Adrenal Tumors, Pituitary Tumors, and Other Pathological Changes in F₁ Hybrids of Strain DE × Strain DBA*

MARGARET M. DICKIE AND PRISCILLA W. LANE

(Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine)

Neonatal gonadectomy has been used in some inbred strains of mice (7, 8, 10, 13-17) as an experimental tool to study the effect of hormonal imbalance. The effect of neonatal gonadectomy in reciprocal hybrid mice of several inbred strains has been described (11, 12). This paper will report the response to gonadectomy of the F₁ animals of strain DE × strain DBA and will compare the response of this hybrid with the response of F₁ animals of strain CE × strain DBA (11).

Strain DE, an inbred strain phenotypically identical with strain CE/Wy and, like strain CE, exhibiting a poor breeding record, was originated by Eaton from a cross of the E strain with strain CE. Offspring with the extreme dilution color were selected and inbred (6). Adrenal cortical carcinomas occur in 100 per cent of CE gonadectomized mice (13-17) but have not been observed in DE gonadectomized animals.¹

MATERIALS AND METHODS

Half of the hybrid offspring from the cross DE/Wy × DBA/²Wy were gonadectomized 1-3 days after birth, and half were raised as unoperated controls. Animals of both sexes, gonadectomized and unoperated, were autopsied at bi-monthly intervals from 2 to 14 months of age. Some mice of older ages were also autopsied.

The adrenals from all animals were saved and were sectioned serially. Dimensions of each gland were measured with an ocular micrometer or with calipers. Size was estimated in the manner previously reported (11, 12). Histological studies were made of the submaxillary gland, thyroid, pituitary, uterus, vagina, accessory sex organs (seminal vesicles and prostates), and of testes and ovaries of unoperated controls. Tissues other than abnormal hypophyses were fixed in Vandergrift's fluid, sectioned at 8 μ, and stained with Mayer's hematoxylin and aqueous eosin. The abnormal hypophyses were fixed in a modified Bouin fluid, sectioned at 4 μ and differentially stained (4). The skins of all animals were preserved so that the mammary glands adherent to them might be examined.

RESULTS

Intact females and males.—Estimated volumes of adrenal glands of DE-DBA F₁ females ranged from 1.5 to 5.5 c.mm., while adrenal volumes of the hybrid males ranged from 1.5 to 8.0 c.mm. Bands of small, dark, subcapsular cells ("A" cells) were present in all female adrenals after 8 months and in male adrenals after 12 months. One "A" cell tumor was diagnosed in a 77-month female and one medullary adenoma in a 18-month female.

The ovaries averaged about 6 c.mm. in size, and many cysts were observed in them after 18 months. No hyalinization of corpora lutea, common in strain DBA, was evident.

A few endometrial cysts were noted in several females after 16 months of age. One interstitial-cell tumor of the testis was found in a 35-month animal. All other endocrine and accessory reproductive organs showed no pathological changes.

Fifteen of 25 animals had lung tumors after 18 months. Other pathological conditions observed were:² one adenoma of the Harderian gland; two epidermoid carcinomas of the skin, one basal-cell type and one squamous-cell type with metastases to liver, kidney, and spleen; three fibrosarcomas; one lymphatic leukemia; two epidermal papillomas; one myoepithelioma of the salivary gland; and three hepatomas.

Longevity was notable in the hybrid males. Several lived to 36-38 months of age, and one lived to 42.5 months.

Gonadectomized females and males.—"A" cells were present in female and male adrenals at 4 months, and "B" cells and nodular hyperplasia were observed at 6 months in females but not until 2 Diagnoses were by the courtesy of Dr. Elizabeth Fekete.

Received for publication June 6, 1955.

¹ Unpublished data.

² Diagnoses were by the courtesy of Dr. Elizabeth Fekete.
10 months in males. Adrenal cortical carcinomas were first observed at 12 months in females but not until 22 months in males. After these ages carcinoma was always present. The histological pattern of the carcinomas was similar to that previously described in strain CE (7) but in some instances was more densely cellular or more cystic. Tumor size was small in comparison with that of strain CE and its hybrids, the largest being only 5,000 c.mm. and the majority 500 c.mm. or less. All adrenal tumors in males were microscopic in size.

Terminal tubule cells of the male and female submaxillaries were heightened at 8 and 12 months, respectively. The stimulation was never sufficient to bring the epithelium to a complete masculine type. The uteri attained the size of those of a mature virgin female by 10 months. Some endometrial cysts but no other pathological changes were observed. The vaginal epithelium showed a diestrous picture until 10 months of age, after which other cyclic stages were noted. There was a slight masculinization of the male accessory sex organs at 6 months, while later stimulation was either of the estrogenic or androgenic type. These accessory sex organs never attained a size comparable to that of an intact male.

Cellular changes were noted in the pituitary glands of the females at 12–14 months, and after 18 months all females studied had basophil adenomas of the anterior lobe. Similar changes and tumors were noted in the pituitaries of the males after 20 months of age. These tumors ranged from 4 to 44 mg. in weight.

Mammary glands were rudimentary in females until 8 months and in males until 10 months, when end buds were seen. Alveoli were present in female glands at 16 months, and by 18 months the alveolar development and secretion into the ducts was extensive. Similar changes were found in the male by 20 months of age. Three females had mammary adenocarcinomas, adrenal cortical carcinomas, and pituitary basophil adenomas.

One basophil adenoma was transplanted into six young hybrid gonadectomized females and males. Twenty-five months later large masses were evident at the sites of transplantation. Histologically, these masses of tissue were comparable to that of the original adenoma. The host animals not only grew this transplant but also developed adrenal cortical carcinomas and basophil adenomas of their own hypophyses, as is characteristic of these F₁ gonadectomized mice over 18 months of age.

