Effects of Anterior Hypophyseal Transplants on Intrasplenic Ovarian Grafts*

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In virgin mice of strain A, anterior hypophyseal transplants increased the incidence of mammary cancers; in castrate males bearing hypophyseal and ovarian grafts, the number of mammary cancers exceeded that noted after transplantation of ovaries alone. These findings were interpreted as owing to the release of gonadotrophins by the hypophyseal transplants, which stimulate the output of estrogen by the animal's own or the grafted ovaries (6, 9, 10). Therefore, it seemed of interest to investigate whether or not hypophyseal transplants might exert effects on intrasplenic ovarian grafts which are already exposed to increased activity of the animal's own hypophysis.

MATERIALS AND METHODS

One hundred and twenty-three mice of the closely inbred strain A were ovariectomized at the age of 3–4 weeks. At the time of ovariectomy, one ovary was transplanted into the spleen. Sixty-two of the animals received no further treatment. One to 3 weeks after ovariectomy, the remaining 61 mice received grafts of four anterior hypophyses obtained from closely related donors 1–3 months of age. Fifty-one mice received anterior hypophyses from male donors, seven mice received hypophyses from female donors, and two mice received combinations of two male and two female, or one male and three female hypophyses, respectively. In one case, the sex of the donors was not recorded. The technic of transplantation has been described previously (7, 9). Transplanted hypophyses may remain alive and function through life; yet, with an increasing interval of time after grafting, many transplants undergo fibrosis or resorption. In 16 of the 36 animals used for the present investigation, hypophyses or remnants thereof could be identified microscopically. The results of cytological and cytochemical studies of such grafts, which are presently being carried out, will be reported at a later date.

MICROSCOPIC EXAMINATION

In Tables 1 and 2, the interval from the time of grafting of the ovaries, the number of mice, the number of ovarian tumors and of hyperplastic ovaries found, and the microscopic findings are given for all animals. The grade of the change is indicated by one or several + signs placed in parentheses behind the number of mice showing the respective change. In Table 3, the tumors are classified according to their microscopic structure. Uniformly enlarged and proliferating ovaries, yet containing the typical ovarian structures, were

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### Table 1
**Ovarian Grafts Not Grossly Enlarged**

<table>
<thead>
<tr>
<th>Months after grafting of ovaries</th>
<th>No. of ovaries showing</th>
<th>Increase of</th>
<th>Presence of</th>
<th>No. of ovaries showing</th>
<th>Increase of</th>
<th>Presence of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>lutein tubules</td>
<td>tissue</td>
<td></td>
<td>lutein tubules</td>
<td>tissue</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Young</td>
<td>Old</td>
<td></td>
<td>Young</td>
<td>Old</td>
</tr>
<tr>
<td>1-5</td>
<td>3</td>
<td>3 (+)</td>
<td>0</td>
<td>3 (+)</td>
<td>1 (+)</td>
<td></td>
</tr>
<tr>
<td>6-8</td>
<td>2</td>
<td>2 (+)</td>
<td>0</td>
<td>2 (+)</td>
<td>1 (+)</td>
<td></td>
</tr>
<tr>
<td>9-12</td>
<td>2</td>
<td>2 (+)</td>
<td>1 (+)</td>
<td>1 (+)</td>
<td>1 (+)</td>
<td></td>
</tr>
<tr>
<td>Irrespective of duration of exp.</td>
<td>7</td>
<td>7 (+)</td>
<td>1 (+)</td>
<td>6 (+)</td>
<td>3 (+)</td>
<td>3 (+)</td>
</tr>
</tbody>
</table>

*One animal had an early mammary tumor.*

### Table 2
**Ovarian Grafts Grossly Enlarged**

<table>
<thead>
<tr>
<th>Months after grafting of ovaries</th>
<th>No. of hyperplastic ovaries showing</th>
<th>Increase of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pres. Young</td>
</tr>
<tr>
<td></td>
<td></td>
<td>lutein tubules</td>
</tr>
<tr>
<td>1-5</td>
<td>7*</td>
<td>5 (+)</td>
</tr>
<tr>
<td>6-8</td>
<td>8*</td>
<td>4 (+)</td>
</tr>
<tr>
<td>9-12</td>
<td>8*</td>
<td>6 (+)</td>
</tr>
<tr>
<td>Irrespective of duration of exp.</td>
<td>23*</td>
<td>10 (+)</td>
</tr>
</tbody>
</table>

*Classification is given in Table 3.*

† One animal had, in addition, a mammary tumor.

