The Fibromatogenic Action of Specific Urinary Estrogens (Metahormones) in the Guinea Pig*†

Alexander Lipschütz, M.D., René Thibaut, M.D., and Luis Vargas, Jr., M.D.

(From the Department of Experimental Medicine, National Health Service of the Republic of Chile, Santiago, Chile)

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Conversion of estradiol and estrone, or ortho- and parahormones, into estriol, a specific urinary metahormone of women, acquires a new interest since, in guinea pigs, toxic phenomena, including tumorigenesis, are elicited by prolonged action of follicular hormones. Further interest is added by the findings of Pincus and Graubard (20) who have reported that cancerous women appear to be unable to convert estrone into estriol to any appreciable extent. It is generally assumed that this conversion signifies inactivation; i.e., that substances of high estrogenic activity (estradiol and estrone) are transformed into substances of low estrogenic activity (estriol in the urine of the pregnant woman; different urinary estrogens of the mare). One may also assume, tentatively, that by means of this conversion the body protects itself against the toxic or tumorigenic action of follicular hormones. This assumption was tested by making a quantitative comparison of the faculty of different ovarian and urinary estrogens to elicit abdominal serosal fibroids in the guinea pig.

**Materials and Methods**

Alpha-estradiol and estrone were used as representatives of ovarian estrogens. Both are found also in the urine, especially estrone, which can be extracted from the human placenta, as shown by Doisy (2) and Marrian (19). Since both are present in the ovary (2), they can be considered as primary ovarian estrogens. Estriol and equilenin were used as representatives of urinary estrogens. The first is present also in the placenta, but both are absent from the ovary. They can be considered as specific urinary estrogens.

Pellets of crystalline hormones were prepared by compression in a suitable hand press as described in detail in the thesis of Thibaut (23). The weighed pellets were implanted subcutaneously in castrated guinea pigs. After the death of the animals the pellets were recovered and weighed again. The animals were killed and autopsied at different intervals after implantation of the pellet. The fibrous reaction was classified in accordance with criteria specified in previous papers by Lipschütz and Vargas (14), and Lipschütz, Bellolio, Chaume, and Vargas (10). The results with respect to absorption and total tumoral effect as obtained in 114 animals are summarized in Table I and Fig. 1.

**Comparative Fibromatogenic Activity of Different Estrogens**

All of the four estrogens were tumorigenic. The localization of tumors was the same as in animals receiving estradiol and its esters, or artificial estrogens, as reported previously by Lipschütz and Vargas (10), Iglesias (6), Lipschütz, Iglesias, and Vargas (11), and Szabo (22). However, there were considerable differences as to the degree of the reaction.

The first manifestations were found as early as 21 to 30 days after implantation of pellets. These were small, barely visible, nodules on the spleen, the stomach, and sometimes at the junction between the uterus and the parametrium. These primary manifestations of a fibrous reaction graded as 0.5 were present with all four estrogens.

The tumoral reaction increased with time. At 80 and 120 days marked differences became evident between estradiol and estrone on one hand, and estriol and equilenin on the other. As noted in our previous reports (6, 11, 15), individual variations were con-
siderable. Resistant animals were present in the groups given each of the four estrogens. Nevertheless, it was a striking finding that, in experiments continued for 50 days or longer, 6 out of 33 animals treated with estradiol and estrone developed high tumoral reactions, it is known that estrone is absorbed more slowly than estradiol. In our experiments, results of which are shown graphically in Figs. 1 and 2, we found that the absorption of estrone was considerably slower than that of estriol or equilenin. Nevertheless, the tumori-

**Table I: Tumoral Effect in 71 Castrated Female Guinea Pigs (210 to 530 gm. When Receiving the Implant) with Subcutaneously Implanted Pellets of Four Different Estrogens**

