Irradiation-induced Erythroleukemia and Myelogenous Leukemia in the Beagle Dog: Hematology and Ultrastructure

By T. M. Seed, D. V. Tolle, T. E. Fritz, R. L. Devine, C. M. Poole, and W. P. Norris

A high incidence of leukemia in adult beagle dogs was induced by continuous whole-body exposure to low doses of $^{60}$Co gamma irradiation. At 5, 10, and 17 R per 22-hr exposure day, 20 animals of 53 died of either myelogenous leukemia (15 of 20) or erythroleukemia (5 of 20); the latter occurred only at 5 R/day. Consistent preclinical changes occurred in the peripheral blood, including (1) a partial recovery from an initial severe leukopenia, (2) a prolonged accommodation-to-irradiation phase, and (3) marked oscillations in platelet values in the preleukemic period. In the terminal condition the dogs were severely anemic, thrombocytopenic, and commonly leukopenic. Peripheral blood buffy-coat preparations contained circulating “blast” cells and juvenile forms. Abnormal erythrocyte and platelet morphology was consistently present. The bone marrow was altered most severely; other organs showed variable degrees of leukemic infiltration and proliferation and loss of normal tissue architecture. The marrow was hyperplastic with little or no fat remaining. Differential marrow cell counts showed increased numbers of immature cell forms. Myeloid:erythroid (M:E) ratios ranged from 2.6:1 to 61.5:1 in the granulocytic leukemias, and 0.2:1 to 1:1 in the erythroleukemias. Juvenile leukemic cells (both circulating and tissue forms) displayed a number of distinctive cytologic features, including asynchronous patterns of nuclear-cytoplasmic maturation, increased incidence of nuclear clefts, coalescence of cytoplasmic granules, and bizarre arrangements of endoplasmic reticulum. These experimentally induced canine leukemias have many hematologic and cytologic features in common with both spontaneous and radiation-induced leukemias of man. Thus, they may provide a useful model for the study of human leukemia.

The incidence of spontaneous myeloproliferative disorders (MPD) in most domesticated animals is quite low. The precise incidence of spontaneous MPD in the dog is unknown, but it is clear that it rarely occurs. Consequently, any case experimentally induced is considered significant. In contrast, MPD appear to occur much more frequently in man. The etiology of spontaneous leukemia in both man and animals is largely unknown, but factors such as chromosomal aberrations, exposure to certain toxic chemicals (e.g., myelosuppressive drugs), oncogenic viruses, etc. appear to be causally related.

There is little question that exposure to certain types of radiation can be leukemogenic to man and lower animals. An increased incidence of MPD has been reported in atomic bomb survivors, radium dial painters, etc.
and patients with ankylosing spondylitis who have been treated by x-irradiation.9

The dog, like man, is susceptible to the leukemogenic effects of irradiation. Continuous experimental exposure either to gamma rays (e.g., $^{60}$Co) or to radionuclides deposited within the body ($^{137}$Cs, $^{144}$Ce, $^{90}$Sr) has produced MPD.5,11,13 In all of these studies, irradiation dose and dose rate seem to be critical factors in determining the incidence.16 The hypothesis presented by Dameshek,17 Cronkite,18 and others11,19 seems to be applicable: i.e., continuous low-level irradiation of the marrow does not completely prevent its hematopoietic function, but puts the marrow under continuous, maximal stress and forces it to accommodate by producing clones of hematopoietic cells with abnormal radioresistant properties.

Whether oncornaviruses play an inductive role in radiation-promoted human and canine MPD, as they seem to do in the rodent models,14 is obscure. Certainly, within the transplantable $^{90}$Sr-induced myelomonocytic leukemias in miniature swine there is strong evidence of C-type virus involvement.20 Similar suggestive evidence is being collected for human spontaneous myelocytic leukemia.21

In this paper we present the terminal hematologic picture of dogs with myeloproliferative disease induced by continuous exposure to a $^{60}$Co gamma-ray source and describe in detail the ultrastructural features of the leukemic cell population and its supportive structures. Our purpose is to explore the potential usefulness of this malignancy as an experimental model of human disease.

**MATERIALS AND METHODS**

**Animals**

Sixty-one anatomically normal, healthy, beagle dogs of both sexes approximately 400 days of age were selected from a large outbred colony. The status, origin, and general management of the Argonne National Laboratory beagle colony has been described in detail elsewhere22. Fifty-three of these dogs were assigned to experimental groups and the remainder served as controls. All dogs were caged starting 2 wk prior to beginning radiation treatment. During this period the animals were given physical examinations, which included obtaining baseline hematologic values. Control dogs were handled in an identical fashion, except they were caged for the duration of the experiment in an anteroom adjoining the radiation facility. The animals had water ad libitum and standard dog food once a day (Rockland Dog Diet, Teklad, Monmouth, Ill.).

