The Effect of Nephrectomy on the Incidence of Breast Carcinoma in Irradiated Parabiosed Rats

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

Cancer Research Institute, New England Deaconess Hospital, Boston, Massachusetts 02215

SUMMARY

The incidence of carcinoma of the breast in parabiosed female NEDH rats is 2.5%. When one member of such a pair is irradiated, the incidence rises to 11% in the irradiated partner and 10% in the nonirradiated partner. If in such a pair of rats one kidney is removed from each partner, the incidence of breast carcinoma rises fivefold in the nonirradiated partner. The factors involved in such an increase probably have to do with alteration in retention, interchange, and feedback of the pituitary and ovarian hormones.

INTRODUCTION

Excessive hormonal stimulation of a target organ such as the breast produces tumors in mice and rats. Numerous methods have been used to accomplish this, such as injections of estrogen (8), increase of endogenous estrogens in parabionts by interference with the feedback mechanism to the pituitary (17), transplantation of normal pituitaries (11, 16) or of high-hormone-secreting pituitary tumors (5), and combinations of mammotropin pituitary hormones with low-level doses of carcinogenic agents (4).

In 1952 when Finerty (2) published his comprehensive review on parabiosis, it had already been established by the early work of Kallas (7), Hill (6), and others that when an intact female rat was joined to an oophorectomized female, excessive gonadotropic hormones generated in the castrate crossed over to stimulate the ovarian follicular epithelium of the intact partner. Zeckwer (17, 18) showed that the excessive endogenous estrogen produced in the intact partner caused secondary changes in its genital system and by increasing luteinizing hormone also caused breast hyperplasia and adenosas.

Essentially similar effects have been noted in this laboratory in the intact nonirradiated partners of parabiosed female rats when the partner received 1000 R of total-body irradiation (PR series). Thus, an incidence of 2.5% of breast carcinoma in control parabiont rats was raised to about 10% in each member of the pair when 1 was irradiated. This paper describes an increase in the incidence of breast carcinoma to 50% in the nonirradiated partner when a kidney was removed from each member of the pair shortly preceding or following irradiation of one member (PRN series).

MATERIALS AND METHODS

Forty-six pairs of inbred virgin female NEDH rats were joined in parabiosis by a modification of the Bunster-Meyer method at the approximate age of 40 days (15). These were divided into 3 groups: (a) the right partner of 32 pairs received 1000 R total-body irradiation following which a kidney was removed from each partner; (b) a kidney was removed from each partner of 10 pairs following which the right partner received 1000 R total-body irradiation; (c) control group. A kidney was removed from each partner of 4 pairs. No irradiation was given. Eleven untreated normal female rats of the same age served as single controls.

Irradiation was given without added filtration at 250 kVp and 15 ma to the right partner, the left partner being shielded with lead. Irradiation and nephrectomies were performed within 13 weeks following parabiosis. The 2 experimental groups were combined (PRN series) since no apparent differences ensued. The rats were fed Purina laboratory chow and water was given ad libitum. Twenty-nine pairs of rats were allowed to live to a natural death. Seventeen pairs were killed in order to assay plasma hormone levels. Four of these pairs were killed at 5 to 7 months of age, prior to the appearance of breast tumors, to detect early changes in hormones and in morphology of the breast, ovary, and uterus. Hormone analyses were also performed on single controls. Standard gross and microscopic examinations were made, the tissues being fixed in Zenker-formalin and stained with hematoxylin and eosin. For comparison, pertinent autopsy data from our series of 842 female parabiont pairs, one member irradiated, one shielded (PR series), were utilized (Table 1).

Blood urea nitrogen analyses were performed on randomly selected rats in the PR and PRN series on the Beckman urea analyzer. Hormonal analysis performed on the plasma of both partners and on single controls included estradiol, progesterone, FSH, and prolactin. Results are recorded in Table 2.

1 This investigation was supported by United States Atomic Energy Commission Contract AT(ll-l)-3017 with the New England Deaconess Hospital and by USPHS Grant RR 05591 from the General Research Support Branch, Division of Research Resources. The research described in the report involved animals maintained in animal care facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care. We are indebted to the National Institute of Arthritis, Metabolism and Digestive Diseases Rat Pituitary Distribution Program for reagents and methods for the rat FSH and prolactin studies.

Received May 1, 1974; accepted October 1, 1974.

1 The abbreviation used is: FSH, follicle-stimulating hormone.
RESULTS

Of the 42 experimental pairs of parabionts in the PRN series, adenocarcinomas occurred in the breasts of 21 of the nonirradiated partners. One pair had an adenocarcinoma in both partners, and in 3 other pairs the irradiated partners had breast carcinoma. None of the controls had carcinoma of the breast (Table 1).

