RADIATION CARCINOGENESIS

PROGRESS REPORT III
15 MARCH 1975 - 15 MARCH 1976

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MEMBERS OF PROJECT

15 March 1975 through 15 March 1976

STAFF

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ABSTRACT

The attention of forensic pathologists has been drawn to the criteria essential for the recognition of radiation death or injury in the hope that such cases will be better recognized and that some false claims of radiation damage might be prevented at the local level.

The results of our experiments have been as follows.

The series of parabiont rats one of which had been irradiated with 1000 R of 250 kVp x-ray, the other shielded, has been completed. With controls and related special studies the total is over 3000 pairs equally divided as to sex. Some specialized histologic studies remain to be completed. Much information on the relative sensitivity of different tissues and organs to tumor induction is available and is being statistically analyzed. These experiments have provided further evidence of the effectiveness of parabiosis in assuring survival after 1000 R x-radiation and have enabled us to learn of the types of problems that might be expected in the way of late effects in heavily irradiated individuals should they be enabled by effective therapy to survive the acute phases of the radiation syndrome.

Additional evidence has been brought out that carcinogenesis in some hormonally dependent tissues and organs is not so much a direct effect of radiation as it is a summation of this effect and of alterations caused by changes in hormonal balance brought about by the radiation.

We have found unilateral nephrectomy of each rat of a parabiont pair followed by radiation of one of the pair to augment the hormonal imbalance. This results in a very high incidence of carcinoma of the breast in females, not in the irradiated animal but in the unirradiated.

Evidence has long existed that functional change occurs in the adrenal cortex following radiation. There has, however, been little evidence of morphologic changes other than a direct destructive effect on cells at high dose levels. In irradiated mice in dose levels from 30-500 rads we have found proliferation of "A" cells, an established preneoplastic change. Not only this change but some tumors of the adrenal cortex have been produced although the adrenal gland is in general radioresistant. This change, which tends to occur in some aging mice, is accelerated and enhanced by radiation.

The interplay of radiation effect on lung and pleura with their inflammatory reaction to asbestos has been carried through the preliminary phases, and a number of the experimental animals have been prepared. Incident to the experiment we hope to obtain data on the relative carcinogenic effects of Canadian and Rhodesian asbestos and other important insulating materials.

Further studies have been made of metabolically active tumors induced by radiation in rats and mice. Two have proved of special experimental value--a pheochromocytoma and an insulinoma. Both are readily transplantable in rats and produce large amounts of the respective hormones.

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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% of his time devoted to the research of this project.

It is expected that during the coming year, Dr. Warren will continue to spend 20% of his time on this project.

INTRODUCTION

The period March 15, 1975 through March 15, 1976 has seen substantial progress in winding up our long-term experiments with irradiated parabiotic rats. Over 6500 histologic slides have been prepared and diagnosed. The data have been assembled on punch cards and several test series analyzed. Some detailed results are given in the articles cited in the list of publications.

We are deeply indebted to Dr. William V. McDermott, Jr., Director of the Cancer Research Institute, for provision to us of the facilities of the Institute, especially the excellent Animal Farm without which we could not function. The animal facilities of the Harvard Medical School have continued to provide us with inbred pathogen-free rats.

Under a grant from the National Cancer Institute special study has been given to the effect of radiation on leukemogenesis in rats. Results thus far indicate a mild inhibitory rather than an inducing effect.

During the year Dr. Warren spent 20 percent of his time on the project. Dr. Gates has continued to devote a major portion of her time as a volunteer which has greatly facilitated our work. Dr. Clark E. Brown has continued to aid us as Consultant in Pathology, a portion of the time as a volunteer. We are deeply indebted to both.

We are grateful for assistance from the General Research Support Grant of the National Cancer Institute to the New England Deaconess Hospital.

