COO-3017-39

RADIATION CARCINOGENESIS

Progress Report VI

Shields Warren and Olive Gates

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

16 May 1978 through 15 May 1979

Prepared for the U. S. Department of Energy
Under Contract No. EY-76-S-02-3017.A004

DISCLAIMER

TABLE OF CONTENTS

Page

U. S. DEPARTMENT OF ENERGY
Contract No. EY-76-S-02-3017.A004

MEMBERS OF PROJECT

16 May 1978 through 15 May 1979

STAFF

Principal Investigator: Shields Warren, M. D.
Pathologist: Olive Gates, M. D.

Consultants:

Clark E. Brown, M. D.
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

Asbestos fibers, Canadian and Rhodesian chrysotile, have induced mesotheliomas and pleural plaques in mice and rats. Most lesions occur in animals dying over 500 days after treatment. The pathogenesis of the lesions is being studied. This experiment is as yet incomplete.

Proliferation of the adrenal cortical "A" cells is accelerated in time and enhanced in extent by ionizing radiation in female RAP mice. The development of cortical tumors was significantly correlated with extensive "A" cell proliferation. Whole-body protracted doses, above 500 rads, were followed by a 9% tumor incidence (none in controls). Endocrine neoplasia in mice appears related to functional ties between several endocrines particularly adrenal, ovary and pituitary.

A study of the spontaneous benign and malignant tumors occurring in control NEDH rats has provided background for the tumorigenic effects of radiation and also of altered hormonal internal milieu.

We have produced benign tumors by radiation in a wide variety of tissues for the first time, providing data as to the relative responsiveness in benign tumor production of various tissues to the dose of 1000 rads used. The hormonal changes secondary to parabiosis and hemi-irradiation of the pair are important.

A radiation-induced functioning pheochromocytoma causing hypertension and death has been found widely useful by investigators. A radiation-induced malignant insulinoma has also been widely used, and insulin-producing DNA from it has served in the hands of others through genetic engineering to provide an insulin-producing strain of the bacillus E. coli.

NOTICE

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 budgetary constraints has charged only 10 percent to the contract.

INTRODUCTION

During this year we have been initiating experiments in carcinogenesis with ionizing radiation and asbestos fibers as physical agents and have made some use of methylcholanthrene, highly potent chemically, to correlate to a degree the relative effectiveness of physical and chemical carcinogens.

The studies of neoplasms induced by 1000 R of total-body radiation to one of a pair of NEDH parabiont rats has been brought virtually to a conclusion. The wealth of data provided by these studies is still being analyzed and is continuing to provide information as to the relative radioresponsiveness of various tissues. In addition, new data are provided as to the late effects of a heavy dose (1000 R) of radiation, obtained from rodents only through the long survival times permitted by parabiosis. In the event of accidental human exposure to heavy doses of radiation, the research of others has suggested means for survival of the acute radiation syndrome induced by doses in this range. The data from our past experiments on radiation of parabiont rats provide an indication of the late effects that might be anticipated. The most important of these is tumorigenesis.

Our systematic study of radiation tumorigenesis in parabiont rats has provided for the first time clear evidence that a number of benign tumors as well as malignant can be caused by radiation. The quantitative data thus provided for a wide variety of tissues permit determining the relative responses by tumorigenesis in organs and tissues to radiation as manifested in the incidences of benign and malignant tumors as compared with those lesions occurring spontaneously.

We are still reviewing and analyzing the data obtained from our long series of hemi-irradiated parabiont rats. We are finding additional useful data as to the relative radioresponsiveness of various tissues. Also, this technique has provided about the only means of providing information as to late effects, particularly carcinogenic, in the rat. These data provide additional evidence as to the carcinogenic effects to be anticipated among survivors of heavy doses of radiation.

While there is recently increased emphasis on the need for dose-response effects from low levels of radiation, such experiments have to be very large scale to yield statistically-significant results. It is important to continue to probe the tumorigenic response of organs and tissues to those radiation doses large enough to produce measureable alterations in incidence with numbers of animals small enough to be economically feasible and yet give clear end results in a reasonable time frame.

