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Mortality in Beagles Irradiated during Prenatal and Postnatal Development. II. Contribution of Benign and Malignant Neoplasia

Stephen A. Benjamin,*[,1] Arthur C. Lee,* George M. Angleton,* William J. Saunders,*[,2]
Thomas J. Keefe† and Craig H. Mallinckrodt‡

*Collaborative Radiological Health Laboratory, †Department of Environmental Health and ‡Department of Statistics, Colorado State University, Fort Collins, Colorado 80523

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To evaluate the lifetime carcinogenic hazards of exposure to ionizing radiation during development, 1,680 beagles received whole-body exposures to ^{60}Co γ rays or sham exposures. Eight groups of 120 dogs each received mean doses of 15.6–17.5 or 80.8–88.3 cGy in early, mid- or late gestation, at 8, 28 or 55 days postcoitus or at 2 days after birth. Another group of 120 dogs received a mean dose of 82.6 cGy as 70-day-old juveniles and one group of 240 dogs received a mean dose of 81.2 cGy as 365-day-old young adults. Sham irradiations were given to 360 controls. Sexes were equally represented. In 1,343 dogs allowed to live out their life span, neoplasia was a major disease, contributing to mortality in 40% of the dogs. There was a significant increase in benign and malignant neoplasms occurring in young dogs (<4 years old), including fatal malignancies, after irradiation in the perinatal (late fetal and neonatal) periods. The lifetime incidence of fatal neoplasms was also increased in dogs irradiated perinatally. Three malignancies—lymphomas, hemangiosarcomas and mammary carcinomas—accounted for 51% of all fatal tumors. There was an apparent lifetime increase and earlier onset of lymphomas in dogs exposed as fetuses. Fatal hemangiosarcomas were increased in dogs irradiated early and late in gestation. Fatal mammary carcinomas were not increased by irradiation, although non-fatal carcinomas were increased after perinatal exposure. Myeloproliferative disorders and central nervous system astrocytomas appeared to be increased in perinatally irradiated dogs. These data suggest that irradiation in both the fetal and neonatal periods is associated with increased early onset and lifetime cancer risk.

[1]Present address: Center for Environmental Toxicology and Technology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523.

[2]Present address: Covance Laboratories Inc., P.O. Box 7545, Madison, WI 53707-7545.

INTRODUCTION

Among the more controversial topics relating to radiation carcinogenesis is the question of prenatal sensitivity. Initial reports by Stewart *et al.* from the Oxford Survey of Childhood Cancers (*1*, *2*) of an association between low-level diagnostic X-ray exposures *in utero* and increased risk for childhood cancer were confirmed by some subsequent epidemiological studies (*3–11*) but not by others (*12*), most notably the study by Jablon *et al.* of the atomic bomb survivors (*13*). Periodic re-evaluations of various sets of data from the Oxford Survey and other studies have either supported (*14–19*) or questioned the validity (*20–22*) of some of the findings relating to fetal radiosensitivity.

There was little information on the lifetime risk for prenatally irradiated persons until the report of Yoshimoto *et al.* (*23*) suggested an increased risk for cancer over a 40-year period among the atomic bomb survivors exposed *in utero*. More recent data have supported the increased lifetime risk for cancer among atomic bomb survivors exposed *in utero*, though there are still relatively few cases reported (*24*).

At the Collaborative Radiological Health Laboratory at Colorado State University, a study of the health effects of low-level radiation exposure during prenatal and early postnatal development was undertaken. The main experiment was a life-span study in beagles given a single whole-body exposure to ^{60}Co γ radiation to determine the role of age at exposure as a factor influencing radiation injury. A variety of end points were identified that were to be evaluated for any relationship between radiation effects and age at exposure, the most prominent being neoplastic disease incidence patterns. A number of reports dealing with the effects of prenatal and early postnatal irradiation in beagles from the life-span study have been published, including effects on neoplasia in the thyroid gland (*25*) and skin (*26*), and neoplasms in juvenile dogs (*27*). A companion paper addresses the non-neoplastic causes of death in this study (*28*). The present paper reports the major findings with respect to neoplastic diseases that contributed to death of dogs from the life-span study.

MATERIALS AND METHODS

Experimental Design and Animal Care

A total of 1,680 beagles (equal numbers of males and females) received either a single bilateral whole-body exposure to ^{60}Co γ radiation or a sham exposure. Exposures were during prenatal development at 8, 28 or 55 days of gestation (days postcoitus) or during postnatal life at 2, 70 or 365 days of age (days postpartum). The gestation period of the dogs was approximately 63–64 days. Details of the experimental design and methods for the study are given in the companion manuscript (*28*).

The procedures for the radiation exposures and the dosimetry have been reported (*29*, *30*). The overall mean doses for dogs exposed at 20 and 100 R were 16.3 and 83.6 cGy, respectively (see ref. *28*). A total of 1,343 dogs were allowed to live out their full life span. Details of the experimental protocols and animal care have been reported (*31*). The dogs were maintained in a federally inspected and approved facility in accordance with the NIH "Guide for the Care and Use of Laboratory Animals". They were observed daily and were given annual veterinary clinical examinations, with appropriate therapy when ill (see ref. *28*). All dogs that lived their full life span either died or were euthanized due to terminal illness or because of humane considerations.

Pathology

Complete gross and histopathological evaluations were performed on all life-span dogs. Samples were taken of all organ systems and any grossly apparent lesions for histology. Tissues were fixed and processed for evaluation by light microscopy. For each dog, a combination of clinical findings and pathological lesions was used to determine diseases which caused or contributed to death. Neoplasms were recognized either clinically or at necropsy. Clinically recognized neoplasms were biopsied after detection or removed surgically. Neoplasms were generally classified according to the International Histological Classification of Tumors of Domestic Animals as reported in the *Bulletin of the World Health Organization* (*32*, *33*).

Disease diagnoses were based on a combination of clinical findings, laboratory data and pathology findings. The cause of death was defined as the major condition that directly resulted in the death of the animal. Any other diseases that may have played a role in the death of the animal were considered as contributing to mortality. To establish a disease as a cause of, or contributing to, death, the disease had to be a condition which by itself had the potential to cause death at the stage found at necropsy. For example, a few dogs had two different primary neoplasms that metastasized widely, either of which could have caused death. The neoplasm determined as more likely to have done so was designated as the cause of death. The second neoplasm was designated as having contributed to death. Thus the neoplasms causing or contributing to death were all considered to be fatal neoplasms. Any condition which was purely secondary to a fatal neoplasm was not listed separately. For example, if an intestinal adenocarcinoma eroded through the intestinal wall and caused peritonitis, the intestinal adenocarcinoma was considered to be the primary cause of death and the peritonitis would not be included in analyses of causes of or contributors to death. Since categorizations of diseases causing or contributing to death were subjective to some degree, combining causes of death and contributors to death as all contributing to mortality was deemed most appropriate for purposes of analysis in this paper.

Statistical Analysis

The analysis of the age-related incidence of fatal neoplasia was performed using the weighted combination of contingency tables described by Peto *et al.* (*34*). This was a unidirectional analysis for positive trend with dose using group mean doses (see ref. *28*). For each specific disease analysis, a dog was counted only once. Some dogs had more than one neoplasm entry (for example, a dog may have had both lymphoma and metastatic mammary carcinoma as the cause of death and contributing disease, respectively). In such a case, the cause of death was the event used for analysis. Table II (all neoplasms causing or contributing to death) lists all fatal neoplasms; therefore, the numbers in Table II may not match the number of affected dogs shown in subsequent tables summarizing results of analyses. The analyses accumulated events over 1-year intervals and took into account dose, competing non-neoplastic risks of death and age of disease occurrence. Neoplasms were categorized according to the context in which they occurred. Thus a neoplasm that was responsible for the death of the animal (fatal context) was categorized and analyzed differently than one found incidentally at necropsy (incidental context) or those diagnosed or removed by surgical intervention (mortality-independent). The rates for cancer mortality, prevalence of incidental tumors and onset of mortality-independent tumors were determined. Separate analyses of these groupings of tumors allowed for evaluation of both carcinogenesis rates and the biological impact (relative malignancy) of the lesions. While most of the analyses reported here were cancer mortality rates, several analyses evaluated prevalence and onset rates for nonfatal neoplasms.

The relative risks for specific diseases with respect to radiation dose was evaluated by application of hazard rate models[3] as described previously (*28*, *35*). The cumulative probability of observing a specific disease in an animal at necropsy by age at death was calculated using the method of Kaplan and Meier (*36*).

Estimated breeding values (*28*, *37*) and heritability[4] (*28*) for binary incidence of specific diseases were obtained. The significance of fixed other effects, such as gender, were estimated by analysis of variance. The specific neoplastic diseases chosen for genetic analyses were those for which a potential radiation effect was seen or might have been expected and there were enough occurrences for an adequate analysis.

RESULTS

Neoplasia was by far the most common disease problem in the dogs in the study, comprising 29% of the diseases that caused or contributed to death. While there were a large number of primary neoplasms found in the study (over 10,000 primary neoplasms in the 1,343 dogs that lived their full life span), this report concentrates on those neoplasms which contributed directly to mortality in these animals. Also included, however, are analyses of non-fatal neoplasms where it was deemed appropriate for comparison with human data.

Early-Occurring Neoplasms

One of the early, and striking, findings was the occurrence of an unusual number of neoplasms in young (0–4-year-old) dogs irradiated in the perinatal period (55 days postcoitus or 2 days postpartum). The findings in these dogs have been reported in detail (*27*) and thus will be discussed only briefly here for completeness. Figure 1 shows the prevalence of early-occurring benign and malignant neoplasms in perinatally irradiated dogs (55 days postcoitus and 2 days postpartum), compared with that seen at the two earlier (8 and 28 days postcoitus combined) and two later (70 and 365 days postpartum combined) irradiation times, and in the controls. Perinatally irradiated dogs accounted for 28.6% of the population but had 56% (10 of 18) of the early-occurring tumors; dogs irradiated at 55 days postcoitus had 33% (6/18) and those irradiated at 2 days postpartum had 23% (4/18). Of

[3]D. L. Preston, J. H. Lubin, D. A. Pierce and M. E. McConney, *EPICURE*. Hirosoft International Corporation, Seattle, WA, 1993.

