Effects of Radiation Type and Dose and the Role of Glucocorticoids, Gonadectomy, and Thyroidectomy in Mammary Tumor Induction in Mammosomatotropin-secreting Pituitary Tumor-grafted Rats

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ABSTRACT

Three experiments on the induction of mammary neoplasms by total-body 11- to 100-rad neutron or 50- to 500-rad γ-radiation of female Fischer or W/Fu rats are reported. Grafts of mammotropin-secreting pituitary tumor were used to elevate mammosomatotropin hormone levels. The results (a) confirm and extend previous reports that neutrons are more efficient in carcinoma induction than are γ-rays (the neutron relative biological effectiveness for first carcinomas was 3.68) and (b) demonstrate that the potentiation of carcinoma induction by adrenalectomy is reversed by glucocorticoid replacement. Statistical analysis of the data by procedures that take into account time at risk as well as tumor frequency indicates that multiple mammary tumors do not occur independently (i.e., a first mammary neoplasm significantly increases the probability of development of another neoplasm). The statistical procedure used in this analysis is presented.

INTRODUCTION

In classic studies Shellabarger and his associates demonstrated the susceptibility of Sprague-Dawley rats to the radiation induction of mammary tumors and that both direct damage to the mammary cells and indirect abscopal ovarian-pituitary-mediated effects are involved (cf. Ref. 20). Vogel (23, 24) and Shellabarger et al. (20, 22) have more recently found that neutron irradiation is surprisingly effective in the induction of mammary tumors. The RBE² of neutrons as compared to that of γ- or X-rays was reported to vary from 100 or more at doses of less than 1 rad neutrons to 10 or less at higher doses (20, 22-24).

Yokoro et al. (25) showed that grafted MTT's and presumably MTH potentiate the radiogenic induction of mammary tumors in W/Fu rats. In recent experiments we have found: (a) that extracranial grafts of single anterior pituitaries increased mammary tumor incidence in female Fischer rats that had been irradiated with γ-rays or neutrons (2); (b) that elevation of MTH levels by grafts of MTT 2 weeks before as opposed to 2 weeks after irradiation did not significantly alter mammary tumor incidence in female W/Fu rats (6); and (c) that adrenalectomy markedly enhanced mammary carcinoma induction in MTT-grafted, irradiated Fischer rats (5).

Preliminary radiation dose-mammary tumor response results in MTT-grafted, adrenalectomized Fischer rats have been presented (6). The results of the experiments summarized herein demonstrate that the type and incidence of mammary neoplasm observed is dependent not only on the dose and type of radiation administered but also upon the levels of MTH and glucocorticoids. The carcinoma-potentiating effect of adrenocortical deficiency in the presence of elevated MTH is demonstrated in the W/Fu rat and is reversed by glucocorticoid administration. The effects of gonadectomy or thyroidectomy on tumor incidence in intact or adrenalectomized, irradiated, MTT-grafted Fischer rats is also described. Finally, a statistical procedure for analysis of tumor risk is summarized.

MATERIALS AND METHODS

Animals. Inbred female Fischer and W/Fu rats, 8 to 11 weeks of age at the initiation of the experiments, were housed throughout in temperature-controlled quarters with a 12-hr light-dark cycle, fed commercial rat chow ad libitum, and given tap water to drink except as indicated below.

Radiation Procedures. For γ-irradiation 2 rats at a time were enclosed in a circular 4-mm-thick Lucite container with an adjustable lid placed firmly against their backs. The container was positioned on a wooden shelf such that its floor was 35.9 cm from a 2000-Ci ¹³⁷Cs teletherapy source, and the animals were irradiated at a dose rate of 45 rads/min to the whole body.

Groups of 4 rats at a time individually held in Lucite tubes were exposed to neutron irradiation in the beam port of the Wisconsin TRIGA reactor. The dose rate was 2.2 rads/min, and the beam was estimated to comprise 95% fast and intermediate neutrons, 5% γ-rays, and less than 0.5% thermal neutrons (6).

