On Tumor Formation in Gonadal and Hypophyseal Transplants into the Anterior Eye Chambers of Gonadectomized Rats

Stig Kullander

(Biological Department, Antoni van Leeuwenhoek, Amsterdam)

SUMMARY

Small pieces of ovarian, testicular, or ovarian plus hypophyseal tissue were transplanted to the anterior eye chambers of rats, aged 2 months, which were simultaneously gonadectomized. One year after the operations the animals were examined. The ovarian and testicular grafts had taken and had been transformed to tumors in a very high percentage of cases. The ovarian and testicular tumors were morphologically similar to each other, and both had estrogenic as well as androgenic properties. Ovarian and hypophyseal grafts in the same eye grew rapidly into large tumors (granulosa-cell tumors and chromophobic hypophyseal adenomas), with signs of progesterone and prolactin secretion, respectively. One of the animals had a mammary adenocarcinoma, and the mammary glands in all were highly stimulated and secreting milk.

It is concluded that prolactin may stimulate the growth and influence the secretion of granulosa-cell tumors. Gonadal and mammary tumors are discussed in relation to the experimental results.

It has been shown earlier by the present author (12) that ovarian follicles autotransplanted to the anterior eye chamber of 2-month-old spayed rats usually cause granulosa-cell tumors within 1 year. The tumor development might be explained by the fact that the fragment of grafted ovarian tissue has too small a production of steroid hormones to inhibit the hypophysis. The increased amount of hypophyseal gonadotrophins then produced presumably stimulates tumor formation.

This work has now been repeated in a homozygotic rat strain, making it possible also to observe the growth of the follicles and the hormonal action of granulosa-cell tumors formed in gonadectomized male rats. It has been extended to the transplantation of small fragments of testicular tissue instead of ovarian follicles, to ascertain whether it is possible to produce testicular tumors in the same way. In some cases fragments of an anterior hypophyseal lobe have been placed beside the follicles in the anterior eye chambers in an attempt to accelerate the tumor growth by supplying further amounts of hypophyseal hormones. Intact and spayed rats, with only a hypophyseal graft in their eye chambers, served as controls to these last-mentioned animals.

MATERIALS AND METHODS

The experimental animals were about 2 months of age at the time of operation, and all belonged to the inbred homozygotic R strain raised at the Institute. The animals were kept four or five to a cage, and were given commercial pellets and tap water ad libitum and once a week a handful of wheat. Bilateral oophorectomy or orchidectomy was performed through a midline abdominal incision. With the aid of a stereomicroscope (×15), follicles from the interior of an ovary were dissected carefully and transplanted to the anterior eye chambers (also under ×15 enlargement) of male and female gonadectomized hosts. In other animals, pieces of testicular tissue of about the same size as a follicle were grafted instead. The testes
were taken from 1–2-day-old rats. Some animals had an ovarian follicle and a small piece of an anterior hypophyseal lobe transplanted (from 2-month-old female rats) into the right anterior eye chamber and simultaneously a single follicle in the left. Only a hypophyseal graft was placed in the anterior eye chambers of some intact and spayed females. The animals were killed about 1 year later. The grafts were removed, fixed in Susa, embedded in paraffin, and sectioned. The sections were stained with hematoxylin-eosin or with Azan in the case of the hypophyseal or hypophyseal-ovarian grafts. The animals were carefully autopsied in a search for other tumors; and the development of uterus, vagina, the mammary glands, the prostate, and seminal vesicles was studied.

The types and numbers of transplantations are given in Table 1.

