The Effect of Exogenous Gonadotrophins on the Development of Experimental Ovarian Tumors in Rats*

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Since our first description of the induction of an ovarian tumor by transplantation of an ovary into the spleen of castrated rats (4), numerous reports have confirmed this result (7–11, 14).

The types of cellular change that occur in the transplanted gonad vary with the experimental animal employed. The ovarian tumor may metastasize in mice but not in rats (9); luteal proliferation without luteoma occurs in the guinea pig (12), but the monkey is refractory to this type of tumor induction (16); and different rat strains may show great resistance to testicular tumor formation, whereas ovarian tumors are readily obtained (6).

In spite of this somewhat variable response, it has been suggested that the development of the tumor is the result of inactivation by the liver of the hormones elaborated by the transplanted ovary (5). This would permit uninterrupted excessive stimulation by the pituitary and the release of the ovary from estrogenic inhibition. The possibility of elaboration of growth-promoting substances by the transplanted ovary must also be considered. Silverberg et al. (15), by subcutaneous transplantation of the hypophysis in mice with intrasplenic ovarian grafts, accelerated tumorigenesis. Miller et al. (13) demonstrated increased gonadotrophin in castrated mice with intrasplenic ovarian grafts by the use of parabionts. Bernstorf (1), however, recently reported that the intrasplenic ovary was refractory to the administration of exogenous gonadotrophins in the mouse.

The experiments to be described show that, in the Long-Evans strain of rats, the administration of exogenous gonadotrophin accelerates the development of both luteoma and granulosa-cell tumors when an ovary is transplanted to the spleen of a castrated rat.

MATERIALS AND METHODS

The rats employed in this study were of the Long-Evans strain, and they were divided into groups as follows:

Forty-eight young, adult, female animals were given intramuscular injections of 5 units of luteotrophin,2 3 times weekly. These were divided into Group A, which consisted of 29 castrated rats in which one ovary was transplanted to the spleen, and Group B, which consisted of nineteen rats in which one ovary was transplanted to the spleen and the other left in situ.

Thirty-nine young, adult, female animals were given intraperitoneal injections of 5 units of pregnant mares' serum (PMS),3 3 times weekly. These were divided into Group C, which consisted of 29 castrated rats in which one ovary was transplanted to the spleen, and Group D, which consisted of ten rats in which one ovary was transplanted to the spleen and the other left in situ.

Group E consisted of 25 young, adult, female rats which were castrated, had one ovary transplanted to the spleen, and received no exogenous hormones.

The rats were maintained on a complete stock diet and water available ad libitum. The animals were sacrificed at intervals of from 14 to 388 days, and a complete autopsy was performed. Histologic sections of the endocrine glands, genital organs, heart, lungs, liver, kidney, and spleen of each animal were examined.

RESULTS

The animals of Group A (Table 1), which received luteotrophin, were sacrificed at intervals of from 14 to 250 days. Eleven of these animals had

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2 Luteotrophin supplied as Luteotrophin Squibb by Squibb & Sons, New York.

3 Pregnant mares' serum supplied as Gonadin by the Cutter Laboratory, Berkeley, Calif.

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adhesions which permitted blood from the spleen to bypass the portal circulation, and the transplants failed to show growth or neoplastic development. Of the remaining eighteen animals, four showed luteomas which were found at 160, 180, 202, and 219 days, respectively. However, in animal No. 3604 (Table 1), a granulosa-cell tumor was found 175 days after transplantation (Figs. 3, 4).

In Groups B and D, in which one ovary was transplanted to the spleen and the other left in situ, the intrasplenic ovary failed to undergo growth, despite the administration of exogenous gonadotrophin (Figs. 1, 2). There was hypertrophy of the ovary in situ, but it was no greater than that reported in a previous series of untreated animals (2, 4).

Of the 29 animals of Group C, which received PMS (Table 2), there were fourteen animals with splenic adhesions that permitted circumvention of the portal circulation, and characteristic growth or neoplastic development of the transplant was not observed in these animals. In the fifteen animals without adhesions, two luteomas were seen at an interval earlier than in any control group, namely, at 90 and 127 days. Three of the granulosa-cell tumors occurred as early as 126, 150, and 194 days, which is half or less of the usual time of previously reported series (3).

The histologic pattern of the luteoma and of the granulosa-cell tumors of this investigation was identical with that described in previous reports (3, 5). No striking cytologic alteration in the pituitary was discerned that could be attributed to the exhibited exogenous gonadotrophins. The staining technic employed was hematoxylin-eosin and Poirrier's blue-eosin. Differential cell counts were not made.