The dose-response study was set up over the course of 10 weeks, with 4 female Fischer rats assigned to each experimental group in each of 7 replicate experiments. Twenty-eight rats were thus accumulated in each of the following treatment groups: unirradiated control (Group C), 500 rads γ-rays (Group GA), 167 rads γ-rays (Group GB), 50 rads γ-rays (Group GC), 99 rads neutrons (Group NA), 33 rads neutrons (Group NB), and 11 rads neutrons (Group NC).
In the glucocorticoid effect study, a single batch of female W/Fu rats was distributed among the following treatment groups: unirradiated untreated controls (Group A), unirradiated MtT-grafted controls (Group B), and 3 groups of rats irradiated with 500 rads $\gamma$-rays as described above (Groups C to E). Groups A and B comprised 5 rats each, while Groups C through E each initially contained 20 rats.

In the endocrine ablation study, over the course of 4 weeks 4 batches of female Fischer rats were irradiated with 500 rads $^{131}$Cs $\gamma$-rays as above and were assigned to each of 6 experimental groups. A total of 16 rats were thus accumulated per treatment group in the 4 replicate experiments as follows: MtT grafted, adrenalectomized (Group A), MtT grafted ovariectomized (Group G), MtT grafted thyroidec tomized (Group T), MtT grafted adrenogonadecto mized (Group AG), MtT grafted adrenothyroidec tomized (Group AT), and pituitary grafted adrenalectomized (Group PA).

Endocrine Treatment. For the achievement of chronic high levels of endogenous MTH (9, 10), all Fischer rats in the radiation dose-response study and all but Group PA in the endocrine ablation study were grafted with MtT strain F4, and Groups B through E of the W/Fu rats in the glucocorticoid effect study were grafted with MtT strain W10. MtT suspensions were prepared for grafting with the aid of a cytosieve and were inoculated i.d. into the back. The first MtT grafts were made on the day following irradiation of the W/Fu rats and on the third and fourth days after exposure of the Fischer rats in the dose-response and endocrine ablation experiments. Rats of Group PA of the endocrine ablation study were each grafted with a single anterior pituitary gland beneath the kidney capsule (4) 3 days after irradiation. Pituitary donors were of the same age and sex as recipients.

All Fischer rats in the dose-response study, Groups D and E of the W/Fu rats and Groups A, AG, AT, and PA of the endocrine ablation study, were adrenalectomized under ether anesthesia, 2 and 3 days after radiation exposure, respectively. Gonadectomies and thyroidecotomies were performed in the appropriate groups in the ablation study on the same day. All adrenalectomized rats were given a single weekly s.c. injection of 2.5 mg deoxycorticosterone acetate in 0.2 ml suspending menstruum and their choice of 0.9% NaCl solution or tap water to drink throughout the remainder of the experiment.

W/Fu rats of Group E also were given s.c. injections of 1.5 mg cortisol in 0.05 ml suspension 3 times/week from the day of adrenalectomy to Day 65 after irradiation and 5 times/week thereafter.

General. The animals were inspected frequently with particular reference to nodules in the mammary glands and to the MtT grafts. For the prolongation of life, MtT grafts were surgically resected when about 1 cm in diameter, and the animals were regrafted. MtT's were resected an average of 4.6 times/rat at an average interval of $46 \pm 31$ (S.D.) days in the dose-response experiment, 4.7 times at intervals of $53 \pm 25$ days in the glucocorticoid study, and 2.5 times at intervals of $50 \pm 27$ days in the endocrine ablation study. Mammary tumors and s.c. MtT metastases were removed soon after discovery, and samples of all were fixed in neutral formalin, embedded, sectioned, and stained with hematoxylin and eosin for histological examination. After surgery the animals were returned to their respective experimental groups.

Animals that died or were killed when moribund were autopsied, with special reference to mammary neoplasms and, when appropriate, to regenerating or accessory adrenocortical tissue. Only data from those rats that survived until the appearance of a mammary neoplasm in the same experiment are considered in the presentation and analyses.

Mammary tumors were histologically classified as fibroadenomas or carcinomas, which were readily distinguishable from each other and from MtT's on both gross and histological bases. Only histologically confirmed neoplasms are, however, included in the data summaries. When a neoplasm was found in a mammary gland from which a tumor of the same histological type had been resected, it was not considered to be a new neoplasm and was excluded from the data summary.

As survival varied among the various treatment groups and as tumor frequency at a given time is a function of the number of rats then alive, data from the larger experiments were analyzed in terms of the number of rats at risk on a given day by the procedure outlined in the "Appendix."

