Estriol Prevention of Mammary Carcinoma Induced by 7,12-Dimethylbenzanthracene and Procarbazine¹

Henry M. Lemon

Section of Oncology, Department of Internal Medicine, The University of Nebraska Medical Center, 42nd Street and Dewey Avenue, Omaha, Nebraska 68105

SUMMARY

The concentration of estrogenic, androgenic, progestational, and adrenocortical steroid hormones in body fluids of mature intact Sprague-Dawley female rats was increased by s.c. implantation of 5 to 7 mg NaCl pellets containing 1 to 20% steroid 48 hr before administration p.o. of either 7,12(dimethylbenz(a)anthracene or procarbazine. The incidence of rats developing one or more mammary carcinomas in each treated group was compared to that observed in simultaneously treated groups receiving only the carcinogen, steroid, or no treatment whatsoever, with weekly observation of all rats until palpably growing tumors were biopsied and proven carcinomatous or until death occurred from other causes determined by autopsy. A total of 105 untreated or steroid-implanted rats followed to death (234 to 256 days median observation) developed no breast carcinomas. Rats fed either of the carcinogens developed initial evidence of breast carcinoma, after 136 to 156 days median observation, in 51 to 57% of 318 total treated rats. Nonbreast carcinomas and sarcomas developed in 5 to 10% of the carcinogen-treated rats.

Estriol administered as 0.15 to 0.60 mg/pellet reimplanted every 2 months reduced breast carcinoma incidence to as low as 5 to 7% of 106 rats given dimethylbenzanthracene or procarbazine (p < 0.001) during a median observation period of 202 to 232 days. The incidence of nonbreast neoplasms was not significantly altered. Breast tumor incidence noted following implantation of 0.50 to 1.14 mg estrone and 17β-estradiol in 80 dimethylbenzanthracene-treated rats was reduced to 22 to 25%, compared to 45 to 47% in carcinogen controls; more significant breast cancer reduction was observed by estrone treatment, in thracene-treated rats was reduced to 22 to 25%, compared to prosca

INTRODUCTION

Multiple genetic and endocrine factors contribute to increased risk of human breast carcinogenesis, such as female sex, Caucasian racial background, breast carcinoma in premenopausal 1st-degree relatives, and duration of ovarian function; pregnancy, marked by a 1000-fold increase in estriol production, is notably protective (26, 34). Controversy exists as to the etiological significance of reduced estriol excretion noted in 20% of nonpregnant healthy Caucasians compared to 60% of patients with precancerous and malignant breast disease that we have reported (27, 32). Premenopausal Asiatic women with one-sixth the breast carcinoma risk of Caucasians commonly excrete a higher proportion of estriol relative to estrone and estradiol in their urine (28, 34). Wide variation in estriol excretion by healthy Caucasian premenopausal women may reflect several phenotypic mutant variants for the endogenous production of estriol (28), which appear susceptible to genotypic quantitation by leukocyte incubation with tritiated precursors of estriol in vitro (29).
The present investigation was planned to explore the possible carcinogenic or anticarcinogenic function not only of estriol but also of other metabolites of estradiol hydroxylated at C-2, C-6, C-16 and of nonestrogenic steroids. Some estrogen metabolites resemble estriol in altering physiological response to estradiol and are classified among "impeded" estrogens (16, 27, 51). Commencing in 1967, we adopted the model system of rat mammary carcinogenesis developed by Dao (5) and Huggins et al. (14, 15) to our needs, by superimposing upon the normal ovarian estradiol secretory activity (44), required for DMBA induction of mammary carcinogenesis, periodic s.c. implantation of 0.06- to 1.3-mg doses of the test steroid (Table 1). Mammary carcinogenesis induced in the female Sprague-Dawley rat by this agent has many similarities to human mammary carcinogenesis, especially relating to the near identity of estrogen receptor proteins and the response of these tumors to various types of endocrine therapy (Table 2). Furthermore, the rat offered an acceptable model for determining the potential chronic toxicity and tolerance of various doses of steroid metabolites, which might have significant inhibitory effect upon mammary carcinogenesis, and also be suitable for human clinical trials. PC was later substituted for DMBA as the carcinogenic agent to determine whether the inhibition of breast carcinogenesis by estriol was related to the chemical structure or possible endocrine activity of either carcinogen (13).

MATERIALS AND METHODS

Rats and Their Maintenance. Throughout the study, intact female Sprague-Dawley rats were obtained from a single source (Sasco, Inc., Omaha, Nebr.) at 50 to 55 days of age. They were randomized upon receipt and housed individually in suspended cages. They were fed Wayne Lab Blox (South Omaha Terminal Co., Omaha, Nebr.) and municipal water, with temperature maintained within 22–23° and 40 to 50% relative humidity. Prophylactic hormone therapy was achieved by implanting s.c. in the posterior nuchal region 5- to 7-mg pellets once every 1 or 2 months under light ether anesthesia. The 1st pellet implantation was 48 hr before administration by gavage of the carcinogen, again under light ether anesthesia. The rats were all examined for tumors and weighed every 1 to 2 weeks. After 1 or more tumors were identified in the mammary areas and their size(s) were measured, they were reexamined after 1 to 2 weeks to confirm the continued growth of the tumor(s). Firm enlarging masses were biopsied and the animals were sacrificed upon pathological verification of mammary carcinomas, as defined by Murad and von Haam (43). Animals with nonbreast tumors were observed until near death, when they were sacrificed and necropsied. Non-tumor-bearing rats were reimplemented with steroids according to protocol, until death, when they were necropsied. At this time, gross examination was conducted of the dissected axillary and inguinal mammary pads, along with gross examination of the heart, lungs, and abdomen. In sacrificed animals, the uterus was dissected free and its weight was recovered as an index of estrogenic response.

Steroid Incorporation into Pellets. The pellets were constructed in a Forbes pellet press (9), from 1 to 20% mixtures of the authentic crystalline steroid with crystalline NaCl. They were prepared a few days before use, weighed, and kept in a dessicator until use. The dynamics of steroid release from such pellets has been described (10) and, in the case of 100% estrogen pellets, monthly for the 1% pellets. The percentage composition of nonestrogenic steroids or synthetic nonsteroidal estrogens in pellets was adjusted to provide equimolar activity compared to estriol. Steroids were obtained from commercial sources. Estrogens were screened for identity and purity by thin-layer silica gel chromatography using 5% ethanol, 47.5% ethyl acetate, 47.5% cyclohexane liquid phase, and UV spotting to measure Rf values.

