Combination Therapy of 3-Methylcholanthrene-induced Mammary Carcinoma in Rats: Effect of Chemotherapy, Ovariectomy, and Food Restriction

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Summary

The growth of mammary carcinoma induced by oral 3-methylcholanthrene in female Wistar rats is retarded by ovariectomy and by food restriction sufficient to produce 12–14% body weight loss. Estradiol benzoate (NSC 9566), 5-fluorouracil (NSC 19893), miracil D (NSC 14574), mitomycin C (NSC 26980), and AB-100 (NSC 37095) showed inhibitory activity. The best tumor inhibition without mortality was elicited by ovariectomy combined with food restriction, and by ovariectomy combined with estradiol benzoate.

Introduction

Female rats that are exposed to carcinogenic polycyclic aromatic hydrocarbons develop adenocarcinomas and other tumors of the breast (6, 7, 10). For this laboratory model, much of the work has involved the use of 2 strains of rats, Wistar and Sprague-Dawley, of 2 carcinogenic hydrocarbons, 3-methylcholanthrene (MCA) and 7, 12-dimethylbenz(a)anthracene (DMBA), and of 2 routes of administration, intragastric or i.v. At higher doses of carcinogens, practically all female rats of the 2 strains develop mammary tumors within 8 months. The great majority of the earlier-appearing tumors are adenocarcinomas, and they are often multiple. Many grow progressively, and a small proportion metastasize to distant organs; others show indolent growth and a few regress. The tumors are partially hormone-dependent, in that ovariectomy retards their appearance and growth. The MCA-induced tumors are also partially nutrition-dependent, since restricted food intake retards their appearance and growth (5, 9) and is about true for DMBA-induced tumors, although specific data on this point are not available. The MCA-induced mammary carcinoma of the female rat has been applied to a series of investigations on carcinogenesis and factors modifying this reaction (4, 9, 10), and, more recently, chemotherapy (1, 2, 5, 8).

In this laboratory, we have examined over 150 chemical compounds for their effect on the MCA-induced mammary carcinoma in rats, and have recorded the results on some 70 compounds (1, 2). By the criteria of decreased tumor growth and appearance of additional tumors as compared with untreated controls, 8 of 64 nonhormonal and 4 of 8 hormonal agents were deemed to have activity. However, none of these agents was more effective than ovariectomy.

It was also noted that significant inhibition of tumor growth was achieved with diets that produced approximately 15% loss of body weight (5). This effect could be dissociated from the effect of chemotherapy with many agents that also produced loss of body weight.

The investigations indicated a study of combining ovariectomy and chemotherapy, and of induced weight loss and chemotherapy. It was also planned to determine the effect of chemotherapy on hormone-dependent as contrasted with nonhormone-dependent tumors.

Materials and Methods

Female Wistar rats, weighing between 90 and 100 gm, were purchased from Carworth Farms, New City, Rockland County, New York. They were housed multiply in plastic shoe box cages and fed Rockland complete mouse/rat diet and water ad libitum.

Employing a No. 8-French soft-rubber catheter and a self-filling syringe, gastric intubation of the carcinogen, MCA at a dose level of 10 mg/ml of olive oil, was given 3 times a week (Monday, Wednesday, Friday) for a period of 9 weeks (27 doses or 270 mg). The MCA-oil mixture was prepared by stirring and heating to a maximum temperature of 80°C for 30–60 min on a hotplate magnetic stirrer. This concentration must be maintained above 30°C to avoid crystallization.

After completion of the carcinogen administrations, the animals were placed in individual metal cages. Breast tumors began to appear on each rat as grossly palpable masses in 1 or more of the 12 breasts as early as 8 weeks following commencement of the carcinogen feedings. Approximately 85–90% of the animals developed tumors within a year. However, the experimental animals were limited to those that developed tumors within 10 weeks after the completion of the feedings. This reduced the number of experimental animals to about 70% of the original population.