RESULTS

$\gamma$-Ray and Neutron Dose-Carcinoma Response Study. All mammary tumors in these Fischer rats were found on histological examination to be carcinomas, and tumor frequency was directly related to radiation dose (Table 1). Survival was insignificantly affected by dose or type of radiation.

In all groups total cumulative mammary tumor frequency per rat had reached its maximum by 300 days after exposure. The total mammary carcinomas at 300 days/total rat days at

$$\text{Table 1}$$

Effect of radiation dose and type on mammary carcinoma frequency in MtT-grafted, adrenalectomized Fischer rats

<table>
<thead>
<tr>
<th>Radiation dose (rads)</th>
<th>No. of rats</th>
<th>Days</th>
<th>No. of tumors</th>
<th>Latency (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>0</td>
<td>25</td>
<td>227 $\pm$ 74</td>
<td>250 $\pm$ 6</td>
</tr>
<tr>
<td>GA</td>
<td>5000</td>
<td>25</td>
<td>205 $\pm$ 81</td>
<td>16 37 215 $\pm$ 89</td>
</tr>
<tr>
<td>GB</td>
<td>167</td>
<td>24</td>
<td>206 $\pm$ 109</td>
<td>7   12 215 $\pm$ 89</td>
</tr>
<tr>
<td>GC</td>
<td>500</td>
<td>25</td>
<td>225 $\pm$ 71</td>
<td>4   4 212 $\pm$ 109</td>
</tr>
<tr>
<td>NA</td>
<td>95n</td>
<td>24</td>
<td>239 $\pm$ 99</td>
<td>14  38 201 $\pm$ 76</td>
</tr>
<tr>
<td>NB</td>
<td>33n</td>
<td>26</td>
<td>266 $\pm$ 98</td>
<td>14  22 214 $\pm$ 98</td>
</tr>
<tr>
<td>NC</td>
<td>11n</td>
<td>25</td>
<td>250 $\pm$ 111</td>
<td>5   7 190 $\pm$ 58</td>
</tr>
</tbody>
</table>

See "Materials and Methods" for explanation of group designations.

Rats that survived $\geq$69 days (day of first carcinoma) after irradiation.

Number of rats that developed 1 or more carcinomas.

Total number of mammary carcinomas.

Days after irradiation when carcinomas were recorded.

Mean $\pm$ S.D.
risk were thus analyzed against type and dose of radiation. The relationship did not markedly deviate from linearity in either case, and the intercepts at the ordinate were insignificantly different (Chart 1, ALL CARCINOMAS).

If, in a given animal, the appearance of 1 mammary carcinoma alters the risk of development of a second or subsequent carcinomas, then an analysis based on all tumors could lead to erroneous conclusions. A version of the log rank test (see "Appendix") was applied in which, within each experimental group, a comparison is made of new carcinoma appearance over time between animals with previous carcinomas and those without. The results were then accumulated over experimental groups, and a $\chi^2$ statistic was calculated. This test indicated that, in those rats that had had 1 carcinoma, a second carcinoma developed significantly more rapidly than would be expected were there no effect of the first tumor ($p < 0.001$).

The survival-adjusted probability of developing a first carcinoma is shown as a function of dose in Chart 2. Taking the risk of development of the first carcinoma only per unirradiated control rat day as 1.0, the relative risk of first carcinoma was calculated for each radiation dose (Chart 1, FIRST CARCINOMAS). Once again, risk increased approximately linearly with dose over the ranges tested.

**Glucocorticoid Effects on Radiogenic Mammary Tumor Induction.** No mammary tumors were observed during the 600-day course of the experiment in the small group of untreated control W/Fu rats, and only 1 mammary neoplasm, an adenofibroma, developed in unirradiated rats with grafted MtT (Table 2). As expected, irradiated, MtT-grafted, intact rats had an increase in total mammary fibroadenomas and a high frequency of mammary carcinomas. Adrenalectomy with mineralocorticoid replacement reduced mean survival ($p < 0.001$) and shortened carcinoma and fibroadenoma latencies. Treatment of such rats with cortisol did not alter survival time or the mean latencies of either type of neoplasm (Table 2). In general, mean latencies of fibroadenomas were greater than were those of carcinomas within a treatment group.

