The Effect of Estrogens and a Carcinogenic Chemical in Stimulating the Secretion of the Mammogenic Duct Growth Factor of the Anterior Pituitary

A. A. Lewis, Ph.D., and C. W. Turner, Ph.D.

(From the Department of Dairy Husbandry, University of Missouri, Columbia, Missouri)

(Received for publication November 16, 1940)

When estrogens are injected into normal immature and castrated male or female animals growth of the duct system of the mammary gland is stimulated. During the past several years increasing evidence has accumulated indicating that this mammary hyperplasia is due not to a direct action of estrogen upon the mammary gland but instead to an increased rate of secretion of anterior pituitary hormones which have been called mammogen (17, 23, 18). Data recently published ¹ indicate that this consists of 2 factors: a duct-growth factor and a lobule-alveolar-growth factor. A technic for the biological assay of the mammogenic duct-growth factor, in which young male mice are used, has been described (22). This method has now been extensively employed in assaying the mammogen content of pituitaries from dairy and beef cattle during several stages of growth, the sexual cycle, pregnancy, and lactation (23).

The object of the present paper is to describe how this same assay technic may be used to advantage in the direct determination of the ability of various estrogenic chemicals to stimulate the secretion of mammogen (duct factor) by the pituitary. Such information is not only of interest as indicating the relative effectiveness on mammary hyperplasia of various types of chemical compounds which have estrogenic effects, but has a far greater value in measuring the effect of carcinogenic chemicals upon the secretion of mammogen.

Lacassagne (21), Gardner, (13, 14), and others (1) have shown that the injection of estrogens into male mice of cancer-susceptible strains will induce the formation of mammary tumors. As mammary growth is a prerequisite for mammary tumor formation, it would appear that the direct cause of such tumors is the secretion of mammogen by the pituitary rather than the action of estrogens. It is conceivable that compounds having little or no estrogenic activity might so stimulate mammogen secretion as to lead eventually to mammary tumor formation. It would appear that in connection with the study of natural and synthetic carcinogenic compounds, the determination of their ability to stimulate the secretion of mammogen would be of great importance. The assay technic might also be applied to extracts of the blood and urine of patients having active breast tumors in order to determine whether there may be secreted unrecognized compounds which act upon the pituitary and cause increased secretion of mammogen.

EXPERIMENTAL

Procedure.—The method for the determination of the ability of various estrogens and carcinogenic chemicals to stimulate the secretion of mammogen by the anterior pituitary consists in the subcutaneous injection of the compound once daily for 6 days into male mice weighing 15 to 25 gm. The animals are sacrificed on the 7th day and the mammary rudiments examined for definite signs of duct development. A mouse unit is defined as the total amount of the chemical compound required per mouse to produce signs of duct growth in 5 or more glands of 10 or more male albino mice weighing 15 to 25 gm.

The chemicals assayed were dissolved in ether, if not already in solution, and were administered in 0.05 to 0.2 cc. sesame or olive oil except for the estriol which was in aqueous solution. Estradiol benzoate was obtained from the Schering Corporation, estrone and estriol from Parke, Davis & Company, diethylstilbestrol and triphenyl ethylene from E. R. Squibb & Sons, anol from the British Drug House, and 1,2,5,6-dibenzoanthracine from the Eastman Kodak Co.

The mice were fed a mixed ground grain ration and Purina dog pellets ad libitum.

Results.—The 3 principal ovarian hormones were assayed in male mice in order to ascertain the dosage required when assayed for mammary hyperplasia under standard conditions. It was found that 200

* This investigation was aided in part by a grant from The International Cancer Research Foundation.
† Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 682.
international units (20 γ) of estrone (theelin) were required for 1 mammogenic mouse unit (Table 1), and 600 γ of estriol (aqueous theelol) gave a similar response. The estrone was therefore 30 times as effective, by weight, as estriol. The fact that the estriol was injected in water probably reduced its effectiveness compared with estrone in an oil carrier.