[4]W. S. Snelling, Stayability. Ph.D. dissertation, Colorado State University, Fort Collins, 1994.

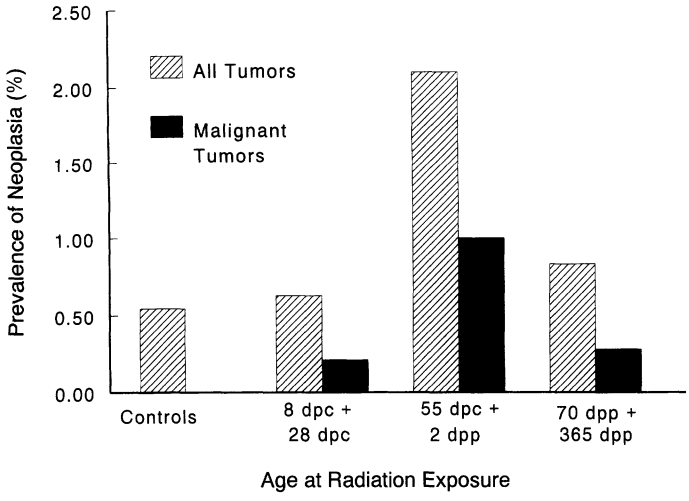

FIG. 1. Prevalence of neoplasia occurring up to 4 years of age in dogs irradiated in early to mid-gestation (8 and 28 days postcoitus, dpc), in the perinatal period (55 days postcoitus and 2 days postpartum, dpp), as juveniles to young adults (70 and 365 days postpartum) and in controls.

malignancies, 5/7 (71%) also were in the perinatally irradiated groups. Furthermore, fatal cancers were seen only in the dogs irradiated in the perinatal period, and all four deaths occurred prior to 2 years of age. These early fatal malignancies were a cerebral astrocytoma in a dog given 90 cGy at 55 days postcoitus, a malignant lymphoma in a dog given 79 cGy at 55 days postcoitus, a fibrosarcoma of the maxilla in a dog given 17 cGy at 2 days postpartum, and an oral squamous cell carcinoma in a dog given 89 cGy at 2 days postpartum. Trend analysis indicated that both fatal malignancies (P = 0.003) and all neoplasms (P = 0.011) in young dogs were increased significantly after exposure as fetuses at 55 days postcoitus. All neoplasms in young dogs were increased after exposure as neonates at 2 days postpartum (P = 0.034), although the increase in fatal malignancies in neonatally exposed dogs only approached significance (P = 0.098).

Neoplasms Contributing to Death throughout the Life Span

Tables I and II provide a summary of the major types of neoplasms, both benign and malignant, that caused or contributed to death in the irradiated and sham-irradiated dogs. In Table I, a dog was counted only once. Thus, if an animal had a specific neoplasm as the primary cause of death, and another of different type as contributing to death, only the former was listed. A total of 454 dogs had a neoplasm which caused the death of the animal. Of these 415 (91.4%) were malignant. A total of 543 dogs had neoplasms that either caused or contributed to death, with 466 (85.8%) being malignant. Of course, these total numbers do not reflect time of appearance, which was taken into account in statistical analyses of age-related incidence.

Three types of malignant neoplasms accounted for 51% of those contributing to mortality. These were lymphoid neoplasia and hemangiosarcomas, both of which were found in a variety of organs and tissues, and mammary gland carcinomas. The "other malignant" category included malignancies originating from a variety of organs and tissues, including but not limited to neoplasms of the endocrine system, skin, respiratory tract, urogenital tract and digestive system. These neoplasms were pooled because no single

TABLE I
Neoplasms Causing Death in Life-Span Study Beagles

Age at exposure	Dose (cGy)	All neoplasia	All malignant	Malignant lymphoma	Hemangiosarcoma	Mammary carcinoma	Other malignant	All benign	Pituitary adenoma	Other benign
Controls	0	73[a]	69	17	6	16	30	4	4	0
8 days postcoitus	15.9	38	36	9	4	9	14	2	1	1
	81.6	35	33	5	8	7	13	2	2	0
28 days postcoitus	16.0	33	31	12	3	4	12	2	2	0
	80.8	31	29	6	1	6	16	2	1	1
55 days postcoitus	15.6	35	33	7	4	5	17	2	1	1
	80.8	36	34	11	6	4	13	2	2	0
2 days postcoitus	17.5	31	27	3	7	5	12	4	3	1
	88.3	43	40	5	2	6	27	3	3	0
70 days postpartum	82.6	32	27	8	3	5	11	5	3	2
365 days postpartum	81.2	67	56	12	7	6	31	11	4	7
Totals		454	415	95	51	73	196	39	26	13

[a]Number of dogs affected (dogs counted only once).

TABLE II
Neoplasms Causing or Contributing to Death in Life-Span Study Beagles

Age at exposure	Dose (cGy)	All neoplasia	All malignant	Malignant lymphoma	Hemangiosarcoma	Mammary carcinoma	Other malignant	All benign	Pituitary adenoma	Other benign
Controls	0	88[a]	77	18	6	20	33	11	10	1
8 days postcoitus	15.9	43	39	9	4	10	16	4	2	2
	81.6	49	42	7	9	7	19	7	6	1
28 days postcoitus	16.0	36	31	12	3	4	12	5	3	2
	80.8	32	29	6	1	6	16	3	1	2
55 days postcoitus	15.6	42	37	8	5	6	18	5	2	3
	80.8	44	37	11	6	5	15	7	5	2
2 days postcoitus	17.5	36	30	3	7	6	14	6	4	2
	88.3	51	45	5	2	7	31	6	6	0
70 days postpartum	82.6	37	31	8	3	6	14	6	3	3
365 days postpartum	81.2	85	68	13	7	8	40	17	7	10
Totals		543	466	100	53	85	228	77	49	28

[a]Number of dogs affected (dogs may have been counted in more than one neoplasm category).

group of fatal malignancies had more than 20 cases which, when spread among the various treatment groups, made meaningful analysis of these neoplasms impossible.

Benign neoplasms that were classed as causing or contributing to mortality were much less frequent, comprising only about 8% of causes of death and 14% of those contributing to death. More importantly, about 69% of all benign neoplasms classified as contributing to mortality were adenomas of the pituitary gland that produced adrenocorticotropic hormone (ACTH) and resulted in hyperadrenocorticism. Other benign neoplasms that were responsible for or contributed to death were quite varied in their nature, including insulin-producing pancreatic islet cell adenomas, space-occupying meningiomas, and neoplasms that became necrotic and led to fatal hemorrhage. The incidence of specific benign tumors other than the pituitary tumors was diverse and too low for meaningful analyses.

All Fatal Malignancy

Table III summarizes the data for all malignant neoplasms contributing to death over the full term of the study. As noted above, there was earlier appearance of fatal cancers in

TABLE III
Malignant Neoplasia Contributing to Death in Life-Span Study Beagles

		Males			Females			Sexes combined		
Age at exposure	Dose (cGy)	Number of dogs in group[a]	Number of dogs affected	*P* value[b]	Number of dogs in group	Number of dogs affected	*P* value	Number of dogs in group	Number of dogs affected	*P* value
Controls	0	138	33	—	138	44	—	276	77	—
8 days postcoitus	15.9	49	14	0.067	49	23	0.035	98	37	0.009
	81.6	49	16		49	22		98	38	
28 days postcoitus	16.0	49	13	0.810	49	18	0.142	98	31	0.524
	80.8	49	11		49	18		98	29	
55 days postcoitus	15.6	48	17	0.049	50	20	0.079	98	37	0.018
	80.8	48	18		48	18		96	36	
2 days postpartum	17.5	49	13	0.024	48	17	0.006	97	30	<0.001
	88.3	49	19		48	25		97	44	
70 days postpartum	82.6	48	15	0.200	48	14	0.443	96	29	0.258
365 days postpartum	81.2	95	30	0.141	96	34	0.040	191	64	0.026

[a]Number of dogs that were not sacrificed at 5, 8 or 11 years of age.
[b]Analysis for positive trend with dose [Peto *et al.* (*34*)].

dogs irradiated at 55 days postcoitus or at 2 days postpartum. The increased risk for fatal cancer over the full lifetime was highly significant by the trend test in both of these perinatally irradiated groups. This was true for males and females, although females at 55 days postcoitus only approached significance ($P = 0.079$). For dogs irradiated at 8 days postcoitus, the increase in fatal cancer risk was significant in both males and females. Dogs irradiated at 365 days postpartum also had a significantly increased risk, with females being the predominant contributors. The lifetime relative risk (RR)/Gy and 90% confidence interval (CI) for combined sexes in the six age-at-exposure treatment groups were 8 days postcoitus (RR 1.26, 90% CI 0.85–1.87), 28 days postcoitus (RR 0.87, 90% CI 0.56–1.35), 55 days postcoitus (RR 1.23, 90% CI 0.82–1.86), 2 days postpartum (RR 1.61, 90% CI 1.13–2.28), 70 days postpartum (RR 1.12, 90% CI 0.72–1.74) and 365 days postpartum (RR 1.30, 90% CI 0.92–1.84). The hazard analysis model indicated a significant increase at the 5% level ($P = 0.029$) only in dogs exposed at 2 days postpartum. The cumulative incidence of fatal malignancies (Figs. 2–7) gives a better picture of the age relationship of tumor occurrence. It is important to recognize that the cumulative incidence depicts the probability of a dog that died at any specific age having a fatal neoplasm. Thus, if the last dog in a group died from a neoplasm, the cumulative incidence would reach 100% for that group. The most striking differences from controls are seen in Figs. 4 and 5 for the dogs irradiated perinatally. In the 55 days postcoitus exposure group, the early onset of fatal malignancies in the high-dose group started at 2 years of age, but the difference from the controls remained evident throughout the full life span. In the low-dose group, the incidence rose above the controls by 8 years of age and remained greater thereafter. For the dogs exposed at 2 days postpartum, the early onset in the high-dose group was evident by 1 year of age and the incidence remained greater than the controls thereafter. In the low-dose group, the early onset was evident by 2 years of age, and the incidence remained above the control values through 8 years but did not differ from the controls thereafter until 16 to 18 years, at the end of the life span. The cumulative incidences in the dogs exposed at 8 days postcoitus (Fig. 2) did not show the early onset. The curve for the high-dose group did not diverge from that for the controls until about 12–13 years, and that for the low-dose group did not diverge until about 16 years. For the dogs irradiated at 365 days postpartum (Fig. 7), there was some increase by about 10 years of age, although the curves for irradiated and control dogs merged at 14 years. After 14 years the curve for irradiated dogs rose above controls again.

