Inhibition of Growth of a Transplanted Adrenal Cortical Tumor by Sex Steroids*

HENRY C. BROWNING, WILMA D. WHITE, and WILLIAM A. SADLER

(University of Texas Dental Branch, Texas Medical Center, Houston, Texas)

Adrenal cortical carcinomas may be induced by early castration of male or female CE (22, 23) or BALB/c (6) mice. Such tumors are often hormonally dependent, i.e., they show faster growth as transplants in castrated than in intact hosts (8, 18). These dependent tumors enter a dormant, or latent, period of 1 or more months after intraocular transplantation, followed by a shorter one during which increased vascularity and moderate enlargement take place (post-latency). Subsequently, active and continuous growth occurs.

Administration of testosterone to the hosts at this time inhibits further growth of the transplants and often induces regression to the post-latency or latency status (8). This action of the steroid may be direct or it may be mediated through the output of pituitary trophic hormones.

This report covers the results of our study of the action of estrogen and progesterone, given systemically, and of testosterone, given either systemically or locally, upon transplants of a dependent adrenal cortical tumor.

MATERIALS AND METHODS

This adrenal cortical tumor had developed in a castrated CE male mouse and, when studied, was in its second subcutaneous transplant generation in a castrated first hybrid male of the DB and CE strains. Intraocular transplants (2) were made into both eyes of nine groups of male and two groups of female first hybrids of the CE and BALB/c strains (each group consisted of six to eight mice with, excluding occasional infected eyes, eleven to fifteen transplants). Initially the transplanted fragments occupied approximately 25 per cent of the anterior chamber. Seven of the nine male groups and one of the two female groups were castrated at the time of transplantation (Table 1). The hosts ranged in age from 3 to 7 months, but age distribution was essentially equal in all groups.

One month after transplantation, when approximately half of the transplants in the castrated male groups had entered the active growth phase, subcutaneous administrations of progesterone, of estradiol dipropionate, of estradiol dipropionate and progesterone, or of testosterone phenylacetate were made at regular intervals to particular groups of castrated males (Table 1, Groups II, III, IV, V). One group (VIII) of intact males also received progesterone. Steroid administrations were continued until the average amount of anterior chamber space filled by transplants in a group was at least 50 per cent (progesterone, Group II; estrogen, Group III; estrogen and progesterone, Group IV), or for 10 months (testosterone, Group V) or 14 months (progesterone, Group VIII). At the end of the 10th month half of the intact males and females (Groups VIII, IX, and X) were castrated. Observations ceased at, or shortly after, the time that the average amount of anterior chamber space occupied by transplants in a group was 90 per cent; or at the end of 9 or 15 months.

At the time that the subcutaneous administration of steroid to Groups II, III, IV, and V commenced (i.e., 1 month after transfer) two groups of castrated male (VI and VII) were combined, and each animal received 0.1 mg. of testosterone phenylacetate in the right anterior chamber. This micro-crystalline steroid was suspended in a cylinder (4) of 2 per cent agar gel, 1.5 mm. in length and 0.5 mm. in diameter. A similar agar cylinder, without the steroid, was placed in each left anterior chamber. This intraocular administration was not repeated.

Observations of the number of actively growing transplants (takes), and of the area of the anterior chamber occupied by them, were made twice a month for the first 6 months and thereafter at monthly intervals.

RESULTS

Untreated groups.—In intact males and females (Table 1, Groups IX, X) transplants diminished...
in size during the first 2 months, occupying approximately 5 per cent of the anterior chamber space. They were without obvious vascularity and retained irregular outlines, and, by the end of the 10th month, some showed little change while others were completely, or almost completely, absorbed. At this time half of the animals in these groups (IX, X) were castrated; 5 months later two out of six transplants in the castrated males (Group IX), but none of eight in the castrated females (Group X), were growing. The remainder of the transplants in castrated hosts, and all those in intact hosts, showed no change.