**Irradiation**

Dogs were irradiated for 22 hr/day for duration of life within a specially designed $^{60}$Co gamma-irradiation facility capable of delivering doses of 5–35 R/day. The dogs received an accumulated dose of either 5, 10, or 17 R/day of gamma rays12 by arranging the cages at appropriate distances from the source.

**Hematology**

Hemograms were performed on each irradiated animal every 14 days and on each control animal every 28 days. Blood samples were collected, via jugular venipuncture, into vacutainer tubes containing EDTA. Erythrocyte and leukocyte counts were performed electronically. Packed cell volumes were determined by microhematocrit centrifugation methods. Peripheral blood platelets were enumerated by direct observation with phase contrast optics. The animals had water ad libitum and standard dog food once a day (Rockland Dog Diet, Teklad, Monmouth, Ill.).

**Hemoglobin**

Hemoglobin was measured spectrophotometrically as cyanmethemoglobin at 540 nm. Differential white blood cell determinations were made by direct microscopic examination of Wright-stained thin films.
When patently leukemic animals became acutely ill, or control tissues were needed from non-irradiated beagles, the animals were sacrificed by exsanguination while under Surital (sodium thiamydal, Parke Davis) anesthesia. Gross pathologic changes were recorded and tissue samples were taken for light microscopic examination. Touch imprints were made from femoral bone marrow, spleen, liver, and various excised lymph nodes. These imprints were stained with Wright's double-strength Giemsa reagent, coverslipped, and interpreted by light microscopy. On selected imprints, peroxidase, Sudan black B, and PAS stains were used. Bone marrow differential cell counts (based on 1000 cells) were performed and myeloid:erythroid (M:E) ratios were calculated.

**Electron Microscopy**

At necropsy, small pieces of spleen, liver, and selected lymph nodes were quickly excised and placed in pools of chilled 2.5%, glutaraldehyde in 0.1 M phosphate buffer, pH 7.25. Bone marrow samples were collected from longitudinally split femurs; bone pieces were gently teased apart and the marrow was flooded with fixative. All excised tissues were cut into 1-mm cubes and placed in fresh fixative. After approximately 8 hr they were rinsed in buffer and postfixed in buffered 1%, osmium tetroxide for 1 hr; then they were dehydrated in ascending concentrations of ethanol and finally in 100%, propylene oxide. Tissues were infiltrated and embedded in Epon Araldite according to standard methods. Multiple blocks of each specimen were cut. Sections were stained with uranyl acetate-lead citrate, coated with evaporated carbon, and examined with a Siemens's 101 electron microscope.

**RESULTS**

**Incidence**

At 5, 10, and 17 R gamma rays per 22-hr exposure day, 20 animals of 53 died of either myelogenous leukemia (15 of 20) or erythroleukemia (5 of 20). The erythroleukemic deaths occurred only at the lowest exposure rate, 5 R/day (Table I). The overall incidence of these two types of leukemia at the various dose rates was 11 of 24 at 5 R/day, 7 of 16 at 10 R/day, and 2 of 13 at 17 R/day. The mean survival times were 1456, 848, and 1038 days for total exposures of 7380, 8480, and 17,646 R, respectively.

Septicemia (7 of 13) and aplastic anemia (4 of 13) accounted for the nonleukemic deaths at 17 R/day. These syndromes occurred early in the course of the experiment with mean times to death of 139 and 270 days, respectively. At 10 R/day the nonneoplastic deaths were predominantly the result of aplastic anemia (7 of 16) occurring fairly early (relative to the induction times of leukemia) with a mean time of 264 days. A late occurring lymphoproliferative disease (1966 days) and a mammary carcinoma (1332 days) accounted for the remaining 2 deaths within the 10 R/day group. At 5 R/day, the nonleukemic

