is clear that other organs besides the spleen allow for at least minimal proliferation of the tumor. This explains why residual tumor cells could be found for several months in mice splenectomized before tumor cell injection. However, these residual cells did not show progressive malignant growth in the absence of a spleen despite their ability to grow progressively after being transferred to normal mice. In addition, progressive tumor growth in animals splenectomized after the development of marked leukocytosis shows that other tissues can support substantial proliferation during the later phases of tumor dissemination. Thus the spleen may modulate tumor growth in other organs and not just provide an obligatory site for the proliferation of BCL_1 cells.

The mechanism of the interaction between the tumor cells and the spleen cells or their products remains to be elucidated. The spleen may provide a specialized microenvironment for lymphoid tumor proliferation similar to that which supports the proliferation and differentiation of hematopoietic stem cells (*7*). Our demonstration that implantation of normal splenic fragments can initiate progressive tumor growth in splenectomized recipients strengthens this hypothesis. Variations of this experimental protocol (for example, implanting dissociated cells or fragments in Millipore chambers) will help clarify the spleen-tumor interaction. The mechanism by which BCL_1 cells grow progressively after splenectomy late in the course of the disease is also unclear. It is possible that the tumor cells alter their growth requirements, or that the other tissues provide a more "fertile soil" after substantial infiltration by tumor cells.

Other murine B cell tumors also localize in the spleen; the process is related to the differentiation stage and to cell surface characteristics (*8*). However, passage of these other tumor cells to splenectomized mice and subsequent transfer to other mice was not investigated. The modulation of BCL_1 growth by the spleen suggests that the tumor still responds to normal growth or to differentiative signals. Thus this experimental system provides a useful model by which the growth of a tumor similar to chronic lymphocytic leukemia or lymphocytic lymphoma in man can be manipulated by altering normal signals in mice.

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Bone Cancer from Radium: Canine Dose Response Explains Data for Mice and Humans

Abstract. *Analysis of lifetime studies of 243 beagles with skeletal burdens of radium-226 shows that the distribution of bone cancers clusters about a linear function of the logarithms of radiation dose rate to the skeleton and time from exposure until death. Similar relations displaced by species-dependent response ratios also provide satisfactory descriptions of the reported data on deaths from primary bone cancers in people and mice exposed to radium-226. The median cumulative doses (or times) leading to death from bone tumors are 2.9 times larger for dogs than for mice and 3.6 times larger for people than for dogs. These response ratios are well correlated with the normal life expectancies. The cumulative radiation dose required to give significant risk of bone cancer is found to be much less at lower dose rates than at higher rates, but the time required for the tumors to be manifested is longer. At low dose rates, this time exceeds the normal life-span and appears as a practical threshold, which for bone cancer is estimated to occur at an average cumulative radiation dose to the skeleton of about 50 to 110 rads for the three species.*

Knowledge of the effects on people of bone-seeking radionuclides is based primarily on studies of the luminous dial of painters and others who accidentally ingested or were given dosages of radium (*1*). However, the best data relating dosages, radiation dose to bone, and observed effects from exposures to radioactive materials have been obtained from animal studies. In particular, the Department of Energy and its nominal predecessor, the Atomic Energy Commission, have long supported work to develop the beagle as a reliable experimental model (*2*) for radiation dose effects, with the intent of extrapolation to human populations. We used beagle dose-response data for the bone-seeking radionuclide radium-226 to evaluate the relations among radiation dose rate, cumulative dose, time until death, and incidence of bone cancer.

To temporally imitate the medical and occupational exposure of people to ^{226}Ra, 243 purebred beagles of the University of California's Davis colony were administered six graded doses of ^{226}Ra (*3*). Intravenous injections were begun when the beagles were 14 months old and were continued fortnightly until they were 18 months old, so that each dog received eight injections. These dogs and 78 unexposed controls have been under study for the past 18 years.

Skeletal burdens of ^{226}Ra were measured during and after the period of injections by means of whole-body radiation counting of radon daughter gamma-ray emissions with NaI(Tl) scintillation crystal detectors in conjunction with multichannel pulse-height analyzers (*4*). Each measured skeletal activity burden of ^{226}Ra was used to calculate a corresponding dose rate to the skeleton from ^{226}Ra and associated ^{222}Rn and progeny by

