# RADIATION CARCINOGENESIS

Comprehensive Final Report

16 May 1979 through 31 December 1980

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## DISCLAIMER

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## ABSTRACT

This abstract covers three main areas of investigation: mesothelioma induction by asbestos, radiation tumorigenesis and transplantable tumors.

Canadian and Rhodesian asbestos fibers have been administered under anesthesia to rats by intratracheal, intrapleural and intraperitoneal injection. Additional groups were given 3-methylcholanthrene or x-radiation along with asbestos. The 1.3% incidence of mesotheliomas in rats receiving intratracheal asbestos was increased to 4.9% with accessory radiation. The 6.3% incidence of mesotheliomas in rats receiving intrapleural injections of asbestos was increased to 7.3% with added radiation and to 10.9% with added 3-methylcholanthrene. The 35% incidence of mesotheliomas from intraperitoneal asbestos and x-rays was increased to 39.6% with asbestos and 3-methylcholanthrene.

A large series of mice also treated as above have displayed mesotheliomas. In addition, glass fiber injections and feeding of asbestos were done and have produced negative results to date.

The carcinogenic effect of whole-body radiation on hemi-irradiated parabiont partners exposed to a single 1000 R dose of x-ray was evidenced by a significant increase in the incidence of malignant tumors in only six tissues: skin, supporting soft tissue, kidney, bone, pancreatic islets and ovary. In the male adrenal medulla, the spontaneous incidence in single rats was higher than in any parabionts, and in the female breast the incidence of spontaneous breast carcinoma in single rats equalled that of the irradiated partners and nearly that of the shielded partners. In these two tissues genetic and parabiotic hormonal factors were judged to exert a significant effect.

The occurrence of incisional (anastomotic) sarcomas in significant numbers in hemi-irradiated and parabiont control pairs suggests the operation of mechanical factors complicating the healing process, only slightly enhanced by radiation.

One of the very valuable but unanticipated developments of the rat radiation program was the isolation of two transplantable endocrine tumors with strong hormonal potentials: an insulinoma of the pancreas and a pheochromocytoma of the adrenal medulla. As both transplantable tumors have maintained a substantial hormonal secretory capacity, a considerable demand has developed in widely scattered research institutions.

## NOTICE

## INTRODUCTION

Approximately 18 months have elapsed since Drs. Warren and Gates in May of 1979 submitted a comprehensive three-year progress report on work supported by U. S. Department of Energy Contract DE-AC02-76EV03017.

The present report covers studies completed at the time of death (July 1, 1980) of the principal investigator, Dr. Shields Warren, others carried to completion by his associates Drs. Clark E. Brown and Olive Gates, as well as unfinished projects.

Studies on the carcinogenic properties of asbestos and the possible cocarcinogenic effects of physical and chemical carcinogens, such as 3-methylcholanthrene and external x-radiation for the NEDH rats have been completed. A report of these findings is due to appear shortly in THE ARCHIVES OF PATHOLOGY AND LABORATORY MEDICINE. Parallel studies on 580 mice are near completion.

The systematic study of radiation tumorigenesis, carried on over the years in a large series of parabiont NEDH rats following a supralethal dose of 1000 R x-radiation to one partner, has been brought one step nearer conclusion by a summary report on cancers recently submitted for publication. This supplemented an earlier report on benign tumors published in 1978 (1).

Under way at the present time is an analysis of collected data on the concurrent development of malignant and/or benign tumors in different tissues of these hemi-irradiated pairs and the effect of ablation of the pituitary or a severance of the partners at 60 or 150 days after radiation, especially those in endocrine glands and their target organs. Such data

would contribute to the long-recognized but only partially elucidated modification by indirect systemic factors of the tumorigenic response of radiation-injured cells. It will also contribute to the questionable relation between benign and malignant neoplasia and the influences suggestive of one or the other prevailing type. One of the chief advantages of this experimental model of hemi-irradiated pairs is the introduction of hormonal disequilibrium by parabiosis per se. Its effect on mammary tumorigenesis and the countervailing action of radiation have been explicitly pinpointed in Dr. Brown's published reports relating hormone levels to mammary tumor incidence in single and parabiont controls and in radiated and shielded partners, correlating these findings with those in parabiosed surgical castrates and their intact partners (2). This line of approach was similarly illuminating in the case of interstitial cell testicular tumors and is currently applied to cancer of the prostate.