<table>
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<th>No. of Dogs</th>
<th>Bone Marrow Interpretation</th>
<th>Mean RBC (x 10^{12}/liter)</th>
<th>Mean WBC (x 10^{9}/liter)</th>
<th>Mean Platelets (x 10^{9}/liter)</th>
<th>Mean 1000-Cell Myeloid:Erythroid Ratio</th>
<th>Incidence of MPD in Descendants</th>
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<td>5</td>
<td>Erythroleukemia</td>
<td>1.43 ± 0.45*</td>
<td>11.2 ± 5.8</td>
<td>3.1 ± 2.9</td>
<td>0.46:1 ± 0.34</td>
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<td>15</td>
<td>Granulocytic leukemia</td>
<td>1.91 ± 1.23</td>
<td>13.8 ± 14.0</td>
<td>7.9 ± 13.6</td>
<td>12.86:1 ± 15.1</td>
<td>28.8 (15/52)</td>
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<td>Normal</td>
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<td>10.3 ± 0.2</td>
<td>375.0 ± 100.0</td>
<td>1.5:1 ± 0.05</td>
<td>0.0 (0/127)</td>
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*Mean ± SD.
syndromes included marrow aplasia (4 of 24), a variety of nonleukemic malignancies (3 of 24) (e.g., osteosarcoma, mammary and ovarian carcinoma), and other degenerative diseases (e.g., hepatic degeneration).

Peripheral Hematology

All dogs that developed leukemia exhibited similar blood responses during the course of irradiation (Fig. 1). All animals irradiated initially developed a progressively severe leukopenia and thrombocytopenia. Leukocyte and platelet values continued to fall until the accumulated dose was approximately 2000 R. At this critical period, a number of dogs died of marrow aplasia, complicated at times by septicemia (e.g., approximately 50% at 10 R/day). In the survivors, a partial hematopoietic recovery occurred, and leukocyte and platelet values slowly rose and stabilized at approximately one-half their normal values. During the initial depression and recovery period abnormal cellular morphology was not observed. There was, however, a slight increase in the number of basophilic lymphocytes during the early part of the recovery phase. Anemia developed slowly and was usually mild, except in the terminal condition.

Between 200 and 400 days before death with leukemia, the majority of dogs exhibited a rather predictable exaggerated oscillation in platelet values, with values in some dogs ranging from $1.5 \times 10^9$ to $1.0 \times 10^{12}$/liter. At this time various morphologic abnormalities in circulating erythrocytes were common.
Fig. 2 Thin buffy-coat film from a dog terminally ill with myelogenous leukemia. Myeloblasts and other immature granulocytes dominate the white cell population. Note the three large myeloblasts with aberrant nuclei containing multiple nucleoli (arrows). Above these cells are two fairly normal segmented neutrophils. The abnormal red cell morphology is indicative of pronounced terminal anemia. Wright-Giemsa. x 1350.

Figs. 3 and 4 Peripheral blood film prepared at the time of death showing abnormal cytologic features of myelocytic leukemic cells. Fig. 3: Immature granulocyte with cytoplasmic blebs (arrows) and atypical granules. Note the nucleated red cell in the upper left-hand corner and again the abnormally shaped red cells. Fig. 4: Immature granulocyte with apparent coalescence of cytoplasmic granules (arrow). Note the multiple nucleoli. Wright-Giemsa. x 1350.

Fig. 5 Impression imprint from bone marrow of a dog with myelogenous leukemia. Note its high cellularity. Field is filled mainly with immature granulocytes, myeloblasts, and promyelocytes, with only a scattering of mature forms. Erythrocytic and megakaryocytic elements are few in number. Wright–Giemsa. x 330.

Fig. 6 Tissue impression imprint of erythroleukemic bone marrow. Hypercellular condition is caused by a proliferation and/or accumulation of immature erythrocytic precursors, especially the basophilic erythroblasts (arrows). Many of these cells are megaloblastic and have bizarre nuclear configurations. Wright–Giemsa. x 450.
(e.g., anisocytosis, poikilocytosis, macrocytosis, target cells, and nucleated forms). A few dogs had increased numbers of immature granulocytes in their peripheral blood, although circulating blast cells were not yet observed.

All leukemic dogs entered an acute phase of patent disease approximately 50–100 days before death. During this period immature granulocytes and circulating blasts were evident in buffy-coat preparations (Fig. 2–4, 7, and 8). Lymphopenia occurred concurrently with a shift in the neutrophil population toward the less mature cell types. Abnormal cell morphology was observed, including asynchrony of the nuclear–cytoplasmic maturation sequence, monocytoid features of some granulocytes (Figs. 2 and 8), neutrophilic agranularity in some cells, and prominent cytoplasmic blebs in others (Fig. 3). Although Auer bodies were not observed, atypical granule formation with coalescence of granules and margination of granules was prominent in a few cases (Figs. 3 and 4). Circulating nucleated red cells (NRBC) were found in all leukemic dogs; however, in cases of erythroleukemia large numbers of NRBC (i.e., 25–325–200 white cells), including basophilic erythroblasts, were observed. Many of the erythroid precursors had megaloblastic features with bizarre, double and triple nucleated forms. Giant platelets and megakaryocytic fragments were common in the peripheral blood of all leukemic animals. Subpopulations of these cells contained large inclusion bodies and a central coalescence of granules, surface-connected channels, and microtubules (Fig. 7). Such changes in circulating cell type and morphology progressed with time and were most severe just prior to death.