<table>
<thead>
<tr>
<th>Estrogen</th>
<th>Duration of experiment in days</th>
<th>Number of animals</th>
<th>Average absorption per day (mgm.)</th>
<th>Total tumoral effect</th>
<th>Number of animals with total tumoral effect not less than grade 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>50</td>
<td>10</td>
<td>75</td>
<td>2.7</td>
<td>9</td>
</tr>
<tr>
<td>Estradiol</td>
<td>80</td>
<td>5</td>
<td>18</td>
<td>4.6</td>
<td>0-12</td>
</tr>
<tr>
<td>Estrone</td>
<td>50</td>
<td>5</td>
<td>13</td>
<td>3.2</td>
<td>0.5-8</td>
</tr>
<tr>
<td>Estrone</td>
<td>80</td>
<td>5</td>
<td>11</td>
<td>3.9</td>
<td>0.5-6.5</td>
</tr>
<tr>
<td>Estrone</td>
<td>120</td>
<td>8</td>
<td>5.5</td>
<td>4.6</td>
<td>1.5-12</td>
</tr>
<tr>
<td>Estriol</td>
<td>50</td>
<td>4</td>
<td>44</td>
<td>1.9</td>
<td>0.5</td>
</tr>
<tr>
<td>Estriol</td>
<td>80</td>
<td>5</td>
<td>20</td>
<td>2.4</td>
<td>0.5-4</td>
</tr>
<tr>
<td>Estriol</td>
<td>120</td>
<td>8</td>
<td>15</td>
<td>3.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Equilenin</td>
<td>50</td>
<td>5</td>
<td>22</td>
<td>0.5</td>
<td>0.1-5</td>
</tr>
<tr>
<td>Equilenin</td>
<td>80</td>
<td>9</td>
<td>19</td>
<td>0.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Equilenin</td>
<td>120</td>
<td>7</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Fig. 1**—Graphs showing degree of fibromatogenic action of estradiol, estrone, estriol, and equilenin in 114 castrated guinea pigs. The estrogens in pellets were implanted subcutaneously. Each point is the average of results in 4 to 10 animals. The numbers near each point indicate the average absorption in mgm. The fibromatogenic action of estriol and equilenin is considerably less than that produced by equal or smaller quantities of estrone or estradiol. For details see Table I and Thibaut (23).

**Fig. 2**—Graphs showing percentage absorption from subcutaneously implanted pellets of estradiol, estrone, estriol, and equilenin in 114 female guinea pigs. All pellets were of the same diameter, 1.6 mm., but not of the same length. The weights of the pellets varied between 1.4 and 6.2 mgm. Rate of absorption of estrone was less than that of other estrogens. For details see Thibaut (23).

not lower than grade 6, while among 38 animals treated with estriol and equilenin only one developed such a reaction, the tumoral reaction generally failing to appear.

The differences in the degree of tumoral reaction were not due to the differences in the quantities of hormones absorbed. From the work of Deanesly (1) the fibromatogenic action was enormously superior with estrone. Our results show, therefore, that specific urinary estrogens are less fibromatogenic than ovarian estrogens. This confirms the statement of Lacassagne (7) who found equilenin to be less active than estrone in eliciting mammary adenocarcinoma in mice, although no exact quantitative data on this point are available.
COMPARATIVE HYSTEROTROPIC ACTION

The question arises as to whether the lower fibromatogenic action of certain estrogens is concomitant with a lesser estrogenic activity. According to general opinion this should be so because, in the Allen-Doisy test, estriol and the urinary estrogens of the mare are less active than estradiol or estrone. The same results are obtained with the uterine tests. When rats are injected once daily for 5 days with estrogens in oil, the highest uterine ratio (ratio of the weight of the uterus in mgm. to body weight in gm.) is obtained with estradiol. With estrone this ratio is somewhat less and with estriol and equilenin it is considerably below the value with estradiol, as shown by the work of Dorfman (3). Similar statements concerning this ratio in the mouse were made by Evans, Varney, and Koch (5). Emmens (4), however, discovered that the differences in the degree of estrogenic effect exerted by the natural estrogens, as evidenced by cornification of the vaginal mucosa, depend in part upon the number of injections made into the same animal for purpose of assay. With two injections the approximate amounts of estradiol, estrone, and estriol in oily solution needed to produce 50 per cent of positive estrous vaginal responses are 1:4:280, whereas with four injections these amounts are only 1:2.7:6.4.

