RADIATION CARCINOGENESIS

COMPREHENSIVE THREE-YEAR PROGRESS REPORT
1 MAY 1972 - 15 MARCH 1976

SHIELDS WARREN AND OLIVE GATES

NEW ENGLAND DEACONESS HOSPITAL
CANCER RESEARCH INSTITUTE
185 PILGRIM ROAD
BOSTON, MASSACHUSETTS 02215

MARCH 1976

NOTICE
This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Energy Research and Development Administration, nor any of its subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

PREPARED FOR
THE U.S. ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION
UNDER CONTRACT E(11-1)-3017

MASTER

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

TABLE OF CONTENTS

	Page
Notice	1
Members of Project	2
Introduction	3
Main Research Accomplishments.	6
The Tumorigenic Effects of Acutely Delivered High Doses in Parabiotic Rats.	6
The Pathologic and Tumorigenic Effects on Specific Tissues of Various Doses of Radiation to Mice and Rats and Analysis of the Influence of Hormonal and other Factors.	9
The Interplay of Radiation and Asbestos as Carcinogens in both Single and Parabiotic Rats	11
Induced Metabolic Alterations.	11
Life-shortening in Rats.	13
Irradiated Cultures of Human Cancer Cells.	13
Comment as to Present State of Knowledge in this Area of Research	14
Publications	15

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Energy Research and Development Administration, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product or process disclosed or represents that its use would not infringe privately owned rights.

U. S. ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION
Contract E(11-1)-3017

MEMBERS OF PROJECT

1 May 1972 through 15 March 1976

STAFF

Principal Investigators

Shields Warren, M. D.
Olive Gates, M. D.

Consultants:

Clark E. Brown, M. D.
Russell F. Cowing
Olive M. Lombard, M. S.

Supporting Personnel:

Rosanna N. Chute, M. A.
Natalie E. Fossati, B. S. in B. A.
Ruth A. Marshall
Robert O'Brien
Carol H. Parker, B. A.
Marilyn W. Porter, B. A.

INTRODUCTION

Emphasis has been placed on completion of long-range studies in this period. The chief of these has been radiation carcinogenesis in parabiont NEDH rats. Over 3000 pairs, control and experimental, have been prepared, one given 1000 R of 250 kVp whole-body x-radiation, the other shielded. The present LD_{50/30} for this strain is about 820 R, varying ± 100 days over a 25-year span. The average lifespan of control parabiont rats is 635 days, while three-fourths of control single rats are alive at that time. One thousand R kills over 80% of single rats within 50 days, whereas with parabiosis few die in this period, and 50% survive over 400 days postirradiation. A wide range of tumors and other lesions are induced in the survivors.

Autopsy and histologic study have enabled us to characterize most of the lesions induced by radiation, and the data are being analyzed in the hope that we may determine the relative sensitivity to tumor induction of most organs and tissues. Such a categorization would be an important aid in evaluating dose-effect relationships. We have demonstrated that imbalance in hormonal relationships may be a major factor in some forms of radiation tumorigenesis, not only in parabiont rats, but in single animals as well.

These and other points will be elaborated in the following pages. Detailed results are given in the articles cited in the list of publications.

Until September, 1975 we had the advantage of the presence of several graduate students in a training program in radiobiology supported by

the National Cancer Institute and administered by the Hospital and the Biology Department of Boston University. All of these have obtained their degrees and several are now independent investigators. We continue to have some assistance from medical students.

An important asset has continued to be the very excellent Animal Farm maintained by the Institute. This has had the advantage of continuing veterinary supervision by staff of the New England Regional Primate Research Center. The Farm is fully accredited by the American Association for Accreditation of Laboratory Animal Care. The Harvard Medical School Animal Farm provides us with pathogen-free WEDH rats.

We have had few changes of personnel. In June, 1974, Dr. Gates resigned but fortunately has continued to work with us as a volunteer. Dr. Clark E. Brown has continued to act as Consultant. Mr. Russell F. Cowing, our Radiation Engineer, received the Award of the Health Physics Society in 1974.

In May of 1972 Dr. Warren was fortunate to receive the Enrico Fermi Award jointly with an old friend and colleague, Dr. Stafford L. Warren.

Dr. Warren was chosen as one of the biographees in the Oral History Program of the National Library of Medicine. He also was chosen to prepare a videotape for the Leaders in American Medicine, a series of autobiographical memoirs sponsored by Alpha Omega Alpha and the National Library of Medicine/National Medical Audiovisual Center. He served as Consultant to the National Academy of Sciences Advisory Committee on the Biological Effects of Ionizing Radiation and was also a member of its Subcommittee on Somatic Effects. He served as Chairman of the Life Sciences Committee of the National Aeronautics and Space Administration

from 1971-74 and is still a member of the Space Program Advisory Council of the National Aeronautics and Space Administration.