In castrated males and females (Table 1, Groups I, XI) the transplants entered a latent phase during which they diminished in size, rounded off and acquired slight vascularity, occupying 10–15 per cent of the anterior chamber space. While there was considerable individual variability, this phase continued for approximately 3 weeks in males and 5 weeks in females. Following this latent phase, the transplants entered a post-latency phase during which there was increased vascularity and moderate enlargement to occupy 20 per cent of the anterior chamber space. This phase continued for a week to 2 months in different individual transplants, tending to be longer in females. This was again followed by a so-called active phase of continuous growth until the anterior chamber was filled. Entry into this active phase was considered certain only when 25 per cent of the space was filled, and transplants were not evaluated as takes until this phase had been reached.

Transplant growth was expressed as the mean percentage of the anterior chamber space occupied

### Table 1

**Effect of Steroid Administration on Transplants of an Adrenal Cortical Tumor of a C57 Mouse in C57BL/6J Hybrid Mice**

<table>
<thead>
<tr>
<th>Group</th>
<th>Host status</th>
<th>Steroid dose and frequency of administration</th>
<th>Admin. time (mo.)</th>
<th>Observ. time (mo.)</th>
<th>No. takes at latency time/No. takes at end obs. time/No. trans. made initially/No. trans. at end obs. time</th>
<th>Latency</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Cast. males</td>
<td>Progesterone (120 mg. every 20 days)</td>
<td>(mo.)</td>
<td>(mo.)</td>
<td>13/14/14/14/14</td>
<td>66 days</td>
</tr>
<tr>
<td>II</td>
<td>Cast. males</td>
<td>Estradiol dipropionate (0.025 mg. every 7 days)</td>
<td>4</td>
<td>4</td>
<td>13/14/14/14/14</td>
<td>55 days</td>
</tr>
<tr>
<td>III</td>
<td>Cast. males</td>
<td>Estradiol dipropionate (0.025 mg. every 7 days) + progesterone (120 mg. every 20 days)</td>
<td>9</td>
<td>9</td>
<td>9/9/13/11*</td>
<td>157 days</td>
</tr>
<tr>
<td>IV</td>
<td>Cast. males</td>
<td>Testosterone phenylacetate (7.5 mg. every 20 days)</td>
<td>9</td>
<td>9</td>
<td>8/8/10/11*</td>
<td>273 days</td>
</tr>
<tr>
<td>V</td>
<td>Cast. males</td>
<td>Testosterone phenylacetate (0.1 mg. once) ↑ Unreated eyes</td>
<td>10</td>
<td>15</td>
<td>−1/14/14</td>
<td>Growth inhibited</td>
</tr>
<tr>
<td>VI and VII</td>
<td>Cast. males</td>
<td>Testosterone phenylacetate (0.1 mg. once) ↑ Treated eyes</td>
<td>10</td>
<td>10</td>
<td>14/15/15/15</td>
<td>63 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14/15/15/15/15</td>
<td>120 days</td>
</tr>
<tr>
<td>VIII</td>
<td>Intact males</td>
<td>Progesterone (120 mg. every 20 days)</td>
<td>14</td>
<td>15</td>
<td>−0/14/14</td>
<td>No growth</td>
</tr>
<tr>
<td>IX</td>
<td>Intact males</td>
<td></td>
<td>15</td>
<td>15</td>
<td>−2/14/14</td>
<td>No growth</td>
</tr>
<tr>
<td>X</td>
<td>Intact females</td>
<td></td>
<td>15</td>
<td>15</td>
<td>−0/14/14</td>
<td>No growth</td>
</tr>
<tr>
<td>XI</td>
<td>Cast. females</td>
<td></td>
<td>9</td>
<td>9/11/13/11§</td>
<td>9/11/13/11§</td>
<td>138 days</td>
</tr>
</tbody>
</table>

* One animal, bearing two transplants, died by 75th day.
† In one anterior chamber of each animal; all other administrations were given subcutaneously.
‡ Growth occurring only after castration of half of the animals in the group after 10 months.
§ One animal, bearing two transplants, dead by the 250th day.
by transplants in the active phase (i.e., the multiple of the percentage of takes in a given group by the mean amount of anterior chamber filled by these active transplants). These data are shown at monthly intervals (Chart 1). The time in days taken for the mean amount of anterior chamber space occupied to reach 50 per cent in a given group has been taken as the index of latency. This index was 66 days for castrated males and 188 days for castrated females (Table 1, Groups I, XI).