These findings of Emmens raise the question as to whether there might be equality in certain actions of estrogens, hitherto different as judged by the results of the Allen-Doisy test and the results of the short-term uterine test, provided a steady flow of hormone be maintained from a given source. Our results with pellets of estrogens appear to be in favor of such an assumption. In our experiments with pellets there was no considerable difference in uterine weights between estradiol, estrone, and estriol. Uterine weights were inferior with equilenin, but the difference between equilenin and the other estrogens on this basis was not so remarkable as the differences between the fibromatogenic actions, data on which are shown in Figs. 1 and 3.

It must be emphasized that, in experiments in which treatment with estrogens is prolonged, the uterine weight does not depend entirely upon an increase in the muscular coats. The increase depends to a large extent also upon the atypical proliferation which the endometrium undergoes under these experimental conditions. In some instances adenomatous polyps may fill the uterine cavity and descend into the cervix and vagina (18). Although we have not yet made a comparative microscopical study of the myometrial and endometrial reactions following implantation of pellets of different estrogens, we have noted that atypical growth of the endometrium, with the formation of polyps, may be obtained also with equilenin. Genital bleeding occurred with equilenin as with other estrogens.

COMPARATIVE FIBROMATOGENIC ACTION OF FOLLICULAR HORMONES ABSORBED FROM PELLETS AND INJECTIONS

In previous experiments we (13, 21) found that 38 to 50 injections of 150 to 200 µgm. of estradiol, or 150 to 300 µgm. of estrone, in oily solution; i.e., a total of 5.7 to 11.4 mgm. given in the course of 3 to 4 months, did not elicit abdominal fibroids in female guinea pigs. Only the first manifestations of a fibrous reaction occurred in the form of tumoral seeds or fibrous strands at various places. Fibroids were produced only when 400 µgm. were injected thrice weekly; i.e., when a total of 16 to 20 mgm. were injected in the course of 3 to 4 months. The results of the experiments we are now reporting provided a basis for comparison of the fibromatogenic action of estrogens administered on the one hand in repeated injections of oily solutions, and on the other by subcutaneous implantation of pellets.
In Table II we have summarized the results obtained in experiments with 18 guinea pigs killed 80 to 120 days after implantation of the pellet. These experiments are fully comparable as to duration of treatment with experiments on a group of 17 animals previously described in Tables I and II of the appendix of the report by Rodríguez (18), each animal in this group having received 40 to 50 injections of 150 to 300 μg of hormone in 95 to 123 days. As is shown in Table II, and Figs. 4A, 4B, and 4C, considerable tumorigenesis was elicited with pellets when only 15 μg of estradiol were absorbed daily in the course of 80 days.

Table II: Fibromatogenic Action of Small Quantities of Estradiol or Estrone Absorbed from Subcutaneously Implanted Tablets in 18 Castrated Female Guinea Pigs