When all mammary neoplasms were pooled, the log rank test revealed that any prior mammary neoplasm increased the risk of subsequent mammary carcinomas ($p < 0.05$) or mammary fibroadenomas ($p < 0.05$). The data were inadequate for more detailed log rank analysis.

**Table 2**

<table>
<thead>
<tr>
<th>Groupa</th>
<th>Radiation dose (rads)</th>
<th>Treatmentb</th>
<th>Survival</th>
<th>Mammary carcinomas</th>
<th>Mammary fibroadenomas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No. of ratsc</td>
<td>No. of tumors</td>
<td>Latency (days)</td>
</tr>
<tr>
<td>A</td>
<td>0</td>
<td></td>
<td>5 600 ± 0$^e$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td></td>
<td>5 456 ± 150</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>500$^g$</td>
<td>Adx + DOC</td>
<td>19 357 ± 123</td>
<td>14 28</td>
<td>271 ± 117</td>
</tr>
<tr>
<td>D</td>
<td>500$^g$</td>
<td>Adx + DOC</td>
<td>16 211 ± 83</td>
<td>13 24</td>
<td>174 ± 79</td>
</tr>
<tr>
<td>E</td>
<td>500$^g$</td>
<td></td>
<td>19 257 ± 109</td>
<td>7 8</td>
<td>185 ± 55</td>
</tr>
</tbody>
</table>

| a See "Materials and Methods" for explanation of group designations. |
| b Adx, adrenalectomized; DOC, deoxycorticosterone; cort, cortisol. All but Group A were grafted with MtT strain W10. |
| c Rats that survived ≥106 days after irradiation (i.e., to time of first tumor) are included. |
| d Survival of rats that lived to the end of the experiment included as 600 days. |
| e Mean ± S.D. |
Further analysis was thus restricted to risk of first tumors. The survival-adjusted probabilities of developing a first mammary neoplasm are summarized for Groups C, D, and E (Chart 3). Taking the risk of first carcinoma development in Group C as equal to 1.0, adrenalectomy with mineralocorticoid replacement increased the risk of first carcinoma by 2.35 times (Group C versus Group D, \( P < 0.05 \)). Glucocorticoid replacement with exogenous cortisol reversed the adrenectomy effect, reducing relative carcinoma risk to 0.71 (Group D versus Group E, \( p < 0.02 \)). The fibroadenoma frequency data are less extensive, but adrenalectomy with mineralocorticoid treatment appeared to increase risk, while glucocorticoid replacement was less effective in reducing risk than it was in the case of carcinomas (Table 2; Chart 3).

Effect of Other Endocrine Manipulations. In the third experiment adrenalectomy in combination with irradiation and MtT grafting (Table 3, Group A) resulted in a greater frequency of first mammary carcinomas as compared to all other groups pooled (\( p = 0.01 \)) and a shorter time to first carcinoma (\( p < 0.001 \)). Pituitary grafts in combination with adrenalectomy (Group PA) resulted in the second highest carcinoma frequency, but both mean survival and mean time to first carcinoma were significantly longer than those of Group A (\( p < 0.02 \) and \( p < 0.05 \), respectively).

Mammary carcinoma frequency was less in ovariectomized than it was in adrenalectomized MtT-grafted rats (Group G versus Group A, \( p < 0.05 \)), and the tumors appeared later (\( p < 0.01 \)). No carcinomas were observed in adrenogonadectomized rats of Group AG, and only 1 was observed in each of the 2 thyroidectomized groups, T and AT.

**DISCUSSION**

These experiments: (a) confirm and extend previous reports (20, 22-24) that neutrons are significantly more efficient in the induction of mammary carcinomas than \( \gamma \)-rays; (b) confirm our previous observation in the Fischer rat (5) that adrenalectomy with mineralocorticoid replacement potentiates mammary carcinoma induction in irradiated, MtT-grafted rats and extend this finding to W/Fu rats; (c) demonstrate that this potentiation of carcinoma induction by adrenalectomy is reversed by glucocorticoid treatment; and (d) indicate that gonadectomy and thyroidectomy of irradiated MtT-grafted Fischer rats does not potentiate mammary carcinoma induction as does glucocorticoid deficiency and, in combination with the latter, may reduce the adrenectomy effect.