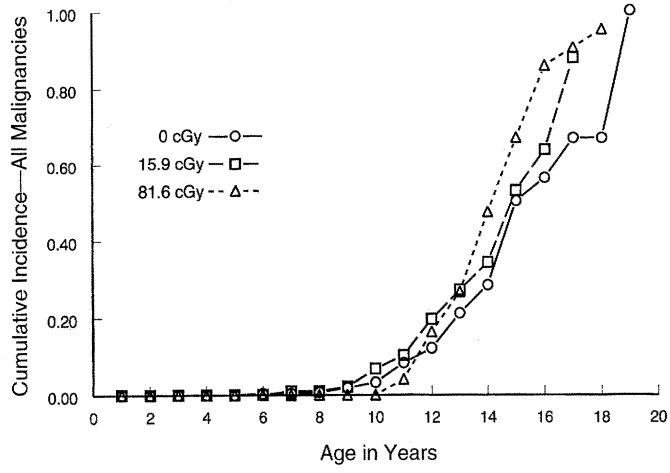

FIG. 2. Cumulative incidence of all malignant neoplasms that contributed to mortality in dogs irradiated at 8 days postcoitus. Sexes are combined.

Lymphoma

Fatal malignancies of the lymphoid system (lymphoma) were a leading cause of death in this study. This complex included lymphomas in a variety of organs, most often in a generalized form, but also as localized, especially intestinal, forms. These included lymphoid leukemias that sometimes accompanied the solid tumors. Most of these appeared as an acute disease syndrome with a relatively short course. Studies with specific immunological markers indicated that most were of B-cell origin (unpublished data). The estimated heritability for lymphoma was not high (0.115), and the influence of sire was not significant ($P = 0.801$). Therefore, genetics did not have a noticeable impact on the incidence of lymphomas. Table IV summarizes the results of the trend analyses for lymphoma showing that dogs irradiated at 55 days postcoitus were the only group in which there was a statistically significant increase. Lymphomas were significantly increased in females but not in males after exposure at 55 days postcoitus, although the latter did show a trend in the same direction. The RR/Gy for dogs exposed at 55 days postcoitus was 1.63 (CI 90% 0.74–3.62), though this increase was not significant at the 5% level. The relative risk for the other treatment groups ranged from 0.65 to 1.27 and none showed evidence of a significant

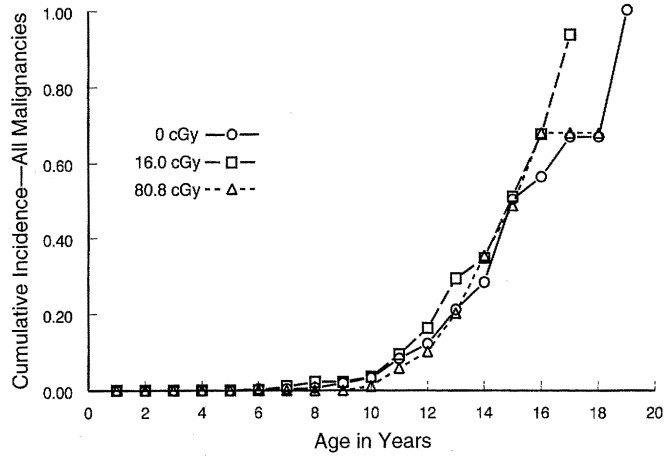

FIG. 3. Cumulative incidence of all malignant neoplasms that contributed to mortality in dogs irradiated at 28 days postcoitus. Sexes are combined.

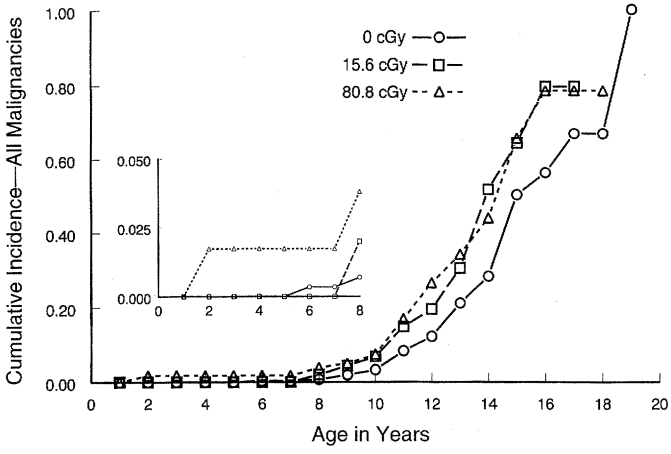

FIG. 4. Cumulative incidence of all malignant neoplasms that contributed to mortality in dogs irradiated at 55 days postcoitus. The inset shows the details of the cumulative incidence through 8 years of age. Sexes are combined.

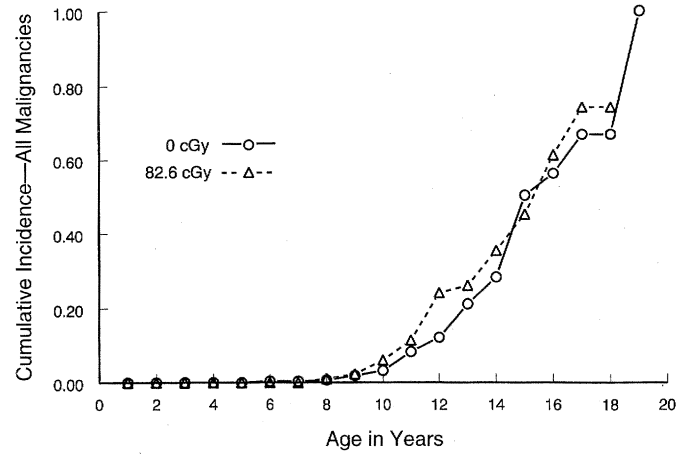

FIG. 6. Cumulative incidence of all malignant neoplasms that contributed to mortality in dogs irradiated at 70 days postpartum. Sexes are combined.

effect. As seen from the cumulative incidence of lymphoid neoplasia in dogs irradiated at 55 days postcoitus (Fig. 8), early appearance of lymphoid neoplasms was contributed to by the death of one of the high-dose dogs irradiated at 55 days postcoitus at less than 2 years of age, but even in the low-dose group, the first lymphomas appeared earlier (at 8 years) than in the controls (first appearance at 11 years).

Hemangiosarcoma

Malignant endothelial neoplasms (hemangiosarcomas) of a variety of organs were common in study dogs. The most common sites were skin, heart, spleen and eye. Genetic analysis of the all hemangiomatous neoplasms (benign and malignant) revealed a definite heritable component. The heritability estimate was 0.179 with a significant sire effect ($P = 0.026$). However, there was no evidence that this genetic component influenced the radiation response ($P = 0.577$). Table V shows the trend analysis results for hemangiosarcomas that contributed to mortality. Dogs irradiated at 8 or 55 days postcoitus had a significantly increased risk for these neoplasms contributing to death. Once again, the main increase was present in females. In fact, in females, there also was a significant increase in dogs irradiated at 365 days postpartum. The large difference between response of the males and females was due to a paucity of cases (only one) in the female controls as well as larger numbers in the irradiated dogs. The RR/Gy for the combined sexes irradiated at 8 days postcoitus was 5.93 (90% CI 1.99–14.61) and at 55 days postcoitus was 2.74 (90% CI 0.92–8.18). This difference was significant at 8 days postcoitus ($P = 0.007$) but not at 55 days postcoitus ($P = 0.144$). The relative risks for the combined sexes in the other treatment groups ranged from 0.37 to 2.39 and were not statistically significant. However, in the females alone, the relative risk was 11.55 at 8 days postcoitus (90% CI

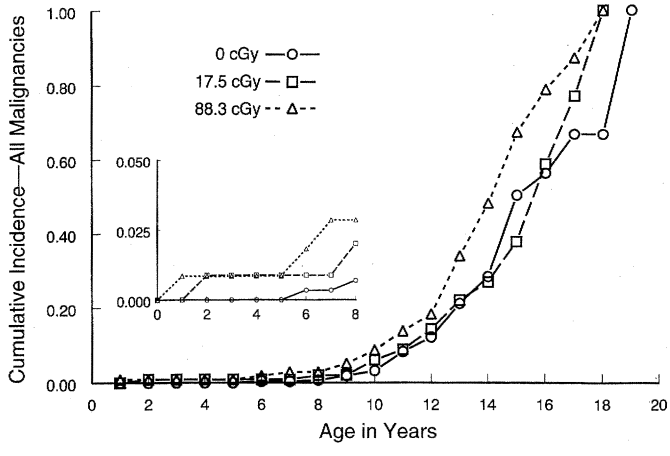

FIG. 5. Cumulative incidence of all malignant neoplasms that contributed to mortality in dogs irradiated at 2 days postpartum. The inset shows the details of the cumulative incidence through 8 years of age. Sexes are combined.