Treated groups; subcutaneous administration (Chart 1; Table 1).—At the time of the initial steroid administration, approximately half of the transplants in castrated males had just entered the active phase of their growth period, when the mean percentage of anterior chamber occupied was between 10 and 15 per cent.

Growth was accelerated in the progesterone-treated group (II), the latency index being 55 days. It was retarded in the estrogen-treated mice (III), the latency index being 157 days (i.e., a longer latency than in untreated castrated females). Growth was even further retarded in Group IV, which received estrogen and progesterone and in which the latency index was 273 days. In this group alone, a number of transplants re-entered the latency phase for a time, so that the percentage of takes was 55 per cent at the end of the first month and 18 per cent at the end of the second. The original percentage of takes was not regained until after the end of the 3d month. Growth was completely inhibited by testosterone (Group V); nearly all active transplants re-entered the latency phase. (Six of fourteen transplants were in the active phase at the end of the 1st month, but only four by the end of the 5th month, and one by the end of the eighth month.) Those that had not become active at the initial administration remained latent. No transplant showed

1 In previous work on intraocular transplants of dependent adrenal tumors (8) the index of latency was taken as the time in days required for 50 per cent of the transplants to enter the active growth phase.

Transplants of another adrenal cortical tumor, which arose in a castrated male C57BL/BALB/c mouse, also showed stimulation by progesterone, the latency period being 85 days in the treated and 115 days in the untreated castrated isologous male hosts. This tumor had absolute dependence.
any signs of growth in the 5 months after testosterone administration ceased.

One group of intact males (VIII) received progesterone. The behavior of their transplants was identical with that of transplants in untreated intact males and females (Groups IX, X). Following castration, at the end of the 10th month, of one half of the animals comprising Group VIII, with continuing progesterone administration, four out of eight transplants entered the post-latency phase. They remained in this phase from the end of the 11th to the end of the 13th month, but regressed to small inactive fragments again by the end of the 15th month.

Treated groups; intraocular administration (Table 1; Groups VI, VII).—The growth curves from the pooled data for the treated and for the untreated eyes are shown in Chart 2, together with the growth curves for untreated castrated males (Group I) and for castrated males receiving testosterone subcutaneously (Group V), extrapolated from Chart 1.

At the time of intraocular administration of testosterone, the mean percentage of the anterior chamber occupied by the tumor was 17 per cent in the injected eyes and 12 per cent in the controls. The testosterone was absorbed slowly but had disappeared by the end of the 2d month after administration. Growth of the transplants in the uninjected eyes was practically identical with that in the untreated castrated male group (I), the latency period being 63 and 66 days, respectively (Table 1). Growth of the transplants in the injected eyes was partially inhibited; this inhibition was most conspicuous at about the time that all the intraocular testosterone had disappeared but continued for the duration of the experiment. The latency period for transplants in the treated eyes was 120 days (Table 1).

DISCUSSION

Within the limits of our observation period this adrenal cortical tumor showed absolute dependency, for it grew only in castrated hosts. This was in contrast to other CE and BALB/c adrenal cortical tumors which exhibited partial dependency, since transplants also grew in intact hosts, although the latency period was twice as long as in castrated hosts (3). Other spontaneous tumors (and those induced by hormonal imbalance) of the adrenal cortex (16, 18), ovary (10, 15, 19), testis (1, 13, 17), and pituitary (8, 9) have shown absolute or partial dependency, although such dependence tended to be lost with serial transfer.