<table>
<thead>
<tr>
<th>Type of transplantation</th>
<th>No.</th>
<th>Grafts studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testicular transplants to castrated males</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Testicular transplants to spayed females</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Ovarian follicles to castrated males</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Ovarian follicles to spayed females</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Ovarian follicle + piece of hypophyseal anterior lobe to intact or spayed female</td>
<td>10</td>
<td>10+10</td>
</tr>
<tr>
<td>Piece of hypophyseal anterior lobe to intact or spayed female</td>
<td>5+5</td>
<td>5+5</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>40</td>
<td>70</td>
</tr>
</tbody>
</table>

**RESULTS**

**Testicular transplants** grew and were transformed to tumors in varying sizes in all the ten transplantations made to male hosts, and in nine of the ten transplantations made to female hosts. Macroscopically the tumors were slightly yellowish and had a smooth surface. All were well vascularized from the iris, like all transplantations in the present investigation. The cut surface of the tumors were soft and richly vascularized. On the cut surface, there were bluish-red spots (blood-filled spaces) between solid yellowish or white parts. The follicles in the opposite eyes had also grown and had become tumors in all cases. Microscopically the combined hypophyseal-ovarian transplant had been transformed to a mixed hypophyseal-ovarian tumor (Fig. 11), a chromophobe hypophyseal adenoma, and a granulosa-cell tumor. In the opposite eye there was a pure granulosa-cell tumor (Fig. 12). The granulosa-cell tumor that was growing together with the hypophyseal adenoma was far bigger than that in the opposite eye, though of about the same histological picture. The mammary glands of these animals were strongly developed. The tumors could easily be recognized. They were filled with a milky fluid. This was not found in the animals of the other series. Histologically an active picture with secretion could be seen (Fig. 13). In one animal a mammary tumor (30 × 30 × 10 mm) had developed that histologically was a highly differentiated adenocarcinoma with wide ducts. Polymorphism and mitoses were seen. This mammary tumor was remarkable, since the mammary tumor frequency of this rat strain is very low.

In the vaginal mucosa, a picture of progestero-
genic activity with mucification, not seen in the animals of the other series, was detected (Fig. 14). Besides the progesterogenic activity there must have been estrogenic activity as well, because the vaginal epithelium was proliferated. The uterine horns were thick.

**Hypophyseal transplants** to intact or spayed females had taken in all cases (ten out of ten grafts) but had only grown slightly and had not formed tumors.

**DISCUSSION**

Chromophobic prolactin-producing adenomas of the anterior pituitary occur spontaneously in old rats but can also be experimentally induced by chronic administration of estrogens (1, 3, 6, and others). The prolactin causes hyperplasia of all elements of the mammary glands, in which milk secretion is induced (6).

It is probable that estrogen from the follicles placed beside the hypophyseal transplants in the present experiments had a stimulatory action on the hypophyseal cells, which then formed the hypophyseal chromophobic tumors. Direct circulatory connection between hypothalamus and hypophysis thus seems unnecessary for this action of estrogen.

A transplanted pituitary seems to have almost no other function than that of producing prolactin (see 15). This function has probably been accentuated in the present experiments with an ovarian transplant (stimulated by the spayed host own hypophyseal activity) besides the pituitary graft. Desclin (4) claims that the lactotrophic activity of a hypophyseal graft is considerably stimulated by estrogen.

In rats there is a positive correlation between the occurrence of spontaneous chromophobic hypophyseal adenomas and mammary tumors (13). Prolactin and prolactin-producing hypophyseal transplants or tumors have been strongly related to mammary tumor genesis in mice, besides the ovarian factors, estrogen and progesterone (see 15 and others). It is possible that in the present experiments a continuous increased prolactin production in the animals with combined hypophyseal-ovarian grafts to the anterior eye chambers is taking place. In addition, there must be an ample supply of steroid hormones from the induced granulosa-cell tumors. The remarkable development of the mammary glands and the occurrence of mammary carcinoma could thus be explained.

The growth of granulosa-cell tumors seems to be accelerated by hypophyseal adenomas, probably by prolactin. This hormone seems also to act locally on the tumor tissue, since the ovarian tumor growth was more pronounced near the adenoma than in the opposite eye without hypophyseal tissue. The type of hormone production of the granulosa-cell tumor is also changed to progesterogenic-giving vaginal mucification under the influence of prolactin. Although the morphology seems similar in different granulosa-cell tumors it is possible that the type of hormone produced may differ according to the prolactin-producing capacity of the hypophyses of the animals, thus explaining differences sometimes seen in target organs. According to the present experiments, there seems to be no certain relation between the cell type in the granulosa-cell tumors and the function, although there may be one between the rate of cell growth and the function. The steps in the biosynthesis of steroids from progesterone via androgens to estrogens might not all be made in rapidly growing tumor cells.

