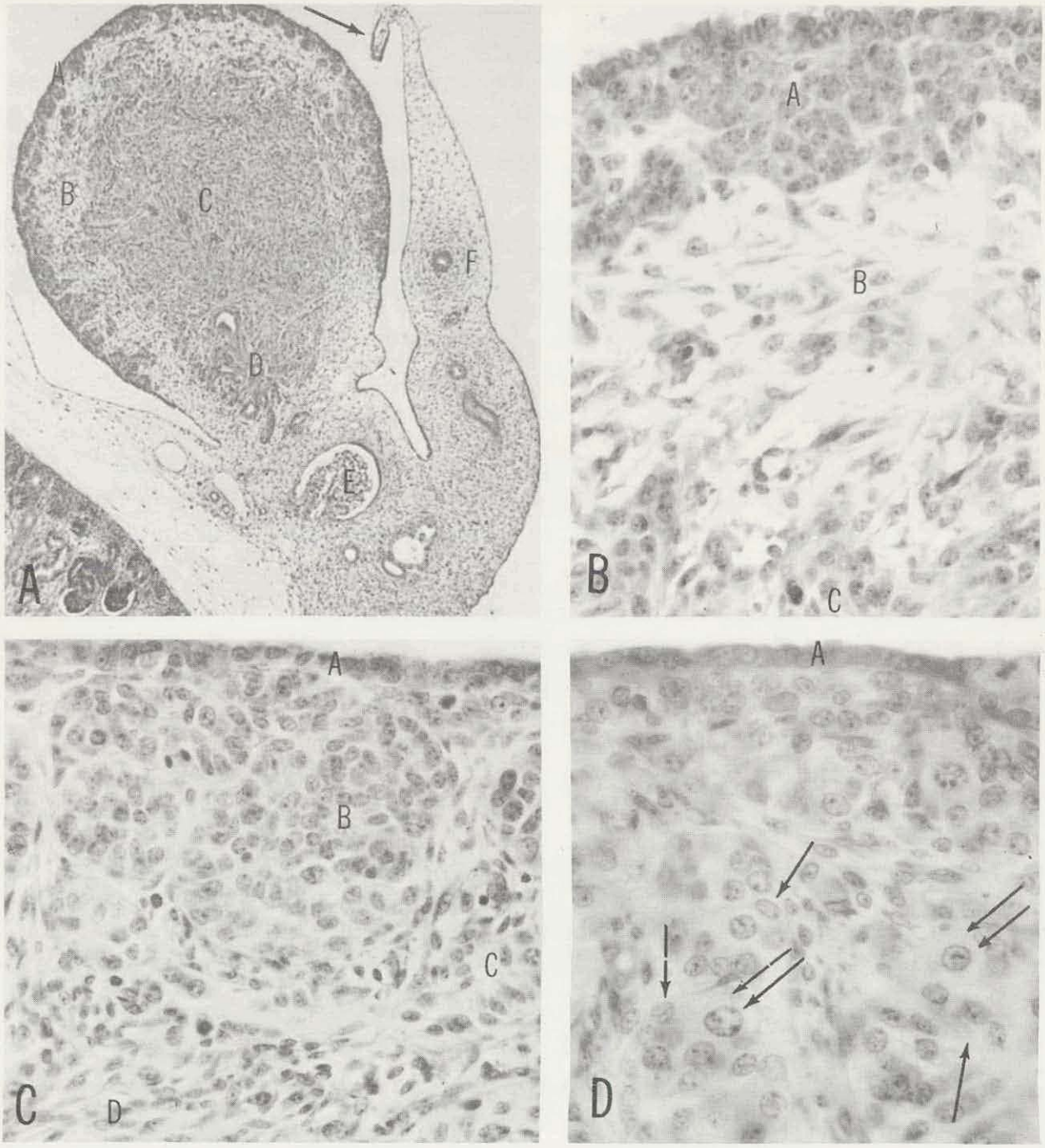


Fig. 2. Cortical lobulation and oogonia proliferation

a. Ovary. (A) cortical rim, (B) primitive tunica albuginea, (C) core, (D) rete ovarii, (E) regressing mesonephros and (F) oviduct. Arrow points to developing fimbria. Metanephros, lower left corner of photograph. Fetus - C-R, 62 mm; 40 days pc; body wt. 12.8 g; H and E; 20X.

b. Higher (350X) magnification of cortex shown in Fig. 2a. (A) cortical rim, (B) primitive tunica albuginea, (C) core.

c. Cortex of ovary revealing lobulation. (A) surface epithelium, (B) lobule, (C) interlobular connective tissue and capillary. Fetus - C-R 109 mm; 43 days pc; body wt. 88.5 g; H and E; 40X.

d. Cortex of ovary showing presence of oogonia (double arrows) and pregranulosa cells (single arrow). (A) surface epithelium. Fetus - C-R 177 mm; 58 days pc; body wt. 268 g; H and E; 350X.

In fetuses between 52 and 60 mm in C-R length, sex could be determined by location of the genital tubercle. The genital tubercle in the female is located immediately ventral to the anus; in the male it is midway between the umbilicus and pubic arch. Identification of sex by position of the genital tubercle was confirmed histologically in 20 specimens ranging from 52 to 65 mm in C-R length (35 to 45 days pc). It was impossible to determine sex by this means in embryos less than 52 mm in length. Therefore, sex must be determined in younger embryos by histological examination of the gonads, the testis being identifiable in embryos 19 to 20 mm in C-R length (27 to 29 days pc), the ovary in embryos 29 to 36 mm in length (34 to 38 days pc).

Fetuses above 60 mm in length show a gradual increase in connective tissue fibers between cell clusters of the cortical rim, and delineation of the core becomes less distinct due to infiltration of cortical cells into the primitive tunica albuginea. These processes are readily apparent in fetuses above 100 mm in C-R length. At this stage an increasing number of blood vessels which stem from the mesovarium can be seen in the less dense cellular areas of the primitive tunica albuginea; lymph vessels were recognized in the 120-mm fetus. Capillaries and extensions of connective tissue surround epithelial clusters except where continuity is maintained with the surface epithelium (Fig. 2c). This arrangement of cells denotes the early formation of cortical lobules, which develop from epithelial ingrowths that proliferate in the periphery of the ovary. As lobules and cords become more distinct in the cortex, a variable but increasing number of oögonia and pregranulosa cells become evident. Oögonia have a large spherical nucleus and lightly staining cytoplasm in contrast to the smaller pregranulosa cells. From about the 100-mm fetus (42 to 44 days pc), and throughout late gestation, the formation of cortical lobules and cords containing oögonia becomes increasingly more prominent. By late gestation, differentiated cortical lobules and cords are composed almost exclusively of oögonia and pregranulosa cells, surrounded by connective tissue and capillaries (Fig. 2d). Mitoses are numerous, and necrosis of oögonia can be detected. The thickness of the cortical rim has increased by late gestation, and the primitive tunica albuginea is practically obliterated. Prior to birth there is no evidence of prophase meiosis or the formation of follicles.

Throughout prenatal development, proliferation of the surface epithelium in the ovary is often referred to as the "second ingrowth." Since the ovary increases in size several fold during prenatal development and the cortical rim remains in the periphery of the gland, it is questionable whether the term "ingrowth" is applicable. To gain insight into the manner in which the ovary develops, the gland and its components were measured in 30 embryos and fetuses ranging in age from 34 to 58 days pc. Measurements obtained from a central section of the ovary were: (a) height of the ovary, (b) overall length of the ovary, (c) average thickness of the cortical rim, (d) height of the core and (e) length of the core. To evaluate proportional changes during growth, the section of the ovary and its components were considered as elliptical and the area of each ellipse was computed (πab)*. It was determined that an abrupt change in growth of the ovary occurs in fetuses between 64 and 109 mm in C-R length; hence the earlier growth rates of the ovary and its components were compared with later growth rates. As shown in Table 1, the rate of growth of the core during the early fetal growth (C-R, 29 to 64 mm) exceeds that of the cortical rim. Hence, the cortical rim during this period is expanding and the proliferation should not be designated as an "ingrowth." As the ovary increases in size the faster growth rate of the core by proliferation pushes the cortical rim outward and epithelioid cells (either singularly or in strands) from the cortical rim are retained within underlying tissues. In contrast to earlier growth of the ovary, fetuses above 109 mm in C-R length show a decided increase in cortical growth rate (Table 1). This increase in cortical proliferation largely accounts for growth of the entire gland, because growth rate of the core decreases, and, in fact, cannot be shown to differ significantly from zero. Thus, it is only during later stages of fetal development that the concept of the cortical rim as an ingrowth could possibly be applied. Even then, however, the term should be used with reservation, because the ovary is increasing in size and cortical proliferation is expanding outward.

Table 1. GROWTH RATES OF THE PRENATAL OVARY AND ITS COMPONENTS[a]

Fetal C-R Length (mm)	Ovary			Cortical Rim			Core		
	b	s_b	r^2	b	s_b	r^2	b	s_b	r^2
29-64	1.13	0.26	0.69	1.04	0.16	0.83	1.46	0.46	0.52
109-177	1.36	0.32	0.61	2.13	0.37	0.78	0.60	0.49	0.12

[a] Growth rates determined by the equation $Y = aX^b$ as given by Huxley, where b is growth rate, s_b = standard error of b. r^2 = coefficient of determination.

* Analyses conducted by Dr. Leon S. Rosenblatt.

EXPERIMENTAL DESIGN OF RADIONUCLIDE TOXICITY STUDIES

Radium-226 Injection Series
(8 Semimonthly IV Injections Starting at 435 Days of Age)

Treatment Code	Multiple of 1 level	Avg. µg/inj.	µg Ra-226/kg	Number of Dogs	
				Lifetime	Sacrifice
R00	0	0.00	0.000	60	5
R05	0.3	0.03	0.003	30	5
R10	1.0*	0.08	0.008	30	5
R20	6	0.5	0.047	30	5
R30	18	1.4	0.14	30	5
R40	54	4	0.42	30	5
R50	162	12	1.25	30	5
				240	35

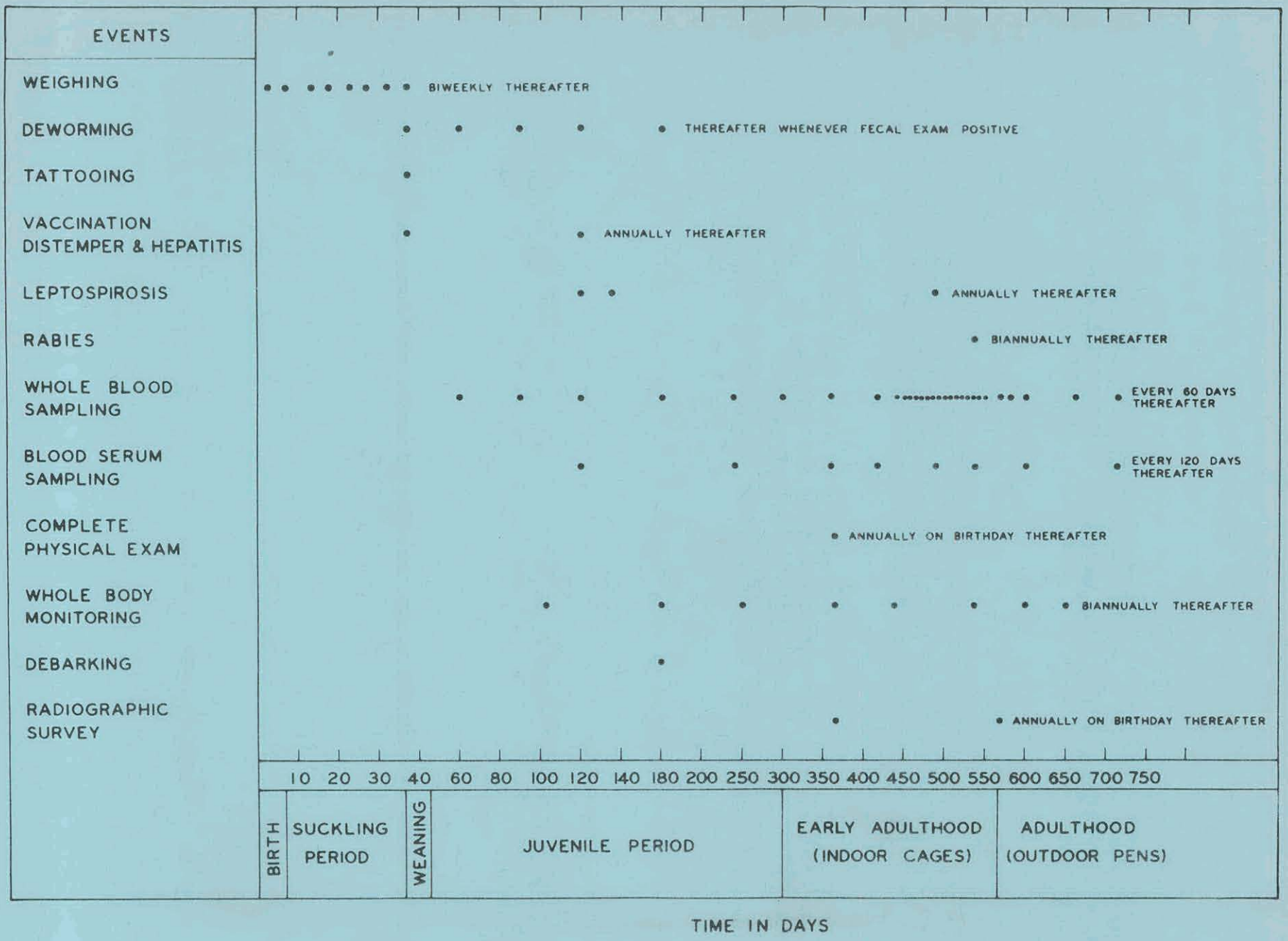
Strontium-90 Ingestion Series
(In utero to 540 Days of Age)

Treatment Code	Multiple of 1 level	Avg. µCi/day	Diet µCi Sr-90/g Ca	Number of Dogs	
				Lifetime	Sacrifice
D00	0	0.00	0.000	60	5
D05	0.3	0.03	0.007	30	10
D10	1	0.08	0.021	30	5
D20	6	0.5	0.123	30	5
D30	18	1.5	0.37	30	5
D40	54	4	1.11	30	5
D50	162	12	3.33	30	5
				240	40

Strontium-90 Injection Series
(Single IV Injection at 540 Days of Age)

Treatment Code	Multiple of 1 level	µCi Sr-90/kg	Number of Dogs	
			Lifetime	Sacrifice
S20	6	3.7	10	5
S40	54	33	10	5
			20	10

* This level was computed to represent the canine equivalent of ten times the Radiation Protection guide value for man (0.1 µg Ra-226).



Chronological events in the life of Radiobiology Laboratory Beagles.

Sr-90/Ca RATIO AND CALCIUM AND PHOSPHORUS CONTENT OF BEAGLE DIET

R. J. Della Rosa
Fiorella Gielow
Nancy Nix
C. D. Abrahams
Adeline Santos
J. P. Wittmier

The Sr-90/Ca ratio and the Ca and P concentrations in six dietary dose levels have been maintained to within 10% of prescribed values for the past 7 yr. The constancy of the dietary Ca (1%) and P (0.7%) was satisfactorily maintained by the selection of natural feed products.

An efficient procedure for preparing diets for the continuous feeding of Sr-90 to Beagles at constant Sr-90/Ca ratios has been in operation since 1961 (Della Rosa et al., UCD 472-108, 1963, p 37; UCD 472-110, 1964, p 31). The prepared food consists of 60% by weight of dry kibble, 20% meat, 20% tap water, plus daily requirements of vitamins. The steps in food preparation and feeding include: mixing of food containing the radionuclide; semi-automatic packaging, quick freezing and storage; thawing, warming, and feeding of daily ration to experimental dogs. Over the past 7 yr, a 2- to 3-month supply of prepared food has been maintained for approximately 200 experimental dogs distributed among six different dose levels plus controls.

The mixed food was packaged in individual 300-g portions. Representative packages were taken randomly at the time of packaging for analysis. Sampling was done frequently in the early years (5 to 10 samples/100 kg total mix) and was reduced (to 2 samples/100 kg) as reproducibility was assured. Fifty-gram aliquots of each package were dried, ashed at 650C for 8 hr, dissolved in hot dilute HCl, and made up to volume. Aliquots were removed and analyzed for Sr-90, Ca, and P using methods previously described (Della Rosa et al., UCD 472-108, 1963, p 37). All pertinent data were recorded on IBM work sheets for subsequent computer analyses. Mean values, standard deviations, and computer plots of all data for the 7-yr period were calculated.

Calcium and Phosphorus. The constancy of the calcium and phosphorus is shown in Fig. 1. Since no attempt was made to adjust mineral content of the food by chemical supplementation, the changes observed are attributed solely to variations in natural materials. Thus, any "adjustment" in calcium-phosphorus content was accomplished through Ca-P rich ingredients, such as bone meal and meat meal, in the kibble preparation (Sturdy Dog Food Co., San Leandro, California). The mean value for calcium in the mixed food was 1%; that for phosphorus was 0.7%.

Strontium-90. The relative specific activities of Sr-90/g Ca in the mixed food for the different levels is shown in Fig. 2. The Sr-90/Ca ratios have been maintained to within 10% of those stated in the experimental design.

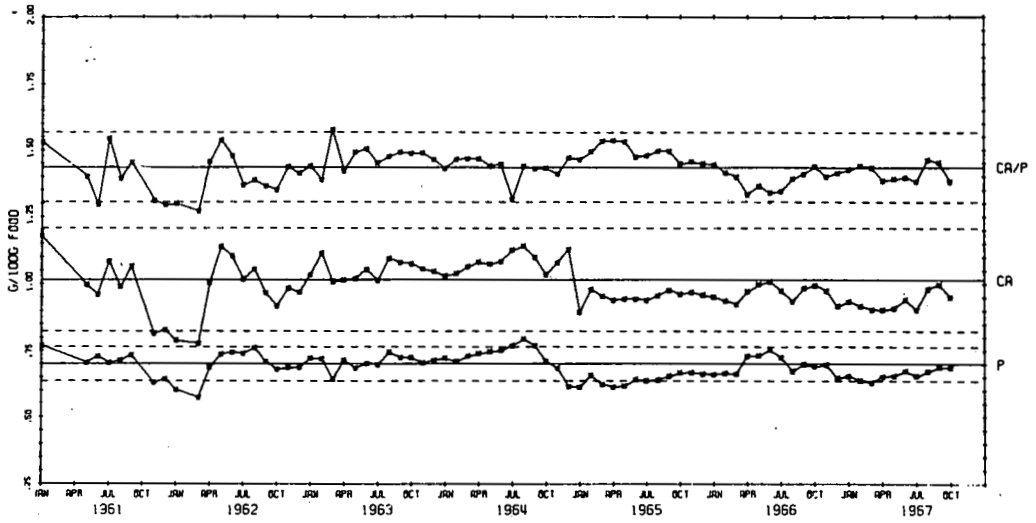


Fig. 1. Calcium and phosphorus content, means, and standard deviations in prepared dog food.

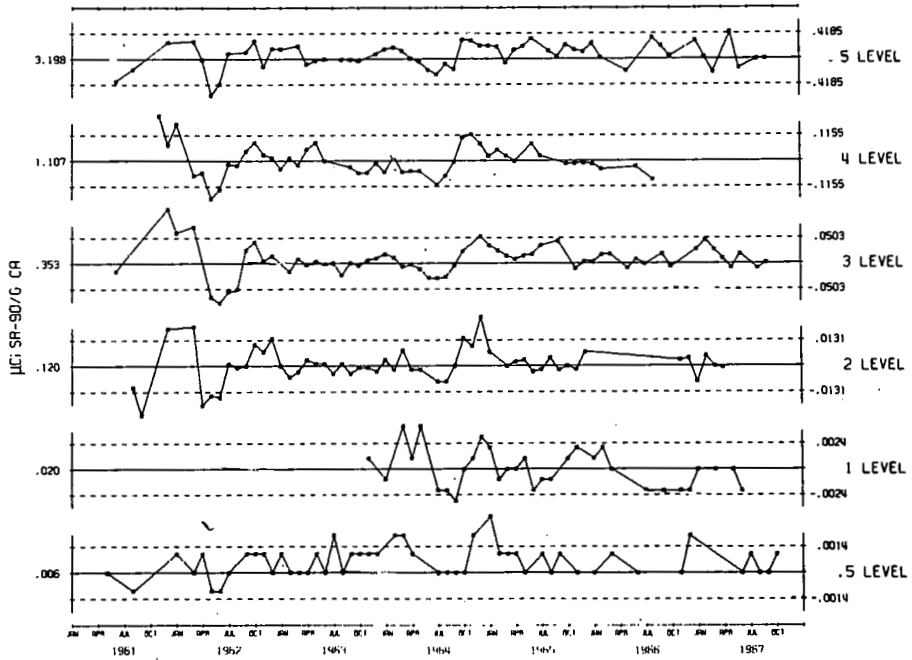


Fig. 2. Sr-90/g Ca ratios, means, and standard deviations for six dietary levels.

RETENTION OF SR-90 BODY BURDENS ACQUIRED VIA GESTATION AND NURSING

R. J. Della Rosa
Marvin Goldman
L. W. Gilman

The retention of Sr-90 body burdens acquired via gestation and nursing was compared with that of dogs exposed for 18 months (main study). It was expressed by fitting the data to two exponentials which indicate an effective half-life of ∿60 days for the fast component and ∿450 days for the slow component.

The fractional translocation of Sr-90 to progeny during gestation and nursing has previously been determined in a special study of Beagles fed approximately 12 μCi Sr-90 per day for about 1 yr during adulthood (Della Rosa et al., UCD 472-114, 1967). The loss of Sr-90 maternal body burdens appeared to have been dependent primarily on litter size and to some extent on the length of time from acquisition of the body burden to breeding.

The body burdens of the pups at weaning were almost entirely a result of Sr-90 absorption and retention during nursing, accounting for nearly one-third of the maternal losses during lactation. As much as 25% of the maternal body burden was lost during lactation; gestational losses were less than 5%. Thus, a dam ingesting ∿12 μCi Sr-90/day for 1 yr might acquire a body burden of ∿30 μCi Sr-90, of which 7 μCi might be lost by the combined influence of gestation and lactation. Her litter of four to five pups would, thus, have acquired body burdens of up to 0.5 μCi each. The retention of body burdens of one such litter was followed by frequent bremsstrahlung monitoring during nursing and after weaning, at about 100, 175, 250, 350, 430, and 570 days of age.

The retention pattern of Sr-90 in the litter is shown in Fig. 1. It is expressed by fitting the data to two exponentials which suggest an effective half-life of ∿60 days for the initial, fast component and ∿450 days for the later, slow component. The time to reduce the body burden to one-half of the maximum following 18 months of continuous Sr-90 feeding is estimated at 5 to 6 yr (Goldman et al., UCD 472-113, 1966, p 31). This difference is a reflection of the mineral kinetics in rapidly growing bone compared to adult bone.

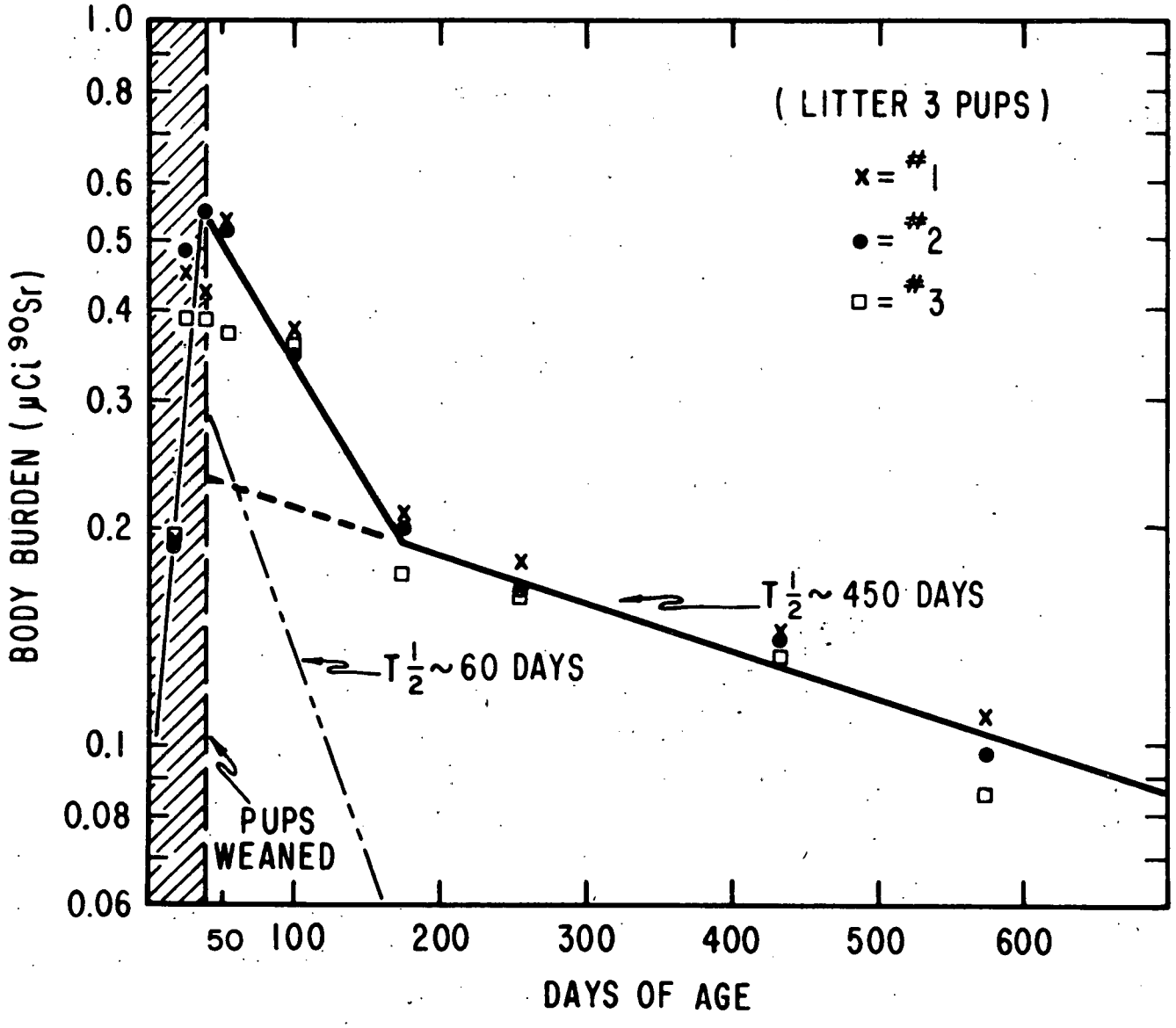


Fig. 1. Retention of Sr-90 body burdens acquired during gestation and nursing.

COLONY STATUS AND CLINICAL OBSERVATIONS IN SR-90 AND RA-226-TREATED BEAGLES

Amy Hosein
Marie Bulgin
D. H. McKelvie

Production of Beagles for fulfillment of the experimental design was completed in 1967. Clinical observations probably attributable to irradiation were: leukemias in Sr-90 fed dogs; lameness, fractures, dental erosion and discoloration, and ocular lesions in Ra-226 treated dogs. Observations not related to irradiation were diarrhea, epilepsy, heartworm incidence, and possible chlordane toxicity. There were 47 preweaning deaths and 21 postweaning deaths for the year.

The production of Beagles for fulfillment of the experimental design (p 15) was completed this year. There are also 122 ancillary dogs being used in special studies. Few problems arose in the maintenance and observation of 847 Beagles in indoor cages and outdoor pens.

Clinical observations probably attributable to irradiation were myelogenous leukemias in the two highest Sr-90-ingestion levels (Goldman et al., p 72). Some dogs in the three highest radium levels exhibited lamenesses due to fractures in bones otherwise normal on radiographs; other dogs showed osteolytic lesions without fractures.

Interesting clinical observations apparently unrelated to radionuclide treatment included intermittent diarrhea in dogs housed in one indoor facility, epilepsy in 11 dogs, one case of dirofilariasis in a Beagle kenneled outdoors, and 10 cases of possible chlordane toxicity.

Diarrhea Cases

Chronic intermittent, sometimes bloody, diarrhea was seen in more than 50% of the animals housed in an indoor facility. Associated with the diarrhea was slight depression, anorexia, and sometimes an elevated body temperature. Spontaneous remission, lasting several weeks, occurred within 2-3 days in all cases. After an exhaustive investigation, a group G beta-hemolytic streptococcus, tentatively identified as Str. canis, was found in 5 of 8 fecal samples cultured. The significance of this finding has not been determined.

Epilepsy Investigation*

Electroencephalography was performed on 11 Beagles with histories of convulsions and on 7 apparently normal dogs during the summer of 1967. Paroxysmal activity, shown by spikes, slow waves or combinations thereof, was found in 5 of the 10 epileptic dogs from which satisfactory records were obtained; the record

* We appreciate the work of Drs. T. A. Holliday and M. J. Gutnick of the UCD Veterinary School.

of one epileptic dog was unsatisfactory because of movement and muscle artifact. Two of the 7 control dogs also yielded abnormal records. The activity seen in these cases was similar or identical to that observed in cases of human epilepsy. The absence of EEG abnormality in some epileptic dogs is not surprising and is consistent with human epilepsy. The control dogs from which abnormal recordings were obtained could be latent epileptics which may later develop clinical signs; the abnormalities could also represent foci of abnormality which are incapable of generating a convulsion, but may be of value in determining the etiology of epilepsy in the Beagle.

Heartworm Examination

During 1967, a 4-yr-old project-bred female dog was found to have a microfilarial infestation. The condition was noticed in a survey using routine EDTA blood samples taken at 7:00 a.m. for complete blood counts. Dr. M. Lavoipierre and Patrick McGreve of the Department of Veterinary Microbiology identified the parasite as Dirofilaria immitis, the heartworm, which had been thought not to occur in this part of the country. Further work by McGreve showed that the microfilaria could be naturally transmitted to noninfected dogs by a common mosquito, which is found in large numbers around irrigation canals near the kennel. Therefore, blood samples from all dogs kenneled outside were taken daily between 5:30 p.m. and 6:30 p.m. and checked for microfilaria. The maximal concentration of the parasite in the blood of the dog occurs at approximately 6:00 p.m., the usual feeding time of the mosquito.

Using the Knott technique, 1 ml of blood was mixed with 10 ml of formalin. The formalin lyses the red blood cells and preserves the parasite, enabling the test to be read 24-36 hr later; tubes are centrifuged and the sediment is examined microscopically. All microfilaria checks were negative.

Possible Insecticide Effects

From November 21 to December 22, 1967, five dogs died of a condition manifested by anorexia, progressive dehydration and emaciation, and convulsions. Animals showing the condition were unrelated genetically, and cases showed no pattern in regard to radiation treatment.

Post-mortem lesions included (1) toxic gastroenteritis; (2) passive congestion of liver; (3) focal hemorrhages in joint capsules; (4) pulmonary edema and hemorrhage including interstitial pulmonary edema; (5) mild reactive hyperplasia of lymph nodes; and (6) generalized emaciation and dehydration.

To combat a seasonal flea infestation, all dogs in the outside colony were given a series of three or, in some cases, four dips in 1% chlordane solution between August and November; outdoor housing facilities were sprayed with the same solution. The subsequent appearance of the lesions described above was limited to dogs that were, in general, "poor keepers." However, as a precautionary measure, the kennel facilities were steam-cleaned and the dogs bathed, to remove all traces of chlordane.

Tissue samples from dipped and undipped (inside) dogs were analyzed for chlordane; results were inconclusive, but an investigation is being made to determine the possible role of chlordane and/or stress in these deaths.

Pre- and Post-Weaning Losses

During the year, 217 dogs were whelped and 121 Beagles died or were culled from the colony. Forty-seven pups died before 38 days of age (weaning); thus, the preweaning mortality rate for the year is 21.6%. The over-all preweaning mortality rate since the beginning of the radionuclide toxicity study is ∿20%. The preweaning mortality rate was not affected by administration of Sr-90 during the gestation and lactation periods.

Of the 47 preweaning deaths, 12 were stillborn, 17 died within 48 hr after birth, and 18 lived at least two days. Approximately 50% of the pups dying prior to weaning showed no significant lesions. All of these died within four days of birth; post-mortem examinations showed presence of bile-stained mucus and gas and absence of ingesta in the gastrointestinal tract. Fifteen of the preweaning deaths were caused by trauma either during parturition or just after parturition. Evidence of trauma at necropsy included ruptured liver, pulmonary hemorrhage, hemoperitoneum, hemothorax, and massive subcutaneous hemorrhages. Four animals died as a result of congenital anomalies. One was born with a cleft palate and died of inhalation penumonia. Another was born with a partial cleft lip and died of starvation due to its inability to nurse. Two pups had urinary tract abnormalities at necropsy; in one the right kidney was hypoplastic and in the other only one kidney was present.

Fifty dogs were culled as excess progeny. One dog was culled for further study of microfilaria of Dirofilaria immitis. Another two dogs from one of the indoor colonies were culled and euthanized in hopes that post-mortem findings might be of value in diagnosing the chronic diarrhea mentioned earlier. The alimentary tract mucosa was irregularly congested in both cases; histopathologically there was uniform loss of luminal epithelium in one case. Culturing of the intestine demonstrated no significant organisms.

The causes of the 21 post-weaning deaths for the year are listed in Table 1.