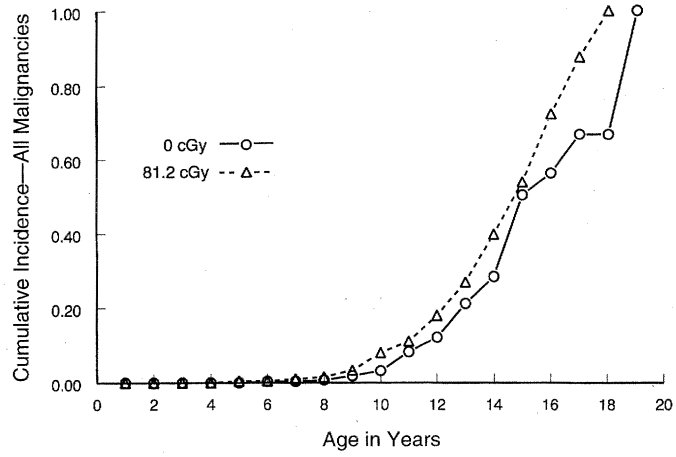

FIG. 7. Cumulative incidence of all malignant neoplasms that contributed to mortality in dogs irradiated at 365 days postpartum. Sexes are combined.

TABLE IV
Lymphoid Neoplasia Contributing to Death in Life-Span Study Beagles

		Males			Females			Sexes combined		
Age at exposure	Dose (cGy)	Number of dogs in group[a]	Number of dogs affected	*P* value[b]	Number of dogs in group	Number of dogs affected	*P* value	Number of dogs in group	Number of dogs affected	*P* value
Controls	0	138	10	—	138	8	—	276	18	—
8 days postcoitus	15.9	49	6	0.355	49	3	0.412	98	9	0.340
	81.6		49	4		49	3	98	7	
28 days postcoitus	16.0	49	7	0.617	49	5	0.584	98	12	0.663
	80.8		49	4		49	2	98	6	
55 days postcoitus	15.6	48	5	0.235	50	3	0.016	98	8	0.026
	80.8		48	5		48	6	96	11	
2 days postpartum	17.5	49	3	0.323	48	0	0.828	97	3	0.621
	88.3		49	4		48	1	97	5	
70 days postpartum	82.6	48	5	0.266	48	3	0.373	96	8	0.236
365 days postpartum	81.2	95	6	0.596	96	7	0.157	191	13	0.304

[a]Number of dogs that were not sacrificed at 5, 8 or 11 years of age.
[b]Analysis for positive trend with dose [Peto *et al.* (*34*)].

2.76–48.28), 5.39 at 55 days postcoitus (90% CI 1.12–26) and 21.37 at 365 days postpartum (90% CI 2.2–207.9), all statistically significant increases. Figures 9 and 10 present the cumulative incidence of fatal hemangiosarcomas in both sexes for dogs irradiated at 8 or 55 days postcoitus. Because of the low incidence of hemangiosarcoma in control females, evaluation of the combined sexes would be less likely to overestimate any possible radiation effect. The first appearances of fatal hemangiosarcomas in the 8 days postcoitus group (Fig. 9) were in a low-dose-treated dog at 10 years of age and a high-dose-treated dog at 12 years, while the first case in the controls was not until 14 years. While there was only small separation between the curves for low-dose and control dogs after 10 years, the difference between the curves for high-dose and control dogs was marked. After exposure at 55 days postcoitus (Fig. 10), again there was earlier onset of fatal hemangiosarcomas in both the low-dose (at 11 years) and the high-dose group (8 years), compared with 14 years in the controls.

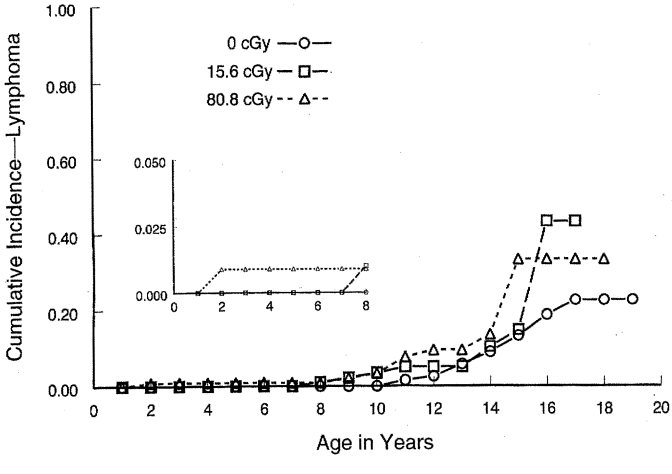

FIG. 8. Cumulative incidence of lymphomas that contributed to mortality in dogs irradiated at 55 days postcoitus. The inset shows the details of the cumulative incidence through 8 years of age. Sexes are combined.

Mammary Carcinoma

Surprisingly, there was no evidence of an increased risk for fatal mammary carcinoma after irradiation at any age at exposure (Table VI). There were several subtypes of mammary carcinoma based on different histological characteristics which had different degrees of malignant and metastatic potential. Carcinomas arising from the small interlobular and intralobular ductules (ductular, or solid, carcinomas) were by far the most malignant of the mammary carcinomas. These lesions comprised only 13% of all mammary carcinomas but accounted for 70% of the fatal tumors. An analysis for fatal mammary carcinoma taking into account the different histological subtypes (ductular or solid carcinoma compared to adenocarcinomas) also did not reveal any evidence of an effect of radiation (data not shown). A further analysis of all mammary carcinomas (fatal plus nonfatal) suggested that irradiation did affect the incidence of these two carcinoma subtypes in the irradiated and control populations when all age-at-irradiation groups were combined ($P = 0.016$). Interestingly, in this case there was a reduction in the incidence of ductular carcinomas in the irradiated dogs, that is, a reduction of the most malignant lesions after irradiation, rather than an increase. In fact, the relative risks for fatal carcinomas in females in all the treatment groups were decreased, ranging from only 0.49 to 0.91, although none was statistically significant. Additionally, the data for non-fatal mammary carcinoma were analyzed. Prevalence of incidental carcinomas (those that were found at necropsy but were not responsible for death) and onset of mortality-independent carcinomas (those diagnosed after biopsy or surgical removal) were increased in

TABLE V
Hemangiosarcomas Contributing to Death in Life-Span Study Beagles

Age at exposure	Dose (cGy)	Males: Number of dogs in group[a]	Males: Number of dogs affected	Males: *P* value[b]	Females: Number of dogs in group	Females: Number of dogs affected	Females: *P* value	Sexes combined: Number of dogs in group	Sexes combined: Number of dogs affected	Sexes combined: *P* value
Controls	0	138	5	—	138	1	—	276	6	—
8 days postcoitus	15.9	49	1	0.113	49	3	<0.001	98	4	<0.001
	81.6	49	3		49	6		98	9	
28 days postcoitus	16.0	49	1	0.931	49	2	0.352	98	3	0.762
	80.8	49	0		49	1		98	1	
55 days postcoitus	15.6	48	2	0.405	50	3	0.004	98	5	0.021
	80.8	48	2		48	4		96	6	
2 days postpartum	17.5	49	2	0.896	48	5	0.205	97	7	0.569
	88.3	49	0		48	2		97	2	
70 days postpartum	82.6	48	2	0.394	48	1	0.208	96	3	0.261
365 days postpartum	81.2	95	2	0.693	96	5	0.005	191	7	0.083

[a]Number of dogs that were not sacrificed at 5, 8 or 11 years of age.
[b]Analysis for positive trend with dose [Peto *et al.* (*34*)].

dogs exposed at 55 days postcoitus or 2 days postpartum ($P < 0.05$). Although the heritability estimate for mammary carcinomas (0.148) was significant and the sire influence was strong ($P = 0.009$), there was no evidence that this affected the radiation response ($P = 0.806$).

Other Fatal Malignancy

As noted previously, the neoplasms classified under "other" fatal malignancies were diverse. While there were too few of any one type for meaningful analysis as specific tumors, the grouping showed a distinct trend for increased risk with increasing dose, most strikingly after exposure at 2 days postpartum (Table VII). Dogs exposed at 8 days postcoitus or 365 days postpartum also exhibited significantly increased risk, and there was a trend toward a similar effect at 28 and 55 days postcoitus. There was no consistency in the response between sexes. While both males and females showed evidence of the effects at 2 and 365 days postpartum, only females showed an effect at 28 days postcoitus, and only males showed a borderline effect at 55 days postcoitus. Because this was such a diverse grouping of neoplasms, specific relative risk values were not derived. Figures 11–13 depict the cumulative probability of occurrence of "other" malignancies in the combined sexes for dogs exposed at 8 days postcoitus and 2 and 365 days postpartum. In the 8 days postcoitus group (Fig. 11), there did not appear to be earlier onset in the irradiated groups compared with the controls, and differences were evident only later in the life span (after 13 years). In the dogs exposed at 2 days postpartum (Fig. 12), however, the unusually early appearance of fatal cancers prior to 2 years of age contributed to the accelerated onset in the irradiated dogs.

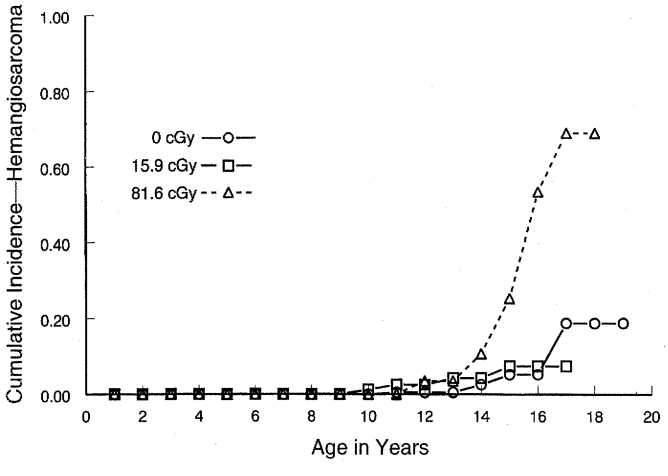

FIG. 9. Cumulative incidence of hemangiosarcomas that contributed to mortality in dogs irradiated at 8 days postcoitus. Sexes are combined.

