

**THE INCIDENCE OF THREE SPLENIC LESIONS
(SARCOMAS, HEMATOMAS, EMH)
IN CHRONICALLY IRRADIATED BEAGLE DOGS**

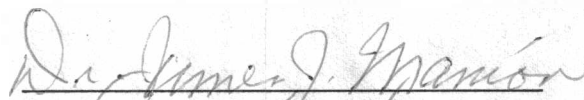
Submitted in Partial Fulfillment of the Requirements for
Graduation with Honors to the Department of Biology at Carroll
College, Helena, Montana.

Robert Gerard McGreevy
March 30, 1987

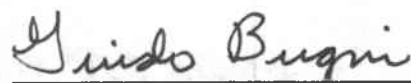


CORETTE LIBRARY
CARROLL COLLEGE

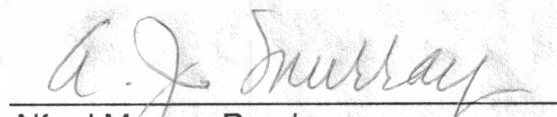
This thesis for honors recognition has been approved for the Department of Biology by:



Dr. James J. Manion, Advisor



Guido Bugni, Reader



Alfred Murray, Reader

TABLE OF CONTENTS

ACKNOWLEDGEMENTS ii

ABSTRACT..... iii

LIST OF TABLES iv

INTRODUCTION AND LITERATURE REVIEW 1

MATERIALS AND METHODS..... 5

RESULTS.....11

DISCUSSION..... 21

SUMMARY.....25

LITERATURE CITED 28

ACKNOWLEDGEMENTS

I would like to express a note of deep appreciation to the Radiation Toxicity group at Argonne National Laboratory for the support they gave me during my research participation at Argonne. Without their help and guidance this thesis would not have been possible.

To my readers, Dr. James Manion, Mr. Guido Bugni, and Mr. Alfred Murray, also goes a heart felt "thank you."

ABSTRACT

Data gathered on beagle dogs who received terminated exposures to protracted whole-body gamma irradiation was reviewed in order to make an evaluation of the incidence of splenic lesions. Three splenic lesions (sarcomas, hematomas, and EMH) were evaluated in terms of the total accumulated dose and dose rate received by the beagle dogs.

The incidence of all three lesions, in the irradiated dogs, was found to be significantly greater than the incidence of the same lesions in the control dogs. The irradiated male dogs differed significantly from the control male dogs in the proportion of both splenic sarcomas and splenic hematomas. The females showed no significant difference between irradiated and control dogs in the proportion of any splenic lesion. No association was found between increasing total dose or dose rate and any of the three splenic lesions.

LIST OF TABLES

TABLE	PAGE
I. SPLENIC LESIONS.....	13
II. INCIDENCE OF SPLENIC SARCOMAS.....	15
III. STATISTICAL ANALYSIS OF SARCOMA GROUPS.....	16
IV. INCIDENCE OF SPLENIC HEMATOMAS.....	17
V. STATISTICAL ANALYSIS OF HEMATOMA GROUPS.....	18
VI. INCIDENCE OF SPLENIC EMH.....	19
VII. STATISTICAL ANALYSIS OF EMH GROUPS.....	20
VIII. FATAL TUMORS IN BEAGLES GIVEN TERMINATED EXPOSURES TO PROTRACTED WHOLE-BODY IRRADIATION.....	26
IX. THE EFFECT OF TOTAL AND DOSE OF PROTRACTED IRRADIATION ON FATAL TUMORS (Controls Deleted).....	27

INTRODUCTION AND LITERATURE REVIEW

Ongoing research entitled Radiation Toxicity Studies in Dogs for Interspecies Comparisons in the Biological and Medical Research Division at Argonne National Laboratory is designed to provide information on the late effect of ionizing radiation in the dog. This information along with existing information on radiation toxicity in rodents will allow for more meaningful extrapolations from laboratory animals to man. The objectives of this program are (1) to determine the relative influence of two major factors, daily dose rate and total accumulated dose; (2) to provide data for estimates of radiation-specific excess mortality rates in the dog to allow interspecies comparisons with existing rodent data; and (3) to study the radiation damage related to life shortening and death. Factors such as target organ sensitivity, tissue responses (especially neoplasia), and the incidence of tumors are being evaluated. Protracted whole-body ^{60}Co is given to adult beagles, 22 hours/day, 7 days/week, at various dose rates down to those allowing a near normal life span, either until death or until predetermined total doses are accumulated.

Over the course of this project it has been discovered that there is a high incidence of splenic lesions in these experimental dogs. The purpose of this research is to evaluate the significance of three types of splenic lesions; (1) sarcomas, (2) hematomas, and (3) extramedullary hematopoiesis (EMH) with regard to the dose and dose rate received by the beagle dogs.

Much attention has been given to splenic pathology in the Argonne beagles. In

particular, primary splenic sarcomas have received extensive investigation. Dr. L. Lombard, of Argonne National Laboratory, after extensive histological and ultrastructural studies proposed that these splenic neoplasms found in the Argonne beagles are neurogenic in origin. Believing that many of these malignancies arose from the Schwann cell of the myelin sheath, a preliminary diagnosis of malignant Schwannoma was assigned [1]. In order to obtain outside opinion on the origin and classification of these tumors, slides from 17 cases of primary splenic sarcomas were submitted to Dr. Ralph Bunte, Venterinary Pathology Section, Armed Forces Institute of Pathology, for review. Generally, the diagnosis indicated that the tumors were highly anaplastic sarcomas that may have their origin in the nerve sheath, but that additional study was required to identify the cell of origin [2]. In a study undertaken at Argonne by Mike Gallagher and Dr. K. Duggal, immunohistochemical staining techniques were utilized to test for the presence of the S100 antigen which is thought to be unique to the Schwann cell. The presence of the S100 antigen was in general not detected; however, the results of this study are believed to be inconclusive due to problems with the tissue preparations [3]. Studies of neoplasms which occur in the future will utilize freeze preserved specimens in order to reduce the problems associated with formalin preservation seen in Gallagher and Duggal's study [4]. It is hoped that future studies will allow for a more definitive diagnosis of these neoplasms.