All dogs had a marked terminal anemia and thrombocytopenia (Table 1). Terminal leukocytosis was not a prominent hematologic feature of the syndrome, as only 30% of the animals had peripheral leukocyte counts above 15,000/cu mm.

**Tissue Pathology**

**Bone marrow.** The most prominent pathologic feature in the 15 cases of myelogenous leukemia was the extensive proliferation of the granulocytic elements within the bone marrow, accompanied by a loss of normal fat deposits and a dramatic reduction in numbers of megakaryocytic and erythrocytic cell types (Fig. 5). These cellular changes gave rise to elevated M:E ratios (Table 2) that, at times, were extreme (e.g., 61.5 in dog 1210).

The erythroleukemias, in contrast to the myelogenous syndrome, were characterized by proliferation in the erythroid cell line and at times in the myeloid cell line as well. The proliferative activity of the granulocytic elements, however, was usually much less exaggerated and maintained normal morphologic patterns of cellular differentiation. Again, fat deposits and megakaryocytic elements were decreased (Fig. 6). These changes in marrow cellularity were quantitatively reflected, in part, in the differential cell counts and by the M:E ratios shown in Table 2.

In both types of leukemia, the marrow samples contained increased percentages of immature cells of the predominant cell line. This response was variable, however, ranging from moderate shifts to immature populations (e.g., in dog
1379) to a rather dramatic shift to populations consisting predominately of blast cells (e.g., in dog 1397, Table 2).

Leukemic marrow characteristically had a great deal more cellular debris, phagocytic activity, and plasma cells than marrow from healthy control dogs (Figs. 5, 6, 9, and 10). In general, the word “disordered” might be applied to the architecture of the leukemic marrow in contrast to the highly ordered marrow and sinus structures of healthy marrow. Tissue derangement was not limited to the free hematopoietic cells but extended to the structural and vascular tissues as well. In each marrow sample examined by electron microscopy from both myelocytic and erythroleukemic animals there were varying degrees of damage to the vascular sinus walls, ranging from a simple widening of the endothelial gaps to a total breakdown of the sinus wall (e.g., Fig. 9). In local areas the supportive reticular cells exhibited abnormal fine structure, including highly vacuolated cytoplasm and elongated, convoluted nuclei with prominent nucleoli (Fig. 10).

Other organs. The gross and light-microscopic tissue pathology of 13 MPD cases has been reported, and the recent cases did not differ from those. Touch impressions of spleen, liver, and lymph nodes always showed varying degrees of leukemic infiltration. The spleen was usually severely affected, exhibiting marked hematopoietic areas and moderate numbers of mitotic figures. In many cases the spleen imprints resembled bone marrow. Leukemic involvement in the liver and lymph node imprints ranged from severe to mild focal reactions.

An intensive search was made by electron microscopy for C-type particles within the tissues of the leukemic dogs. No conclusive particles were found.

Leukemic Cell Ultrastructure

The characteristic ultrastructural features of the leukemic cells of bone marrow were the same in the circulating leukemic cells and in the cells of myelo- or erythropoietic areas of the spleen, liver, and lymph nodes. The myelocytic leukemic cell population exhibited a variety of distinctive cytologic features related to the various stages of differentiation at which maturation difficulties occurred. The canine leukemic myeloblast was most often like its normal counterpart, in that it was a large cell, approximately 10 μm in diameter, with a large centrally placed blast-type nucleus and a high nuclear:cytoplasm ratio (Figs. 7, 8, and 11). In both, the nucleus usually had only a thin band of condensed chromatin along the nuclear membrane and a scattering of small condensates throughout the nucleoplasm. However, at times the periphery of the myeloblast nucleus from the leukemic animal became highly irregular and large chromatin condensates assumed highly abnormal patterns (Fig. 8). Large plump mitochondria with well-defined cristae were found in close association with these nuclear membrane convolutions, as were nuclear clefts, or pockets (Figs. 11 and 13A), and sessile bodies (Fig. 8). One or more prominent nucleoli were usually seen. The broad rim of cytoplasm contained relatively few organelles and a scant amount of rough endoplasmic reticulum (ER).