One of the accessory but by no means minimal accomplishments of the radiation project was the isolation of two functioning transplantable tumors, an insulinoma and a pheochromocytoma. These have been maintained over the years and proved to be an indispensable adjunct in research conducted by Dr. William L. Chick at the Joslin Diabetes Foundation and Dr. William M. Manger at the National Hypertension Council.

Personnel

During the current term of the project, up to July 1, 1980, the Principal Investigator, Dr. Shields Warren, Member at the Cancer Research Institute, has donated 20% of his time to the research under this contract. Dr. Olive Gates contributed to the research as a fulltime volunteer.

Dr. Clark E. Brown has been involved as a parttime consultant and as Principal Investigator since July 1, 1980. Dr. Kenneth J. Ryan of the Laboratory of Human Reproduction and Reproductive Biology of Harvard Medical School continued to cooperate in studies on the relation of radiation to the development of tumors of the reproductive system requiring hormonal assay. This study, initiated by Dr. Brown and originally supported by the Department of Energy, is now funded by the National Cancer Institute.

Members of the staffs of the Cancer Research Institute, the Shields Warren Radiation Laboratory and the Department of Pathology of the New England Deaconess Hospital participated in the program "Recent Advances in Clinical Science" at the annual meeting of the Association of Clinical Scientists held in Boston May 8-11, 1980.

Dr. Warren participated in the International REAC/TS Conference, "The Medical Basis for Radiation Accident Preparedness," held at Oak Ridge, Tennessee, October 18-20, 1979.

A professorship honoring Dr. Warren was established shortly prior to his death at the New England Deaconess Hospital and the Harvard Medical School. The Shields Warren-Mallinckrodt Professorship of Clinical Research is a fitting recognition of Dr. Warren's many contributions to science, medicine and medical education.

Safety for Personnel and Environment

Careful attention to the safety guidelines for handling carcinogenic substances drawn up by the National Cancer Institute and recommendations of the Nuclear Regulatory Commission have resulted in no accidents.

Facilities

We have been provided by the New England Deaconess Hospital with three adequately-equipped laboratories, three offices, facilities for conventional preparation of tissue for microscopic study, for electron microscopy, through the cooperation of Dr. Micheline Federman and the New England Deaconess Hospital Laboratory of Pathology, and the use of the Animal Farm under the veterinary supervision of Dr. Robert Hopkins, II, Director, Division of Laboratory Animal Medicine, Tufts-New England Medical Center.

## MAIN RESEARCH ACCOMPLISHMENTS

Our experiments during the past 18 months may be grouped as follows:

I. Carcinogenesis by Asbestos in Rodents

- A. Chrysotile Fibers Injected Intratracheally, Intrapleurally and Intraperitoneally
- B. Cocarcinogenesis
  - 1. Asbestos and methylcholanthrene--lung and pleura
  - 2. Asbestos and ionizing radiation

II. Tumorigenesis by X-radiation of NEDH Rats

- A. Spontaneous Incidence of Benign and Malignant Tumors
- B. Effects of Parabiosis Alone
- C. Effect of 1000 R Whole-Body X-radiation on One Partner of Parabiosed Pairs
- D. Comparison of Incidence of Benign and Malignant Tumors Ascribed to Irradiation
- E. Review of Etiologic Factors Influencing Tumorigenesis

III. Tumor Transplantation

- A. Propagation of Insulinomas and Their Distribution to Other Institutions
- B. Propagation of Pheochromocytomas and Their Distribution to Other Institutions

## I. Carcinogenesis by Asbestos in Rodents Including Synergistic Effects of 3-Methylcholanthrene and Radiation

### A. Rats

The established carcinogenicity of asbestos fibers for man promoted by cigarette smoking prompted this investigation of a possible cocarcinogenic effect of a physical carcinogen, such as x-irradiation, or a chemical carcinogen, such as 3-methylcholanthrene, on the known inducive effect of asbestos in rats.