Table 1. CAUSE AND NUMBER OF POST-WEANING DEATHS

Cause of Death	Number of Animals
Blood dyscrasia	1
Anesthetic overdose	3
Clostridial septicemia	1
Epilepsy	4
Leukemia	5
Osteosarcoma	2
Possible chlordane toxicity	5

SERUM CHEMISTRY VALUES IN BEAGLES TREATED WITH SR-90 AND RA-226

D. H. McKelvie
Susan Munn
Susan Bentley

Serum chemistry values were obtained for Beagles labeled with Ra-226 between 14 and 18 months of age and those uniformly labeled with Sr-90 from 3 weeks in utero to 18 months of age. No changes from control values were noted in the Sr-90 labeled dogs up to 44 months of age. Ra-226 dogs showed significant increases in phosphorus, BUN, amylase, and transaminase (SGOT and SGPT) values to 44 months of age.

Since the beginning of the main experiment, routine serum chemistry analyses have been done at 120-day intervals on all dogs. Tests include analyses for bilirubin, glucose, cholesterol, total protein, calcium, chloride, inorganic phosphorus, blood urea nitrogen (BUN), uric acid, creatinine, alkaline phosphatase, amylase, and transaminases (glutamic oxalacetic [SGOT] and glutamic pyruvic [SGPT]). In addition, serum protein electrophoresis was performed to obtain percent values for albumin and the globulins. The serum chemistry values were obtained by use of the autoanalyzer (Technicon Corporation, Yardsley, N.Y.). Sufficient data have been gathered to statistically analyze values for each dose level up to 44 months of age (just over 2 yr post-treatment).

Figures 1-4 are graphic representations of mean serum chemistry values for at least 20 dogs at each of three dose levels up to 44 months of age. The dose levels are: controls; R5 (1.25 μg Ra-226/kg or ∿12 μg per dog total in 8 semi-monthly injections between 14 and 18 months of age); and D5 (3.33 μCi Sr-90/g Ca or ∿12 μCi/day) in the diet from 3 weeks in utero to 18 months of age.

Statistical analyses showed no significant differences in serum chemistry values between the D5 dogs and the controls except in BUN values at 44 months of age, when the D5 value was significantly higher ($P < 0.01$) than controls'.

The Ra-226 dogs showed some changes during and after the treatment period, including a sustained increase in inorganic phosphorus values beginning at 24 months of age. At 28 months of age the Ra-226 dogs' values were significantly higher than controls', $P < 0.01$. The significance for the next three test periods was $P < 0.001$; at the 44-month test the value was returning to normal, but significance was still $P < 0.05$. This increase is possibly due to the development of the osteoporosity in the bone in the Ra-226-labeled dogs. It could be due to renal damage, however, since the BUN values in the Ra-226-labeled dogs were significantly higher beginning at 32 months of age, when $P < 0.10$; by 40 months of age the significance was $P < 0.005$.

Amylase values of the R5 dogs were significantly higher ($P < 0.01$) at 36 months of age, but returned to normal thereafter. No explanation is noted at this time. SGOT values increased at 20 months of age ($P < 0.05$) and at 24 months of age ($P < 0.001$). The values then dramatically decreased and at 32 and 36 months were significantly less than controls at 44 months of age ($P < 0.005$). The SGPT values were significantly greater in the Ra-226-labeled dogs at 20 months of age, indicating hepatic damage during the injection period. The increase in SGOT values at this time could be related to the liver and kidney damage. Since increased SGOT values are indicative of a variety of necrotic processes, elevation is not pathognomonic for any specific entity. The decrease in SGOT in R5 and increase in the controls at the 36-month age period is unexplained.

Values of other serum constituents, including serum protein percentages, were not statistically different between controls and the highest level Ra-226- and Sr-90-labeled dogs. Additional tests that have been initiated include lactic dehydrogenase, protein bound iodine, sodium, and potassium. Not enough data have been accumulated at this time to compare values statistically.

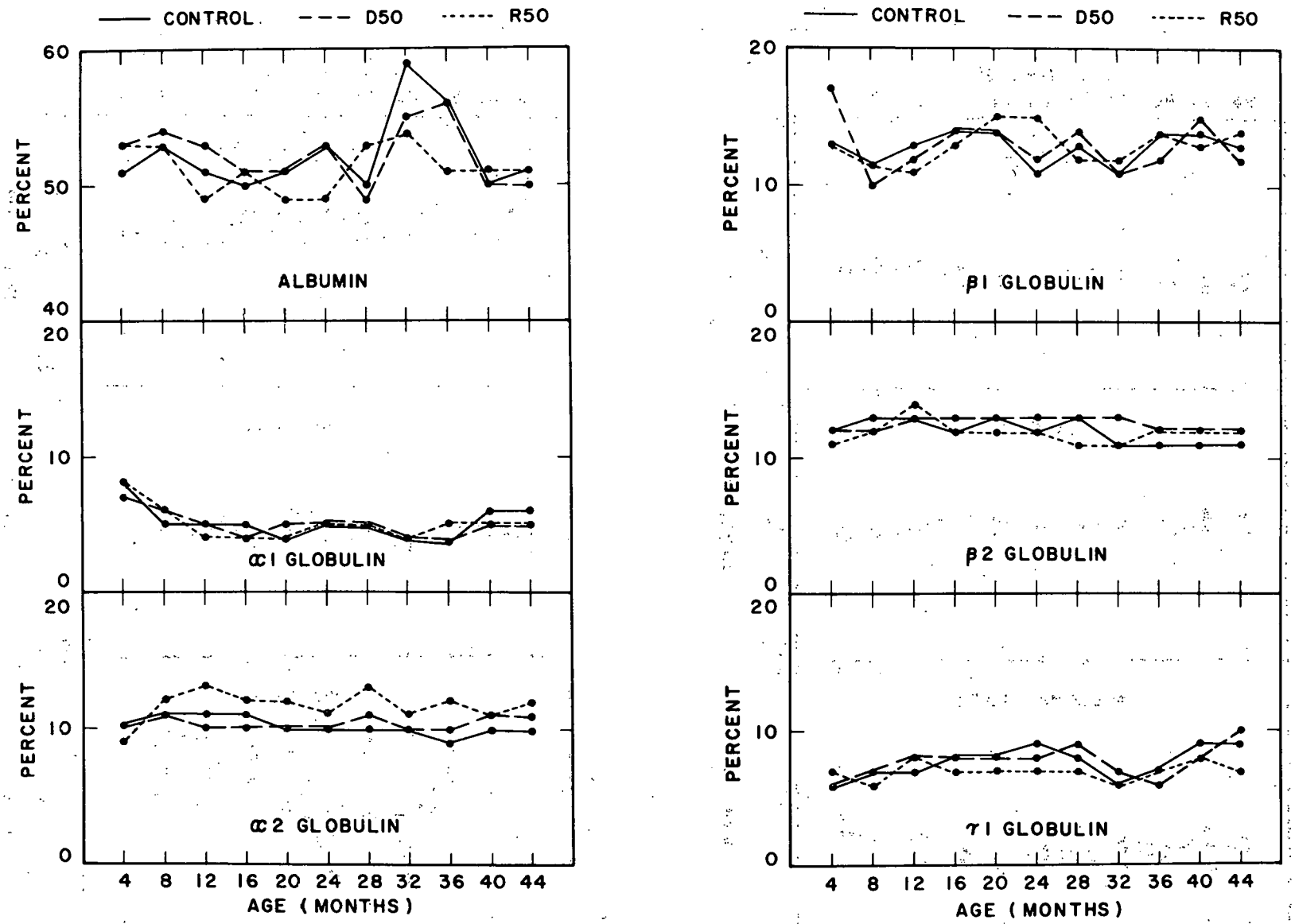


Fig. 1. Serum protein values in Beagles (% by electrophoresis).

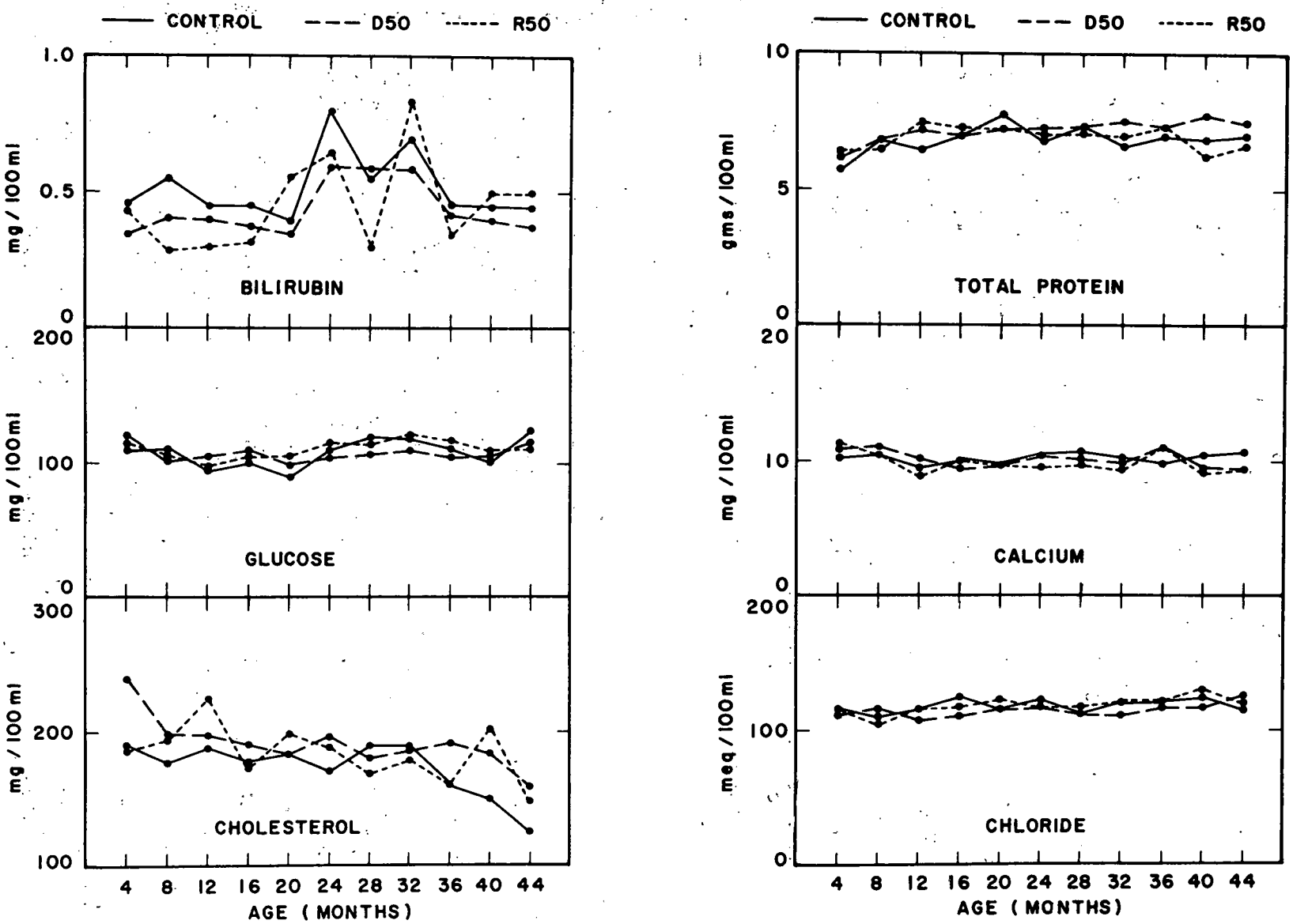


Fig. 2. Serum chemistry values in Beagles.

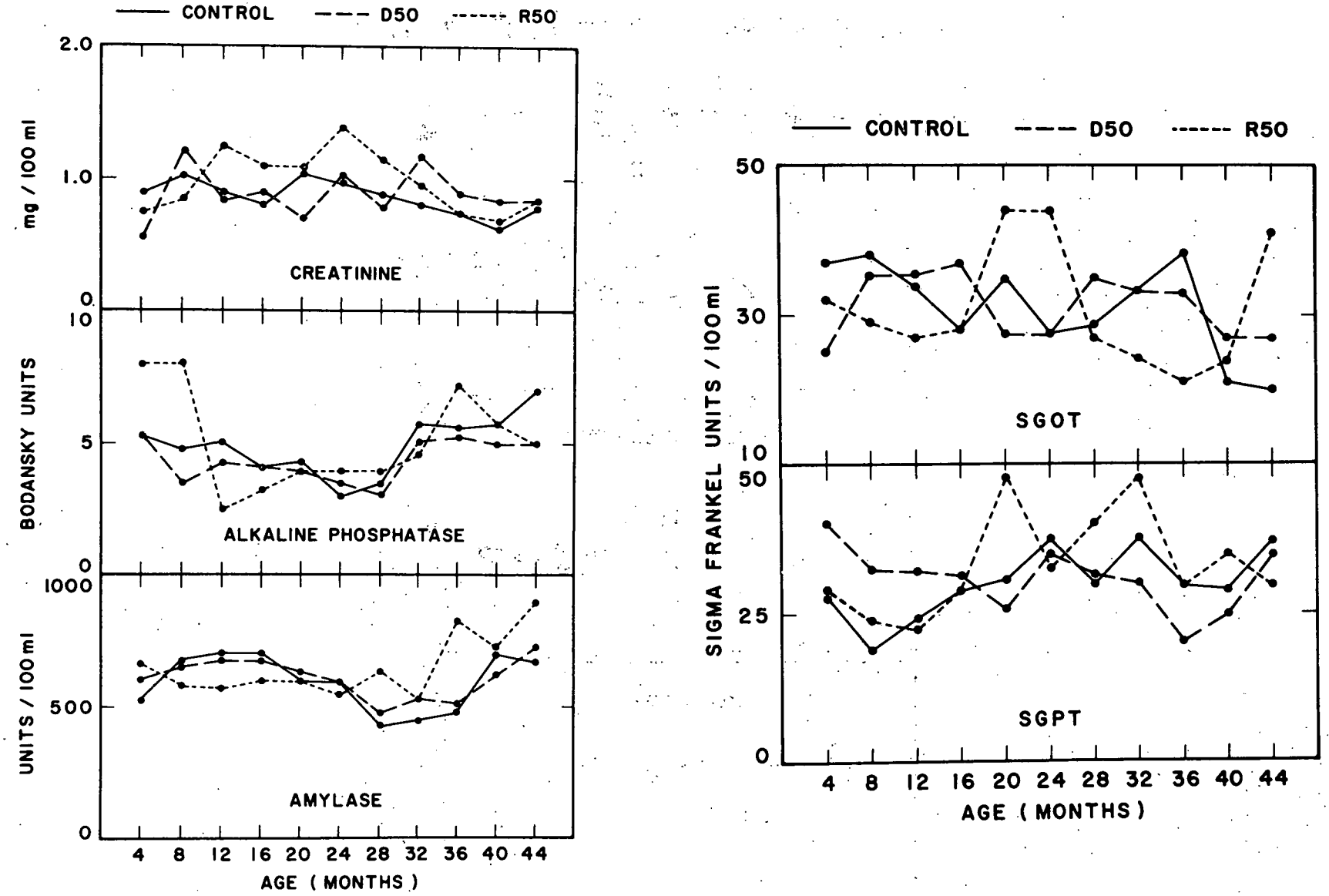


Fig. 3. Serum chemistry values in Beagles.

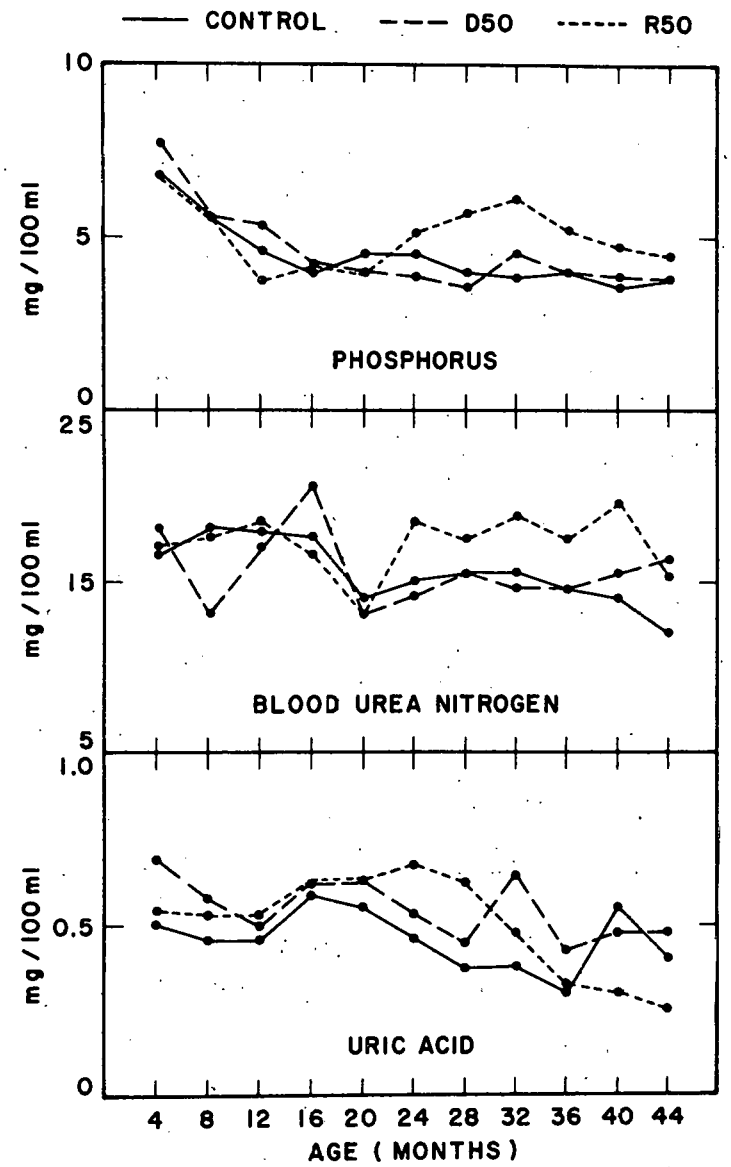


Fig. 4. Serum chemistry values in Beagles.

RADIUM EFFECTS ON TEETH

Marie Bulgin

Abnormally colored teeth were observed among the high level radium dogs; the carnassial teeth were most frequently affected. After several months these teeth showed marked erosion. There appeared to be a dose-time relationship.

During routine oral examinations in August 1967, "pink" teeth were seen for the first time in 11 Beagles in the three highest radium groups. The discoloration was seen most frequently in the upper carnassial (fourth premolar) tooth, as a definite pink color in the enamel starting at the gum line and diffusing downward toward the point of the teeth. None of the teeth was totally pink but both upper carnassials usually were affected. Occasionally canines, other premolars, or molars were affected.

Four months later 16 more dogs were discovered with pink teeth, and the teeth of the previously affected dogs were showing various stages of erosion (Fig. 1). The pink color did not appear to have progressed further but, at the gum line, where it was first noticed, the enamel and dentine had disappeared. The cavities were filled with a very hyperemic tissue which bled easily and profusely when disturbed.

Fig. 1. An eroded carnassial tooth.

The erosion of these teeth is rapid; several such teeth have virtually crumbled, leaving only a smooth-appearing gum. There seems to be a dose-time relationship. The average time for the appearance of the condition is after 900 days of age in the R5 level dogs, after 1200 days of age in the R4 level dogs, and after 1500 days of age in the R3 level dogs. The number and age of affected animals in each group are presented in Table 1.

Table 1. INCIDENCE OF ABNORMAL TOOTH COLOR

Age in Days When Condition First Observed	Number of Dogs R50	R40	R30	R20
900 — 1000	2	0	0	0
1000 — 1100	0	1	0	0
1100 — 1200	4	0	0	0
1200 — 1300	5	6	1	0
1300 — 1400	2	5	2	0
1400 — 1500	1	1	0	0
1500 — 1600	1	2	0	0
1600 — 1700	0	0	2	0
1700 — 1800	0	0	1	0
Total Affected	15	15	6	0
Total Dogs in Group Over 900 Days	18	23	20	21
Total Dogs in Group	37	40	39	37

PRELIMINARY OPHTHALMOLOGIC OBSERVATIONS IN THE BEAGLE

Amy Hosein

A special study on ocular changes with age in the Beagle and one on changes with serial administration of radium, begun at two different ages, were done to provide a basis for evaluation of Ra-226-induced ocular changes.

To provide a basis for assessment of Ra-226-induced ocular changes in experimental Beagles, two preliminary studies were performed.

In the first study, 24 Beagles that had not received any radionuclide were assigned to four experimental groups of six animals each. Group 1 was composed of dogs 4 to 6 months of age; Group 2, of dogs 6 to 12 months of age; Group 3, of dogs 1 to 3 yr of age; and Group 4, of dogs 3 to 7 yr of age.

The pupillary responses in all groups were normal. The irides were variable in color in all age groups. The pupillary margin of the iris (the collarette) was tan to dark brown and the stroma ranged in color from gold to very dark brown.

The predominant color of the tapetum in the eyes of Group 1 pups was purple-blue or lavender, with green-blue coloration of the dorsal area of the tapetal fundus. The non-tapetal fundus was deep brown in color.

Very little purple-blue coloration remained in the eyes of the 6- to 12-month-old (Group 2) dogs, except at the junction between the tapetal and the non-tapetal fundus. The predominant tapetal color was yellow or yellow-green. A fine, brown-gray stippling was present throughout the tapetal fundus.

The tapetal coloration in 1- to 7-yr-old dogs (Groups 3 and 4) was essentially the same as in Group 2 dogs: a deep brown pigmented non-tapetal fundus, a narrow blue or purple-blue area at the tapetal/non-tapetal border, a finely stippled green-yellow to yellow tapetum with a regularly shaped, pinkish optic disc lying usually just within the ventral border of the tapetal fundus.

No corneal, iridic, lens, vitreous, tapetal, or retinal irregularities were found in Groups 1, 2, or 3. One dog in Group 4 had irregularly shaped optic discs. The tapetum in two other Group 4 Beagles showed a surface alteration consisting of a coarseness or granularity, with absence of stippling and an increased blueness of the tapetal area; one of these dogs also showed bilateral asteroid hyalitis.

In a separate study, gross ophthalmologic changes were noted in six Beagles which were given Ra-226 intravenously in eight semi-monthly injections, receiving 1.25 μg Ra-226/kg body weight per injection. Three of the Beagles received their first injection at 60 days of age; the other three began at 120 days of age.

The eyes were examined prior to the first injection of Ra-226, 1 to 2 days before each subsequent injection, and periodically after completion of the injection series. Findings through 2 months post-injection are included here; observation of these animals is continuing.

Ocular changes occurred earlier in the dogs that were 60 days of age at the beginning of the injection series. Appearance of similar alterations in the other three dogs showed a definite time lag (Fig. 1).

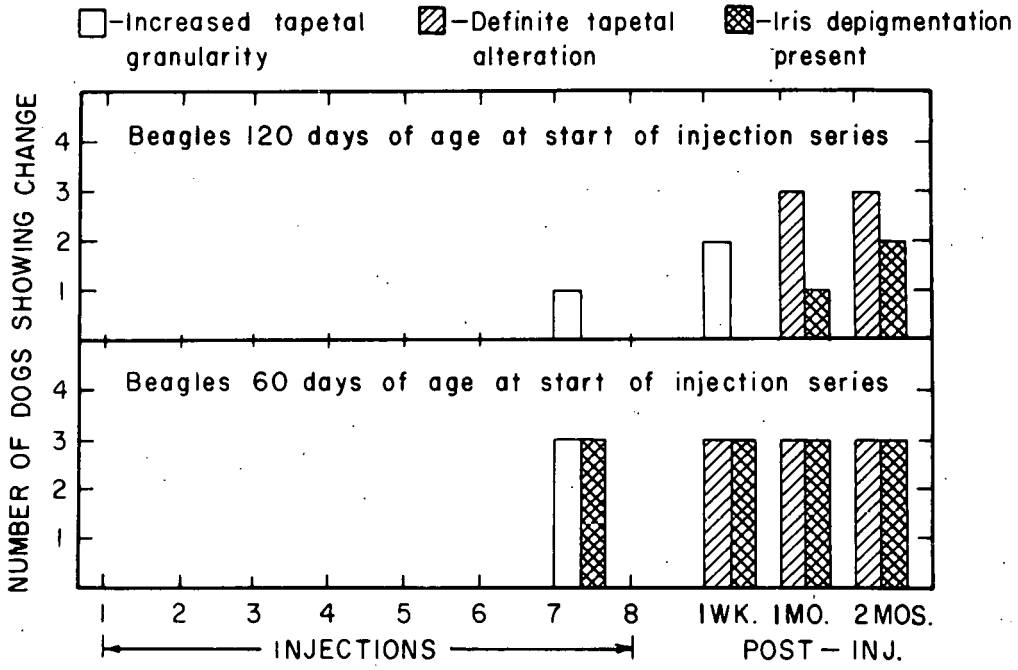


Fig. 1. Incidence of alterations in eyes of six Ra-226-treated Beagles

The first definite changes--initial depigmentation of the iris and a suggestion of increased tapetal coarseness and granularity--were seen in the younger dogs after the seventh injection. One week after completion of the injections there was a definite tapetal alteration and the iridic depigmentation had progressed. Also at this time, increased tapetal coarseness was observed in two of the older dogs. (See p 34 for specific description of ocular alterations.)

One month after completion of the injection series, definite tapetal alteration was observed in the three older dogs; one of them also showed initial iridic depigmentation. Two months after the last injection, initial iridic depigmentation was observed in another of the older dogs.

Tapetal alteration is more advanced in the younger dogs; however, much of the tapetum remains even in these Beagles 2 months post-injection. The choroidal pigment is becoming visible in patches through the remaining tapetum but the choroidal vessels are not yet visible. Pupillary responses are normal in all animals. All six dogs, because of their youth, were difficult to restrain for ophthalmic examination; therefore, their photosensitivity was not ascertained.

GROSS OPHTHALMOLOGIC OBSERVATIONS OF CONTROL AND RA-226-EXPOSED BEAGLES

Amy Hosein

Gross ophthalmologic examinations were made of control and Ra-226-exposed Beagles. Iridic and tapetal alterations, observed in some control dogs, were much more frequent in Ra-226-exposed Beagles. Tapetal alterations appeared to precede iridic changes at the higher dose levels; at the lower dose levels, the reverse was true. Alterations appeared earlier in Beagles receiving higher dose levels of Ra-226.

Among the earliest clinical alterations seen in Beagles following the intravenous injection of Ra-226 are ocular changes which appear to be limited to the uveal tract (vascular tunic) of the eye. The uvea is composed of the iris, the ciliary body, and the choroid. The changes in the four highest levels of radium-treated Beagles consist of iridic depigmentation and tapetal alteration.

The Normal Beagle Iris. The normal Beagle iris is a structure consisting of a spongy, connective tissue stroma, muscular fibers, and an abundance of vessels and nerves. The iris functions to regulate the amount of light admitted to the interior of the eye. It is covered anteriorly by endothelium and posteriorly by retinal pigment layers. On its irregular posterior surface, the iris is deeply pigmented with heavy radial striations. The color of the iris depends partly upon the variable pigment in the stroma cells and partly upon that in the cells of the retinal layers (Magrane, Canine Ophthalmology, Lea and Febiger, 1965).

There is much variation in the pigmentation of the Beagle iris. Most Beagles' irides are of a brown to golden hue with the collarette, or pupillary border, being darker than the stroma. Some Beagles have irides of only one color, usually dark brown. Some irides appear to be etched with thin blue-grey to grey-white streaks at the junction of collarette and stroma. This may be mistaken at first glance as the beginning of iridic depigmentation (threadbare iris) seen in Ra-226-treated Beagles. Some Beagles normally have completely unpigmented irides; in these dogs the tapetum is also absent and a red fundus reflex due to exposed choroidal vessels is seen. Others normally have well-defined areas of vitiligo in one or both irides; tapetal changes may or may not be associated.

Iridic Alterations in Ra-226-Treated Beagles. The progressive iridic depigmentation that has been observed in dogs at the four highest radium levels appears initially at the collarette-stromal junction of the iris and extends both

outward and inward toward the margin of the pupil. In the earliest stages of this change the irides take on a threadbare appearance. The pigmentation progresses until the transition is made from normal dark brown or golden irides to irides that are a faded chalky blue-grey in appearance.

The Normal Beagle Tapetum. Another component of the uveal tract is the choroid, which is located between the sclera and the retina, extending from the ciliary body to the opening for the optic nerve. The choroid is composed mainly of blood vessels and pigmented tissue. Its major function is to supply nutrients for the retina, vitreous, and lens.

The tapetum is a reflective cellular layer of tissue situated behind the retina between the choriocapillaris and the larger vessel layer of the choroid (op. cit.). It reflects light that has already passed through the retina and returns it to the receptors. The choroid absorbs any light that passes through the retina or that is not reflected by the tapetum. The tapetum lucidum (tapetal fundus) varies in color and is responsible for the fundus reflex seen when exposing a dilated pupil to a point light source in a darkened room. The tapetum nigrum (non-tapetal fundus) is dark brown with choroidal pigment; it surrounds the crescent-shaped tapetal fundus in the dorsal quadrants and completely fills the ventral quadrants.

The tapetum lucidum in the normal adult Beagle is usually blue to blue-green at its ventral border, becoming more green to yellow-green, and finally brilliant yellow, dorsally. Seen uniformly over the area is a fine grey-brown stippling. In some normal Beagles, yellow-brown "donut-like" shapes are present within the tapetal fundus. The normal tapetum lucidum is highly reflective.

The pigment of the non-tapetal fundus of normal Beagles is usually quite dense so that underlying choroidal vessels are not visible.

The optic disc in the normal Beagle usually lies just within the ventral border of the tapetal fundus. It is pale pink in color with usually 4 or 5 large retinal vessels leaving the head of the optic nerve. In some Beagles, it is situated far down into the non-tapetal area. Sometimes it is surrounded by a "halo" of brilliant yellow tapetum lucidum.

Tapetal alterations resembling those characteristic of early Ra-226-induced changes have been observed in some control Beagles in the colony. The reason for such alterations is not known. Also, in some control Beagles, the tapetum and choroidal pigment are absent, exposing the choroidal circulation. Such Beagles have been of no value in evaluating the ocular effects of Ra-226.

Tapetal Alterations in Ra-226-Treated Beagles. The Ra-226-induced tapetal alteration observed in Beagles at the four highest dose levels begins as a slight increase in coarseness, granularity, and blueness of the tapetum, with a decrease in reflectivity and a loss of the fine stippling pattern (mild alteration). The optic disc appears to become less pink in color. The coarseness and increased granularity appears to be due to a gradual and progressive loss of tapetum. As the reflective tapetum is lost, patchy areas of the underlying choroidal pigment become visible (moderate alteration). The loss of tapetum progresses gradually until it is entirely gone; as a result, the tapetum lucidum and tapetum nigrum look alike. The choroid then becomes progressively depigmented until finally the underlying choroidal circulation is exposed (extensive alteration).

The mode of action by which Ra-226 induces such ocular alterations is not known. One suggestion is that, since Ra-226 is retained in the pigmented portions of the canine eye for long periods and since analyses showed Ra-226, the alterations are the result of a direct radiation effect (Rehfeld et al., J. Am. Vet. Med. Assoc. 136: 562, 1960).

Gross ophthalmologic observations were made of Beagles treated intravenously with Ra-226 and of control Beagles. The Ra-226-treated Beagles received eight semimonthly injections of radionuclide, at six different levels, starting at 435 days of age. The experimental design (p 15) shows the amount (μg) of Ra-226/kg that was administered at each dose level. The radium dogs were compared with Beagles that received either Sr-90 in the diet to 540 days of age, a single i.v. injection of Sr-90 at 540 days of age, or no radionuclide; the latter three groups are considered as controls.

Initially, test and control animals were examined at 6 months and 1 yr of age, after the fourth and eighth Ra-226 injections, 3 and 6 months after completion of the injection series, and every 6 months thereafter. The ophthalmologic examination schedule has since been revised. Ra-226-treated Beagles are now examined at 6 months and 1 yr of age; after the fourth and eighth injections; 1, 2, 3, 6 months, and 1 yr after completion of the injection series; and annually thereafter. Controls are examined annually starting at 6 months of age.

Examinations are conducted in a darkened room using a pen-light and the naked eye and, in some cases, a binocular loupe. After dilating the pupil by instilling a few drops of a mydriatic, the ophthalmoscopic examination is performed.

Observations are recorded in Table 1. In control dogs, cases of initial iridic depigmentation and mild tapetal alteration were observed, but at a very low frequency. Very few cases of ocular alterations were seen in dogs treated with Ra-226 at the two lowest levels.

Ocular alterations appeared sooner after completion of the Ra-226 injection series in the highest level dogs than in any other group; initial tapetal changes were seen at 1 month post-injection. In R4, R3, and R2 level dogs, the first changes were seen at 8, 12, and 14 months post-injection, respectively.

In the two highest radium levels, tapetal alteration appeared to precede iridic changes. In the highest level, initial tapetal alteration was first observed at 1 month post-injection; moderate tapetal alteration and initial iridic depigmentation at 6 months post-injection; and extensive tapetal alteration and iridic depigmentation at 18 and 21 months, respectively.

In the dogs treated with Ra-226 at the R2 and R3 levels, iridic depigmentation appeared earlier than mild tapetal alteration. No cases of extensive iridic depigmentation or extensive tapetal alteration have been observed in these two groups.