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.

A friend of the Hospital has aided our work with an emergency grant to meet in part unforeseen laboratory costs due to inflation. Additional support has been obtained from a number of sources including the National Cancer Institute and private donors.

Personnel

As in the past, Dr. William V. McDermott, Jr., Director of the Cancer Research Institute has been most helpful and provided the Department of Experimental Pathology with excellent facilities.

Notable were the services of Dr. Olive Cates who has contributed as a volunteer full-time effort to this research and Dr. Clark E. Brown who has aided with part-time endeavor to a significant degree as Consultant in Pathology.

Radiation safety and protection have been maintained with the aid of the Harvard University Environmental Health and Safety Services. Mr. Russell F. Cowing, who had long served as Consultant in radiation safety, died in January, 1979.

The New England Deaconess Hospital is one of the teaching hospitals of the Harvard Medical School, and the staff of the Cancer Research Institute has direct ties with the Departments of Biochemistry, Pathology, Surgery and the Laboratory of Human Reproduction and Reproductive Biology at the School.

Our cooperation with Prof. K. J. Ryan and the Laboratory of Human Reproduction and Reproductive Biology is continuing focussed on hormonal assay of experimental animals in relation to the development of tumors of the reproductive tract. This study originated in pilot form with Department of Energy support. It is now funded by the National Cancer Institute.

Dr. Warren has served as a member of the National Academy of Sciences--National Research Council Panel on the Extent of Radiation from the PAVE PAWS Radar System. This report was made available to the public this Spring. He also served as Chairman of Scientific Committee 58 on Radioactivity in Water of the National Council on Radiation Protection.

Dr. Warren participated in and gave a paper at the meeting of the Association of Clinical Scientists, delivered the Ronald C. Sniffen Memorial Lecture at the Memorial Hospital in Worcester, MA on "The Late

Effects of Radiation at Hiroshima and Nagasaki" and presented a seminar on "History of Pathology in Medicine" at the National Naval Medical Center.

A pilot study under the guidance of Dr. Brown has been completed, and continuing research funding has been provided by the National Cancer Institute Grant CA 24893. We acknowledge gratefully support from the Biomedical Research Support Grant RR 05591 for aid in meeting the costs of animal care and histologic preparations.

The recent verdict in the Silkwood case has again demonstrated the importance of familiarizing pathologists and particularly forensic pathologists with the effects of ionizing radiation on man. Dr. Warren has been long interested in this field and is continuing his educational efforts here in the Hospital of providing a better-balanced view of the subject as a whole and in providing objective criteria that can be utilized in any given case.

Safety for Personnel and Environment

In the handling of carcinogenic agents the appropriate safety guidelines recommended by the National Cancer Institute for carcinogenic substances are carried out as well as appropriate recommendations of the Nuclear Regulatory Commission. There has been no lost-time accident involving personnel of the Experimental Pathology Division during this period.

Among the available specialized facilities that we are using are those for accurate measurement and safe handling of a considerable range of radioactive substances. The Deaconess Hospital has a general license for the biomedical use of a considerable range of radioactive isotopes. The Cancer Research Institute, in addition to its own 250 kVp x-ray

apparatus for small animals, has other modalities through the cooperation of the Department of Therapeutic Radiology. A considerable additional range of devices for measurement of radiation are available through the Joint Center for Radiation Therapy.

There has been no incident of excessive exposure to radiation or uncontrolled escape of radiation to the environment.

Facilities

The New England Deaconess Hospital has provided for us three adequately equipped laboratories, three offices, a facility for preparation of pathologic slides and use of the Animal Farm, an invaluable asset. Veterinary supervision of our experimental animals was provided in the first part of this period by the staff of the New England Regional Primate Research Center. Recently to meet the increasing needs of supervision of animals, through a joint service with several other Boston hospitals and the Animal Rescue League of Boston, we have been fortunate in obtaining as Consultant Dr. Richard W. Fite, Staff Veterinarian of the Animal Rescue League of Boston. Dr. Fite has had extensive experience in the care of small animals. An Animal Care Committee of the Cancer Research Institute staff and administration is responsible for the operation of the Farm. The Farm is fully accredited by the American Association for Accreditation of Laboratory Animal Care.

