COO-3017-35

RADIATION CARCINOGENESIS

Progress Report V

Shields Warren and Olive Gates

New England Deaconess Hospital
Cancer Research Institute
185 Pilgrim Road
Boston, Massachusetts 02215

NOTICE
This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Department of Energy, 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.

16 May 1977 through 15 May 1978

Prepared for the U. S. Department of Energy
Under Contract No. EY-76-S-02-3017.A002

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

U. S. DEPARTMENT OF ENERGY
Contract No. EY-76-S-02-3017.A002

MEMBERS OF PROJECT

16 May 1977 through 15 May 1978

STAFF

Principal Investigator: Shields Warren, M. D.
Pathologist: 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
Roberta O'Brien, B. A.

ABSTRACT

Experiments are underway as to the cocarcinogenic effects of asbestos and carcinogenic hydrocarbons using rats and mice as test objects. None of the protocols of these experiments have as yet been completed.

The study of tumorigenesis in irradiated parabiont rats has been completed. Study of the benign tumors indicates that radiation is an effective neoplastic stimulus for only a limited number of organs and tissues, chiefly ovary, adrenal, mammary tissue, islands of Langerhans and liver. In general the benign tumors did not seriously affect health, and in only a very few animals did they become malignant. The incidence of malignant tumors in the parabiont series has been tabulated and analyzed.

Parabiosis alone appears to increase the incidence of leukemia and solid lymphoid tumors in NEDH rats.

Our study of radiation tumorigenesis in the adrenal cortex in the mouse has been completed. The cortex is highly resistant to tumor induction by irradiation. Cortical tumorigenesis is strongly influenced by changes in pituitary and ovarian hormones. Proliferation of capsular "A" cells appears an important early factor in carcinogenesis.

Hormonal imbalance continues to be an important factor in tumorigenesis in the parabiont pairs. If each of a pair of parabiont rats is irradiated sequentially at intervals, 30 days after a lethal dose of radiation is enough to permit the irradiated rat to support its partner through a like dose as effectively as would an unirradiated animal.

The transplantable radiation-induced functioning pheochromocytoma and insulinoma of rats continue to be valuable research tools.

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Department of Energy, 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.

STATEMENT OF TIME OR EFFORT OF THE PRINCIPAL INVESTIGATOR

During the current year of the project, the Principal Investigator, Shields Warren, M. D., Member at the Cancer Research Institute, has spent 20 percent of his time devoted to the research under this contract, but due to the restriction of funds caused by inflation has charged only 10 percent to the contract.

INTRODUCTION

Our work in Experimental Pathology has continued to have the advantages of excellent facilities provided through Dr. William V. McDermott, Jr., Director of the Cancer Research Institute, and the willingness of our investigators to volunteer appreciably more time and effort than provided for in the contract.

An unusual emergency which might well have been disastrous was the crippling February snowstorm in this area. Had it not been for the determined willingness of those able to reach the hospital to do whatever type of work had to be done, patients would have suffered, and heavy losses might have occurred in our animal colony. We are most grateful to all who were physically able to be present. In addition to the meeting of this emergency, volunteer efforts were contributed by a number on the staff, particularly Drs. Olive Gates and Clark E. Brown.

Rapidly rising costs against a fixed budget have forced us to cut down the number of experimental animals carried at any given time and otherwise protract the work schedules.

Work under Grant 5 R01 CA 12944 from the National Cancer Institute on radiation leukemogenesis in rodents was completed on January 31, 1978 and resulted in five publications.

Assistance from the Biomedical Research Support Grant of the National Cancer Institute to the New England Deaconess Hospital enabled us to pursue more effectively two pilot research projects undertaken under the direction of Dr. Brown and with the cooperation of Professor Kenneth Ryan, Director of the Laboratory of Human Reproduction and Reproductive Biology,

Harvard Medical School. One is concerned with the development of an animal model for the production of cancer of the prostate, hormonally induced in parabiont rats, the other with a model of interstitial tumor of the testis. In addition, special provision was made for prompt preparation of a number of histologic slides from our series of parabiont rats, essential to the timely completion of this experiment.