In a number of the myelocytic leukemias, promyelocytes accumulated in ab-
Table 2. Marrow Differential Cell Counts (%) of Dogs Dying With Either Erythro- or Myelogenous Leukemia

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<tr>
<th>Dog No.</th>
<th>Myeloblast</th>
<th>Pro-myelocyte</th>
<th>Myelocyte</th>
<th>Metamyelocyte</th>
<th>Band</th>
<th>Segmented</th>
<th>Basophilic Erythroblast</th>
<th>Erythroblast</th>
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Contral111 (mean ± SE) 0.4 ± 0.1 1.1 ± 0.2 10.6 ± 0.6 9.1 ± 0.5 15.6 ± 0.8 20.3 ± 1.2 0.2 ± 0.1 2.9 ± 0.3 16.2 ± 0.3 20.6 ± 0.8 1.5 ± 0.1

Values do not total 100% because not all cell types were counted; only granulocytic and erythrocytic elements are included here. Dogs 1–5 were classified as erythroleukemic; dogs 6–20 were classified as myelogenous leukemias.

*Autolysis did not permit differential cell count.
†Thirty-five untreated clinically normal dogs.
Fig. 7. Electron micrograph of buffy coat from a dog in the terminal stages of myelogenous leukemia. Three granulocytes at different stages of maturation are shown: (1) myeloblast (MB) with dispersed chromatin, a prominent nucleolus, and broad rim of cytoplasm containing many mitochondria and a little rough ER; (2) myelocyte (M) with a reduced nuclear:cytoplasmic volume ratio, cytoplasmic granules at varying stages of differentiation, and a moderate quantity of rough ER; and (3) a number of mature segmented neutrophils with primary and secondary cytoplasmic granules. Note the large platelet (PT) with centrally coalesced surface-connected channel system. × 5,300.

Fig. 8. Portion of a circulating myeloblast with abnormal cytologic features, including: peripheral cytoplasmic vacuoles, a highly convoluted nuclear membrane (arrows) with associated mitochondria, and an abnormal pattern of condensed chromatin. Sessile bodies (SB) are also evident. × 13,500.
Fig. 9. Bone marrow from a dog with myelogenous leukemia. In contrast to marrow from healthy dogs, this marrow is singularly granulocytic, with conspicuous absence of megakaryocytes, erythrocytic precursors, and fat elements. These marrows consistently have localized areas where cellular debris is abundant, as well as evidence of increased phagocytic activity and degeneration of structural elements in sinus walls (arrows). × 4800.

Fig. 10. Bone marrow from a dog with erythroleukemia. As is characteristic of this type of leukemia, the predominant erythroid population is megaloblastic. A typical basophilic erythroid blast (EB) with large, convoluted open nucleus is seen; heterochromatin is abnormally distributed and a nuclear cleft is present (arrow). Other erythrocytic and granulocytic elements are also present at various stages of differentiation. Also characteristic are the increased numbers of plasma cells (PC) and the abnormal appearing, highly vacuolated reticular cell (RC). × 3600.
normal numbers and comprised a major portion of the leukemic infiltrate. Some of these cells exhibited unique ultrastructural features (Fig. 12). These leukemic promyelocytes were usually large cells (e.g., 10–16 μm) with rather bizarre arrangements of rough ER, various types of structural abnormalities of the cytoplasmic granules (e.g., Fig. 13B, granule coalescence), and asynchronous patterns of nuclear–cytoplasmic differentiation characteristic of the leukemic syndrome (Fig. 12).

More mature granulocytic cells had certain cytologic abnormalities as well, including loose bundles of perinuclear fibrils (Fig. 13C), an absence of primary and secondary granules, and the presence of mitotic centrioles in normally non-dividing, late-stage granulocytes (Fig. 13D).

Dogs with erythroleukemia had atypical megaloblastic erythroid cell types. Ultrastructurally, these distinctive basophilic erythroblasts were the most abnormal cell type within the erythroleukemic marrow, with blast-type nuclei, heavy and irregular condensates of chromatin, and prominent nuclear pockets (Fig. 10).

**DISCUSSION**

The high incidence of leukemias reproducibly induced by continuous gamma-irradiation in our beagle dogs is consistent with the concept that radiation is a potent leukemogen. Both chemical leukemogens and ionizing radiation have the common feature of usually producing chronic bone marrow damage that may be partially repaired, but frequently terminates in aplasia or in leukemia. This feature was also characteristic of our canine system.