Surprisingly, estradiol benzoate proved to be many times more potent for mammary duct hyperplasia than estrone. The extremely small amount of 0.083 γ of the former gave a 41 per cent response in 17 mice. The most pertinent assay groups are given in Table I. The assay of estradiol benzoate actually involved 104 mice. This chemical appears to be 241 times as active per unit weight as is estrone for mammary duct growth.

On assay by the mammogenic mouse unit technic 0.05 γ of stilbestrol (4,4-dihydroxy a β diethyl stilbene) produced a 69 per cent response in 16 male mice. It is apparently at least 400 times as effective as estrone for mammogenic stimulation, and even more effective than estradiol benzoate.

Anol (p-hydroxyprophenyl), assayed by the mammogenic mouse unit technic, gave a 53 per cent response at 0.6 mgm. per mouse. Thus it appeared to be 1/30 as effective by weight as estrone and equal to aqueous estriol in promoting the production of mammogen in male mice and thus causing mammary hyperplasia.

Administration of triphenyl ethylene to 14 male mice at a total dosage of 0.5 mgm. caused a 36 per cent response, while 1.0 mgm. given to each of another group of 14 mice resulted in a 79 per cent response. One mammogenic mouse unit should, therefore, require about 0.7 mgm. Triphenyl ethylene is thus possibly 1/35th as effective as estrone and practically equal to aqueous estriol and to anol.

One mgm. of 1,2,5,6-dibenzoanthracene administered to each of 9 male mice caused a positive mammary response in 56 per cent, indicating 1 mouse unit. This compound is possibly 1/75th as effective as estrone for mammogenic stimulation.

**Discussion**

In previous experiments, dosages of estrogenic compounds used to induce mammary hyperplasia have been based on comparative assays of genital potency.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>No. of mice</th>
<th>Dosage in γ</th>
<th>Percentage response (positive)</th>
<th>Effectiveness (estrone = 2)</th>
<th>One estrogenic mouse or rat unit in γ</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol benzoate (Progynon-B)</td>
<td>6</td>
<td>0.017</td>
<td>17</td>
<td>241 X</td>
<td>0.1-0.25</td>
<td>(11, 20, 31)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.033</td>
<td>33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>0.085</td>
<td>41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.165</td>
<td>80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4,4-dihydroxy a β diethyl stilbene (Stilbestrol)</td>
<td>10</td>
<td>0.2</td>
<td>60</td>
<td>At least 400</td>
<td>0.5-0.37</td>
<td>(26, 20, 9, 8)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.1</td>
<td>70</td>
<td></td>
<td>0.1-1.5</td>
<td>(11, 31, 20)</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>0.05</td>
<td>69</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrone (Theelin)</td>
<td>20</td>
<td>20</td>
<td>55</td>
<td>1</td>
<td>0.1-1.5</td>
<td>(11, 31, 20)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>10</td>
<td>33</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>0.25 to 2.5</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estriol, aqueous (Theelol)</td>
<td>14</td>
<td>500</td>
<td>21</td>
<td>1/30</td>
<td>15.0</td>
<td>(31)</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>600</td>
<td>42</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>600</td>
<td>53</td>
<td>1/30</td>
<td>100-1,000</td>
<td>(26, 35, 15)</td>
</tr>
<tr>
<td>P-hydroxyprophenyl (Anol)</td>
<td>14</td>
<td>500</td>
<td>36</td>
<td>1/35</td>
<td>1,000</td>
<td>(26)</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>1,000</td>
<td>79</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,2,5,6-Dibenzoanthracene</td>
<td>9</td>
<td>1,500</td>
<td>56</td>
<td>1/75</td>
<td>0</td>
<td>(24, 5)</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>2,000</td>
<td>77</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The present observations show that such evaluation is not of much advantage in studies on growth of the mammary gland and stimulation of mammogenic secretion in the anterior pituitary.