A friend of the hospital has aided our work with an emergency grant to meet in part unforeseen laboratory costs that have escalated with inflation.

Staff members participated in and gave papers at the meetings of the American Academy of Forensic Sciences, American Association for Cancer Research, American Industrial Hygiene Association, New England Society of Pathologists and the Radiation Research Society.

In addition Dr. Warren accepted the Chairmanship of the ad hoc Committee on Radioactivity in Drinking Water of the National Council on Radiation Protection and Measurements.

Dr. Warren continued his work with the Historical Advisory Committee of the Department of Energy, particularly stressing the early years of the Division of Biology and Medicine of the Atomic Energy Commission.

The New England Society of Pathologists established through the Shields Warren Club an Annual Lectureship in Dr. Warren's honor. The new Pharmaceutical Research and Development Laboratories of Mallinckrodt, Inc., St. Louis, where Dr. Warren is a Consultant and Director, were named for him. Dr. Warren served as the Michelson Visiting Professor at the University of Tennessee School of Medicine at Memphis.

Radiation safety has been maintained with the aid of the Harvard University Environmental Health and Safety Services. In addition,

Mr. Russell F. Cowing has served as a Consultant in radiation safety and in the planning and dosimetry of radiation experiments.

This year the compendium Forensic Medicine: A Study in Trauma and Environmental Hazards was published and contains a chapter on "Effects of Occupational and Environmental Exposures to Ionizing Radiation," a further step in our efforts to inform medical examiners and others concerned with industrial hazards of radiation and to counter undue fears of occupational and environmental exposure to radiation.

MAIN RESEARCH ACCOMPLISHMENTS

1) Asbestos and Cocarcinogenesis

Because of the ubiquity of asbestos fibers in the modern industrial environment and the known carcinogenic effects of asbestos in human tissues, we had undertaken a study of the effects in rats and mice. Secretary Califano has just called added attention to the major importance of asbestos as an industrial and environmental hazard. The great utility of asbestos as a fireproof insulator together with its essentially indestructible physical character have brought about its wide dissemination in the environment. Asbestos fibers in small amount are often found in the lungs of urban dwellers. Inhalation appears to be the most effective means of access to the body tissues in man, and the effects of inspired asbestos have been extensively studied.

We have made our initial experiments with two types of chrysotile asbestos fiber--one Canadian, the other Rhodesian. Chrysotile is the most commonly used form of asbestos, largely because of availability and the positive charge on the surface of the fibers which facilitates their binding with a wide variety of materials. We are using Grade 7 fibers from the standard UICC samples supplied through the kindness of the Medical Research Council, Pneumonoconiosis Unit, Penarth, Wales.

Miller et al. (1) have demonstrated high rates of carcinogenicity for asbestos combined with benzopyrene in the respiratory tract of animals. Because we have had past experience in the use of methylcholanthrene, we had decided to use this substance as a cocarcinogen with asbestos. In a limited number of the animals we have used localized x-radiation of the

lung and pleura as an added potential cocarcinogen exposing a limited field of the lung to radiation after intratracheal or intrapleural injection of the fibers. Because of the known danger of synergism in exposure to multiple carcinogenic agents, we are attempting to define the effects of asbestos and glass wool fibers commonly used in insulation when combined with those of radiation and selected known chemical carcinogens. The organs and tissues being exposed are the lungs, pleura, peritoneum and digestive tract.

We had hoped to carry out a series of experiments using glass wool, obtained from commercial sources, in place of asbestos because of somewhat similar physical properties of the two substances and because of the widespread use of glass wool as a substitute for asbestos. The experiments with glass wool have not yet been begun so as to keep cost of animal care within the budget.