Carcinogens. DMBA was obtained from Eastman Kodak Co., Rochester, N. Y., and PC was from the National Cancer Institute, Bethesda, Md. Both were administered by flexible gavage tubes under light ether anesthesia. DMBA was given in a 20-mg dose in 1.0 ml sesame oil for most of the experiments, producing a 10 to 40% mortality from adrenocortical necrosis (17). Increasing the sesame oil vehicle to 2.0 ml without changing the DMBA dose abolished any mortality consequent upon carcinogen administration within the 1st 2 to 3 weeks of observation and this volume was adopted. PC was kept in crystalline form until just before use at 4°, when it was made up to 50–70-mg/ml individual rat doses in distilled H2O, prepared in 5.0-ml batches immediately before administration. No mortality resulted from PC administration at any time, but...
### Table 2

**Comparison of induced breast carcinomas in rodents with human mammary carcinoma**

<table>
<thead>
<tr>
<th>Rat</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex incidence</strong></td>
<td>Rare in males (1, 4, 5, 7, 8)</td>
</tr>
<tr>
<td><strong>Effect of prior castration on carcinogenesis</strong></td>
<td>Reduced tumor incidence by 80-90% (5, 7, 8, 14, 19)</td>
</tr>
<tr>
<td><strong>Estrogen replacement therapy in castrate</strong></td>
<td>Restored carcinogenic activity in young ovariectomized rats (7, 33)</td>
</tr>
<tr>
<td><strong>Pregnancy</strong></td>
<td>Pregnancy soon after DMBA reduces risk (7, 15)</td>
</tr>
<tr>
<td><strong>Role of pituitary in carcinogenesis</strong></td>
<td>Required (5, 7, 40, 42, 45, 50)</td>
</tr>
<tr>
<td><strong>Evidence of viral cocarcinogenesis</strong></td>
<td>Not proven</td>
</tr>
<tr>
<td><strong>Tumor histogenesis</strong></td>
<td>Origin from both myoepithelial cells and duct epithelium (43)</td>
</tr>
<tr>
<td><strong>Pathology</strong></td>
<td>Adenoid cystic carcinoma, adenocarcinoma, medullary, papillary, follicular, and myxoid carcinoma; rare in situ ductular carcinoma resembling human (43)</td>
</tr>
<tr>
<td><strong>Multiple primary tumors</strong></td>
<td>In 40-80% (4, 7, 43)</td>
</tr>
<tr>
<td><strong>Metastasis</strong></td>
<td>Common (1, 4, 5, 14, 15, 43)</td>
</tr>
<tr>
<td><strong>Spontaneous regression</strong></td>
<td>Rare but documented</td>
</tr>
<tr>
<td><strong>Estrophile proteins</strong></td>
<td>In 50-70% of tumors (21, 23, 38, 41) similar to those isolated in human (37, 48) bind to estriol as well as 17β-estradiol (30)</td>
</tr>
<tr>
<td><strong>Tumor response to castration or adrenalectomy</strong></td>
<td>Tumors with estrophile proteins regress (23, 40, 41)</td>
</tr>
<tr>
<td><strong>Hypophysectomy</strong></td>
<td>Induces regression (5, 7, 40, 45)</td>
</tr>
<tr>
<td><strong>Prolactin</strong></td>
<td>Restores tumor growth after castration-induced regression (39, 40, 44)</td>
</tr>
</tbody>
</table>

the interval required for mammary carcinogenesis was considerably longer than that required for DMBA activity.

**Experimental Design.** Initial experimental protocols included groups of 4 to 12 rats assigned as untreated controls, steroid-implanted controls, carcinogen-treated rats, and steroid-implanted rats followed by carcinogen feeding. As experience developed, and no breast carcinomas were noted in over 100 rats observed during their natural life-span in the 1st 2 control groups, these were no longer included in the routine design of experiments. Most of the later experiments involved comparison of 2 to 4 groups composed of 8 to 18 rats each, 1 of which was fed carcinogen, with the others given steroid pellets plus carcinogen.

The development of 1 (or more) breast carcinoma(s) per rat sufficed for our objectives to determine cancer incidence rats, so that these rates were sacrificed to save expense. The results are reported as the number of rats in each group developing 1 (or more) breast carcinoma(s) out of the total number treated and alive 30 days after carcinogen administration. The median duration in days of observation of all rats in each group was also determined; premature mortality from infectious disease was the major factor reducing tumor incidence in carcinogen-treated groups, owing to the 120- to 150-day median latent period of carcinogenesis. All breast carcinomas were diagnosed between the 40th and 370th day after administration of either carcinogen. The mean of mean weights of steroid in pellet implants every 1 to 2 months has also been included in the tables when appropriate, to indicate the actual dosage administered to each group of rats.

All histological sections of tumors were initially read without knowledge of the treatment received by the rat. They were again reviewed at the time of this report to confirm the pathological variants of breast carcinoma recognized according to the classification developed by Murad and von Haam (43). Fibroadenomas and lactational hyperplasia, which was usually grossly readily distinguishable from carcinoma, were not included among the malignancies. The interval required for mammary carcinogenesis was considerably longer than that required for DMBA activity. The median duration in days of observation of all rats in each group was also determined; premature mortality from infectious disease was the major factor reducing tumor incidence in carcinogen-treated groups, owing to the 120- to 150-day median latent period of carcinogenesis. All breast carcinomas were diagnosed between the 40th and 370th day after administration of either carcinogen. The mean of mean weights of steroid in pellet implants every 1 to 2 months has also been included in the tables when appropriate, to indicate the actual dosage administered to each group of rats.

All histological sections of tumors were initially read without knowledge of the treatment received by the rat. They were again reviewed at the time of this report to confirm the pathological variants of breast carcinoma recognized according to the classification developed by Murad and von Haam (43). Fibroadenomas and lactational hyperplasia, which was usually grossly readily distinguishable from carcinoma, were not included among the malignancies.
nant neoplasms. Between 5 and 10% of mammary nodules less than 5 mm in diameter failed to grow during several weeks of observation or regressed totally, a common finding in these tumors (Table 2). On the other hand, once a tumor had reached 5 to 10 mm in diameter, we seldom encountered regression except after castration, and it was at this size that most biopsies were made. Only pathologically verified carcinomas were included in the statistics. No unusual incidence of tumor regression was noted in most of the steroid-treated groups, suggesting that the end results were significantly affected by noninclusion of spontaneously regressed neoplasms.