Dr. Warren served as an Alternate Delegate from the United States to the XIth International Cancer Congress in Florence, Italy, and served as Cochairman of the Founding Committee of the Radiation Effects Research Foundation together with Dr. Yanagisawa, the Japanese Cochairman. This Founding Committee served as the transition vehicle from the Atomic Bomb Casualty Commission to the new Foundation which is to continue much of the work of the Atomic Bomb Casualty Commission.

Dr. Warren called the attention of medical examiners, coroners physicians and forensic pathologists during the period to the pathologic criteria essential for the recognition of radiation death or injury, a highly specialized field which has not until now been very much developed.

MAIN RESEARCH ACCOMPLISHMENTS

Our experiments during this period may be grouped as follows.

A. Radiation Carcinogenesis in Rodents

1. The tumorigenic effects of acutely delivered high doses in parabiotic rats.
2. The pathologic and tumorigenic effects on specific tissues of various doses of radiation to mice and rats and analysis of the influence of hormonal and other factors.
3. The interplay of radiation and asbestos as carcinogens in both single and parabiotic rodents.

B. Incidental Radiation Effects

1. Induced metabolic alterations.
2. Life-shortening in rats.
3. Irradiated cultures of human cancer cells.

A. 1. The Tumorigenic Effects of Acutely Delivered High Doses in Parabiotic Rats--Littermate NEDH rats of the same sex were parabiosed, and after recovery the right-hand animal received 1000 R of 250 kVp x-radiation while the left-hand animal was shielded. The left-hand animal supported the irradiated one during the stage of acute response to injury and thus permitted recovery to take place. The irradiated animals developed a high proportion of malignant tumors of various tissues and organs.

This series of parabiont animals has now been completed from the experimental standpoint, and most of the data have been placed on punch cards for statistical analysis.

Parabiosis alone increased the incidence of neoplasms somewhat, perhaps attributable in part to the hormonal imbalance between partners and the absence of complete histocompatibility. Three fourths of the irradiated partners 800 days of age or over had one or more tumors present when they died, nearly twice as many as were found in control parabiont pairs of comparable age. In this strain of rat single control animals develop almost no tumors until 500 days of age, the incidence eventually increasing in them to about 50% at 900 days of age. Tumor incidence in control parabiotic pairs was 43% which did not differ significantly from the control single rats, but they did tend to develop tumors earlier than the control singles. The irradiated partners developed more tumors than single or parabiont controls and also developed them about two months earlier than either.

Mammary tumors in the female rats presented a rather special case in that many are hormonally influenced. Also, at least some of the mammary tumors may be viral in origin. We would comment that in this strain of rat it is extremely difficult to differentiate between some benign and some malignant mammary tumors. Mammary tumors were often multiple. Control parabiont female rats developed only half as many mammary tumors as did either the irradiated parabiont partners or the single controls. This suggests that the control parabiont pairs which, of course, have double endocrine systems may have less hormonal disturbance with aging than do either single rats or irradiated parabiont pairs which have only one set of fully functioning endocrine glands because of radiation damage to the other set. It should be remarked that this NEDH strain is less likely to develop mammary tumors than is the

Sprague-Dawley strain studied by Shellabarger and others. The incidence of carcinoma of the breast in control parabiosed female NEDH rats was 25%.

An unexpected finding was that following unilateral nephrectomy prior to radiation in each parabiosed partner the incidence of breast carcinoma rose sharply in the nonirradiated partner. This increase was apparently related to alterations in retention, interchange between joined rats and feedback of pituitary and ovarian hormones. Interestingly enough, this correlates with the earlier observation of Zeckwer^{1,2} that parabiosis of an intact female rat to a castrate produced excess luteinizing hormone which was associated with mammary hyperplasia and adenomas. Our data support the involvement of endogenous gonadal steroids and pituitary hormones in the occurrence of breast carcinoma in the rat.

Malignant mesenchymal tumors, sarcomas, either undifferentiated, fibrosarcomas or rhabdomyosarcomas, tend to arise sporadically at the anastomotic line between the irradiated and unirradiated members of parabiont pairs. In occasional lots of animals as high as 50% have shown this tumor; at other times none have appeared. About 500 days is required for their development. They sometimes metastasize before

¹Zeckwer, I. T.: Correlation of the Alterations in Mammary Glands, Pituitary Body, and Ovaries of Parabiologic Rats. Arch. Path. 38:99-109, 1944.