The animals of Group E (Table 3), which received no gonadotrophin, served as a control group and reaffirmed the time relationships for the appearance of the luteoma that were described in the original reports (3, 5). These animals were sacrificed at fairly regular intervals during a 270-day

### TABLE 1

<table>
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<tr>
<th>Transplant</th>
<th>Uterus</th>
<th>Pathological Findings</th>
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<td>14</td>
<td>2</td>
</tr>
<tr>
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<td>2</td>
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</tr>
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<td>167</td>
<td>2</td>
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<tr>
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<tr>
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* Luteotrophin administered 5 units 5 times weekly, intramuscularly.
† A single figure denotes the diameter of a spheroidal mass in the spleen.
‡ The adhesions that circumvented the portal circulation are graded 1 to 4.
§ a = atrophic; e.s. = estrogenic stimulation.
& c.l. = corpora lutea; fol. = follicles.
|| In animal 3063 the adhesions did not bypass the portal circulation.
period in order to re-establish the earliest time for the development of the tumor. This was found to be 210 days for the luteoma. No granulosa-cell tumors were seen in the 270-day experimental period. In a previous larger group in which the animals were examined at shorter intervals, the usual time for the appearance of the luteoma was 6–7 months; however, in one animal it was found as early as 157 days (3). Fourteen animals with adhesions from the spleen to the parietal abdominal wall that circumvented the portal circulation showed a few partially involuted corpora lutea, but no evidence of luteal proliferation or neoplastic change in the ovarian transplant.

**DISCUSSION**

The observations of this and other laboratories indicate that a characteristic series of changes occurs after the transplantation of an ovary into the spleen in the castrated rat. The transplant first shows necrosis; regeneration is then evidenced by the development of showers of primordial follicles which undergo luteinization. The outstanding feature is the continuous appearance of primordial follicles and the failure of involution of the corpora lutea. The process continues for about 6 or 7 months and seems to change abruptly when there is an overgrowth of luteal tissue, which rather quickly pushes aside the remaining corpora lutea and takes on a uniform cellular appearance that fits the commonly described pattern of a luteoma. This tumor generally increases in size and may sometimes be cystic. After another interval, which usually is 10–12 months following transplantation, there appear in the luteoma clusters of cells of the granulosa type. These are arranged in the varying patterns ordinarily noted in the human granulosa-cell tumor. At present there is no explanation for

### TABLE 2

**EFFECT OF PMS* ON INTRASPLENIC OVARY IN CASTRATED FEMALE RATS (GROUP C)**

<table>
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<th>Transplant Age</th>
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<tr>
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<td>150</td>
<td>5</td>
<td>4+</td>
</tr>
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</table>

*PMS administered 5 units 3 times weekly, intraperitoneally.
† A single figure denotes the diameter of a spherical mass in the spleen.
‡ The adhesions that circumvented the portal circulation are graded 1 to 4.
§ e.s. = estrogenic stimulation.
¢ c.l. = corpora lutea; fol. = follicles.
£ Luteoma with early granulosa-cell tumor in a cystic transplant that retained corpora lutea (See Figs. 5, 6).
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the appearance of the luteoma before the granulosa-cell tumor. If adhesions which develop following transplantation permit blood from the spleen to bypass the liver, the above sequence of events does not occur.

Exceptions to this usual pattern of development of the tumor seem to occur under stimulation by exogenous hormones. This is illustrated in Figures 5 and 6, taken from animal No. 3211, that had been treated with PMS. There is a small area of luteoma separate from the intact, well defined corpora lutea. The transplant also has a cystic zone lined by compressed corpora lutea. Small nests of granulosa-cell elements are scattered through the luteoma. This is in contrast to the findings in control animals where corpora lutea are no longer recognizable by the time the granulosa-cell tumor appears.

In the present work it is apparent that PMS accelerated the development of the ovarian tumors, as evidenced by a shortened interval for the appearance of both the luteoma and granulosa-cell tumor in the intrasplenic ovary of castrated Long-Evans female rats. Luteotrophin did not stimulate luteoma production, but in one rat a granulosa-cell tumor was found as early as 175 days (Figs. 3, 4). The relative lack of effect by luteotrophin may be owing to either the commercial preparation per se or the route of administration. However, the possibility of an antihormone effect must also be considered, as it will be noted that in two animals of the PMS group, namely, No. 3465 and No. 3449, and in all four animals of the luteotrophin group, namely, Nos. 3986, 3748, 3982, 3985, the luteomas were 3–7 mm. in diameter, which is considerably smaller than that usually found in the controls.