Statistical analysis by $\chi^2$ evaluation based upon a 4-fold table of the number of rats with and without breast carcinoma in the carcinogen versus steroid-plus-carcinogen-treated group was used to determine significance of differences. Since cancer induction rates by these agents vary with time and each batch of animals, only those groups simultaneously receiving the 2 treatment protocols were included for analysis of significance of differences in incidence rates. The Yates' correction for small numbers was used throughout in $\chi^2$ analysis.

RESULTS

Estrogenic Steroids

17β-Estradiol. This primary ovarian estrogen (27) was tested in 5 experiments in 1 to 10% pellet concentration for its effect upon DMBA carcinogenesis (Table 3). No breast tumors appeared in untreated or steroid implanted controls observed for as long as 300 days median observation. The incidence of DMBA-induced mammary tumors was reduced by nearly 50% in rats implanted with 10% but not 1% pellets, and the median time of tumorigenesis was delayed by 34 days, compared to those receiving only the carcinogen. This difference in incidence, while suggestive, did not achieve statistical significance. In 20% pellets, 17β-estradiol did not reduce significantly breast carcinoma incidence in a single experiment, but it delayed onset of tumors by 147 days to a median of 221 days.

When 17β-estradiol in 10% pellets was implanted only a single time 2 days prior to DMBA administration, 7 of 10 recipients developed mammary carcinomas in a median period of 241 days (range of 65 to 297 days).

Estrone. Estrone equilibrates reversibly with 17β-estradiol in vivo, depending upon the oxidation-reduction potential and availability of pyridine nucleotide respiratory cofactors, and might be expected to have a somewhat similar degree of activity affecting carcinogenesis as estradiol (27). In 6 experiments with 1 to 10% pellet concentration, estrone closely resembled 17β-estradiol in its antitumorigenic effect upon DMBA induction of breast cancers, with a 50% reduction of their incidence compared to DMBA controls (Table 3). Highly significant inhibition of PC-induced mammary carcinogenesis was observed after 10% estrone pellets with minimal prolongation of median life-span of the implanted animals beyond that of untreated controls. No spontaneous tumor regressions were observed either in rats receiving estrone or 17β-estradiol in the DMBA- or PC-treated groups.

Estrone 3-Sulfate. This conjugate is the principal estrogen recovered from human plasma. Tested in 10% pellet concentration (equivalent to 7.4% estrone), it was not effective in preventing breast neoplasms (Table 3). In 14.1% pellet concentration (equivalent to 10.4% estrone), possible inhibition of breast tumor development after PC feeding was noted in a single experiment.

All 3 of these estrogenic steroids similar to other estrogens reduced the rate of body growth by 3 to 6.6% compared to that noted in the rats receiving only carcinogens (Table 4). No significant correlation existed between reduction in weight and inhibition of breast tumor formation by these and other estrogens. Neither of the 2 carcinogens used affected the rate of growth of the rats, in comparison with simultaneously observed untreated controls. No other systemic effects were noted from these small estrogen doses, with the longevity of the steroid-implanted controls equivalent to that observed among untreated rats (Table 3).

Estradiol. The 2 principal pathways for estradiol or estrone metabolism involve hydroxylation at C-16 to produce estriol or an epimer and at C-2 yielding 2-hydroxyestradiol, 2-hydroxyestrone, or 2-hydroxyestriol, which are methylated in the kidney and liver prior to excretion (22, 27). Estradiol in 10% pellet concentration reimplanted every 2 months proved to be the most inhibitory compound of any of the 17 tested in the entire series of experiments, reducing the development of palpable breast carcinoma to only 5 to 7% of the treated rats (Table 5). No inhibition of tumor formation followed 1% pellet implantation. Single implantation of 10% estriol pellets 2 days before DMBA administration in 2 experiments resulted in breast cancers in 20 out of 34 rats, within a median period of 83 to 111 days (range, 37 to 187 days), similar to the incidence of breast carcinomas observed in the DMBA-treated rats. Four multiple primary breast carcinomas occurred. Serial implantation of 10% estriol pellets every 2 months commencing 24 hr after DMBA administration has resulted in 4 of 11 rats with breast carcinomas after 270+ days in 1 experiment, with another rat with 3 primary breast carcinomas.

Estriol was most inhibitory against PC-induced mammary carcinoma development, with highly significant reduction of breast tumors after implantation of 2.5% pellets yielding only a 16% incidence of rats with cancer. At this dosage no inhibition of DMBA-induced tumors occurred.

The median survival of estriol-protected rats treated with either carcinogen was in all cases somewhat shorter than that noted in either untreated or steroid-treated controls. In all cases the survival of the protected rats considerably exceeded that noted in the carcinogen-treated rats, which was shortened by the earlier biopsy and sacrifice of the larger number of tumor-bearing animals.

Excluded from these incidence figures are 5 transient mammary tumor in the PC-treated rats and 8 transient tumors that totally regressed spontaneously within a few weeks after discovery in the estriol implanted-plus-carciogen-treated (5 after DMBA and 3 after PC) rats. Five of these 8 transient regressing tumors were noted in rats implanted with the lowest estriol doses of 1.0 to 2.5% per pellet.
Table 3  
Mammary carcinoma incidence after estrone and 17β-estradiol implantation

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Carcinogenic agent</th>
<th>No. of experiments</th>
<th>Untreated controls</th>
<th>Mammary carcinoma incidence after estrone and 17β-estradiol implantation</th>
<th>10% pellet only</th>
<th>χ²**</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>17β-Estradiol</td>
<td>DMBA, 20 mg</td>
<td>5</td>
<td>0/10,* 236 days</td>
<td>8/17 (47), 136 days</td>
<td>0/4, 300 days, 0.53 mg</td>
<td>1.6</td>
<td>NS</td>
</tr>
<tr>
<td>DMBA, 20 mg</td>
<td>1</td>
<td>5/11 (45), 74 days</td>
<td>4/12 (33), 221 days,* 1.14 mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrone</td>
<td>DMBA, 20 mg</td>
<td>7</td>
<td>0/13, 228 days</td>
<td>9/20 (45), 159 days</td>
<td>0/6, 207 days, 0.63 mg</td>
<td>2.2</td>
<td>NS</td>
</tr>
<tr>
<td>PC, 50 mg</td>
<td>2</td>
<td>10/15 (66), 137 days</td>
<td>1/14 (7), 147 days, 0.54, 0.66 mg</td>
<td></td>
<td></td>
<td>8.5</td>
<td>0.004</td>
</tr>
<tr>
<td>Estrone 3-sulfate</td>
<td>DMBA, 20 mg</td>
<td>2</td>
<td>4/5, 80 days</td>
<td>8/12 (67), 142 days, 0.65 mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC, 60 mg</td>
<td>1</td>
<td>4/7, 179 days</td>
<td>2/8, 135 days, 0.64 mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC, 50 mg</td>
<td>1</td>
<td>5/13 (38), 187 days</td>
<td>1/12 (8), 179 days, 0.88 mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total incidence</td>
<td>0/23</td>
<td>45/88 (51)</td>
<td>4/12 (33)</td>
<td>28/114 (26)</td>
<td>22/35 (63)</td>
<td>0/10</td>
<td></td>
</tr>
</tbody>
</table>