$$D(t) = \frac{51.2A(t)[\bar{E}_{Ra} + R(t)\bar{E}_{Rn}]}{m(t)} \quad (1)$$

(*5*), where t is time; $D(t)$ is the dose rate in rads per day; $A(t)$ is activity in microcuries: $\bar{E}_{Ra}$ is 4.86 MeV, or the total energy deposited by decay of ^{226}Ra alpha particles (from recoil nuclei and 4.78- and 4.60-MeV alpha particles); $\bar{E}_{Rn}$ is 20.36 MeV, the average energy deposited by ^{222}Rn with the daughters ^{218}Po, ^{214}Pb, ^{214}Bi, and ^{214}Po in secular equilibrium (of which 19.16 MeV is from alpha particles, 0.34 MeV from recoil nuclei, and 0.86 MeV from beta particles); $R(t)$ is the radon-to-radium activity ratio given by Parks *et al.* (*4*); and $m(t)$ is the mass of the skeleton in grams (*6*). Since the irradiation is primarily alpha, it can be expected to have a quality factor (QF) of about 10 to 20 (*7*). Dose rate values were smoothed numerically with time to yield an average daily dose rate, $\bar{D}$, from the beginning of exposure until death for each dog. For comparison, the unexposed controls were assumed to have re-

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ceived average dose rates between 3×10^{-4} and 1×10^{-3} rad/day.

To elucidate the dose-response relations with respect to time until death, we developed a graphic representation of the three-dimensional dose-response phenomenon (*8*). The logarithmic coordinates are the fundamental variables of time from exposure until death, t, and the average dose rate to the skeleton, $\bar{D}$ (Fig. 1). The beginning time for exposure was taken as the midpoint of the period of repeated injections. Each dog is represented as a point that identifies the dog as living on 30 June 1978 or identifies primary cause of death. The third perpendicular dimension, although not plotted, is the response or incidence distribution (probability density), whose magnitude is indicated by clustering of individual values.

Natural, nonradiogenic deaths were more frequent with increasing age. Using control dogs living longer than 3000 days, we characterized the old-age-related peak of the natural deaths as having a lognormal distribution with median age 5350 days [geometric standard deviation (G.S.D.), 1.12]. This yielded the median time to death t_L from the midpoint of the exposure interval of 4865 days for these old-age-related deaths. The relation is assumed to be independent of the dose rate (Fig. 1).

No deaths could be attributed to exposure of the skeleton to average dose rates below 0.05 rad/day, equivalent to 0.5 rem/day (with QF = 10)—about 1000 times greater than background levels. On the other hand, all dogs receiving > 1 rad/day died prematurely. Eighty percent of these deaths were from primary bone cancer (in > 97 percent of the cases, osteosarcoma); the remainder were primarily from concurrent competing risks, especially complications of pathologic fractures of the bone.

A clear dose-response relation for bone cancer is indicated by these data. To evaluate this function separately, the distribution of deaths from primary bone tumors was approximated by a linear function of the natural logarithms of average daily dose rate and time until death

$$\ln t = \ln K - S \ln \bar{D} \qquad (2)$$

with K and S characteristic parameters to be determined. A linear regression in the logarithmic coordinates was then performed. With this relation, the distribution of deaths is implicitly treated as being lognormal at a given average dose rate or time, with standard deviations ln (G.S.D.) and [ln (G.S.D.)]/S, respectively. The fitted value of K is the median of values about the regression line. Standard least-squares analysis for the 116 bone cancer cases yielded a median $K = 2500$ [geometric standard error (G.S.E.), 1.02; G.S.D., 1.24] and $S = 0.29$ (S.E., 0.01), with $r = .92$ ($P < .001$ for zero correlation). Hence, much smaller cumulative doses are involved in the occurrence of bone cancer at lower dose rates, and the cumulative dose alone is not an accurate indicator of risk. The average cumulative dose to the