Table 1. OPHTHALMOLOGIC OBSERVATIONS

	Controls								
	Sr-90 Ingestion Dogs	Sr-90 Injection Dogs	R00	R05	R1	R2	R3	R4	R5
Total No. Dogs Examined	208	39	44	29	21	27	36	40	44
Initial									
Iris Depigmentation Present									
Total Affected	4	0	0	2	0	5	17	25	22
Mean Age First Seen (yrs)	4.84	--	--	2.19	--	2.68	2.47	2.32	2.01
Mos. Post-Injection Ra-226	--	--	--	8	--	14	12	10	6
Percent Unaffected	98	100	100	93	100	81	53	38	50
Extensive Iris Depigmentation									
Total Affected	0	0	0	0	0	0	0	6	13
Mean Age First Seen (yrs)	--	--	--	--	--	--	--	3.51	3.23
Mos. Post-Injection Ra-226	--	--	--	--	--	--	--	24	21
Percent Unaffected	100	100	100	100	100	100	100	85	70
Mild Tapetal Alteration									
Total Affected	4	0	4	2	2	6	21	10	8
Mean Age First Seen (yrs)	4.07	--	3.75	2.42	2.42	3.08	2.58	2.18	1.58
Mos. Post-Injection Ra-226	--	--	--	11	11	19	13	8	1
Percent Unaffected	98	100	91	93	90	78	42	75	82
Moderate Tapetal Alteration									
Total Affected	0	0	0	0	0	1	3	17	12
Mean Age First Seen (yrs)	--	--	--	--	--	3.04	3.45	3.41	1.98
Mos. Post-Injection Ra-226	--	--	--	--	--	19	24	23	6
Percent Unaffected	100	100	100	100	100	96	92	58	73
Extensive Tapetal Alteration									
Total Affected	0	0	0	0	0	0	0	4	21
Mean Age First Seen (yrs)	--	--	--	--	--	--	--	3.63	3.01
Mos. Post-Injection Ra-226	--	--	--	--	--	--	--	26	18
Percent Unaffected	100	100	100	100	100	100	100	90	52

URINARY HYDROXYPROLINE AS AN INDEX OF BONE METABOLISM IN CONTROL, SR-90-, AND RA-226-TREATED BEAGLES

R. B. Baggs
R. J. Della Rosa
L. S. Rosenblatt

Hydroxyproline was measured in the urine of dogs fed Sr-90 or injected with Ra-226, and corresponding controls. In controls the total daily excretion of hydroxyproline appears to be highly correlated with nitrogen excretion, and is determined primarily by urine volume rather than hydroxyproline concentration. Stepwise linear regression equations have been developed for normal dogs, allowing evaluation of normalcy of individuals' hydroxyproline excretion. Most D5 dogs appear to be slightly-to-markedly less than normal; R5 dogs are more variable.

The organic matrix of bone and the dermis of skin are composed largely of collagen. Hydroxyproline is found almost exclusively in collagen, at a constant level of 13.5% regardless of mammalian source (Newman and Logan, J. Biol. Chem. 184: 299, 1950). The breakdown of mature collagen and/or collagen precursors releases free hydroxyproline and hydroxyproline-containing polypeptides into the blood; some of these substances subsequently appear in the urine.

A preliminary study on hydroxyproline excretion (µg hydroxyproline/ml bladder urine) was performed in June-September 1966 utilizing 18 Sr-90-fed dogs and 19 control dogs. A significant difference ($P < 0.05$) between control and Sr-90-fed dogs was noted. On the basis of these data a larger experiment was performed during summer 1967.

A total of 75 Beagles selected from the colony were assigned to treatment groups as shown below:

Radionuclide Level	No. Males	No. Females
D0	10	10
R0	10	11
D3	4	4
D5	4	4
R3	5	5
R5	4	4
Total	37	38

The dogs were housed randomly in 20 identical metabolism cages in a thermostable room and acclimatized to their cages for 3 days. A total urine collection was then performed for 48 hr, and the following parameters were evaluated: water volume consumer per 48 hr, urine volume per 48 hr, and body weight at end of trial.

The urine was mixed with an equal volume of concentrated HCl (reagent), and determinations were made on duplicate samples for hydroxyproline

concentration (colorimetry); calcium concentration (flame spectrophotometry); creatinine concentration (AutoAnalyzer); and total nitrogen (microKjeldahl). Differences between mean levels of µg hydroxyproline/ml and of µg hydroxyproline/day of pooled controls were tested against those of the highest level strontium and radium groups independently. Simple t-tests indicated lack of statistical difference. A two-way correlation matrix was then constructed for control male and female dogs. Coefficients obtained for the correlation of variables and µg hydroxyproline/day are shown in Table 1.

Table 1. CORRELATION COEFFICIENTS FOR INDICATES VARIABLE AND HYDROXYPROLINE EXCRETION (mg/day)

Variable	Control Females (n = 21)	Control Males (n = 20)
mg N/day	0.92	0.70
Urine volume (ml)	0.88	0.68
mg Ca/day	0.67	0.55
ml H_2O consumed/48 hr	0.66	0.45
Body weight (kg)	0.65	0.25
Creatinine (mg/100 ml)	-0.64	-0.34
mg creatinine/day	0.63	0.63
Calcium (mg/100 ml)	0.41	0.24
K^{40} count (uncorrected)	-0.39	-0.06
Percent nitrogen	0.20	-0.12
Age (days)	-0.16	0.01
µg hydroxyproline/ml	-0.12	0.23

The high degree of correlation between urinary nitrogen and hydroxyproline suggests that, in the normal dog, connective tissue (which contains hydroxyproline) is associated with the dog's protein metabolism status.

The patterns of correlation coefficients involving total urinary output of hydroxyproline and of creatinine are remarkably symmetrical, even though each is the endpoint of a very different metabolic parameter--bone and muscle, respectively. A schematic representation of the correlation pathways of the variables involved for the control females is shown in Fig. 1. A similar diagram could be constructed for the males.

The data in Fig. 1 show that urine volume is negatively correlated with both hydroxyproline and creatinine concentrations, as expected on the basis of dilution. Concentrations, however, are weakly correlated with total output.

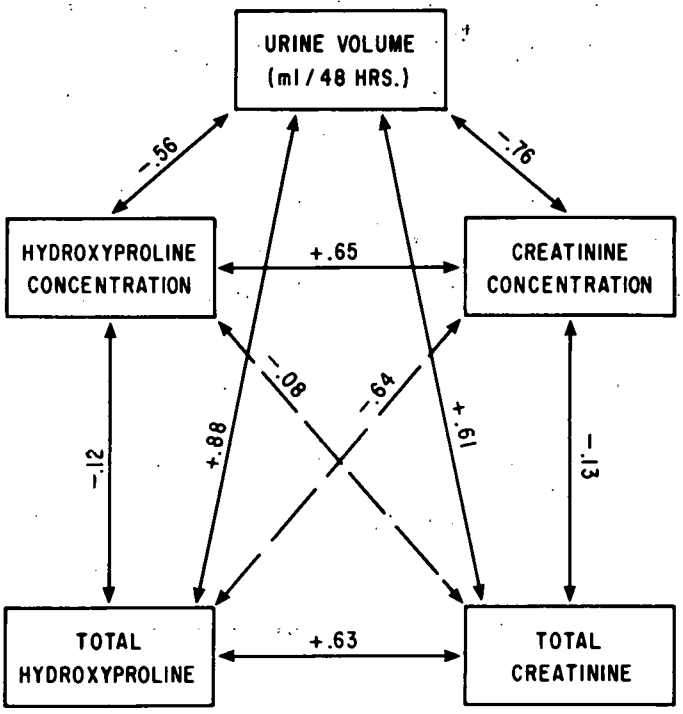


Fig. 1. Interrelationships among urine volume, hydroxyproline and creatinine excretion for control female dogs.

Urine volumes are positively correlated with total output; concentrations and total outputs are positively correlated with each other. These latter findings indicate that creatinine and hydroxyproline are related, perhaps through the general metabolic rate of the animal. The total excretion of creatinine is thought to be reasonably constant (Cornelius and Kaneko, Clinical Biochemistry of Domestic Animals, Academic Press, 1963) since it is an endproduct of muscle metabolism and thus determined by the amount of muscle tissue in the animal. Our data suggest that total hydroxyproline excretion is also reasonably constant. Thus the high correlations of the two total outputs suggest that both variables reflect a stable metabolic state. Although the role of urine volume relative to total excretion of hydroxyproline and creatinine cannot be said to be causal, the data indicate that the high positive correlation of the two metabolites with urine volume depends upon an unknown factor that influences urine volume.

Figure 1 shows a lack of symmetry in the correlations between total outputs and concentrations. The correlation between creatinine concentration and total hydroxyproline excretion is negative, while that between total creatinine with hydroxyproline concentration is nonexistent. This inconsistency is being investigated.

Correlation coefficients of greater than ±0.45 and ±0.55 indicate significance at the $P < 0.05$ and $P < 0.01$ levels, respectively. Much information can be deduced from these coefficients. In general, the males are a more variable group, as indicated by their lower correlation coefficients.

A stepwise linear regression analysis was performed in an attempt to identify which parameters were most directly associated with the amount of hydroxyproline excreted per day. The following equations were generated for the control animals:

Females

μg hydroxyproline/day = -7714 + 33.7 (ml urine/48 hr) + 125 (μg hydroxyproline/ml) - 11 (mg% creatinine) + 5.5 (mg creatinine/day)

R^2 = 99%, Standard Error = 407 μg hydroxyproline/day

Males

μg hydroxyproline/day = -6486 + 21.7 (ml urine/48 hr) + 118 (μg hydroxyproline/ml) - 20.3 (mg% creatinine) + 23.3 (mg creatinine/day)

R^2 = 96%, Standard Error = 827 μg hydroxyproline/day

Males and Females

μg hydroxyproline/day = -7711 + 28.0 (ml urine/48 hr) + 122 (μg hydroxyproline/ml) - 13.6 (mg% creatinine) + 14.6 (mg creatinine/day)

R^2 = 97%, Standard Error = 680 μg hydroxyproline/day

R^2, the coefficient of multiple determination, indicates the percentage of the variance of μg hydroxyproline/day which is accounted for by the association of μg hydroxyproline/day with the other variables listed. When R^2 = 100%, complete determination is indicated. It is clear from these equations that almost all of the variation is accounted for by the four variables given. The equations indicate that, to ascertain if a dog's hydroxyproline excretion differs from normal, it is necessary to measure the concentration of hydroxyproline and creatinine in the collected urine and the volume of urine excreted in 48 hr.

It was of interest to utilize these sex-specific equations to determine the percentage of error $\left[100 \times \left(\frac{\text{observed - predicted}}{\text{observed}}\right)\right]$ for the control, D3,

D5, R3, and R5 dogs. Since the urine volume (ml/48 hr) was indicated by the correlation coefficient to be of great importance in determining the total daily excretion of hydroxyproline, it was plotted against the percentage of error for the control females (Fig. 2), control males, and treated dogs. The males exhibited somewhat more scatter than the females.

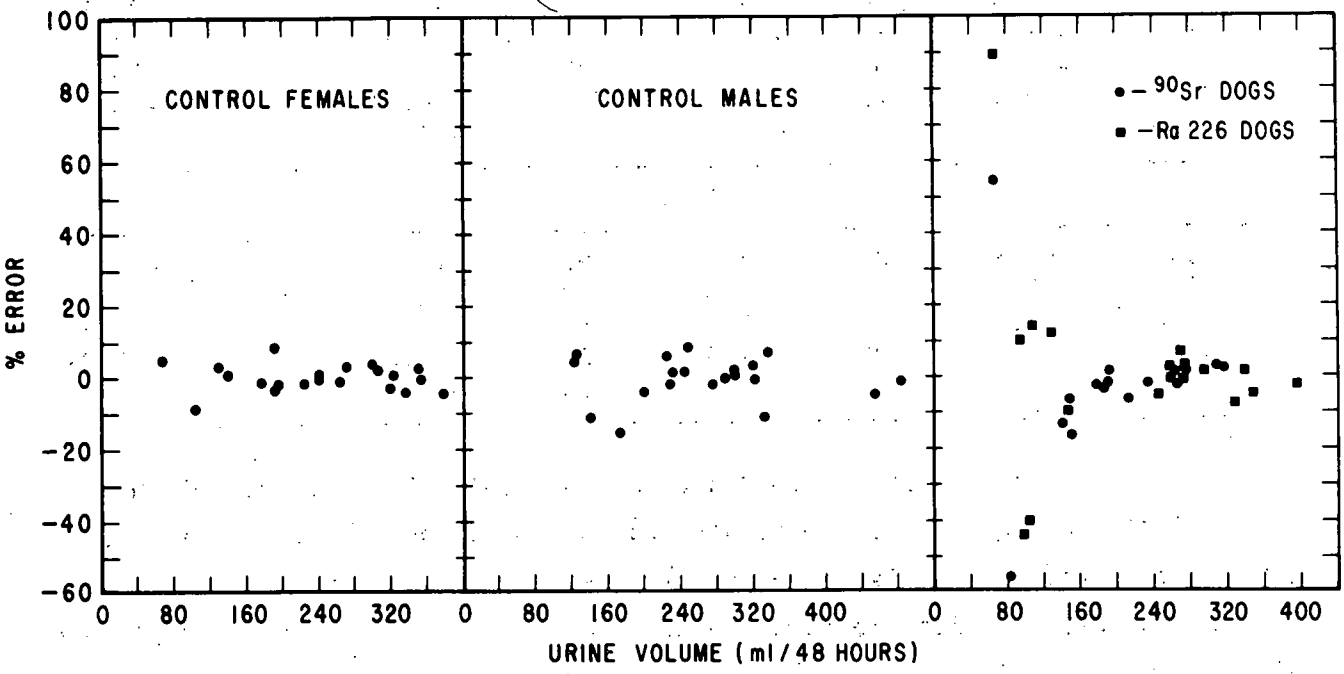


Fig. 2. Percentage of error in hydroxyproline excretion as a function of urine volume.

If the appropriate regression equation (by sex) is used to predict the total hydroxyproline excretion per day for the Sr-90 and Ra-226 dogs, and the percentage of error is calculated, some very impressive deviations from "normalcy," i.e., predictability by the equations of normal dogs, develop. If the error values are ranked by increasing urine volume (Table 2), the high errors are seen to occur especially in dogs with a low 48-hr urine volume. If these dogs are excluded, the pattern of errors in sign and magnitude is similar to that observed for the controls, except in the highest-level strontium group, for which the errors are generally negative. Thus, these dogs appear to have lower bone metabolism rates than expected on the basis of the control data.

The very high (>50%) errors are invariably associated with a low total urine volume; i.e., the regression equation as stated does not accurately predict the total hydroxyproline excretion of Ra-226 and Sr-90 dogs with a total urine volume of <200 ml/day. In the control males this could be due to the

Table 2. PERCENTAGE OF ERROR RANKED BY URINE VOLUME (ml/48 hr)

D3		D5		R3		R5	
Urine (ml)	% E	Urine (ml)	% E	Urine (ml)	% E	Urine (ml)	% E
			Males				
70	55.6	144	12.7	125	13.3	70	91.1
81	-55.7	150	-16.7	247	- 4.5	110	14.0
192	- 1.0	180	- 2.1	275	7.1	155	-10.7
320	2.9	262	- 3.5	275	0.0	230	- 6.0
				350	- 4.1		
			Females				
188	- 1.3	150	- 6.0	98	10.2	100	-43.4
198	0.4	215	- 6.3	112	-40.4	268	0.4
255	0.5	238	- 1.1	250	- 0.7	270	2.2
310	3.2	278	1.3	330	2.6	300	2.2
				400	- 1.3		

fact that only 4 of the 20 control males had a urine volume of <200 ml and none had <100 ml/48 hr. Of the treated males, 10 of the 17 had <200 ml, and 3 had <100 ml/48 hr. It is these latter three males that exhibit errors >50%.

The control females are more predictable, with all values being ±10%, and all but two ±5%. Eight of the 21 female controls had a urine volume of <200 ml/48 hr, presumably due to generally smaller body size. However, in the treatment groups (R3, R5, D3, D5), the greatest error is associated with the lower urine volume.

It is apparent that some individual dogs within the treatment groups exhibit total hydroxyproline excretion patterns that are at considerable variance with those predicted by an equation derived from control dogs. The association of large error with low urine volume and the higher incidence of low urine volume in Ra-226 and Sr-90 dogs indicate the desirability of including some evaluation of renal function when measuring urinary hydroxyproline. These data also suggest the possibility of some radiation-induced nephropathy.

A more sophisticated stepwise linear regression analysis now being performed includes all dogs with the 13 variables considered earlier, plus a body burden value. The importance of body burden (i.e., radiation) as a determinant of urinary hydroxyproline will, thus, be evaluated.

BIOCHEMICAL STUDIES ON BONE AND OTHER CONNECTIVE TISSUES. 1. BIOCHEMICAL SURVEY OF THE ORGANIC MATRIX OF BONE AND CARTILAGE.

Huan-Chang C. Tsai
R. J. Della Rosa
Nancy Nix

Collagen, neutral sugars, and mucopolysaccharides were determined in rib, costal, and articular cartilage of Sr-90- and Ra-226-treated dogs. Hexosamines and hexuronic acid levels were markedly reduced in the cartilage of Ra-226-treated dogs; this effect appeared to be dose-related. A slight decrease in the neutral sugars was also observed. No such changes were seen in rib bone. Only preliminary data are available from Sr-90-treated dogs.

The purpose of this study was to determine the effect of radionuclide treatment on the biochemical composition of the organic matrix in bone and cartilage. The organic fraction of compact bone constitutes up to 35% of the dry fat-free weight. Only a small part of this is contributed by the cells; the remainder, impregnated with the bone salt, is the bone matrix. The organic matrix has two main components: the most prominent is fibrillar in nature and is chemically a collagen. Between the fibers is a ground substance whose best characterized components are mucopolysaccharides (MPS), all of which contain hexosamines. Reducing substances such as neutral sugars have been found in bone and cartilage matrix.

Radium-Treated Dogs. The following analyses were done on bone and cartilage of control dogs and dogs receiving various levels of Ra-226 as they became available over the past year: (1) collagen, (2) carbohydrate (neutral sugars), and (3) mucopolysaccharides (hexosamines and hexuronic acids).

Six dogs with very low body burdens of radionuclide (five with Sr-90 and one with Ra-226) were used as controls. Most of the dogs used were about the same age (27 months). One R5 dog was approximately 4 yr of age; two of the Sr-90 dogs were 20 months of age. Results are summarized in Fig. 1, a-d.

Collagen. No differences were detectable in the collagen content of costal or articular cartilage or bone.

Neutral Sugars (carbohydrates). A minimal variation in neutral sugars was observed in the biochemical composition of costal and articular cartilage. No changes were detectable in rib bone.

Mucopolysaccharides (hexosamines and hexuronic acids). A marked reduction in the content of hexosamines and hexuronic acids of costal cartilage was observed. These changes appear to be dose-related: there was little or no difference at the R2 level, R3 being the crucial level. In the R5 dogs, the concentration of hexosamines and hexuronic acids of costal cartilage was about 60% of control values. A similar trend was observed in articular cartilage, but to a much lesser degree. Rib bone showed no changes in hexosamines or hexuronic acids.

When cartilage and bone samples were taken less than one month after the last injection of Ra-226, one each from the R5 and the R4 level, essentially normal or baseline values were obtained.

The preliminary data presented here were obtained from material from only a limited number of dogs; however, a few tentative conclusions can be made:

1. Costal cartilage has the highest concentration of MPS of all tissues sampled, and is metabolically active. These studies can be extended employing a rib cartilage biopsy technique, with the option of repeated sampling in the same dog.
2. The changes in MPS content of costal cartilage suggest an age-treatment effect, with a time lag factor of perhaps several hundred days before biochemical changes can be measured. These changes further suggest an acceleration of normal biochemical "breakdown" associated with aging.

Few reports on the effect of radiation on the metabolism of MPS in vivo are available in the literature. S-35 uptake studies have been reported in epiphyseal cartilage of mice after high doses of X-rays. S-35 uptake studies in vitro and in vivo are contemplated.

Strontium-90-Treated Dogs. Data available from Sr-90-treated dogs are still too few and variable for comment. Measurements in a limited number of dogs suggest only minor changes in bone and/or cartilage biochemistry. Further studies will be carried out as material becomes available, either by rib cartilage biopsy or necropsy.

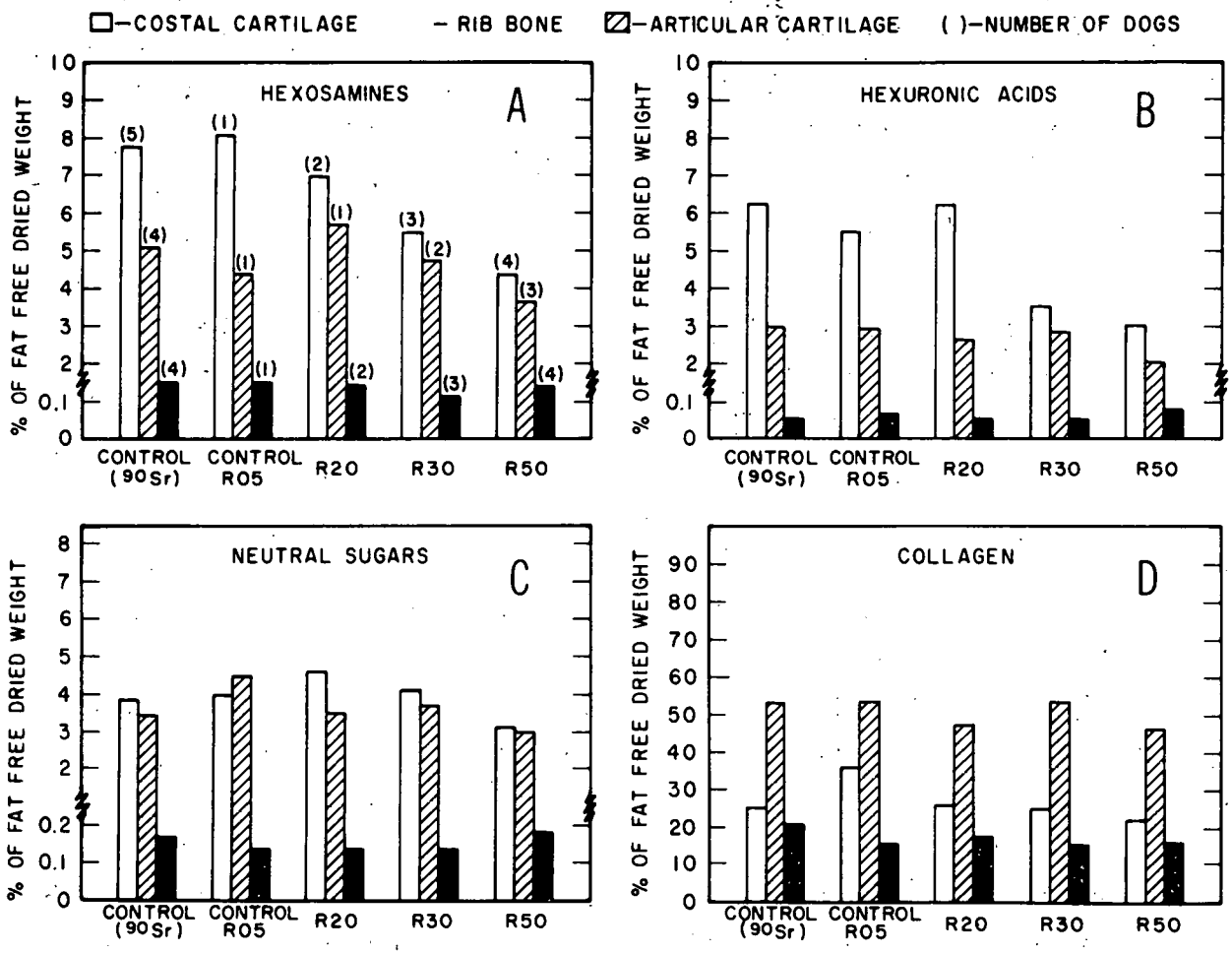


Fig. 1. Biochemical composition of bone and cartilage.

BIOCHEMICAL STUDIES ON BONE AND OTHER CONNECTIVE TISSUES.
2. EFFECT OF Ra-226 ON THE MUCOPOLYSACCHARIDES OF CARTILAGE.

Huan-Chang C. Tsai
R. J. Della Rosa
Nancy Nix

The hexosamines of cartilage were fractionated into galactosamine and glucosamine. An increase in glucosamine and a decrease in galactosamine content was observed in the highest-level radium-treated dogs.

Various connective tissues contain different types of mucopolysaccharides (MPS), whose metabolism is affected by nutritional, genetic, and pathologic factors. Aging has a profound effect on these compounds. In human costal cartilage, galactosamine increases with age and glucosamine decreases with age. The neutral MPS such as keratosulfate increases with age, then remains constant after maturity (Kaplan and Meyer, Nature 183: 1267, 1959).

As shown previously (Tsai et al., p 45), Ra-226 caused a reduction of total hexosamines. In this study, the hexosamines were fractionated into galactosamine and glucosamine to determine the relative changes in distribution.

The results of the fractionation of the hexosamine of cartilage are shown in Figs. 1 and 2. A decided trend in the distribution of these amino sugars is noted as a function of treatment level: there is an increase in glucosamine content and a decrease in galactosamine of both costal and articular cartilage at the highest levels of radium. Thus, the Ra-226 effect on cartilage produced not only a quantitative but also a qualitative change of hexosamines. With age, there is a reduction of total hexosamines and a lowering of the ratio of galactosamine to glucosamine. These changes further suggest the possibility of an accelerated aging effect due to protracted irradiation. Further studies are contemplated to provide an age vs. MPS curve from which one might define an age-treatment effect in Beagles.

At this stage we have insufficient data to enable correlation between the biochemical effects of radium on cartilage and the radiographic changes seen in the bone of radium-treated dogs (Williams et al., p 63). Cartilage, rather than rib-bone, is apparently more sensitive as a tissue indicator of biochemical changes, due partly to its higher concentration of MPS. Cartilage (articular and costal) may contain 20 to 40 times more MPS than rib bone. Also, the bone sites showing radiographic changes have thus far been taken in toto for pathology observations. Samples from pathological sites will be analyzed biochemically as they become available. Further, rib cartilage is metabolically more active than other skeletal sites at the ages examined, as evidenced radiographically, i.e., by dense lead lines. The decalcification of new bone forming beyond these dense lines may be due to a defect in ground substance (osteoid).

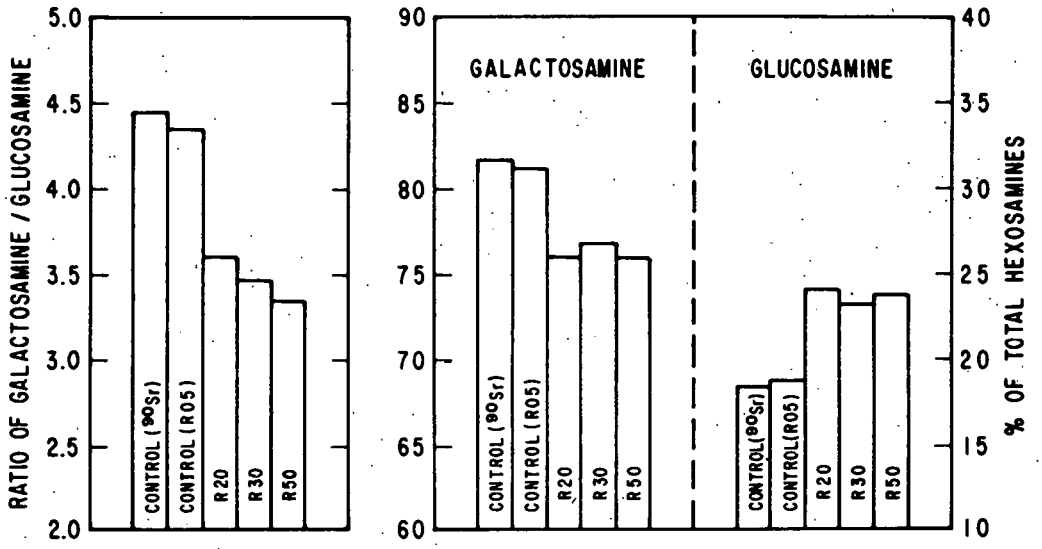


Fig. 1. Hexosamines in costal cartilage.

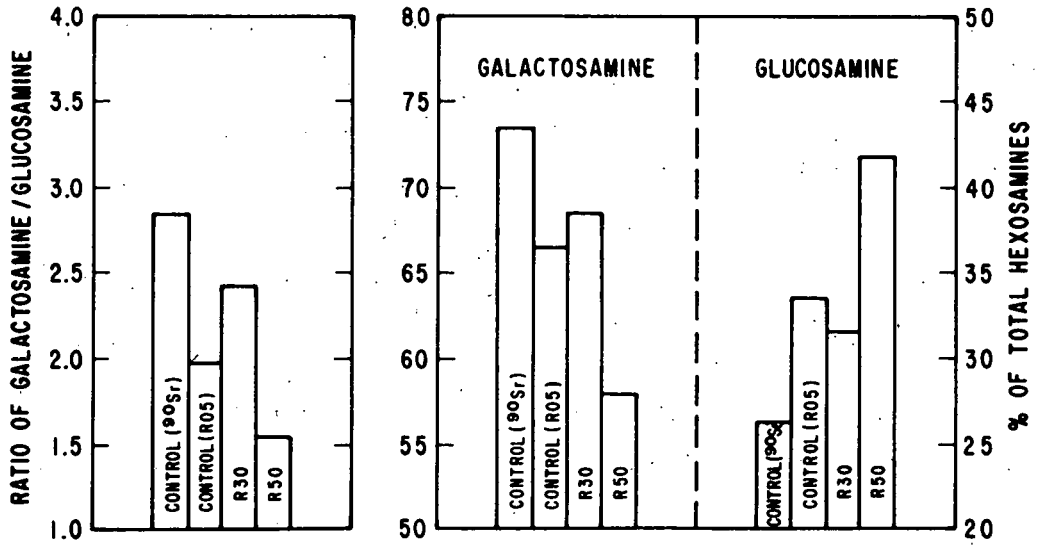


Fig. 2. Hexosamines in articular cartilage.

METABOLIC STUDIES OF BONE IN VITRO: PRELIMINARY REPORT

R. B. Baggs
R. J. Della Rosa
C. D. Abrahams

The rationale and methods for in vitro investigation of bone metabolism are presented. It is suggested that, as Sr-90 specific activity is increased, the cell density decreases, but the remaining cells may be more active metabolically.

Bone as a tissue may be divided into an organic and an inorganic phase. The organic phase may be further subdivided into cellular and acellular fractions. The acellular fraction, or matrix, consists of a formed fibrillar moiety, collagen, and an amorphous "filler," mucopolysaccharide. The biosynthesis of the organic matrix is a basic function of the osteoblast, the inorganic phase, or bone salts, being deposited secondarily in the pre-existing organic matrix.

There is little positive information to define a single mechanism at the cellular level to explain the processes of formation, maintenance, and destruction of bone. There is, however, little doubt that the osteoblast-osteocyte-osteoclast series is intimately concerned.

The rate of remodeling of bone can be approximated in vivo by observing the Sr-90 accretion-retention kinetics. Up to 3.5 yr, dogs with higher body burdens of radionuclide exhibit a slightly increased turnover time (Goldman et al., UCD 472-113, 1966, p 31). Accelerated repair, possibly due to radiation-induced injury, would increase the rate of loss of Sr-90. If any of the cell series were more sensitive to radiation, it should be possible to note excessive or deficient matrix production, mineralization, or resorption. In addition, there is unquantitated evidence pointing to abnormal shaft size and relative hardness of the long bones in some dogs with higher body burdens (McKelvie, p 60).

It is the purpose of this on-going experiment to ascertain if there is an effect of Sr-90 or Ra-226 on bone cell viability or metabolism that can be measured in vitro.

The metabolism of bone chips in vitro is measured using oxygen consumption per unit time as an index of metabolic rate. This is a convenient parameter because both the viability and aerobic metabolic rate of the tissue can be measured directly The oxygen utilization rate, or oxygen uptake (μliter O_2/hr) is measured with a Warburg constant volume respirometer.

Using the manometric approach, Flanagan and Nichols (J. Clin. Invest. 44: 1795, 1965) found a significant increase in oxygen uptake in bone of patients suffering from hyperparathyroidism. We have instituted a cooperative experiment with Dr. A. Heusner of the Department of Physiological Sciences, using a coulometric

method for the measurement of oxygen consumption (Heusner, et al., Med. Electron. Biol. Engrg. 3: 34, 1965). The cellularity, or cell density, varies greatly within any one bone. To correct for this, deoxyribonucleic acid is measured in the bone sample (Webb and Levy, J. Biol. Chem. 213: 107, 1955), as the amount of DNA per cell is quite constant for a given species. The oxygen uptake values are thus expressed as μliter O_2/hr/mg DNA.

Data are being collected on bone from three groups of animals:

1. dog rib by biopsy, with periodic resampling
2. dog rib by sacrifice (control, Ra-226, and Sr-90)
3. mouse calvaria by sacrifice (control, Sr-90, X-ray).

The radioactivity of each sample of bone is routinely determined. To date, data collected from any one group are insufficient to allow conclusions regarding the effect of radiation on bone metabolism. There is some indication that as the relative specific activity of Sr-90 increases (μCi Sr-90/g dog bone), the cell density decreases (mg DNA/g dog bone), but that the metabolic activity per cell increases (μliter O_2/hr/mg DNA).

A METHOD FOR MICRORADIOGRAPHY OF BONE SAMPLES

D. H. McKelvie
Shirley Coffelt
John Schwind

To study structural alterations of bone resulting from irradiation from internal emitters such as Sr-90, a microradiography system was developed. The bones are sectioned, polished to 80μ and stored. Microradiographs are made using emulsion-coated glass slides; the resultant X-ray is easily studied microscopically.