*a Incidence of tumors compared in carcinogen-treated to that in 10% steroid pellet-plus-carcinogen-treated using Yale's correction.

*b Number of rats with tumor/total number of rats treated.

c Median days of observation.

d Numbers in parentheses, percentage of rats with tumor.

e mg estrogen per pellet.

f NS, no significant difference in incidence between carcinogen-treated and estrogen-plus-carcinogen-treated.

g One survivor free of tumor after 310 days.

h 14.1% pellet concentration equivalent to 10.4% estrone.

Multiple breast carcinomas verified by biopsy were noted in 10 to 75% of DMBA-treated tumor-bearing animals, at the time of initial biopsy and sacrifice of the tumor-bearing rats. Twelve of the 48 rats fed only DMBA had 2 to 4 multiple primary breast adenocarcinomas in the estriol experiments (Table 5). No multiple primary breast adenocarcinomas were noted among rats protected by 10 to 20% estriol pellet implants prior to DMBA therapy, in the 4 developing breast tumors. Six multiple primary neoplasms with 2 to 6 breast tumors per rat were noted in the 26 tumor-bearing rats receiving 1.0 to 2.5% estriol pellets prior to DMBA feeding.

After PC feeding, 7 out of 29 tumor-bearing rats developed 2 to 3 primary breast cancers, and 1 spontaneous regression of a breast tumor was noted. Two rats with multiple primary breast carcinomas were observed in the 6 developing breast cancers after estriol pellet implantation plus PC treatment; both of these were noted in rats receiving 2.5% estriol pellets. Therefore, adequate circulating concentrations of estriol sustained by 10 to 20% pellet implantation every 2 months not only reduced the incidence of breast cancer-bearing rats by 90% following either of these carcinogens compared to the carcinogen-treated controls but also abolished the appearance of multiple palpable primary carcinomas that grew progressively.

16-Epiestriol. Following either DMBA or PC administration, only 18 to 19% of rats implanted with 0.6 mg 16-epiestriol developed breast cancers; however, the carcinogen-treated controls developed breast cancers in only 33 to 40%, requiring further tests to establish significance of
the difference in incidence rates (Table 5).

This steroid caused the least reduction of body growth, compared to that seen in recipients only of the carcinogen used, of any of the estrogenic compounds (Table 4). Neither 16-epiestriol nor estriol altered the incidence of rats with breast carcinomas, following monthly reimplantation of 1% pellets. If the stress of repeated anesthesia and pellet implantation in any way were involved in the tumor-inhibitory activities of these compounds, one would have expected some reduction in tumor incidence in these groups.

17α-Estradiol. This unnatural stereoisomer of 17β-estradiol may displace the latter, from intranuclear binding of the estrogen receptor complex to uterine DNA (2). In the 10% pellet concentration tested, breast carcinomas developed rapidly following DMBA administration in all recipients except 1 in the 3 experiments (Table 6). Multiple primary breast carcinomas were noted in several animals.

17α Epimers of Estriol. Neither of the unnatural stereoisomers of estriol with a 17α hydroxyl group inhibited the incidence of mammary carcinoma induced by DMBA (Table 6). These findings, along with the negative results observed with 17α-estradiol, suggest that the 17β-hydroxyl group that is required for uterine and mammary estrophile protein binding is essential for mammary carcinogenesis inhibition by estrogens. Five separate primary breast cancers developed in 1 rat receiving 16,17-epiestriol and a double primary in one of the carcinogen controls in this group of experiments.

16-Keto-17β-estradiol. This oxidation product of estradiol is one of the precursors of estriol and 16-epiestriol in human metabolism, but it was completely devoid of inhibitory activity against DMBA mammary carcinogenesis (Table 6). These results suggest that only a C-16 hydroxyl group will satisfy the steroid-protein structural requirements for optimum suppression of DMBA-induced mammary carcinogenesis, with the 16α position providing maximal inhibition.

2-Hydroxy-17β-estradiol and 2-Hydroxyestriol. Oxidation of the 2nd carbon atom on ring A of the estrogen nucleus modifies greatly the physiological activity of this representative of the 2nd major pathway of estradiol metabolism in man (27). Despite retention of some physiological activities classifying it among the "impeded" estrogens, 2-hydroxyestradiol was inactive in 10% pellet concentration in altering breast tumor incidence or in delaying tumorigenesis (Table 6). 2-Hydroxyestriol in similar dosage was also inactive.

D-Equilenin. This major equine urinary estrogen resembles estrone except for the presence of 2 unsaturated C=C bonds in ring B of the steroid nucleus. Either alone in 10% pellet concentration or in 21% concentration combined with its l isomer, it has not significantly decreased breast carcinoma incidence following DMBA administration (Table 6); further experiments in PC-treated rats will be needed to assess its possible inhibition of breast neoplasia. However, 6-dehydroestrone with a single unsaturated C=C bond in ring B exhibited no antimammary carcinogenic activity in PC-treated rats.

Hexestrol. This nonsteroidal estrogen binds with high affinity to rat uterine estrogen receptors and to some neoplastic human breast carcinomata in vivo (26, 27). In pellet concentrations equimolar to estriol, it has not significantly reduced DMBA-induced breast carcinoma incidence (Table 6). Multiple breast cancers appeared in 3 hexestrol-treated rats and in 1 DMBA-treated control; 1 spontaneous breast tumor regression was noted in the latter group.