The 1st change noted in these rats was abdominal swelling which developed about 3 to 4 months postnephrectomy. Uterine enlargement was identified and often progressed to pyometra and occasionally to peritonitis (Fig. 1). Nodular breast tumors appeared 5 to 14 months postnephrectomy. Many of these became hemorrhagic and some of them ulcerated (Fig. 2). Nineteen of the 42 experimental pairs were dead at the end of the 1st 12 months, 38 were dead at 18 months, and none survived by 22 months. In the PR series the life-span was considerably longer with a much lower breast carcinoma incidence.

Microscopically, the most consistent change in the breasts of the nonirradiated partners in the PRN series was a lobular type of acinar hyperplasia usually with secretion in the acini (Fig. 3). Foci of neoplastic breast epithelium characterized by pleomorphism and partial loss of acinar structure appeared within the lobules suggesting a transformation from hyperplasia to neoplasia (Fig. 4). Solid sheets of tumor cells all but completely obliterated the acinar and lobular structure in some regions (Fig. 5). Rapid growth of some tumors is suggested by numerous mitoses. In the breasts of the nonirradiated partners without tumors, alveolar hyperplasia and hypersecretion were nearly always present; whereas in the breasts of the irradiated partners without tumors, a simple ductal structure with rare acini was encountered. The 4 breast carcinomas, occurring in the irradiated partners tended to be well differentiated (Fig. 6). In 2 of the 4 irradiated partners with breast carcinomas, 1 had a granulosa cell tumor of the ovary (Fig. 7), and the other had a proliferating mass of granulosa cells suggesting tumor. These were the only 2 instances of tumor-like granulosa cell proliferation in the ovaries of the 42 irradiated partners, although Sertoli cell proliferations frequently occurred. Sertoli cell and granulosa-theca cell tumors have been reported in the large PR series of irradiated parabionts as the rats aged (4).

The 4 nephrectomized nonirradiated control pairs had no breast tumors, but all of them showed lactational or cystic change. One died at 23 months, and the other 3 survived over 24 months. The ovaries and uteri were not unusual.

The ovaries of the nonirradiated partners with breast carcinoma were consistently enlarged, some reaching diameters of 1 cm (Fig. 2). The ovaries were riddled with cysts lined with either granulosa cells, luteinized cells, or mixtures of both in single or multiple layers (Fig. 8). Over one-half of the cysts were lined with luteinized cells (Fig. 9). In 2 of the 4 nonirradiated partners killed before the appearance of breast carcinomas, the ovaries contained many solid, hypertrophied, highly vascularized corpora lutea. In the remaining 2, luteinized cysts had already been formed. Since no follicles gave the appearance of having ruptured, the direct luteinization of follicular lining cells may have occurred. Failure of resolution of the lutein cysts suggests that progesterone secretion was continuing during the early stages of breast carcinoma development. Granulosa cell proliferation appeared to continue in the walls of many follicular cysts. The ovaries of the irradiated partners were small, and the follicles were atrophic. Occasional strands of

<table>
<thead>
<tr>
<th>Parabionts</th>
<th>Rats reviewed</th>
<th>With breast carcinoma</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irradiated partner</td>
<td>842</td>
<td>83</td>
<td>11</td>
</tr>
<tr>
<td>Nonirradiated partner</td>
<td>842</td>
<td>74</td>
<td>10</td>
</tr>
<tr>
<td>Irradiated-nephrectomized</td>
<td>42</td>
<td>4*</td>
<td>10</td>
</tr>
<tr>
<td>partner</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonirradiated-nephrectomized</td>
<td>42</td>
<td>21*</td>
<td>50</td>
</tr>
</tbody>
</table>

*One breast carcinoma in each member of the pair.

<table>
<thead>
<tr>
<th>FSH* (μg/ml)</th>
<th>Estradiol* (pg/ml)</th>
<th>Progesterone* (μg/100 ml)</th>
<th>Prolactin* (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left animal</td>
<td>Right animal</td>
<td>Left animal</td>
<td>Right animal</td>
</tr>
<tr>
<td>Single controls</td>
<td>0.22 (4)*</td>
<td>85.7 (6)</td>
<td>0.95 (9)</td>
</tr>
<tr>
<td>Nephrectomy control pairs</td>
<td>0.21 (3) 0.23 (3)</td>
<td>85.7 (3) 117.2 (3)</td>
<td>3.3 (3) 1.4 (3)</td>
</tr>
<tr>
<td>Irradiated nephrectomized pairs</td>
<td>0.24 (2) 0.38 (2)</td>
<td>332.5 (4) 171.1 (4)</td>
<td>1.51 (5) 0.95 (6)</td>
</tr>
</tbody>
</table>

* Radioimmunoassay methods. Estradiol and progesterone determinations were performed as recommended in the New England Nuclear kit procedure with modifications for rats. FSH and prolactin were assayed with kits provided by the National Institute of Arthritis and Metabolism and Digestive Diseases.