The method of treating dogs with Sr-90 results in uniform distribution of the radionuclide in the bone; consequent pathological effects on bone may result. To adequately observe such changes, microradiography of thin bone sections was necessary. A brief description of the method and equipment used to produce the microradiographs follows:

Acetone-fixed pieces of bone are defatted by placing in five changes of acetone (3 hr per change) and ten changes of ether, or by defatting for 6 hr in an ether fat extract (LabCon Co; Kansas City, Missouri). They are then dried and embedded in Ward's Liquid Bioplastic (Ward's Natural Science Establishment, Inc., Rochester, N.Y.). Embedded specimens are trimmed to desired size and mounted with Duco cement on plexiglas plates (4" x 1.25" x 0.25") which are attached to the table of a Gillings-Hamco thin-sectioning saw (Hamco Machines, Inc., Rochester, N.Y.). Specimens are sectioned using a diamond edged jewelers' slitting sawblade (diam, 4"; arbor, 0.5"; thickness, 0.012"; 50 teeth/in.) (Circular Tool Co., Inc., Providence, R.I.). Sections are cut to a thickness of 100μ to 120μ and then ground to 80μ between two glass plates roughened with 120-grit aluminum oxide. The grinding material is a mixture of levigated alumina and 50% alcohol. The section thickness is measured with a dial indicator (L.S. Starrett Co., Athol, Mass.). The polished sections are rinsed in 50% alcohol, dried, and placed under a cover slip glued to a microscope slide. The section is not glued, so that it can be removed as needed for microradiography.

Microradiography is done by means of an assembled X-ray unit. The power supply is a Sorensen Model 1030-20-M1 high voltage unit (Sorensen Raytheon Co., Norwalk, Conn.), attached by means of a 4-ft shockproof cable to a Machlett A-2 X-ray diffraction tube with tungsten anode; 1.0-mm sq focal spot; 2 windows, each with 0.5 mm beryllium; and a water cooled jacket (Machlett Laboratories, Inc., Springdale, Conn.). The tube is mounted upright in a metal cabinet with a mounting bracket and an extension tube attached at each window post.

Exposures are made using two cameras, specially constructed of cast aluminum and designed as shown in Figs. 1 and 2, in a modification of a design furnished

by Dr. Robert Rowland, Biophysics Division, Argonne National Laboratory, Argonne, Ill. The camera is loaded with Kodak type 649-0 emulsion-coated spectroscopic plate (1" x 3"). The specimen holder is prepared by gluing a tightly stretched piece of 0.00025-inch thick mylar to the camera end. A plastic washer with external diameter the same as the internal diameter of the specimen holder is fitted with five successively smaller pieces of aluminum foil (7.0 mg/cm^2) glued to one edge to form a density wedge. The washer is then inserted into the port opening of the specimen holder until the aluminum wedge is in direct contact with the mylar. The specimen--an 80μ thick section of bone--is then glued to the outer surface of the mylar (Fig. 3). The specimen holder is attached by means of a setscrew to the port end of the extension tube (Fig. 4). The cameras are placed over the specimen holders at the port (Fig. 5). Exposures are made at 11 Kv and 16-19 ma for 5 min. The cameras are removed after exposure, making sure that no photographic plate is in contact with the port when the camera is removed from the extension tube. Slides are developed in groups of 40 or more in a special container. Developing is done with Kodak D19 developer and rapid fixer.*

* Appreciation is extended to Dr. W.S.S. Jee of the University of Utah for his advice on preparation of specimens.

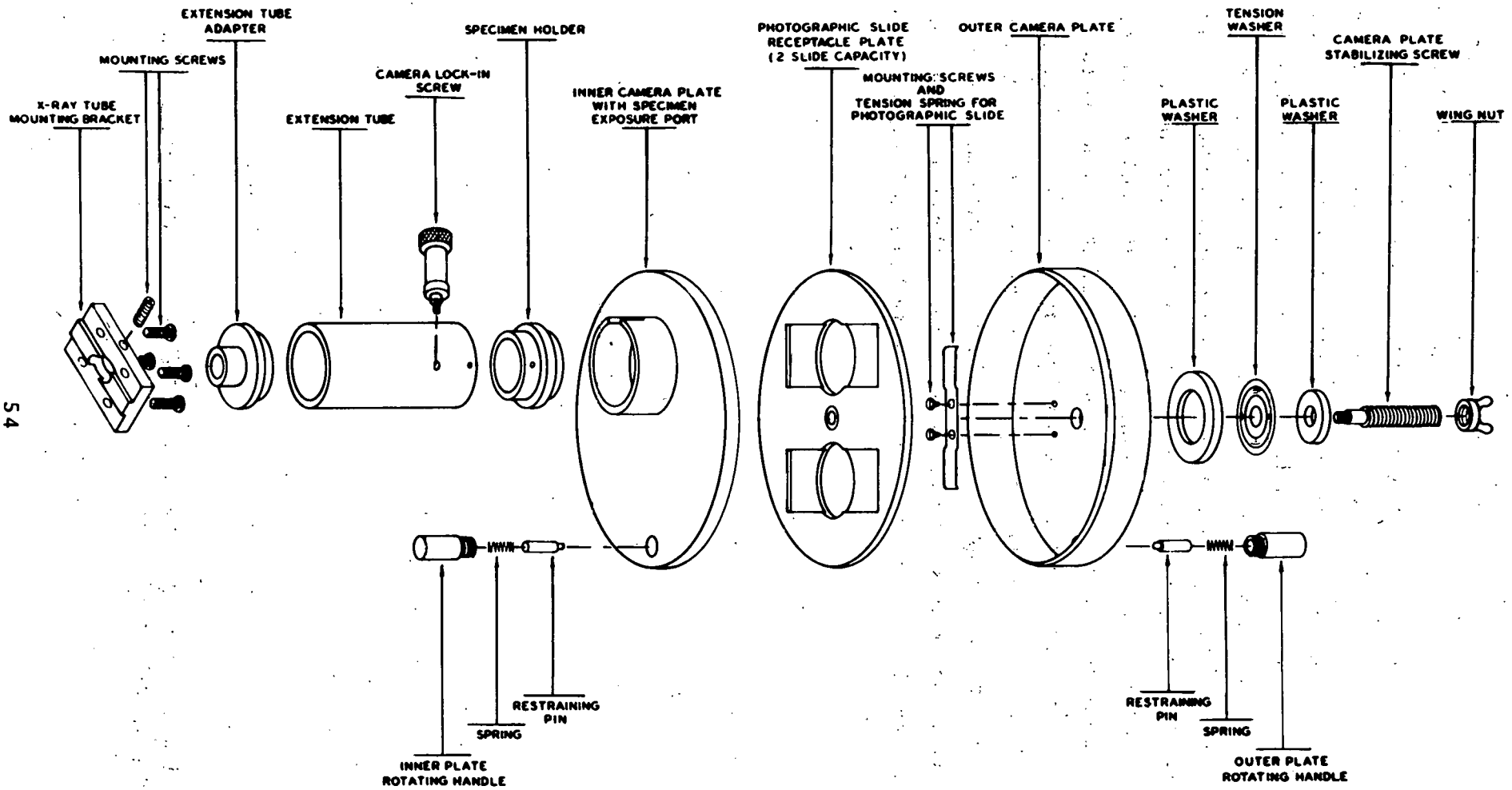


Fig. 1. Schematic drawing of microradiograph camera.

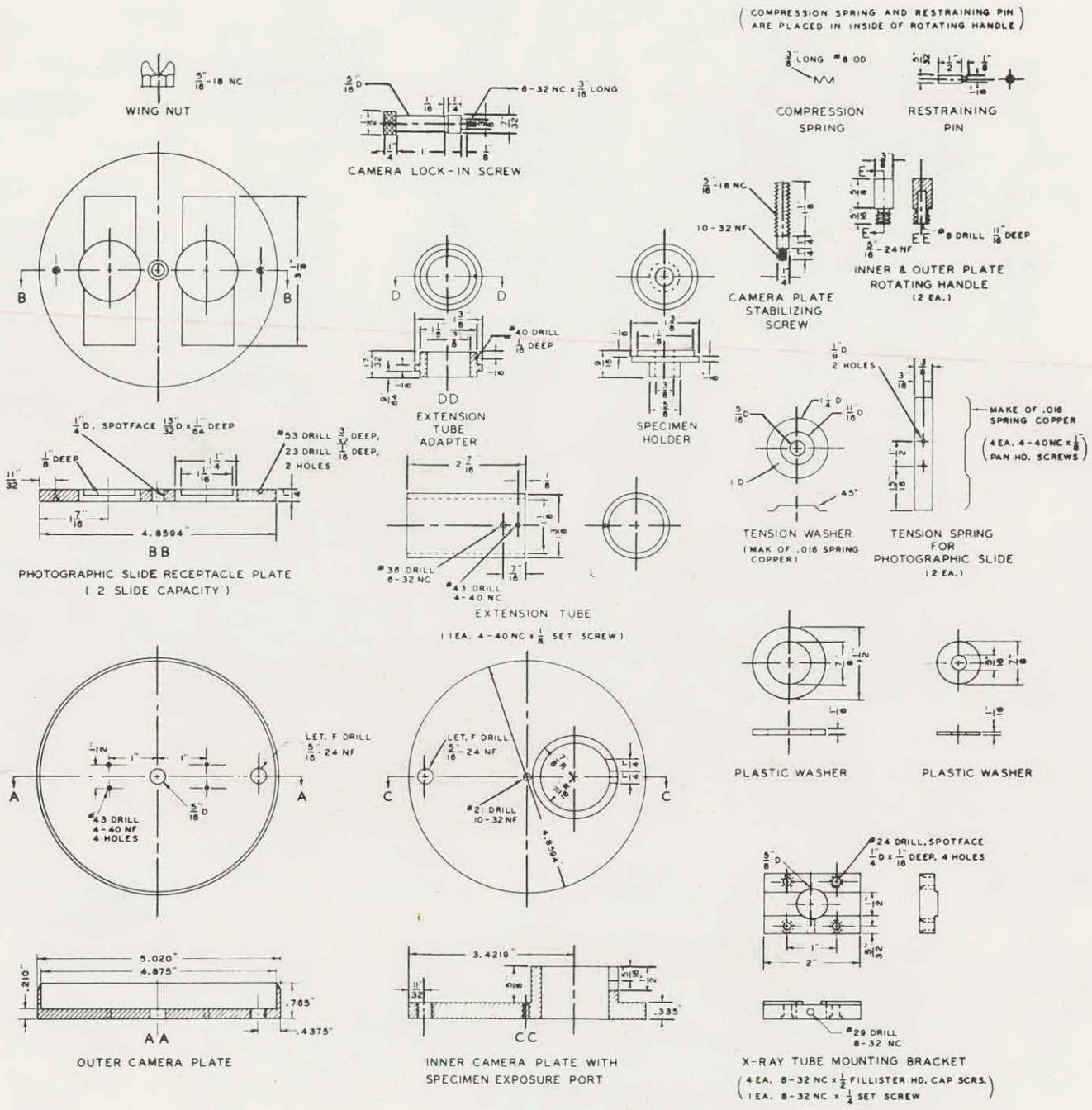


Fig. 2. Plans for a microradiograph camera.

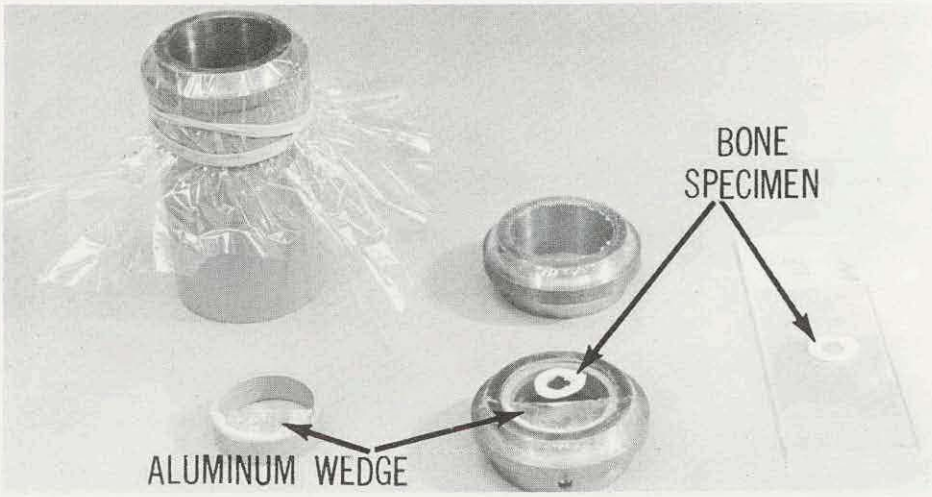


Fig. 3. Specimen holders, density wedge and specimens.

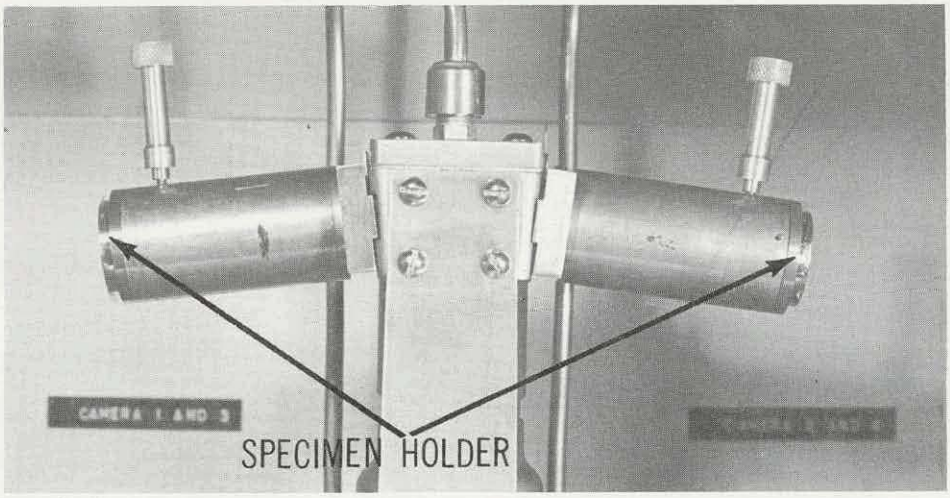


Fig. 4. Specimen holder in place.

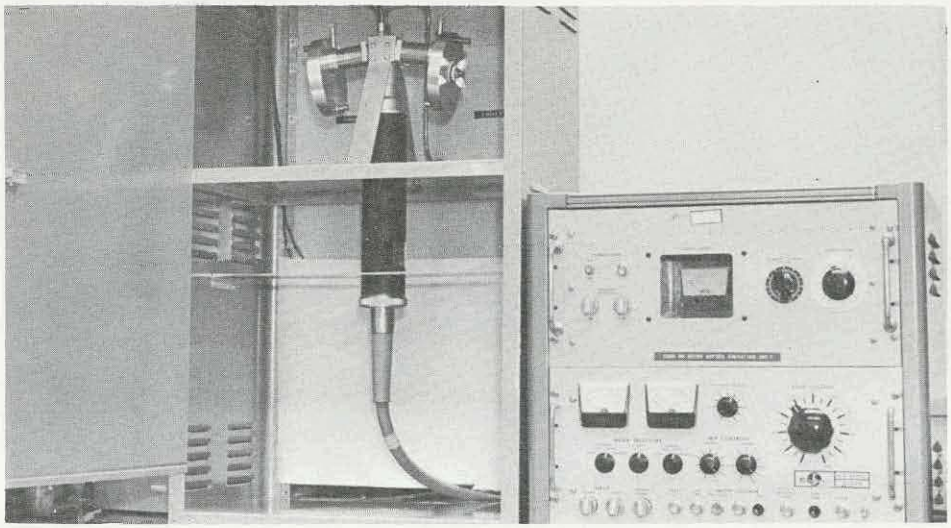


Fig. 5. Power supply and X-ray tube with cameras in place.

BONE DENSITY EVALUATIONS BY MICRORADIOGRAPH SCANNING

D. H. McKelvie
Shirley Coffelt

Density variations in 80 μ bone samples were estimated by microspectrophotometric scanning of microradiographs. The technique was facilitated by use of aluminum foil step wedges as density controls for microradiographs of the bone sections.

It was observed that dogs exposed, from birth, to daily irradiation from Sr-90 administered in the diet developed flint-like bones that were difficult to fracture. This finding prompted a determination of density variations, which was accomplished by the microspectrophotometric scanning of microradiographs to observe variations in light transmission.

To quantitate the beam passing through the microradiograph plate, a series of five aluminum foil step wedges was used as a control for each microradiograph. The aluminum foil weighed 7 mg/cm^2. Figure 1 shows a microradiograph of a cross-section of bone with the radiograph of the step wedge pattern that had been taken simultaneously.

A system for scanning as shown in Fig. 2 was used. A Leitz-Femco Ameda Stage Drive (Femco, Inc., Irvin, Pa.) was attached to the stage assembly of the microscope. The beam for the scope was channeled through a Leitz microspectrophotometer (E. Leitz, Inc., New York) and then through a phototube to a photometer (Photovolt Corp., New York) and to a Linear/Log Varicord 43 (Photovolt Corp.) to be graphed. Each specimen was scanned in comparable areas at 250X at 25 μ per second. Eight 2-mm passes were made with a distance of 400 μ between each. The field of light reaching the phototube represented a circle of bone about 100 μ in diameter; this is the approximate diameter of most of the larger haversian canals. Density was recorded on

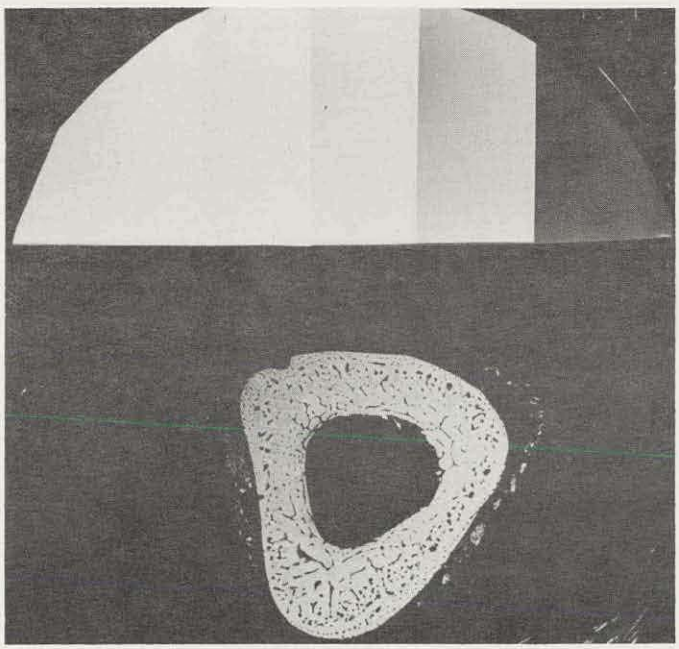


Fig. 1. Microradiograph of cross-section of tibia with density controls in form of radiographs of aluminum step wedges.

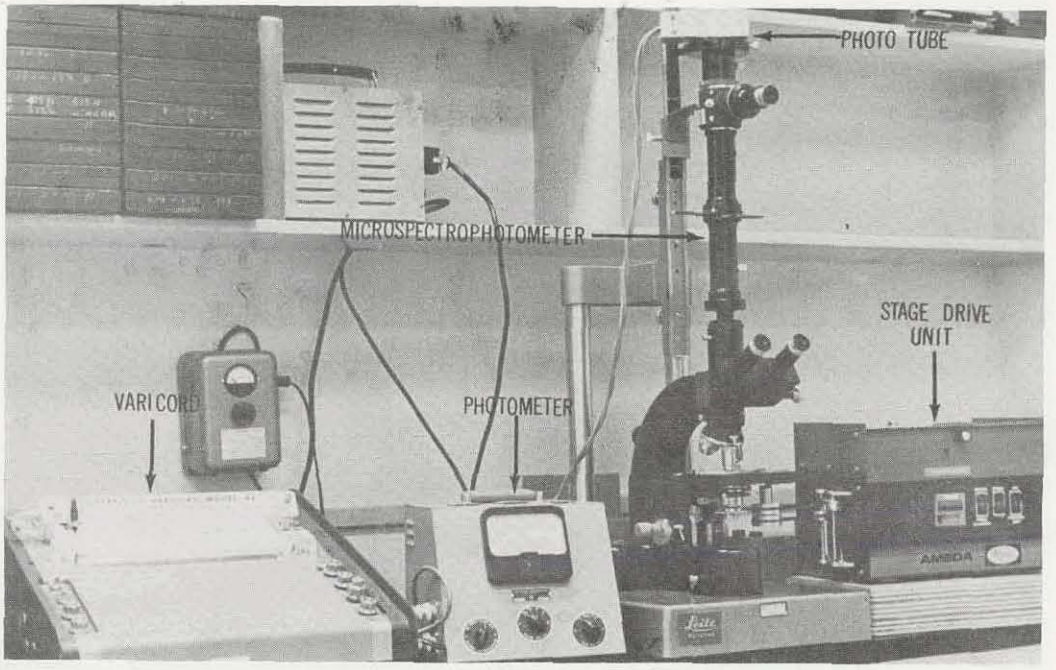


Fig. 2. Scanning system for density measurements from microradiographs.

a running sheet of graph paper. Since each slide had its own standard in the form of radiographs of the aluminum wedges, each set of data from one slide also included a scan of the density variances from the wedges. This allowed for variance in thickness of slides and/or emulsion. Determination of density at maximum height and at lowest peak values was made. Lowest values represented scanning of haversian canals and/or other nonbone areas. Average peak densities were determined by summing all peak values and finding the means (Fig. 3).

This system, as shown, gives some indication of density variations. Refinement of apparatus is being undertaken, primarily by collimating the light beam from beneath the stage of the microscope.

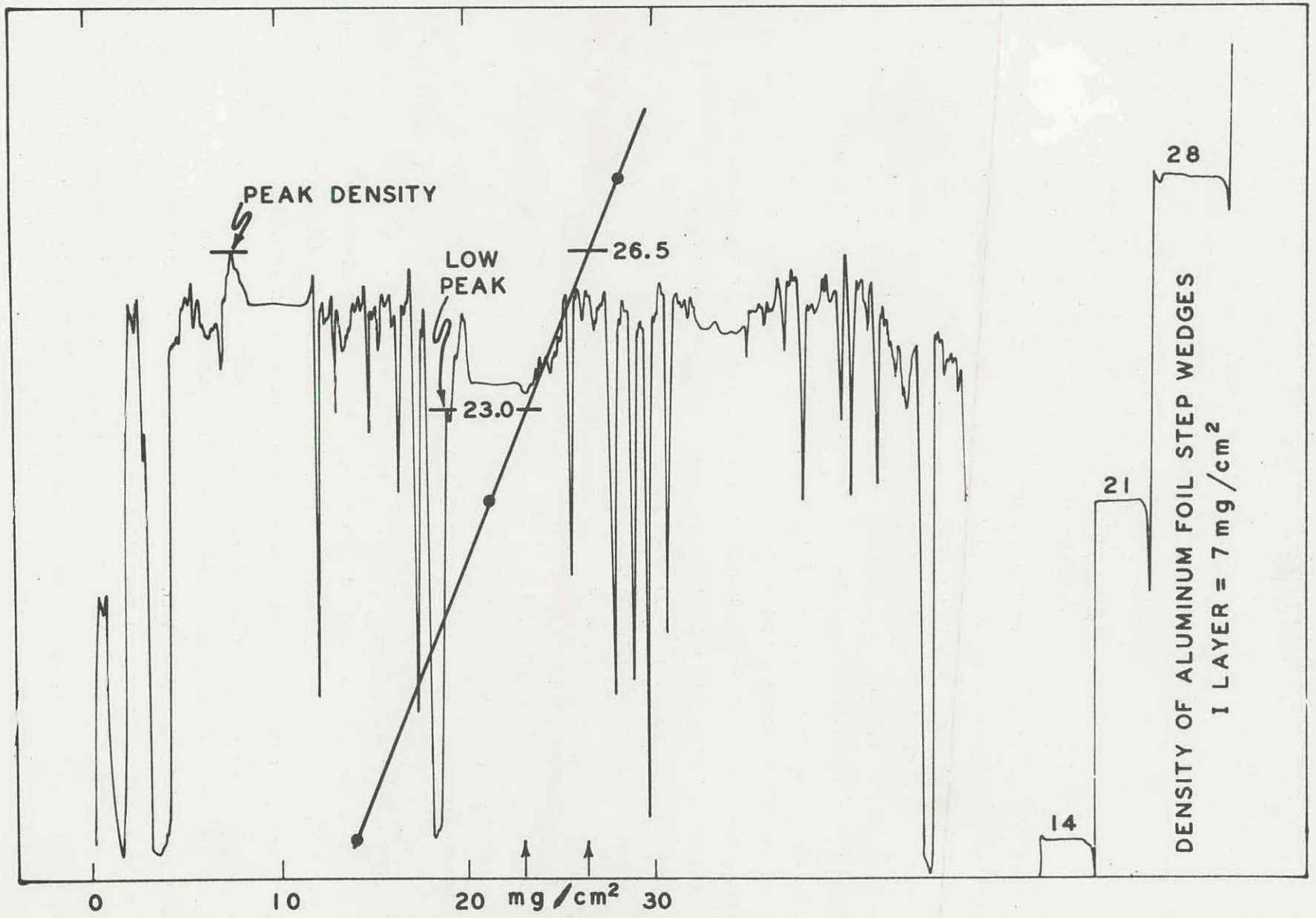


Fig. 3. A scan from a section of a microradiograph representing the humeral shaft of a Sr-90-treated Beagle.

"PACHYOSTOSIS" RESULTING FROM β-IRRADIATION IN BONE UNIFORMLY LABELED WITH SR-90

D. H. McKelvie

A study of microradiographs of bones from dogs uniformly labeled with Sr-90 showed an increased percentage of nonactive osteons and decreased percentages of hypomineralized osteons and resorption cavities; medullary indices were lower than controls. Because the resultant bone is more compact, with a smaller medullary cavity, resembling the bones of pachyderms, the condition is termed "pachyostosis."

Preliminary gross observations were made on bones derived from Beagles maintained from 3 weeks in utero to 18 months of age on a diet containing Sr-90. The overall effect was the production of a "flintlike" consistency in the bone, together with narrowing of the marrow cavity and a possible increase in density. Such effects are not the same as those seen with high levels of X-irradiation or from irradiation from nuclides such as Ra-226 deposited in the bone by intravenous injections at puberty and early adulthood.

An extensive study was done to determine if the uniform deposition of internal emitters such as Sr-90 has a differential effect on osteoclasts and osteoblasts. Thirty-two pups from eight litters were used. Four of the litters (18 pups) were maintained on 10 μCi Sr-90/g Ca in the diet from 3 weeks in utero to 5 months of age; the remaining litters were controls. The dogs were then sacrificed and their bones were sectioned for microradiography.

The microradiographs were examined to determine the medullary index (UCD 472-114, 1967, p 63), which gives relative area of medullary cavity to cortical bone, and for analysis of hypomineralized osteons (with or without enlarged haversian canals), resorption cavities, and nonactive osteons (Fig. 1). Percentages of such osteons and the medullary indices were determined in control and Sr-90-labeled humeri, femora, tibiae, and metatarsals. In Sr-90 dogs there was a general decrease in hypomineralized osteons (both with and without enlarged canals) and in resorption cavities; there was an increase in percentage of nonactive osteons. Since the hypomineralized osteons and resorption cavities are indicative of remodeling, it was concluded that the resorption process had been depressed. This was further verified by the fact that the medullary index was less in the Sr-90-labeled bones as a result of depression of endosteal resorption and a consequent increase in the thickness of the cortical bone. The result is the formation of an average-sized bone with a narrower medullary cavity and a denser compacta. Densitometry of microradiographs also verified that the Sr-90-labeled bone was more dense than controls' (McKelvie and Coffelt, p 57). This type of bone is not unlike that seen in

elephants and hippopotami and in aquatic animals such as seacows (dugongs), whales, otters, and beavers (Jowsey, Cornell Vet. 58: 74, 1968). The condition is therefore referred to as "pachyostosis" and characterizes bone uniformly labeled with high levels of Sr-90. Figure 2 shows cross-sections of femurs from various animals as drawn by Foote in 1915 (J. S. Foote, A Contribution to the Comparative Histology of the Femur, Smithsonian Institute, Washington, D.C., 1916).

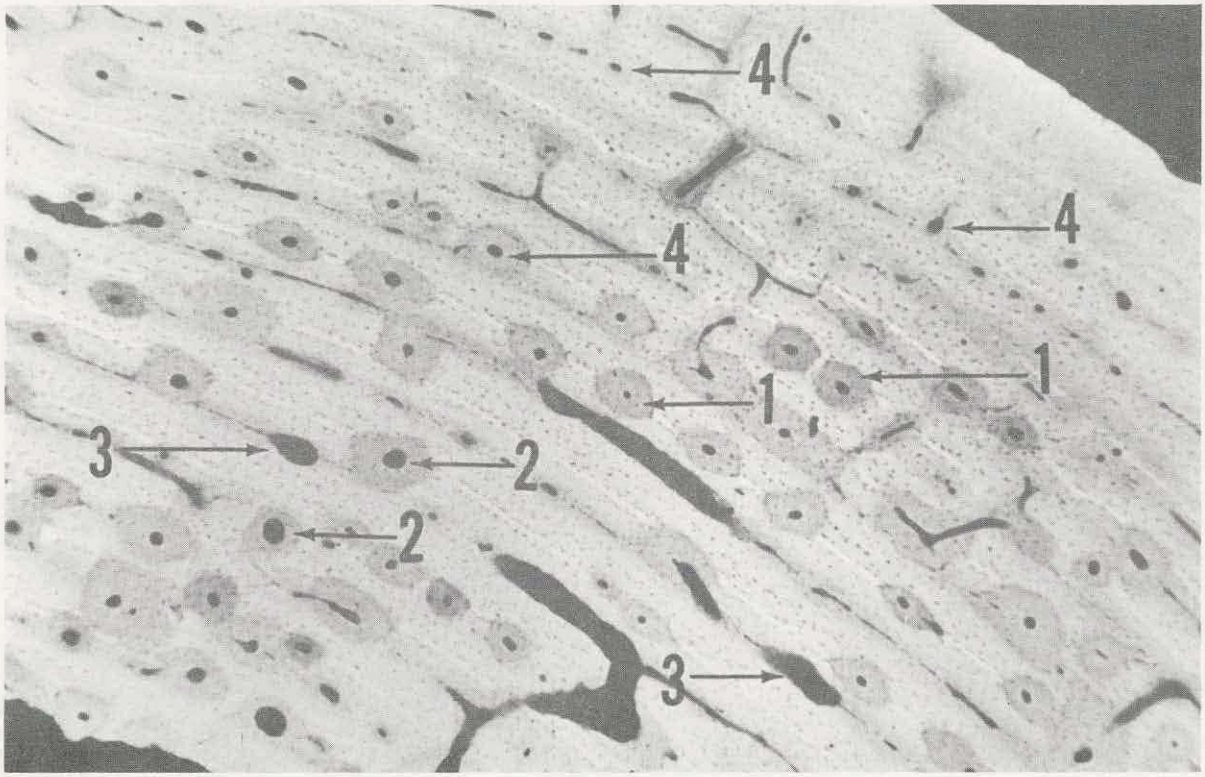


Fig. 1. Microradiograph of normal femur from 3-yr-old Beagle (250X).
1. Hypomineralized osteon
2. Hypomineralized osteon with large canal
3. Resorption cavity or porosity
4. Nonactive or normally mineralized osteon

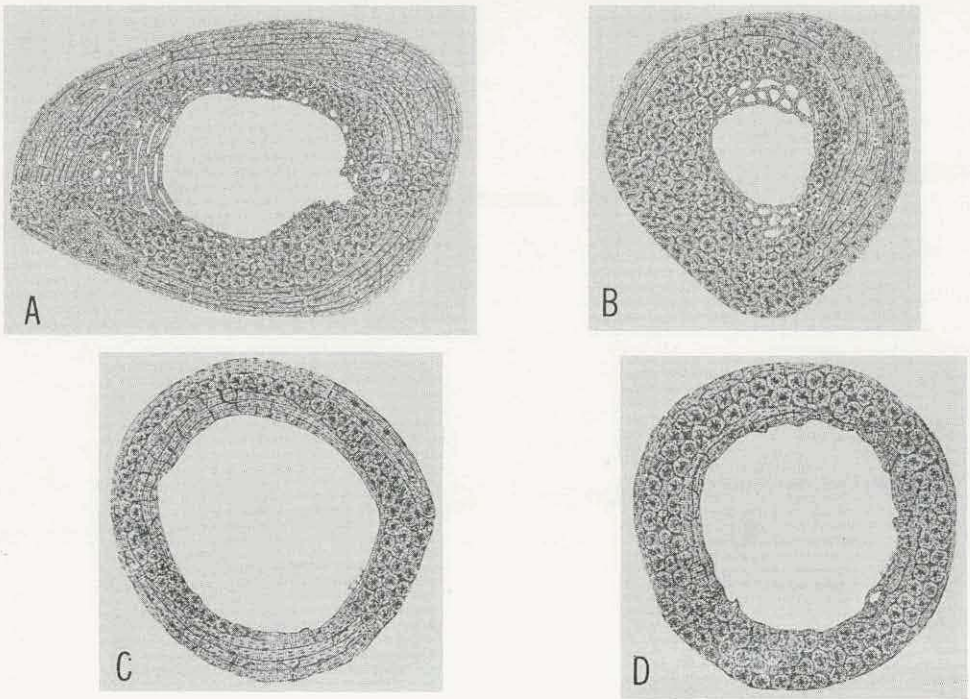


Fig. 2. Cross-sections of femurs from (A) Asiatic elephant, (B) hippopotamus, (C) mongrel dog, (D) spaniel dog. (Taken from Foote.)