²Zeckwer, I. T.: The Passage of Endogenous Estrogens across the Parabiologic Union in Rats. Endocrinology 28:249-257, 1946.

killing the animals. The etiology has as yet not been determined though it tends to be associated with certain technical procedures. Even though our parabiosed rats are from an intensively inbred strain, there may be slight lack of histocompatibility between members of pairs, and it is possible that the tumors originate because of the failure of proliferating cells along the anastomosis to recognize the adjacent cells of the partner.

Spontaneous osteogenic sarcoma is extremely rare in the NEDH strain, a spontaneous incidence of 0.2%. (Incidentally, this is the same incidence as shown by the control beagles in the Utah study.) The incidence rose to 0.8% in the control parabiont pairs, but in the irradiated parabiont partner of the experimental pairs the incidence rose to about 5% from 600 days of age on.

Leukemia, interestingly enough, had a lower incidence in the irradiated parabiont rats than in their partners, or in controls, parabiont or single. Our work in leukemia has been largely financed by a grant from the National Cancer Institute. In general, it may be said that in the rat in sharp contrast to the mouse, radiation (at least in doses over 500 R) tends to inhibit rather than to favor the development of leukemia.

A. 2. The Pathologic and Tumorigenic Effects on Specific Tissues of Various Doses of Radiation to Mice and Rats and Analysis of the Influence of Hormonal and other Factors—We studied breast tumors in mice as related to exposure to radiation and attempted to analyze the influence of hormonal and other factors. For this study female RAP mice were used. Surviving mice were killed at 500 days in order to avoid

the high incidence of mammary tumors that occurs spontaneously after that age. Both generalized and localized radiation was used. Localized radiation serves to demonstrate specific tissue sensitivities to tumor induction. The mammary gland in responding to whole-body radiation is highly resistant to direct effect and rather serves for all practical purposes as an end organ of the female endocrine system which expresses the disturbance by radiation of the sensitive pituitary-ovarian axis. Analysis of our results leads us to believe that mammary cancer in RAP mice also results from the interaction of several factors no one of which was either clearly or solely decisive. These factors are direct effects of radiation, hormonal imbalance and the possible presence of mammary tumor viruses.

We have long been interested in factors predisposing to the development of carcinoma of the breast in women and had encountered a case of carcinoma of the breast years following multiple fluoroscopies similar to those reported by Mackenzie.³ We estimated the total dose to the skin over the breast to have been about 4000 R. As a result of this case and the interest in the subject of Dr. Norman C. Telles who was studying with us at the time, an epidemiologic study is being supported by the Bureau of Radiologic Health. This is now being carried out by Mr. John Boice, a graduate student in the Harvard School of Public Health working with Dr. Brian MacMahon. This study entails followup of several hundred girls repeatedly fluoroscoped during treatment of

³Mackenzie, I.: Breast Cancer Following Multiple Fluoroscopies. Brit. J. Cancer 19:1-8, 1965.

pulmonary tuberculosis years ago. Over 80% of these patients have been identified.

For some time there has been evidence that changes occur in adrenal cortical function following radiation. There has been little in the way of morphologic changes to correlate with these observations. A study of adrenals of a large number of RAP mice, 700 controls and 900 irradiated, has shown certain cellular changes to be induced, particularly "A" cell proliferation in the cortex, predominantly seen in females. This change is a precursor to tumor formation. It occurs spontaneously with aging to a limited extent. It follows gamma and x-radiation in a range of dose levels (30-500+ rads). The evidence reinforces the view the adrenal cortex has a high degree of radioresistance. The "A" cell proliferation, increased in degree and advanced in time in females by radiation, is strongly influenced by the hormonal milieu. This is currently being analyzed.

A. 3. The Interplay of Radiation and Asbestos as Carcinogens in both Single and Parabiotic Rodents--A number of rats have been injected with standard UICC samples of Rhodesian and Canadian asbestos intrapleurally, intraperitoneally or intratracheally and then given local radiation to portions of the injected tissues. Good distribution of the fibers has been obtained. Results are not anticipated for some months. Other types of insulating fibers will be used.

B. 1. Induced Metabolic Alterations--RAP mice, possibly harboring mammary tumor virus (MTV), of representative ages were exposed to limited or lifetime whole-body continuous gamma and to fractionated x-radiation. Doses varied from 30-2100 R and dose rates from 0.062-121 R/day in the

two series GA and PN in which mammary cancer incidence was elevated. Induction of mammary cancer was found to be related to the dose range for ovarian tumorigenesis, 30-500 R, but there was no statistically significant correlation between incidence of mammary cancer and ovarian tumors. Active MTV infection was suggested by cancer-prone litters in one irradiated (PN) and one unirradiated series (OCB). Unirradiated descendants of perinatally-irradiated mice with a radiation-induced inherited hormonal disorder had a lower incidence of mammary cancer than did stock virgin mice. The balance of evidence pointed to induction of mammary cancer through systemic change set in motion by irradiation. Further evidence supporting this interpretation is provided by the disturbance in pituitary and estrogenic hormone levels in our series of parabiont and partially nephrectomized rats now being studied.