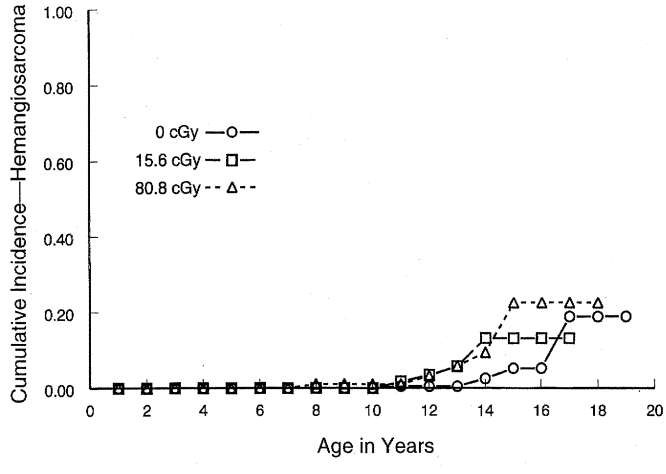

FIG. 10. Cumulative incidence of hemangiosarcomas that contributed to mortality in dogs irradiated at 55 days postcoitus. Sexes are combined.

TABLE VI
Mammary Carcinomas Contributing to Death in Life-Span Study Beagles

Age at exposure	Dose (cGy)	Females		
		Number of dogs in group[a]	Number of dogs affected	*P* value[b]
Controls	0	138	20	—
8 days postcoitus	15.9	49	10	0.472
	81.6	49	7	
28 days postcoitus	16.0	49	4	0.609
	80.8	49	6	
55 days postcoitus	15.6	50	6	0.616
	80.8	48	5	
2 days postpartum	17.5	48	6	0.493
	88.3	48	7	
70 days postpartum	82.6	48	6	0.536
365 days postpartum	81.2	96	8	0.806

[a]Number of dogs that were not sacrificed at 5, 8 or 11 years of age; all dogs in this group were females.
[b]Analysis for positive trend with dose [Peto *et al.* (*34*)].

After this early onset, the difference between the low-dose group and the controls was negligible until very late (17 years). The difference between the high-dose group and the controls remained marked throughout the life span. For the dogs irradiated at 365 days postpartum (Fig. 13), again there was no evidence of early onset of fatal tumors and the increased incidence was not evident until after 12 years of age.

One group of neoplasms that falls in this "other" category represents an important subset for evaluation. Myeloproliferative disorders are a general category of abnormal bone marrow proliferation that encompass a continuous spectrum from myeloid dysplasia and myelofibrosis through frank granulocytic leukemia (*38*). Because of this continuum, and because our cases showed maturation defects in bone marrow cell populations, we classified all such syndromes as malignant neoplasia; three cases were frank granulocytic leukemias. These are rare spontaneous diseases in the dog but are highly sensitive to induction by ionizing radiation (*38*). In our study, there were five cases of myeloproliferative disorders, all in dogs irradiated in the mid-prenatal to neonatal period. One of the five affected dogs received 16.1 cGy at 28 days postcoitus, one dog received 15.7 cGy at 55 days postcoitus, one dog received 17.5 cGy at 2 days postpartum, and two dogs received 88.0 and 89.5 cGy, respectively, at 2 days postpartum. The dogs with myeloproliferative disorders and leukemias died between 9.4 and 16.7 years of age. No cases were seen in any other groups, including the controls.

Benign Neoplasms

Benign neoplasms were also evaluated for their role in contributing to mortality (Table VIII). For the combined

TABLE VII
Other Malignancies Contributing to Death in Life-Span Study Beagles

Age at exposure	Dose (cGy)	Males			Females			Sexes combined		
		Number of dogs in group[a]	Number of dogs affected	*P* value[b]	Number of dogs in group	Number of dogs affected	*P* value	Number of dogs in group	Number of dogs affected	*P* value
Controls	0	138	18	—	138	15	—	276	33	—
8 days postcoitus	15.9	49	7	0.120	49	7	0.075	98	14	0.033
	81.6	49	9		49	8		98	17	
28 days postcoitus	16.0	49	5	0.571	49	7	0.022	98	12	0.151
	80.8	49	7		49	9		98	16	
55 days postcoitus	15.6	48	10	0.058	50	8	0.656	98	18	0.160
	80.8	48	11		48	3		96	14	
2 days postpartum	17.5	49	8	0.003	48	6	<0.001	97	14	<0.001
	88.3	49	15		48	15		97	30	
70 days postpartum	82.6	48	8	0.288	48	4	0.537	96	12	0.347
365 days postpartum	81.1	95	22	0.036	96	15	0.026	191	37	0.004

[a]Number of dogs that were not sacrificed at 5, 8 or 11 years of age.
[b]Analysis for positive trend with dose [Peto *et al.* (*34*)].

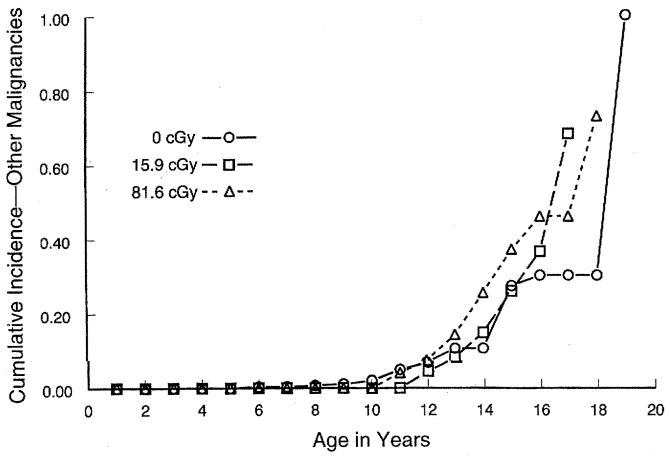

FIG. 11. Cumulative incidence of "other" malignant neoplasms that contributed to mortality in dogs irradiated at 8 days postcoitus. Sexes are combined.

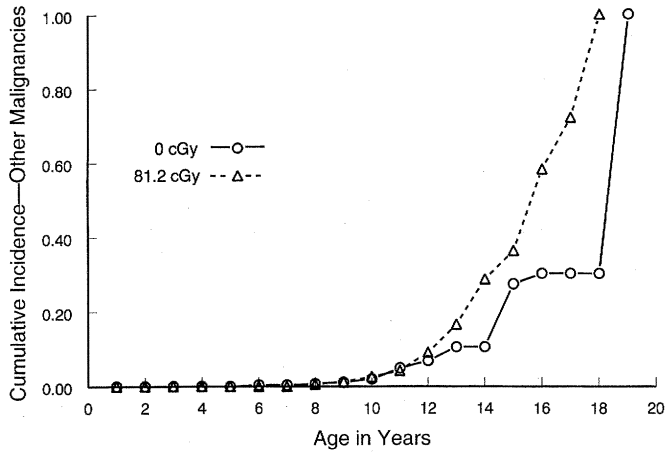

FIG. 13. Cumulative incidence of "other" malignant neoplasms that contributed to mortality in dogs irradiated at 365 days postpartum. Sexes are combined.

sexes, there was an increased positive trend for fatal tumors after irradiation at 55 days postcoitus or 365 days postpartum. Females had a higher incidence than males at these exposure times. The relative risk for the combined sexes for the group irradiated at 55 days postcoitus was 2.01 (90% CI 0.76–5.36) and at 365 days postpartum was 2.20 (90% CI 0.97–4.96). The relative risks in the remaining groups ranged from 0.52 to 1.53. In none of the groups was the risk significantly increased by the hazard test. Figures 14 and 15 show the cumulative incidence of benign neoplasms contributing to mortality in dogs irradiated at 55 days postcoitus (Fig. 14) and 365 days postpartum (Fig. 15). In both cases, the differences between the irradiated and control dogs was not evident until after about 13 years.

Of the benign neoplasms that contributed to mortality, nearly 70% were pituitary adenomas which were immunohistochemically positive for ACTH production, and which were associated with severe adrenal cortical hyperplasia and clinical and laboratory signs consistent with generalized hyperadrenocorticism (Cushing's syndrome). Considering the overall effect seen for benign tumors, it is notable that in the analysis of pituitary adenomas for the sexes combined (Table IX), there was no evidence of a significant increase in any irradiated populations. Analysis for each sex suggested an effect for these tumors in males irradiated at 8 days postcoitus and in females at 2 days postpartum; however, these numbers are relatively small with no evident consistency within or between sexes. No other specific tumor types were present in sufficient numbers to enable determination of the specificity for the increases seen in benign fatal neoplasms.

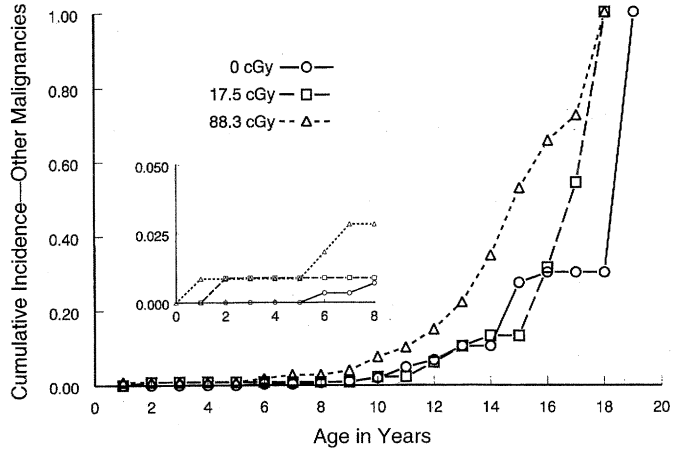

FIG. 12. Cumulative incidence of "other" malignant neoplasms that contributed to mortality in dogs irradiated at 2 days postpartum. The inset shows the details of the cumulative incidence through 8 years of age. Sexes are combined.