RADIOGRAPHIC CHANGES IN SKELETONS OF BEAGLES ADMINISTERED SR-90 AND RA-226

R. Jean Romer Williams
R. J. Hanson

Pathologic changes in the skeletons of Sr-90 and Ra-226-treated dogs are compared. Only dogs at the highest Sr-90 dose level show more than random incidence of injury. A dose-related pattern of injury persists in higher levels of Ra-226-injected dogs up to 4 yr of age. Two dogs at the highest level of radium have developed osteosarcomas; a third dog is believed to have a similar osteosarcoma.

Use of the code system previously described (UCD 472-114, 1967, p 60) was continued for quantitative evaluation of radiographic observations of dogs administered Sr-90 and Ra-226. Routine annual skeletal surveys were evaluated for 473 dogs in the Sr-90 group and 278 in the Ra-226 group. In addition, 43 special examinations were done. Comparisons of the controls and dogs in each dose level in the Sr-90 and Ra-226 groups were again made by calculation of an Index of Injury, per attained age, for each level.

One radium control dog showed periosteal and endosteal changes in the radii, ulnae, and tibiae after 18 months of age; these lesions were similar to those seen in the radium-treated dogs.

No lesions were noted in strontium-injected dogs at the highest (S4) level. However, the long bones of 2 of the 28 dogs in the S2 group showed minimal lesions which are apparently unrelated to the radiation history.

The strontium ingestion group showed only random evidence of injury. There might have been an increase in bone lesions by 4 yr of age at the highest dose level (D5); these dogs showed an Index of Injury of 2.3, which is similar to that of the R2 level. (Of the original D5 pilot study dogs over 4 yr of age, none has developed a significant skeletal lesion.)

The radium-injected dogs show the same pattern of injury described last year: trabecular coarsening of the distal femora; multiple areas of endosteal cortical sclerosis and thickening in the long bones (especially distal to the knees and elbows); fairly frequent periosteal reaction in long bones; early fractures in the ribs and spinous processes of vertebrae (mainly thoracic); and spiral fractures in femora and, occasionally, humeri, the latter developing rather late. The rib-end changes referred to as "lead lines" last year have developed very poorly calcified new bone beyond the dense lines. At the higher levels there has been a surprising development in many of the humeri and some

other long bones that showed rather exuberant periosteal reaction: they show a tendency to "heal," with marked improvement in the appearance or even complete return to normal. This factor may help account for the apparent dip at 3.5 yr in the R5 curve in Fig. 1. Many of the oldest dogs in the two highest-level radium groups show a peculiar type of dental caries (Bulgin, p 31) as well as tooth root abscesses and loss of some teeth.

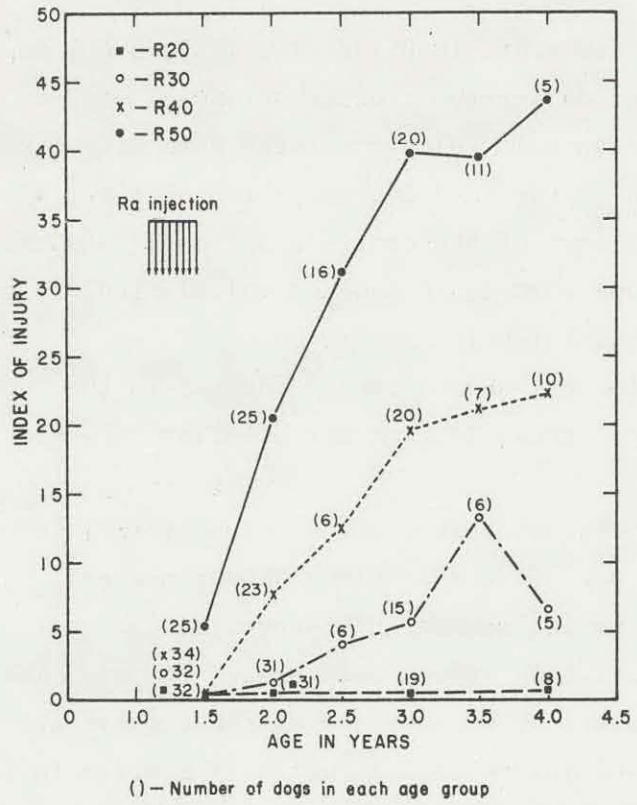


Fig. 1. Comparison of Index of Injury with age in Ra-226 dogs.

A few of the oldest dogs in the highest radium group have shown tumors, two of which are proven osteosarcomas. One of these was a nasal tumor that extended into the maxilla; in the other case nearly half of the pelvis disappeared due to an increasing osteolytic lesion with obvious soft tissue extension and either a secondary or a metastatic lesion in one radius. A rather large osteolytic lesion in the ulna of a third dog is a suspected osteosarcoma.

The incidence of the various types of lesions seen in the radium-injected dogs is shown in Figs. 2 to 4. Generally there is increasing injury with age, the higher dose levels showing earlier onset and greater incidence, both in numbers of animals and bone areas involved. Therefore, the Ra-226-injected dogs show a dose-related pattern of injury, whereas the Sr-90-injection and ingestion groups still have a notable paucity of skeletal lesions identifiable by radiography.

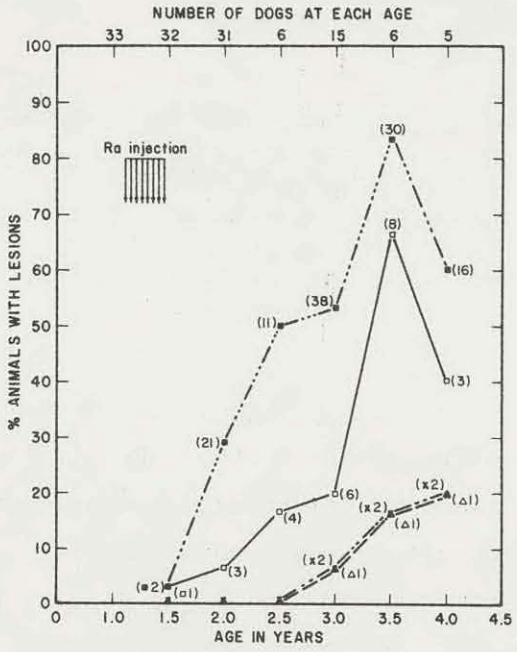


Fig. 2. Skeletal lesions in Beagles treated with 0.14 µg Ra-226 per kg per injection (R3).

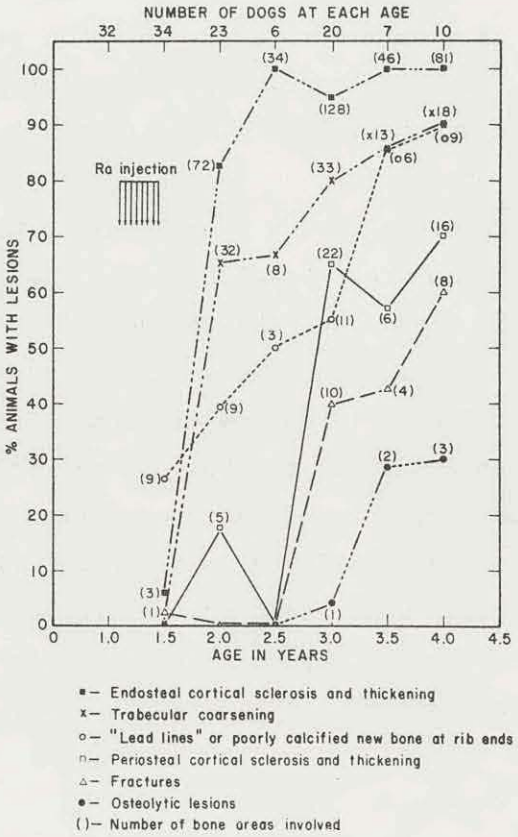


Fig. 3. Skeletal lesions in Beagles treated with 0.42 µg Ra-226 per kg per injection (R4).

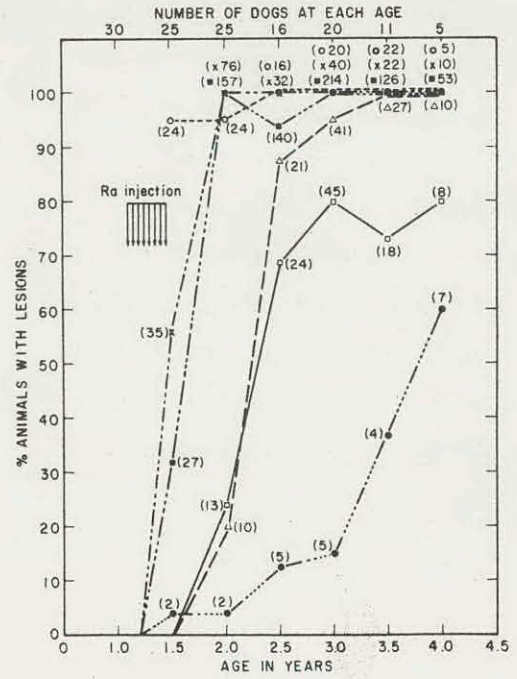


Fig. 4. Skeletal lesions in Beagles treated with 1.25 µg Ra-226 per kg per injection (R5).

BONE-SEEKING RADIONUCLIDE EFFECTS AS DEMONSTRATED BY SCINTILLATION CAMERA SCANNING

Marvin Goldman
D. C. Van Dyke*

Total body scintillation camera scanning has demonstrated the distribution of Sr-90 and Ra-226 in Beagles. Scanning of deposition sites of Fe-52 and Tc-99 indicate no significant changes due to Sr-90 treatment. Bone blood flow visualized with F-18 indicated no Sr-90 effects, but demonstrated Ra-226 impairment of the normal pattern and localized an active endosteal resorption site one month before radiographic confirmation.

Beagles reared on diets containing Sr-90 or receiving high levels of Ra-226 exhibit radiation injury to the skeleton and hematopoietic system. Results to date indicate that minimal injury to bone of Sr-90-treated animals can be detected by gross radiography, although a pattern of pachyostosis is seen on autopsy and microradiography. The pattern of strontium deposition by continuous feeding has been demonstrated to result in a uniformly labeled skeleton. However, after 18 months of Sr-90 feeding, the radiostrontium is lost from the skeleton at rates which seem to be proportional to the degree of trabeculation of the particular region of the skeleton (i.e., the turnover rate in compact bone is less rapid). It is also known that Sr-90 effects on the hematopoietic system have resulted in chronic leukopenia and in the induction of leukemias.

The distribution of active marrow in the Beagle is primarily along the axial skeleton, while the bulk of the Sr-90 body burden is distributed, at least at long times after the cessation of the labeling period, within the appendicular skeleton. It is reasonable to expect that, with time, marrow absorbs a lower β dose rate compared with the mean skeletal dose rate based only on total skeletal mass and body burden. By contrast, the alpha irradiation dose delivered from Ra-226 injections into adults is distributed to a large extent in "hot spots," such that the highest dose rates would be found in the regions of bone closest to active marrow (i.e., in trabecular and epiphyseal bone). A comparison of the temporal dose-effect relationships upon leukocyte concentrations in blood relative to the two patterns of radionuclide administration tends to support such a hypothesis. Furthermore, in a limited number of dogs studied, the Fe-59 ferrokinetic pattern in Sr-90-fed dogs did not appear to show any dose-effect relationship. This report presents some preliminary studies designed to compare spatial distribution of deposited bone-seeking radionuclides with in vivo dynamic tests of bone

* Donner Laboratory, UC Berkeley

and marrow functions. Scintillation scanning was initiated to obtain further insight into the distribution of cells at risk, relative to the skeletal distribution of radioactivity from these two bone-seeking nuclides. Figure 1 shows the total body bremsstrahlung distribution pattern in vivo and was obtained from a 4-yr old dog with a body burden of approximately 100 μCi Sr-90. Since external bremsstrahlung from Sr-90 + Y-90 is a continuum whose intensity logarithmically scales toward the lower energies, tissue absorption of bremsstrahlung intensity can be quite marked as demonstrated by comparing the dorsal (1a) and (1b) ventral views of the dog: the viscera severely attenuate the bremsstrahlung output from the vertebrae. By contrast, in the gamma ray emission pattern from a dog containing about 25 μCi Ra-226, the body distribution is readily observable in the scintillation scan of the energetic gamma-emitting daughters of Ra-226 (Fig. 2).

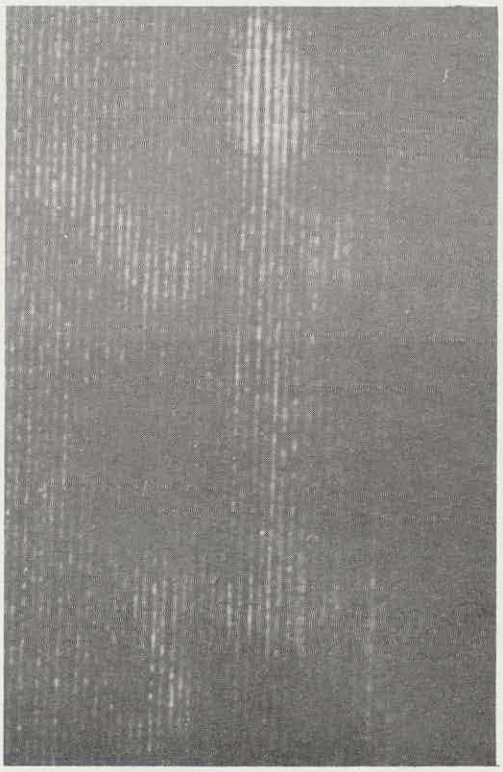


Fig. 1a. Bremsstrahlung scan of Sr-90-labeled Beagle, dorsal surface to detectors.

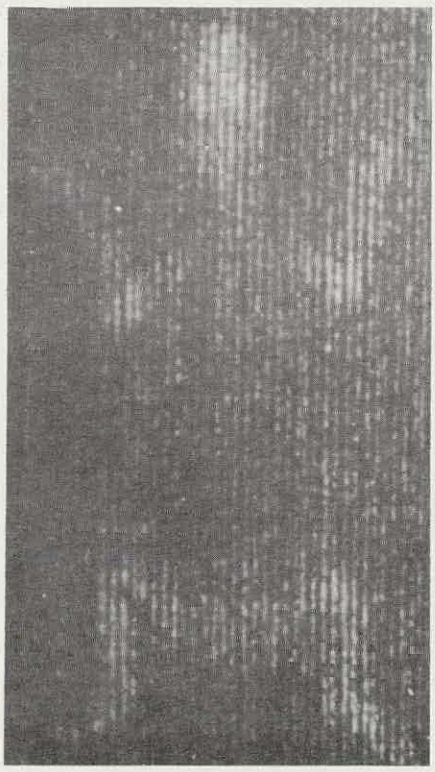


Fig. 1b. Bremsstrahlung scan of Sr-90-labeled Beagle, ventral surface to detectors.

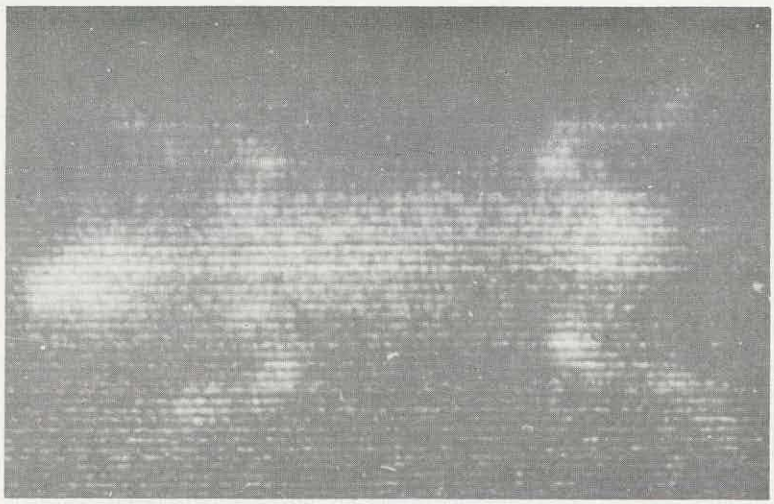


Fig. 2. Whole body scan of Ra-226 Beagle.

The qualitative distribution of active hematopoietic sites was compared between a high level strontium dog (100 μCi Sr-90 body burden) and one at our lowest level (<0.1 μCi Sr-90 body burden, essentially equal to control values). Positron-emitting Fe-52 was injected 12 hr before the scintillation scan to allow for maximum erythroid marrow uptake. Figure 3 shows that the distribution of the iron in the marrow is qualitatively the same in the two dogs. Essentially all the iron was deposited in marrow, liver, and spleen. The distribution of reticuloendothelial cells, as demonstrated by the uptake of colloidal Tc-99, was similar and also demonstrated the lack of a difference between the two dogs.

The pattern of bone blood flow was evaluated in a high level strontium, a high level radium and a control dog (Fig. 4), utilizing the skeletal incorporation of F-18. Following intravenous injection, fluorine that has not been adsorbed onto bone crystals is rapidly cleared by the kidneys. The control and strontium dogs exhibited essentially the same pattern; however, the high level radium animal demonstrated a poorer skeletal resolution, suggesting that a lesser fraction of the fluorine was adsorbed on bone and was still circulating so that a higher fraction was excreted into the bladder. In addition, a hot spot on the left humerus of the radium dog suggested a localized region of increased bone blood flow which could have indicated a fracture (Fig. 4). Subsequent radiographic examination demonstrated no abnormal lesion in this humerus; however, one month later there was radiographic evidence of active endosteal resorption, a common characteristic of radium poisoning. The fluorine "hot spot" demonstrated

the location of an abnormal process prior to its recognition radiographically. Since the dose commitment from F-18 is quite low, due to a short half-life (112 min), this method may have considerable utility in diagnosis and therapy evaluation of osteologic diseases. Future studies will utilize such tests at earlier ages and during, as well as after, the period of radionuclide administration.

Acknowledgment is made to the UC Donner Laboratory for cooperation in scintillation scanning.

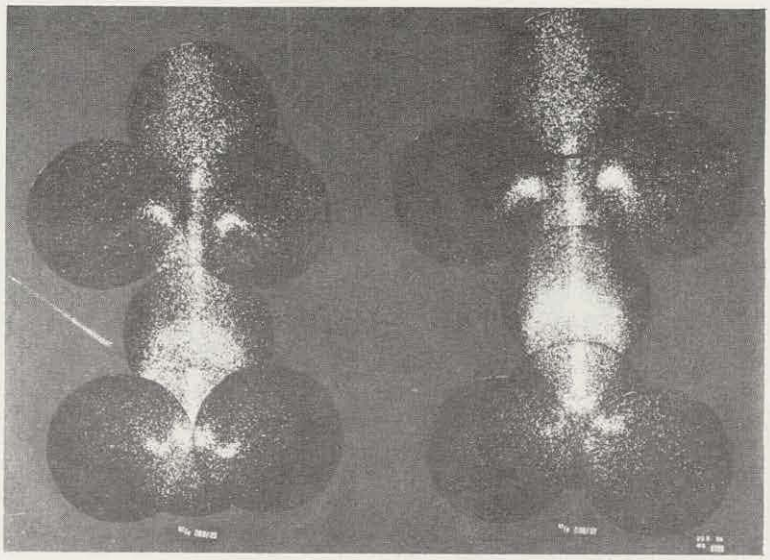


Fig. 3. Erythropoietic marrow distribution in low (<0.1 μCi burden, left) and high (>100 μCi burden, right) Sr-90-labeled Beagles.

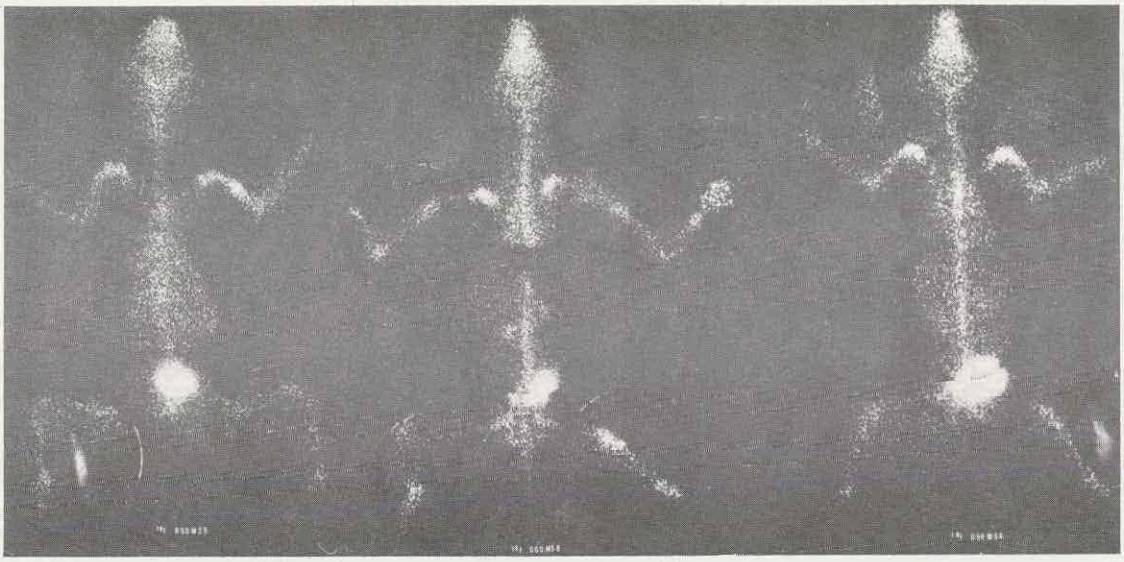


Fig. 4. Bone-blood flow pattern in Sr-90- (right) control (center), and Ra-226- (left), treated Beagles.

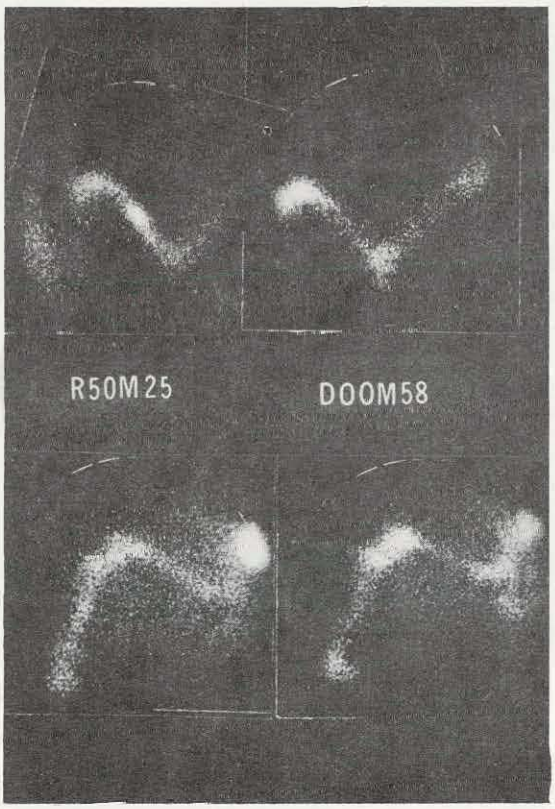


Fig. 5. Left fore (top) and hind limbs (bottom) in Ra-226-treated (left), and control Beagles. Note humeral "hot spot" and poorer limb resolution, especially about the stifle.

HEMATOLOGICAL EFFECTS OF RADIUM

Marie Bulgin

Immediately after radium injection, the number of circulating white cells decreases. The count begins to rise after the last injection and approaches control levels. Radium showed a small effect on circulating red cell numbers at the highest dose level; no effect was noted at lower dose levels.

The series of eight Ra-226 injections, given at 15-day intervals, begins at 435 days of age and ends at approximately 540 days of age. At the highest level (R5), immediately after the first radium injection there is a decrease in the white cell count. After the last injection the number of circulating white cells begins to increase. The count stabilizes at 4,000 to 7,000 (about half of normal) at around 900 days, however, and remains more or less constant, not showing the further decline with age demonstrated by the control dogs (Fig. 1). The greater variability noted at higher ages may be due to the smaller number of dogs at those ages.

Effects at the R4 level are less marked but show the same pattern--a decrease in white cell count during injection and a rise afterward--reaching stability at 900 days. Values at that time are slightly below controls' and remain at this level rather than demonstrating the slow decline with age shown by the control dogs.

Radium has less of an effect upon the erythropoietic system. Although a transient drop is noted in the packed cell volume during injection in the R5 level, no change from the normal is seen in the R4 level.

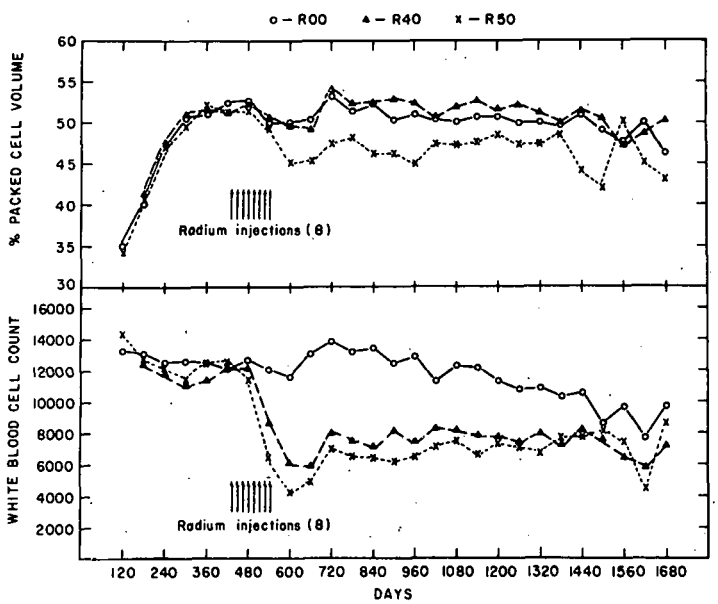


Fig. 1. Changes in packed cell volume and total leukocyte counts with age in Ra-226-treated Beagles.

MYELOPROLIFERATIVE DISORDERS IN SR-90-BURDENED BEAGLES

M. Goldman
D. L. Dungworth
J. F. Wright
J. E. West
J. W. Switzer
H. Tesluk

Fourteen Beagles in the two highest Sr-90 treatment levels have developed varying degrees of myeloproliferative disorders. The incidence rates range from 2 to 7% per year between 1 and 5 yr of age; the most florid cases of granulocytic leukemia are generally in the younger dogs. Clinically, anemias signal the onset of the disease with variable leukemic response, rare abnormal cells in peripheral blood, and a spectrum of organ infiltration and architectural disruption. The severity of the disease in these cases is correlated with changes in marrow, spleen, liver, lymph nodes, and lung, and other tissues to a lesser degree, consistent with the concept of progressive stages in the development of granulocytic leukemia.

An apparent dose-related incidence of myeloproliferative disorders occurred in Beagles between 1.5 and 4 yr of age at the two highest levels of Sr-90 feeding (∿4 or 12 μCi/day for their first 18 months of life) (UCD 472-114, 1967, p 53). At present there are 14 cases. To date, no myeloproliferative disease has been observed in any of the Sr-90-treated animals in the lower dose levels. Table 1 summarizes the population at risk at various ages and indicates the incidence of the disease in the colony. Intensive study and comparative evaluation, particularly by pathologists at the Radiation Laboratories at Oak Ridge, Utah, Argonne, Battelle Northwest, and the Lovelace Foundation, have substantially corroborated the diagnosis and its salient characteristics.

Table 1. VARIATION WITH AGE OF THE NUMBER OF BEAGLES AT RISK IN THE VARIOUS Sr-90 DOSAGE LEVELS

		Number of Beagles at Risk in Each Age Grouping								
Dose Level	μCi Sr-90/day	0.10 to 0.99 Yr	1.00 to 1.49 Yr	1.50 to 1.99 Yr	2.00 to 2.99 Yr	3.00 to 3.99 Yr	4.00 to 4.99 Yr	5.00 to 5.99 Yr	6.00 to 6.99 Yr	7.00 to 7.99 Yr
D0	0.00	96	79	71	50	40	6	--	--	--
D05	0.03	104	88	68	67	59	38	26	14	4
D1	0.08	56	40	35	28	22	3	--	--	--
D2	0.5	79	67	54	46	41	24	16	10	--
D3	1.5	83	65	59	59	46	33	13	6	--
D4	4	67	54(1)*	50	47(2)	37	23	12(1)	10	--
D5	12	69	54(2)	52(2)	44(3)	31(3)	12	4	1	--

* Number of dogs with myeloproliferative disorder is in parentheses.

As a group, the 14 cases seem to manifest a spectrum of involvement that may characterize the development of myelogenous leukemias. This spectrum ranges from the earliest indications of myeloid hyperplasia and metaplasia through intermediate cases to those that can be classified as undoubtedly neoplastic. There were two acute deaths; in the remaining 12 cases, a progressive or precipitous terminal anemia was the first clinical evidence of the disease process.

A wide range of terminal peripheral blood leukocyte numbers (3,000 to 45,000) was observed. These are illustrated by four cases in Table 2. An interesting characteristic of these Beagles was the appearance of transient variations in peripheral leukocyte count during the disease. The disorder is generally characterized by the appearance of hypercellularity of bone marrow, splenomegaly and, frequently, mild enlargement of lymph nodes. There is an apparent decrease in marrow space in the long bones of the high-level strontium dogs, but the importance of this finding has yet to be evaluated. Histologically, the marrow manifests a hypercellularity of granulopoietic elements, usually with a shift to the left and sometimes including abnormal blast cells with a great reduction in erythroid precursors. In some cases there is also a mild fibrosis.

The spleen usually has an excess of granulopoietic cells in the vessels. There are varying degrees of architectural disruption in the spleen and to a lesser extent, the lymph nodes. The myeloid infiltration is the predominant feature. There appears to be a good correlation between the severity of the disease involvement and the degree of terminal splenomegaly. Changes in the liver also reflect the severity of the disease, cellular infiltrations being predominantly in central and sublobular vein regions. Lung capillaries frequently appear to be infiltrated with blast cells. Rarely are abnormal blast cells seen in smears of peripheral blood.

Table 2 summarizes some of the salient features of the dogs studied to date, arranged to show a pathologic progression from myeloid metaplasia to myeloid neoplasia. In view of the continuum represented, it appears difficult to separate these cases except by degree; therefore, one is forced to conclude that these cases represent varying stages in the potential development of granulocytic leukemia. This affords a unique opportunity for the study of the pathogenesis of granulocytic leukemia as related both to myeloid metaplasia with myelofibrosis and the inductive effects of marrow irradiation.

Table 2. REPRESENTATIVE TERMINAL HEMOGRAMS OF Sr-90-IRRADIATED BEAGLES THAT DEVELOPED A MYELOPROLIFERATIVE DISORDER

Dog Number	D50M32	D50F14	D50M64	D40F81
Age at Death (yrs)	2.3	1.5	3.5	2.1
Duration of Anemia (dy)	24	76	386	174
Hematocrit	17	5	9	3
Erythrocytes ($10^6/mm^3$)	2.60	0.70	1.10	0.50
Hemoglobin (g%)	7.5	1.6	3.0	1.0
Leukocytes ($/mm^3$)	38,900	3,300	7,100	4,100
Leukocyte Differential				
Myeloblasts (%)	12	0	0	2
Progranulocytes (%)	26	0	0	3
Myelocytes (%)	8	18*	5	17
Metamyelocytes (%)	10	4	21	8
Bands (%)	18	2	22	25
Seg. Neutrophils (%)	23	14	45	28
Lymphocytes (%)	1	62	4	11
Monocytes (%)	0	0	3	0
Eosinophils (%)	2	0	0	6†
Erythrocyte Morphology				
Poikilocytosis	+	+	+	+
Anisocytosis	+	+++	+	+
Hypochromasia	-	++	-	++
Macrocytosis	-	++	-	++
Platelets	few	rare	rare	rare

* Fine chromatin, prominent nucleoli
† Immature-myelocytes, metamyelocytes, and bands

The mean marrow dose rate diminishes with increasing post-Sr-90-feeding time, possibly resulting in an incidence that is highest in the younger dogs. In addition, it is estimated that the marrow dose rate is relatively high at the time of maximum hematopoietic development; this may also play a role which is reflected by the incidence. The data from Table 1 indicate about a three-fold difference in total cases per level, which is approximately proportional to the difference in dosage between the two levels. There also appears to be an annual doubling of the age-specific incidence rate between 1 and 5 yr of age, starting at ∿2%/yr for D4-level dogs and ∿4%/yr for D5-level dogs. Although the data are limited, the incidence trend at this time correlates roughly with the period of maximum leukopenia and may be related to the period of maximal efficiency of irradiation in perturbing marrow cells (including stem cell pool).

EFFECTS OF X-IRRADIATION ON NUCLEIC ACID METABOLISM OF BEAGLE LYMPHOCYTES IN CULTURE

M. Goldman
K. K. Wolf
A. Kimi Klein
Angela T. Foin

Phytohemagglutinin-stimulated Beagle blood lymphocytes begin transformation toward blastoid forms within 24 hr in vitro and proceed with a doubling time of about 12 hr. Initial X-irradiation (300R) reduces the cell number to about half, but does not change the transformation rate. Labeled thymidine and uridine uptake rates are similar with respect to time and X-ray effect. After exposures to 0, 30, 50, 100, 300, or 500R, uridine uptake was depressed about 50% per 100R; cell survival and thymidine uptake were minimally affected at doses less than 100R.

We have previously reported that graded doses of X-rays from 30R to 500R decreased survival and reduced transformation efficiency following phytohemagglutinin (PHA) stimulation (UCD 472-114, 1967, p 68). This report summarizes attempts to correlate some of the parameters of nucleic acid metabolism with those of cell transformation and survival in vitro. The assumption was made that tritiated thymidine, a DNA precursor, would be incorporated only into the nucleic acids of leukocytes stimulated by PHA; i.e., cells undergoing division. In addition, the incorporation of C-14-uridine into the RNA of cells may not be related primarily to cell replication and may not show the cyclic phenomenon anticipated for DNA synthesis.