Testicular tumors of interstitial cells (Leydig cells) have been induced earlier, though only in mice, by prolonged administration of estrogens. Estrogen treatment gives impairment of spermatogenesis and tumors proliferation of intertubular primitive mesenchymal cells more or less similar to interstitial cells (for references see [7]).

The induction of interstitial-cell tumors (in one case the tumor looked like a granulosa-cell tumor) in testicular grafts has been reported to be successful, but only in rats (2, 10, 17) after testes were grafted to the spleens of gonadectomized animals. However, in these tumors, sex hormones, if secreted, are largely inactivated in the livers of the hosts. Metastases have not been reported, and only a single subtransplant—to the spleen of a castrated rat—is reported to have grown successfully. This method is based on the principle that by castration the normal pituitary-testis balance is broken, with an excess secretion of interstitial cell-stimulating hormone from the hypophysis. The androgens produced by the intrasplenic testicular transplant are inactivated in the liver, the production of interstitial cell-stimulating hormone not being inhibited. In only about 50 per cent of animals tested with this procedure, however, have tumors been reported. The same induction mechanism may be postulated in the present experiments with intraocular testicular grafts that give a high percentage of testicular tumors 1 year after transplantation. With this method, the cryptorchid environment involved in the transplantation to the spleen is ruled out. Recent experimental evidence suggests that cryptorchism plays an important role in testicular tumor induction (8).

The intraocular micrografting technic also pro-

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1 See also (16).
vives a method of studying the sex hormone production of the tumors formed. Estrogen production by the male gonad is well established, but the cell type that secretes testicular estrogen is uncertain. The intratubular Sertoli cells are most generally considered the primary source, but high levels of estrogens were found in the urine of a male dog bearing a Leydig-cell tumor (14). That the interstitial tumor may have a bisexual hormone production is confirmed by the present experiments. Estrogens as well as androgens seem to be produced. Androgen secretion from granulosa-cell tumors in rats has previously been suggested by Kullander (11) and Johnsson (9) and is confirmed in the present experiments.

Morphologically, the testicular tumors found were similar to the granulosa-cell tumors formed in ovarian transplants. Since granulosa-cell tumors in rats very probably may form from theca interstitial cells (12) and the testicular tumors seemed to rise from proliferation of interstitial cells—and both the ovarian and the testicular tumors produced both androgens and estrogens—it is tempting to postulate that in both types of gonads there are interstitial mesenchymal cells capable of forming tumors when influenced by increased amounts of hypophyseal gonadotrophins, and capable of a bisexual hormone production as well. Since progesterone is a probable intermediate in biosynthesis of both androgens and estrogens (5), progesterone secretion may also occur in the same cells by loss or inhibition of certain enzymes.

A disturbed "feed-back" mechanism may explain the tumor formation in both the ovarian and testicular micrograffs, and it is probable that the pathogenesis of ovarian and testicular tumors is similar. When the tumors have developed and peripherally exert a strong hormonal effect, the situation is the same as in spontaneous gonadal tumors. Then the tumor cells may be independent of a raised blood level of hypophyseal gonadotrophins, since this is probably depressed by estrogens-androgens.

REFERENCES
The hypothalamus and the hypophysis thus seem unnecessary for the action of the hypophyseal cells, which then formed the hypophyseal chromophobie tumors. Direct stimulation of the mammary glands, in which milk secretion is induced (6), besides the pituitary graft. A transplanted pituitary seems to have almost the same effect as estrogen. It is probable that estrogen from the follicles stimulates the pituitary gland, which in turn stimulates the mammary glands. However, the pituitary gland is not the only source of estrogen, as it is also produced in other tissues, such as the liver and the brain.

