Studies on Hypophyseal Isografts in Mice

I. Biologic Aspects

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Summary

Experiments are reported on the induction of mammary carcinoma in intact, mammary tumor agent (MTA)-free female (♀ O20 × ♂ DBAf)F₁ mice by single and multiple (4) prolactin-producing hypophyseal isografts in the kidney. Tumor incidences varying between 67 and 88% were recorded in the treated groups as against 2% in virgin controls. Identical treatment of intact and castrated males proved totally ineffective. In the female hosts all grafts became extremely enlarged—some weighing up to 2000 mg or even more—whereas in the male animals they seldom exceeded 10 mg. Evidence is put forward which suggests a difference in hypothalamic control over the ectopic hypophyses between female and male animals due to a basic difference in hypothalamic prolactin-inhibiting factor (PIF) potency, which is enhanced by the suppression of PIF by estrogens. The neoplastic transformation of the enlarged grafts is questioned.

Introduction

There is general agreement that the adenohypophysis disconnected from the hypothalamus produces prolactin continuously, at least in female animals. This phenomenon has been used to explain the high incidence of mammary carcinoma in intact female mice bearing heterotopic multiple or single hypophyseal isografts (3, 5, 11, 13–16). In a previous publication, in which a number of variables in the experimental procedure were introduced, it has been shown that the kidney is one of the more favorable sites for implantation of hypophyseal tissue (5).

The experiments reported here were performed with the following objects in mind: (a) to confirm, if possible, part of the findings published earlier (5) in another F₁ hybrid combination of which data on the mammary tumor incidences under a number of experimental conditions are available; (b) to extend the experimental range by studying also the effects of hypophyseal grafts in male hosts; (c) to study more thoroughly the remarkable growth potential of hypophyseal isografts under certain conditions as first described by us (15), and later confirmed by others (1, 2, 7–9, 11, 14).

Materials and Methods

The experimental animals were (♀ O20 × ♂ DBAf)F₁ hybrids. The O20 strain, developed at our institute, was in the 125th–132nd generation of inbreeding during the period of these experiments. This strain is naturally free of the mammary tumor agent (MTA), and details on the frequency of mammary tumors in normal animals and animals infected with the MTA have been published (17). Our DBA strain (with MTA) was obtained from Dr. C. C. Little, Jackson Memorial Laboratory, Bar Harbor, Maine, in 1931. After a further 32 generations of inbreeding at our institute a MTA-free DBAf subline was established in 1947 by the usual procedure of cesarian section and foster-nursing of the artificially delivered young on C57BL. Calculated from 1947, this subline passed the 33rd–42nd generation of inbreeding during the period of these experiments. Data on the mammary tumor incidences in the DBA strain and its DBAf subline have been published, too (18).

For details on maintenance of the animals, the technic of the vaginal smear studies, the operative technic of transplantation of hypophyseal to the kidney, the gross and microscopic diagnosis of the mammary tumors, and the histologic technics used in the study of the grafts, we refer to our previous publications (5, 15). The operations on the hosts of the grafts were performed at an age of 6–8 weeks. The donors of the hypophyses were either adult (7 weeks to 5 months) or infantile (5–10 days).

In the present experiments the 1st mammary tumor was observed at an age of 248 days. Animals which died or had to be sacrificed before reaching this age were disregarded in the calculations on the mammary tumor percentages; whenever possible data on graft size were recorded, however.

At sacrifice the size of the hypophyseal isografts was determined: (a) by measuring length, width, and height to the nearest 0.25 mm, and (b) by weighing to the nearest mg on a torsion balance. Generally it was found that the weight in mg was equivalent to 80% of the product of length, width, and height in mm. In those cases in which it was impossible to determine the weight of the grafts accurately, either because of their small size or because of blood loss from the larger grafts during preparation, the weight was calculated from the linear measurements on this basis.

Results

Female hosts

Six experimental groups were studied: 1 and 2, 1 hypophysis of a male or female adult donor was implanted in the left kidney; 3 and 4, 1 hypophysis of a male or female infantile donor was implanted in the left kidney; 5 and 6, 4 hypophyses of male or...
female adult donors were implanted in the kidneys, 2 on each side.