Dr. Warren has called the attention of those concerned with forensic pathology to the criteria essential for the recognition of radiation death or injury in the hope of properly recognizing such cases and of

preventing at the grassroots the initiation of mistaken claims for radiation injury. He has also continued to serve as a member of the National Aeronautics and Space Administration's Space Program Advisory Council. Dr. Warren, together with Dr. John Z. Bowers and Dr. Walter D. Claus, was honored at the Workshop on the Biological Effects and Toxicity of ^{239}Pu and ^{226}Ra sponsored by the University of Utah Division of Radiobiology and the Energy Research and Development Administration.

Dr. Warren and Dr. Gates presented a paper on "Leukemogenesis by Low-Level Radiation" at the International Atomic Energy Agency Symposium on Biological Effects of Low Level Radiation Pertinent to Protection of Man and His Environment.

Dr. Warren presented a Seminar in Radiobiology at the University of Iowa.

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 parttime as a Consultant in radiation safety and in the planning and dosimetry of radiation experiments. Miss Olive M. Lombard has continued as Consultant in biostatistics. Her book, "Biostatistics for the Health Professions," was published in 1975.

MAIN RESEARCH ACCOMPLISHMENTS

Our experiments during this period have been 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 parabiont rodents.

B. Incidental Radiation Effects

1. Physiopathologic effects of some radiation-induced endocrine tumors.
2. Irradiated transplants of human cancer cells.

A. 1. The Tumorigenic Effects of Acutely Delivered High Doses in Parabiotic Rats--This series is now virtually complete--over 3000 pairs. The parabiotic pairs dying during the year provided further evidence of the effectiveness of parabiosis in assuring survival and of heavy-dose (1000 R) radiation in increasing the incidence of neoplasms. About three fourths of the data have been transferred to punch cards for tabulation and analysis.

In one subgroup, 1000 rads of acute whole-body 250 kVp x-rays had been delivered to each member of the pair successively with an interval of 60 days between. The long-term survival of the pairs has demonstrated the ability of a supralethally-irradiated rat not only to recover with

the aid of parabiosis, as is well known, but in turn to be able to support its subsequently irradiated partner. Interestingly enough, these pairs of animals have developed several of the anastomotic line tumors described by us recently.¹

A number of parabiosed and irradiated pairs which were separated at various time intervals after irradiation have died and are being studied to attempt to differentiate between the effects of parabiosis alone and those of radiation. Some of these animals have developed tumors, but not a sufficient number have died as yet to permit conclusions to be drawn.

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—Cataracts were produced in 75% of the irradiated members of parabiont pairs one of which had received 1000 R of whole-body x-radiation. The incidence of cataract in their shielded partners was 0.2%, the same as in control parabiont pairs. In addition to cataract this dose of radiation produced extensive ophthalmic pathology, including degeneration of the retina with loss of cells in all layers, marked hyaline thickening of the walls of intraocular blood vessels, and increased deposition of collagen in interstitial tissues. This fact of damage to other tissues of the eye, the lens, has not previously been adequately stressed.

¹Warren, S., Hurst, E. E., Jr., Brown, C. E. and Chute, R. N.: Anastomotic Sarcoma of Irradiated Parabiont Rats. Cancer Res. 32:983-987, 5/1972.

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 by Dr. Gates 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 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) in many animals, especially females. The evidence reinforces the view that 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.

We have continued to give special attention to the development of mammary tumors in female parabiont WEDH rats, particularly in carrying further our study of the effect of unilateral nephrectomy in each of the parabiosed partners prior to radiation. Evidence continues to accumulate that the induction of mammary cancer by radiation is strongly influenced by hormonal changes. Preliminary results by radioimmune assay techniques suggest that the increased incidence of mammary tumor observed in the nonirradiated partner is related to high prolactin levels, perhaps incident to impaired pituitary feedback from the damaged ovaries in the irradiated partner.

Many of our recent experiments in leukemogenesis have been carried out with the aid of funds granted by the National Cancer Institute.