Our data on mammary tumors in mice and rats which had received gamma or x-radiation do not support a linear dose-effect hypothesis, again suggesting the importance of the hormonal environment. Perinatal whole-body radiation up to 2100 rads increased the incidence of breast cancer above that of controls. However, the unirradiated offspring of mice under force breeding had a tumor incidence of 34%, not significantly different from that of the perinatally irradiated mice--27%.

Another type of induced metabolic alteration is that produced by transplantation of a functionally active radiation-induced tumor of endocrine gland origin. We have two such tumors that are particularly useful and have been supplied to several investigators. One is a malignant pheochromocytoma which when transplanted produces hypertension and toxic myocarditis in the host. Not only are the tumor cells able

to produce large amounts of catecholamine but in addition some of the cells have been demonstrated to respond to nerve growth factor when grown in tissue culture. The other tumor is an insulinoma which produces a marked degree of hyperinsulinism. This tumor is currently being studied by electron microscopy. In addition, its insulin content is being determined.

B. 2. Life-shortening in Rats--The lifespan of control and of irradiated rats demonstrates clearly the protection provided by parabiosis. Of the single rats exposed to 1000 R, one half died within 13 days, while the parabiosed and irradiated rats survived an average of 452 days. The average lifespan of single control rats is 700 days, of control parabionts 635 days.

B. 3. Irradiated Cultures of Human Cancer Cells--The irradiated cultures of human cancer cells transplantable to the hamster cheek pouch will be maintained in the frozen tissue bank.

COMMENT AS TO PRESENT STATE OF KNOWLEDGE IN THIS
AREA OF RESEARCH

Carcinogenesis is probably the most important single endpoint as far as radiation effects are concerned when population dose-effect relationships are under consideration. This was a major consideration in arriving at the conclusions in the Biological Effects of Ionizing Radiations Report. In assessing the risk of carcinogenesis from whole-body or localized radiation of various tissues, factors other than the direct effect of radiation on cells must be weighed. Prominent among these factors are hormonal balance, carcinogenic viruses and immunologic influences. Because of the importance of hormonal and immunologic factors in carcinogenesis, study of cells in tissue culture alone cannot be expected to provide adequate information.

As the environment changes with new types of industrial and agricultural development, the opportunity for cocarcinogenic effect from new or more abundant physical or chemical agents tends to increase. A case in point is the recent greater need for energy conservation and the consequent increasing use of asbestos and other types of insulating fibers, asbestos being a recognized carcinogen.

Among future investigations needed are first those that can discriminate between direct effect of radiation on cells and the indirect effect of radiation-induced hormonal changes and, second, ones leading to clarification of the role of radiation as a cocarcinogen with other physical and chemical agents.

PUBLICATIONS SUPPORTED BY
ERDA CONTRACT E(11-1)-3017
NEW ENGLAND DEACONESS HOSPITAL
1 MAY 1972 - 15 MARCH 1976

Brown, C. E., Chute, R. N.,
Porter, M. W. and Warren, S.:

Abstract: The Effect of Nephrectomy
on the Incidence of Breast Carcinoma
in Irradiated Parabiosed Rats.
Proc. Am. Assoc. Cancer Res. 15:3,
3/1974.

Brown, C. E., Chute, R. N.,
Porter, M. W. and Warren, S.:

The Effect of Nephrectomy on the
Incidence of Breast Carcinoma in
Irradiated Parabiosed Rats. Cancer
Res. 35:37-44, 1/1975.

DeLellis, R. A., Merk, F. B.,
Deckers, P., Warren, S. and
Balogh, K.:

Ultrastructure and in Vitro Growth
Characteristics of a Transplantable
Rat Pheochromocytoma. Cancer
32:227-235, 7/1973.

Gates, O. and Warren, S.:

Abstract: Adrenal "A" Cell Prolif-
eration as a Radiation Response.
Federation Proc. 34:825, 3/1/75.

Porter, M. W., Chute, R. N. and
Warren, S.:

Abstract: The Incidence of
Radiation-Induced Cataract in
Single and Parabiocic Rats.
Radiation Res. 62:544, 6/1975.