<table>
<thead>
<tr>
<th>Series XXXVIII guinea pig No.</th>
<th>Duration of experiment in days</th>
<th>Weight of pellet mgm.</th>
<th>Quantity of estradiol absorbed Total mgm.</th>
<th>Per day mgm.</th>
<th>Total tumoral effect</th>
<th>Uterine subserous and parametral</th>
<th>Uterine apical</th>
<th>Digestive tract and parietal</th>
<th>Splenic</th>
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<tr>
<td>Estradiol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>96 (Fig. 4, A-C)</td>
<td>80</td>
<td>2.2</td>
<td>1.2</td>
<td>15</td>
<td>12.0</td>
<td>3 pm.</td>
<td>3</td>
<td>3 pr., ms.</td>
<td>3</td>
</tr>
<tr>
<td>97</td>
<td>80</td>
<td>3.3</td>
<td>1.2</td>
<td>15</td>
<td>5.0</td>
<td>0</td>
<td>1</td>
<td>3 pr.</td>
<td>1</td>
</tr>
<tr>
<td>98</td>
<td>80</td>
<td>2.4</td>
<td>1.4</td>
<td>18</td>
<td>0.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>102</td>
<td>80</td>
<td>3.4</td>
<td>2.3</td>
<td>28</td>
<td>3.0</td>
<td>2 ss.</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>95</td>
<td>80</td>
<td>4.4</td>
<td>2.4</td>
<td>30</td>
<td>3.0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Estrone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>67</td>
<td>80</td>
<td>1.0</td>
<td>0.3</td>
<td>4</td>
<td>4.0</td>
<td>0</td>
<td>3</td>
<td>0.5 f., ms.</td>
<td>0.5 f.</td>
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<tr>
<td>69</td>
<td>80</td>
<td>2.1</td>
<td>0.5</td>
<td>4</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>60 (Fig. 5)</td>
<td>80</td>
<td>5.2</td>
<td>0.4</td>
<td>5</td>
<td>6.5</td>
<td>0</td>
<td>3</td>
<td>3 ms.</td>
<td>0.5 f.</td>
</tr>
<tr>
<td>24</td>
<td>80</td>
<td>3.3</td>
<td>1.1</td>
<td>14</td>
<td>4.5</td>
<td>1 pm.</td>
<td>3</td>
<td>0.5 f., ms.</td>
<td>0</td>
</tr>
<tr>
<td>22</td>
<td>80</td>
<td>5.2</td>
<td>2.1</td>
<td>27</td>
<td>4.0</td>
<td>0</td>
<td>3</td>
<td>0.5 f., ms.</td>
<td>0.5 f.</td>
</tr>
<tr>
<td>74</td>
<td>120</td>
<td>2.1</td>
<td>0.2</td>
<td>1.7</td>
<td>2.0</td>
<td>0</td>
<td>0</td>
<td>0.5 f., ms.</td>
<td>0</td>
</tr>
<tr>
<td>86</td>
<td>120</td>
<td>1.7</td>
<td>0.4</td>
<td>3.4</td>
<td>1.3</td>
<td>1 pm.</td>
<td>0.5 f.</td>
<td>0</td>
<td>0</td>
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<tr>
<td>70 (Fig. 6)</td>
<td>120</td>
<td>1.8</td>
<td>0.5</td>
<td>4.2</td>
<td>3.0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>71</td>
<td>120</td>
<td>3.0</td>
<td>0.5</td>
<td>4.3</td>
<td>2.5</td>
<td>0</td>
<td>0.5 f.</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>85</td>
<td>120</td>
<td>1.7</td>
<td>0.6</td>
<td>5.6</td>
<td>3.0</td>
<td>1 ss., pm.</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>68 (Fig. 7, A and B)</td>
<td>120</td>
<td>1.9</td>
<td>0.9</td>
<td>7.5</td>
<td>4.0</td>
<td>2 ss., pm.</td>
<td>1</td>
<td>1 int., ss.</td>
<td>0</td>
</tr>
<tr>
<td>72 (Fig. 8)</td>
<td>120</td>
<td>2.4</td>
<td>0.9</td>
<td>7.5</td>
<td>12.0</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>66 (Fig. 9, A-C)</td>
<td>120</td>
<td>3.8</td>
<td>1.3</td>
<td>10.9</td>
<td>9.0</td>
<td>3 ss., pm.</td>
<td>3</td>
<td>1 pr.</td>
<td>2</td>
</tr>
</tbody>
</table>

Abbreviations: f.=fibrous strands; s.=tumoral seed; ss.=subserous tumors of uterus or intestine; pm.=parametrial tumors of uterus; ms.=mesenteric tumors; int.=tumor on intestine.

With pellets of estrone, 5 to 7.5 μg. per day were sufficient to produce large fibrous tumors in the course of 80 to 120 days. The distribution and sizes of these tumors are indicated in Figs. 5 to 9C.

The comparative results obtained with implanted pellets and with injections of oily solutions demonstrate the fundamental importance of timing in tumorigenesis elicited by estrogens. Quantities as small as 15 μg. of estradiol or 8 μg. of estrone, or less, absorbed daily over a period of time, were equivalent in their fibromatogenic action to 400 μg absorbed from an oily solution injected every second day. The following explanation is suggested: Estradiol and estrone when given by subcutaneous injections are rapidly absorbed and partly inactivated in the liver. Hence injections every second day are not likely to maintain a stable level of estrogen in the body. Accordingly, it has been assumed by Lipschütz (9) that the estrogen level in the blood will drop rapidly below the tumorigenic threshold unless enormous quantities are injected repeatedly. On the contrary, when there is a continuous supply of estrogen from a subcutaneously implanted pellet it is very likely that the level of concentration of estrogen in the blood is maintained constant. The height of this level will probably depend on the quantity of estrogen absorbed in unit time; i.e., on the surface area of the tablet or pellet, as indicated by the studies of Lipschütz and Vargas (15, 16). The quantity sufficient to maintain the fibromatogenic threshold level of estrogens administered as pellets is evidently much smaller than when these hormone is administered by injection.