### Table 3
**Microscopic Classification of the Tumors**

<table>
<thead>
<tr>
<th>Months after ovarian grafting</th>
<th>Total no. of tumors and cysts</th>
<th>Types of growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-5</td>
<td>3</td>
<td>3 granulosa-cell tumors (2 cystic)</td>
</tr>
<tr>
<td>6-8</td>
<td>4</td>
<td>4 granulosa-cell tumors (2 cystic)</td>
</tr>
<tr>
<td>9-12</td>
<td>6</td>
<td>3 granulosa-cell tumors (2 cystic)</td>
</tr>
<tr>
<td>Irrespective of duration of exp.</td>
<td>15</td>
<td>10 granulosa-cell tumors (6 cystic)</td>
</tr>
</tbody>
</table>

*Measurements: 3X3X3 mm.
† One animal had, in addition, a mammary tumor.
‡ Measurements: 5X5X5 mm., 4X4X5 mm.
¶ Measurements: 6X7X8 mm.*
considered as hyperplastic; tumors were diagnosed when proliferating nodules free from pre-existing ovarian tissue were seen. The following description and symbols apply to the findings in the grossly nonenlarged as well as hyperplastic ovaries.

**Follicles.**—(+) stimulation: numerous mitotically proliferating primordial and small or medium-sized follicles are seen; (+ +) stimulation: many large proliferating follicles are present with or without follicular fluid.

**Tubules.**—(+) some tubules lined by cuboidal epithelium are found—they are often situated near the surface or the hilum of the ovary; (+ +): several foci of tubular glands are noted; (+ + +): small adenomatous formations are observed.

**Young lutein tissue.**—An increase of (+) means the presence of large foci of eosinophilic lutein cells; of (+ +) that of coalescent layers of lutein tissue; of (+ + +) that of large lutein bodies occupying most of the ovary.

**Old lutein tissue.**—Consists of large, vacuolated, clear cells or of cells containing fine, yellowish, granular "ceroid" pigment. An increase of (+) is characterized by small isolated groups of cells, one of (+ +) by coalescent foci of old lutein tissue, and one of (+ + +) by diffuse replacement of the ovarian tissue by ceroid cells.

**Intrasplenic Ovarian Grafts without Hypophysial Transplants**

- **Grossly nonenlarged ovarian grafts (7 of 30 mice).**—Proliferating follicles were present in all grafts removed 1–12 months after transplantation. A few tubules were noted in a single ovary out of seven removed 12 months after grafting. Increased numbers of young lutein tissue were seen in all ovaries of the younger age groups, whereas old lutein tissue was found only in one of three grafts 5 months old, and in one of two grafts 7 months old. Only after 9–12 months of intrasplenic growth did the ovaries contain abundant old lutein tissues.

- **Grossly enlarged ovarian grafts (23 of 30 mice).**—One to 5 months after transplantation, focal hyperplasia was noted in four, and granulosa-cell tumors in three of seven mice, 5 or 6 months old. Two of the tumors showed cysts, and one disclosed tubules and teleangiectases. Six to 8 months after transplantation, granulosa-cell tumors were present in four of eight grafted ovaries. Two of these growths contained blood, phagocytosed blood pigment or small cysts, and some tubules. The longer the transplant was allowed to grow, the more conspicuous the latter elements as well as old lutein tissue became. After 9–12 months, tumors were found in six of eight ovarian grafts: three were of granulosa-cell type, two were mixed tumors, and one was a luteoma. In all hyperplastic ovaries, follicular growth was stimulated, and in half it was marked. Tubules were observed in one of four grafts each 5 or 8 months old, and they were conspicuous in one of two grafts 10 months of age. A great deal of young lutein tissue was present in the ovaries of all age groups. One of four grafts 5 months old, and three of six grafts 6–12 months of age showed old lutein tissue.