The experiment involving the carcinogenicity of chrysotile asbestos fibers (Canadian and Rhodesian) for the mesothelium of the pleura and peritoneum of NEDH rats was completed by us in 1980. This involved the instillation of 2 mg of asbestos fibers suspended in saline intratracheally, intrapleurally or intraperitoneally with or without ancillary x-radiation treatment (1000 R whole-body radiation to one partner of parabiont pairs or 2000 R to the right thorax of single rats) or alternatively instillation of asbestos plus one milligram 3-methylcholanthrene suspended in 0.1 ml sesame oil.

The carcinogenicity of asbestos alone, intratracheally instilled, for pleural mesothelium was 1.3% in 80 rats as against none in 1417 controls. Direct instillation of fibers into the pleural cavity resulted in 6.3% mesothelioma in 79 rats. The addition of radiation or 3-methylcholanthrene increased the incidence of mesotheliomas of the pleura to 7.3% and 10.9% respectively. In contrast, the rates of peritoneal mesothelioma in rats with direct instillation of fibers into the peritoneal cavity were quite high, 35% with ancillary radiation and 39.6% with added 3-methylcholanthrene. These incidences were far above the 0.1% rate

of spontaneous mesothelioma in the peritoneum and significantly above the correspondingly-induced incidences of mesothelioma in the pleural cavity. Table I summarizes these experiments.

Table I

Incidence of Mesotheliomas and Carcinomas of Lung

Relative to Routes of Injection of Asbestos and Adjuvant Treatments

| | | Asbestos | | Asbestos and Radiation | | Asbestos and 3-Methylcholanthrene | |
|---|---|---|---|---|---|---|---|
| Route of Injection | | # | % | # | % | # | % |
| Mesothelioma | Intratracheal | 1/80 | 1.3 | 2/41 | 4.9 | ----- | ---- |
| | Intrapleural | 5/79 | 6.3 | 3/41 | 7.3 | 5/46 | 10.9 |
| | Intraperitoneal | ---- | --- | 7/20 | 35.0 | 19/48 | 39.6 |
| Carcinoma | Intratracheal | 0/80 | 0 | 3/41 | 7.3 | ----- | ---- |
| | Intrapleural | 1/79 | 1.3 | 2/41 | 4.9 | 2/46 | 4.4 |

In view of the similar posttreatment mean survival times of rats bearing pleural or peritoneal mesothelioma, 541 and 528 days respectively, the higher rates of mesothelioma in the peritoneum cannot be attributed to differences in lifespan. They may be due to the larger number of cells at risk in the peritoneal cavity or possibly to opportunity for local aggregation provided by the peritoneum.

The 0% incidence of lung adenocarcinoma in the intratracheally-instilled asbestos fibers in 159 rats was unexpected. In rare animals there were suspicious foci of bronchiolar hyperplasia. The colony in general has a 0.4% incidence of spontaneous lung carcinoma, consisting of undifferentiated and epidermoid carcinoma and no adenocarcinoma. Radiation in a dose of 1000 R to one partner of parabiont pairs induced a one percent incidence of lung cancer ($P < 0.05$) almost equally undifferentiated, epidermoid and adenocarcinoma in type. The carcinogenicity of 3-methylcholanthrene for tissues in general is well established, although not for mesothelioma specifically. In the present series, eight rats injected intrapleurally or intraperitoneally with 3-methylcholanthrene failed to develop mesothelioma. The incidence of pleural or peritoneal mesothelioma in rats receiving 1000 R x-radiation was zero for pleural and 0.1% for peritoneal.