Short-term vaginal smear studies on all animals, started the day after operation and lasting 60 days, showed that all grafts were successful. In experimental Groups 1, 2, 5, and 6 the 1st pseudopregnancy induced by the prolactin secreted by the grafts occurred either immediately after implantation or following a single normal estrous cycle, depending on the stage of the estrous cycle at which the operation was performed. In Groups 3 and 4 there was a latency period of 16–25 days (3–5 normal cycles) before the 1st pseudopregnancy was found. This relative insufficiency of grafts of infantile donors early after implantation is of great significance, and the prolactin production from the beginning in Groups 5 and 6.

The mammary tumor incidences found are listed in Table 1. For comparison Table 2 summarizes the data of older experiments on this particular hybrid combination with and without MTA (15, 17).

Again the hypophyseal isograft technic proved to be highly effective as a method for the induction of mammary cancer. Except for the fact that the animals with multiple grafts developed their tumors somewhat earlier than the animals with single grafts, no significant differences between the groups were found.

Of the total of 187 mammary tumors in the graft-bearing animals, 7, chosen at random, were studied with the electron microscope. No virus-like particles associated with the MTA were found, whereas these were readily detected in tumors of the DBAb and C3Hf sublines induced by the same isograft technic. This conflicts with the report of Pitekla ef al., who found that the so-called electron microscopist’s viral particles (EMV) can be transmitted by the male parent as well, at least in (♀ BALB/c x ♂ C3Hf)F1 mice (20). In the latter experiments hyperplastic nodules were studied, however, and not mammary tumors. In an earlier experiment 12 mammary tumors of (♀ O20 x ♂ DBAf)F1 hybrids, induced by subcutaneous hypophyseal isografts were bioassayed for the presence of the MTA with completely negative results (15).

**TABLE 1**

<table>
<thead>
<tr>
<th>No. hypophyses implanted and age of donors</th>
<th>SEX OF DONORS</th>
<th>NO. OF ANIMALS WITH TUMOR</th>
<th>NO.</th>
<th>%</th>
<th>平均年龄(天)</th>
<th>Average age at death(天)</th>
<th>EXPERIMENTAL GROUP</th>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>1, adult</td>
<td>♂</td>
<td>50</td>
<td>1</td>
<td>2</td>
<td>619</td>
<td>730</td>
<td>Controls, virgin</td>
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<tr>
<td>1, adult</td>
<td>♀</td>
<td>51</td>
<td>38</td>
<td>75</td>
<td>575</td>
<td>579</td>
<td>1</td>
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<tr>
<td>1, infantile</td>
<td>♂</td>
<td>24</td>
<td>16</td>
<td>67</td>
<td>587</td>
<td>646</td>
<td>3</td>
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<tr>
<td>1, infantile</td>
<td>♀</td>
<td>27</td>
<td>18</td>
<td>67</td>
<td>569</td>
<td>661</td>
<td>4</td>
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<tr>
<td>2 + 2, adult</td>
<td>♂</td>
<td>49</td>
<td>37</td>
<td>76</td>
<td>520</td>
<td>619</td>
<td>5</td>
</tr>
<tr>
<td>2 + 2, adult</td>
<td>♀</td>
<td>49</td>
<td>43</td>
<td>88</td>
<td>522</td>
<td>601</td>
<td>6</td>
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**TABLE 2**

<table>
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<tr>
<th>Treatment</th>
<th>NO. OF ANIMALS</th>
<th>WITH TUMOR</th>
<th>WITHOUT TUMOR</th>
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<tr>
<td></td>
<td></td>
<td>%</td>
<td>平均年龄(天)</td>
</tr>
<tr>
<td>Without MTA*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virgins</td>
<td>93</td>
<td>3</td>
<td>700</td>
</tr>
<tr>
<td>Breeders</td>
<td>54</td>
<td>4</td>
<td>691</td>
</tr>
<tr>
<td>Force-bred</td>
<td>84</td>
<td>19</td>
<td>577</td>
</tr>
<tr>
<td>With MTA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virgins, foster DBA</td>
<td>73</td>
<td>96</td>
<td>438</td>
</tr>
</tbody>
</table>

* MTA, mammary tumor agent.