Over 200 B6AF$_1$/J mice and over 100 NEDH rats have been injected intratracheally, intrapleurally or intraperitoneally with asbestos fiber and methylcholanthrene in sesame oil, each a potent carcinogen, to determine whether or not there is a cocarcinogenic effect and to study the cellular reactions to asbestos. Some of the animals have died as a result of induced cancers. The oldest animals of this series are now 765 days of age. A number are still surviving over 500 days after treatment with the combination of asbestos and methylcholanthrene.

The respiratory route of access of asbestos to the human body is by far the most important. However, asbestos in water and adhering to foodstuffs may provide risk to the gastrointestinal tract as suggested by Selikoff (2). We fed one series of mice a diet in which over 2 mgm of

asbestos fiber were ingested daily for over eight weeks, and the mice have been subsequently followed. Twenty-eight of the 40 so treated are alive and in good health. No gastrointestinal tumors have been found in those that have died thus far.

2) Parabiont Irradiated Rats: Aspects of Carcinogenesis

Very little attention has been paid to ionizing radiation as a cause of benign tumors. Our series of parabiont rats has provided extensive information on this point and has clearly established that in the dosage used, 1000 R of 250 kVp x-rays, radiation does not induce benign tumors in most tissues. In a limited number of organs, high incidence rates of benign tumors were found: ovary 49.7%, adrenal medulla 23.9% in males, 15.2% in females, mammary tissue 19.6% in females, islands of Langerhans 15.3% in males, and liver 7.4% in males, 13.8% in females. In addition to direct effects of radiation, hormonal imbalance induced by either parabiosis or radiation appear to be important. Benign mammary tumors appeared earlier in the hemi-irradiated pairs than in the unirradiated control pairs by about 200 days.

In general the benign tumors did not seriously affect health. In only a very few animals did the benign tumors become malignant.

A tumor-like lesion, a polyp of the endometrium, occurred in 30.2% of the irradiated partners, but this lesion was probably more related to hormonal than to direct radiation changes.

In a small series of parabiont rats each partner was given sequentially a dose of 1000 R after varying time intervals between the treatments. With an interval of 30 days between sequential radiations of the two partners

the same degree of protection was afforded to the most recently irradiated partner by its previously-irradiated partner as was afforded by an unirradiated partner indicating that complete recovery of its protective powers had occurred in 30 days. At 10-day and even at 5-day intervals partial protection was afforded.

Data as to the incidence of tumors in a series of parabiont rats surgically separated soon after irradiation are now being analyzed.

The incidence of malignant tumors in the parabiont series has been tabulated and analyzed and is now being prepared for publication.

Further analysis of the incidence of leukemia and solid lymphoid tumors in parabiont and hemi-irradiated pairs of rats indicates that in this species parabiosis in itself increases the incidence of these diseases. In sharp contrast to the effect of radiation in single mice, radiation tends to have an inhibitory effect on the development of these diseases in the rat.

In our study of parabiont pairs of rats, we found that some of the neoplastic diseases, particularly leukemia, were shared between the two partners. In order to determine better when such tumors arose and whether they arose in the irradiated or the shielded partner, the partners were surgically separated from one another, one series of 30 males and 29 females 60 days after the radiation of one partner; another series of 34 males and 30 females 150 days after irradiation of a partner. In those pairs separated 60 days after radiation, both partners of one former pair developed leukemia, suggesting that the disease was already present at the time of separation. Leukemia also occurred unilaterally in two former partners,

one irradiated, one shielded. In the partners separated at 150 days, each member of two former pairs died of leukemia. One partner only of former pairs became leukemic in seven instances.

3) Hormonal Aspects of Carcinogenesis

Our study of radiation tumorigenesis in the adrenal cortex of the mouse has been completed, and the results have been analyzed and are now being prepared for publication. Proliferation of capsular "A" cells and their progression to "B" cells precedes the development of tumors. Cortical tumor development is strongly influenced by pituitary and ovarian hormones.