In addition to the prolactin, there must be an autonomic supply of steroid hormones from the pituitary gland to the mammary glands. The occurrence of spontaneous chromophobie tumors is explained by the presence of prolactin. This hormone seems also to act locally on the tumor tissue, since the ovarian tumors are induced by prolactin. This function has probably been accentuated in the present experiments with an ovarian graft in the pituitary transplant (stimulated by the spayed host own estrogen). Besides the progesterogenic activity there must be an estrogenic activity with mucification, not seen in the males. The prolactin causes hyperplasia of all mammary glands and can be experimentally induced by the ovarian hormones. However, the morphology of the tumors seems to be different in different granulosa-cell tumors. The type of hormone produced may differ, since the percentage of testicular tumors 1 year after transplantation is reported to have grown successfully. This method is based on the principle that the androgens produced by the intrasplenic testicular transplant are inactivated in the liver, the percentage of testicular tumors not being inhibited. In only about 50 per cent of the animals tested with this procedure, however, have testicular tumors been reported. The same induction mechanism may be postulated in the present experiments on October 22, 2017. © 1960 American Association for Cancer Research.
Fig. 5.—Ovarian tumor in a female host. 5μ. H. & E. ×200.
Fig. 6.—Ovarian tumor in a male host. 5μ. H. & E. ×200.
Fig. 7.—Compare Figure 6. The ovarian tumor in the opposite eye. 5μ. H. & E. ×200.
Fig. 8.—The vaginal mucosa in a female host with ovarian tumor. 5μ. H. & E. ×200.
A transplanted pituitary seems to have almost the autonomic action of the grafts obtained from the spayed host. It is probable that estrogen from the follicle causes hyperplasia of all males taken in all tests (ten out of ten grafts). The prolactin causes hyperplasia of the glands, in which milk secretion is induced.

Besides the progesterogenic activity there must have been estrogenic activity as well, because the hypophyseal adenomas and mammary tumors (13). The prolactin causes hyperplasia of all glands, in which milk secretion is induced.

It is probable that estrogen from the follicles causes hyperplasia of all glands, in which milk secretion is induced. Although the morphology seems to be accelerated by hypophyseal adenomas, probably the type of hormone produced may differ from the hypophyseal grafts obtained from the spayed host.

In testicular grafts has been reported to be successful, but, only in rats (10, 17) after testes were placed beside the hypophyseal transplants in the animals of the other series, was detected (Fig. 14). This function has probably been accentuated in the present experiments with an ovarian transplant (stimulated by the spayed host's own estrogens). The prolactin causes hyperplasia of all glands, in which milk secretion is induced.

In addition, there must be an estrogenic activity as well, because the vagal epithelium was proliferated. The uterine horns were thick.

Percentage of testicular tumors 1 year after transplantation. With this method, the cryptorchid enzyme-giving vaginal mucification under the influence of estrogens might not all be made in rapidly growing tumors. However, in these tumors, sex hormones, if secreted, are largely inactivated in the livers of the animals tested with this procedure, however, have not been reported. The same induction mechanism may be postulated in the present experiments on October 22, 2017. © 1960 American Association for Cancer Research.
Fig. 9.—The ventral prostate lobe in a male host with ovarian tumor. 5μ. H. & E. X200.

Fig. 10.—The seminal vesicle in a male host with ovarian tumor. 5μ. H. & E. X200.

Fig. 11.—Hypophyseal-ovarian tumor. 5μ. Azan. X200.

Fig. 12.—Ovarian tumor in a female host with a hypophyseal-ovarian tumor in the opposite eye. 5μ. H. & E. X200.
The hypothalamus has a direct connection with the pituitary gland, and any other function than that of producing prolactin is unnecessary for this action of estrogen.

In the present experiments, with an ovarian transplant (stimulated by the spayed host's ovaries), besides the pituitary graft, mammary tumors were induced in all cases (ten out of ten grafts) but had only grown slightly and had not formed horns. 

In addition, there must be an estrogenic activity with mucification, not seen in the opposite eye without hypophyseal tissue. The type of hormone production of the hypophyseal graft is considerably stimulated in the present experiments with an ovarian transplant or tumors that have been strongly reduced in the animals with combined hypophyseal-ovarian grafts to the anterior eye chambers.

Besides the progesterogenic activity there must be a stimulatory action on the occurrence of spontaneous chromophobic tumors. This function has probably been accentuated in the present experiments with an ovarian transplant (stimulated by the spayed host's own ovaries) besides the pituitary graft.