- **Intrasplenic Ovarian Grafts with Subcutaneous Hypophysial Transplants**

- **Grossly nonenlarged ovarian grafts (11 of 36 mice).**—One to 5 months after transplantation, four of five animals showed living hypophysal grafts. Three mice had received male and one had received female hypophyses, the fifth being the one in which the sex of the donors had not been recorded. Follicular growth was slightly stimulated in two, and markedly intensified in three of five ovarian grafts; in four ovaries tubules were present. All ovarian transplants contained increased numbers of young lutein cells; in one graft, there were some, and in another numerous foci of old lutein cells. One ovarian graft was fibrosed in spite of the presence of living hypophysal tissue (male donors). The one mouse examined 7 months after the transplantation of male hypophyses showed, in the ovarian graft, stimulation of follicular and tubular growth and large amounts of young and old lutein tissue. Hypophysal grafts were not identified microscopically in this case. Nine to 12 months after transplantation, follicular stimulation was accentuated in three, moderate in one, and absent in another of five ovarian grafts. Two ovaries contained clusters of tubules. Increased layers of young lutein tissue were noted in three grafts, and in four of five ovaries large areas were replaced by old lutein tissue. Hypophysal grafts were not found except for one mouse in which a small lutein cyst had developed. In all experiments of this group, male hypophyses had been grafted.

- **Grossly enlarged ovarian grafts (25 of 36 mice).**—One to 5 months after transplantation, hyperplastic granulosa cells were seen in one ovary in association with viable hypophysal tissue (male donors). There were tubules and abundant young and old lutein tissue in this ovary. Mitoses of young lutein cells were particularly impressive in areas directly underneath the surface epithelium of the ovary. Tumors were found in five of six mice 5 or 6 months old. Three of the neoplasms were granulosa-cell tumors (male hypophyses had been transplanted in two, and female hypophyses in one mouse), one (Fig. 1) was a mixed tumor (female hypophyses had been grafted), and one was a lu-
teoma (female hypophyses had been grafted). Hypophyseal grafts were identified microscopically in four tumor-bearing mice. In one animal with a granulosa-cell tumor, no hypophyseal grafts were found. Small cysts, hemorrhages, phagocytes of blood pigment, and thin-walled dilated vessels were observed frequently. Six to 8 months after transplantation of hypophyses (male hypophyses had been grafted into all recipients), four ovarian tumors were found, while three transplants were hyperplastic. In the latter, follicular growth was stimulated; tubular growth was present in three grafts, and it had reached major proportions in one of them; large amounts of fresh and old lutein tissue were noted in two ovaries. Of the tumors, one was a granulosa-cell tumor, one a mixed tumor (Fig. 2), one a luteoma (Fig. 3), and one was a large cyst with lutein tissue at the periphery. In the mouse bearing a granulosa-cell tumor, viable hypophyseal tissue was seen, while in the three remaining animals the hypophyseal grafts had undergone fibrosis. All the mice sacrificed 9–12 months after transplantation of the hypophyses had received pituitaries from male donors. Hyperplastic ovaries were found in three and tumors in nine grafted ovaries. These grafts disclosed follicular stimulation, and two of them showed adenoma-like formations of tubules and focal fibrosis. Abundant young and old lutein tissue was seen in three grafts. Of the tumors, three were mixed tumors, three were luteomas, and three were large cystic growths, two of them containing lutein tissue at the periphery. Viable hypophyseal tissue was found with one luteoma and with one large cyst containing lutein tissue at the periphery.

DISCUSSION

The present findings regarding tumor growth in intrasplenic ovarian grafts are essentially in agreement with those of other investigators (1–5). Moreover, we made some additional observations by studying the changes in non-neoplastic or pre-neoplastic grafts. There occurred in these transplants changes similar to those found in aged ovaries of untreated mice, namely, formation of epithelial tubules, cysts, and clear or ceroid-laden lutein cells. In mice of strain A, these findings become common between the ages of 14 and 16 months but are rare in ovaries less than 1 year old (6). In intrasplenic ovarian grafts of mice receiving no further treatment, these age changes began to appear as early as 5 months after transplantation, i.e., in ovaries about 6 months old. In 8-month-old ovaries, grafts left in the spleen for 7 months, these changes were seen regularly. Presumably, the same factors that are involved in the pathogenesis of tumors are also responsible for the acceleration of age changes in the nontumorous intrasplenic ovarian grafts.