The relationship between various rates of continuous gamma radiation exposure and the induction of pathology in the dog is of interest and is phenomenologically quite revealing. Four general types of disorders, namely septicemia, anemia, leukemia, and nonleukemic malignancies, accounted for the vast majority of deaths in the irradiated animals. The induction and incidence of specific syndromes were seemingly time and dosage dependent. At 17 R/day there was a predominance of early occurring septicemic deaths. At 10 R/day aplastic anemias and the later occurring leukemias were the major causes of death. Leukemias and other types of malignancies occurred with greatest incidence at 5 R/day. Placing these syndromes on a relative time scale, irrespective of dose rate, septicemias were the first to occur, shortly followed by aplastic anemias, and then later by the leukemias. The hematopoietic disorders were followed much later by nonleukemic neoplasias (e.g., osteosarcoma, marrow and ovarian carcinoma) and degenerative diseases (e.g., hepatic degeneration).

Observation of different types and incidences of pathologies at given dose rates pointed out the inherent variation in sensitivity and response of individuals to protracted radiation. Such variation surely is based on a differential response of cellular components of target organs (e.g., hematopoietic system). For example, at 17 R/day, the most sensitive animals (the majority) developed absolute granulocytopenias and died of fulminating microbial infections. At this dosage immune competence and related granulopoietic functions of the marrow were seemingly irreparably damaged. The more resistant animals were
Fig. 11. Myeloblast within bone marrow of a dog with myelogenous leukemia. Nucleoli (NU) and a nuclear cleft (NC) are present. A broad rim of cytoplasm contains scant amounts of rough ER and numerous plump mitochondria (MT). × 12,300.

Fig. 12. Early promyelocyte in the bone marrow of a dog with myelogenous leukemia. The large cell has a blast-type nucleus and two nucleoli (NU). The cytoplasm contains a rather bizarre polar distribution (arrows) of rough ER and a scattering of immature granules. × 5000.
Fig. 13. Distinguishing ultrastructural features of the canine myelocytic leukemic cell. (A) Nuclear pocket or cleft (arrow), found in high incidence within myeloblasts and promyelocytes. In this particular case centrioles (CT) are noted within the pocket. × 22,000. (B) Coalescence of cytoplasmic granules (arrows) within peripheral blood and bone marrow cells have occasionally been observed. Granule coalescence might represent a phenomenon analogous to Auer body formation in man. × 15,800. (C) Perinuclear fibrillar material (arrow) within a myelocytic leukemia granulocyte. × 15,400. (D) Increased numbers of centrioles (CT) within segmented neutrophils suggest delayed, post-myelocyte phase mitotic activity. × 30,000.
spared from these early septicemic deaths, but later succumbed to either aplastic anemia or eventually to leukemia.

From this result it would seem that a proportion of animals managed to maintain, in the face of continuous radiation insult, a necessary protective level of immunocompetence, but failed to accommodate hematologically. Furthermore, a small segment of the irradiated population consistently proved to be much harder than the rest, escaping both the terminal septicemic and anemic phases. The percentage of dogs responding in this positive fashion to continuous irradiation increased dramatically at the lower dose rates (i.e., 15% at 17 R/day, 50% at 10 R/day, and 83% at 5 R/day). These responding hardy animals were not spared, however, from the long-term mutagenic (leukemogenic) effects of chronic irradiation. It is interesting to note that in this particular case the induction of marrow hyperplasia and associated leukemia could be thought of as a positive effect, since it spared the animal from early anemic death and substantially increased longevity.

The leukemogenic effect of continuous irradiation was greatly increased at the lower exposure rates. At 10 R/day about one-half of the animals developed leukemia, almost exclusively of the granulocytic variety. The other half of the group fell subject to aplastic anemia and died relatively early. At 5 R/day, there was a definite early sparing of hematologic function, as only a minimal number (~17%) of animals died of anemia. In contrast to the 10 and 17 R/day dose rates, at 5 R/day the erythropoietic elements were not significantly depressed during the first 2000 R of accumulated exposure (representing 400 days). The granulocytic and thrombocytic elements were depressed initially, and when hematopoietic accommodation did occur (evidenced by a partial recovery of peripheral blood values), it did so at a slower rate and at periods later than those seen at the higher daily doses. Leukemia accounted for nearly one-half of the deaths at the 5 R/day dose rate. It was quite interesting, however, that half of these cases were erythroleukemias. This leukemia subtype was found only at this particular dose-rate level, suggesting that the 5 R/day dose-rate represents an optimal erythroleukemogenic stimulus. A significant number of dogs within the 5 R/day group totally escaped terminal hematologic disorders. These animals survived for extended periods and died of either nonhematologic malignancies or degenerative diseases. Requirements such as extended induction time, large total radiation dose, age of the animal, etc. were some of the more plausible explanations for the late appearance of this last wave of non-leukemic pathologies.