These results provide new evidence that the estrogen exerts fibromatogenic action when experimental conditions provide for its continuous action on the effecto tissues. Subcutaneous implantation of tablets or pellet is the most effective method for obtaining this effect. Lipschütz and his associates (17) have shown also that esterification, especially with caprylic acid in the 17-position, is another important means of securing continuity of action.

In contrast with the effects of 5 to 8 μg. of estrone absorbed from pellets, quantities of equilenin twice or three times greater, administered in the same manner, had no tumorigenic action or only an insignificant effect (Table I).
Summary and Conclusions

The ovarian estrogens, estrone and estradiol, and the specific urinary estrogens, estriol and equilenin, were implanted subcutaneously, in the form of pellets, into castrated female guinea pigs. Observations were made upon the rates of absorption and the comparative effects of these estrogens upon uterine weights and their fibromatogenic activities.

Specific urinary estrogens, such as estriol and equilenin, elicited abdominal serosal fibroids similar to those following subcutaneous implantation of pellets of estradiol and estrone. The fibromatogenic action of estriol was less than that of estradiol and estrone. The fibromatogenic action of equilenin was insignificant.

The stronger action of estradiol and estrone as compared with that of the specific urinary estrogens was not due to absorption of larger quantities of the ovarian estrogens. On the contrary, estrone was absorbed more slowly and was more effective than twice the quantities of absorbed estriol and equilenin.

Estriol produced increase of uterine weight almost equal to that produced by estradiol and estrone; the
Fig. 6.—Photograph showing a large tumor at the apex of the left uterine horn in contact with the abdominal wall in a castrated guinea pig (Table II, No. 70) implanted subcutaneously with a pellet of 1.8 mgm. of estrone. Absorption was at the rate of 4.2 μgm. daily, or a total of 0.5 mgm. in 120 days.

Fig. 7A.—Photograph of several subserous uterine tumors, an apical tumor of the right uterine horn, and adhesions between the tumors and the omentum, in a castrated female guinea pig (Table II, No. 68) implanted with a pellet of 1.9 mgm. of estrone. Absorption was at the rate of 7.5 μgm. daily, or a total of 0.9 mgm. in 120 days.

Fig. 7B.—Photograph of a tumor of the mesocolon in contact with the serosa of the intestine, in the same animal as in Fig. 7A.

Fig. 8.—Photograph of fibrous tumors in castrated female guinea pig (Table II, No. 72) implanted subcutaneously with a pellet of 2.4 mgm. of estrone, showing tumors in the mesentery of the ileum and uterus embedded in large tumoral masses to which the colon is firmly adherent. Absorption was at the rate of 7.5 μgm. daily, or a total of 0.9 mgm. in 120 days.

Fig. 9A.—Photograph of the ventral surface of the uterus of a castrated female guinea pig (Table II, No. 66) implanted subcutaneously with a pellet of 3.8 mgm. of estrone, showing several subserous uterine tumors at typical locations. Absorption was at the rate of 11 μgm. daily, or 1.3 mgm. in 120 days.

Fig. 9B.—Dorsal view of the uterus shown in Fig. 9A, showing a chain of parametrial fibroids.

Fig. 9C.—Tumor at the hilum of the spleen in the same animal as in Fig. 9A.
increase with equilenin was smaller. The differences between the hysterotrophic actions were less significant than the differences between the fibromatogenic effects.

When a steady flow of estrogen is established from a subcutaneously implanted pellet, abdominal fibroids can be elicited with about 5 μgm. of estrone absorbed per day, as compared with 400 μgm. injected thrice weekly in the course of 4 months. This difference is explained by the assumption that the tumorigenic faculty of estrogens is dependent upon a continuous action upon the effector tissues.

Under the same experimental conditions which provided for continuous action quantities of equilenin, two or three times as large as the effective amounts of estrone, had no fibromatogenic action or only an insignificant effect.

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