Benign and Malignant Neoplasms

An analysis also was performed for all neoplasms, both benign and malignant combined, as causes of or contributors to death (Table X). Overall, trend test evaluation of neoplasia showed significant increases in dogs irradiated at 8 or 55 days postcoitus, and at 2 or 365 days postpartum, similar to what was seen for malignancies alone. Relative risk estimates for the combined sexes for the six age-at-exposure groups were 8 days postcoitus (RR 1.23, 90% CI 0.84–1.78), 28 days postcoitus (RR 0.83, 90% CI 0.55–1.26), 55 days postcoitus (RR 1.34, 90% CI 0.91–1.95), 2 days postpartum (RR 1.59, 90% CI 1.14–2.21), 70 days postpartum (RR 1.19, 90 % CI 0.79–1.78) and 365 days postpartum (RR 1.39, 90% CI 1.01–1.92). The hazard analysis found a significant increase in risk only in the 28 days postcoitus group ($P = 0.024$). Genetic analysis for all neoplasms indicated a definite sex difference ($P = 0.021$) which was not surprising considering that mammary carcinomas occurred only in females. There was a fairly strong overall genetic effect with a heritability for all neoplasms of 0.227 along with a significant sire effect ($P = 0.001$). Genetic background did not influence the effect of radiation on total

TABLE VIII
Benign Neoplasms Contributing to Death in Life-Span Study Beagles

		Males			Females			Sexes combined		
Age at exposure	Dose (cGy)	Number of dogs in group[a]	Number of dogs affected	*P* value[b]	Number of dogs in group	Number of dogs affected	*P* value	Number of dogs in group	Number of dogs affected	*P* value
Controls	0	138	6	—	138	5	—	276	11	—
8 days postcoitus	15.9	49	1	0.106	49	2	0.412	98	3	0.142
	81.6	49	4		49	2		98	6	
28 days postcoitus	16. 0	49	3	0.820	49	2	0.478	98	5	0.753
	80.8	49	1		49	2		98	3	
55 days postcoitus	15.6	48	3	0.120	50	2	0.138	98	5	0.047
	80.8	48	4		48	3		96	7	
2 days postpartum	17.5	49	3	0.714	48	2	0.021	97	5	0.149
	88.3	49	1		48	5		97	6	
70 days postpartum	82.6	48	3	0.315	48	3	0.160	96	6	0.153
365 days postpartum	81.2	95	6	0.243	96	9	0.011	191	15	0.017

[a]Number of dogs that were not sacrificed at 5, 8 or 11 years of age.
[b]Analysis for positive trend with dose [Peto *et al.* (*34*)].

incidence of fatal neoplasms, although this did approach significance ($P = 0.077$).

An astrocytoma was the cause of death in one of the dogs that died prior to 2 years of age, and increased risk for nervous system neoplasms has been reported in irradiated animals and humans (*39*). Therefore, we examined the occurrence of both benign and malignant nervous system neoplasms in the study dogs. Thirty-seven primary neoplasms involving the nervous system were found (see Table XI). An analysis for the prevalence of all nervous system neoplasms indicated that there was a significant increase in dogs irradiated at 28 days postcoitus ($P = 0.015$) or 365 days postpartum ($P = 0.010$), and increases approached significance in dogs irradiated at 55 days postcoitus ($P = 0.080$) or 2 days postpartum ($P = 0.096$). As can be seen from Table XI, however, this included many different types of neoplasms, the majority being meningeal and glial in origin. The number of astrocytomas in dogs irradiated at 55 days postcoitus was striking, with five of seven neoplasms being this type. Only five other astrocytomas were seen, with no more than two in any age-at-exposure group. All but two of the astrocytomas were fatal, one of the incidental lesions in a dog exposed at 55 days postcoitus and the other in a dog exposed at 365 days postpartum. Considering that one of the astrocytomas in the dogs exposed at 55 days postcoitus was one of the neoplasms fatal prior to 2 years of age, these findings suggest a radiation effect after fetal exposure. However, with the small number of astrocytomas, the increase only approached significance ($P = 0.065$) with the trend test.

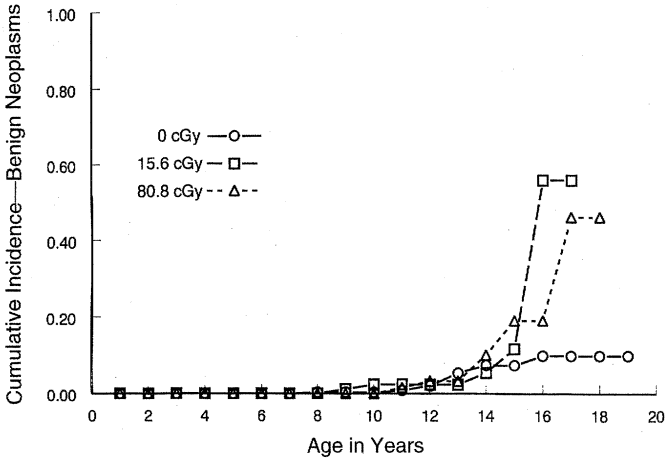

FIG. 14. Cumulative incidence of benign neoplasms that contributed to mortality in dogs irradiated at 55 days postcoitus. Sexes are combined.

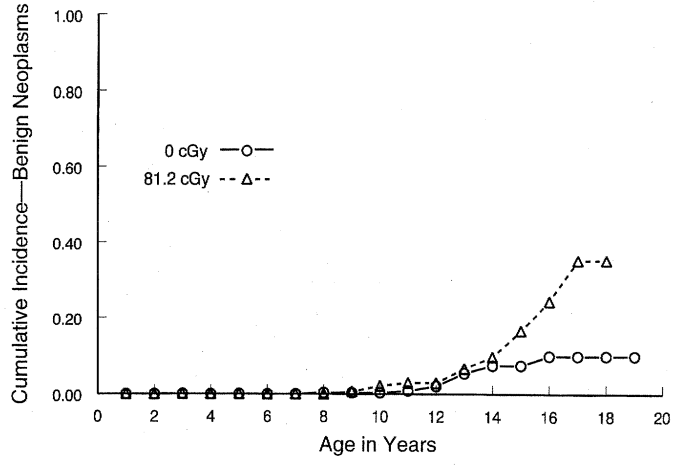

FIG. 15. Cumulative incidence of benign neoplasms that contributed to mortality in dogs irradiated at 365 days postpartum. Sexes are combined.

TABLE IX
Pituitary Adenomas with Hyperadrenocorticism Contributing to Death in Life-Span Study Beagles

		Males			Females			Sexes combined		
Age at exposure	Dose (cGy)	Number of dogs in group[a]	Number of dogs affected	*P* value[b]	Number of dogs in group	Number of dogs affected	*P* value	Number of dogs in group	Number of dogs affected	*P* value
Controls	0	138	5	—	138	5	—	276	10	—
8 days postcoitus	15.9	49	0	0.048	49	2	0.412	98	2	0.092
	81.6	49	4		49	2		98	6	
28 days postcoitus	16.0	49	1	0.926	49	2	0.731	98	3	0.923
	80.8	49	0		49	1		98	1	
55 days postcoitus	15.6	48	2	0.357	50	0	0.091	98	2	0.120
	80.8	48	2		48	3		96	5	
2 days postpartum	17.5	49	2	0.652	48	2	0.021	97	4	0.105
	88.3	49	1		48	5		97	6	
70 days postpartum	82.6	48	1	0.709	48	2	0.373	96	3	0.563
365 days postpartum	81.2	95	4	0.398	96	3	0.466	191	7	0.404

[a]Number of dogs that were not sacrificed at 5, 8 or 11 years of age.
[b]Analysis for positive trend with dose [Peto *et al.* (*34*)].

Analysis for prevalence of the meningiomas correlated well with the overall analysis of nervous tumors with the group exposed at 365 days postpartum being significantly increased ($P = 0.043$) and the group exposed at 28 days approaching significance ($P = 0.055$). This is not surprising since the meningeal tumors accounted for about 50% of all the nervous system lesions.

Relatively few fatal thyroid carcinomas were found in the study, and there was no effect of irradiation on thyroid cancer mortality, as reported previously (*25*). Also as reported, dogs exposed as 2-day-old neonates or as 70-day-old juveniles showed evidence of significantly increased risk of incidental benign and malignant follicular cell neoplasms, including multiple neoplasms. This effect was stronger in the dogs exposed as juveniles, which is consistent with the high risk for radiogenic thyroid neoplasia in humans after exposure during early childhood (*39*).

Combined Dose Groups

A trend analysis for malignancies contributing to death was run after combining all the age-at-exposure groups to get three pooled dose groups (0, 16.3 and 82.6 cGy). Table XII summarizes the numbers of dogs and the results. There was a statistically significant trend for increasing cancer risk with increasing dose for the combined sexes ($P = 0.012$), with *P* values for males and females being 0.040 and 0.067, respectively. Figure 16 depicts the cumulative incidence of fatal tumors in the combined dose groups.

TABLE X
All Neoplasms (Benign and Malignant) Contributing to Death in Life-Span Study Beagles

		Males			Females			Sexes combined		
Age at exposure	Dose (cGy)	Number of dogs in group[a]	Number of dogs affected	*P* value[b]	Number of dogs in group	Number of dogs affected	*P* value	Number of dogs in group	Number of dogs affected	*P* value
Controls	0	138	39	—	138	48	—	276	87	—
8 days postcoitus	15.9	49	15	0.050	49	25	0.046	98	40	0.009
	81.6	49	19		49	23		98	42	
28 days postcoitus	16.0	49	16	0.885	49	20	0.128	98	36	0.595
	80.8	49	12		49	20		98	32	
55 days postcoitus	15.6	48	20	0.022	50	22	0.037	98	42	0.004
	80.8	48	22		48	21		96	43	
2 days postpartum	17.5	49	16	0.053	48	19	0.001	97	35	<0.001
	88.3	49	20		48	29		97	49	
70 days postpartum	82.6	48	18	0.166	48	17	0.284	96	35	0.147
365 days postpartum	81.2	95	35	0.136	96	42	0.005	191	77	0.005

[a]Number of dogs that were not sacrificed at 5, 8 or 11 years of age.
[b]Analysis for positive trend with dose [Peto *et al.* (*34*)].