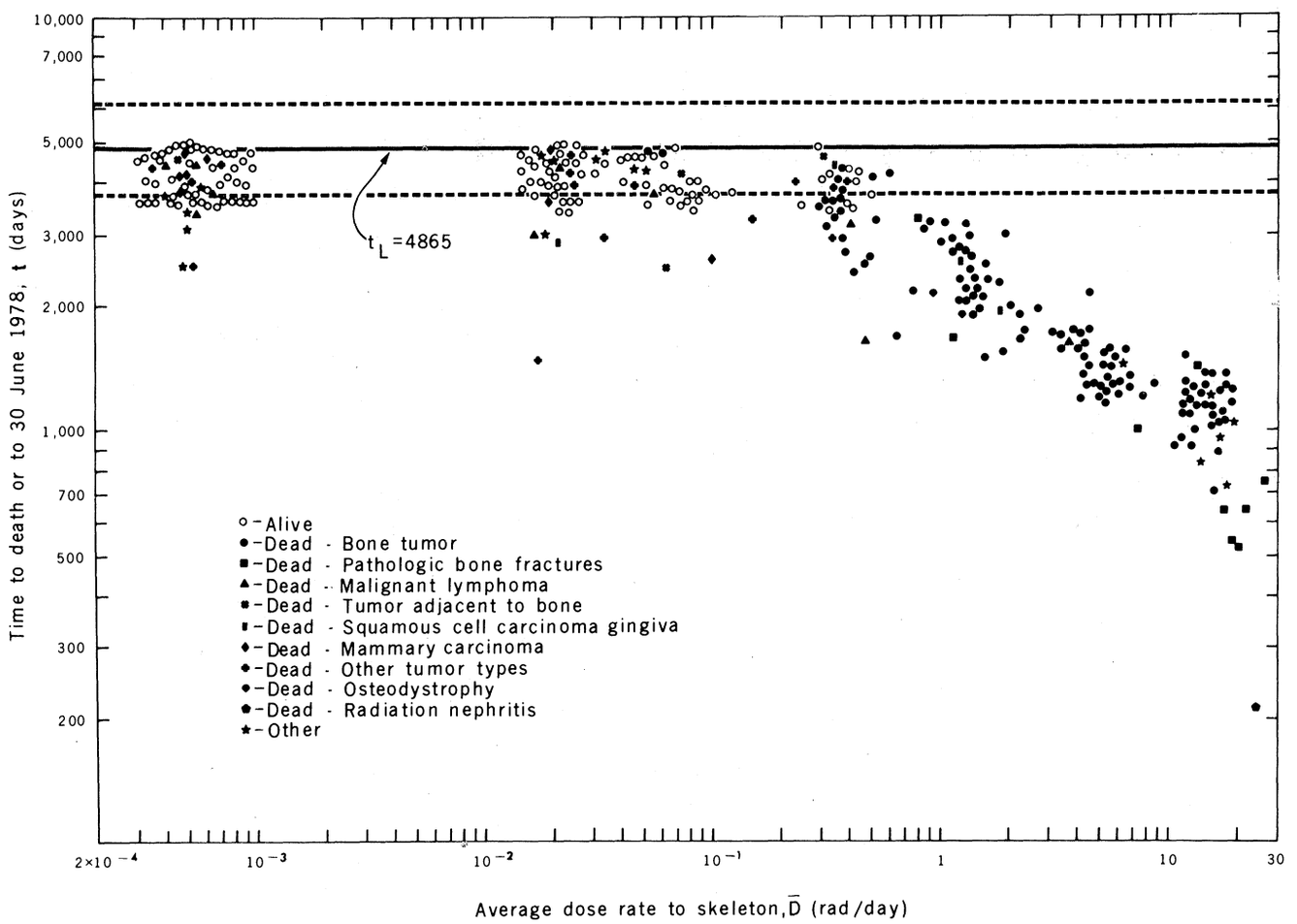

Fig. 1. Fate of the Davis beagles injected with ^{226}Ra. Each individual is represented by a symbol plotted with the logarithmic coordinates of (i) time until death (from the midpoint of the exposure period) or to 30 June 1978, and (ii) average skeletal dose rate from radium and radon in equilibrium with the short-lived progeny. The solid line shows the median time of spontaneous deaths among controls after 3000 days of life, and each dashed line indicates two geometric standard deviations from the median.

skeleton is only 1300 rads at the exposure rate of 0.4 rad/day compared to 13,000 rads at 10 rad/day, and the relative dose effectiveness in this case is 10 (*9*).

We similarly analyzed data on female CF_1 mice injected with ^{226}Ra (*10*) and on humans who had been exposed to radium (*11*) (Fig. 2). Our analysis of the latter was limited to those whose skeletal burden was mostly (two-thirds or more) associated with ^{226}Ra, since ^{228}Ra was also present in some cases. Most individuals with the higher radium burdens (about 80 percent of whom were women) have since died prematurely, primarily from cancer. Of 84 deaths among persons with average dose rates to the skeleton exceeding 0.1 rad/day, 34 were from bone cancer, 23 were from other cancers, and the remainder were from unclear causes, possibly including spontaneous fractures. However, these deaths were concurrent with bone cancer deaths, so we evaluated the apparent bone cancer dose-response relation separately. The median age at initial exposure for the persons who developed bone cancer was ~ 20 years, and all but three were women. We assumed for the sake of comparison that the median time for the peak of natural nonradiogenic deaths would have been 20,000 days from the initial exposure.