The rats were weighed weekly and palpated twice a week for the presence of breast tumors. When 1 tumor, or more, attained a diameter of 6–10 mm, the animal was assigned to an experimental or control group by randomization, using the index card method. At that time, the mean weight of the rats was 212 gm, with the means of groups varying between 192 and 237 gm.
MCA-induced Mammary Carcinoma in Rats

TABLE 1

<table>
<thead>
<tr>
<th>NSC No</th>
<th>Name</th>
<th>Vehicle</th>
<th>Source*</th>
<th>Dose (mg/kg/day)</th>
<th>Total dose (mg/kg)</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>9166</td>
<td>Androst-4-en-3-one, 17β-hydroxy-, propionate; testosterone propionate</td>
<td>5% alcohol</td>
<td>43</td>
<td>2.5</td>
<td>90</td>
<td>1-42</td>
</tr>
<tr>
<td>9566</td>
<td>Estra-1,3,5(10)-triene-3,17β-diol, 3-benzoate; 17β-estradiol, 3-benzoate</td>
<td>5% alcohol</td>
<td>43</td>
<td>10</td>
<td>360</td>
<td>1-42</td>
</tr>
<tr>
<td>9704</td>
<td>Pregn-4-ene-3,20-dione; progesterone</td>
<td>5% alcohol</td>
<td>43</td>
<td>100</td>
<td>3600</td>
<td>1-42</td>
</tr>
<tr>
<td>14574</td>
<td>Thioxanthen-9-one, 1-(2-[diethylamino]-ethyl)amino)-4-methyl-, hydrochloride; miracil D</td>
<td>5% alcohol</td>
<td>1</td>
<td>50</td>
<td>1800</td>
<td>1-42</td>
</tr>
<tr>
<td>19893</td>
<td>Uracil, 5-fluoro-</td>
<td>0.85% saline</td>
<td>217P</td>
<td>20</td>
<td>720</td>
<td>1-42</td>
</tr>
<tr>
<td>23519</td>
<td>Uracil, 5-diazoe-, hydrate; 5-diazouracil</td>
<td>Distilled water</td>
<td>63P</td>
<td>4</td>
<td>144</td>
<td>1-42</td>
</tr>
</tbody>
</table>

Experiment 2: Injections every other day for 5 doses

<table>
<thead>
<tr>
<th>NSC No</th>
<th>Name</th>
<th>Vehicle</th>
<th>Dose (mg/kg/day)</th>
<th>Total dose (mg/kg)</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>9566</td>
<td>Estra-1,3,5(10)-triene-3,17β-diol, 3-benzoate; 17β-estradiol, 3-benzoate</td>
<td>Distilled water</td>
<td>6</td>
<td>0.8</td>
<td>4</td>
</tr>
<tr>
<td>26980</td>
<td>Mitomycin C; azirino[2',3':3,4]-pyrrolo-[1,2-ajindole-4,7-dione, 6-amino-1,1a-</td>
<td>Distilled water</td>
<td>147</td>
<td>13</td>
<td>65</td>
</tr>
<tr>
<td>37095</td>
<td>Carbamic acid, [bis(1-aziridinyl)-phosphinyl]-, ethyl ester; (AB-100)</td>
<td>Distilled water</td>
<td>147</td>
<td>13</td>
<td>65</td>
</tr>
</tbody>
</table>

* See Addendum.

The agents (Table 1) were supplied by the Cancer Chemotherapy National Service Center (CCNSC), National Cancer Institute.

The dosages were selected from previous information (1, 2) on chemotherapeutic screening. The drug solutions were freshly prepared and 0.01 mg/gm body weight of the solutions were injected i.p.

Two experiments were performed. They consisted of 24 and 12 groups, respectively, with 10–12 rats/group; there were 6 groups of untreated or ovariectomized controls, each of 12–30 rats. Of the total of 490 rats with tumors, there were 383 experimental and 107 control animals.

In Experiment 1, injections of the 6 chemicals indicated in Table 1 were given daily, 6 days/week (Monday through Saturday) for 6 or 3 weeks, starting on Day 1 or Day 21, respectively, of the 42-day period. In Experiment 2, the 3 chemicals listed in Table 1 were administered every other day from Day 1 to Day 9 for a total of 5 injections.

Ovariectomies were performed through bilateral incisions, with the rats under ether anesthesia.

Body weight loss was produced by limiting the food per rat to 10 gm daily and 20 gm or less over the weekend, depending upon the body weight change during that week.

Each rat was weighed and the tumor diameters were measured 3 times a week for 42 days, by means of externally applied calipers. The greatest diameter and the diameter at right angle to it were averaged, and the volume of each tumor was calculated for an assumed sphere by the formula $\frac{4}{3}\pi r^3$. Record was made of all additional tumors detected on the animal, including its location.