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**THE HYPOPHYSEAL GRAFTS.** At the time of sacrifice of the animals, all hypophyseal grafts were found to be enlarged, mostly very markedly so. The variations in graft weight between the groups were relatively small. At an age of 600 days, around which age many of the animals had to be killed, the rough average weight of the grafts was 100, 100, 300, and 200 mg in Groups 1–4, respectively. The combined weight of the multiple grafts in Groups 5 and 6 was 600 and 500 mg, respectively, at the same age. In animals older than 600 days the grafts sometimes weighed 2000 mg or more. No differences between the grafts on the right or left side were noted. Within each group the variations in graft weight observed could nearly all be brought back to 1 single factor, the time elapsed between implantation and sacrifice. This is most clearly demonstrated when the graft weights are plotted semilogarithmically against time as has been done for Groups 1 and 5 in Chart 1. The histology and cytology of the enlarged grafts have been discussed extensively elsewhere (5, 9, 14, 15, 22), so no further comment is necessary here. The ultrastructure of the grafts and some biochemical properties are discussed in the following paper (4).

**Male hosts**

Three experimental groups were studied: 7, intact host implanted with 1 hypophysis of adult male donor (48 animals); 8, intact host implanted with 1 hypophysis of adult female donor (41 animals); 9, castrated host implanted with 1 hypophysis of adult male donor (46 animals). The site of implantation was again the left kidney.

The average survival time of the animals of these groups was 595, 599, and 650 days, respectively. No mammary tumors were observed. Contrary to the findings in the female hosts, the hypophyseal grafts were not markedly enlarged in any of the male host groups. In but a very few of the animals surviving to an age of 600–800 days the grafts weighed more than 10 mg (but
because of ill-health. Indirectly this phenomenon proved to be absent in castrated male hosts. Frequently the intact male hosts acting synergistically with the testicular secretion, as they were tory lesions, either on 1 or on both sides. These effects were due to the presence of ovarian hormones.

However, studies of the mammary glands failed to reveal any growth stimulation, which indicates either that the prolactin secretion is insufficient or that the prolactin released will induce mammary growth stimulation (and tumor formation) only in the presence of ovarian hormones.

One additional point of interest was that in both groups of intact male hosts the seminal vesicles and coagulation glands were highly enlarged and distended with secretion in most animals. Frequently these organs also showed severe inflammatory lesions, either on 1 or on both sides. These effects were caused through an action on the testes, or through a mechanism acting synergistically with the testicular secretion, as they were absent in castrated male hosts. Frequently the intact male hosts were affected in such a degree that they had to be sacrificed because of ill-health. Indirectly this phenomenon proved to be a favor in that it allowed the conclusion that all grafts in the intact males had been successful, simply by judging the state of the accessory glands at sacrifice.

Discussion

In the experiments with intact female mice bearing hypophysal isografts described here, the following points pertinent to mammary gland carcinogenesis already observed in animals with a different genetic make-up (5, 15) could be confirmed: (a) the effectiveness of the hypophysal isograft technique in inducing mammary gland tumors even in animals free of the mammary tumor agent; (b) the suitability of the kidney as site of implantation; (c) the efficacy of grafts of infantile donors, in spite of the small amount of tissue implanted; (d) multiple grafts are more effective than single grafts.

The slight superiority of male donors over female donors, previously described (5, 13, 15), was not observed here, because near-maximum tumor incidences were obtained already with grafts of the adult female donors. The (♀ O2O x ♀ DBA)F1 hybrids with grafts of infantile donors in the kidney did not show a higher average tumor age when compared to the animals treated with a single hypophysis of an adult donor. Such a longer induction period was observed originally in the hybrid (♀ O2O x ♀ 1F)F1 with grafts of infantile donors in the spleen (6). Clearly the lower initial activity of the infantile grafts in the kidney is compensated by the more rapid growth rate of these grafts, a phenomenon which is much less pronounced when the spleen is used as implantation site.

Except for the effect on the mammary gland, the hormone production from the grafts did not lead to pathologic changes anywhere in the body. However, a number of animals had to be sacrificed before a mammary tumor occurred, owing to ill-health caused by the extreme enlargement of the grafts themselves, especially in the groups implanted with infantile hypophysal or bearing multiple grafts. In the latter groups the serious deformations of both kidneys sometimes led to disturbances of renal function as additional complication.

Only very occasionally did the hypophysis in situ show some slight enlargement. This occurred only in very old animals (above 2 years), and the incidence was the same in the treated groups and the control group.