The earliest mesothelioma was observed in a rat dying 253 days after intratracheal injection of asbestos and radiation and the latest in a parabiont rat dying 899 days after intrapleurally-injected asbestos.

Some insight into the pathogenesis of mesotheliomas is provided by a review of the histology of the lesions caused by asbestos. Polymorphonuclear leukocytes first appear around the fibers followed shortly by macrophages. At some point red cells appear in the exudative foci. These cellular components disappear by lysis and are replaced by a rather sparce fibroblastic proliferation in abundant mucinous material, which eventually becomes hyalinized into plaques. Foci of hemosiderin indicate red cell lysis, but ferruginous bodies fail to develop in the rat lesions. The

mesothelial cells adjacent to these plaques begin to show hyperplasia and pile up over the plaques in papillary conformations. The surface mesothelial cells in places appear to grow downward in and around the granulomatous plaques. The critical point in the pathogenesis of these mesotheliomas is whether the neoplastic mesothelial cells, as judged by their nuclear enlargement and atypism, develop in foci removed from the asbestos plaques initially, or whether they develop on the surface of the plaques in response to the presence of the plaque itself. In the present series, numerous plaques are scattered about in most of the areas of proliferating mesothelial tumor cells. In other areas, however, many nobs and papillae of mesothelial proliferation are separate from the plaques. Certainly the magnesium silicate of the asbestos fiber appears to have the capacity for producing lytic changes in the mesothelium, red cells and macrophages, but whether it produces a neoplastic change on a chemical basis or on a physical basis secondary to plaque development remains unanswered. One feature apparently not encountered previously is the formation of intrabronchial polypoid projections of edematous hyalinized tissue which extend intraluminally from the bronchial wall following intratracheal injections of asbestos fibers. Bronchial mucosa covers these. If the bronchial mucosa over these polypoid plaques has the same neoplastic potential as the mesothelial pleural plaques, one might have expected a higher incidence of bronchial carcinoma. On the other hand, it could be supposed that the higher incidence of adenocarcinoma of the lung in series other than ours may have developed for this reason.

Our results, in summary, show that injection of Canadian or Rhodesian chrysotile asbestos alone or in combination with x-radiation or

3-methylcholanthrene produce mesotheliomas of the pleura or peritoneum. The increased incidence of mesothelioma in groups in which combinations with physical or chemical carcinogens occur appears to produce an additive effect. Additional information on the rat experiments with asbestos will soon appear in the ARCHIVES OF PATHOLOGY AND LABORATORY MEDICINE.

B. Mice

The parallel experiment on 580 $B6AF_1$ mice omitting radiation and adding implantation of glass fibers as well as ingestion of asbestos has been completed. Morphologic observation of tissues on all but 100 mice yet to be studied do not confirm a significant cocarcinogenic effect of 3-methylcholanthrene with asbestos in the production of mesotheliomas. Nine point eight percent of 150 mice treated with 3-methylcholanthrene and asbestos developed mesothelioma while 8.5% of 129 mice with asbestos alone developed mesothelioma. No mesotheliomas were seen in 20 3-methylcholanthrene-treated mice, in 58 glass fiber-treated mice, in 25 untreated mice nor in 40 mice fed asbestos. However, the high incidence of lymphoma and lung adenomatosis occurring in this strain of mice introduced a complicating factor, and firm conclusions await final assessment of all data.