In this study the minimum total exposure to produce leukemia during continuous irradiation was about 4000 R, with 383 days being the minimal induction time. While this minimal induction time was accurate, the minimal total exposure was high since other data from this laboratory have shown that terminating continuous radiation exposure after accumulated doses as low as 1700 R (17 R/day for 100 days) can produce leukemia.

The role oncornaviruses play in the etiology of these canine leukemias remains to be demonstrated. We found no evidence of budding C-type particles within any of the leukemic tissues. Others also have failed to find viral par-
particles on electron microscopy within both spontaneous and experimentally induced canine neoplasms. Possibly the virus particles in dogs are in very limited numbers. Alternatively, the virus might be incorporated into the genome of the host cell rather than expressed as an intact particle. Although a $^{90}$Sr-induced beagle myelomonocytic leukemia has been successfully transmitted to neonatal pups by leukemic cell transfer, neither nonviable, irradiated leukemic cells nor cell-free filtrates of neoplastic tissues have successfully transmitted the disease. Preliminary attempts to detect viral-specific reverse transcriptase within lymphoid and granulocytic leukemic tissues have also failed. The dog, therefore, continues to be a notable exception to the many species in which some form of oncornavirus expression has been found associated with various types of malignancies, in particular the leukemias.

The course of the developing leukemic syndrome in the beagle can be divided into four phases (Fig. 1): (1) the initial leukopenic and thrombocytopenic stage; (2) a partial recovery and accommodation phase occurring after a total exposure to approximately 2000 R; (3) a preleukemic phase with oscillatory thrombocytosis; and (4) the patent leukemic stage.

The partial hematopoietic recovery period, phase 2, is potentially the most informative for elucidating the early sequences of leukemogenesis. During this phase, the dog's hematopoietic system adapts to the continuing radiation stress and the gamma-irradiation becomes apparently less cytotoxic. The partial recovery is seen as a rise in peripheral granulocyte and thrombocyte values to approximately $50\%$ of the baseline values. Clonal selection and repopulation of the marrow with radioresistant committed stem cell populations is an attractive explanation for this hematopoietic recovery. Another possibility is that the hematopoietic microenvironment might be modified, affecting its role as a regulator of hematopoiesis via the granulopoietins. Such modifications of extrinsic processes might occur singularly or in concert with intrinsic cellular events in regulating hematopoiesis. In any case, the adaptive hematopoietic response might represent an early result of the initial neoplastic event. This concept is supported by our finding that only dogs that show this hematopoietic recovery phase in the face of continuing irradiation go on to develop overt leukemia.

In studies now in progress using serial bone marrow biopsies, we have verified and extended the observation that hematopoietic accommodation to continuous radiation stress occurs at approximately 2000 R (200 days at 10 R/day). The adaptive response within the marrow is initially seen as an increased cellular proliferation, particularly within the erythroid series. It is reasonable to expect that this heightened proliferative activity of immature hematopoietic elements allows for an increased cell production and is responsible for the noted partial recovery of peripheral blood values. As such, this period of increased proliferative activity, particularly as it occurs within the stem cell compartment, might be an obligatory preleukemic phase.

Following the recovery phase there is a prolonged period in which red cells, leukocytes, and platelets oscillate in numbers. This oscillation is similar to that seen in human chronic myelogenous leukemia.

The radiation-induced canine leukemias described here have many clinical
and histologic features that are closely analogous to both the spontaneous and
the radiation-induced syndrome in man.\textsuperscript{7,8,38} This conclusion also applies
to many of the prognostic, preclinical abnormalities of the peripheral blood
and bone marrow defined as preleukemia.\textsuperscript{42,43} Both canine and human leukemias are known to have extended latent periods after initiation of radiation.