Growth of transplants of a partially dependent adrenal cortical tumor of a BALB/c mouse in castrated hosts was inhibited by testosterone administered for a 1- or 2-month period (3). However, growth was resumed from 2 to 3 months after administration ceased. In the present study transplant growth was inhibited over a 10-month period of testosterone administration, and it was not resumed over a period of 5 months after cessation of administration. Similarly, growth did not occur in intact males or females and was seen only rarely in such hosts after castration 10 months later. Prolonged treatment with testosterone or residence in intact hosts apparently resulted in the loss of neoplastic properties by most, or all, of the transplants.

This very obvious inhibiting effect of testosterone may have been the result of a direct action or of an indirect one, promoted by the reduction of the elevated output of pituitary FSH in the castrated rodent (11, 20). This elevated gonadotropin may initiate the development of adrenal cortical tumors in mice (7) and may also be responsible for the dependency of transplants of such tumors upon castration of the hosts. However, the moderate inhibition of transplant growth in anterior eye chambers receiving an amount of testosterone (0.1 mg.) insufficient to affect the transplant in the untreated eye of the same host (or to alter atrophy of the seminal vesicles) suggests that the steroid acts directly upon the tumor. The moderate slowing (rather than complete inhibition) of growth in animals receiving systemic estrogen, or estrogen and progesterone, again suggests direct action rather than one mediated via the pituitary, since the high nonphysiologic dosages used should have been as effective in suppressing elevated gonadotropin output of the castrate pituitary as the similarly high dosages of testosterone. Estrogens, androgens (21), and deoxycorticosterone (12) have been shown to inhibit the development of adrenal carcinomas in castrated CE mice. However, the inhibition of development of a neoplasm is not necessarily equivalent to inhibition of growth of its transplants.

With the intraocular transplantation technic, distinction can be made between the inhibition by a steroid of a growing transplant after it has entered the active phase, and the inhibition of entry into the active phase from latency, i.e., inhibition of growth versus inhibition of take. Systemic testosterone induced actively growing transplants to re-enter latency. Systemic estrogen slowed development and growth but did not cause regression of active transplants, and local testosterone had a similar effect. In the estrogen-progesterone group, four of six transplants in the active phase, when steroid administration commenced, returned to the post-latency or latency phase; and growth of transplants when again active was slowed. After 9
months three of eleven transplants were still latent. It is possible that a steroid may affect the assumption of the active phase and the growth rate independently.

In hosts such as castrated CE/BALB/c mice, spontaneous adrenal cortical tumors might be expected to arise, since both parental strains are susceptible (6, 29, 23) and, in other hybrids with CE mice, the susceptibility is genetically dominant (14). The sex steroids usually secreted by such tumors (5, 6, 14) might then affect transplant growth. Actually, in only two animals (both in the pooled groups receiving intraocular testosterone) were such tumors found at autopsy at the end of 10 months, one being 0.5 cm. in maximum diameter and the other 1.2 cm. On the other hand, the untreated castrated female group showed evidence of estrogenic stimulation of the uterus, either from the host adrenals or from the growing transplants. The untreated castrated male group showed no evidence of androgenic stimulation of the seminal vesicles.

SUMMARY

1. Intraocular transplants of a dependent adrenal cortical tumor of a CE mouse were made into both eyes of intact or castrated male and female CE/BALB/c hybrids. Certain groups were treated with progesterone, estrogen, estrogen and progesterone, or testosterone systemically or with testosterone intraocularly in one eye only. Other groups were left untreated.

2. Growth was slower in untreated castrated females than in untreated castrated males, the latency period being 138 and 66 days, respectively. Growth did not occur over a 15-month period in intact males, intact males receiving progesterone, or intact females.

3. In castrated males progesterone accelerated growth (latency 55 days), estrogen moderately slowed growth (latency 157 days), estrogen with progesterone markedly slowed growth (latency 273 days), and testosterone completely inhibited it.

4. In castrated males with intraocular testosterone in one eye, growth in the other eye was unaffected (latency 63 days), while that in the treated eye was retarded (latency 120 days).

REFERENCES


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Henry C. Browning, Wilma D. White and William A. Sadler