The finding that an animal that has suffered 1 mammary neoplasm is at greater risk of developing a second has been observed in other experiments in our laboratories (13) and is best established for carcinomas. In the dose-response study in which this effect was most prominent, all animals were subjected to repeated surgery for removal of grafted MtT's, and it seems unlikely that the additional surgery necessary to remove the first carcinoma can account for the effect. As the mammary carcinomas were surgically removed soon after they became palpable, it is also unlikely that the rats that developed 1 carcinoma suffered a significantly greater "tumor burden" than those that had not yet developed a carcinoma. In any event this result indicates that multiple mammary carcinomas should not be considered as independent events in this system.

Within the confines of the neutron and \( \gamma \) doses used in the dose-response study, both total mammary carcinomas/rat day and risk of first carcinoma were approximately linear with dose for both modalities, and there was thus no clear evidence of a marked increase in RBE at the lowest dose used. The risk of carcinoma was increased by about 2%/HUNDREDS OF DAYS AFTER IRRADIATION

![Chart 3. Probabilities of development of a first mammary neoplasm in irradiated female W/Fu rats grafted with MtT strain W10. C, intact animals; D, adrenalectomized, mineralocorticoid-supplemented animals; E, adrenalectomized, mineralocorticoid- and glucocorticoid-supplemented rats. Vertical dotted lines, S.E.](chart)

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Mammary neoplasia in hormonally modified Fischer rats irradiated with 500 rads ( \gamma )-rays</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatment</strong></td>
<td><strong>Survival</strong></td>
</tr>
<tr>
<td>Group&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Graft</td>
</tr>
<tr>
<td>A</td>
<td>MIT</td>
</tr>
<tr>
<td>G</td>
<td>MIT</td>
</tr>
<tr>
<td>T</td>
<td>MIT</td>
</tr>
<tr>
<td>AG</td>
<td>MIT</td>
</tr>
<tr>
<td>AT</td>
<td>MIT</td>
</tr>
<tr>
<td>PA</td>
<td>Pituitary</td>
</tr>
</tbody>
</table>

<sup>a</sup> See "Materials and Methods" for explanation of group designations.
<sup>b</sup> First mammary neoplasms only.
<sup>c</sup> Mean ± S.D.
rad by γ-rays and 7.5%/rad by neutrons. As tumor frequencies after exposure to the 2 radiation types were within the same range, the RBE of the modified fission neutrons as compared to 137Cs γ-rays is the ratio of the slopes. For total carcinomas per rat day, the neutron RBE was 4.90 (95% confidence limits: 3.52, 6.80), and for first carcinomas it was 3.68 (95% confidence limits: 2.25, 5.65). The neutron RBE might well increase in this system, of course, at doses below 10 rads, as observed by Vogel (23, 24) and Shellabarger et al. (20, 22).

In the dose-response experiment all mammary neoplasms were diagnosed as carcinomas. In a previous study in which neutrons- or γ-irradiated intact Fischer rats were each grafted with a single pituitary gland, both fibroadenomas and carcinomas were observed (2). Furthermore, in the current glucocorticoid study in W/Fu rats, carcinoma frequency was high and carcinoma latency was less than fibroadenoma latency in MtT-grafted irradiated rats, whereas carcinomas are rare and fibroadenomas are infrequent in unirradiated rats of this age and strain. Thus, the type as well as the frequency of mammary neoplasm observed is dependent on the hormonal milieu after irradiation. Specifically, the results are consistent with the conclusion that carcinoma development is potentiated more and carcinoma latency is shortened more in relationship to MtH titer than is fibroadenoma development and latency. This is reminiscent of the results of Shellabarger et al. (21), who observed that mammary carcinoma latency was shortened more by radiation exposure than was fibroadenoma latency in intact Sprague-Dawley rats.

Potentiation of mammary carcinogenesis by adrenalectomy was observed in irradiated Fischer rats grafted with MtT strain F4 (5), and Chen et al. (1) observed stimulation of carcinogen-induced mammary tumor growth by adrenalectomy in line-bred Sprague-Dawley rats. The latter authors attributed the effect to an elevation in serum MtH, which occurred following adrenal ablation and was reversed by glucocorticoid replacement. It does not seem probable that this explanation applies in rats grafted with MtT. Serum MtH levels are elevated by a factor of 10- to 100-fold in MtT-grafted animals (5), and it is improbable that any further modest increase that might result from adrenalectomy would be significant.