TABLE XI
Nervous System Neoplasms in Life-Span Study Beagles

Age at exposure	Dose (cGy)	Number of dogs in group[a]	Number of dogs affected	Tumor type
Controls	0	276	3	Meningioma, brain Meningeal sarcoma, brain Choroid plexus carcinoma, brain
8 days postcoitus	15.9	98	2	Chondroma, meninges, spinal cord Meningioma, brain
	81.6	98	1	Malignant schwannoma, brachial plexus
28 days postcoitus	16.0	98	1	Meningioma, cranial nerve
	80.8	98	5	Hemangioma, brain Meningioma, brain (2 dogs) Meningeal sarcoma, brain Astrocytoma, brain
55 days postcoitus	15.6	98	4	Ependymoma, brain Astrocytoma, spinal cord Astrocytoma, brain (2 dogs)
	80.8	96	4	Meningioma, brain (2 dogs) Astrocytoma, brain (2 dogs)
2 days postpartum	17.5	97	3	Meningioma, brain (3 dogs)
	88.3	97	3	Neurofibrosarcoma, peripheral nerve Meningioma, brain Astrocytoma, brain
70 days postpartum	82.6	96	3	Hemangioma, brain Meningioma, brain Astrocytoma, brain
365 days postpartum	81.2	191	8	Meningioma, spinal cord Meningioma, brain (2 dogs) Meningeal sarcoma, spinal cord Meningeal sarcoma, brain Oligodendroglioma, brain Astrocytoma, brain (2 dogs)

[a]Number of dogs that were not sacrificed at 5, 8 or 11 years of age.

DISCUSSION

The two sets of analyses used to evaluate the effect of irradiation on neoplasia contributing to mortality both provided evidence of increased risk, but differed with respect to the statistical significance of this risk. The test for positive trend with increasing dose (*34*) indicated that there were significant dose responses for all fatal neoplasms, benign and malignant, and for fatal malignant neoplasms alone in dogs irradiated at 8 or 55 days postcoitus and 2 or 365 days postpartum. The corresponding hazard analysis (AMFIT) showed a clear statistically significant increase in risk only for dogs exposed at 2 days postpartum both for all fatal neoplasms and for fatal malignant neoplasms. The findings were similar for specific types of neoplasm. The increased risk of lymphomas in dogs irradiated at 55 days postcoitus was statistically significant based on the trend test, but not with the hazard analysis. The trend test indicated significantly increased risk of hemangiosarcomas after exposures at 8 or 55 days postcoitus, but while the hazard analysis agreed with the change at 8 days postcoitus, the change at 55 days postcoitus was suggestive at best. Both of these analytical procedures are accepted for data of this type, but clearly differ in their statistical power. However, it should be noted that the trend test was based on a one-sided alternative hypothesis (i.e. positive trend), whereas the likelihood ratio test employed in the hazard analysis was based on a two-sided alternative hypothesis.

Interpretation of the above findings must be cautious in light of the somewhat different results of the statistical analyses, but several conclusions are warranted. First, there certainly appears to be an increase in risk of both benign and malignant neoplasms in young dogs after perinatal irradiation. Because the number of neoplasms was relatively small in this group of young dogs, it is useful to compare the findings in our dogs to those of the general canine population. The most comprehensive data for dogs (*40*) indicate that the rate for malignant tumors in all breeds of dogs from 0 to 2 years of age is 1.53 cases/1,000 animal-years at risk. In our study, the rate for malignant

TABLE XII
Malignant Neoplasia Contributing to Death in Life-Span Beagles: All Ages at Exposure Combined

	Dose (cGy)[a]	Number of dogs in group[b]	Cause of death: Number of dogs affected	Cause of death: *P* value[c]	Cause of and contributing to death: Number of dogs affected	Cause of and contributing to death: *P* value
Sexes combined	0	276	69		77	
	16.3	391	127	0.021	135	0.012
	82.6	676	219		240	
Males	0	138	32		33	
	16.3	195	53	0.110	57	0.040
	82.6	338	100		109	
Females	0	138	37		44	
	16.3	196	74	0.042	78	0.067
	82.6	338	119		131	

[a]Mean doses for all dogs irradiated in the low- and high-dose groups.
[b]Number of dogs that were not sacrificed at 5, 8 or 11 years of age.
[c]Analysis for positive trend with dose [Peto *et al.* (*34*)].

tumors in perinatally irradiated dogs from 0 to 2 years of age was 4 cases/943 animal-years at risk or 4.24/1,000. While this is almost three times higher than the spontaneous rate in the canine, the fatality rate is even more impressive. Priester and McKay (*40*) estimated that only 21.5% of the malignancies seen in young dogs had metastasized. Of course, not all tumors that metastasize are fatal and tumors that do not metastasize can also lead to death. Even if we assume that 25% of all spontaneous malignancies seen in young dogs of all breeds cause death, this translates to a fatality rate of 0.38/1,000 animal-years at risk. Considering that all of the malignancies in the young dogs that had been irradiated were fatal, the spontaneous rate is less than one-tenth that seen in the perinatally irradiated dogs in our study.

Second, an important question to be asked is whether prenatal and/or postnatal irradiation in the dogs was associated with an increased risk for neoplasia throughout life.

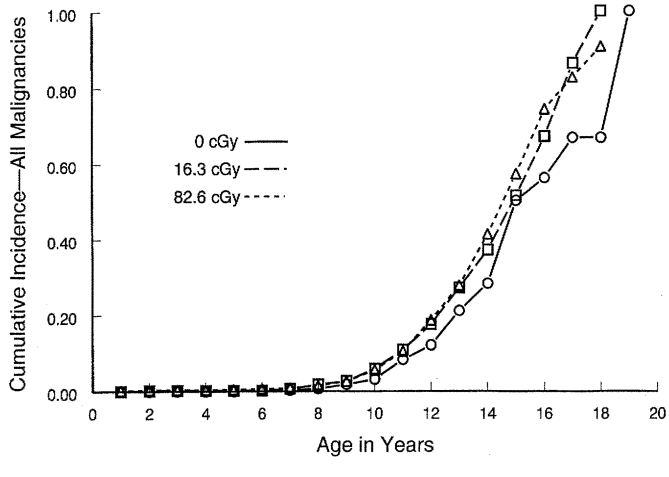

FIG. 16. Cumulative incidence of all malignancies that contributed to mortality in irradiated beagles. All exposure ages and both sexes are combined to create three dose groups.

The trend test for an increased risk for all fatal malignancies and all fatal neoplasms related to death was significant in four of six experimental groups, though this was found for only one group (neonatally exposed dogs) with the hazard analysis. For both methods of analysis, this increased risk was most pronounced in dogs irradiated in the neonatal period. Considering the increased risk in the young dogs, especially after fetal exposure, this suggests that the perinatal period might be a particularly sensitive time for carcinogenesis. Such a conclusion is supported by the data on the earlier onset of all fatal tumors in perinatally irradiated dogs. While the numbers of tumors are smaller for each of the major individual types of neoplasms examined (lymphomas, hemangiosarcomas and "other" malignancies), there was advancement in the time of occurrence of the fatalities, i.e. a shortening of the latent period, only among the groups of dogs irradiated in the perinatal period. While this was certainly contributed to by the fatalities that occurred before 2 years of age, later-occurring neoplasms also had an impact on this finding. While there appeared to be some increased risk in dogs irradiated at 8 days postcoitus and at 365 days postpartum, a similar shortening of the latent period was not seen. If the radiation effect was not real, one would not expect such a consistent finding across tumor types with respect to a reduced latent period after irradiation. Further, the finding of increased risk for astrocytomas and for nonfatal neoplasms, including incidental mammary carcinomas, after exposure in the fetal and early postnatal periods tends to support the validity of the findings with regard to fatal neoplasms. Thus, while the overall statistical evaluations do not indicate a strong radiation effect, we nonetheless believe that the data do demonstrate a real effect of the radiation exposures on increasing cancer risk, especially after late prenatal and neonatal exposures.

The finding of increased risk for lymphoid neoplasia in the dogs exposed as fetuses is interesting. The disease categorized as lymphoma in the dog is actually a complex of

syndromes including localized or multicentric solid lymphocytic neoplasms of lymph nodes and a variety of other organs, with lymphocytic leukemia in about 20% of the cases (*41*). Certainly, lymphomas are well documented to occur in a variety of other species after irradiation, most prominently in rodents (*42*, *43*). The data for humans for radiation-induced lymphoma have been inconsistent (*39*). One report on the atomic bomb survivors suggested a significant excess in males but in not females (*44*), but the most recent data do not support an increase in either sex (*45*). Some recent epidemiological studies have reported increases in lymphoma in patients with ankylosing spondylitis who had been irradiated (*46*) and in prenatally irradiated children (*8*), but others have shown no such effect (*47*, *48*). Lymphoma is one of the most common fatal neoplasms of the canine (*41*, *49*). The beagle, which has a relative risk for lymphoma of 1.1 compared with all other breeds (*40*), is not particularly prone to lymphoma occurrence. Two smaller studies of beagles that received protracted whole-body irradiation from injected ^{137}CsCl did not show any evidence of an effect on lymphoid neoplasia even though juvenile dogs as well as adults were exposed (*50*, *51*).