A negative pressure room with laminar flow hood is available for handling of hazardous materials.

In addition, through the cooperation of Dr. Micheline Federman and the Laboratory of Pathology of the Hospital, facilities for electron microscopy are available.

Results of our research from May 16, 1973 through May 15, 1979 will be summarized in the following pages. Detailed results may be found in the articles cited in the list of publications.

MAIN RESEARCH ACCOMPLISHMENTS

Our experiments during the past year may be grouped as follows:

A. Carcinogenesis by Asbestos in Rodents

1. Chrysotile Fibers Injected Intratracheally, Intrapleurally and Intraperitoneally
2. Cocarcinogenesis
   a. Asbestos and methylcholanthrene -- lung and pleura

B. Tumorigenesis by Ionizing Radiation

1. Adrenal
   a. Cortex
   b. Medulla
2. Pancreas
   a. Insulinoma
3. Effect of Radiation of the Female Breast
4. Hormonal Changes in Parabiont Males and Neoplasms of Testis and Prostate

C. Relative Sensitivity of Tissues to Induction of Benign and Malignant Tumors by Radiation

D. Miscellaneous

1. Transplantable Tumors
   a. Functional endocrine tumors
   b. Irradiated human cancers transplantable to hamsters
2. Radiation Leukemogenesis

A. Carcinogenesis by Asbestos in Rodents

1. Chrysotile Fibers Injected Intratracheally, Intrapleurally and Intraperitoneally

The first hundred NEDH rats injected with a physiological saline suspension of asbestos fibers have completed their lifespan and are now being studied. We have found in them pleural mesothelioma as well as the fibrous and calcified pleural plaques often associated with asbestosis in man. These pleural plaques are very similar to those in man with sharp, raised edges and generally smooth surface. They are yellowish white or gray in color. Microscopically there are dense fibers with abundant collagen. The fibrocytes are mature. The wavy fibers of collagen tend to parallel the pleural surface. The plaques are relatively avascular. Occasionally they show slight infiltration with lymphocytes and plasma cells. Calcification, usually patchy, is not unusual. They are not associated with adhesions. These pleural plaques are not an unique reaction to asbestos (1). Most tumor-bearing animals had lived over 500 days postinjection. Since not all of the test animals have died, the results of the experiment have not yet been analyzed and reported.

These experiments with chrysotile asbestos are still in progress. Single NEDH rats have been injected intrapleurally or intratracheally, and in addition a segment of one lung was given a local dose of 2000 R. Of these 43 animals, 11 have thus far shown evidence of tumor. In 45 other rats injected intratracheally or intrapleurally with asbestos 11 have thus far shown related tumors.

The asbestos was injected in physiologic saline solution, suspended by agitation. Two types of asbestos were used--Canadian and Rhodesian. Grade 7 of the UICC standardized sample has been used throughout our work.

Because glass is closely related chemically to asbestos and its fibers used for insulation (glass wool) are somewhat similar to those of asbestos, although in general coarser, we have injected these fibers similarly into NEDH rats and mice to test their carcinogenicity. Also, glass wool is widely used in industry. The finer washed fibers eluted from the glass wool have rather similar fibrogenic effects to those of asbestos, but we have not yet found any tumors following injection of this material.