Nonestrogenic Steroids. None of the nonestrogenic steroids tested thus far in 10% pellet concentrations have
shown any promise of altering experimentally induced breast cancer incidence in our rats (Table 7). None of these significantly altered body weight from that observed in carcinogen-treated controls. In 20% concentration, testosterone pellets reduced tumor incidence in a single experiment and increased mean body weight by 8% at 2 months. Further experiments in progress with 20% pellets of testosterone and progesterone have not reduced breast cancer incidence.

Effect of Castration upon Breast Carcinogenesis Modified by Estrogen Pellet Implantation. Castration prior to DMBA administration reduced breast tumor incidence markedly in Sprague-Dawley females (Tables 2 and 8). The reduced incidence of breast carcinomas was not significantly altered by 10 to 20% estril pellet implantation prior to DMBA administration in castrate females. Simultaneous implantation of 10% estrone or 17β-estradiol pellets, along with 20% estril pellets in castrate females, did not significantly change the low incidence of tumors observed in these experiments (Table 8). The interval of 1 to 3 days from castration to pellet implantation was probably too brief entirely to remove ovarian influence upon breast carcinogenesis. Further exploration of ratio of the steroids used is needed to ascertain the dosage required to reproduce an ovarian type of hormonal sensitivity of the breast to this carcinogen and to establish whether estriol is equally inhibitory under these circumstances.

Incidence of Nonbreast Carcinomas and Sarcomas. Both DMBA and PC-induced 5 to 10% incidence of sarcomas and nonbreast carcinomas, especially epidermoid carcinomas of the ear duct (1, 4, 5, 13) (Table 9). No significant alteration in the incidence of these tumors occurred following estrogenic steroid implantation prior to DMBA treatment, regardless of the inhibitory activity that any of these steroids possessed for mammary tumorigenesis. Nonestrogenic steroids tested thus far also did not significantly affect nonbreast tumor incidence.

Following the use of breast tumor-inhibitory doses of estril in PC-treated females, nonbreast neoplasms occurred more frequently (16.7%). These were similar in type

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Carcinogenic agent</th>
<th>No. of experiments</th>
<th>Untreated controls</th>
<th>Carcinogen-treated</th>
<th>20% pellet + carcinogen</th>
<th>10% pellet + carcinogen</th>
<th>2.5% pellet + carcinogen</th>
<th>1% pellet only</th>
<th>10-20% pellet only</th>
<th>χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estriol</td>
<td>DMBA, 20 mg</td>
<td>13</td>
<td>0/23.6 254 days</td>
<td>29/48 (60), 155 days</td>
<td>2/16 (13), 237 days, 1.30 mg</td>
<td>2/27 (7), 202 days, 0.62 mg</td>
<td>6/7, 75 days, 0.16 mg</td>
<td>20/36 (56), 114 days, 0.07 mg</td>
<td>0/25, 249 days, 17.9 &lt; 0.001</td>
<td>9.2 &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>PC, 50–70 mg</td>
<td>5</td>
<td>18/29 (62), 156 days</td>
<td>2/41 (5), 232 days, 0.63 mg</td>
<td>5/31 (16), 224 days, 0.16 mg</td>
<td>24.5 &lt; 0.001</td>
<td>11.5 &lt; 0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16-Epistriol</td>
<td>DMBA, 20 mg</td>
<td>3</td>
<td>4/10 (40), 123 days</td>
<td>4/21 (19), 101 days, 0.60 mg</td>
<td>6/7, 68 days, 0.06 mg</td>
<td>0/4, 205 days, 0.64 mg</td>
<td>0.6 NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PC, 60 mg</td>
<td>2</td>
<td>7/21 (33), 213 days</td>
<td>4/22 (18), 263 days, 0.59, 0.63 mg</td>
<td>0.6 NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total breast tumor incidence</td>
<td>0/23</td>
<td>58/108 (54)</td>
<td>2/16 (13)</td>
<td>12/111 (11)</td>
<td>11/38 (29)</td>
<td>26/43 (61)</td>
<td>0/29</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Number of rats with tumors/total number treated.
* Yate's correction used for all X² determinations for all tables.
* Median days of observation.
* Numbers in parentheses, percentage of rats with tumors.
* mg estrogen per pellet.
* X² comparing incidence of tumors in carcinogen-treated to that in 20% steroid pellet-plus-carcinogen-treated.
* X² comparing incidence of tumors in carcinogen-treated to that in 10% steroid pellet-plus-carcinogen-treated.
* X² comparing incidence of tumors in carcinogen-treated to that in 2.5% steroid pellet-plus-carcinogen-treated.
Table 6
Mammary carcinoma incidence after implantation of 9 other estrogens

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Carcinogenic agent</th>
<th>No. of experiments</th>
<th>Un- treated controls</th>
<th>Carcinogen- treated</th>
<th>10% pellet + carcinogen</th>
<th>1% pellet + carcinogen</th>
<th>10% pellet only</th>
<th>$\chi^2$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>17α-Estradiol</td>
<td>DMBA, 20 mg</td>
<td>3</td>
<td>0/1*</td>
<td>6/7, 99 days</td>
<td>18/19 (94), 116 days, 0.59 mg</td>
<td>0/5, 200 days, 0.67 mg</td>
<td>NS*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PC, 60 mg</td>
<td>1</td>
<td>2/2, 243 days</td>
<td></td>
<td>4/10, 149 days, 0.62 mg</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16,17-Epiestriol</td>
<td>DMBA, 20 mg</td>
<td>1</td>
<td>5/10, 82 days</td>
<td></td>
<td>8/14 (57), 107 days, 0.59 mg</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-Epiestriol</td>
<td>DMBA, 20 mg</td>
<td>1</td>
<td>8/12 (67), 110 days</td>
<td></td>
<td>6/12* (50), 193 days, 0.52 mg</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16-Keto-17β-estriol</td>
<td>DMBA, 20 mg</td>
<td>3</td>
<td>0/2</td>
<td>7/14 (50), 130 days</td>
<td>12/19 (63), 126 days, 0.59 mg</td>
<td>0/2, 135 days, 0.56 mg</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Hydroxy-17β-estriol</td>
<td>DMBA, 20 mg</td>
<td>3</td>
<td>4/7, 161 days</td>
<td></td>
<td>13/18 (72), 99 days, 0.60 mg</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d-Equilenin</td>
<td>DMBA, 20 mg</td>
<td>1</td>
<td>0/1</td>
<td>6/7, 67 days</td>
<td>7/10 (70), 86 days, 0.58 mg</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PC, 60 mg</td>
<td>1</td>
<td>3/6, 192 days</td>
<td></td>
<td>4/18 (22), 175 days, 0.61 mg</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-Dehydroestrone</td>
<td>PC, 60 mg</td>
<td>1</td>
<td>3/6, 192 days</td>
<td></td>
<td>3/10 (30), 208 days, 0.60 mg</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Hydroxyestriol</td>
<td>DMBA, 20 mg</td>
<td>1</td>
<td>5/11 (45), 74 days</td>
<td></td>
<td>8/12 (67), 74 days, 0.62 mg</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexestrol</td>
<td>DMBA, 20 mg</td>
<td>2</td>
<td>10/22 (45), 107 days</td>
<td></td>
<td>12/25 (48), 100 days, 0.61 mg</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total incidence</td>
<td></td>
<td>0/4</td>
<td>56/98 (57)</td>
<td></td>
<td>95/167 (57)</td>
<td>0/7</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Number of rats with tumors/total number tested.
* Median days of observation.
* Numbers in parentheses, percentage of rats treated.
* mg estrogen per pellet (mean).
* NS, no significant difference between incidence in carcinogen-treated versus estrogen-plus-carcinogen-treated by $\chi^2$.
* One survivor free of tumor after 360 days of observation.
* Five survivors free of tumor after 210 days of observation.