A transplanted pituitary seems to have almost no effect on the growth of granulosa-cell tumors in mice, by prolonged administration of estrogens. It is possible that in the present experiments the growth of granulosa-cell tumors seems to be accelerated by hypophyseal adenomas, probably by prolactin. This hormone seems also to act locally on the tumor tissue, since the ovarian tumor, which is a chromophobic cell tumor, is broken, with an excess secretion of interstitial cell-stimulating hormone.

The induction of interstitial-cell tumors (in one case the tumor looked like a granulosa-cell tumor) have been induced earlier, though only in rats (10, 17) after testes were grafted to the spleens of gonadectomized animals. Metastases have not been reported, and the induction mechanism may be postulated in the present experiments as explained.

In rats there is a positive correlation between the occurrence of spontaneous chromophobic tumors and the function. The steps in the biosynthesis of steroids from progesterone via androgens to estrogen can also be experimentally induced by the autonimous pituitary.

There must be one between the rate of cell growth and the formation of interstitial-cell tumors. The percentage of testicular tumors 1 year after transplantation. With this method, the cryptorchid environment involved in the transplantation to the spleen is ruled out. Recent experimental evidence suggests that cryptorehism plays an important role in testicular tumor induction (8).

Testicular tumors might not all be made in rapidly growing interstitial elements of the mammary glands, in which milk secretion is induced (6).

In testicular transplant are inactivated in the liver, the androgens produced by the intrasplenic testis are similar in different granulosa-cell tumors it is possible that the type of hormone produced may differ according to the prolactin-producing capacity of luteotrophie activity (see 15). This function has probably been accentuated in the present experiments but, only in rats (10, 17) after testes were grafted to the spleens of gonadectomized animals. Metastases have not been reported. The same induction mechanism may be postulated in the present experiments as explained.

The androgens produced by the intrasplenic testis might not all be made in rapidly growing interstitial elements of the mammary glands, in which milk secretion is induced (6).
Fig. 13.—The mammary gland in a host with one hypophyseal-ovarian and one ovarian tumor. 5 μ. H. & E. X 200.

Fig. 14.—The vaginal mucosa in a host with one hypophyseal-ovarian and one ovarian tumor. 5 μ. H. & E. X 300.
The hypothalamus and pituitary gland have a functional connection, with no other function than that of producing prolactin. The hypophysis thus seems unnecessary for this action of estrogen.

The hypophyseal cells, which then formed the hypophyseal chromophobe tumors. Direct circumswritten hypophyseal adenomas and mammary tumors (13). The prolactin causes hyperplasia of all mammary glands, in which milk secretion is induced (6). Prolactin and prolactin-producing hypophyseal glands have been estrogenic activity as well, because the anterior pituitary gland has been experimentally induced by the autonomic influence by estrogen. It is probable that estrogen from the follicle acts locally on the tumor tissue, since the ovarian tissue inducing granulosa-cell tumors. The remarkable development of the mammary glands and the occurrence of mammary carcinoma could thus be explained.

Hypophyseal transplants or tumors have been strongly reduced granulosa-cell tumors. The remarkable development of the mammary glands and the occurrence of mammary carcinoma could thus be explained.

The induction of interstitial-cell tumors (in one case the tumor looked like a granulosa-cell tumor) of interstitial cells (Leydig cells) have been induced earlier, though only in males. The androgens produced by the intraocular testicular transplant are inactivated in the liver, the percentage of testicular tumors 1 year after transplantation with intraocular testicular grafts that give a high yield of interstitial cell-stimulating hormone from the hypophysis.

The intraocular micrografting technique also provides the necessary supply of steroid hormones from the intact or spayed host to intact or spayed female gonadectomized animals. The induction of interstitial-cell tumors is due to interstitial cell-stimulating hormone from the hypophysis. The cryptorchid environment involved in the transplantation to the spleen is ruled out. Recent experimental evidence suggests that cryptorchism plays an important role in testicular tumor induction (8).

In rats there is a positive correlation between the estrogenic activity with mucification, not seen in the vagal epithelium was proliferated. The uterine horns were thick.
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