2. Cocarcinogenesis

a. Asbestos and methylcholanthrene -- lung and pleura

Methylcholanthrene is an appreciably faster acting carcinogen than is asbestos, apparently by 100 to 200 days in rats. Consequently, the mice and rats thus far available for study have shown cancers induced by the chemical carcinogen rather than those expected to be induced by the asbestos fibers. The dosage used was 2 mgm of asbestos suspended in saline combined with 1 mgm of methylcholanthrene suspended in sesame oil. This combination was injected intrapleurally and intraperitoneally in 88 rats, 29 of which have thus far shown evidence of related tumor. The methylcholanthrene is partly suspended in its carrier agent, sesame oil, and consequently its tumors contain macrophages and foreign body giant cells that have phagocyted some methylcholanthrene crystals. One hundred sixty $B6AF_1/J$ mice were injected with 0.4 mgm asbestos combined with 0.2 mgm methylcholanthrene intrapleurally and intraperitoneally, and of these 51 have shown evidence of related tumors. Electron microscopic studies have shown that the larger diameter fibers tend to break down to very fine fibers, about 1.0 μm in our preparations. These are within the diameter of fibers considered to be active in induction of asbestosis,

where the diameters are given as 0.5 to 2.5 μm (2). We have not controlled the length of the fibers which are highly variable in our samples, ranging from two micra to several millimeters in length. Because man is at risk from a wide variety of fiber sizes, we have not attempted to standardize the size of fiber used. Methods for such standardization are given by Spurny et al. (3).

In addition, we are following 80 $B6AF_1/J$ mice injected intracavitarily with 0.4 mgm of Rhodesian asbestos and a similar group using Canadian asbestos. Another group of 80 mice has been injected with 2 mgm of a saline suspension of glass wool prepared by elution to provide the finer fibers in the sample. These range over 10 times the diameter of the asbestos fibers.

B. Tumorigenesis by Ionizing Radiation

1. Adrenal

a. Cortex

Proliferation of adrenal cortical "A" cells, which normally occurs as a response to aging in mice, particularly in females, is the precursor of cortical tumors. We have studied this proliferation of "A" cells in the adrenals of 695 untreated RAP male and female mice and in 915 that had received gamma or x-radiation. This proliferation was both accelerated in time of development and enhanced in extent by radiation, particularly in females. The response tends to be slight following doses in the range from 30 R to 300 R of x-radiation, moderate after continuous lifetime gamma radiation, and most marked in perinatally-irradiated females who had received gamma radiation in the range of 332 to over 1200 rads. This latter group alone developed cortical tumors. The development of

tumors was significantly correlated with extensive "A" cell proliferation. The incidence of cortical tumors was influenced by the total dose of gamma radiation; those who had received less than 500 rads had about 3% incidence while those who had received over 500 rads had 9% tumor incidence.

We have reviewed extensively the published data on tumorigenesis in the adrenal cortex and have been impressed by the extent to which hormonal imbalance appears to be a factor. In female mice, the sex we have studied more carefully, the hormones of ovary, adrenal medulla and pituitary appear to have important effects on the neoplastic behavior of cortical cells. Studies of endocrine neoplasia, a field brilliantly advanced by Furth, Upton and numerous others, suggest that this group of lesions may be a "disease of senility" developing in organs undergoing involution. Such changes may be simulated to some extent in young mice by adventitious cell injury; for example, radiation or other means of altering the mutually responsive functional ties between the several endocrines. This is perhaps seen best in the ovary where moderate doses of radiation injure or kill oocytes without obviously affecting the residual cells. These may respond to the reversed ovarian-pituitary feedback by the production of a variety of tumors. Interestingly enough, the morphologic sequence of change in the adrenal or the ovary tends to involve undifferentiated stem cells and may culminate in neoplastic metaplasia. The course of development of the tumors appears to be the same whether the initial impetus is natural (senescence) or imposed (radiation). If one accepts the normal stem cell as the key target in carcinogenesis, it would appear that in the adrenal cortex the capsular blastomeres appear to be the target of radiation that results in the ultimate formation of adrenal tumors.

b. Medulla

There is a high spontaneous incidence (over 35%) of pheochromocytomas in single and parabiosed male NEDH rats, over 20% in females. One of these tumors, found in an irradiated partner of a parabiosed pair, has been readily transplantable and actively functioning, producing fatal hypertension, often associated lesions of arterioles and myocardium.