Some idea of comparative mammogenic potency could, however, be obtained on growth of the mammary glands. Turner et al. (32), for example, found that 2.5 rat units of theelin (urine extract) injected daily for 20 days into castrate female rats gave good response in growth of the mammary duct system. Similarly, 16.7 rat units of estrone daily for 12 days in male rabbits brought about considerable duct proliferation. Five rat units of theelin daily for 15 days produced no response in male mice, nor was any response obtained with 0.5 ml. of theelol (estriol). Gardner et al. (12), however, were able to produce well developed mammary glands in male mice with 2.0 rat units of estrone daily for 15 days. Turner and Gomez (33) found that 10 rat units of estrone injected daily for 20 to 30 days into adult male albino mice would...
cause extensive growth of the duct system. At that time no standard assay technic for mammary growth was available, so that the comparative evaluation of various compounds from such experiments is difficult.

The comparative effectiveness of estrone and estriol observed in this study agrees rather well with the effect of the 2 estrogens on the vagina as determined by Thayer et al. (31). They assayed estrone and estriol by observation of the production of vaginal estrous changes in spayed female mice. Twenty times as much estriol was required for a 90 per cent response as of estrone for a 100 per cent response. For mammary growth, however, 40 times as much estrone or estriol was required as for genital stimulation.

Van Heuverswyn et al. (34) found that 16 γ of estradiol benzoate caused a + + mammary response in 5 male mice when given in 8 injections over a 16-day period. A + response indicated an increased number of small, broader, branching ducts, usually terminating in enlarged end buds. Plus two glands were further developed.

Lewis and Turner (23) found that a daily dose of 3.3 γ of estradiol benzoate gave a good response (+, +++) in mammary duct stimulation in male mice when administered for 6, 8, and 10 days. In the present study it was found possible to decrease the total dosage to 0.083 γ per mouse and still obtain a 41 per cent response. This is a far greater potency, compared to estrone, than has previously been reported with comparisons on the basis of vaginal smears or uterine response. In the latter case estradiol was found to be only 3 to 5 times as active as estrone (31, 20). Our assay involves a large number of mice, however, and was characterized by consistent results. This dosage of 0.083 γ per mouse is very close to that required for genital response, whereas with most of the other estrogens tested the dosage required for minimum mammary response by this technic was considerably higher than that reported as necessary for genital response.

Folley and Watson (10) reported that stilbestrol produced changes in the chemical composition of cows' milk similar to changes produced by estrogen. Breast hypertrophy and genital changes were reported by Guldberg (19) in a castrate woman given 20.25 mgm. of diethyl-stilbestrol in 18 days followed by 30 rabbit units of progesterone in 6 days. MacBryde (25) reported similar results. Proliferation of the mammary epithelium was also revealed by biopsy specimens obtained before and after oral administration of 280 mgm. of stilbestrol to a castrate woman. No malignancy was apparent. One mgm. a day given orally for 14 days to another woman caused painful swelling of the breasts (Bishop, Boycott, and Zuckerman, 3).

Van Heuverswyn et al. (34) found that groups of 5 male mice given 0.05, 0.2, and 2.0 mgm. of stilbestrol, respectively, gave +++, +++, and + responses after 8 injections in 16 days. Stilbestrol was found to be about 1/5 as active as estrone in causing proliferation of the mammary glands of spayed rats (Felding and Möller-Christensen, 9) and of guinea pigs (Dodds, Lawson, and Noble, 6).

Assays of the effect of stilbestrol on the genital organs show that 0.5 γ produced estrus in 70 per cent of spayed rats weighing 175 gm (26). Stilbestrol was found to be equal to or 5 times as active as estrone in producing vaginal cornification in rats and mice (Morrell, 26; Emmens, 8; Sealy and Sondern, 29). Orally stilbestrol was 20 times as active in mice as estrone and 16 times as active as estradiol (Sealy and Sondern, 29).

In contrast to these reports, stilbestrol proved in our experiments to be at least 400 times as active as estrone in causing mammary proliferation in male mice. It was even more effective than estradiol benzoate. In view of this great activity, overdosage should be avoided in human therapy because of the danger of cancer promotion in susceptible individuals, as indicated by Auchincloss and Haagensen (2).