## II. Tumorigenesis by Ionizing Radiation

A large part, but by no means all, of the experimental effort in this laboratory has been concerned with a comprehensive approach to radiation carcinogenesis (3-12). The study of long-term tumorigenic effects of a whole-body supralethal dose of 1000 R x-rays to NEDH rats (a modified Wistar strain) was facilitated by permanent parabiosis to a shielded

partner, which not only mitigated the acute radiation syndrome but provided evidence of the modifying effect of hormonal disequilibrium introduced by parabiosis on the tumorigenic response of radiation-injured cells of hormone-dependent tissues. The survival time following 1000 R was only slightly shortened; whereas, the $LD_{50/30}$ for single rats in this colony was 820 rads. Mortality from the parabiotic procedure itself has been less than one percent.

Methods: NEDH rats of the same sex and age, syngeneic or littermate, were parabiosed (7) at about 35 days of age. The right-hand members of the experimental pairs were irradiated at about 110 days of age with a single whole-body exposure of 1000 R of 250 kVp x-rays from a G. E. Maximar apparatus, while the left-hand members of the pairs were shielded by lead. Three series of controls were used: single untreated rats, parabiosed but otherwise untreated rats, and the parabiosed shielded partners of the irradiated partners (Table II).

Parabiosis was maintained throughout life. All pairs were kept in individual cages and given Purina Chow and water ad lib. Only moribund pairs were killed. Complete autopsies were performed and representative samples for histologic study were taken of all tissues thus allowing for detecting tumors not visible to the naked eye. The sample tissues were fixed in Zenker-formol, embedded in paraffin, sectioned, and stained with hematoxylin and eosin supplemented by special stains as required for histologic diagnosis.

Results: The use of the above model over a period of years has allowed the accumulation of a large body of data from 404 single male controls, 389 single female controls, 322 male and 302 female parabiont control

TABLE II

Tissues in Which the Spontaneous Cancer Incidence was Significantly Altered

Either by Radiation (1000 R) or by Parabiosis Per Se

| | Radiated Partner | | | | Shielded Partner | | | | Control Parabiont | | | | Single Control | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ♂ | | ♀ | | ♂ | | ♀ | | ♂ | | ♀ | | ♂ | | ♀ | |
| Total No. | 1252 | | 1366 | | 1252 | | 1366 | | 322 | | 302 | | 404 | | 389 | |
| | # | % | # | % | # | % | # | % | # | % | # | % | # | % | # | % |
| Carcinoma of skin | 135 | 10.8 | 59 | 4.3 | 8 | 0.6 | 1 | 0.1 | 1 | 0.3 | 4 | 1.3 | 1 | 0.3 | 7 | 1.3 |
| Sarcoma, soft supporting tissue | 130 | 10.4 | 92 | 6.7 | 10 | 0.8 | 3 | 0.2 | 8 | 2.5 | 2 | 0.7 | 17 | 4.2 | 6 | 1.5 |
| Renal cell carcinoma | 68 | 5.4 | 32 | 2.3 | 4 | 0.3 | 1 | 0.1 | 1 | 0.3 | 0 | | 1 | 0.3 | 1 | 0.3 |
| Osteosarcoma | 64 | 5.1 | 51 | 3.7 | 1 | 0.1 | 3 | 0.2 | 1 | 0.3 | 1 | 0.3 | 0 | | 0 | |
| Carcinoma, islets of Langerhans | 37 | 3.0 | 6 | 0.4 | 2 | 0.2 | 0 | | 1 | 0.3 | 0 | | 0 | | 0 | |
| Carcinoma of ovary | | | 54 | 4.0 | | | 10 | 0.7 | | | 1 | 0.3 | | | 4 | 1.0 |
| Malignant pheochromocytoma | 40 | 3.2 | 8 | 0.6 | 14 | 1.1 | 1 | 0.1 | 9 | 2.8 | 6 | 2.0 | 46 | 11.4 | 3 | 0.3 |
| Carcinoma, mammary gland | 13 | 1.0 | 148 | 10.8 | 6 | 0.5 | 179 | 13.1 | 2 | 0.6 | 17 | 5.6 | 3 | 0.7 | 45 | 11.6 |
| Lymphoid tissue | 21 | 1.7 | 17 | 1.2 | 44 | 3.5 | 24 | 1.8 | 12 | 3.7 | 19 | 6.3 | 20 | 5.0 | 8 | 2.1 |
| Leukemia | 35 | 2.8 | 19 | 1.4 | 36 | 2.9 | 28 | 2.1 | 16 | 5.0 | 10 | 3.3 | 8 | 2.0 | 1 | 0.3 |
| Anastomotic fibrosarcoma | 181 | 14.5 | 116 | 8.5 | 153 | 12.2 | 105 | 7.7 | 32 | 9.9 | 22 | 7.3 | | | | |