On Day 42 all survivors were killed and the tumors excised and weighed. The tumors were fixed and prepared for histologic examination. All were found to be adenocarcinomas, as previously described (3) for the earlier-appearing tumors in female Wistar rats.

Analysis of the data included examinations of the derived tumor volumes and the appearance of additional tumors in all animals, as reported in previous investigations (1, 2). It became clear, however, that except in some instances where such data were relevant, the results were adequately expressible in terms of the total tumor weight per rat at 42 days, and that none was significantly altered when tumor volumes or additional tumors were used as the parameters of effect. For simplicity of presentation, therefore, tumor volumes are reported herein only for Experiment 1. The relationship of tumor volume to tumor weight in Experiment 1 is indicated in Chart 1.

Statistical tests for variability and significance of differences between groups were performed. The standard errors of the individual groups are presented. "Significance" is indicated when the difference met the criterion of $P < 0.05$. For these calculations, rats grossly free of tumor at Day 42 were given values of 0.01 gm.

EXPERIMENT 1. The 3 purposes of this experiment were to study (a) the effect of 6 chemotherapeutic agents, 3 hormonal and 3 nonhormonal, on MCA-induced mammary carcinoma, as compared with untreated and with ovariectomized controls, (b) the effect of combining ovariectomy and chemotherapy, and (c) the effects on hormone-dependent and hormone-independent tumors.

For the 3rd purpose, the design was to withhold chemotherapy
Results

Table 2 presents the results of the first experiment. For its interpretation, the following information on the growth of the tumors during the first 21-day period and the second 21-day period is necessary. This information is derived from tumor volumes calculated from external measurements of the tumor diameters.

At Day 1 of the experiment, the total tumor volumes in the 24 groups varied from 0.16 to 0.44 ml. The means for untreated controls and the ovariectomized controls were 0.23 ml; for the treated groups, the mean was 0.24 ml. At Day 21, the means of the total tumor volume for untreated controls and ovariectomized controls was 4.20 and 1.27 ml, respectively. Among the treated intact groups in which treatment was withheld until Day 21, the tumor mean volume was 5.27 ml, and among the treated ovariectomized groups in which treatment was withheld until Day 21, it was 1.43 ml. The values are reasonably similar, and indicate that during the first 21-day period there was an 18- to 22-fold increase in tumor volume among the untreated intact animals, and a 5- to 6-fold increase in tumor volume among the ovariectomized rats. Thus, ovariectomy reduced the growth rate of the tumors to approximately 1/4 observed in intact animals.

During the second 21-day period, the volumes of the tumors increased to a mean of 9.63 ml for the untreated, intact rats, and to a mean of 3.76 ml for the untreated ovariectomized controls. The increment between 21 and 42 days was approximately the same for the 2 groups: 2.3-fold and 3.0-fold, respectively, again substantiating that the major tumor-inhibiting effect of ovariectomy is complete by 21 days. For the total 42-day period, the tumor volume increased by over 40-fold in the untreated intact animals, and by less than 20-fold in the ovariectomized rats. In terms of volume doubling-time, this can be expressed as 5 versus 4 doublings over a 42-day period, or 8 versus 10 days per doubling.

The growth rate of the tumors is, therefore, more rapid during the first 21-day period than during the second 21-day period, in both the intact and the ovariectomized animals. This factor must be considered in the interpretation of the data on the effects of chemotherapeutic agents.

Examination of data on tumor weights at 42 days among the intact animals shows that significant inhibition of tumor growth was achieved in one or both groups treated with estradiol benzoate (Group 3), 5-fluorouracil (Group 8) and miracil D (Groups 12 and 13). However, inhibition with 5-fluorouracil and miracil D was evident at dose levels that produced mortality of over 30% and, in Group 12, a 32% body weight loss. Only miracil D actually caused significant tumor regression by Day 42. With 5-fluorouracil and estradiol benzoate, there was only retardation in the growth of the tumors.

Testosterone propionate and 5-diazouracil, at the doses used, had no significant effect on tumor growth; if anything, the tumors were larger than among the untreated controls. With progesterone there was a significant increase in tumor weight in Group 6.

