Lack of Effect of Norethynodrel (Enovid) on Methylcholanthrene-induced Mammary Carcinogenesis in Female Rats*

M. GRUENSTEIN, H. SHAY,† AND M. B. SHIMKIN
(Fels Research Institute, Temple University School of Medicine, Philadelphia, Pennsylvania)

SUMMARY
Norethynodrel with mestranol (Enovid) fed to female Wistar rats, 3 mg. 6 times per week for up to 50 weeks, produced no neoplasms of the breast or other tissues and did not enhance or retard mammary carcinogenesis evoked by gastric instillations of 3-methylcholanthrene.

Oral progestogens for contraception (8) have become a feature of our culture, exposing a significant proportion of the female population to exogenous hormonal agents for protracted periods. Despite their importance, there is a paucity of published data on the effects of these agents on carcinogenesis in laboratory animals.

For this reason, we are recording the results of an investigation of Enovid® in female Wistar rats. The carcinogenic stimulus was 3-methylcholanthrene, given by gastric instillation, which produces mammary adenocarcinomas in these animals (1).

MATERIALS AND METHODS
Female Wistar rats, 5-6 weeks of age and weighing 60-80 gm., were used. The animals were maintained individually in metal cages, on a Rockland mouse/rat diet and an unlimited supply of water.

Enovid was obtained as a powder without a binder. The powder was dissolved in sesame oil with the aid of heating to 100° C. at a concentration of 3 mg/0.5 cc. The carcinogen, 3-methylcholanthrene, was dissolved in olive oil, 2-5 mg/0.5 cc. These solutions were given to the animals via a rubber tube introduced into the stomach. Methylcholanthrene was given in the mornings and Enovid in the afternoons, 4-5 hours later, daily except Sundays. The animals were observed for the appearance of tumors and were weighed once a week.

Three experiments were performed:

Experiment 1.—A group of 21 rats were given gastric instillations of 3 mg. of Enovid for up to 50 weeks, 6 times per week, for a total maximum dose of 900 mg. At 40 weeks half the animals were sacrificed for the purpose of obtaining material for histological studies. A concurrent group of 54 rats, used as controls for another experiment (6), was watched as untreated comparands.

Experiment 2.—Female Wistar rats received daily gastric instillations of 2 or 5 mg. of methylcholanthrene. In each of these two dose levels half the animals also received daily gastric instillations of 3 mg. of Enovid. Subsequent analysis of mammary cancer occurrence and the body weight increment showed similar responses, so that data on both doses of carcinogen were combined.

Experiment 3.—Rats were given 5 mg. of methylcholanthrene for 9 weeks, for a total dose of 270 mg. The animals were then divided into two groups, one of which received 3 mg. of Enovid 6 times a week for the subsequent 43 weeks, for a total dose of up to 738 mg.

For comparative purposes our data on the effects of progesterone and estradiol on the methylcholanthrene-induced mammary carcinoma in female Wistar rats are also reported here. Seven groups of rats, under experimental conditions similar to those in the Enovid series, were treated as follows:

The first group received no carcinogen and received subcutaneous injections of 4 mg. progesterone, 6 times per week, for 4 months.

The second and third groups received ten daily gastric instillations of 5 mg. methylcholanthrene. The third group, starting with the 1st day, also was given daily subcutaneous injections of 4 mg. progesterone. The injections were given 6 times per week and continued for the duration of the experiment—52 weeks.

The fourth and fifth groups were given gastric instillations of 2 mg. methylcholanthrene for 4 months, 6 times per week, for a total dose 204-214 mg. The fifth group received injections subcutaneously, starting with the 1st day, of 4 mg. progesterone, 6 times per week, for 4 months.

The sixth and seventh groups were given gastric instillations of 2 mg. of methylcholanthrene 6 times per week during the year's course of the experiment. The seventh group also received subcutaneous injections of 4 mg. progesterone, 6 times per week, during the course of the experiment; and on the first day it was also given a solid pellet of 25 mg. of estradiol, implanted subcutaneously.
RESULTS

The results of the three Enovid experiments are given in Table 1. No mammary carcinomas or other tumors appeared in the rats of Experiment 1, receiving Enovid alone, although half the animals were sacrificed at 40 weeks and the rest at 50 weeks. Mortality did not appear to be increased in the Enovid-treated group, but there was a sustained decrease in weight increment of approximately 20 per cent, as compared with that in the controls.

In the Enovid-treated animals the most consistent finding at autopsy was a decrease in the weight of the ovaries, expressed either as absolute weights or as related to body weight. Histologically, the prominent feature at 40 and at 50 weeks was the absence of corpora lutea. There was no notable hyperplasia of the mammary glands or of the endometrium. In general, the effects were consistent with those described by Holmes and Mandi (2).

In rats of Experiment 2, that had received daily gastric instillations of 2 or 5 mg. of methylcholanthrene, there was no increase in the proportion or total number of mammary tumors when Enovid was administered concurrently. Histologic examination of the tumors verified that these were mammary adenocarcinomas, as described previously (7). There was again a significant depression in the weight gain in animals on Enovid, as compared with that of the controls. Since dietary restriction does depress mammary carcinogenesis in the rat (5), the quantitative effect of this factor would have to be resolved by matched-feeding experiments. It is clear, however, that Enovid had no significant depressive effect on mammary carcinogenesis evoked by methylcholanthrene, at least at the dose levels of the hydrocarbon that were used.