Radiation induced neurofibrosarcomas (malignant Schwannomas) have been extensively reported in patients who have received radiation treatment for prior cancers [5-8]. Therefore, if the tumors seen in the Argonne beagles are indeed malignant Schwannomas they show an important parallel with the human condition.

Although the diagnosis of these neoplasms remains doubtful, it is clear that they are definitely of the nonhemangiomas type [1]. This fact is particularly important inasmuch as soft tissue tumors not arising from vascular endothelium have been a rare occurrence in the dog [9,10].

Rupture of the spleen due to hematoma formation caused by physical trauma is not an uncommon accident in the dog [10]. However, radiation induced hematomas of the spleen have generally not been reported in the open literature[18].

In most adult mammals, lymphocytes are normally formed in the lymphatic tissues (eg. lymph nodes, spleen) and myeloid elements in the bone marrow. Ectopic or extramedullary hematopoiesis represents an abnormal condition in which myeloid cells may occur in tissues and organs other than the bone marrow. During embryonic development the spleen is a site of normal myeloid hematopoiesis, but by birth the myeloid cells have been suppressed and hematopoiesis no longer takes place in the post natal spleen. Some researchers believe that EMH of the spleen represents reactivation of the multipotential mesenchymal cells that persist throughout life in the spleen [11,12,15]. However, recent evidence suggests that EMH results from migration of marrow stem cells [12-14]. EMH may be a compensatory response by the animal to damage to blood forming elements of the bone marrow. Whole body irradiation given in sufficient amounts has been shown to result in death due to damage of the hematopoietic tissues [16,17]. However, studies have shown that shielding the spleen against X-rays prevents death after an otherwise lethal dose by producing a great increase in hematopoiesis in the spleen [12]. EMH is often associated with other pathological conditions, particularly the myeloproliferative disorders (MPD) including

myeloid leukemia [11,12]. Although MPDs have occurred frequently in the Argonne beagles, EMH has not been reported in conjunction [16,17]. It is important to note that the formation of granulocytes, erythrocytes, and megakaryocytes in the spleens of some experimental animals (particularly the rodents) is so frequent that EMH can be regarded as a normal condition [12]. Some investigators believe that EMH is even a common occurrence in the canine spleen [18].

Although much work has gone into gathering data on splenic pathology over the course of the radiation toxicity studies, no evaluation has been made of the association between dose/dose rate and the frequency of splenic lesion occurrence. In making this evaluation it is hoped that additional information will be added to what is already known about radiation damage of the spleen in the Argonne beagles.

MATERIALS AND METHODS

Dogs used in these studies are from the purebred beagle colony at Argonne National Laboratory. The colony was derived from a nucleus selected in 1959-60 and has been an outbred closed breeding colony since then. The dogs are fed Purina Lab Chow ad libitum, unless restricted to prevent excess weight gain. Water is available at all times from self waterers. The colony and its management have been described in detail by Norris et al. (1968) [19].

Records are kept on each dog over the entire course of its life. The data which is gathered comes from various sources. Sources which were directly applicable to evaluating splenic lesions include clinical observations, necropsies, surgeries, histopathology, and cytopathology.

Clinical observations are made by a veterinary doctor assigned to the Argonne beagle colony. Complete physical examinations are performed regularly (approximately 90 day intervals). Splenic neoplasms or other lesions are often found first by palpation of the abdomen at the time of a physical examination. X-ray examinations are available for diagnostic use. Surgical exploratories may be performed to detect or confirm the presence of lesions with the spleen.

Necropsies are given to each dog according to a standard protocol either at death, or when the dog is moribund or regarded as having a serious life threatening disease. At necropsy the weights of all major organs and any pathological conditions are described and recorded. A preliminary cause of death may be assigned at necropsy, but final diagnoses is deferred until histological evidence is reviewed.

Tissues for histopathological studies are collected either by surgical biopsy or at

necropsy. The tissues are fixed in neutral formalin and microscopic slides are prepared using standard procedures (parafin embedding, hemotoxylin and eosin staining).

At surgery or necropsy specimens are also gathered for cytological examination. This entails imprinting tissues or depositing aspirated fluids onto microscopic slides. The slides are fixed using MeOH and stained with Wrights and Giemsa stains. Cells indicative of neoplasia or other pathological condition are noted under light microscopy.

The data which is gathered on each dog for the radiation toxicity studies is computerized into Oracle, a relational data base management system (RDBMS). Data from the several sources is stored in separate areas or tables within the Oracle system. In order to evaluate the data on splenic lesions it was necessary to merge the tables; creating a new single table, "SPLPROJ". SPLPROJ was created using UFI (user friendly interface) which allows the user to write programs to arrange data stored in Oracle.

Two separate but related radiation toxicity experiments are in progress at Argonne National Laboratory. In both studies dogs are exposed to protracted whole-body gamma irradiation from a Gammabeam Model 150A unit (Atomic Energy of Canada, Limited) fitted with a ^{60}Co source of appropriate strength for periods of 22 hours/day, 7 days/week. In one experiment dogs are exposed at dose rates of 0.4, 1.0, and 2.5 R/day for the entire duration of their lives. The second experiment involves exposures at dose rates of 5, 10, 17, and 35 R/day but exposures are terminated and the dogs are removed from the gamma field when a prescribed total dose is accumulated (600,