Udupa, K. B., Warren, S. and
Chute, R. N.:

Variations in LD_{50/30} in a Strain
of Rats Over 20 Years. Health
Physics 26:319-322, 4/1974.

Warren, S.:

A Radiation-induced Breast Cancer.
Cancer 32:991-993, 10/1973.

Warren, S.:

Iatrogenic Effects in Radiation
Carcinogenesis. In Modern Radiotherapy
Malignant Diseases in Children,
Ed. by T. J. Deeley, Chap. 26,
Butterworths, London, 1974,
pp. 484-494.

Warren, S.:

Pathologic Criteria of Radiation
Injury. J. Forensic Sci. 19:709-
714, 10/1974.

Warren, S.:

Skeletal and Tissue Lesions
Resulting from Exposure to Radium
and Fission Products. Ann. Clin.
Lab. Sci. 5:75-81, 3-4/1975.

- Warren, S.: Autopsy Techniques for Recognition of Radiation Injury. Bull. Soc. Pharmacological & Environmental Pathologists III:9, 6/1975.
- Warren, S.: Effects of Low Levels of Radiation on Rodents and Potential Effects in Man. Health Physics 29:251-255, 8/1975.
- Warren, S., Brown, C. E. and Chute, R. N.: Abstract: Parabiosis, Radiation and Carcinogenesis in the Rat. Panel 20-Radiobiology, Xth International Cancer Congress, Florence, Italy, October 20-26, 1974, pp. 210-211.
- Warren, S., Brown, C. E. and Porter, M. W.: Abstract: Renal Tumors in Parabiosed Irradiated Rats. Radiation Res. 51:494, 8/1972.
- Warren, S., Brown, C. E. and Porter, M. W.: Renal Tumors in Parabiosed Irradiated Rats. Radiation Res. 53:153-163, 1/1973.
- Warren, S., Chute, R., Brown, C. and Porter, M.: Abstract: Radiation-Induced Life Shortening in the Parabiont Rat. Fifth International Congress of Radiation Research, Seattle, Washington, July 14-20, 1974, p. 131.
- Warren, S., Chute, R. N. and Porter, M. W.: The Effect of Parabiosis on Lifespan of Rats Stressed by Radiation. J. Geront. 30:15-21, 1/1975.
- Warren, S., Chute, R. N. and Udupe, K. B.: Abstract: Variations in the LD_{50/30} in a Strain of Rats over 20 Years. Health Physics 25:337, 9/1973.
- Warren, S. and Gates, O.: Abstract: Mammary Tumorigenesis through Perinatal Radiation of Mice. Federation Proc. 32:834, 3/1973.
- Warren, S. and Gates, O.: Mammary Tumorigenesis through Irradiation of Mice. Radiation Res. 57:488-507, 3/1974.
- Warren, S., Gates, O. and Chute, R. N.: Abstract: Breast Tumors and Radiation in Man and Rodents. Proc. Am. Assoc. Cancer Res. 13:26, 3/1972.

Warren, S., Udupa, K. B. and
Chute, R. N.:

Abstract: Leukemia in Irradiated
Parabiotic Rats. Proc. Am. Assoc.
Cancer Res. 14:8, 3/1973.

Warren, S., Udupa, K. B. and
Chute, R. N.:

Leukemia in Irradiated Parabiotic
Rats. Radiation Res. 57:67-72,
1/1974.

Warren, S., Udupa, K. B., Chute,
R. N. and Brown, C. E.:

Abstract: The Crossover of
Estradiol in Parabiosed Female
Rats. Federation Proc. 33:274,
3/1974.

Publications in Press

Porter, M. W., Chute, R. N. and
Warren, S.:

Incidence of Radiation-Induced
Cataract in Single and Parabiotic
Rats. Radiation Res.

Warren, S.:

Effects of Occupational and
Environmental Exposures to Ionizing
Radiation. Chapter in Forensic
Medicine; A Study in Trauma and
Environmental Hazards. Ed. by
C. G. Tedeschi, L. G. Tedeschi
and W. G. Eckert, W. B. Saunders
Co., Philadelphia.

Warren, S.:

Safe Handling of Tissues Containing
Radioactive Substances. Ann. Clin.
Lab. Sci.

Warren, S., Brown, C. E. and
Chute, R. N.:

Parabiosis, Radiation and Carcino-
genesis in the Rat. I. Tumori.

Warren, S. and Gates, O.:

Leukemogenesis by Low-Level Radi-
ation. Proceedings of IAEA/WHO
International Symposium on Biological
Effects of Low-Level Radiation
Pertinent to Protection of Man
and His Environment, Chicago, Ill.,
11/1975.