The finding of an increase in fatal hemangiosarcomas was unexpected since this neoplasm has usually been associated with induction by relatively high-dose radiation. Hemangiosarcomas have been reported in humans after therapeutic radiation doses (*52*). Hemangiosarcomas have been reported in beagles exposed to a variety of internally deposited radionuclides, resulting in very high doses (of the order of tens of grays) to the target organ systems (*53*, *54*). We previously reported an increase in risk of hemangiosarcoma associated with ultraviolet-radiation-induced solar dermatosis on the unpigmented abdominal skin in our dogs (*26*). There was an apparent interaction between the ultraviolet exposure and ionizing radiation delivered at 365 days postpartum, but this would not influence the increases in fatal hemangiosarcomas we saw after prenatal exposures. Hemangiosarcomas are neoplasms which are particularly common in the dog, often occurring in a range of sites, including skin, heart, spleen and liver (*55*). One difference in our study was the frequent occurrence of hemangiomas and hemangiosarcomas of the conjunctiva and cornea as reported previously (*56*) and which was thought to be associated with solar ultraviolet radiation. However, these were not associated with mortality and thus do not have an impact on the current analyses. Since the dog is clearly more prone to the development of hemangiosarcomas than most species, this might have the effect of increasing the sensitivity to induction of these neoplasms by radiation. Our genetic analyses did indicate that there was a significant degree of heritability of these tumors; however, there was no evidence for an interaction between the genetic factors and radiation dose in this study.

The breast is one of the most sensitive organs to radiation in humans (*39*), and increased mortality from radiation-induced breast cancer is well documented (*57–62*). The lack of evidence for increased mortality from mammary cancer after irradiation in our dogs was unexpected. Other studies of irradiation in the beagle have demonstrated some sensitivity of the mammary gland to carcinogenesis. A study of whole-body X irradiation found increased breast cancer risk and mortality (*63*). A study of whole-body irradiation from injected ^{137}CsCl showed an increase in malignant mammary neoplasms which was related to an earlier onset of these tumors, though there was no dose response (*51*). Both studies involved irradiation of young adult dogs. There also are two reports of increased risk for mammary tumors in dogs injected with radium. Dogs injected with ^{226}Ra had a significant increase in malignant tumors (*64*), while dogs injected with ^{224}Ra had an increased age-specific incidence of all mammary tumors (*65*). Our data on non-fatal mammary neoplasia suggesting a radiation effect, particularly after exposures in the perinatal period, are consistent with the previous canine studies.

Myeloproliferative disorders and leukemias are among the most extensively studied radiogenic neoplasms. The evidence for induction by radiation in the human is strong (*39*, *44*, *45*, *66*) and includes evidence for increased risk after prenatal exposures (*2*, *3*, *6*, *9*, *11*). Beagles exposed to chronic ^{60}Co γ radiation (11 R/day), starting at either 21 days of gestation or 50 to 150 days after birth, had a dramatically higher risk for myeloproliferative disorders in the group initially irradiated as fetuses (*67*). Studies of beagles chronically irradiated with ^{60}Co γ rays (7.5 cGy/day) during the fetal period, or both fetally and postnatally, found that continuous irradiation over a long period was a more important determinant of myeloid leukemogenesis than whether the exposure started in the fetal or postnatal period (*68*). It is noteworthy that all the cases of myeloproliferative disorders/leukemias seen in our study were in dogs exposed in mid- to late gestation or as neonates and did not appear until the dogs were well into adulthood. Other studies of dogs which were exposed to a variety of internally deposited radionuclides including ^{144}Ce (*69*), ^{137}Cs (*50*) and ^{90}Sr (*70–72*) have confirmed this sensitivity to induction of myeloproliferative disorders. Priester and McKay (*40*) reported that the occurrence of all non-lymphocytic leukemias (including "miscellaneous" and unspecified types) was 0.18 cases/1,000 animal-years at risk. In our dogs irradiated at mid- or late gestation or as neonates, the myeloproliferative disorder/leukemia rate was approximately 0.75 cases/1,000 animal-years at risk, four times the "spontaneous" rate. These data suggest a radiogenic origin in our cases. The fact that leukemias in the dogs were increased after both prenatal and neonatal irradiation actually fits well with the latest data on the atomic bomb survivors (*24*), where it was reported that leukemia risk was raised comparably after either *in utero* or childhood exposure.

The suggested increase in astrocytomas and meningiomas in irradiated dogs is of interest in view of the evidence for similar effects in humans. There have been numerous reports of radiation-induced nervous system

neoplasms in humans, particularly gliomas, including astrocytomas, after therapeutic irradiation early in life (*73–76*). Prenatal human exposures have also been reported to increase central nervous system tumors (*2*, *9–11*). There is also extensive evidence for radiation-induced meningeal tumors after childhood irradiation (*73*, *77–80*). Animal studies, including one study of neutron-irradiated beagles (*81*), have reported increased brain tumors after irradiation, including gliomas and meningiomas (*82–84*). In our dogs, there was evidence for a particular sensitivity of the fetal animal to induction of astrocytomas, but the overall analysis of nervous system neoplasms did not reveal a particular early age sensitivity, since the dogs irradiated as young adults had the highest incidence.

The inference from epidemiological data that the human fetus has a relatively high sensitivity to radiation carcinogenesis has often been challenged on several grounds, including a purported lack of such prenatal sensitivity in experimental animals (*21*, *39*). There have been numerous studies of age-related sensitivity using external radiation exposure and internally deposited radionuclides in a variety of animals, primarily rodents [reviewed by Sikov (*85*, *86*)]. Studies have demonstrated both increases and decreases in specific neoplasms after prenatal or neonatal exposures, all of which must be considered in light of several factors. First, there are clearly differences in sensitivity among species and strains of animals used. Second, both dose and the specific time of irradiation with respect to gestation can influence tumorigenic responses. Third, whether animals are killed at predetermined times or allowed to live out their full life spans can influence the tumorigenic end point being evaluated. Finally, relatively few studies have compared concurrently the oncogenic susceptibility of animals exposed as fetuses and as adults.

Upton (*87*) reported that myelogenous leukemia was increased in mice irradiated as adults but not in those irradiated as fetuses. Other studies in mice have also shown a lack of carcinogenesis after fetal irradiation (*88–90*). Reductions in the incidence of neoplasms of the reticulum cell sarcoma/histiocytic lymphoma type in mice have also been reported after fetal irradiation (*91*, *92*). However, in some of the same experiments, the fetus was shown to be more sensitive to radiation-induced tumors of a variety of other organ systems, including lung, liver and pituitary gland (*92*), as well as other solid tumors (*91–94*). It also should be noted that decreased tumor incidence was often the result of high-dose radiation exposure in the fetal period, raising the question of the competing effects of cell killing and neoplastic transformation. A few rodent studies also have addressed differences in sensitivity between the fetus and the neonate, as well as between the neonate and the adult. In some studies carcinogenic responses were similar between fetal and neonatal exposures (*87*, *95*). Data from other studies suggested that the neonatal sensitivity of the mouse was actually greater than that of the fetus or the adult, at least for some neoplasms (*91*, *92*, *96*). Some organs, like the pituitary gland, show the highest sensitivity in the rodent fetus (*84*, *92*). Thus the data for rodents are conflicting and are difficult to interpret with respect to prenatal and neonatal sensitivity.

Doll and Wakeford (*19*) recently pointed out several grounds for the continuing controversy over the effects of *in utero* irradiation. Among these was the concern that the excess risk associated with fetal exposure is substantially higher than that derived from childhood exposure. It has been stated that there is no obvious reason why the susceptibility of the human to radiation should suddenly change at birth (*21*). Data from the dogs studies reported here support the thesis that there is *not* a sudden drop in radiosensitivity at birth and, if anything, the neonatal period had an even greater responsiveness to carcinogenesis than the fetal period. It is true that the average doses to the dogs exposed as neonates were about 10% higher than those for the other groups, but this was taken into account in the analysis and would not change the response enough to alter this conclusion significantly. It is worth noting, however, that the dogs exposed in the juvenile period (70 days postpartum) showed little evidence for increased risk except for thyroid neoplasia (*25*). Thus, though a dramatic change in radiation sensitivity may not take place at birth, sensitivity might change significantly during the first months of postnatal life. The recent report on the atomic bomb survivors exposed *in utero* or during early childhood and followed through 1992 did not support such a drastic change in radiosensitivity from prenatal to postnatal life. Risk for both solid cancers and leukemia was increased comparably in persons irradiated either *in utero* or during the first 6 years of childhood (*24*).

A second long-standing objection to the idea of a causal relationship between prenatal irradiation and childhood cancer is the fact that in prenatally exposed infants both the leukemias and all the major groups of solid tumors are increased almost equally, "a situation quite uncharacteristic of any other human or animal exposure" where leukemias predominated (*21*). From the data for our dogs, there does appear to be evidence of a radiation-induced increase in a broad spectrum of neoplasms, including both leukemia and a variety of solid neoplasms, including those of the nervous system, both early in life and throughout the lifetime of the exposed dogs. This contradicts the "animal exposure" part of this objection. Further, the recent data for atomic bomb survivors exposed *in utero* indicated a predominance of solid cancers occurring in later life (*24*).

Another issue that has been cited as an inconsistency (*97*) in the data for humans is the extraordinarily high fetal risk factor for exposures in the first trimester of pregnancy reported for the Oxford Survey (*17*, *98*). The recent data on the atomic bomb survivors are interesting in this regard as they show some evidence for a higher risk after *in utero* exposure during the first trimester (*24*). The first trimester of human pregnancy encompasses all events from fertilization through the end of major organogenesis. In the dog, both 8 and 28 days postcoitus would fall within

the equivalent developmental period of the first trimester in humans. The dog study is interesting in that the results suggest that there was increased risk after exposures very early in gestation at 8 days postcoitus, or preimplantation, but not at 28 days postcoitus, late in organogenesis. It is not clear why there should be an effect before implantation of the conceptus but not during organogenesis.

Finally, one of the major remaining questions relating to prenatal radiation exposure is whether there is an increased lifetime risk for carcinogenesis. The results of the dog study reported here suggest that there is a significant lifetime risk for cancer associated with both fetal and neonatal irradiation. The initial 1988 report (*23*) of increased adult risk after prenatal exposure in the atomic bomb survivors has been supported by the more recent data, as noted above (*24*). However, because the populations irradiated *in utero* and during childhood are just now reaching the ages where cancer incidence will rise sharply, and because the data for exposures *in utero* are based on only 10 cases, the full impact is yet to be determined.

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