In Experiment 3, Enovid was introduced after the completion of the carcinogenic stimulus of methylcholanthrene. No effect was evident upon the carcinogenic response, as measured by the proportion or the number of breast cancers. In these groups the body weights were not significantly different, so that this factor is not present to cloud the issue.

Data on the effects of progesterone and estradiol are presented in Table 2. Progesterone, given daily in doses of 4 mg, for 4 months produced no tumors. At the 50-mg. dose of methylcholanthrene, the addition of

| TABLE 1 |
| LACK OF EFFECT OF NORETHYNODREL (ENOVID) ON MAMMARY CARCINOGENESIS IN FEMALE WISTAR RATS |

<table>
<thead>
<tr>
<th>EXPERIMENT</th>
<th>TREATMENT</th>
<th>DAYS</th>
<th>RATE WITH MAMMARY CANCER</th>
<th>TOTAL MAMMARY CANCERS</th>
<th>OTHER TUMORS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Untreated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. rats alive</td>
<td>54</td>
<td>54</td>
<td>54</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>No. rats with mammary cancer</td>
<td>75</td>
<td>186</td>
<td>210</td>
<td>222</td>
</tr>
<tr>
<td></td>
<td>Mean body weight (gm.)</td>
<td>21</td>
<td>21</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Enovid (3 mg. 6 x/wk for 50 wk)</td>
<td>73</td>
<td>156</td>
<td>172</td>
<td>182</td>
</tr>
<tr>
<td>2</td>
<td>3-Methylcholanthrene (2-5 mg., 6 x/wk for 52 wk)</td>
<td>53</td>
<td>53</td>
<td>48</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>No Enovid</td>
<td>72</td>
<td>161</td>
<td>189</td>
<td>201</td>
</tr>
<tr>
<td></td>
<td>No. rats alive</td>
<td>47</td>
<td>46</td>
<td>43</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>No. rats with mammary cancer</td>
<td>75</td>
<td>138</td>
<td>155</td>
<td>161</td>
</tr>
<tr>
<td></td>
<td>Mean body weight (gm.)</td>
<td>30</td>
<td>30</td>
<td>26</td>
<td>21</td>
</tr>
<tr>
<td>3</td>
<td>3-Methylcholanthrene (5 mg. for 9 wk; 270 mg.)</td>
<td>69</td>
<td>165</td>
<td>184</td>
<td>203</td>
</tr>
<tr>
<td></td>
<td>No Enovid</td>
<td>24</td>
<td>24</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>No. rats alive</td>
<td>72</td>
<td>168</td>
<td>189</td>
<td>202</td>
</tr>
<tr>
<td></td>
<td>No. rats with mammary cancer</td>
<td>1</td>
<td>1</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Mean body weight (gm.)</td>
<td>1</td>
<td>1</td>
<td>11</td>
<td>4</td>
</tr>
</tbody>
</table>

* Nine sacrificed for histology studies.
† Lymphoma.
‡ Fibroadenoma of mammary gland.
progesterone did not increase the proportion of animals that developed mammary tumors, but the mean appearance time of the tumors may have been shortened. At the 204- to 214-mg. dose of methylcholanthrene, the addition of progesterone did not yield significant effects. The combination of progesterone and estradiol sharply inhibited the occurrence of mammary cancer in the animals. The high dose of estradiol produced a significant decrease in weight increment, so that at least part of the cancer-inhibiting effect may be attributable to this factor until match-feeding data become available.

DISCUSSION

Under the conditions of the experiment, norethynodrel (Enovid) did not induce neoplasms in female Wistar rats and produced no effect upon mammary carcinogenesis initiated by gastric instillations of 3-methylcholanthrene.

Huggins, Moon, and Morii (3) recorded that mammary cancers were induced in all Sprague-Dawley female rats following a single feeding of 20 mg. of 7,12-dimethylbenzanthracene. Pregnancy or injections of progesterone accelerated the growth of the tumors. Treatment with 30 daily doses of 4 mg. of progesterone and 20 µg. of estradiol, starting 15 days after the carcinogen, prevented the appearance of mammary cancer in 52 of 100 animals. Such inhibition was not observed with estradiol or with progesterone alone.

Our data in regard to the effects of progesterone and estradiol are similar to those of Huggins et al. (3), although the higher dose of estrogen introduces the problem of decreased body weight increment. A previously published summary (4) of the effect of estradiol on methylcholanthrene-induced tumors in Wistar rats showed that tumor occurrence was reduced by approximately 50 per cent but that the mean appearance time was not changed. The present results suggest that progesterone may enhance this inhibition.

The appearance and growth of methylcholanthrene-induced mammary cancers in Wistar rats are markedly influenced by certain hormonal alterations, especially ovariectomy (6). In view of the results of Huggins et al. (3), we would have anticipated a reduction in mammary carcinogenesis by Enovid, if norethynodrel is similar to progesterone and mestranol to estradiol, given in daily doses of approximately 3 mg. and 45 µg., respectively. It is obvious that the conditions of experiments, as well as the dose levels and the specific steroids, were not the same and produced different results.

The above discussion emphasizes the narrow limitations that must be imposed on the interpretation of these and similar data. Specifically, they must not be extrapolated to man. Such knowledge must be based on direct epidemiologic studies of long duration. Nevertheless, findings in various laboratory animal models provide additional information that may be relevant and, we feel, should be available in the literature.

REFERENCES


Lack of Effect of Norethynodrel (Enovid) on Methylcholanthrene-induced Mammary Carcinogenesis in Female Rats

M. Gruenstein, H. Shay and M. B. Shimkin

Cancer Res 1964;24:1656-1658.

Updated version Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/24/9/1656

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.