Anol, or p-hydroxyprophenyl, is a synthetic compound which has been found to have estrogenic activity of about 1 mouse unit per mgm. (26, 35). Dodds and Lawson (7) found that pure anol had no estrogenic properties, whereas the activity of the impure material was considerable. Bottomley and Folley (4) reported that anol stimulated growth of the nipples of guinea pigs. The condition of the mammary glands was not described.

Gomez and Turner (15) found that anol caused the production of mammogen in the pituitaries of rabbits. Thirteen mgm. of pituitary from young virgin does which had been given anol contained 1 mammogenic mouse unit, while pituitary tissue from untreated females and males was negative at 80 and 60 mgm., respectively. Anol plus estradiol appeared to be more effective than either alone.

Anol in amounts of 0.05 to 0.5 mgm. daily was reported by these same workers (16) to cause mammary duct development in male mice in 15 and 30 days. Female rabbits and rats also responded. Some abnormal development occurred.

With the assay technic anol was found to be fairly effective in causing stimulation of mammogen secretion and minimal signs of duct hyperplasia. Six-tenths mgm. was equivalent to 1 mammogenic mouse unit. This is 6 times the amount required for 1 estrogenic mouse unit of this material according to the assays made by Gomez (15).

Van Heuverswyn et al. (34) found that triphenyl ethylene administered to 3 groups of 7 to 10 male mice in doses of 5, 10, and 40 mgm. caused exten-
sive development of mammary rudiments (+ + to + + + +). Eight injections were given in 16 days.

Noble (27) reported that, although the estrogenic activity of triphenyl ethylene was low, it did have estrogenic properties as manifested by a lowered growth rate in rats and reduction of the gonadotropic activity of the pituitary following subcutaneous implantation of crystals.

Triphenyl ethylene was found to be fairly active in causing mammary duct hyperplasia in male mice. Interpolation of the 36 per cent response with a 0.5 mgm. dose, and 79 per cent response with a dose of 1 mgm. indicated that approximately 0.7 mgm. were required for 1 mouse unit response. This dose is within 0.1 mgm. of that required by estriol and anol.

Loeb (24) reported that 1,2,5,6-dibenzanthracene, which is actively carcinogenic (Shear, 30), is without estrogenic potency. Noble (27) found that crystals of 1,2,5,6-dibenzanthracene implanted subcutaneously did not reduce the growth rate of rats. The compound did not cause any decrease in pituitary gonadotropic activity, nor did it have estrogenic activity in male rats (Cook, et al. 5).

Perry and Ginzton (28) found that normal female mice from a low-tumor strain did not develop mammary cancer when 1,2,5,6-dibenzanthracene alone was applied cutaneously, but among spayed females similarly treated 4 per cent developed mammary cancer. When theelin was also given, tumors developed in 43 per cent, both of normal and spayed animals. This would indicate that 1,2,5,6-dibenzanthracene alone failed to produce tumors because of lack of mammary growth stimulation, which was supplied by estrone.

In spite of this lack of estrogenic and mammary carcinogenic properties, 1,2,5,6-dibenzanthracene was found by us to cause mammary duct hyperplasia in male mice. It was, however, the least potent of the compounds tested, 1.5 mgm. being required for a mouse unit response. The interesting point is that, though nonestrogenic, it will cause mammary growth. In this particular it compares with the mammogenic duct growth factor of the anterior pituitary.

Summary

Of various estrogens promoting mammary gland proliferation, the most active, as judged by the mammogenic mouse unit technic, was found to be stilbestrol, which proved to be at least 400 times as effective as estrone. Next in activity, among the compounds studied, was estradiol benzoate, which showed 240 times the effectiveness of estrone, by weight. Anol and triphenyl ethylene compared favorably with aqueous estriol but were only 1/30 as effective as estrone. 1,2,5,6-Dibenzanthracene was only 1/75 as effective as estrone.

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