The acceleration of tumorigenesis by PMS lends credence to the hypothesis that increased or uninhibited pituitary stimulation of the intrasplenic gonad is of prime importance in the genesis of these experimental tumors. However, pituitary stimulation may not be the only factor involved in the formation of these tumors, because the intrasplenic ovary of the animals of Groups B and D, where one ovary was left in situ, did not show any stimulation by exogenous gonadotrophin. Current similar investigations on hypophysectomized animals may elucidate this problem.

SUMMARY

In castrated female rats with one ovary in the spleen that received 5 units of luteotrophin intramuscularly, 5 times weekly, a luteoma appeared at 160 days, and one granulosa-cell tumor was found.

TABLE 3

<table>
<thead>
<tr>
<th>RAT NO.</th>
<th>AGE (days)</th>
<th>SIZE (mm.)</th>
<th>ADHESIONS</th>
<th>VAGINAL</th>
<th>PATHOLOGICAL</th>
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<td>1820</td>
<td>30</td>
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<td>0</td>
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<td>c.l., developing fol.</td>
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<td>a</td>
<td>c.l., developing fol.</td>
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<td>4</td>
<td>e.s.</td>
<td>atrophy of c.l.</td>
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<tr>
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<td>90</td>
<td>3</td>
<td>4</td>
<td>e.s.</td>
<td>atrophy of c.l.</td>
</tr>
<tr>
<td>1813</td>
<td>120</td>
<td>5</td>
<td>1</td>
<td>a</td>
<td>large c.l., developing fol.</td>
</tr>
<tr>
<td>1811</td>
<td>120</td>
<td>6 X 7</td>
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<td>1800</td>
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<td>4</td>
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<tr>
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<td>3</td>
<td>4</td>
<td>e.s.</td>
<td>occasional c.l.</td>
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</table>

* A single figure denotes the diameter of a spheroidal mass in the spleen.
† The adhesions that circumvented the portal circulation are graded 1 to 4.
‡ a = atrophic; e.s. = estrogenic stimulation.
§ c.l. = corpora lutea; fol. = follicles
in 175 days. In similarly prepared animals injected intraperitoneally with 5 units of pregnant mares' serum, 3 times weekly, luteomas appeared as early as at 90 and 127 days, and granulosa-cell tumors at 126, 150, and 194 days. Unilaterally castrated female rats with one ovary in the spleen served as controls to each of the above groups, and the exogenous hormones in both cases did not stimulate the intrasplenic ovary.

These findings are evidence that exogenous gonadotrophins may accelerate the development of both luteomas and granulosa-cell tumors in the rat ovary transplanted to the spleen of castrates. This lends credence to the hypothesis that increased or uninhibited pituitary stimulation of the intrasplenic ovary may be an important factor in the development of such tumors in the rat.

REFERENCES

6. ———. Tumor of Rat Testes Produced by Heterotransplantation of Infantile Testes to Spleen of Adult Castrate. Ibid., 59:4—8, 1945.

Fig. 1.—The atrophied ovarian tissue in the spleen shows a single corpus luteum composed of a few luteal cells surrounded by fibrous tissue. Above it are distorted follicles, two with deeply stained follicular cells (X50). Animal No. 3196 with right ovary in situ, left ovary in spleen, received PMS for 196 days.

Fig. 2.—The corpus luteum and follicles of Fig. 1 are enlarged and show that both retain an essentially normal cytological pattern (X200).

Fig. 3.—Distinct clusters of granulosa-type cells are invading through a background of luteal tissue, in the typical pattern of a granulosa-cell tumor (X100). Castrated animal No. 3604 with intrasplenic ovary received luteotrophin for 175 days.

Fig. 4.—An enlargement of Fig. 3 shows granulosa cells arranged in dense clumps and tubular spaces in a background of luteal tissue (X250).

Fig. 5.—This transplant is unusual because an early granulosa-cell tumor is seen in the presence of well defined corpora lutea (X20). Castrated animal No. 3211 with intrasplenic ovary which received PMS for 126 days.

Fig. 6.—A zone of the granulosa-cell tumor in Fig. 5 is enlarged to show a background of luteal cells (X200). In this tumor the cords and clumps of granulosa cells differ in their arrangement from that noted in Figs. 2 and 3. The two tumors illustrate variations in cellular pattern that may occur.
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