This tumor has been widely used in studies of hypertension, and transplants have been provided to 26 investigators in the United States and overseas.

2. Pancreas

a. Insulinomas

Insulinomas, both benign and malignant, have been induced by radiation of parabiont pairs of rats. The incidence in controls is 1% or less, but over 15% in irradiated male partners.

Dr. William L. Chick of the Joslin Clinic has been working with this radiation-induced insulinoma of the rat, which we had supplied to him. He and his colleagues had obtained insulin messenger RNA from the insulinoma cells, later converted to insulin DNA. Dr. Walter Gilbert and associates of the Biology Department of Harvard successfully linked this DNA with a penicillinase gene of E. coli, found on the bacterial plasmids. It should be made clear that our group was not involved in the gene transplantation experiments in which we have no competence. However, without the special properties of the tumor (4) which we developed this brilliant transplantation work could not have been done.

3. Effect of Radiation of the Female Breast

The study of girls whose breasts were repeatedly exposed to radiation incident to fluoroscopy during the course of treatment of tuberculosis

mentioned in the last Progress Report, a study begun under this research program, has been completed by Drs. John D. Boice and Norman C. Telles, now of the Bureau of Radiological Health (5). This study, carried out in the Department of Epidemiology of the Harvard School of Public Health, with the cooperation of Dr. Gerald Parker of the Massachusetts Department of Public Health, was supported by the Bureau of Radiological Health. The estimated total dose to the skin of the breast was about 4000 R in some cases, much less in others. The incidence of carcinoma of the breast in the repeatedly fluoroscoped group was twice that found in matched controls. The followup was highly successful in identifying and obtaining the cooperation of the survivors although the time from exposure to the followup study was as long as 40 years in some cases. This further evidence that cancer of the breast may be induced by radiation and that radiation of the prepubertal nonfunctioning breast may result in subsequent development of an excess incidence of cancer leads us to give further attention to the relationship of the internal hormonal milieu and exposure.

We found that when hemi-irradiation of paired female parabiosed NEDH rats was done carcinomas of the breast were far more frequent in the shielded than the irradiated partners. This difference was further emphasized by unilateral nephrectomy, which aided in retention of gonadotropic hormones.

Parabiosis of female NEDH rats with castrate males of oophorectomized females produces carcinoma of the breast in the majority of intact females. When unilateral nephrectomy of each partner is added to the procedure, the incidence is nearly 100 percent.

Radioimmunoassays for luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, estradiol and progesterone have been performed on the sera of these rats. The levels were found generally to be high, though progesterone remained at normal levels.

An attempt to weigh various factors thought to be involved in causing human and experimental rat cancer of the breast suggests significant differences of a quantitative nature (6). As yet no virus has been established as causative in man. Heredity is a much clearer etiologic factor in rodents than in man.

4. Hormonal Changes in Parabiont Males and Neoplasms of Testis and Prostate

Another aspect of the role of the internal hormonal milieu is brought out by a pilot study (7, 8) undertaken first with parabiotic hemi-irradiated rats indicating that following hemi-irradiation of the pair mammary cancers developed in the unirradiated partner of female pairs as well as in the irradiated partners. Unilateral nephrectomy intensified this effect. With this and other indications of the importance of hormonal alterations brought about by parabiosis with or without irradiation as a lead, we found that parabiosis of normal male rats and castrate male rats, some unilaterally nephrectomized led to high levels of some gonadotropic hormones and also to development of interstitial cell tumors of the testis and adenocarcinomas of the prostate in the normal male partners. These initial pilot studies are being elaborated with the aid of funding from the National Cancer Institute.