* Numbers in parentheses, number of rats in sample.

* Right animal irradiated.
attenuated thecal cells lay in a matrix of fibrillar stroma and thickened vessels (Fig. 10). As noted above, an initial atrophic phase in the irradiated ovaries of our PR series often preceded the development of Sertoli and granulosa cell tumors in senescence.

The uteri of the nonirradiated partners in the PRN series were dilated, some to an extreme degree (Fig. 1). The endometrium of these uteri showed hyperplastic as well as secretory and cystic change, many terminating in infection, ulceration, and squamous metaplasia (Figs. 11 and 12). Many of these uteri appeared to have progressed from a stage of pseudopregnancy to persistent retrogression with terminal infection. Myometrial fibers were thick, edematous, and swollen in spite of the uterine distention. The cervical epithelium in some nonirradiated partners was thickened and hyperplastic, at times undergoing mucification. These changes were attributed to a combined effect of estrogen and progestin (18). In others it was thick and cornified with desquamation, having the appearance of estrus. Lesser degrees of endometrial hyperplasia and myometrial hypertrophy were seen in the uteri of the nonirradiated partners of our large PR series. The uteri of the irradiated partners of both series in general were small and compact with quiescent endometrium. Many contained fibrovascular endometrial polyps.

In the light of the foregoing observations on the ovaries and breasts, the hypophysis requires comment. The glands of the irradiated partners showed no consistent changes in the PRN series. Occasionally, adenomatous nodules and often focal or diffuse mild hyperplasias occurred in the nonirradiated partners with breast tumors. Older animals of the large PR series tended to develop pituitary tumors or hyperplasias as did 2 partners of our 4 nephrectomized control pairs living 746 and 779 days, respectively.

The kidneys removed from each partner shortly before or after irradiation were normal for age. The kidneys removed at autopsy many months later showed interesting variations. As would be expected, the average kidney weight of irradiated nephrectomy partners did not show an increase comparable to that of the control nephrectomy partners (1.28 versus 2.03 g). The average weights of kidneys of nonirradiated nephrectomized partners with breast carcinoma were comparable to those of the irradiated partners (1.30 versus 1.29 g), whereas the average kidney weights of the nonirradiated nephrectomized partners without breast carcinoma were higher than those of the irradiated partners (1.56 versus 1.27 g). The majority of the remaining kidneys of the nonirradiated partners with or without breast carcinoma showed various degrees of chronic pyelonephritis, whereas those of the irradiated partners showed slight to moderate mesangial glomerular thickening. A few also had pyelonephritis. The results of the blood urea nitrogen determinations on each partner of 18 pairs of the PRN series showed a mean concentration of 30 ± 1.2 mg/100 ml (S. E.). In a comparable group of 11 nonnephrectomized parabions the mean concentration was 24 ± 1.6 mg/100 ml (S. E.).

The ovaries, uterus, and kidneys of the 4 control nephrectomized, nonirradiated parabions showed none of the above changes except early pyelonephritis in the older rats and cystic and secreting changes in the breast.

The data on hormonal analyses are shown in Table 2. Correlation from animal to animal is not close, but the differences in blood levels between average value for hormonal levels in the irradiated and nonirradiated partners are in the expected direction. Estradiol and progesterone are higher in the nonirradiated partners than in the irradiated while FSH is higher in the irradiated partner. Average progesterone and estradiol levels were higher in both irradiated and nonirradiated nephrectomized partners than in single controls. A nearly 4-fold increase in the average progesterone concentration occurred in the nonirradiated partners with breast carcinoma compared to their partners' average or to the average of the nonirradiated partners without breast carcinoma. Average prolactin levels in the nonirradiated partners with breast carcinoma exceeded those of their irradiated partners and nephrectomy controls but were below average levels in comparable nonirradiated partners without breast carcinoma. In general, prolactin levels were high.

DISCUSSION

Parabiosis of intact with castrated female rats has provided a valuable means for studying the interplay of endogenous hormones. Cross-over of gonadotropic hormone from the castrate causes increased secretion of estrogen, prolactin, and progesterone in the intact partner with secondary effects on its breast and genital system.