Up to 8 months following transplantation, hypophyseal transplants were identified in eight of nine mice with intrasplenic tumors. Nine to 12 months following transplantation, only two of nine tumor-bearing mice showed hypophyseal grafts. These findings are in harmony with the previously reported progressive fibrosis and resorption of such transplants (7, 9, 10). In the absence of living grafts it is obviously impossible to state whether or not the grafts had originally taken and functioned. There is, however, indirect evidence in support of these possibilities, such as the microscopic appearance of the mammary glands. Nine to 12 months after transplantation, all mice with intrasplenic tumors showed impressive stimulation of the mammary glands, ranging in degree from ductal proliferation and secretion to acinar growth. Estrogen can be ruled out as the cause of this stimulation, since intrasplenic tumors would not grow in the presence of estrogen. On the other hand, pituitary secretions, as produced by grafted hypophyses, have been shown to enhance mammary growth (7, 9, 10).

In spite of the comparatively limited number of experiments, the following conclusions may be made:

Under the influence of subcutaneous anterior hypophyseal grafts, the intrasplenic ovarian transplants underwent changes that distinguished them from similar transplants not exposed to equally excessive hypophyseal stimulation. The differences between these two types of ovarian grafts were quantitative or concerned the time of appearance of age changes within the grafts. The processes of growth and aging were hastened in intrasplenic ovarian grafts of mice that had received hypophyseal transplants: follicular growth and development were stimulated; proliferation of theca cells was accentuated, and the latter cells were, at an early date, converted into lutein cells. An early increase of lutein tissue ensued, and, within the latter, old lutein tissue became rapidly predominant over young lutein cells. Whether or not androgenic hormone is produced by the clear cells (8) has no bearing on the present investigation. The increased growth of lutein cells is probably attributable to the action of the luteinizing hormone produced by the transplanted hypophyses, which in 31 out of 36 recipients were derived from male donors. Moreover, under the influence of anterior hypophyseal transplants, epithelial tubules proliferated and appeared prematurely. These findings may likewise be interpreted as an acceleration.
of age changes within the grafted ovaries caused by the transplanted hypophyses. The present experiments were terminated after 1 year of observation. This may represent too early a stage to determine whether or not all nontumorous intrasplenic ovaries will ultimately undergo cyst formation—possibly a manifestation of exhaustion after excessive growth—as do untreated senile ovaries. The increased number and size of cysts appearing in grafts toward the end of the first year of observation certainly suggest such a possibility.

Regarding the intrasplenic ovarian tumors, there was no evidence that the ultimate amount of newly formed neoplastic tissue was larger in the presence than in the absence of additional hypophyses. There was, however, in mice carrying hypophyseal grafts a trend toward an accelerated rate of tumor growth during the early stages following transplantation. In correspondence with our findings in nontumorous grafts, the neoplasms themselves showed, under the influence of hypophyseal transplants, an increased occurrence of lutein tissue—an effect that has recently been observed following injections of pregnant mare serum into mice bearing intrasplenic ovaries (4). In the present experiments, a mixed tumor and a luteoma were seen as early as 5 months after transplantation; and after 9–12 months, three mixed tumors, three luteomas, and two cysts with lutein tissue were found. This was in marked contrast to the findings in intrasplenic ovarian tumors of mice not receiving hypophyseal grafts in which luteomas are rare (4, 5), and in which we observed only one as late as 9 months after transplantation. Conversely, granulosa-cell tumors were noted in mice of all age groups not receiving hypophyseal grafts, whereas in the presence of the latter we found no granulosa-cell tumors in old ovarian grafts.

SUMMARY

In ovariectomized mice of strain A, the effects of subcutaneous anterior hypophyseal transplants on intrasplenic ovarian grafts were studied. The hypophyseal transplants promoted growth and age changes in the grafted ovaries: proliferation of follicles and lutein tissue was stimulated; epithelial tubules and old lutein tissue appeared prematurely and were increased in amount; intrasplenic tumors tended to appear early; and the ratio of granulosa-cell tumors to luteomas shifted in favor of the latter. These effects are considered to be caused by the action of both the follicle-stimulating and luteinizing hormones given off by the grafted hypophyses.

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Effects of Anterior Hypophyseal Transplants on Intrasplenic Ovarian Grafts

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