Data on tumor volumes, as given in Table 2 and Chart 2, show that the chief augmentation effect of progesterone was during the earlier period, up to 21 days. In contrast, the chief inhibiting effect of miracil D was expressed during the latter half of the treatment period.
When ovariectomized rats were used as the comparative control, as given in Table 2 and in Chart 3, estradiol benzoate (Groups 15 and 16) and 5-fluorouracil (Groups 21 and 22) showed significant inhibition of tumor growth. The effect of 5-fluorouracil was reached at the combined 15% mortality. The inhibition with miracil D in ovariectomized rats was not significant, and there was a combined 40% mortality.

Progesterone produced larger tumors than the ovariectomized controls, but the increase was not significant. Testosterone propionate in the doses used (Group 17) showed no significant effect. Given over the later 21-day period (Group 18) there appeared to be a significant retardation of tumor growth. Further analysis showed, however, that this was an artifact occasioned by the preceding effect of ovariectomy. 5-Diazouracil did not affect tumor growth in ovariectomized rats.

Chart 3 shows that both estradiol benzoate and 5-fluorouracil exerted inhibitory activity during the 21- to 42-day period that was equal or better than observed during the first 21-day period. This is interpreted as evidence that these 2 agents affected tumors that are relatively non-hormone-dependent to a degree equal to or exceeding the effects on hormone-dependent tumors. This observation was not anticipated with estradiol benzoate.

As in the intact animals, progesterone enhanced tumor growth in ovariectomized rats during the earlier 21-day period, but not during the later period of administration.

Table 2 includes observations on rats that were tumor-free at gross autopsy at the end of the 42-day test. Two such animals were seen in each of 2 intact groups receiving estradiol benzoate or 5-fluorouracil during the test period. In the ovariectomy series, 23% had clinically complete regression of tumor. The 6 tumor-free rats on testosterone propionate (group 18) and 3 on miracil D (group 26) given during the 21- to 42-day period are attributed to ovariectomy rather than to the chemotherapy.

Table 3 presents the results of Experiment 2. Shorter courses, consisting of 5 injections over 9 days, were used of estradiol benzoate, AB-100, or mitomycin C. The tumor weights of the 2 control groups (27 and 35) exceeded those of Experiment 1 (Groups 1 and 14), but within variation expected by chance. In intact animals, food restriction sufficient to yield a 12% loss in body weight produced a reduction in tumor weight, but not sufficient to yield statistical significance ($P = 0.09$). With estradiol benzoate, the total tumor weight was reduced as compared with the untreated controls (Group 29 vs. 27) and with food-restricted intact animals (Group 30 vs. 28), but statistical significance was reached only in the former. AB-100 produced a significant increase in inhibition when supplemented with food restriction (Group 27 vs. 33), than with food restriction (Group 28 vs. 34).

The most interesting results were evident in the ovariectomy series. Food restriction resulting in a 14% weight loss (Group 36 vs. 35) produced a significant additional retardation in tumor growth.
CHART 2. Tumor volume at 21 and at 42 days of intact rats treated with progesterone (P), estradiol benzoate (EB), 5-fluorouracil (FU), and miracles D (MD). Numbers in parentheses refer to groups of Table 2.

CHART 3. Tumor volume at 21 and 42 days in ovariectomized rats treated with progesterone (P), 5-fluorouracil (FU), and estradiol benzoate (EB). Numbers in parentheses refer to groups of Table 2.

growth. All 3 chemotherapeutic agents also produced tumor-weight values below those achieved by ovariectomy alone, but none exceeded the results obtained by ovariectomy and food restriction.