A similar hormonal effect was achieved in the nonirradiated partner of our PR series when 1 member of the parabiont pair was irradiated instead of being surgically castrated. When nephrectomy was performed on each member of the pair in addition to 1 member having been castrated (PRN series), the ovarian and uterine changes in the nonirradiated partners became more pronounced, the degree of lobular hyperplasia and secretory activity with cystic change in the breasts was accentuated, and the incidence of breast carcinoma was increased from 10 to 50%.

Rats of the same series killed for early studies showed solid or cystic corpora lutea consistent with progesterone increase. The uterine enlargement and mucification of cervical and vaginal epithelium reflect a marked estradiol-progesterone effect. This coincided approximately in time with the height of proliferative and secretory activity in the breast acini, with the breast carcinomas appearing a short time thereafter.

The preliminary assay data (Table 2) on progesterone showing a conspicuous increase in the nonirradiated partners are consistent with the morphological changes in the ovaries, uterus, and breast. The continuing luteinization of the ovarian follicles in the nonirradiated parabiont partner when breast tumors were presumably in the formative stage is in accord with an increase in prolactin. The double effect of prolactin on the ovaries and on the breast implicates it at least partially in the genesis of these breast carcinomas. Once a continuing elevation of progesterone or prolactin level was established, a vicious circle could well have
resulted. Gonadal steroids have been shown to stimulate prolactin release (9), and prolactin causes a progesterone buildup through its luteinizing effect on the ovary.

Our slightly lowered prolactin results in the 2 nonirradiated partners with breast carcinomas as compared with those in females without carcinoma are unexplained. Increased prolactin receptors at the site of rapidly proliferating breast tumors might exert a lowering effect on prolactin blood levels (13).

Alternative explanations for increased prolactin and progesterone might include a break in the prolactin inhibition mechanism from the central nervous system to the pituitary. Various nonspecific stresses to the central nervous system which could include nephrectomy and impaired renal function have been shown to initiate lactation in the rat (12). In fact, all the nephrectomy control rats without irradiation showed varying degrees of secretory change and cyst formation in the acini.

It is unlikely that the breast tumors in the PRN series arose from the hormonal effects of spontaneous pituitary tumors which were occasionally found at autopsy, although this remains a possibility since multiple sections were not made. Pituitary tumors in the rat characteristically appear late, and some of our breast tumors were noted before 1 year of age. Hyperplasias of the pituitary, which were also seen, may have resulted from hormonal imbalance aggravated by the nephrectomy.

To relate a sustained prolactin increase to nephrectomy, one might consider an interference with excretion. Prolactin is a protein and FSH is a glycoprotein. Perhaps the finding of Vaitukaitis et al. (14) with FSH also applies to prolactin. They found that when radioactive FSH was injected into tail veins of rats the amounts recovered from the kidney substantially exceeded concentrations in the plasma, ovary, liver, and spleen. Since prolactin and FSH have molecular weights between 20,000 and 30,000, it is possible that their complex molecules might encounter filtration difficulties in the kidneys when 50% of the filtration bed was removed, and further reduction was incurred by pyelonephritis and irradiation. Bott and Richards (1) in 1951 showed that glomerular permeability to protein in the frog kidney varied according to molecular weight. Thus, purified protein derivative of tuberculoprotein (M.W. 14,500) was 85% filtered, while amorphous insulin (M.W. 34,000) was only 15% filtered.

Impairment of renal function is suggested by the low average kidney weights of the nonirradiated nephrectomized females with breast carcinoma as compared with the average kidney weights of those without breast carcinoma. Blood urea nitrogen analyses on a group of nephrectomized parabionts showed a slight increase over those in the nonnephrectomized group. The marginal degree of renal insufficiency as regards urea excretion could become greater in spite of hypertrophy of the remaining kidney when applied to a protein molecule. Significant retention of gonadal steroids by impaired renal function is less likely in the rat since they are mostly secreted or destroyed in the liver (L. L. Engel, personal communication).

If a hormonal etiology is excluded or only partially operative, activation of the mammary tumor virus in rats debilitated by parabiosis, nephrectomy, and irradiation is a possibility. If so, however, one would expect the major increase in incidence of breast carcinoma to occur in the irradiated partners. We have no reason to suspect the operation of a mammary tumor virus in our colony.

Our data support the implication of endogenous gonadal steroids and gonadotropins, particularly progesterone and prolactin, in the genesis of breast carcinoma in the rat. Failure of the irradiated partners to develop breast carcinoma in the same percentage as the nonirradiated can be explained by the lack of sufficient estrogen reaching the breast epithelium to prime it for the subsequent action of progesterone and prolactin. The fact that 2 of the 4 irradiated partners developed breast carcinoma is explained by the coexistence of granulosa cell proliferations (1 a tumor) in their ovaries.