The large numbers of mice necessitated plotting the data by dosage groups (*10*). Radiation doses for the groups were calculated by assuming the metabolic data of Miller and Finkel (*12*); the average mouse skeleton was assumed to weigh 3.7 g. The values agreed with those of Goldman *et al.* (*8*). Using control data, we calculated a median time until death of 655 days for mice 70 days old at first exposure.

Neither the data for the humans nor those for the mice were characterized by the precision of the data from the Davis beagle study, but the same basic dose-response function displaced by a species-dependent response ratio was apparent (*13*). Therefore the functional relation of Eq. 2 was applied to the human and mouse data, with S equal to 0.29 as for the dogs. This yielded median values of K = 850 (G.S.E., 1.11; G.S.D., 1.3) for mice and K = 9000 (G.S.E., 1.06; G.S.D., 1.39) for people (Fig. 2). With beagles as the reference species, the response ratio for people is 3.6; therefore, about 3.6 times more total radiation exposure was required to achieve the median bone tumor response in people as in dogs. Likewise, for mice the response ratio is 0.34; only one-third as much total radiation was required to produce the median bone tumor response in mice as in dogs.

We regressed the values for K (which are proportional to the response ratio values) with respect to the approximate normal life expectancies of 70, 15, and 2

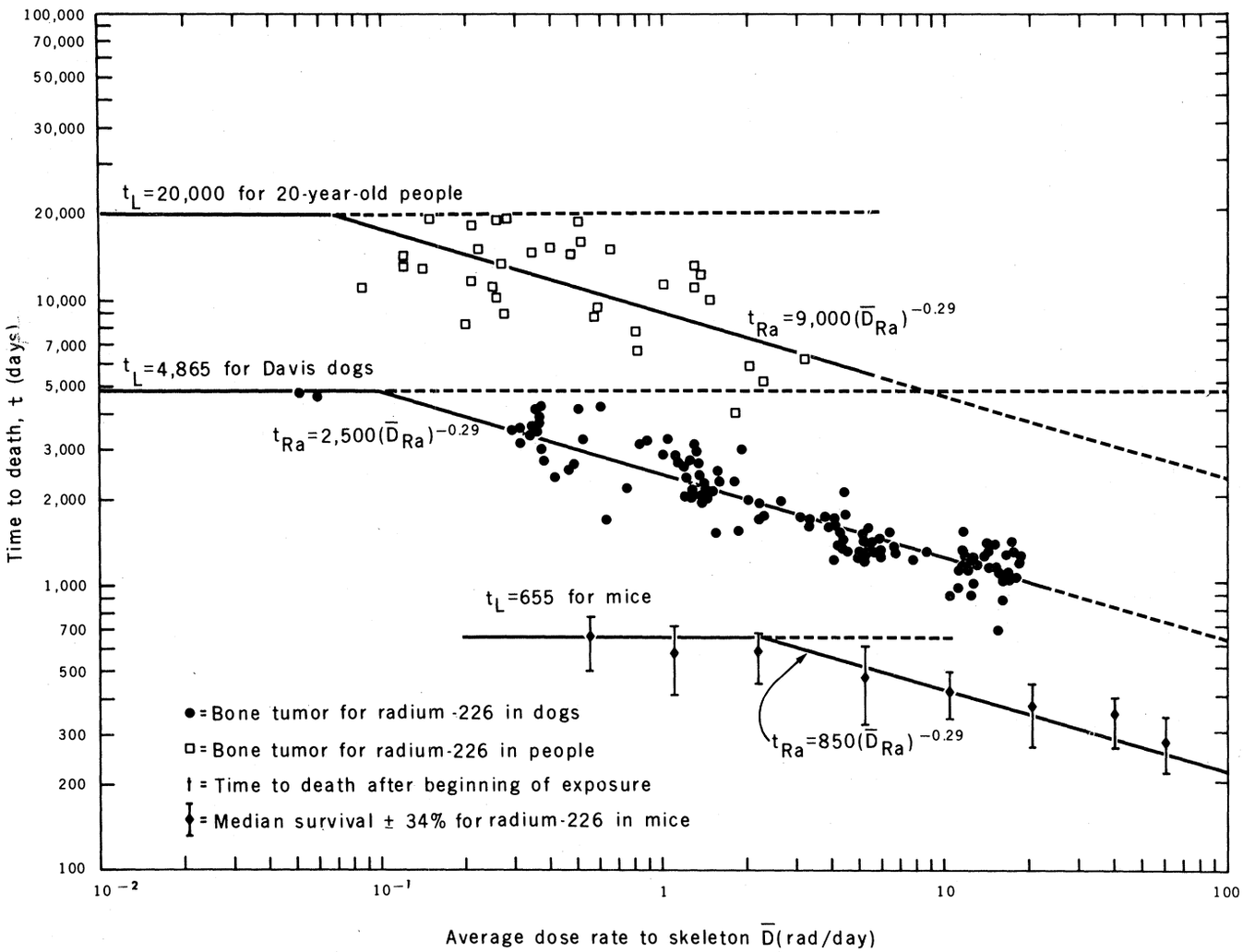

Fig. 2. Primary bone tumor deaths from ^{226}Ra for people, Davis beagles, and female CF_1 mice. Similar dose-response functions are indicated by parallel lines with negative slope, which represent the medians of the respective distributions of death from bone cancer. Also shown by horizontal parallel lines are median times of the occurrence of deaths related to old age.

years for people, Davis beagles, and female CF_1 mice, respectively, and obtained

$$K = (655 \pm 57) + (119 \pm 1)\,L \quad (3)$$

where L is the normal life expectancy in years, with $\chi^2 = .7$ and $r = .9999$ ($P < .01$ for zero correlation). Hence, the species-dependent response ratios are also linearly related to the respective life expectancies.

Figure 2 shows the dose responses and similar relations describing the competing risk of natural death from old age. As individuals age, they enter regions of increasing risk. At high dose rates, the region of high risk for bone tumors is encountered before the region of high risk for natural death, so that premature death from bone cancer is more probable than death from aging processes. At low dose rates, on the other hand, natural deaths occur before bone tumors develop.

The intersection of these two risk distributions, shown in Fig. 2, explains the practical threshold for bone cancer in people noted by Evans *et al.* (*1*). We speculated that this practical threshold occurs for each species at a dose rate that corresponds to the intersection of t_L and a cancer risk that occurs three geometric standard deviations earlier than the median. For the skeleton of man, dog, and mouse, this yielded calculated practical threshold dose rates of 0.0039 rad/day (0.039 rem/day), 0.011 rad/day (0.11 rem/day), and 0.16 rad/day (1.6 rem/day), respectively, and cumulative doses of 80 rads (800 rem), 50 rads (500 rem), and 110 rads (1100 rem), respectively (with QF = 10).