DISCUSSION

Previous investigations of steroid action upon chemically induced rat mammary carcinoma incidence utilized larger intermittent doses over a shorter period of time than given in this study. Few have demonstrated as marked inhibition of mammary carcinogenesis as noted herein following adequate estriol therapy, despite shorter postcarcinogen observation than the natural life-span follow-up of cancer-free rats in this study (5, 7, 11, 14, 15, 19, 36, 45, 47, 49, 50). Huggins et al. (18) noted that 10 to 20 µg estriol injected daily stimulated growth of a transplantable mammary fibroadenoma, while 100 µg/day inhibited its growth. Terentius (47) observed that 100 µg estriol injected every 12 hr for 12 days reduced the volume of DMBA-induced mammary tumors from 2670 cu mm in sham-injected carcinogen-fed rats to 110 cu mm after 120 days. Progestosterone-treated rats averaged 1460 cu mm tumor volume and testosterone-treated rats averaged 4370 cu mm. The smallest tumor volume of 31 cu mm occurred after treatment with an estrogen antagonist, Mer 25, which like estriol decreased somatic growth rate. Progesterone pretreatment of rats during 25 days prior to carcinogen administration to those observed after DMBA administration. The increase in incidence was not significant by $\chi^2$ and may be attributed partly to the longer observation period of the protected rats.
Estriol Prevention of Induced Rat Breast Carcinoma

Table 7
Mammary carcinoma incidence after implantation of nonestrogenic steroids

The carcinogenic agent was DMBA, 20 mg.

<table>
<thead>
<tr>
<th>Steroid</th>
<th>No. of experiments</th>
<th>Untreated controls</th>
<th>Carcinogen-treated</th>
<th>20% pellet + carcinogen</th>
<th>10% pellet + carcinogen</th>
<th>1% pellet + carcinogen</th>
<th>20% pellet only</th>
<th>x²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydroepiandrosterone</td>
<td>2</td>
<td>0/1*</td>
<td>4/6, 112 days</td>
<td>12/15 (80), 106 days, 1.14 mg</td>
<td>0/7.172 days, 1.23 mg</td>
<td>NS*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone</td>
<td>2</td>
<td>3/5, 266 days</td>
<td>1/9, 184 days, 1.15 mg</td>
<td>4/5.66 days, 0.63 mg</td>
<td>4/6.67 days, 0.060 mg</td>
<td>0/2.230 days, 1.15 mg</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progesterone</td>
<td>2</td>
<td>3/5, 145 days</td>
<td>9/17 (53), 146 days, 0.64 mg</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corticosterone (12.7%)</td>
<td>1</td>
<td>6/7.67 days</td>
<td>4/7.92 days, 0.77 mg</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total tumor incidence | 0/1               | 16/23 (70)         | 0/9               |

* Number of rats with tumor/total number of rats.
* Median days of observation.
* Numbers in parentheses, percentage of rats with tumors.
* mg steroid per pellet (mean).
* NS, no significant difference in incidence between carcinogen-treated and steroid-plus-carcinogen-treated.
* Corrected to equimolar concentration at 10% pellet concentration for estriol.
* One survivor free of tumor after 300+ days.

Table 8
Mammary carcinomas induced in females 24 to 72 hr after castration

The carcinogenic agent was DMBA, 20 mg.

<table>
<thead>
<tr>
<th>Steroid</th>
<th>No. of experiments</th>
<th>Untreated controls</th>
<th>Carcinogen-treated</th>
<th>10-20% steroid pellets + carcinogen</th>
<th>10-20% steroid pellets only</th>
<th>x²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estriol</td>
<td>8</td>
<td>0/10, 333 days*</td>
<td>3/21 (14); 180 days</td>
<td>3/12 (25), 203 days, 0.61-1.27 mg</td>
<td>0/16, 301 days, 0.62-1.19 mg</td>
<td>NS*</td>
<td></td>
</tr>
<tr>
<td>10% estrone + 20% estriol</td>
<td>3</td>
<td>6/20' (30), 204 days</td>
<td></td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% 17β-estradiol + 20% estriol</td>
<td>3</td>
<td>2/22* (9), 292 days</td>
<td></td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total tumor incidence | 0/10               | 3/21 (14)          | 0/16               |

* Number of rats with tumor/total number of rats treated.
* Median days of observation.
* Numbers in parentheses, percentage of rats with tumor.
* mg estrogen per pellet (mean).
* NS, no significant difference in incidence rates between carcinogen-treated and steroid-plus-carcinogen-treated.
* Also epidermoid carcinoma of the ear.
* Also epidermoid carcinoma of the ear and parotid neoplasm.