TABLE 3

EXPERIMENT 2

<table>
<thead>
<tr>
<th>GROUP No.</th>
<th>DRUG USED</th>
<th>NO. OF RATS SURVIVING AT DAY 42/ DAY 1</th>
<th>MEAN BODY WT. CHANGE (%) BY 42 DAYS</th>
<th>TUMOR WT. AT 42 DAYS</th>
<th>NO. OF RATS GROSSLY TUMOR-FREE AT DAY 42</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>Untreated control</td>
<td>12/12</td>
<td>12.5</td>
<td>4.02</td>
<td>0</td>
</tr>
<tr>
<td>28</td>
<td>Untreated, diet restricted</td>
<td>12/12</td>
<td>-12</td>
<td>1.65</td>
<td>1</td>
</tr>
<tr>
<td>29</td>
<td>Estradiol benzoate</td>
<td>12/12</td>
<td>+6</td>
<td>2.27</td>
<td>2</td>
</tr>
<tr>
<td>30</td>
<td>Estradiol benzoate, diet restricted</td>
<td>11/11</td>
<td>-14</td>
<td>0.69</td>
<td>3</td>
</tr>
<tr>
<td>31</td>
<td>AB-100</td>
<td>12/12</td>
<td>+9</td>
<td>1.82</td>
<td>0</td>
</tr>
<tr>
<td>32</td>
<td>AB-100, diet restricted</td>
<td>12/12</td>
<td>-14</td>
<td>0.72</td>
<td>3</td>
</tr>
<tr>
<td>33</td>
<td>Mitomycin C</td>
<td>10/12</td>
<td>-6</td>
<td>0.25</td>
<td>2</td>
</tr>
<tr>
<td>34</td>
<td>Mitomycin C, diet restricted</td>
<td>12/12</td>
<td>-13</td>
<td>1.19</td>
<td>3</td>
</tr>
<tr>
<td>35</td>
<td>Ovariectomized control</td>
<td>12/12</td>
<td>+20</td>
<td>1.94</td>
<td>3</td>
</tr>
<tr>
<td>36</td>
<td>Ovariectomized, diet restricted</td>
<td>12/12</td>
<td>-14</td>
<td>0.25</td>
<td>7</td>
</tr>
<tr>
<td>37</td>
<td>Estradiol benzoate, ovariectomy</td>
<td>12/12</td>
<td>-3</td>
<td>0.69</td>
<td>3</td>
</tr>
<tr>
<td>38</td>
<td>Estradiol benzoate, ovariectomy, diet restricted</td>
<td>12/12</td>
<td>-19</td>
<td>0.46</td>
<td>4</td>
</tr>
<tr>
<td>39</td>
<td>AB-100, ovariectomy</td>
<td>12/12</td>
<td>+13</td>
<td>0.85</td>
<td>5</td>
</tr>
<tr>
<td>40</td>
<td>AB-100, ovariectomy, diet restricted</td>
<td>11/12</td>
<td>-15</td>
<td>0.71</td>
<td>6</td>
</tr>
<tr>
<td>41</td>
<td>Mitomycin C, ovariectomy</td>
<td>12/12</td>
<td>-5</td>
<td>1.21</td>
<td>3</td>
</tr>
<tr>
<td>42</td>
<td>Mitomycin C, ovariectomy, diet restricted</td>
<td>12/12</td>
<td>-12</td>
<td>0.85</td>
<td>3</td>
</tr>
</tbody>
</table>
The proportion of rats clinically free of tumors by 42 days was increased in all groups on food restriction. In the intact control animals of both experiments, no complete regressions were seen in 41 rats. Of the rats receiving estradiol, AB-100 or mitomycin C, 4 complete regressions occurred in 36 rats, whereas with food restriction, there were 9 such animals. In the ovariectomized series, 10 of 42 rats, or 24%; were tumor-free at 42 days. With ovariectomy and food restriction, the proportion was 7 of 12, or 58%; this difference is significant. Among the rats receiving chemotherapy, however, the difference is minimal: 11 of 36 versus 13 of 35.

Discussion

The patterns of response to the 8 chemotherapeutic agents of this study, and their relationship to ovariectomy and diet restriction, do not allow any single interpretation. Thus, in Experiment 1 estradiol benzoate and 5-fluorouracil exerted most of the inhibitory activity in intact animals during the earlier half of treatment, whereas the effect of miracil D was evident primarily during the latter half of 42 days. Treatment of ovariectomized rats, on the other hand, seemed as effective with estradiol benzoate or 5-fluorouracil during the 2nd half of the 42-day period. The difference in the size of the tumors between the 2 series may be a factor, but does not appear to explain adequately the difference in the response pattern. Progesterone enhanced the growth of the tumors in both intact and ovariectomized series during the earlier half of the observations and not during the latter half, although the weights of the tumors involved in both the control and the progesterone groups differed by a factor of 2 or more.