If it is assumed that the lognormal risk distribution we observed can be extrapolated to the limit and that competing radiogenic risks occur concurrently, then the risk associated with the currently accepted maximum permissible bone burden of 0.1 μCi of ^{226}Ra (0.0082 rad/day) for industrial workers (*1*) can be estimated. If 400 workers remain at the maximum permissible bone burden for 50 years beginning at age 20, we estimate that only one would die from radiogenic bone cancer or other abnormality, 200 would die from nonradiogenic causes, and 199 would still be alive. The time required to reach the median of the bone cancer risk distribution function would be 99 years.

We believe that this analysis provides an improved perspective of the relations among radiation dose, time, and response in animals and man. Further, it elucidates the effect of dose rate on the incidence of biological effects and shows that the cumulative radiation dose alone is not an accurate indicator of risk with respect to the smaller cumulative doses required at lower dose rates to yield a specific bone cancer risk. Dose responses were clearly nonlinear at any time after initial exposure or at a specific dose rate and were satisfactorily represented as lognormal. The resultant interspecies comparison provides response ratios that may be applicable to injury from other radioactive materials or carcinogenic agents.

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Diphenylhydantoin: Pre- and Postnatal Administration Alters Diazepam Binding in Developing Rat Cerebral Cortex

Abstract. *Close correlations between the development of the anticonvulsant effects of diphenylhydantoin and increases in tritiated diazepam binding were observed in rats from fetal day 16 to maturation. In contrast, significant decreases in tritiated diazepam binding were observed in 2- and 3-week-old rats that were exposed in utero to diphenylhydantoin. These changes can be correlated with reported increases in seizure susceptibility after prenatal exposure to diphenylhydantoin.*

Studies on the maturation of the central nervous system and the development of seizures suggest that excitatory and inhibitory systems in the rat develop with a characteristic time sequence (*1*). From these ontogenic studies it appears that diphenylhydantoin (DPH) has a biphasic effect in the brains of maturing rats: excitatory effects in rats of less than 12 days of age and then increasing inhibitory effects that can be correlated to the maturation of inhibitory systems by 17 to 21 days of age (*2*).

We have reported that treatment of adult rats with anticonvulsant, but not subanticonvulsant, doses of DPH significantly increases (*3*) specific high-affinity benzodiazepine binding (*4*) in rat brain tissue. We have now examined the effects of DPH on benzodiazepine binding at various stages of neuronal maturation and compared these effects to the devel-

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