Reduced DMBA-induced tumor growth (50), but others noted stimulatory activity upon mouse and rat mammary carcinogenesis (19, 42). Estradiol benzoate, 20 μg/day for 20 days, completely suppressed further growth of DMBA-induced mammary tumors, reversible by daily injections of 28 IU ovine prolactin (39). Similar estradiol doses given both before and after DMBA completely inhibited breast carcinogenesis for as long as 150 days of observation, with marked impairment of weight gain (24). Breast cancer onset has been delayed as long as 241 days (median) (range, 65 to 297 days) by a single implant of 17β-estradiol 48 hr prior to DMBA treatment in this investigation. Since breast carci-
**Table 9**

Incidence of nonbreast neoplasms induced by carcinogens in relation to steroid therapy

<table>
<thead>
<tr>
<th>Steroid inhibition of mammary carcinogenesis</th>
<th>Carcinogen administered</th>
<th>Steroid implanted 48 hr before carcinogen</th>
<th>Nonbreast neoplasms</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None (Tables 3, 5-7)</td>
<td>0/51 0</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>DMBA (1.2-0.60 mg estrone, estrone 3-sulfate, 17β-estradiol (Table 3))</td>
<td>7/92 7.6</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>DMBA (0.15-0.060 mg estrone, 17β-estradiol, estriol, 16-epiestriol (Tables 3, 5))</td>
<td>3/76 4.0</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>DMBA (0.60 mg 16-epiestriol (Table 5))</td>
<td>0/21 0</td>
<td></td>
</tr>
<tr>
<td>Significant (p &lt; 0.001)</td>
<td>DMBA (0.60 mg other estrogen metabolites (Table 6))</td>
<td>4/129 3.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DMBA (1.20-0.60 mg nonestrogenic steroids (Table 7))</td>
<td>2/59 3.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Subtotal</td>
<td>16/377 4.2 (mean)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>PC (0.60 mg 17α-estradiol, estrone 3-sulfate (Table 3, 6))</td>
<td>6/52 11.5</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>PC (0.60 mg 16-epiestriol (Table 5))</td>
<td>12/72 16.7</td>
<td></td>
</tr>
<tr>
<td>Significant (p &lt; 0.001)</td>
<td>PC (0.15-0.60 mg estriol (Table 5))</td>
<td>2/43 4.7</td>
<td></td>
</tr>
</tbody>
</table>

- **Nonbreast neoplasms**: Incidence Pathological diagnosis
  - Epidermoid carcinoma of ear: 5
  - Leukemia: 1
  - Sarcoma of uterus: 1
  - Other: 3
  - Endometrial carcinoma: 1
  - Epidermoid carcinoma of ear: 1
  - Fibrosarcoma of ear: 2
  - Sarcoma of lymphoma: 3
  - Epidermoid carcinoma of ear: 1
  - Lymphosarcoma: 1
  - Endometrial carcinoma: 1
  - Epidermoid carcinoma of ear: 1
  - Sarcoma: 2
  - Epidermoid carcinoma of ear: 1
  - Lymphosarcoma: 1
  - Misc. sarcomas: 2
  - Adenocarcinoma? gut: 1
  - Sarcoma of uterus, ovary: 3
  - Adenocarcinoma of lung: 1

Nomas may develop up to 370 days after carcinogen, the value of shorter observation periods in evaluating breast cancer prophylactic agents is dubious.

With the technique used in this investigation, which permitted continuous absorption of varying doses of free steroids into body fluids, a 75 to 90% reduction in incidence of palpable or visible mammary carcinomas was achieved by 10 to 20% estriol pellets reimplanted every 2 months, commencing prior to feeding of either DMBA or PC. Tumor-free rats were observed until death from natural causes, usually bronchopneumonia, which occurred usually long after the age peak when most breast carcinomas become manifest. A threshold of cancer-inhibitory activity was noted when estriol concentration in pellets was reduced to 1.0 to 2.5% with pellet reimplantation every 1 to 2 months, respectively (Table 5). PC-induced mammary carcinoma was more sensitive to estriol inhibition than that induced by DMBA, since significant inhibition of breast tumorigenesis occurred after implantation of 2.5% pellets of estriol in PC-treated rats. With decreasing estriol dosage, the incidence of rats developing single as well as multiple primary breast tumors increased to equal the incidence observed in rats fed carcinogens only. Every enlarging breast tumor was biopsied, and simple hyperplasia with or without lactation as a cause of breast enlargement was ruled out by histological examination. Therefore, the final observed incidence of mammary carcinoma in various rat groups was conservative and was minimally influenced by lactational changes that develop in older virgin females. The incidence of mammary carcinoma in our carcinogen control groups fed a single 20-mg dose of DMBA was similar to that observed by others who histologically confirmed all malignant tumors (1, 4, 5, 7, 13, 36, 39, 47, 50). No difference was observed in the histological types of mammary carcinomas between estriol-treated and non-estriol-treated rats, nor was there any significant incidence of spontaneous remission of unbiopsied tumors to contribute materially to the differences noted in tumor incidence.

The 5 to 10% incidence of rats with sarcomas and nonbreast carcinomas (chiefly squamous neoplasms arising from the ear ducts) induced by both carcinogens was not significantly altered by implantation of maximal doses of estriol or any other of the 17 tested steroids (Table 1). This suggests that the anticarcinogenic activity of estriol was specific for mammary neoplasms only and that estriol did not alter the endogenous metabolism, if any, of the carcinogens, thereby reducing their carcinogenic activity. Continued implantation of estriol pellets every 2 months was required for inhibition of carcinogenesis, since in 2 experiments no reduction of breast cancer incidence was demon-
Carcinogen administration: pellet absorption was estimated to occur over a 90-day period (10). Estriol, therefore, probably does not block initiation of mammary carcinogenesis by an agent such as DMBA which acts within 1 to 3 days upon the breast to induce carcinogenesis (6).

Estriol probably interferes with the 2nd or promotional stage of mammary carcinogenesis, in which continued estradiol stimulation of mammary duct and myoepithelial cell proliferation (3, 35) is the major cocarcinogenic factor. Illustrating this was the similar degree of reduction of mammary carcinogenesis achieved by preliminary castration as achieved by estriol treatment (Table 8). In castrate females, substitution therapy with estrone or 17β-estradiol was ineffective in restoring the expected incidence of DMBA-induced breast tumors, as long as estriol was coadministered, in contrast to the results of substitution therapy with estradiol or diethylstilbesterol alone (5, 7, 14, 19, 33).