C. Relative Sensitivity of Tissues to Induction of Benign and Malignant Tumors by Radiation

An opportunity to determine the relative sensitivity of the various cell types of the NEDH rat to heavy doses (1000 R) of whole-body radiation is afforded by our past experiments with hemi-irradiation of parabiont pairs of rats. While practically every cell type produces an occasional tumor spontaneously, either radiation or the hormonal changes incident to radiation produce a measureable increment in tumor incidence in only a relatively few cell types or organs. Incidentally, the control animals for these studies of tumor incidence have provided very extensive documentation of the spontaneous incidence of both benign and malignant tumors of all types in this strain. Such documentation is of major importance to provide background information for enhanced incidence of different types of tumor with the investigation of potential carcinogens.

We have clearly established for the first time the induction by radiation of benign tumors in a wide variety of organs and tissues. By using parabiosis of rats and irradiating one of the partners while shielding the other, it has been possible to determine the response of the irradiated partner to a heavy dose of whole-body radiation, 1000 R. This procedure enables obtaining information that could not otherwise have been obtained without the vital support of the shielded animal through the period necessary for recovery of the irradiated partner from the acute radiation syndrome. This study has not only demonstrated the induction of benign tumors by this dose of radiation but has further enabled us to determine the relative response of the various tissues. This has indicated that ovarian tumors were the most frequently induced. The endocrine system in general was quite susceptible.

Our studies of the induction of benign tumors by radiation in the parabiont pairs has been completed and published (9). The corresponding study of the incidences of malignant tumors is nearing completion.

Although there has been great interest in the carcinogenic effects of ionizing radiation in recent years, relatively little attention has been focused on its effectiveness as an inducing agent for benign tumors. There has been valuable work done particularly by Shellabarger et al. (10) with regard to the induction of mammary tumors, both benign and malignant, and a number of other articles have called attention to the potential role of radiation as a factor in the induction of benign neoplasms. However, very little attention has been given to the types and sites of neoplasms induced.

Our study of parabiont rats has produced an adequate volume of data to permit the conclusion that spontaneous benign tumors are induced by radiation and that certain organs and tissues respond more readily than others by forming benign tumors. In the NEDH rat following a whole-body dose of 1000 R 250 kVp x-ray to one partner of a pair while the other was shielded to permit survival of the pair following this lethally-potential dose to the partner, large numbers of benign tumors were induced.

Spontaneous benign tumors are frequent in NEDH rats; the ovary is the most frequent site. The adrenal medulla is the next highest, where the incidence of benign pheochromocytoma is 35.1% in males, 22.5% in females. Another tissue frequently involved is the mammary in females with a spontaneous benign tumor incidence of 11.2%.

Following radiation, the ovary was the most frequently involved organ, the incidence of benign tumors being 49.7%. The commonest type of benign

ovarian tumor was the relatively undifferentiated tubular adenoma. In mammary tissue of females the benign tumor incidence was 19.6%.

Another frequent tissue to be involved was the adrenal medulla. While benign pheochromocytomas occurred frequently in single control rats--35.1% in males, 22.1% in females, the spontaneous incidence was sharply reduced by parabiosis alone to 11.8% in males, 11.9% in females. This reduction is in part related to the shorter lifespan of the parabiotic controls, an average of 647 days as against 710 days for the single controls. This decrease may well be related to life shortening since most pheochromocytomas developed after 500 days of age in the single control animals whose average lifespan was 710 days, while the average lifespan of the hemi-irradiated pairs was 561 days. Radiation proved to be tumorigenic for the irradiated partner of the hemi-irradiated pairs, increasing the incidence to 23.8% in males and 14.9% in females, nearly overcoming in a shortened lifespan the apparent protective effect of parabiosis. In the case of the malignant variant of this lesion, radiation was ineffective in cancelling this suppressive effect of parabiosis.

Interestingly enough, although the liver is in general a radiation-resistant organ, cystadenomas and cholangiomas, which were not found in single controls and extraordinarily rare in control parabiont pairs, were found in 7.4% of the male irradiated partners and 13.8% of the female. Their incidence in the shielded partners of the hemi-irradiated pairs was 0% for males, 0.2% for females. The hepatic cells proper are virtually unresponsive to induction of benign tumors, the only series in which they occurred being that of the irradiated male partner, and here the incidence was extremely small, 0.1%.