In the dog under continuous irradiation the mean latent period is about 3 yr
(383–1949 days), whereas in man it is estimated to be about 7 yr.\textsuperscript{38} However,
relative to life expectancy, the latent periods are similar in the two species. The
terminal canine myelocytic leukemia syndrome has striking similarities with
the terminal stages of acute myelogenous leukemia or chronic myelogenous
leukemia patients in “blast crisis.” Clinically there is anorexia, progressive
weakness, loss of appetite, and weight loss in both species.\textsuperscript{7,27,39,41} The terminally ill are severely anemic, thrombocytopenic, and more often than not leukopenic
(only 30\% of the leukemic dogs exhibited elevated peripheral blood leukocyte
counts). Petechial and ecchymotic hemorrhages are seen in a wide variety of
tissues. Splenomegaly, mild hepatomegaly, and lymphadenopathy are common
clinical findings. In both species, there is a general relationship between spleen
weight, ectopic splenic myelopoiesis, peripheral blood leukocyte counts, and
general physical condition at the time of death.

Histologically, the disease(s) is characterized in both dog and man by varying
degrees of myeloid cell infiltration and proliferation within both hematopoietic
and nonhematopoietic tissues. The bone marrow is the tissue most severely al-
tered. Its hypercellular condition distorts normal tissue architecture via deple-
tion of fat deposits and the expansion of either the erythroid or myeloid cell
population, especially the younger, less mature elements, at the expense of the
other cell lines.

Significant alterations of supportive elements of the bone marrow occur in
the dog leukemias. In focal areas, the walls of the vascular sinuses appear al-
tered and/or degenerate. Since these structures are thought to regulate the re-
lease of formed hematopoietic cells into the peripheral circulation,\textsuperscript{44} we antici-
pated finding a correlation between the extent of this focal pathology within
the marrow vasculature and the degree of terminal leukocytosis. We were,
however, unable to substantiate this thesis. Retention of immature granulocytic
and erythroid cells by the marrow, under the condition of pancytopenia and
marrow hyperplasia, suggests that the basic sinus wall structure of the marrow
remains functionally intact with respect to its regulatory role in cell egress.

In the leukemias of both species, the invasive malignant cell line often dis-
plays aberrant sequences in cell differentiation. Our observation of a number of
distinct subclasses of myelogenous disease (i.e., blastic, promyelocytic, and
myelocytic) suggests that several different steps in cell differentiation are sus-
ceptible to being blocked. The latter might be related to either intrinsic altera-
tions of the leukemic cell itself or modifications of extrinsic factors (e.g., hu-
moral regulators of differentiation), or both.\textsuperscript{45,47} Morphologic evidence of cell
maturation defect(s) include asynchronous patterns of nuclear–cytoplasmic dif-
ferentiation and various types of organelle anomalies. By electron microscopy
we observed nuclear clefts, sessile bodies and bizarre distributions of heterochro-
matin only in the malignantly transformed hematopoietic cells. In man similar
structural alterations of the nucleus are known to be associated with specific chromosomal aberrations. This correlation in the canine leukemias remains to be established. It has been reported, however, that a Philadelphia-like chromosomal marker, pathognomonic for chronic myelogenous leukemia in man, is associated with myelomonocytic leukemia in dogs (transplanted Sr-induced leukemia).

Improved laboratory models for the study of leukemias, particularly models for myelogenous and erythroleukemias, are needed. The commonly used rodent models, although useful at times for both pathogenic and chemotherapeutic studies, do not simulate the disease(s) in humans by all criteria. There are differences in the course of the disease, clinical syndromes, and certain etiologic features (e.g., the obvious involvement of oncornavirus), and to a much lesser extent, in the cytology of the leukemic cell population. More importantly, because the conventional test animal is so small, many types of new therapeutic and supportive protocols cannot be adequately tested and evaluated.

We have produced a high incidence of leukemia in the beagle, a large, easily manageable laboratory animal. With precise control of the myeloproliferative stimulus, namely, gamma-irradiation, we can reproducibly select for a predominance of either myelogenous or erythroleukemia. The recognition of a rather precise time for anticipating the critical event or events that mark a commitment of the bone marrow to a neoplastic course is a particularly provocative and stimulating concept. Stage 2 in this syndrome seems to be significant in this way, wherein a partial recovery and accommodation to continuing irradiation is a measurable event and a prerequisite to development of the neoplastic eventuality. It seems important, therefore, to investigate further, at subcellular levels, the integrity of the myelocytic cells at this point using more definitive techniques for markers that are prognostic of the long-term future development of leukemia. We believe, therefore, that these canine leukemias offer a new alternative laboratory model to the research oncologist, especially those interested in developing and testing new clinical methods.

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Irradiation-induced erythroleukemia and myelogenous leukemia in the beagle dog: hematology and ultrastructure

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