The data contribute little to the controversy as to whether estradiol, progesterone, or prolactin are exclusively and solely responsible for the development of breast carcinomas in the rat (10). That a combined hormonal effect is operative in our series cannot be excluded.

When 1 member of a pair of parabiosed female rats is irradiated and a kidney is removed from each rat, the incidence of adenocarcinoma of the breast is sharply increased in the nonirradiated partner. The mechanism by which this occurs is not clear, but interference with excretion of endogenous gonadotropins by the kidney or stress-induced suppression of prolactin inhibition may have played a role in sustaining a prolactin-progesterone increase with subsequent increase in breast carcinoma.

REFERENCES

Nephrectomy and Breast Cancer in Parabiosed Rats


Fig. 1. Parabiont females of the PRN series. Right animal irradiated, left nonirradiated. Age at death 314 days, postirradiation of partner at 211 days, postnephrectomy at 178 days. Massive enlargement of 1 cornu of nonirradiated partner’s uterus (arrow). Cystic ovaries are obscured.

Fig. 2. Nonirradiated left partner of the PRN series, both animals unilaterally nephrectomized. Age at death 435 days, postirradiation of partner at 322 days, nephrectomy at 338 days. Both ovaries enlarged and cystic (arrows). Between them is an enlarged uterine cornu to which adjacent structures are adherent. Hemorrhagic breast carcinomas are seen in the right axilla and left groin.

Fig. 3. Breast of nonirradiated partner of the PRN series. Age at death 523 days, postirradiation of partner at 408 days, postnephrectomy at 423 days. Hyperplastic lobule, many acini distended with secretion and lined with hyperchromatic epithelium suggesting early neoplastic change. × 130.

Fig. 4. Breast of nonirradiated partner of the PRN series. Age at death 389 days, postirradiation of partner at 283 days, postnephrectomy at 338 days. Early adenocarcinoma with acinar pattern. Peripheral dilated acini suggest that the tumor may have arisen in the central portion of a lobule. × 160.

Fig. 5. Breast of nonirradiated partner of the PRN series. Age at death 435 days, postirradiation of partner at 322 days, postnephrectomy at 338 days. Few acinar remnants in a sheet of adenocarcinoma. Adjacent acini are distended with secretion scalloped at edges. × 130.

Fig. 6. Breast of irradiated partner of the PRN series. Age at death 638 days, postirradiation at 523 days, postnephrectomy at 538 days. This breast carcinoma was well differentiated and slightly infiltrative. It occurred in the same female at which the granulosa cell tumor of the ovary was found (Fig. 7). × 160.

Fig. 7. Ovary of irradiated partner of the PRN series. Age at death 638 days, postirradiation at 523 days, postnephrectomy at 538 days. Typical granulosa cell tumor in an older parabiont. This animal also had carcinoma of the breast (Fig. 6). × 160.

Fig. 8. Ovary of nonirradiated partner of the PRN series. Age at death 377 days, postirradiation of partner at 260 days, postnephrectomy at 275 days. Many cystic follicles lined with luteinized cells, granulosa cells, and mixtures of both. This partner had a well-established breast carcinoma. × 30.

Fig. 9. Ovary of nonirradiated partner of the PRN series. Age at death 519 days, postirradiation of partner at 406 days, postnephrectomy at 421 days. The most common type of cyst lining was with luteinized cells as shown here, although at least a few cysts in most ovaries were lined with granulosa cells. This female had a breast carcinoma. × 30.

Fig. 10. Ovary of irradiated partner of the PRN series. Age at death 372 days, postirradiation at 266 days, postnephrectomy at 233 days. Follicles atrophic, vessels thickened, fibrillar stroma with scattered strands of thecal cells. Carcinoma of breast in nonirradiated partner. × 160.

Fig. 11. Uterus of nonirradiated partner of the PRN series. Age at death 242 days, postirradiation of partner at 136 days, postnephrectomy at 101 days. Uterus dilated, myometrium thickened, endometrium secretory and partially ulcerated. Breast carcinoma also in this rat. × 130.

Fig. 12. Uterus of nonirradiated partner of the PRN series. Age at death 417 days, postirradiation of partner at 314 days, postnephrectomy at 281 days. Uterus distended with keratinized debris, myometrium hypertrophied and infiltrated with lymphocytes. Endometrium has undergone squamous metaplasia. This female also had a breast carcinoma. × 130.
The Effect of Nephrectomy on the Incidence of Breast Carcinoma in Irradiated Parabiosed Rats


Cancer Res 1975;35:37-44.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/35/1/37

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.