The time course of lymphocyte transformation to prolymphocytes and lymphoblasts, as shown in Fig. 1, was determined on Beagle peripheral blood lymphocytes over a three-day period. The effect of an initial exposure to 300R of 250 kvp X-rays was also determined. Minimal transformation occurred during the first 24 hr of culturing, and transformation proceeded exponentially, with a lymphoblast doubling time of about 12 hr, during the next two days of culturing. Exposure to 300R of X-irradiation depressed the absolute number of cells transformed (by a factor of almost three) but not the rate.

Fig. 2 summarizes the results of leukocyte cultures in which the rate of incorporation of C-14-labeled uridine into PHA-stimulated lymphocytes was compared on a per culture basis. Uridine incorporation in non-irradiated and 300R-exposed cultures was essentially parallel, indicating a depression similar to that noted in cell survival. Corrected for the number of cells in each of the cultures, uridine incorporation on a per cell basis increased essentially exponentially for the first 60 hr of culture and then seemed to reach a plateau,

whereas 300R-exposed cells showed little incorporation until after 24 hr. The data also suggest that, on the average, some X-irradiated cells were initially capable of partial uridine incorporation prior to death.

Fig. 3 illustrates a comparable pattern for the incorporation of tritium-labeled thymidine. Since only blood lymphocytes survive in culture, and are assumed to be the only cells stimulated by PHA, the initial per culture incorporation of thymidine may represent some of the other blood leukocytes which were destined to die in culture but which were still capable of some DNA synthesis. The X-irradiated cultures clearly show the 24-hr lag prior to thymidine incorporation, and the fact that rate of incorporation levels off between 48 and 72 hr. For the number of cells in culture, the control and irradiated cultures showed essentially parallel patterns with a decrease of approximately 50% due to irradiation.

X-ray exposure-effect relationship was tested on cultures sustained for 66 hr, the time at which the rate of change of DNA incorporation appeared to be minimal. In Fig. 4, the effects of X-ray exposures up to 500R on cell survival, thymidine incorporation, and uridine incorporation are compared as percentages of unirradiated cultures. At low doses (30, 50, or 100R) little effect on cell count was seen; at doses of 300R to 500R, more than 50% of the cells appeared to survive, at least to 66 hr. The thymidine incorporation per culture appears to show a similar absence of marked effects at doses below 100R and an exponential depression at the higher doses. The most marked radiation effect was noted in uridine, which showed an exponential decrease in incorporation at all exposures.

In Fig. 5, radiation effects on the thymidine and uridine incorporation per cell are summarized. The 0 to 100R shoulder is evident for the average cellular activity of thymidine, whereas the greater radiosensitivity described above for uridine uptake is shown to follow a pattern parallel to that of thymidine at the higher doses. The cellular RNA:DNA was seen to decrease only through the first 300R of exposure. An explanation might be that, as DNA synthesis is a cyclic phenomenon and the mechanism is not equally radiation sensitive at all stages of the cycle, the effectiveness of a single radiation exposure (in terms of cellular thymidine incorporation) is a function of the number of cells in each phase of the DNA synthesis cycle. In the case of RNA synthesis, if the process is continuous, then all cells may be equally sensitive to a given radiation exposure.

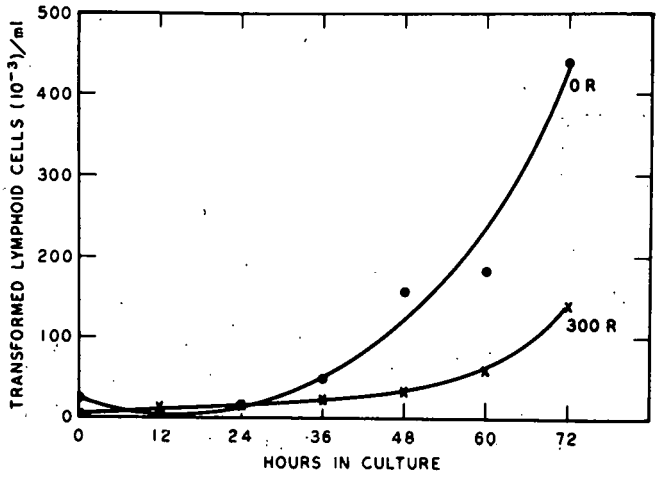


Fig. 1. Lymphocyte transformation following PHA stimulation. Transformation begins after ~24 hr in culture and is further delayed by prior X-irradiation.

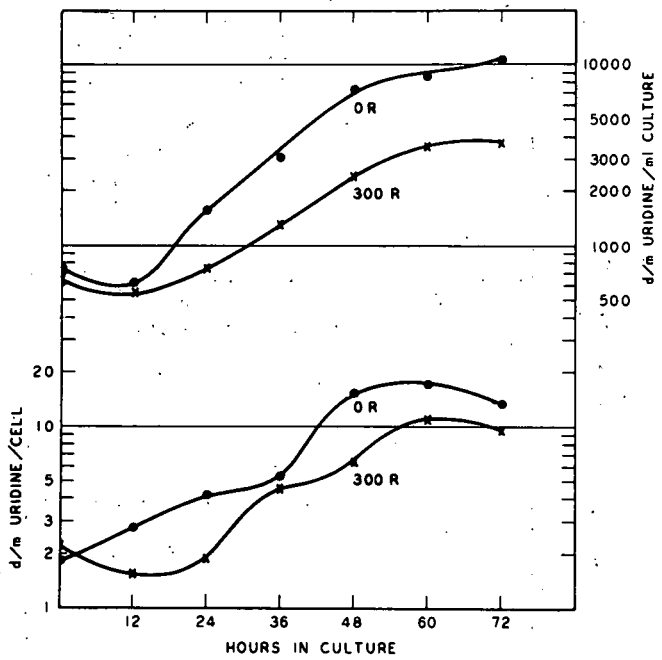


Fig. 2. Uridine-C-14 incorporation into PHA-stimulated Beagle lymphocytes.

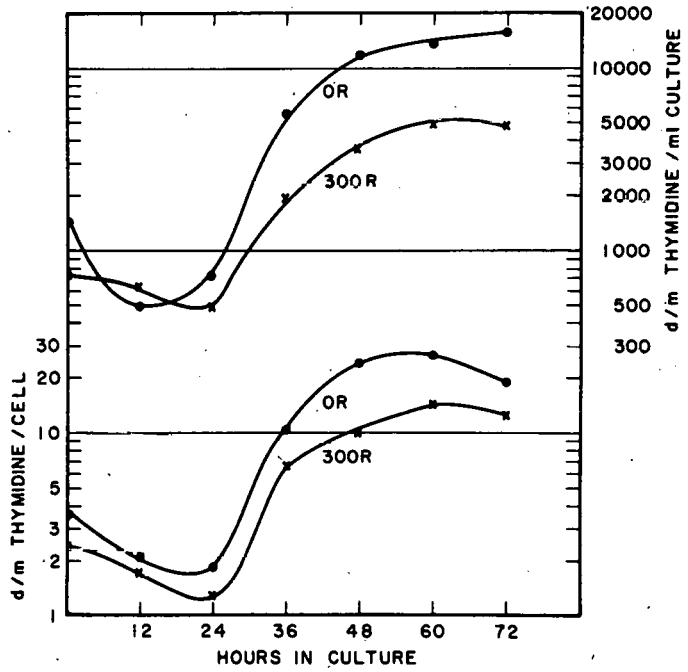


Fig. 3. Thymidine-H-3 incorporation into PHA-stimulated Beagle leukocytes.

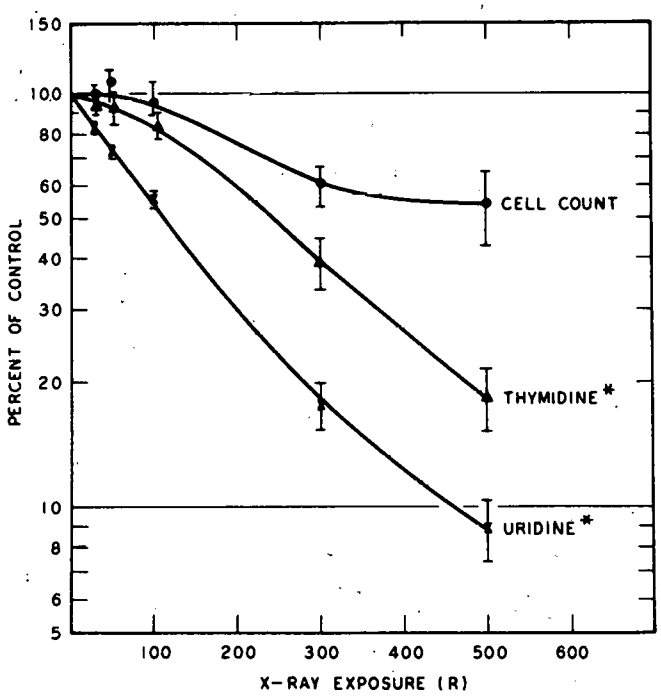


Fig. 4. X-ray effects on lymphoid cell survival and labeled nucleic acid precursor incorporation.

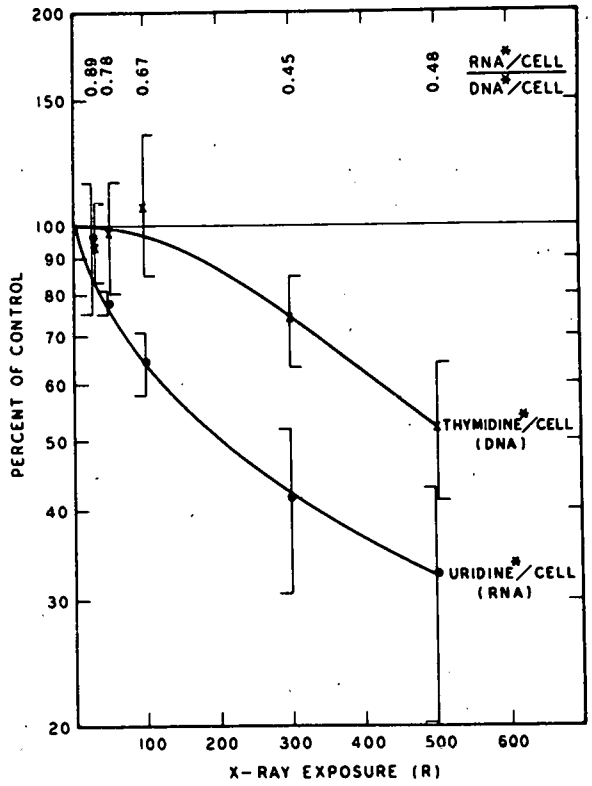


Fig. 5. Average cellular nucleic acid incorporation.

We are currently investigating these metabolic characteristics, utilizing both lymphocytes and marrow cells derived from dogs that have received chronic irradiation of bone marrow by continued maintenance of body burdens of Ra-226 and Sr-90. Based on the findings in three high-level Sr-90-treated animals relative to controls, there is a suggestion of a twofold increase in the ability of the peripheral lymphocytes to respond to PHA stimulation. Further studies are required to determine whether this is a consistent pattern in Sr-90-treated animals, and to evaluate this response in Ra-226-treated dogs.

The immediate and 24-hr uptake of labeled uridine and thymidine by aspirated bone marrow cells in culture is under study, utilizing the relative efficiency of nucleic acid metabolism to a test dose of 300R X-irradiation to the cultures. At this time too few animals have been tested to form any firm conclusions. Preliminary findings would support the hypothesis that marrow cells derived from radium-treated animals were slightly more resistant to additional acute in vitro radiation than comparable controls.

INFLUENCE OF BIOCHEMICAL PARAMETERS ON METABOLIC RESPONSES OF CELLS IN CULTURE

Sally D. Huff
A. Kimi Klein
Angela Foin
Marvin Goldman

Nucleic acid synthesis by Beagle lymphocytes was measured to determine the effects of changing substrate concentration, cell concentration, and other components of the incubation medium. Maximum cellular uptake of H-3-thymidine was obtained with an exogenous thymidine concentration of 6 μM; maximum C-14-uridine incorporation occurred at 25 μM. Isolation of tritiated cellular products after incubation of intact cells with H-3-thymidine indicated that the major portion of intracellular tritium is bound to DNA. Comparison of metabolism of H-3-thymidine by cell-free preparations shows that all enzymatic steps involved in the incorporation of thymidine into DNA are decreased by 300R X-irradiation, but suggests that DNA synthesis may be reduced to a greater extent than is the phosphorylation of thymidine, which precedes its incorporation into nucleic acid.

Recent research has utilized the uptake of nucleic acid precursors as an index of radiation effect on bone marrow and peripheral blood cells in vitro. The response obtained depends not only upon the treatment (irradiation) but also upon the test environment. To make reliable and meaningful comparisons between treated and control groups, the influence of other variables on the response must be considered. The effects of some of these factors are reported here.

Except for the variable being tested, the conditions of cell culture and incubation with H-3- or C-14-labeled nucleic acid precursors were identical to those routinely employed. Briefly, peripheral blood lymphocyte cultures were stimulated by phytohemagglutinin (PHA) and incubated for 66 hr prior to metabolic measurements. Bone marrow cells were cultured for 24 hr before addition of labeled substrates. H-3-thymidine or C-14-uridine uptake was allowed to proceed for 90 min. Total volume per culture tube was 5 ml. Following incubation, cells were isolated and washed three times. After appropriate sample preparation, uptake of radioactivity was measured in a liquid scintillation counter.

1. Concentration of Substrate

Thymidine concentration was from 0.1 to 20 μM. Radioactivity (H-3-thymidine) was maintained at 1.2 μCi/culture tube. Cellular uptake of exogenous thymidine, as a function of thymidine concentration in the medium, is shown in Fig. 1. Thymidine concentration influenced the response up to about 6 μM, after which little change in uptake was observed. The mean concentration of mononuclear cells (MNC) per culture tube was 342,000/ml. The maximum uptake of thymidine per MNC was 1.5×10^{-10} μmoles.

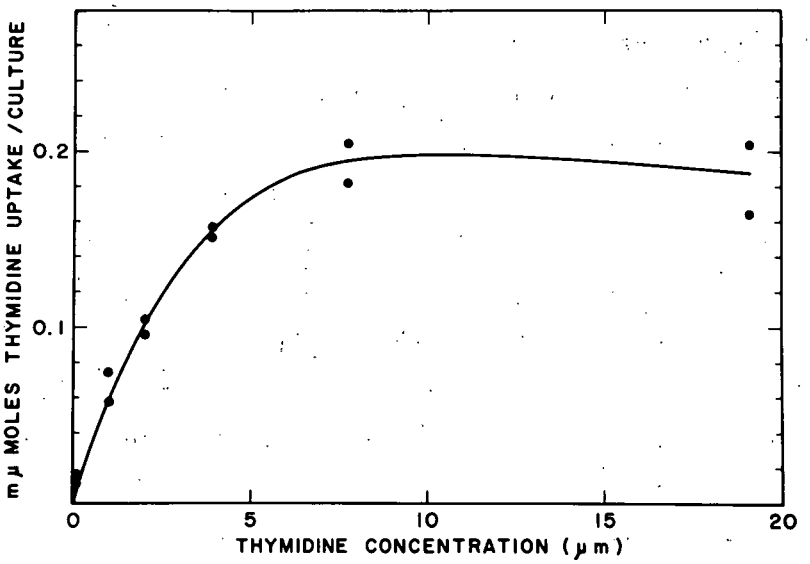


Fig. 1. Thymidine uptake by cultured peripheral blood lymphocytes as a function of exogenous thymidine concentration.

Uridine: Figure 2 shows the effect of varying the concentration of uridine in the medium, from 1 to 60 μM. Each culture tube contained 0.2 μCi of C-14-uridine. Marked increases in uridine uptake as a function of concentration were observed up to about 25 μM. The mean number of MNCs was 634,000/ml; uptake of uridine per cell was 7.5×10^{-10} μmoles--five times as great as the uptake of thymidine.

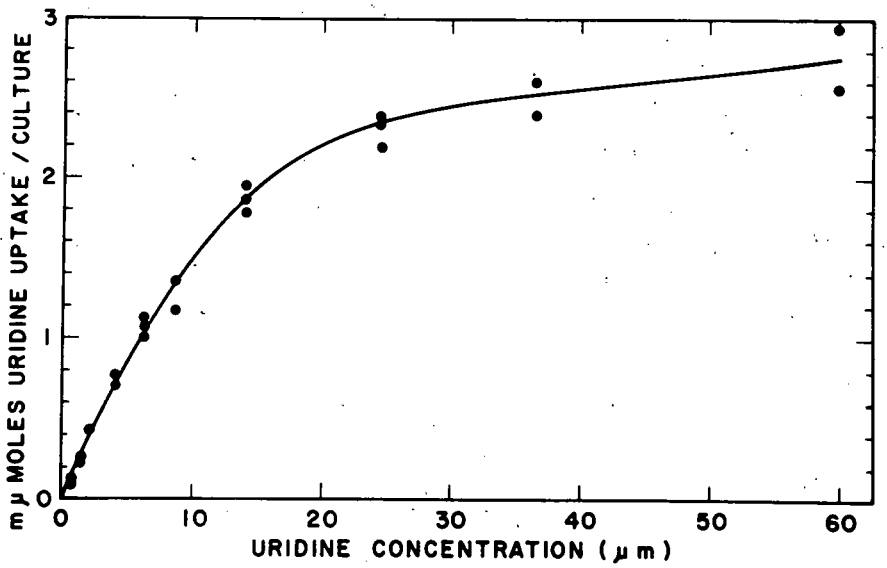


Fig. 2. Uridine uptake by cultured peripheral blood lymphocytes as a function of exogenous uridine concentration.

2. Cell Concentration

Since X-irradiation generally results in cultures containing fewer cells than controls, and since cell numbers may vary between control cultures, it was important to determine whether uptake of substrate per cell was the same regardless of the concentration of cells in the medium. A group of cultures was combined, redistributed to culture tubes in concentrations covering the range ordinarily encountered, and incubated with H-3-thymidine. The results are shown in Fig. 3 (MNCs from peripheral blood) and Fig. 4 (bone marrow leukocytes). The average response in terms of uptake of H-3-thymidine appears linear in relation to cell concentration.

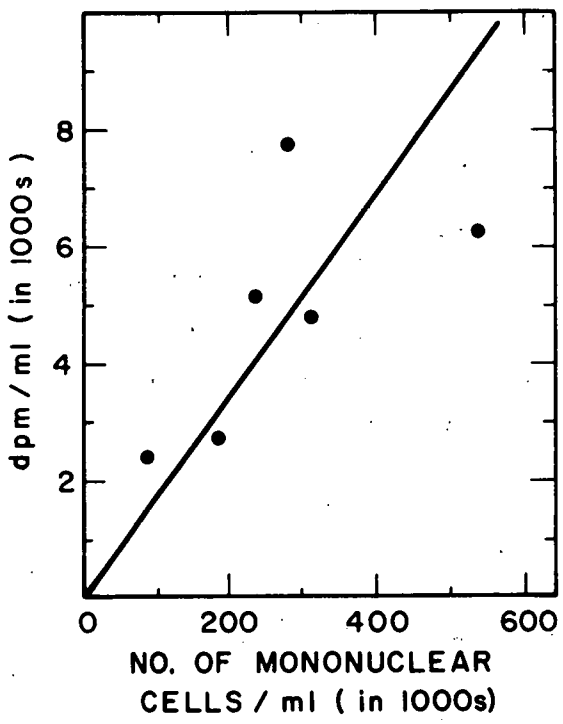


Fig. 3. Thymidine uptake by peripheral blood lymphocytes as a function of concentration of mononuclear cells.

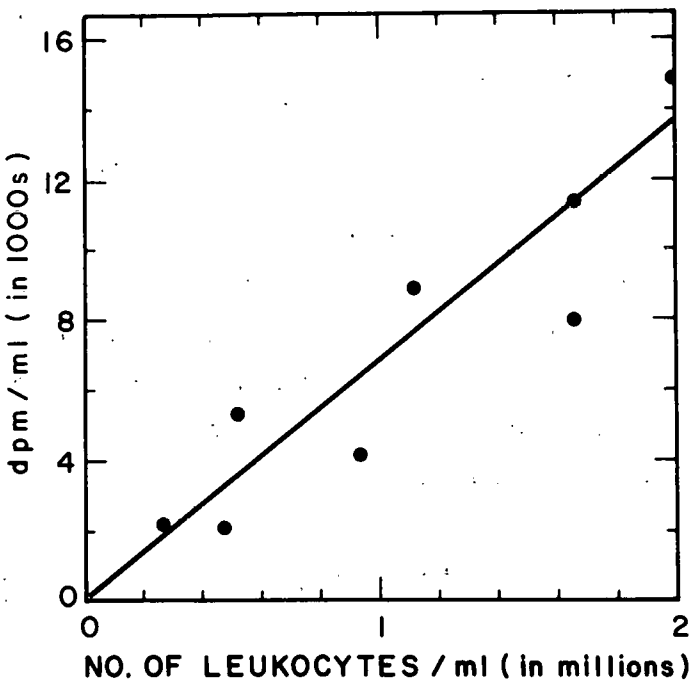


Fig. 4. Thymidine uptake by bone marrow cells as a function of concentration of leukocytes.

3. Change of Medium Prior to Incubation with Labeled Substrate

During the 66 hr of cell culture, the medium changes constantly because of cellular utilization of its components and release of cell products. Cellular breakdown following X-irradiation is a source of disparity in environment between intact cells from control cultures and irradiated cultures. Therefore, comparisons of H-3-thymidine uptake were made between cells remaining in the original

medium and cells transferred to fresh medium. Results indicated that H-3-thymidine uptake per cell was approximately 40% greater in all cell cultures supplied with fresh medium, but that the effect of X-irradiation on H-3-thymidine uptake was the same, regardless of whether or not the medium was changed.

4. Presence of Other Deoxyribonucleosides

DNA is formed from deoxyribonucleoside derivatives other than thymidine. In in vitro systems, the presence of these other precursors often enhances incorporation of H-3-thymidine. Since no nucleosides are present in the synthetic medium used for cell cultures, 3 μM/each of deoxyadenosine, deoxycytidine, and deoxyguanosine was added to the medium, and the effect on the cellular incorporation of H-3-thymidine (6 μM) was measured using transformed peripheral blood lymphocytes. No change in H-3-thymidine incorporation was observed in the supplemented cultures, compared to cultures containing only H-3-thymidine.

Fate of H-3-Thymidine in Transformed Peripheral Blood Lymphocytes

Thymidine (TdR) is successively phosphorylated to thymidine-monophosphate (TMP), thymidine-diphosphate (TDP), and thymidine-triphosphate (TTP) prior to incorporation into DNA. It was important to determine what amount of the H-3 isolated in the cells represented DNA-bound H-3. Therefore, cells were incubated in the usual manner with H-3-thymidine. After washing, the cells were homogenized in 0.14 M NaCl:0.014 M sodium citrate with salmon sperm DNA carrier added. DNA was separated from its acid-soluble precursors by the method of Schneider (J. Biol. Chem. 161: 293, 1945); more than 90% of the radioactivity was found in the DNA fraction. Thus, H-3-TdR uptake by cultured cells appears to be a reliable index of DNA synthesis.

H-3-Thymidine Metabolism in a Cell-free System

DNA was the major isotopically-labeled product of H-3-TdR metabolism isolated from intact, non-irradiated cells in culture. However, incubations of cell-free homogenates of transformed peripheral blood lymphocytes yield H-3-labeled nucleotide intermediates as well as DNA. Comparison was made between metabolism of H-3-TdR (10 mμmoles in 1 ml) in a cell-free system derived from non-irradiated peripheral blood lymphocytes, precultured for 70 hr, and a homogenate derived from cells exposed to 300R prior to culture. Heated salmon sperm DNA was used as primer, and other components required for DNA synthesis were included in the reaction mixture. In addition to DNA separation, acid-soluble products were chromatographed on paper in a descending butanol:acetic acid:water system, which separated the compounds into TdR; TMP; and TDP + TTP; with the latter two compounds observable peaks of radioactivity were obtained but they could not be separately quantitated.

Table 1 indicates the quantities of metabolites isolated, in terms of percentage of original H-3-TdR substrate (total radioactivity). The ratio of control enzymes to irradiated enzymes, in terms of quantity of MNCs homogenized and incubated, was 1.4 to 1. When the data were corrected for this difference, irradiated cells produced 66% as much total metabolism as controls (indicating formation of H-3-TMP from H-3-TdR). Further phosphorylations were decreased to a similar degree by irradiation; however, incorporation of tri-phosphates into DNA showed a larger decrement following X-irradiation. Thus, using a TdR assay system, the nucleotidyltransferase system was more limited by X-irradiation than were the phosphorylation mechanisms. However, it is yet unknown whether this is a direct effect on DNA nucleotidyltransferase or an indirect kinetic effect involving the lower quantities of H-3-TTP available for incorporation into DNA by enzymes from irradiated cells.

Table 1. EFFECTS OF 300R X-IRRADIATION ON PRODUCTS OF H-3-THYMIDINE METABOLISM IN A CELL-FREE SYSTEM

	Products of H-3-TdR Metabolism[a] (% of Total Radioactivity)		
Homogenate	TMP →	TDP;TTP →	DNA
Control	6.8	13.0	1.4
X-irradiated	3.8	6.0	0.3
Irradiated/Control (Corrected %)	66	61	29

[a] Arrows indicate probable sequence of product formation. Thus, calculations of metabolism by irradiated enzymes as a percentage of controls for successive metabolic steps are for the product in question plus all those to the right of it.

EFFECT OF THYROID HORMONE ON CULTURED BEAGLE LYMPHOCYTES

A. Kimi Klein
L. K. Bustad

Thyroid hormone increased the rate and extent of transformation of Beagle lymphocytes to lymphoblasts in culture. The response was similar when T-3 in concentrations one-tenth of that of T-4 were used.

In earlier studies a depression in leukocytes was observed after surgical thyroidectomy or radiothyroidectomy (Bustad et al., Radiation Res. 6: 380, 1957; Hackett et al., Am. J. Physiol. 200: 1011, 1961). There are several possible explanations for the observed leukopenia; however, the direct effect of thyroid hormone on lymphocyte production was a possibility that was readily investigated using cultured Beagle lymphocytes. Heparinized blood was drawn into disposable syringes containing prewarmed (38C) 5% dextran in Hanks' Balanced Salt Solution (BSS). The syringes were kept vertical, needle up, at 38C for 1.5 hr. At the end of that time the red cells had settled, leaving most of the leukocytes suspended in the dextran-plasma supernatant fluid. This fluid was forced out of the syringe through a crescent-shaped needle into a sterile centrifuge tube until the red cell interface reached the top of the syringe barrel. Autologous plasma and dextran were removed by washing the cells in prewarmed (38C) Hanks' BSS. After the final wash, the cell pellet was resuspended in prewarmed tissue culture medium (RPMI-1629, GIBCO) and cell counts were made. The final resuspension volume of RPMI-1629 was adjusted to yield a cell concentration of 4.580×10^3 mononuclear cells (MNC) in 5 ml of final tissue culture medium.

The medium contained RPMI-1629, autologous plasma, fetal calf serum, streptillin (procaine penicillin-G and dihydrostreptomycin sulfate, Trico Pharm. Corp.), and L-glutamine. The inoculum was added to the medium, and the culture was then divided into three portions.

Triiodo-L-thyronine (T_3) in a concentration of 0.807×10^{-6}M was added to one portion; L-thyroxine (T_4) at 0.477×10^{-5}M concentration was added to the second portion; the third portion was the control. The portions were divided into 5-ml aliquots, placed in 25-cm^2 disposable flasks, and loosely capped.

Cultures from each group were incubated at 38C in a 5% CO_2 atmosphere at 90% humidity and incubated for 24, 48, or 72 hr. Cell counts and viability determinations were made after incubations. Differential counts to determine lymphocyte, prolymphocyte, and lymphoblast numbers were made on air-dried smears stained with Wrights' stain. The index of response was calculated by the following equation:

$\frac{1000\ n}{N}$

where n = no. lymphoblasts at harvest per 5 ml of tissue culture and
N = no. MNCs inoculated per 5 ml tissue culture.

There was no significant difference in cell counts or viability between the control and thyroid-hormone groups. But when the index of response was calculated, the degree of transformation from lymphocyte to lymphoblast was significantly greater for the thyroid hormone cultures (Fig. 1).

In addition, there seemed to be a potency difference between T_3 and T_4 as observed by Siegel and Tobias (Nature 212: 1418, 1966). An identical effect on degree of transformation was observed when T_3 concentration was only one-tenth that of T_4 concentration. It appears that T_3 and T_4, in conjunction with PHA, further stimulate transformation of lymphocytes to lymphoblasts in vitro, possibly through the stimulation of nuclear synthesis of RNA.

Further studies to be performed include those regarding the effect of thyroid hormones on the RNA and DNA synthetic mechanisms of lymphocytes growing in vitro and the specific hormonal effect on lymphocytes.

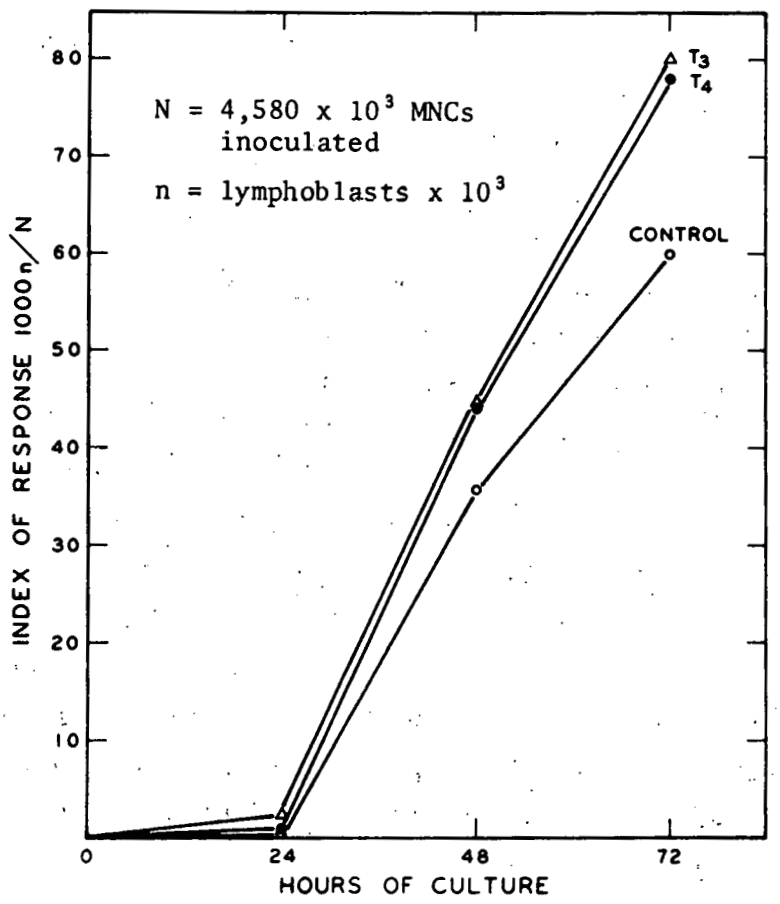


Fig. 1. Effect of triiodothyronine and thyroxine on rate and extent of transformation of lymphocytes to lymphoblasts in culture.

SOME RADIO- AND THERMOLUMINESCENT PROPERTIES OF TISSUES

Marvin Goldman
Edwina Beckman
J. M. Stone

Preliminary measurements on thermal ash derived from normal and neoplastic soft tissues show an induced radioluminescence following X-ray spectrometric analysis. Radioluminescence and thermoluminescence decay slowly following irradiation. Neoplastic tissue is characterized by high Ca and Br concentrations without decay of the thermoluminescence and, in some cases, quantitative retention of Br following thermal ashing.

During analysis of tissues for distribution of trace elements by means of X-ray emission spectrometry, it was noted that samples of thermally ashed tissues were phosphorescent immediately upon removal from the X-ray spectrometer, and that the phosphorescence decayed rather slowly. This property has been reported for bone tissue (Frazier et al., J. Dental Res. 46: 731, 1967) but has not, to our knowledge, been investigated in ashed soft tissue. Ten-milligram portions of the ash were measured in a thermoluminescence dosimetry (TLD) reader within 30 min after a 5-min X-ray exposure in the spectrometer (50 Kvp, 30 ma); the radioluminescence intensity fell off exponentially, with characteristic half-periods varying between 4 and 24 hr. When the aliquots were subsequently heated in the TLD reader, using the technique established for lithium fluoride TLDs, a 100-fold increase in light intensity was noted. The temperature-specific light output during the heating cycle--i.e., the glow curve--appeared to be different for each of the tissues tested. In addition, the integrated light intensity in some cases did not diminish with increased storage time after X-irradiation.

As some of the tissues sampled were neoplastic and some normal, one might speculate on the possible metabolic differences within organs and various pathologic states that could result in a qualitative as well as a quantitative difference in the mineral composition of the thermal ash. Our initial assumption is that the "abnormal" tissues may contain higher quantities of alkaline earth halides than are normally present, and that 24 hr of thermal ashing of the previously lyophilized tissue resulted in a mixture with some of the characteristics of thermoluminescence crystals. Calcium concentrations in neoplastic tissues were higher than normal, a finding consistent with the literature. However, very high bromine levels were also found; surprisingly, only in normal tissues was Br volatilized at 550C. Neoplastic tissue quantitatively retained its Br, which may contribute to the thermoluminescence; the reason for the increased retention of Br is not clear at this time.

Since these analyses were performed on 10-mg quantities of ash, the possible application to assistance in pathologic diagnoses, as well as in investigating certain physical properties of abnormal tissues should be investigated. In particular, the relationship between the quantity and ratios of the various elements in abnormal tissue relative to the difference in glow curve peak, radioluminescence, and thermoluminescence may provide a unique opportunity for the study of the etiology of certain disease states.