Only 2 publications have come to our knowledge on the successful induction of mammary tumors by hypophysal isografts in male mice. In the 1st report castrated agent-free mice were used (3), and in the 2nd intact MTA-infected animals (10). Our results were completely negative in this respect; none of the 89 intact and 46 castrated male agent-free (O2O x ♀ DBA)F1 hybrids developed a mammary tumor. Moreover, histologic study of the mammary glands failed to provide positive evidence of hormonal stimulation by the viable grafts in these animals. No comment on mammary gland histology was given by the authors mentioned above.

This leads to the problem of the difference in behavior of the hypophysal grafts in male and female hosts, respectively. In a preliminary communication (6) we have stated our arguments supporting the working hypothesis that in male animals the ectopic hypophysal might still be under some degree of hypothalamic control. This would mean that enough hypothalamic prolactin-inhibiting factor (PIF) might reach the ectopic hypophysal to keep the prolactin production in check. We further hypothesized that PIF also might inhibit the proliferation of the prolactin-producing cells, resulting in small grafts in male hosts, both intact and castrated. If indeed the growth rate of the grafts is conversely related to the levels of PIF in the general circulation, 2 points would follow.

1. Because of the fact that the grafts still grow markedly in ovarectomized mice, at least when compared with the growth...
rate in orchidectomized animals, a basic sex difference in hypothalamic function, also in respect of PIF production, seems likely.

2. The production of PIF seems to be controlled inhibitorily by estrogens. This can be deduced from the maximum growth rate of the grafts observed in intact females, and in gonadectomized males and females receiving high-dose estrogen treatment (2 mg estrone/liter drinking water). This conclusion fits in nicely with literature data on the depletion of PIF activity from the hypothalamus after estrogen treatment (21). The alternative explanation, favored by others (12, 13), that estrogens act directly on the grafts seems somewhat less likely, because whereas high-dose estrogen treatment also induces uninhibited proliferation of the prolactin-producing cells of the hypophysis in situ—which, in our line of reasoning, simply means elimination of PIF even from the hypothalamo-hypophyseal portal system—continuous low-dose estrogen treatment (0.25 mg estrone/liter drinking water) and discontinuous estrogen treatment (2 mg estrone/liter drinking water every other 5 days) still allow the grafts to grow extensively even in male animals without affecting the hypophysis in situ, at least initially (Röpke and Boot, unpublished). In case of a predominantly direct action of estrogens on the hypophyseal cells a differential susceptibility of graft and hypophysis in situ would have to be postulated in intact females as well as following certain types of exogenous estrogen treatment.

Summarizing this part of the discussion and taking into account the results given in the preliminary communication (6), where it was concluded that neither macroscopic nor microscopic signs of real malignancy could be observed in the enlarged hypophyseal isografts, it would seem permissive to assume that the prolactin-producing cells may proliferate uninhibitedly—just as they do in tissue culture (19)—simply by absence of hypothalamic control. The criterion of transplantability used by others (1, 2) cannot be used to demonstrate a neoplastic change in the grafts, because the prolactin-producing cells as such seem to be readily transplantable. Thus, unless direct proof of an irreversible change in the enlarged hypophyseal grafts—and, though this might be more controversial, be it only for historical reasons, in enlarged hypophyses in situ following estrogen treatment—is forthcoming, it is believed that the use of terms such as “tumors,” “neoplastic changes in,” and so on for these structures had better be avoided. We regret to have been among the 1st to state that a hypophyseal isograft may develop into a “tumor” only on the basis of analogy and size (15).

After this more or less theoretical discussion, the discrepancy between the success of others in inducing mammary cancer in male mice by the hypophyseal isograft technique and the failure (at least in this respect) in the present experiments needs some more elaboration. Various explanations can be envisaged, such as:

1. Strain differences, including the susceptibility of the mammary glands to the hormones ultimately secreted by the grafts, the presence or absence of the MTA, as well as differences in graft function, may cause the discrepancy.

2. Whatever the mechanism of action, the presence of endogenous or exogenous estrogens will greatly affect the results obtained, not so much through their direct effect on the mammary glands, but by stimulating the prolactin production by the grafts. In 1 of the reports cited above (10) the possibility of the presence of estrogenic activity in the food could not be ruled out.

Where castrated male mice were used (3) the possibility of adrenal estrogen production has to be taken into account. In respect of these points we have taken into study the question of the minimal effective dose in stimulating graft growth.

3. The site of implantation might have an effect. Both positive results were obtained by s.c. isografts, whereas in this and yet another negative report (11) renal grafts were used.

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References


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