In our experiments adrenalectomized animals were treated with minimal mineralocorticoid replacement therapy. We first postulated that potentiation of carcinogenesis by adrenalectomy was due to inhibition of milk secretion resulting from glucocorticoid deficiency. In the presence of MtH, milk secretion occurs only if glucocorticoids are present. In glucocorticoid-deficient rats with elevated MtH, proliferation without secretion predominates (3). We suggested that, in the presence of high MtH and glucocorticoids, the population of potential cancer cells in irradiated mammary gland was reduced by diversion to secretion. In glucocorticoid-deficient animals more potential cancer cells retained high proliferative capacity and more carcinomas resulted (5).

The current data illustrate that glucocorticoid replacement reverses potentiation of radiogenic carcinoma induction by adrenalectomy but do not establish the postulate. Indeed, recent results suggest that further investigation is required concerning the time period after radiation exposure during which glucocorticoids must be present to produce the effect. In these latter studies carcinomas were as frequent in irradiated, MtT-grafted, adrenalectomized rats treated with glucocorticoid for 4 weeks after irradiation as they were in similar rats given no glucocorticoid replacement (13).

Finally, it seems to us unlikely that the protective action of glucocorticoids is due to a catabolic effect on protein synthesis. Indeed, milk protein synthesis requires the presence of glucocorticoids (3).

As concerns fibroadenoma induction glucocorticoid deficiency also appeared to increase tumor risk. Perhaps because the data are meager, statistically significant reversal of this effect by glucocorticoid replacement was not demonstrable.

The lack of potentiation of carcinoma induction in irradiated MtT-grafted Fischer rats by gonadectomy or thyroidectomy is in accord with expectation from studies with other strains (cf. Refs. 4 and 20). Our results further indicate that both ablative procedures antagonize tumor potentiation by glucocorticoid deficiency. Finally, presum-ably as a result of higher MtH levels, grafts of MtT were more efficient in potentiating radiogenic mammary carcinoma induction in adrenalectomized rats than were single grafted pituitary glands.

The long-range goal of our program is to quantitatively relate the late radiation effect expressed as tumor formation to the acute effect expressed as cell death and to determine the effect of hormones on both processes. We have used hormonal manipulations similar to those used here in the development of an end point dilution assay for survival of monodispersed mammary cells (6, 11, 12). γ-Ray dose-mammary cell survival response curves generated with this system follow the classic mammalian cell sensitivity pattern, with an initial shoulder followed by an exponential decrease. Assuming the classic multiple target-single hit model equation (8)

\[ S = 1 - (1 - e^{-\alpha})^n \]

where \( S \) is the surviving fraction of the cell population, \( D \) is the dose applied, and \( D_0 \) and \( n \) are constants characteristic of the cell type and conditions; the \( D_0 \) for mammary cells was estimated as 129 rads, and \( n \) was estimated as 5 (12). More recent studies (M. N. Gould and K. H. Clifton, unpublished data) indicate that this \( n \) value is an underestimate when the mammary cells are left in situ during postirradiation recovery. For illustration, however, assuming the above \( D_0 \) and \( n \) and γ doses of 500, 167, and 50 rads, as used in the dose-response study, one would expect survival of about 10, 80, and 100% of the mammary cells, respectively. If the potential carcinoma cells are as sensitive to radiation killing as is the rest of the cell population, these results imply that the observed carcinoma incidence after 500 rads γ-rays reflected only about one-tenth of the potential carcinoma-inducing events that occurred. In contrast, because cell survival was higher after 167 and 50 rads γ-rays, the carcinoma incidence may reflect 80 to 100% of such potential carcinogenic events. The near-linear relationship of
carcinoma incidence with dose may thus be fortuitous and may well not accurately reflect the neoplasia-initiating events. Neutron dose-cell survival data are not yet available. More detailed studies of the relationship between the acute and late effects of radiation clearly are desirable.