RADIOIODINE AND X-IRRADIATION EFFECTS ON BEAGLE PUPS

L. K. Bustad
J. M. Fuller

Prior to a long-term program on the effects of various isotopes of radioiodine and X-irradiation on the thyroid, a comparative study was performed on the effects of radioiodine with and without whole-body X-irradiation. Three sets of three littermate female pups were randomly assigned to three treatment groups: 1500 rads from I-131 to the thyroid; 1500 rads I-131 + 200R whole-body X-irradiation; and 4500 rads from I-131. The peak thyroidal uptake varied from 9 to 15%, and the pattern of uptake varied considerably, even between littermates. An accurate method was developed for studying thyroidal metabolism of I-131 in dogs.

As a result of continued general interest in the radiosensitivity of the thyroid gland, and in view of the paucity of quantitative data regarding the dose-effect relationships of various qualities and intensities of radiation, an experiment was undertaken to study the effect of specific dose levels of radioiodine and to compare the effectiveness of I-131 and whole-body X-irradiation in causing thyroidal damage.

This is a report on the initial phase of an experiment designed to define minimal effective dose levels, and as a test of methods and equipment. Additional dogs and new treatment groups employing some of the shorter-lived radioiodine isotopes are scheduled.

Three female pups from each of three litters were randomly assigned to three treatment groups (Table 1). Thyroid uptake studies using trace doses of I-131 were performed to furnish a basis for estimation of dose levels that would provide thyroid exposures of 1500 or 4500 rads. One group of pups was exposed to whole-body X-irradiation immediately after administration of the experimental doses of I-131. Both the thyroidal uptake and the whole-body retention of the I-131 doses were monitored.

Table 1. NUMBER, AGE, AND WEIGHT OF DOGS IN EACH TREATMENT GROUP

Treatment	Litter and Pup Number	Age	Weight (kg)	Dose (rad)
1500 rads	42C	128	7.00	1640
I-131	43B	122	6.15	1900
	44B	121	6.70	1780
1500 rads	42A	128	4.90	1570
I-131 -	43A	122	5.75	1550
200R X-ray	44A	121	6.60	1950
4500 rads	42B	128	6.00	4500
I-131	43C	122	2.50	4150
	44C	121	5.10	4490

Blood samples for the determination of serum protein-bound iodine (PBI) and free T_4 were taken at the time of dosage and will be taken periodically for the duration of the experiment.

Further thyroid uptake studies and radio-PBI determinations will be performed on all dogs after 6 months and at 18 months or later, depending on the progress of the study. The dogs will then be sacrificed for histological studies of the thyroid glands. Prior to sacrifice, their response to thyroid stimulating hormone (TSH) will be determined.

The I-131 used was sodium iodide in a sodium hydroxide carrier. Doses were pipetted into gelatin capsules (interiorly coated with silicone grease), counted, then administered orally.

For thyroid counting a 7.5 x 7.5-cm NaI crystal enclosed in a 2.5-cm thick lead cylinder and coupled to a single-channel analyzer was used. Collimation provided a 5-cm diameter sensing area with a detector to skin distance of 30 cm, and a 10-cm diameter window when a 40-cm counting distance was used.

Standards were counted in a cylindrical lucite neck phantom. Dimensions of the phantom were determined by administering I-131 to a dog, counting the thyroid region, then surgically removing the thyroid, placing it in the same type of plastic tube used for the standard doses, and counting it in the neck phantom. The neck region of the dog was then counted without the thyroid, and the phantom was adjusted so that thyroid counts in the phantom were equal to thyroid counts in vivo minus neck background counts.

The adjustable dog restraining device, designed at this laboratory, is shown on p 93. The pups were positioned with their heads moderately extended. A spring clamp was used on the nape of the neck to gather excess skin so that skin thickness over the thyroid was constant. Specific adjustments were required to position each pup with the ventral surface of the neck perpendicular to the crystal; these adjustments were repeated each time for each pup, so positioning was standardized. The crystal was mounted on a geared track, facilitating vertical adjustment of the counting distance.

Twenty minutes after administration of the experimental dose of I-131, each pup was monitored in a whole-body counter. The count obtained was used as a 100% retention value for construction of the whole-body retention curves. A standard was also prepared, consisting of 200 μCi of I-131 in a 2-liter plastic bottle filled with water. The diameter of the bottle closely approximated the diameter of the pups' abdomens.

Immediately after whole-body counting, three pups were exposed simultaneously to whole-body X-irradiation in a flat holding box with a ventilated lucite lid. Tube settings were 250 KVP and 15 ma. A Thoraeus II filter with an HVL of 2.63 mm Cu was used. Dose delivery rate, as determined by ionization chambers, was 7 R/min at 114 cm target-to-skin distance.

The 28.5-min confinement in the holding box noticeably stressed the pups, so other pups not exposed to X-irradiation were similarly stressed in holding boxes. One pup (43A) vomited during X-ray exposure; it was given an additional dose of I-131, equal to the amount lost in the vomitus.

Whole-body counts were usually for 5 min; for the initial thyroid uptake studies values from two 2-min counts, taken at 30-cm counting distance, were averaged to derive each of the points plotted in Fig. 1. Following the experimental radioiodine administration two 1-min counts at 40-cm distance were averaged. After about two effective half-lives, counting levels were marginally low. The pups were very cooperative and moved very little if not held longer than 5 or 6 min.

Dosages were estimated by utilization of the following equation:

$$\text{Dose} = \frac{51 \times E \times Q \times t}{w}$$

where: 51 = (disintegrations/μCi I-131/day) (ergs/MEV) (rad/erg)

E = average energy of β-particles, MEV (0.2)

Q = mean No. μCi deposited in the thyroid

t = days exposed to I-131

and w = wt of thyroid gland (g)

By plotting the uptake curves on linear paper and using a compensating polar planimeter to determine total area under the curve, the number of μCi days was estimated; this value was substituted in the formula for Q x t. The formula ignores the loss of beta particle energy to tissues outside the thyroid, as well as the gamma component of I-131.

The dose received from the 9 μCi I-131 administered for the initial uptake studies was utilized to determine the necessary quantity of I-131 to be used.

The "dose received" column in Table 1 is the sum of the doses received from the tracer dose and the experimental dose. No immediate effect of either I-131 or X-ray was noted, other than a slight decrease in the effective half-life of I-131 in the thyroid; this may have been due to a subtle, undetected change in environment or to increased age and growth of the pups.

In Fig. 1 the thyroid uptake of the tracer dose and the experimental dose are compared and the whole-body retention of the experimental dose is illustrated. The points plotted are actual, and indicate that with proper care and equipment, uniform data on thyroid uptake in animals may be obtained.

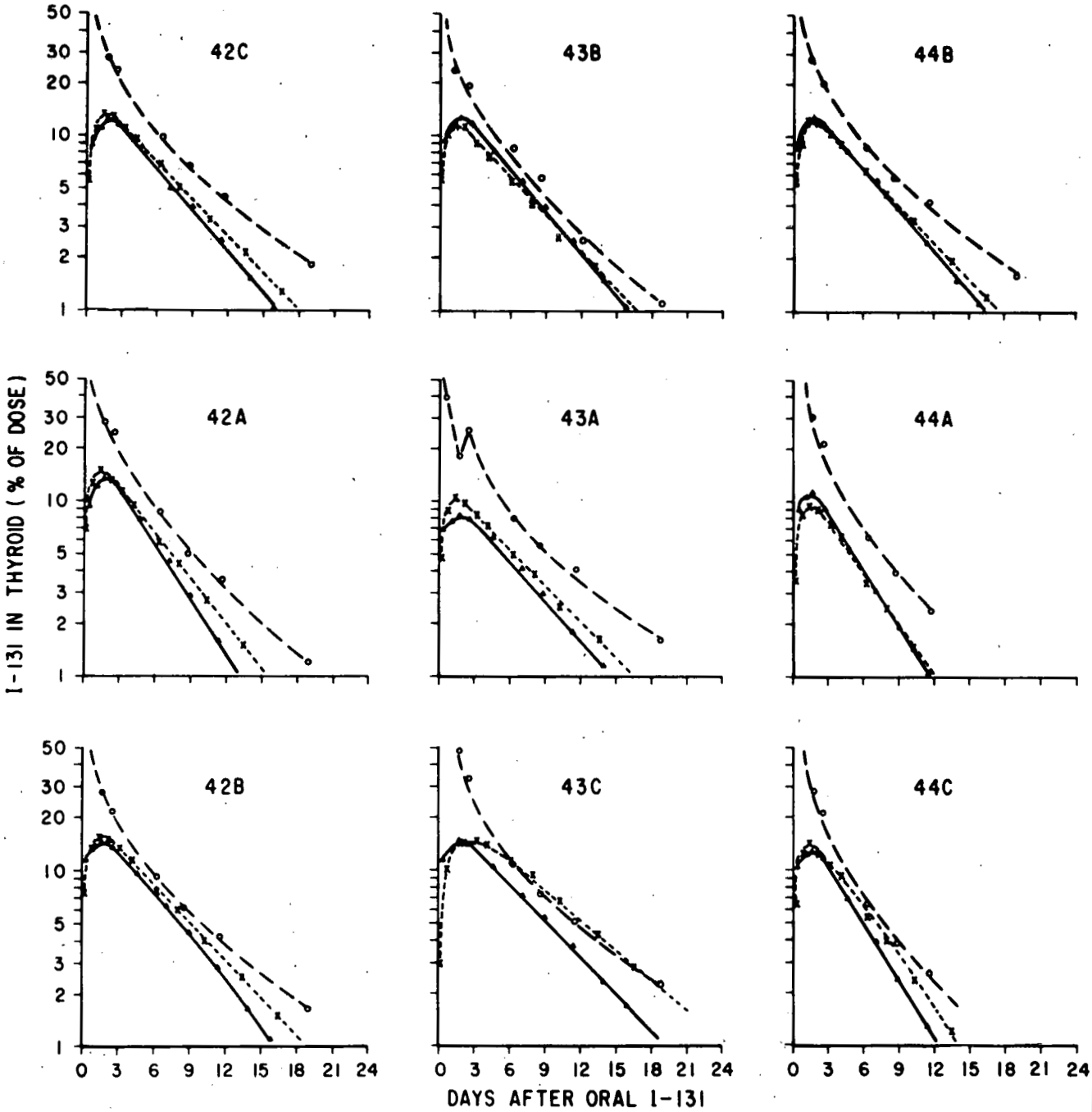


Fig. 1. Thyroid uptake following tracer dose (-x-x-) and experimental dose (-Δ-Δ-) and whole-body retention (-o-o-) of orally administered I-131. (Uncorrected for physical decay.)

The nine preliminary uptake curves are given in composite in Fig. 2 along with a curve of the mean values. Of interest is the degree of variability in thyroid function seen among similar dogs housed under uniform conditions.

These pups are maintained on the standard diet used for all dogs at this laboratory; their daily ration contains from 500-600 μg of stable iodine. This moderately high level probably accounts for the rather low maximum uptake values obtained.

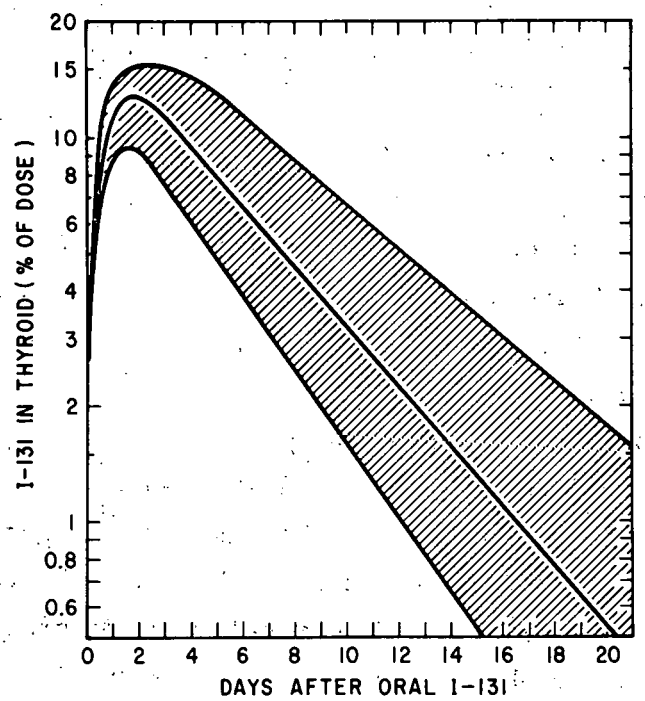


Fig. 2. Range and mean thyroid I-131 uptake curves in nine Beagle pups. Effective half-life = ∿4 days.

RADIOIODINE METABOLISM IN THE ADULT BEAGLE

Ove Wilson
J. M. Stone
D. E. Monty
L. K. Bustad

In a radioiodine metabolism study of 21 adult Beagles, the thyroid accumulation and disappearance rates of I-125 were measured for 3 to 5 months. Maximum thyroid uptake occurred 48 to 72 hr after administration of the single dose and ranged from 9 to 25% of the dose. Maximum PBI-125 values occurred 2 to 3 days after dosing, ranging from 0.27 to 1.9% of the administered dose/liter of plasma. A mean stable PBI of 3.8 µg/100 ml was found. The biologic half-time of thyroidal iodine was a series of decreasing exponential functions indicating a multicompartmental model for iodine turnover in the thyroid of the dog. At least three compartmental effects were observed: a fast component giving an initial short biological half-life of about 7 days, one or more intermediate components successively increasing the biologic half-time, and a late, slow component of about 50 days.

Prior to a study on the comparative effects of X-irradiation and the various radioiodines on the thyroid gland of Beagles, a series of experiments was performed in adult Beagles to develop a standard procedure for thyroid monitoring and to define some metabolic parameters. Previous studies of thyroid uptake and release rate of radioiodine in dogs have utilized I-131, and have been limited to 2 to 3 weeks due to the short half-life (8.05 days) of that isotope. The use of I-125, which has a half-life of ∿60 days, permitted the radionuclide measurements to be extended to 4 to 5 months. Iodine-125 decays completely by electron capture to the 35.4 kev excited state of Te-125. The peak radiation is effectively measured with a 2-mm thin x 5 cm wide NaI (T) crystal assembly, coupled to a gamma spectrometer. A double radionuclide study, using I-131 and I-125, showed identical uptake and release curves with slightly lower values for I-125. Continuous measurements of the I-125 standards throughout the study showed minimal deviations from a linear regression fit to the decay data, insuring that no drift occurred in the thyroid monitoring apparatus.

Twenty-one adult female Beagles and 1 male, ranging from 198 to 614 days of age, and from 6.0 to 10.9 kg in weight, received a single oral dose of ∿50 µCi I-125 in carrier-free NaOH. From birth, the dogs were housed in identical, air-conditioned cages with constant temperature and humidity, and maintained on a constant diet (access to 400 g feed daily, providing 540 µg iodine/day).

The thyroid monitoring set-up that was developed (Fig. 1) permitted precise duplication of results and flexibility in handling animals of various sizes.

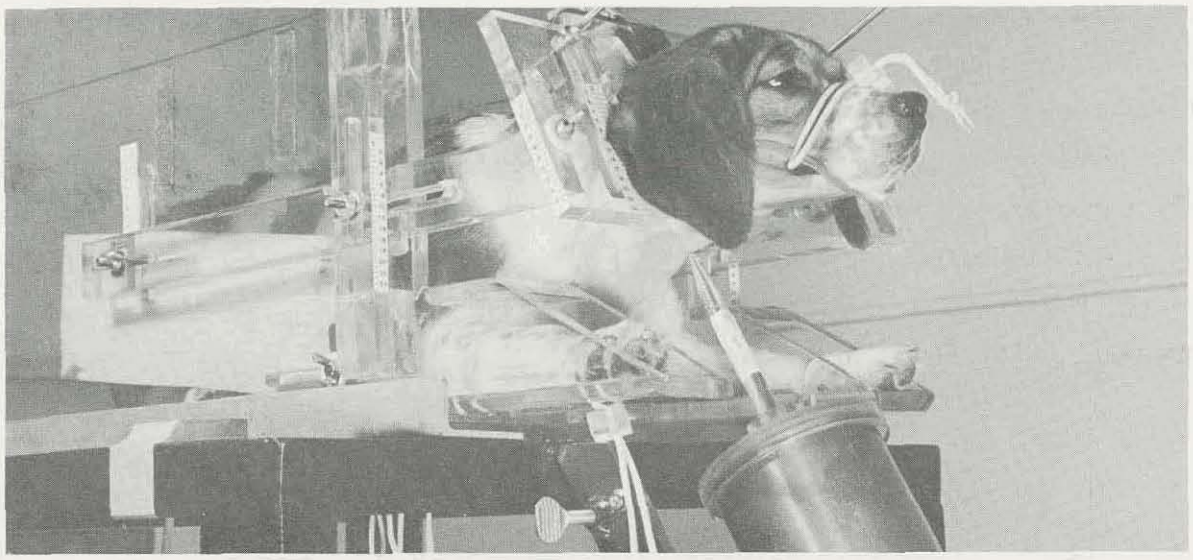


Fig. 1. Thyroid monitoring unit with Beagle in position for measurement at 30-cm thyroid-to-crystal distance. Note numbering index for positioning.

The most noteworthy features of this monitoring procedure were:

(1) A special restraining unit allowed accurate and reproducible position of the shaved and cleaned neck at right angles to the collimated detector.

(2) Duplicate thyroid measurements were made at two different distances (30-40 cm or 20-30 cm) between the thyroid and the detector, giving a check on counting geometry between successive measurements.

(3) The dosing standards were measured in a lucite neck phantom that duplicated the geometry of the Beagle's thyroid permitting correction for counting losses from attenuation and backscatter variations.

(4) A clamp held the dorsal skin of the neck to insure smooth, tight skin over the thyroid region.

The accumulation and disappearance rates of I-125 were measured daily for the first week, then at frequent intervals for 3 to 5 months. Total radioiodine in plasma and protein-bound radioiodine (PBI-125) were determined for evaluation of the fractional release of the administered dose; repeated stable PBI determinations were also made.

To evaluate environmentally induced changes in thyroid function, a study of acute cold exposure was carried out on six dogs. Thyroid and blood measurements were made before and after a 3-hr exposure, on three successive days, to a temperature of -20C. The effect of repeated blood sampling and cold on hemodynamics was also investigated.

The maximum thyroid uptake, which occurred 48 to 72 hr after administration of the dose, ranged from 9 to 25% of the dose with a mean of 15% and a median of 14%. Representative uptake curves corrected for decay are shown in Fig. 2. It is interesting to note the great individual variation in the retention curves of the six littermates. The maximum PBI-125 values occurred 2 to 3 days after dosing, ranging from 0.27 to 1.9% of the administered dose per liter plasma, with a mean of 0.76% and a median of 0.51%.

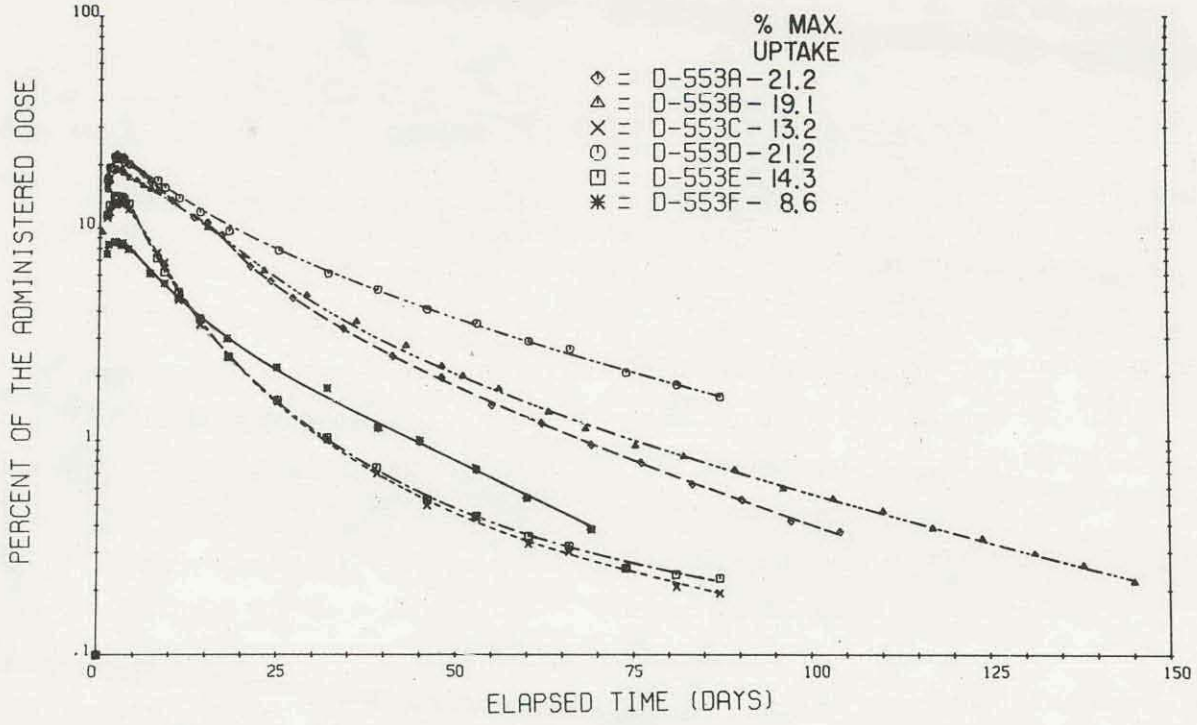


Fig. 2. Thyroid uptake in six littermate Beagles.

Representative curves for total radioiodine, radioactive PBI, and radioiodide in plasma of one dog are shown in Fig. 3.

A mean stable PBI of 3.8 µg/100 ml (range 3 to 4.4 µg/100 ml) was found. Changes in hematocrit, hemoglobin, and total protein due to taking of large blood samples (∿8 ml each time) are shown in Tables 1 and 2. The effect of frequent blood sampling is clear; therefore, the daily samples were reduced from three to two.

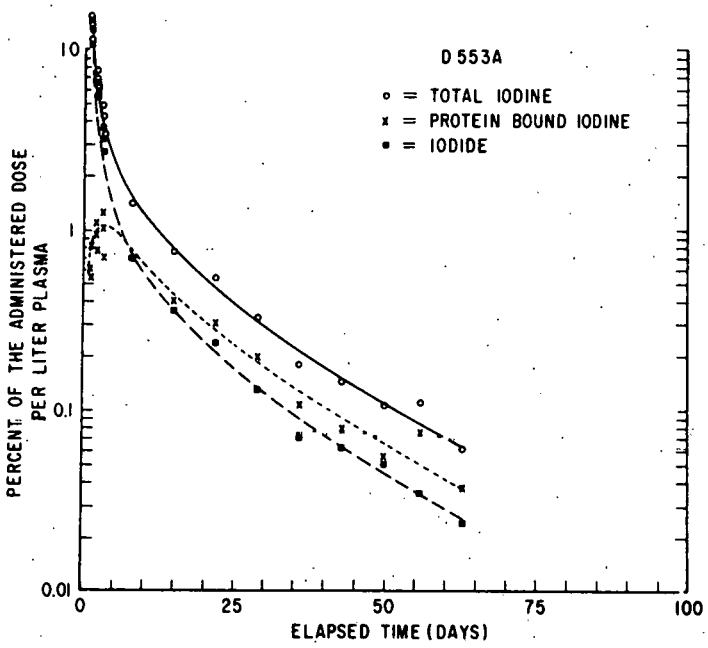


Fig. 3. PBI, total iodine and iodide excretion for one female Beagle

The most important finding, as evident from Fig. 2, is that the retention of radioiodine in the thyroid cannot be described by a single exponential function as has usually been assumed. Instead, the release curve is best described as a series of exponentials whose rate of change decreases with time, so that the final component suggests a minimum rate of change. The release rate is most rapid during the first two weeks and then decreases gradually by 7 to 8 weeks. The retention curve seems to flatten out considerably after 7 to 10 weeks and then approaches linearity when plotted semilogarithmically. The initial average "biological half-life" was 7 days (5 to 15 days); however, with time the rate of loss decreased to one-seventh of the initial value. In two pregnant dogs, the marked flattening of the release curve had already occurred after 20 to 25 days. The clear change in the slope of the release curve may be a function of different release phases and rate constants within various thyroidal compartments.

The release rate of radioiodine from the thyroid does not seem to be correlated to the peak uptake and differs markedly even in littermates (Fig. 2).

The slow component could increase the predicted thyroid dose based on a single exponential model, but the increase would not be great since the flattening of the curve occurs late and would not be affected by the short-lived radioiodines.

No differences in thyroidal uptake or in radioactive PBI were observed in the cold-exposed dogs as compared to the controls. A clear hemoconcentration occurred during the cold exposure; a rapid hemodilution at the termination of the exposure was evident within half an hour, most marked after 1 hr, and persisted after 3 hr, as shown in Table 3.

Table 1. MEAN CHANGES IN BLOOD CONCENTRATION OF 5 CONTROL DOGS

Whole blood sample (8 to 10 ml) taken 3 times daily on 3 consecutive days.

Blood Constituent	Day 1			Day 2			Day 3		
	0 hr	2 hr	6 hr	0 hr	2 hr	6 hr	0 hr	2 hr	6 hr
Hematocrit (%)	52.0	48.7	49.3	49.1	46.1	47.7	47.6	46.3	45.5
Hemoglobin (g/100 cc)	17.7	16.9	17.1	17.1	16.3	16.5	16.3	16.4	15.9
Total protein (g/100 cc)	7.5	7.4	7.4	7.2	7.1	7.2	7.4	7.4	7.2

Table 2. MEAN CHANGES IN BLOOD CONCENTRATION OF 6 CONTROL DOGS

Whole blood sample (∿8 ml) taken twice daily on 3 consecutive days.

Blood Constituent	Day 1		Day 2		Day 3	
	0 hr	4 hr	0 hr	4 hr	0 hr	4 hr
Hematocrit (%)	49.6	48.4	48.1	46.3	45.9	44.3
Hemoglobin (g/100 cc)	16.7	16.3	16.0	16.0	15.3	15.0
Total protein (g/100 cc)	6.4	6.2	6.3	6.3	6.3	6.1

Table 3. MEAN CHANGES IN BLOOD CONCENTRATION OF 5 DOGS EXPOSED TO COLD (-20C) FOR 3 HOURS

Whole blood samples (∿3 ml) taken from 0 to 6 hr at intervals shown. First sample taken immediately before cold exposure, 3-hr sample immediately before termination of exposure.

Blood Constituent	Cold Exposure							
	0 hr	1 hr	2 hr	3 hr	3.5 hr	4 hr	5 hr	6 hr
Hematocrit (%)	45.9	48.1	47.6	47.0	44.5	42.1	43.0	43.3
Hemoglobin (g/100 cc)	16.2	16.6	16.7	16.6	15.5	15.4	15.4	15.6
Total protein (g/100 cc)	6.2	6.5	6.5	6.4	6.4	6.2	6.2	6.4

PULMONARY FUNCTION STUDIES ON BEAGLES

J. R. Gillespie*

Several parameters of pulmonary function were measured in awake and anesthetized Beagles. Further functional and structural studies are under way.

Data were obtained from 15 control Beagles 66 ± 6 months of age. Measurements were made on the same dogs in states of awakeness and anesthesia, as shown in Table 1. Characterization was attempted by measurements of body size (9.6 ± 2 kg), heart girth (50 ± 4 cm), shoulder-to-rib distance (24 ± 3 cm), and chest X-rays. Blood-gas tensions (PaO_2, $PaCO_2$) and pHa values obtained under anesthesia were markedly different from awake values. These parameters were also studied with positive pressure ventilation with oxygen.

Pulmonary dead space (V_D) was determined in anesthetized dogs. Results of inflation compliance (C_I) and deflation compliance (C_E) measurements were particularly rewarding, and were strengthened by the observations of functional residual capacity (FRC). Values for one dog did not fit well with those of the other dogs, and it is suspected of having lung disease.

Table 1. PULMONARY FUNCTION VALUES FOR ADULT BEAGLES

Parameter	Mean ($\bar{X}$)	S.D. (s)	S.E. ($s_{\bar{x}}$)
C_I (L/cm H_2O)	0.033	0.009	0.001
C_E (L/cm H_2O)	0.041	0.013	0.002
FRC (ml)	345	85	17
$P_A - PaO_2$ (on AIR)	27	5.7	1.6
Awake PaO_2	85	12	3.2
Awake $PaCO_2$	38	5.1	1.5
Awake pHa	7.4	0.032	0.008
Anesthetized PaO_2	62	8.1	2.2
Anesthetized $PaCO_2$	49	8.0	2.1
Anesthetized pHa	7.3	0.045	0.011
O_2 PaO_2	532	88	23
O_2 $PaCO_2$	37	10	2.7
O_2 pHa	7.4	0.083	0.021
V_D	44	11	2.2

Further functional and structural studies are under way, with the goal of evaluating changes with age and radiation dose.

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MATERNAL TRANSFER AND FETAL ABSORPTION AND RETENTION OF INJECTED RADIONUCLIDES IN DEER AND SHEEP

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H. G. Wolf
J. M. Stone
C. D. Abrahams
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Fetal absorption and retention of injected Ca-47, Cs-137, Sr-85, and I-131 (to simulate biospheric contamination) was studied in Columbian black-tailed deer and domestic sheep. The amount of radionuclide transported across the placenta and deposited in the fetus was dependent upon the total mass of the conceptus, the physiological demands of fetus, and placental discrimination. Accumulation of radionuclide in the upper GI tract was presumably due to parotid gland salivary secretion. Only a slight accumulation of Cs-137 in fetal tissue was observed in contrast to maternal tissues. Notably higher concentration of I-131 in fetal deer thyroid gland was observed compared to maternal thyroid gland.

The following report summarizes, in part, our current collaborative studies with Dr. William Longhurst, Zoology Department, (UCD Hopland Field Station) on the intraruminal metabolism and kinetics of radioactive Sr, Ca, Cs, and I in sheep and deer. Of primary interest was the extent of transfer of these labeled elements into the rumens of deer and sheep following intravenous administration, and their transfer via the placenta and uptake by the developing fetus in pregnant ewes and does. These data can serve as a basis for the evaluation of possible radiation hazards to natural populations.

Nine gravid deer (Columbian black-tail) and 11 gravid sheep were injected i.v. (via the jugular vein) with a mixture of radionuclides and placed in a specially designed pen area overlaid with plastic sheeting and wood shavings to minimize environmental contamination from urine and feces. One each, non-injected control sheep and deer were kept in a similar, but separate, pen area. Both sheep and deer were fed alfalfa hay, pellets, and water ad libitum. The study on sheep was done in mid-January when the fetuses were at least mid-term or in the last trimester of gestation (150 days); the deer study was carried out during the latter part of May when the fetuses were again assumed to be in mid-gestation. Successful breeding was assumed to have taken place during the previous September for the sheep (range breeding), and during the months of November and December when the buck was present in the deer enclosure.

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* A portion of this material was used as a dissertation for a master's degree, University of California, Davis.

† Dept. of Zoology, UCD Hopland Field Station

The sheep were killed with an overdose of pentobarbital and the deer were shot according to the following schedule: one each at 4, 8, and 24 hr post-injection, and two each at 3, 7, 10, and 14 days post-injection. Only one deer was killed at 7 and one at 14 days. The control animals were killed at day three of the respective experiments.

The radionuclide dose to each animal was ∿40 μCi each of Ca-47 and Sr-85 and 15 μCi of Cs-137 in an appropriate volume (∿8 ml) of 0.1N HCl and saline solution. I-131 (40 μCi) was administered only to deer. Appropriate injection standards were prepared for analytical use and subsequent differential gamma-ray spectroscopy. The gamma rays were sorted using a 400-channel pulse height analyzer employing a 20 x 10 cm NaI(Tl) detector in a whole-body counter at specified constant geometry (Goldman et al., UCD 472-108, 1963, p 90).

The data analysis required spectrum stripping, using computer programs supplied by Mr. Harry M. Murphy, Kirtland Air Force Base, Kirtland, New Mexico. The spectrum stripping involved separation and identification of the major gamma peaks and quantitation of the counts in each peak channel.

The amount of radionuclide transported across the placenta and deposited in the fetus appeared to be dependent upon the total mass of the conceptus. The pattern of uptake and deposition (Table 1 and 2) when expressed per kilogram of fetus was similar in both species, regardless of the total number of fetuses; e.g., in deer No. 4, which had one fetus, and deer No. 5, which had four. Thus, the greater the mass of the conceptus, the greater the placental transfer and resulting deposition *in utero*.

Table 1. PERCENTAGE OF INJECTED DOSE/kg DEER FETUS AT SACRIFICE

Radio-nuclide	4 Hr	8 Hr	24 Hr	3 Day		7 Day	10 Day		14 Day
	#1	#2	#3	#4	#5	#6	#8	#9	#10
Ca-47	1.3	2.9	4.2	6.8	6.2	8.5	7.0	7.7	7.3
Sr-85	0.6	1.5	2.3	3.7	3.2	4.3	3.9	4.1	3.6
I-131	4.8	4.3	5.2	6.7	2.7	3.7	2.1	3.3	2.2
Cs-137	nd*	0.1	0.3	0.5	0.4	0.6	0.7	0.6	0.6

* Not detectable

Table 2. PERCENTAGE OF INJECTED DOSE/kg SHEEP FETUS AT SACRIFICE

Radio-nuclide	4 Hr	8 Hr	24 Hr	3 Day		7 Day		10 Day	14 Day	
	#1	#2	#3	#4	#5	#6	#7	#9	#10	#11
Ca-47	1.5	4.9	9.3	7.9	10.0	9.9	8.5	8.3	5.6	5.6
Sr-85	0.8	1.9	3.4	2.8	2.8	3.3	4.6	3.0	1.5	2.0
Cs-137	0.8	0.2	0.3	0.3	0.3	0.3	0.2	0.2	0.2	0.2

A maximum concentration was observed for Ca-45 and Sr-85 in both deer and sheep from days 3 through 10. The deer fetus showed a relatively high concentration of I-131 during the first 24 hr. The Cs-137 concentration was essentially constant, indicating a small but steady placental transfer of cesium. In the deer, the Cs-137 accumulation suggests an increasing concentration in the early time periods, but the implications are not clear.