Teller et al. (11) reported on the chemotherapy of mammary tumors induced in Sprague-Dawley female rats given single intragastric doses of 15–30 mg of DMBA. Sixteen androgens and 10 nonhormonal agents were used. When compared with untreated controls, at least 6 androgens, given at doses of 40 mg/kg, were active, especially 5α-androstan-3α,17β-diol dipropionate (NSC 23758), 2α-methylhydroxyprogesterone (NSC 26198), and its dipropionate (NSC 12198). 5-Fluorouracil was inactive in 5 rats. Ovariectomy produced regressions of tumors that were in the range of the effects of androgens.

In our investigations, we used MCA-induced mammary tumors in female Wistar rats. Testosterone propionate (NSC 9166) in doses of up to 25 mg/kg showed no significant effect on established tumors (2), although there was inhibition of appearance of additional tumors (5). In the MCA-Wistar system, 5-fluorouracil (NSC 19893) is definitely active (2).

These comparisons, of course, are indirect and involve differences in dose-schedules. The data, nevertheless, suggest that the 2 bioassay systems may be dissimilar, either on the basis of the animal strains or of the carcinogen and the dose schedules. In a comparative study on carcinogenesis we (3) found that the growth rate of mammary tumors induced by a single DMBA administration was slower than that of tumors appearing after multiple MCA instillations. In addition, there were definite differences in the effects of DMBA and of MCA on the adrenal gland, and, therefore, on the hormonal status of the host. A direct comparison of the response of MCA- and DMBA-induced tumors to several chemotherapeutic agents appears desirable, and is being performed. It is clear that the population of these induced tumors is quite mixed, even in the same animal. Some tumors grow progressively, others remain stationary and still others regress with or without hormonal manipulations. Differences in tumor response in different strains and induction procedures may allow better selection of bioassay systems. For example, it may be found that DMBA-induced tumors are more androgen-sensitive, and MCA-induced tumors may be more estrogen-sensitive.

From a practical standpoint, it is of interest that the MCA-induced mammary tumors of rats are inhibited by an estrogenic compound, and that this effect is evident on tumors that continue to grow after ovariectomy and are thus presumably hormone-independent. The tumors were not inhibited by testosterone propionate given in doses of 2.5 mg/kg. Tumor growth was enhanced by progesterone. Previous experiments (4) show that addition of a small dose of estrogenic material to a progestational compound (Enovid) abolishes this enhancement.

We concluded previously (1, 2, 5) that among the agents tested by us, none exceeded the effect of ovariectomy in inhibiting the growth of these tumors. With more prolonged courses of treatment used in Experiment 1, miracil D and, perhaps, 5-fluorouracil may be shown to be more effective, but at the price of a significant mortality. Under the conditions of the 1st experiment, ovariectomy plus estradiol benzoate achieved the best results and without mortality.

Experiment 2 is of particular interest, in that it demonstrated again that the MCA-induced mammary tumors of Wistar rats are nutrition-dependent as well as hormone-dependent, and that these characteristics can be exploited toward therapeutic aims. The best results in inhibiting tumor growth were achieved by ovariectomy and food restriction sufficient to cause a 14% body weight loss. Such body weight loss produced no adverse effects on the rats; they were lighter but healthy at the termination of the experiment, and 7 of 12 were grossly free of tumors. Mitomycin C and miracil D produced similar effects, but with significant loss of weight and mortality.

On the basis of these investigations are derived 2 possible clinical applications worthy of consideration. The first is the treatment of premenopausal women with recurrent breast cancer by a combination of ovariectomy and weight reduction. The second is the trial of estradiol benzoate in addition to ovariectomy and weight reduction. These procedures also may be considered in further combination with more effective chemotherapeutic agents that hopefully will become available in the future.

Acknowledgments

We are indebted to Mrs. Margot Gruenstein and Mrs. Dolores Thatcher for assistance, and to the following technical staff for their efforts: Louise Jackson, Joan Krassenstein, Sheila Horton, and Sheila Lessick.

Addendum

Explanation of codes; source of compounds

"P" following a source code number indicates that the compound was purchased by the Cancer Chemotherapy National Service Center.
References


10. Shimkin, M. B., Gruenstein, M., Thatcher, D., and Acuff, M. Descriptive Summary on Breast Tumors and Leukemia Induced in Female Wistar Rats Receiving Gastric Instillations of 3-Methylcholanthrene. NCI Monograph, in press.

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