ACKNOWLEDGMENTS

The authors are indebted to Joan Mitchen, Joan Eggert, and Irene Meinholz for excellent technical assistance. Greg Hudak, Ben Bates, and Steve Dahlberg (Statistics) helped perform the calculations, and Russ Stry (Wisconsin Clinical Cancer Center) did the programming.

APPENDIX: STATISTICAL METHODS

The end points used for analysis include percentage of animals with tumors, time at risk, time to first tumor, and time to subsequent tumors. Times at risk were analyzed between groups by t tests; analyses of percentage of animals with tumors were done by Fisher's exact tests when times at risk did not differ markedly between groups.

The time-to-first-tumor data were assessed with methods commonly used in clinical trials. Graphs of the probabilities of first tumor versus time (Charts 2 and 3) were derived from their complements, the probabilities of being tumor free, which were constructed by the product limit method (14). This procedure adjusts for the differing times at risk and the fact that some animals did not develop tumors. Differences between these curves were examined by the log rank test (15, 17, 18). This test compares the whole curve and includes adjustment for varying numbers at risk during the entire observation period. It is thus not limited to tumor incidence at a single time point.

The relative risks of first tumors in the experimental groups as compared to the controls were found by methods described by Mantel (15). This relative risk represents the ratio of probabilities of developing a tumor during a small increment of time, assuming the unirradiated control risk as 1.0. In the dose-response experiment, relative risks were plotted against dose for each radiation type, and lines were fit by the least squares method. The ratio of slopes is taken as the RBE; a confidence interval was constructed using Fieller's theorem (cf. Ref. 19). The RBE was similarly estimated from the multiple tumor data, with the total number of tumors by Day 300 per cumulative rat days at risk as the response variable. In this case preliminary F tests were performed to see whether the fitted lines had a common origin (consistent with the controls) so that the RBE could be defined independently of dose.

If multiple tumors in a given animal arise independently (i.e., as the result of a Poisson process), then the additional data on times to subsequent tumors can be used to gain more statistical power. For example, for the log rank test each animal would remain in the analysis until death or the end of the study, rather than being removed at the time of the first tumor. However, if tumors arise independently (i.e., from a single locus or as a result in part of a general systemic process), then use of multiple tumor data could be misleading. To test for independence (that is, to determine whether appearance of 1 tumor changes the risk of development of subsequent tumors), a version of the log rank test developed for analysis of heart transplant survival data was used (7, 16). Within a given experimental group, those animals without prior tumors (Category 0) are compared to those with prior tumors (Category 1) as to development of subsequent tumors. The numbers in the 2 categories change as animals develop tumors and therefore move from 1 category to the other; this fact is accounted for in the analysis. The results can then be accumulated across experimental groups to yield an overall comparison.

The calculations proceed as follows. For a given experimental group, a $\times 2$ table is constructed at each time a tumor appears. The i" such table can be represented symbolically:

$$N_0\begin{bmatrix}1-Z_1 & N_0 - (1-Z) \\ Z_1 & N_1 - Z_1 \\ 1 & 1\end{bmatrix}$$

The marginals $N_0$ and $N_1$ represent the animals at risk at that time, without or with prior tumors, respectively. The $Z_1$ is 1 if the tumor is from an animal in the group with prior tumors (0 otherwise). Multiple tumors at the same time can be resolved conservatively by considering those for animals with tumors to have happened first; within a category they may be resolved arbitrarily.) Then define:

$O =$ observed tumors in the group with prior tumors

$E =$ expected tumors in the group with prior tumors

$$E = \sum N_0 \times N_1 / \sum N_1$$

$V =$ variance of $O - E$

$$V = \sum \frac{N_0 \times N_1}{\sum N_1} \times \frac{1}{N_1}$$

The test statistic is

$$t = \frac{O - E}{\sqrt{V}}$$

To accumulate across experimental groups, define $O$, $E$, and $V$ for each, then calculate

$$T_2 = \sum \frac{(O - E)^2}{V}$$

These statistics can be referred for testing to tables of the $x^2$ distribution with 1 degree of freedom, provided there are several tumors in each category.

REFERENCES

Radiation Type, Glucocorticoids, and Mammary Cancer


Effects of Radiation Type and Dose and the Role of Glucocorticoids, Gonadectomy, and Thyroidectomy in Mammary Tumor Induction in Mammotropin-secreting Pituitary Tumor-grafted Rats

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