The retention ratios of Sr-85/Ca-47 in the fetuses (Tables 3 and 4) were less than 1, reflecting the selection against radiostrontium due to the placental discrimination process.

Table 3. RETENTION OF Sr-85/Ca-47 IN SHEEP MATERNAL AND FETAL BONE AT SACRIFICE

	Deposition and Retention of Sr-85/Ca-47						
	4 Hr #1	8 Hr #2	24 Hr #3	3 Day #4,#5	7 Day #6,#7	10 Day #8, #9	14 Day #10, #11
Fetal							
Limbs	0.59	0.39	0.34	0.32	0.47	0.34	0.31
Skull--Mandible	0.48	0.38	0.36	0.33	0.45	0.36	0.31
Maternal							
Femur	1.41	1.40	1.00	0.83	0.84	0.75	0.53
Mandible	1.22	1.33	0.94	0.85	0.89	0.81	0.54

Table 4. RETENTION OF Sr-85/Ca-47 IN DEER MATERNAL AND FETAL BONE AT SACRIFICE

	Deposition and Retention of Sr-85/Ca-47						
	4 Hr #1	8 Hr #2	24 Hr #3	3 Day #4,#5	7 Day #6	10 Day #8, #9	14 Day #10
Fetal							
Limbs	0.43	0.54	0.57	0.54	0.53	0.55	0.49
Skull--Mandible	0.43	0.56	0.57	0.54	0.51	0.57	0.53
Maternal							
Femur	1.01	1.06	1.18	1.03	0.98	0.96	0.76
Mandible	1.11	1.16	1.09	1.00	0.97	0.97	0.94

The accumulation of radionuclides in the upper gastrointestinal tract of both species is summarized in Tables 5 and 6. In the deer, a peak concentration was reached in the fore-stomachs within the first 4 hr; it decreased rapidly thereafter. As much as 26% of the I-131 injected dose was found in the fore-stomachs of the deer at the first sampling. The peak concentration may have occurred much earlier. For the sheep, the peak concentrations of the radionuclides occurred between 8 and 24 hr. The explanation of these differences between sheep and deer is not readily apparent, but could be due to different metabolic rates as well as lesser or greater excitability.

Table 5. PERCENTAGE OF INJECTED DOSE FOUND IN DEER STOMACHS (RUMEN-RETICULUM, OMASUM, ABOMASUM, AND CONTENTS)

Radionuclide	4 Hr	8 Hr	24 Hr	3 Day		7 Day	10 Day		14 Day
	#1	#2	#3	#4	#5	#7	#8	#9	#10
Ca-47	3.4	2.8	2.2	1.5	1.5	0.6	0.5	0.5	0.4
Sr-85	2.6	1.8	1.5	0.4	0.4	0.1	0.1	0.1	0.1
I-131	25.9	20.8	20.7	11.7	1.6	0.6	0.3	0.2	0.2
Cs-137	7.2	3.9	3.8	2.3	2.8	1.6	1.1	1.0	0.8

Table 6. PERCENTAGE OF INJECTED DOSE FOUND IN SHEEP STOMACHS (RUMEN-RETICULUM, OMASUM, ABOMASUM, AND CONTENTS)

Radio-nuclide	4 Hr	8 Hr	24 Hr	3 Day		7 Day		10 Day		14 Day	
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11
Ca-47	0.5	3.2	3.4	0.8	1.6	3.9	0.6	0.3	0.5	0.4	0.3
Sr-85	0.6	3.5	2.1	0.4	0.4	0.8	0.1	0.1	0.1	nd*	nd*
Cs-137	1.8	8.6	8.4	3.9	5.2	6.0	2.4	1.0	1.1	0.5	0.6

* Not detectable

The accumulation of intravenously injected radionuclides in the forestomachs of deer and sheep may be largely due to salivary gland secretion. Blair-West and co-workers (Physiology of Digestion in the Ruminant, Butterworths, Washington, 1965, p 198) reported total parotid gland secretions of 10-15 liters/day in the sheep. These observations have been confirmed in this laboratory with a limited number of sheep. Parotid gland secretion recently determined in one deer is about 1 liter/day. Radionuclide concentration in saliva is considerably higher than that of circulating plasma in both species and higher in the deer than in the sheep.

Relatively little work has been done on the concentration of I-131 in maternal and fetal thyroid glands of deer. Table 7 gives results of such a study over a 14-day period. The fetal thyroid glands had a greater uptake per gram of tissue than the maternal glands, by a factor of up to five. The generally low uptake in maternal glands might be a reflection of a relatively high level of stable iodine in the diet, which was approximately 1.5 mg I/day based on one analysis of rumen content. This can be attributed to the high iodine content of coastal vegetation plus the inclusion of supplemental (1%) iodized salt in the alfalfa diet.

The higher concentration of I-131 in fetal thyroid gland might also be explained by recycling and re-ingestion of excreted I-131 by the fetus via the amnionic and chorionic fluids. Experiments utilizing surgical preparations, whereby the fetal urine can be collected externally are contemplated (H. Parker and F. Buddingh, UCD School of Veterinary Medicine, Personal Communication, 1968).

Table 7. CONCENTRATION OF I-131 IN MATERNAL AND FETAL THYROID GLANDS OF THE DEER AT SACRIFICE (EXPRESSED AS PERCENT ADMINISTERED DOSE/G THYROID)

	4 Hr	8 Hr	24 Hr	3 Day		7 Day	10 Day		14 Day
	#1	#2	#3	#4	#5	#6	#8	#9	#10
Maternal	0.6(1.7)*	0.8(2.1)	1.4(2.6)	3.2(2.2)	1.0(2.7)	3.6(1.9)	1.1(2.6)	1.5(1.6)	3.2(3.1)
Fetal	1.3(0.3)	1.6(0.5)	3.5(0.2)	15.3)0.3)	4.1(0.7)	5.6(0.5)	3.8(0.7)	5.2(1.2)	3.3(0.8)
		1.3(0.3)	4.1(0.2)		5.4(0.5)		4.0(0.7)	6.9(0.8)	
					4.7(0.6)				
					5.5(0.4)				
Fetal Thyroid Glands	2.1	2.0	2.5	4.8	4.1	1.6	3.5	3.5	1.0
		1.6	2.9		5.4		3.6	4.6	
Maternal Thyroid Glands					4.7				
					5.5				

* Number in parentheses denotes weight (g) of thyroid gland.

MARMOSA MITIS, A SMALL MARSUPIAL FOR STUDIES IN RADIATION BIOLOGY

L. K. Bustad
H. G. Wolf
R. D. Barnes

Marmosa mitis (also known as Marmosa robinsoni, Bangs), a small, pouchless South American marsupial, is a recommended subject for study of the effects of external X-irradiation and of Sr-90 and other internal emitters because of its simple chromosome pattern and the accessibility of fetuses.

Marmosa is a very widely distributed genus consisting of 49 species and 100 subspecies (Tate, Bull. Am. Mus. Natl. History 66: 1, 1933). Several species of marmosa, as well as other marsupials, were introduced to the University of California, Davis, by one of us (RDB) in 1966, in the hope of adapting one or more of them to laboratory conditions (Barnes, Lab. Animal Care 18: 251, 1968). The prime objectives were the development of a bioassay for testing the carcinogenic properties of drugs and the performance of developmental embryological studies.

Of the original species introduced, M. mitis has proven to be the most successful in adapting to the laboratory. In addition, it has the desirable characteristics of being pouchless, small, readily available from the wild, and of having a simple karyotype.

The chromosome pattern, described by Scott and Barnes on p 106, was most attractive to us (see also Reig, Experientia 24: 185, 1968). The karyotype is one of the most simple of mammalian chromosome patterns (2N = 14), and metaphase preparations are easily viewed under the light microscope. The small number and large size of the chromosomes should permit adaptability to machine analysis. The species should prove useful for quantitating cytogenetic radiation damage, including that from a variety of internal emitters. The response to Sr-90, radioiodines, and tritium will be studied first. The nature of the reported deleterious effects of radiostrontium on the gonads could be checked in marmosa and in other larger marsupials with simple karyotypes to determine the contribution of extragonadal radiation to the reported effects in mice.

In early 1967 some M. mitis were made available to us for a pilot study on the cytogenetic effects of X-irradiation and of Sr-90 in bone marrow and peripheral blood. On the basis of encouraging preliminary results a colony of M. mitis was established; eight females have produced young (Fig. 1).

Preliminary studies involving exposure of M. mitis to Sr-90, performed following development of a method for peripheral lymphocyte culture, are described on p 108 by Wolf and associates. The exposure to levels of Sr-90

that are leukemogenic to other animals is currently being studied in young marmosa. The use of these animals in viral transfer and immunologic studies is also under way.

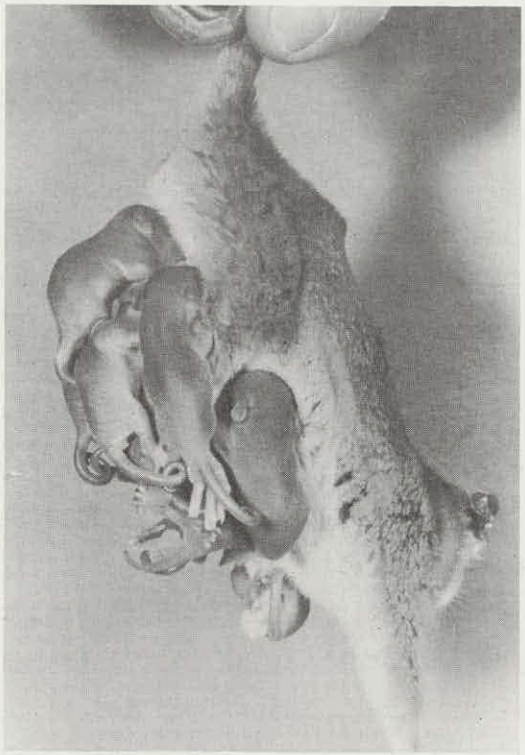

Fig. 1. Female Marmosa mitis with 32-day-old young.

CYTOGENETIC STUDIES OF *MARMOSA MITIS*

C. D. Scott
R. D. Barnes

The mitotic chromosomal pattern of bone marrow preparations from colchicine-injected Marmosa mitis (Marsupialia) is shown and is characterized.

Karyotype analyses for 13 adult Marmosa mitis were carried out in order to obtain standards against which experimental mutagenic influences might be judged. Metaphase spreads are abundant in both bone marrow and spleen cell preparations. The large size and small number of the individual chromosomes combine to render rapid visual scanning and evaluation for frequency of aberrations, and contribute materially to the reliability of such scoring.

The 13 animals subjected to analysis were removed from a colony of over 40 breeding marmosa. Selected animals were injected intraperitoneally with colchicine at 1 mg/kg body weight. (The average weight for adult female marmosa is 65 g; for adult males, 94 g). Bone marrow was washed from both femurs 60 to 90 minutes post-injection.

A cell suspension of marrow was held in 1% sodium citrate for 20 minutes and then fixed in a fresh mixture of methanol-acetic acid as a pellet. Air- and flame-dried preparations were made for staining in Giemsa stain to which 5% by volume of 0.15 N ammonium hydroxide had been added. None of the chromosomes illustrated was derived from a culture.

For karyogram analysis, photographs of metaphase chromosomes were paired according to size, shape, and position of the centromere. Four groups--A, B, C, and the sex chromosomes--have been identified and arranged according to their morphology and comparative size (Fig. 1).* Within group A, all large submetacentrics, one pair may generally be separated visually from the other two pairs. Group B consists of a single pair of metacentrics, and Group C of two pairs of readily distinguished telocentrics. The X chromosome is sub-metacentric and the Y is acrocentric. The sex chromosomes were determined on the basis of the heteromorphic pair found in the males.

The karyogram of 14 chromosomes contains only two pairs that cannot be visually distinguished with certainty. They may yet be separated by means of the more sophisticated methods of analysis such as microdensitometry and statistical and computer analyses. These studies preceded one on the effects of external irradiation.

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* The karyotype is arranged in accordance with the recommendations made at the 3rd Mammalian Cytology and Somatic Cell Genetics Conference, San Juan, Puerto Rico, 1964.

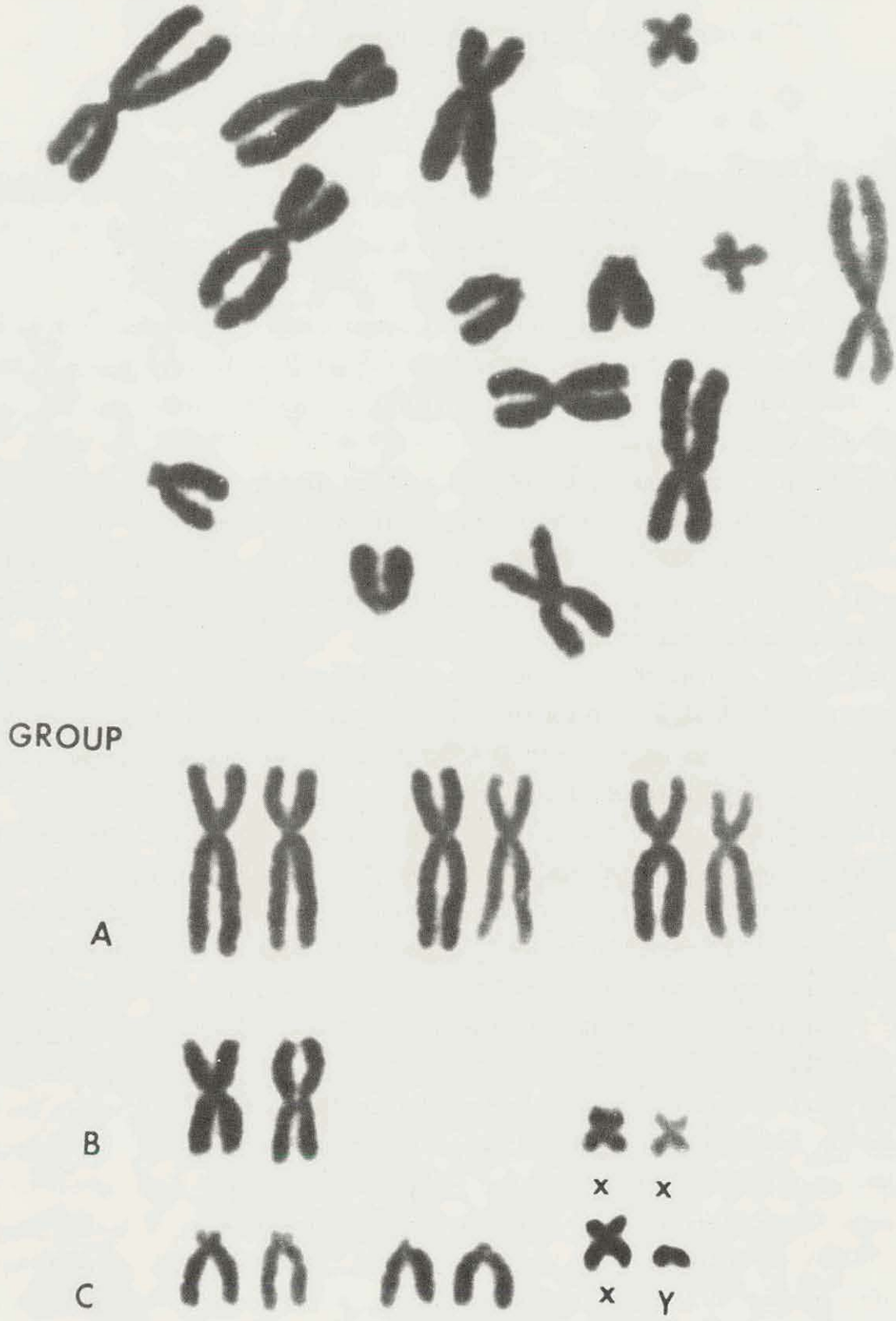

Fig. 1. Karyotype of Marmosa mitis.

A METHOD FOR CULTURING PERIPHERAL LYMPHOCYTES FROM *MARMOSA MITIS*

H. G. Wolf
Leslie Siemon
A. L. Philbrick
Angela Foin

A method for culturing the peripheral lymphocytes from Marmosa mitis has been developed. Increased concentration of PHA-P is required to induce transformation to the primitive cell type. Culture for 72 hr at 37C produces an adequate number of dividing cells for chromosome analysis. Metaphase spreads are prepared according to standard techniques.

The technique of culturing peripheral lymphocytes from humans for subsequent chromosome analysis has been described by Moorhead (Exptl. Cell Res. 20: 613, 1960). Modifications of the standard culturing media have been made to adapt it for use with Marmosa mitis.

Four to six capillary tubes of venous blood are collected via tail vein puncture. The tubes are centrifuged for 5 min at ∿1/2 speed in a microcapillary centrifuge equipped with a rheostat. The capillary tubes are then cut below the buffy coat and the buffy coat and plasma are introduced into 5 ml of medium in a 15-ml Falcon tissue culture flask.

The culture medium that has been used most successfully in our laboratory consists of: 74% RPMI-1629 (GIBCO), 20% fetal calf serum (GIBCO), 2% L-glutamine (GIBCO), and 2% phytohemagglutinin (PHA-P) (Difco); 100 units procaine penicillin, and 0.125 mg streptomycin/ml of medium is added.

Cells obtained from the capillary tube procedure are inoculated into the medium and grown for 72 hr at 37C in an atmosphere of 95% air and 5% CO_2. At 69 hr, 1 ml of 0.05% colchicine solution (Turtox) is added to each culture flask, the cells are gently resuspended using a capillary pipette, and incubation is continued for 3 hr. Cells are harvested at 72 hr according to standard techniques. Slides are stained with Wright's stain and metaphase figures are examined microscopically.

The final concentration of PHA-P is 0.15 mg/ml of culture medium, four times that used in culturing peripheral lymphocytes from other species. Our data indicate that at the recommended dosage of PHA-P, there is little or no transformation of marmosa lymphocytes to the blast stage. Concentrations of two and three times normal result in increasing yields of metaphase figures. PHA-P concentrations of four times normal (0.15 mg/ml culture media) give more than adequate numbers of metaphase figures for examination. Since a higher than usual concentration of PHA-P was required, cells were cultured at concentrations up to 10 times normal to determine whether the PHA-P level was related to chromosome abnormalities. The data are presented in Table 1.

Table 1. PERCENTAGE OF ABNORMAL CHROMOSOMES* VS PHA-P CONCENTRATION

PHA-P Concentration x Normal	Number Cells Examined	Number Abnormal Cells	Number Abnormalities	Abnormal Cells (%)	Abnormalities (%)
1	0	0	0	0	0
2	91	3	5	3.3	5.5
3	406	16	24	3.9	5.9
4	1011	26	35	2.6	3.5
6	500	17	34	3.4	6.8
10	442	24	29	5.4	6.6

* Gaps not included in analysis

There does not seem to be any correlation between the concentration of PHA-P and the percentage of abnormalities observed in the metaphase preparations. An incidence of 3.5% scored abnormalities is low enough to indicate that this system will be a material aid in our attempts to evaluate the cytogenetic effects of irradiation.

CYTOGENETIC EFFECTS OF X-IRRADIATION AND SR-90 IN *MARMOSA MITIS*

H. G. Wolf
Leslie Siemon
A. L. Philbrick
C. D. Scott
Marvin Goldman
L. K. Bustad

The cytogenetic effects of X- and β-irradiation on Marmosa mitis (2N = 14) were evaluated in a pilot study. Three animals were X-irradiated (120R) and 8 were injected i.p. with Sr-90, 4 each at 0.3 μCi/g and 0.03 μCi/g body weight. Peripheral blood and bone marrow were collected periodically after exposure, and mitotic preparations were examined. Chromosome abnormalities were found in all treatment groups. Incidence of damage was higher in X-irradiated animals than in those treated with Sr-90. Marrow preparations showed a higher incidence of damage than preparations from peripheral blood.

The possible cytogenetic effects of Sr-90, a bone-seeking radionuclide, and those of external X-irradiation were compared in Marmosa mitis (2N = 14). Eight male and three female marmosa were randomly assigned to treatment groups as shown in Table 1.

Table 1. RADIATION TREATMENT AND SAMPLING SCHEDULE FOR MARMOSA

Treatment	Time (days) Post-Treatment			
	0.1	1.0	10.0	10+
Sr-90 Injection				
0.03 μCi/g body wt	1♂	1♂	1♂	1♀
0.3 μCi/g body wt	1♂	1♀	1♂	1♂
X-Irradiation				
120R	1♀	1♂	1♂	

Strontium-90 in 0.1 N HNO_3 was injected i.p. at 0.03 μCi and 0.3 μCi/g body weight. The calculated total body dose from the higher level in a 100-g marmosa was ∿14 rads/day; skeletal dose was calculated to be ∿12 rads/day. Since the lower level was 0.1 of the higher level, the corresponding doses were ∿1.4 and 1.2 rads/day.

Exposure to 120R X-irradiation was given using a 250-Kvp therapeutic X-ray unit operated at 10R/min.

One animal from each treatment group was killed at the times indicated in Table 2, and bone marrow was obtained from the femora. The three animals sampled at each period were anesthetized with ether. Blood was drawn via heart puncture for inoculation of peripheral blood cultures, total white cell counts, and

differential counts. Peripheral blood was taken via tail vein puncture at each time period from all animals not scheduled for sacrifice. The blood was cultured according to the method previously described (Wolf et al., p 108). Bone marrow was incubated for 3 hr in Earl's salt solution containing 0.0085% colchicine (Turtox).

Table 2. PERCENTAGE OF ABNORMAL CHROMOSOME SPREADS* IN PERIPHERAL BLOOD AND BONE MARROW OF *MARMOSA MITIS*

Treatment (μCi/g body wt)	Time (days) Post-treatment		
	0.1	1	10
	Peripheral Blood		
0.03	1.6 (2/125)	2.1 (4/188)	11 (11/99)
0.3	3 (4/136)	7.8 (4/51)	11.5 (3/26)
X-Ray (120R)	10 (33/325)	9 (9/102)	8 (1/12)
	Bone Marrow		
0.03	10 (34/346)	6.4 (16/250)	2 (4/225)
0.3	9 (13/150)	12 (29/250)	7 (24/352)
X-Ray (120R)	71 (101/142)	11 (24/214)	0 (0/131)

* Gaps not included in the analysis

Metaphase spreads were prepared by treatment with hypotonic Na-citrate and stained with Wright's stain.

The percentage of abnormal spreads in the peripheral blood (Table 2) indicates that there was a trend toward increasing abnormalities with time post-injection in the Sr-90-treated animals, while the X-irradiated group remained constant. This pattern suggests that continual irradiation from the deposited Sr-90 caused an increase in damage with time, whereas the effect of a single X-irradiation exposure was damage to a given portion of non-dividing cells in circulation; the latter effect persisted as long as the original population survive.

Data obtained from bone marrow preparations are also given in Table 2. In contrast to peripheral blood lymphocytes, the bone marrow is an area of cellular proliferation, the percentage of damaged cells would be expected to decrease

with time as the lethally irradiated cells are lost from the population, and as a result of repair. The data for both the Sr-90-treated and X-irradiated marmosa follow this general trend, suggesting that the low-dose rate from deposited Sr-90 is continually injurious during the period studied.

This preliminary effort has indicated that Marmosa mitis is a particularly useful laboratory animal for studies of the cytogenetic effects of irradiation. Work is now in progress to assess the effects of lower doses of irradiation.

NUTRITION OF *MARMOSA MITIS*

H. G. Wolf
R. D. Barnes*

A basic diet that is acceptable to marmosa and that sustains growth and limited reproduction has been formulated. There is evidence that trace mineral deficiency may be responsible for reproductive difficulties; dietary supplementation has been initiated, and growth and reproduction are being carefully observed for response.

Development of a satisfactory nutritional regimen for a species of animals new to laboratory colonization presents many problems. In the case of marmosa there is virtually no knowledge of their natural feeding habits. Gut morphology and dentition, however, suggest that these animals are mainly carnivorous or insectivorous. It is known that they eat tropical fruits, including figs and bananas. In the formulation of a diet for these animals, palatability plays an important role. A moist, blended diet, approximately 20% dry matter, has proven to be the most acceptable form. Over the past two years several dietary formulations have been tried with varying degrees of success. The present diet, formulated in August 1967, is composed of: 36.4% canned horsemeat, 27.4% peeled bananas, 4.6% whole cooked eggs, 29.5% water, 0.8% U.S.P. 14 salt mix, 0.8% supplemental salt mix (3.4 g $MnSO_4 \cdot H_2O$, 2.20 g $ZnSO_4$, 0.78 g $CuSO_4$/liter) added as of April 1968, and 0.4% Vitamin Diet Fortification mixture (Nutritional Biochemical Corporation). Percentages are expressed on a wet weight basis.

To test the adequacy of this dietary formulation, two groups of mice--one fed the marmosa diet and one fed standard Purina Mouse Breeder Chow--are being maintained and bred. The third generation of mice fed the marmosa diet were smaller than the controls, and had a rough hair coat. They also showed the classical sign of manganese deficiency--congenital absence of otoliths with resulting inability to orient with respect to gravity--which is best demonstrated in a swimming test. These signs are characteristic of manganese and zinc deficiencies as described by Hurley (J. Nutr. 91: 2, 1967). Swimming tests of the mice, conducted by Dr. Hurley, and examination of the otic capsule for otoliths confirmed the diagnosis of manganese deficiency. Second generation Marmosa mitis also revealed loss of equilibrium while swimming. Therefore, supplemental salts were added as noted above; the current diet contains ~60 ppm manganese, 40 ppm Zn, and 10 ppm Cu.

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Reproductive efficiency in a colony maintained since 1966 by Barnes has declined, with almost complete failure to wean young of the second captive generation. Table 1 shows the available data on reproduction of wild-trapped and colony-bred marmosa to date. Although sufficient evidence is not yet available to prove that the reproductive decline was caused by trace mineral deficiencies, preliminary findings suggest that possibility. Since the problem is correlated with time in captivity, absence of environmental stimuli or behavioral pattern changes cannot be discounted as possible causes.

The major nutritional requirements--i.e., protein, fat, carbohydrate, vitamins, and the major elements--appear to be satisfied by the present diet. Proximate analysis of the diet is as follows; 31% protein, 13% fat, 9.8% ash, and 46.2% nitrogen free extract. The diet contains 2% Ca and 1% P. Work is presently under way to define more precisely the nutritional requirements of this species and to determine optimum nutrient levels.

Table 1. REPRODUCTION IN *MARMOSA MITIS*, 1966-1968

Item	Wild-trapped 1966	F_1 Colony-bred 1967	Wild-trapped 1968*	F_2 Colony-bred 1968*
Breeding period	May 11 - Oct 9	Mar 1 - Nov 30		
Females of breeding age	24	38	41	10
Females with litters when received	NR†	NR	14	--
Number of breedings	64	226	55	9
Females without litters	12	21	8	9
Females producing litters	12 (50%)	17 (47%)	19 (46%)	1 (10%)
Litters born	13	26	36§	
Average litter size	7	5	NR	0
Litters nursing	--	--	14	1
Litters weaned	10	1	13	0
Percent young weaned	91	28	NR	0

* Data are for 130-day period (1 Jan-21 May 1968). The females of both groups are cycling, and breeding will continue for ∿2 months

† NR = not recorded

§ Of 117 young born, 108 (92%) were weaned. Six litters nursing when received at Davis were lost in toto and were therefore not used in this calculation.

A MAGNETIC TAPE OUTPUT COUPLER SYSTEM

John Schwind
Larry Gilman
Marvin Goldman

A magnetic tape output coupler system was developed to enable data from various instruments to be recorded for direct processing by a computer.

To record instrument readings automatically in a form amenable to direct processing by a computer, a magnetic tape system was designed. The instrument's data output may be in analog form, a chart recording, or in a quasibinary form such as binary-coded decimal (BCD). Since most instruments do not have provisions for driving a digital incremental tape recorder, a coupler system (Fig. 1) was designed and built to permit the use of various instruments with a digital incremental tape recorder. The coupler has the following characteristics:

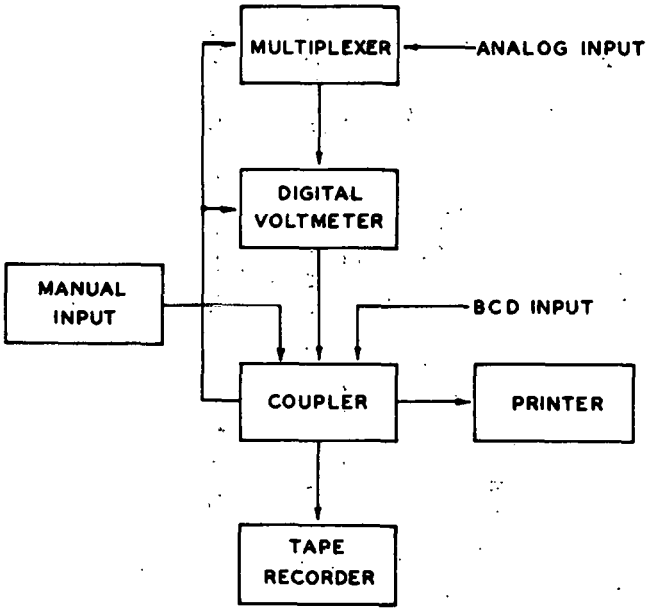

Fig. 1. Coupler System Diagram

1. accepts either positive or negative logic
2. accepts BCD data from instrumentation and has provisions for manual entry of data such as date and experiment number
3. drives a digital incremental tape recorder
4. drives a high-speed parallel printer
5. with the addition of a digital voltmeter, can handle two channels of analog data.

The coupler accepts six characters of BCD (8-4-2-1) information in parallel form. This is considered one coupler word. The digital output is recorded by the magnetic unit in standard IBM 7-channel NRZI code. The parallel data are routed through the interface by a series of "NAND" gates, which are fanned serially by a timing system that also steps the tape transport. The serialized output is connected to the tape input. The tape transport is advanced in synchrony with the flow of characters from the coupler, in response to stepping commands from the coupler timing system. When a complete word is transferred from the coupler to the tape recorder, the coupler is ready to accept a new word. The logic is shown in Fig. 2. An inter-record gap can be entered either automatically or manually at the end of each record. Data are entered manually by setting the data into the unit's thumbwheel switches and depressing the manual entry push button.

The coupler is constructed primarily of integrated circuits. All digital logic is performed by DTL NAND gates. Some complex function TTL integrated circuits are used in the timing section.

The coupler is being used with a 400-channel analyzer and an autoanalyzer. This method eliminates hand-recording of data as well as errors in key-punching and data handling.

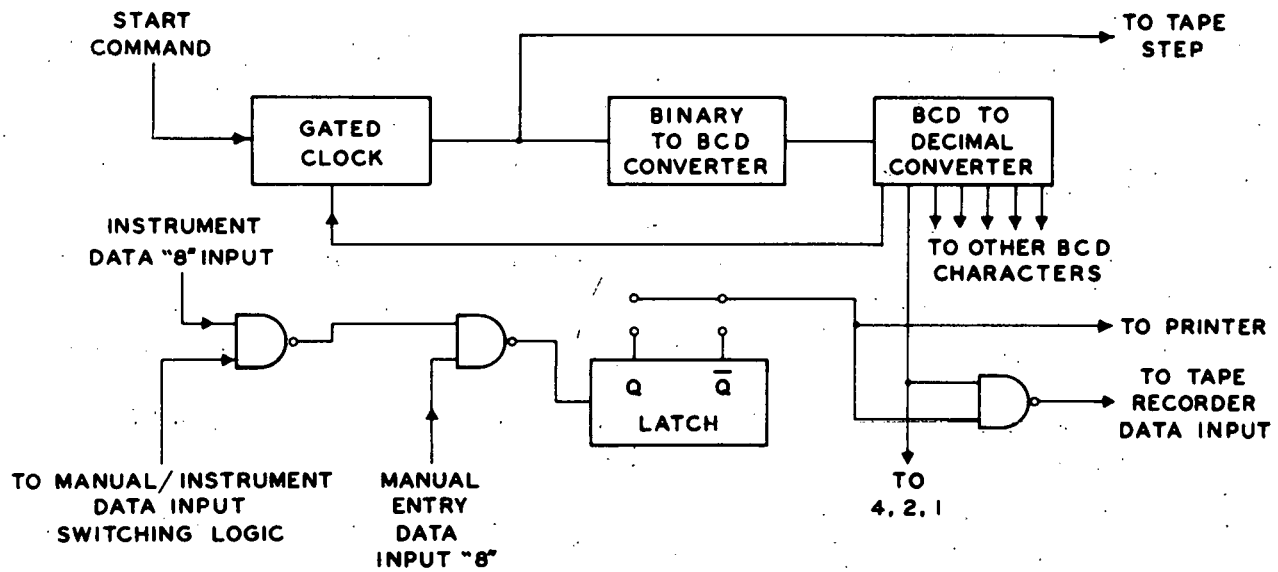

Fig. 2. Simplified logic diagram showing logic for one bit on one BCD character.

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UNIVERSITY OF CALIFORNIA – DAVIS

LOCATION OF

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