partners, 1252 parabiont experimental shielded male control partners, 1252 parabiont experimental irradiated male partners, 1366 parabiont experimental shielded female control partners and 1366 parabiont experimental irradiated female partners. The various factors involved in the tumorigenic effects of whole-body radiation on certain tissue has been recorded in previous reports on smaller series (3-12). A comprehensive report on benign tumors was published in 1978 (1) and summarized in a progress report in 1979. The recently completed study of all malignant tumors throws some light on conditions obtaining for the radiation induction of the two forms in different tissues.

While practically every cell type develops an occasional cancer spontaneously, radiation produces a measurable increment of tumor incidence in only a relatively few tumor cell types or organs. In NEDH rats this was found to be true for skin, soft supporting tissue, kidney, bone, islet cells and ovary, in which the cancer incidences were significantly above control rates at the 0.01 level of probability (Table II), but not for cancers in 26 tissues where the rates in radiated partners were only slightly above or the same as those in control rats, or for cancers of hematopoietic tissues. The spontaneous development of leukemia in males was inhibited and that of leukemia and lymphoma in females was unaltered.

The failure to induce cancers in the two tissues with the highest spontaneous incidences--adrenal medulla of males and mammary gland of females--was related to the inhibitory effect of parabiosis per se. The 2.8% incidence of malignant pheochromocytoma in parabiont controls, the 1.1% incidence in shielded partners and the 3.2% incidence in radiated

partners were significantly below the 11.4% spontaneous rate. In the case of the mammary gland, a similar depressive effect of parabiosis on malignant formation was counteracted by radiation: the 5.6% incidence in parabiont control females was raised to 10.8% in radiated and 13.1% in shielded partners, approximating the 11.6% spontaneous incidence in single controls (Table II). These incidences were related to lower or higher prolactin levels as determined by bioassay. Subsequent serum hormone studies on female parabiont pairs, one member of which was oophorectomized, confirm this hypothesis (2, 14). Thus, the hormone stimulus to the unirradiated mammary gland of the shielded partner, resulting from the well-known chain of events set in motion by diminished secretion of the radiated ovary of its partner was as effectively carcinogenic as was radiation injury of mammary tissue exposed to slightly lower levels of prolactin.

In contrast, parabiosis appeared to enhance the development of leukemia and lymphoma in females, while radiation had no discernible effect. However, the data is vitiated by the involvement of both partners of control and hemi-irradiated pairs in an appreciable number of instances and the impossibility of determining precisely the times of appearance of lesions in one or the other partner.

Cancers arising at the site of anastomosis of experimental and control pairs, primarily fibrosarcomas, although rhabdomyosarcomas, osteogenic sarcomas and occasionally cutaneous and mammary carcinoma also occurred, may be attributed to motion trauma. Frustrated opposition of fibroblasts from constant motion at the healing site of the joined parabionts was thought to be the cause of the so-called anastomotic fibrosarcomas which

occurred in 9.9% and 7.3% of male and female control partners respectively. Their pathogenesis was considered to be similar to that of sarcomas arising adjacent to implanted plastic membranes (13). The slightly increased incidence, 14.5% in irradiated male parabiont partners and 8.5% in females, may indicate an additional complication of the healing process. Radiation-induced incidences of malignant and benign tumors differed slightly in some tissues, markedly in others (Table III). In four of the