Curiously enough, the islet cell adenoma of the pancreas is extremely rare spontaneously with an incidence of 1% or less in all the control series but occurring in 15.3% of male irradiated mice, 2.9% of female irradiated mice. The endometrium showed the presence of polyps fairly frequently--6.7% in control single rats, 9.6% in control parabiont pairs, and 3.1% in the shielded partners of the hemi-irradiated pairs, while the irradiated partners developed 30.2%, suggesting a direct effect of the radiation on the endometrium.

The skin and subcutaneous tissue of the irradiated partners showed distinctly more benign tumors--cysts, fibromas, papillomas--than did the controls.

Mammary tissue also was responsive to radiation, adenomas being the commonest benign tumor induced. The incidence in females was 19.6% as contrasted with 11.2% in single controls. It was only 1.3% in hemi-irradiated parabiont males contrasted with 1.2% in single male controls.

Cholangiomas, usually small, were induced in the liver of a number of rats--7.4% in males and 13.8% in females.

The benign tumors only rarely adversely affected the health of the animals. Occasional endocrine tumors were functional and rarely benign tumors of other tissues became sufficiently large to be locally obstructive or to cause ulceration.

Our study of malignant tumors in hemi-irradiated parabiont pairs is now nearing completion. All animals have been autopsied and all cancers have been identified. Those tissues most susceptible to radiation carcinogenesis in the irradiated partner of parabiont rats appear to be soft tissue, kidney, bone and pancreatic islands, in that order.

As noted in an earlier paper (11), renal cells appear highly susceptible to radiation carcinogenesis at the dose level we have used (1000 R). Recent studies by others of the effects of radiation on the kidney did not report the occurrence of renal cancers, perhaps because the lifespan of the animals used was too short. Madrazo et al. (12) in their study of late effects after high doses of radiation (doses of 4800 and 9600 rads to one kidney) did not find any renal tumors, probably because these doses killed many of the epithelial cells and also because the rats were killed relatively early--six weeks to nine months after radiation. With smaller doses (1500 and 2000 rads) a few rats were allowed to live twelve months. They did not note any tumors (13) in these either. Jordan et al. (14) irradiated the remaining kidney of mice after unilateral nephrectomy with divided doses of 1250 to 5000 rads. Some of the animals survived for up to 31 weeks. Highly abnormal tubular epithelial cells were seen, but no tumors occurred as would be expected with so short a latent period. Jordan et al. (15) noted that mice receiving 1000 rads showed no significant histologic renal abnormalities, but that slight changes in tubular epithelium occurred after doses of 1100 rads.

D. Miscellaneous

1. Transplantable Tumors

a. Functional endocrine tumors

Among the tumors induced in this laboratory several have been extremely efficient producers of specific hormones as well as being readily transplantable. Two (mentioned above) in particular have been widely used by investigators--the pheochromocytoma which produced hypertension and an insulinoma (islet cell carcinoma) which produces both proinsulin and insulin.

Cells of the latter tumor have been successfully used by investigators at the Joslin Diabetes Foundation and the Department of Biology at Harvard University to develop through genetic engineering an insulin-producing strain of E. coli.

Our transplantable rat insulinoma RNC-233 has been used by 16 investigators in this country and three overseas. The rat pheochromocytoma RNC-259 has been provided to 26 investigators in this country and to two overseas.

With relation to insulinoma, passing reference should be made to the brilliant work of Chick, Gilbert and their colleagues since we supplied the radiation-induced insulinoma which made their experiments possible and which has opened up brilliant opportunities for the ultimate production of insulin and other hormones by bacterial cultures.

b. Irradiated human cancers transplantable to hamsters

Several transplantable human cancers that had been heavily irradiated in the hamster cheek pouch have been carried in the tissue bank but are not being actively studied.