Our collaborative studies of hormonal carcinogenesis in mammary tissues undertaken with the help of Dr. Kenneth Ryan, Director of the Laboratory of Human Reproduction and Reproductive Biology, Harvard Medical School, have been continued. The radioimmune assay technique for determination of the sex hormone levels in the parabiont rats has been useful in our studies of mammary cancer. In addition, studies of sex hormone levels in male parabiont rats have been undertaken, particularly as related to prostatic cancer and interstitial cell tumors of the testis.

4) Leukemogenesis

For the first third of the year the bulk of our work in leukemogenesis was supported by the National Cancer Institute. Radiation tends to inhibit rather than favor the occurrence of leukemia and solid lymphoid tumors in rats. The rat, therefore, reacts very differently than the mouse when used as a test object. The thymus, so often and intimately involved with leukemia in the mouse, is much less often involved in the rat.

5) Transplantable Tumors

The transplantable and actively functioning radiation-induced endocrine tumors in rats, particularly a pheochromocytoma and an insulinoma, have continued to be useful to a number of investigators. Transplants of these tumors and breeding stock of NEDH rats have been supplied during this past year to investigators in Israel and West Germany as well as to several in the United States. Through their use much biochemical information has been gained as to factors influencing secretion of the hormones involved. These tumors are being transplanted and further investigated in NEDH rats as well as maintained in reserve in the Institute's frozen tissue bank. The Division has also collaborated with Drs. Greengard and Herzfeld of the Institute's Growth Laboratory, supplying transplantable tumors for enzyme studies.

REFERENCES

1) Miller, L., Smith, W. E. and Berliner, S. W.: Tests for Effect of Asbestos on Benzo(a)pyrene Carcinogenesis in the Respiratory Tract. Ann. N. Y. Acad. Sci. 132:489, 1965.

2) Selikoff, I. J., Churg, J. and Hammond, E. C.: Asbestos Exposure and Neoplasia. JAMA 188:22, 1964.

PUBLICATIONS

DOE CONTRACT EY-76-S-02-3017.A002
NEW ENGLAND DEACONESS HOSPITAL
16 MAY 1977 - 15 MAY 1978

Brown, C. E. and Warren, S.:	Carcinoma of the Prostate in Irradiated Parabiotic Rats. Cancer Res. 38:159-162, 1/1978.
Warren, S.:	Effects of Occupational and Environmental Exposure to Ionizing Radiation. Chapter 18, Section 5. Injury by Ionizing Radiation, In Forensic Medicine. A Study in Trauma and Environmental Hazards. Vol. I, Mechanical Trauma. Ed. by C. G. Tedeschi, W. G. Eckert and L. G. Tedeschi, W. B. Saunders Co., Philadelphia, 1977, pp. 677-685.
Warren, S. and Brown, C. E.:	Mammary and Other Tumors as a Response to Radiation and Multiple Stresses. Arch. Path. Lab. Med. 102:224-226, 5/1978.
Warren, S., Chute, R. N., Porter, M. W., Brown, C. E. and Gates, O.:	Leukemia and Lymphoma in Irradiated Parabiont Rats. Radiation Res. 72:512-518, 12/1977.

Publications in Press

Herzfeld, A., Greengard, O. and Warren, S.:	Tissue Enzyme Changes in Parabiotic Rats with Subcutaneous Lymphoma. J. Nat. Cancer Inst.
Warren, S., Chute, R. N., Porter, M. W. and Brown, C. E.:	Late Effects of Successive Massive Doses of Radiation on Parabiont Rat Pairs. Internat. J. Rad. Biol.
Warren, S., Gates, O., Brown, C. E., Chute, R. N. and Porter, M. W.:	Spontaneous and Radiation-Induced Benign Tumors in Parabiont Rats. Radiation Res.