TABLE III

Tumor Incidences (%) in Radiated Partners

| | Benign Tumors | | Malignant Tumors | |
|---|---|---|---|---|
| | ♂ | ♀ | ♂ | ♀ |
| Skin | 2.8 | 3.2 | 10.8 | 4.3 |
| Soft supporting tissues | 4.0 | 1.2 | 10.4 | 6.7 |
| Bone | 0 | 0 | 5.1 | 3.7 |
| Kidney | 3.1 | 1.1 | 5.4 | 2.3 |
| Islet cells | 15.3 | 2.9 | 3.0 | 0.4 |
| Ovary | | 44.5 | | 4.0 |
| Adrenal medulla | 23.8 | 14.9 | 3.2 | 0.6 |
| Mammary gland | | 17.9 | | 10.8 |

eight tissues malignant tumors predominated--to a greater or lesser degree in males and females--in those tissues responding to the direct action of radiation: skin, soft supporting tissue, kidney and bone. In the remaining four, three of which were hormone secretors with high functional

activity and the fourth a target organ--islet cells, ovary, adrenal medulla and mammary gland--the benign form was by far more frequent in each instance. In two of these tissues, adrenal medulla of males and mammary gland of females, the tumorigenic response to radiation was modified by the hormonal disequilibrium incident to parabiosis per se. The inhibitory effect of parabiosis on the spontaneous development of malignant pheochromocytoma in males and mammary carcinoma in females resulted in incidences in radiated partners below or on a par with rates in single controls (Table IV). Contrarily, incidences of benign pheochromocytoma in radiated partners were elevated to a significant degree above the reduced rates in parabiont controls and of mammary adenoma above rates in single and parabiont control females.

The hormonal role in the induction of mammary cancer in NEDH hemi-irradiated females (14) described on Page 18 of this report is reflected in the variation of tumor incidence in the pituitary in radiated and unirradiated females. In contrast to the low cancer incidences, from 0.22% to 0.73% without significant differences in radiated and control females, pituitary adenomas developed in 11.2% of single control females, 7.6% parabiont control female partners, in 4.2% of radiated partners and, as would be expected, in 16% of shielded partners. This high incidence of adenomas in the shielded partner parallels that of carcinoma in its mammary gland (13.1%), each eventuating from successive functional changes in the ovary and pituitary in the two partners subsequent to sterilization of the radiated partner. Radiation tumorigenesis in the ovary, predominantly of a benign nature, appears to be a prime example of the indirect mechanism by which radiation of one type cell provides the hormonal

TABLE IV

% Incidence of Benign and Malignant Tumors of Four Secretory Organs in Experimental and Control Rats

| | | Adrenal Medulla (Pheochromocytoma) | | Mammary Gland | | Islet Cell | | Ovary | |
|---|---|---|---|---|---|---|---|---|---|
| | Sex | Benign | Malignant | Benign | Malignant | Benign | Malignant | Benign | Malignant |
| Radiated | ♂ | 23.8 | 3.2 | | | 15.3 | 3.0 | | |
| | ♀ | 14.9 | 0.6 | 17.9 | 10.8 | 2.9 | 0.4 | 44.5 | 4.0 |
| Shielded | ♂ | 7.7 | 1.1 | | | 0.8 | 0.2 | | |
| | ♀ | 5.8 | 0.1 | 11.9 | 13.7 | 0.1 | 0 | 2.3 | 0.7 |
| Parabiont Control | ♂ | 11.8 | 2.8 | | | 0.6 | 0.3 | | |
| | ♀ | 11.9 | 2.0 | 6.6 | 5.6 | 0.7 | 0 | 2.7 | 0.3 |
| Single Control | ♂ | 35.1 | 11.4 | | | 1.0 | 0 | | |
| | ♀ | 22.1 | 0.8 | 10.8 | 11.6 | 0.6 | 0 | 2.0 | 1.0 |

stimulus provocative of tumor in other cells for which direct radiation injury may or may not be an essential factor. Tumors of the uterus may plausibly be placed in this category if uterine polyps are accepted as such; 30% of radiated partners, as compared with 6.7% of single controls developed polyps--the corresponding cancer incidences, 2.1% and 1.8%.