2. Radiation Leukemogenesis

Until February of 1973 the bulk of our work in leukemogenesis was supported by the National Cancer Institute. We found that hemi-irradiated pairs of parabiont NEDH rats react very differently to a heavy dose (1000 R) of x-radiation than do single mice to smaller doses. Whereas in many strains of mice the incidence of leukemia following irradiation is high and the leukemia is usually of lymphoid type and associated with thymic involvement, lymphoid leukemia is relatively rare in rats. In the NEDH rat spontaneous leukemia is rare and the monocytoid and myeloid forms

predominate. The thymus is only rarely involved in the leukemic process. The heavy dose of 1000 R to one partner reduced the incidence of leukemia by about one half, but about one half from that which prevailed in the parabiont control animals.

References

1) Preger, L.: Asbestos-Related Disease. Grune & Stratton, New York, 1973, P. 67.

2) Pott, F.: Arbeitsmed. Sozialmed. Präventivmed. 12:172, 1977.

3) Spurny, K. R., Stöber, W., Opiela, H. and Weiss, G.: Size-selective preparation of inorganic fibers for biological experiments. Am. Ind. Hyg. Assoc. J. 40:20-38, 1979.

4) Chick, W. L., Warren, S., Chute, R. N., Like, A. A., Lauris, W. and Kitchen, K. C.: A transplantable insulinoma in the rat. Proc. Nat. Acad. Sci. 74:628-632, 1977.

5) Boice, J. D., Jr., Rosenstein, M. and Trout, E. D.: Estimation of breast doses and breast cancer risk associated with repeated fluoroscopic chest examinations of women with tuberculosis. Radiation Res. 73:373-390, 1978.

6) Warren, S., Brown, C. E. and Chute, R. N.: Is breast cancer a result of endocrine targeting? (How women differ from rats or mice). Ann. Clin. Lab. Sci. 9:87-93, 3-4/1979.

7) Brown, C. E. and Warren, S.: Carcinoma of the prostate in irradiated parabiotic rats. Cancer Res. 38:159-162, 1978.

8) Brown, C. E., Warren, S., Chute, R. N., Ryan, K. J. and Todd, R. B.: Hormonally-induced tumors of reproductive system of parabiosed male rats. Cancer Res., In press.

9) 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. 75:98-105, 1978.

10) Shellabarger, C. J., Cronkite, E. P., Bond, V. P. and Lippincott, S. W.: The occurrence of mammary tumors in the rat after sublethal whole-body irradiation. Radiation Res. 6:501-512, 1957.

11) Bjarnason, O. and Warren, S.: Late effects on kidneys in whole-body-irradiated parabiosed rats. Radiation Res. 33:357-363, 1968.

12) Madrazo, A., Suzuki, Y. and Churg, J.: Radiation nephritis. II. Chronic changes after high doses of radiation. Am. J. Path. 61:37-56, 1970.

13) Madrazo, A. A. and Churg, J.: Radiation nephritis. Chronic changes following moderate doses of radiation. Lab. Invest. 34:283-290, 1976.

14) Jordan, S. W., Key, C. R., Gomez, L. S., Agnew, J. and Barton, S. L.: Late effects of radiation on the mouse kidney. Exp. Molecular Path. 29:115-129, 1978.

15) Jordan, S. W., Yuhas, J. M. and Key, C. R.: Late effects of unilateral radiation on the mouse kidney. Radiation Res. 76:429-435, 1978.

PUBLICATIONS

DOE CONTRACT EY-76-S-02-3017.A004
NEW ENGLAND DEACONESS HOSPITAL
16 MAY 1978 - 15 MAY 1979

Herzfeld, A., Greengard, O. and Warren, S.:	Tissue Enzyme Changes in Parabiotic Rats with Subcutaneous Lymphoma or Fibrosarcoma. J. Nat. Cancer Inst. 60:825-823, 4/1978.
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., Brown, C. E. and Chute, R. N.:	Is Breast Cancer a Result of Endocrine Targeting? (How Women Differ from Rats or Mice). Ann. Clin. Lab. Sci. 9:87-93, 3-4/1979.
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. 34:265-271, 1978.
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. 75:93-107, 7/1978.