In intact female rats, neither of the 2 major estriol precursors, estrone and 17β-estradiol, inhibited carcinogenesis in the breast as effectively as estriol. 17α-Estradiol was completely ineffective against carcinogenesis in spite of its binding to uterine estrogen receptor proteins (27). Estrone and 17β-estradiol (0.50 to 0.76 mg) reduced breast tumor incidence to 22 to 25% in DMBA-treated rats, but 20% estradiol (1.14 mg) was even less inhibitory in 1 experiment. Estrone decreased breast cancer incidence more significantly after PC treatment (Table 3). Rat liver, intestine, and kidney incubated in vitro with tritiated 17β-estradiol along with the oxidative cofactors, NADPH and nicotinamide, yield estradiol as a major metabolite (31), so that part of the anticarcinogenic activity of estradiol or estrone may be attributed to metabolism to estradiol following implantation. Other estrone and 17β-estradiol metabolites such as 16-ketoestradiol or 2-hydroxyestriadiol possessed no anticarcinogenic activity (Table 6), and 16-epiestriol had lesser anticarcinogenic activity (Table 5). Since these and other inactive metabolites of estrone and estradiol implants were probably formed in vivo in addition to estradiol, the lesser anticarcinogenic activity of estrone and estradiol in the dosage tested may reflect the net response of tissues to a variety of metabolites, many of which were inactive. Estradiol metabolism to 2-hydroxyestradiol occurs in vitro by mouse liver (22), which also is ineffective as an antimammary carcinogen. The protective activity of estradiol implantation therefore results from circulation of the native steroid along with inactive metabolites until renal and biliary excretion.

If estradiol indeed accounted for most of the anticarcinogenic activity observed in this study, the mechanism might involve either local mammary effects or effects upon the pituitary or other endocrine organs or both. Sustained high estradiol concentrations in extracellular fluid would probably displace much of the ovarian-secreted estradiol bound to mammary estrophile receptor proteins and thereby alter the nature of the nuclear estrogen-receptor complex bound to DNA (51). Estradiol retains 70 to 100% of the binding activity of equimolar concentrations of 17β-estradiol in vitro for rat mammary estrophile proteins [H. M. Lemon. Estradiol and Estradiol-17β Binding to 7,12-Dimethylbenz(a)anthracene

Induced Rat Mammary Carcinomas, Autologous Muscle and Uteri, in preparation]. Dehydroepiandrosterone has no binding activity and was inactive in 20% pellet concentration in altering mammary carcinogenesis (Table 7). Although estriol probably binds to mammary receptor protein in vivo, suppression of DMBA-induced mammary carcinogenesis at the site of receptor-complex binding to DNA appears unlikely. Polycyclic hydrocarbon carcinogens interfere with estrogenic cellular responses (7), but there has been no close association noted between these compounds and estrogens for nuclear protein binding (12).

High chronic doses of estrone and 17β-estradiol alone are carcinogenic in rodents (33, 46). Estriol lacks any mammary carcinogenic activity resulting from maximally sustained duct epithelial proliferation (3) in castrate or intact rats or mice, but renal tumors have resulted (32, 46). In castrate C3Hf mice free of mammary tumor virus, 10 to 200 µg estriol applied cutaneously weekly for 60 weeks failed to elicit breast carcinomas, while 10 µg estrone weekly for a similar period of time resulted in 25% incidence of mice with breast cancers, comparable to the incidence rate in older breeding females of the same inbred strain (46). Weekly administration of 5 µg estrone along with 5 µg estradiol resulted in a single breast carcinoma (2.9% incidence); when a mixture of 1.4 µg 17β-estradiol, 4.3 µg of estrone, and 4.3 µg estriol was similarly applied, the incidence of breast carcinomas increased to that noted in breeding females in Pullinger’s studies, suggesting the critical role of 17β-estradiol in rodent mammary carcinogenesis. Estradiol maximally increases mitotic activity with increasing dosage in the castrate mouse uterus, while estradiol exhibits a flat dose-response curve, beyond which mitotic activity does not increase (35). No studies are reported using similar techniques for the rodent breast.

The effects of estradiol implantation upon prolactin or gonadotropin activity are unknown as yet; frequently observed lactation in castrate and estrone-implanted rats was uniformly minimal at necropsy in our estriol-treated groups. The paucity of grossly detectable mammary tissue in estradiol-implanted rats was noteworthy.

DMBA-induced mammary carcinomas are highly prolactin dependent, and in the work of Meites (39) and Welsh et al. (50) it has been suggested that estradiol blocks the mammary end-organ response to prolactin, which should be elevated by high doses of estradiol. In women, lactational activity begins after blood estrogen concentration decreases toward normal following delivery. Prolactin as a cofactor in human mammary carcinogenesis is currently being investigated.

The reduction of somatic growth observed herein following estrogen implantation, which did not correlate with breast cancer inhibition, has been noted previously in rats and mice receiving estrogen therapy, including estriol (33, 46). Appetite and/or somatotropin secretion may be suppressed to account for reduction of body growth by estrogens.

In spite of numerous similarities between human mammary carcinoma and chemically induced rat mammary carcinoma (Table 2), the suppressive effects of estradiol on mammary carcinogenesis may not be similar in the 2
species. However, the dose of estriol required for human clinical trials of breast cancer prophylaxis may be estimated from the concentrations used in this investigation. If linear absorption of estriol is assumed from the 10% pellets which ranged between 500 to 700 μg initial estriol content during their estimated 90- to 100-day life, a minimum absorption rate of 5 to 7 μg/day was released into extracellular fluid, or about 20 to 30 μg/kg/day for a 250-g rat. One-quarter of this dose was adequate for 75% inhibition of PC-induced breast carcinomas (Table 5). If allowance is made for differences between parenteral and enteric absorption, estriol, 2.5 to 5.0 mg/day p.o., might be a suitable minimum dose for prophylactic therapy in premenopausal women. Estriol, 2.5 to 5.0 mg/day p.o., has been well tolerated in menopausal and postmenopausal breast cancer patients, for as long as 11 months. Such a dosage is far less than the daily dose for prophylactic therapy in premenopausal women.

These observations afford a basis for clinical trials of estriol therapy for prevention of breast cancer in Caucasian women, especially when nulliparity, familial history, precancerous breast disease, or reduced endogenous estriol production suggest excessive risk.

REFERENCES

35. Martin, L., and Finn, C. A. Oestrogen-Gestagen Interactions on


Estriol Prevention of Mammary Carcinoma Induced by 7,12-Dimethylbenzanthracene and Procarbazine

Henry M. Lemon


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/35/5/1341

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, use this link http://cancerres.aacrjournals.org/content/35/5/1341. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.