Tumors of islet cells are of particular interest as they were markedly sex determined, and, like tumors of the ovary, their development was not obviously affected by parabiosis. A low but significant incidence of malignant tumors, 3.0%, developed only in radiated males. A significant incidence of benign tumors was also confined to radiated males, 15.3%, but the 3.0% incidence in females was just short of significance as determined by the critical ratio of 2.53 instead of 2.56* between incidences in radiated partners and single controls.

There were two other examples of marked differences in the occurrence of benign and malignant tumors. Cholangiomas developed in 13.8% of radiated females, 7.4% in males and cancers of the bile ducts in 0.2% of females and 0% of males. However, anastomotic fibrosarcomas, essentially a response to persisting trauma, were far more frequent than fibromas, 14.5% and 2.3% respectively in radiated male partners, 8.5% and 2% in female radiated partners. In parabiont control partners there was a like

*Significant incidences have been determined on the basis of critical ratios between pairs of rates obtained by dividing the difference between two rates by the standard error of this difference. A critical ratio of 2.6 was considered as the probability of such a difference occurring by chance is once in a hundred.

disparity between the occurrence of malignant and benign tumors at a slightly lower incidence level.

In the present experimental scheme the usefulness of permanent rather than temporary parabiosis in bringing out the qualifying effect of hormones on the tumorigenic response of certain tissues to radiation, notably that of the female mammary gland and the adrenal medulla of both sexes rests on the effect of parabiosis itself, possibly peculiar to NEDH rats. The slight shortening of lifespan, the disturbed estrus cycles and altered serum hormone levels have not so far as we are aware been reported in permanently parabiosed rats of other strains.

The conditions under which benign and malignant tumors develop are suggestive of their independence as distinct entities despite ambiguities of evidence based on incidences unsupported by temporal data. Nevertheless, these findings and the clear-cut distinction brought out between the direct effect of radiation on certain tissues, whether or not promoted by local environmental changes, and the qualifying effect of hormones on radiation-injured cells of other tissues demonstrate the utility of parabiosis in the elucidation of the diverse and complex mechanisms of radiation tumorigenesis following a supralethal whole-body dose of x-ray.

III. Tumor Transplantation

Pheochromocytoma RNC 259 became established in 1969 and is still being maintained in this laboratory under the auspices of Dr. William Manger, President of the National Hypertension Council, who is continuing the experimental work which he and Dr. Warren started in January 1979. This pheochromocytoma has been sent to 24 laboratories in 11 states and two foreign countries. Fifteen of these research groups received NEDH rat breeders from us with which to establish their own colonies in order to maintain the tumor.

Insulinoma RNC 288 became established in this laboratory in 1975 and has been maintained in cooperation with Dr. William L. Chick of the Joslin Diabetes Foundation. We have sent rats bearing transplants of this tumor to 15 laboratories in 11 states and three foreign countries. Six of these have received NEDH rat breeders from us for propagation of the tumor.

We continued to maintain the pheochromocytoma and insulinoma until January 1981. At this time, arrangements started by Dr. Warren in the spring of 1980 were completed. Rats bearing the tumor as well as tumor tissue stored in the liquid nitrogen tissue bank of the Cancer Research Institute of the New England Deaconess Hospital during the past four years were transferred to the Mason Institute, Worcester, Massachusetts, a subsidiary of Edgerton, Germeshausen & Grier.

The three human tumors, a carcinoma of the thyroid, an epidermoid carcinoma and a malignant melanoma carried for more than a decade by serial transplants in the hamster cheek pouch and thereafter stored in the tissue bank, were also transferred to the Mason Institute.

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