Intermittent x-radiation of 30 R administered in 3 R doses for ten successive weeks to RAP mice did not increase the incidence of leukemia, but a total dose of 100 R given at the rate of 10 R weekly significantly increased the level. The incidence was only slightly further increased by a threefold greater dose. Continuous perinatal exposure to gamma rays of ^{60}Co in doses ranging from 200-1000 rads yielded similar and increased incidences of leukemia.

In contrast with the mouse where leukemia was readily induced by radiation, the rat appears to be resistant. Single rats irradiated within a range of 700-1100 R did not develop more leukemia than the controls. Unirradiated parabiotic rats showed a slight increase (about 4%) in incidence of leukemia, but irradiation with 1000 R of one of the pair decreased the incidence of the disease rather than increased it. Our findings are congruent with the suggestions made by Mole² and by other authors summarized by Mayneord³ that with doses higher than those giving the peak responses of leukemia the leukemogenic effect of radiation may be diminished through the killing of cells that have either been made leukemic or are susceptible of becoming leukemic.

We called attention to several examples of variation in susceptibility to radiation-induced leukemia, mammary cancer and other tumors

²Mole, R. H.: In "Proceedings of the Second United Nations International Conference on the Peaceful Uses of Atomic Energy," Vol. 22, "Biological Effects of Radiation," United Nations, New York, 1958, pp. 145-148.

³Mayneord, W. V. and Clarke, R. H.: Carcinogenesis and Radiation Risk: A Biomathematical Reconnaissance. Brit. J. Radiol. Suppl. 12, 1975, 112 pp.

in different experimental rodent populations further emphasizing that intrinsic factors inherent in any given population group must be considered as well as the radiation stimulus applied.

A. 3. The Interplay of Radiation and Asbestos as Carcinogens in Both Single and Parabiont Rodents--UICC standard Canadian and Rhodesian chrysotile samples suspended in physiological saline solution have been injected intratracheally and intrapleurally into single and parabiont NEDH rats. Intrapleural and intraperitoneal inoculation of asbestos can be readily done, but intratracheal is difficult because of resultant respiratory problems.⁴ In spite of the difficulties the number of animals required by the protocol has been achieved. It has been shown by Sanders at Battelle Pacific Northwest Laboratories that fewer lung tumors developed in rats given particulate plutonium and asbestos than in rats given plutonium alone. We hope to determine the effect of asbestos injection or aspiration plus external radiation, each a carcinogen in its own right. Also, we are comparing the carcinogenic effects of Canadian and Rhodesian asbestos.

B. 1. Physiologic Effects of some Radiation-induced Endocrine Tumors--A large range of tumors of endocrine tissue have been induced in our irradiated rats and mice. A number have proved transplantable, and two of these have been particularly serviceable for studies of hormonal effects. One is a functioning pheochromocytoma that reproduces the disease as seen in man characterized by hypertension and myocardial

⁴Sanders, C. L., Jr.: Dose Distribution and Neoplasia in the Lung Following Intratracheal Instillation of ²³⁹PuO₂ and Asbestos. Health Physics 28:383-386, 4/1975.

damage. Tischler and Greene⁵ have demonstrated that some of these pheochromocytoma cells respond to nerve growth promoting factor by developing neural processes. This tumor has been supplied to several groups of investigators including one in Switzerland. We also have available a highly functioning insulinoma which produces a marked degree of hyperinsulinism in the animals into which it is transplanted. This is currently being studied by Dr. Chick of the Joslin Diabetes Foundation in collaboration with us and by Dr. Like of the University of Massachusetts Medical School. Among other aspects the insulin content of the tumor is being determined.

B. 2. Irradiated Transplants of Human Cancer Cells—A number of transplantable human tumors that have been irradiated heavily in the past have been maintained in the Institute's frozen tissue bank. Samples are periodically tested for viability and constancy of morphology.

⁵Tischler, A. S. and Greene, L. A.: Nerve Growth Factor-induced Process Formation by Cultured Rat Pheochromocytoma Cells. *Nature* 258:341-342, 11/27/75.

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