Other pathological conditions observed were: primary lung tumors in ten of 21 mice over 10 months of age; one giant-cell sarcoma, one sarcoma, one fibrosarcoma, and one papilliferous adenoma of the Harderian gland.

DISCUSSION

This experiment was conducted to determine the type of response to gonadectomy of the DE-DBA hybrids. Four types of response to neonatal gonadectomy have been described (8, 10, 11–17). They are (a) typical castrate response, (b) feminizing nodular hyperplasia response, (c) adrenal cortical carcinoma response, and (d) adrenal-pituitary syndrome response.

The latter response occurred in these DE-DBA hybrids and in the CE-DBA hybrids (11). The syndrome consists of neoplastic adrenal changes and basophil adenomas of the pituitary. The DE-DBA hybrids have smaller adrenal tumors and more consistent occurrence of basophil adenomas than do the CE-DBA hybrids.

Both these hybrid groups have a strain DBA male as the father and either a CE or a DE strain female as the mother. Since CE and DE are related (6), differences in the hybrids would presumably be due to factors introduced by the E strain or by interaction of factors in the hybrid CE × E that have become fixed in the new strain DE by inbreeding.

The F₁ intact virgin females of the two groups differed more than the F₁ intact males (Table 1). Notable differences were: (a) "A" cells were present after 3 months in adrenals of CE-DBA F₁ females but not until after 8 months in DE-DBA F₁ females; (b) CE-DBA F₁ ovaries averaged 15.65 ± 11.78 c.mm.³ and became hyalinized after 12 months of age, while DE-DBA F₁ ovaries averaged only 5.97 ± 3.34 c.mm.³ and did not become hyalinized; (c) a hyperovarian or hyperestrogenic syndrome characterized by cystic glandular hyperplasia, adenomyosis, and adenomatous hyperplasia of the endometrium occurred regularly after 6–8 months in CE-DBA F₁ females (1, 2), while, except for an occasional endometrial cyst, no pathological changes of the uterus were observed in the DE-DBA F₁ females; and (d) lung tumor occurrence was higher, and a wider variety of tumor types were found in DE-DBA animals than were found in CE-DBA mice.

Following gonadectomy, differences between the two hybrids were again apparent (Table 2). (a) The adrenals of CE-DBA mice were not only larger, but nodular hyperplasia and neoplasia occurred earlier. (b) Only DE-DBA mice had 100 per cent occurrence of pituitary basophil adenomas, although both groups had adrenal cortical carcinomas. (c) CE-DBA females had pathological changes in the uteri (3), while DE-DBA females did not show any such changes. (d) Masculinizing stimulation, as shown by height of terminal tubule

\[ ± = \text{standard deviation}. \]
epithelium of the submaxillary gland, was more pronounced in CE-DBA than in DE-DBA mice. (e) Lung tumor occurrence and variety of tumor types were again higher in DE-DBA mice than in the CE-DBA animals.

It was postulated in a report on the occurrence of basophil adenomas in CE-DBA and other CE gonadectomized hybrids that "the pituitary may be thrown into imbalance primarily by gonadectomy, exert its effect on the adenals in the absence of the gonads, and the adrenal in turn then becomes abnormal. The abnormal adenals then logically in its own environment or when transplanted to a foreign one (9). The neoplastic response of this gland is thus seen to be genetically determined. These experiments, designed to study the effect of gonadectomy on the adrenal gland of hybrids, have shown that development of carcinoma of the adrenal is the dominant type of response (12), whether or not this response is characteristic of either parent strain.

Another genetic factor that is apparent in both groups of hybrids is the distinct sex difference in time of occurrence of endocrine changes. It would appear that in these hybrids, without gonads during their entire postnatal life span, the response is different because of factors either present or absent on the Y chromosome.

The two hybrid groups reported here are more closely related than other hybrids studied previously. Their responses show that certain characteristics fixed now in strain DE are unlike those in strain CE. Hybridization of both DE and CE with another strain, DBA, makes these differences apparent.

**TABLE 1**

**COMPARISON OF INTACT CE X DBA F1 AND DE X DBA F1 MICE**

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Sex</th>
<th>No. mice</th>
<th>Av. adrenal size after 6 mo.*</th>
<th>1st A cell tumors (months)</th>
<th>No. nodular hyperplasia</th>
<th>Av. ovary size*</th>
<th>Uterus changes†</th>
<th>Lung tumor types</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE-DBA</td>
<td>♀</td>
<td>28</td>
<td>4.40 ± 1.20</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>15.65 ± 11.78†</td>
<td>CGH, AD, AH</td>
</tr>
<tr>
<td>DE-DBA</td>
<td>♀</td>
<td>25</td>
<td>3.58 ± 0.56</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>5.97 ± 3.34</td>
<td>normal</td>
</tr>
<tr>
<td>CE-DBA</td>
<td>♂</td>
<td>28</td>
<td>2.95 ± 0.14</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>DE-DBA</td>
<td>♂</td>
<td>20</td>
<td>2.29 ± 0.48</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

* Measured in c. mm.
† CGH = Cystic glandular hyperplasia; AD = Adenomyosis; AH = Adenomatous hyperplasia.
‡ ± = standard deviation.

**TABLE 2**

**COMPARISON OF GONADECTOMIZED CE X DBA F1 AND DE X DBA F1 MICE**

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Sex*</th>
<th>No. mice</th>
<th>Av. adrenal size after 6 mo.†</th>
<th>Nodular hyperplasia (months)</th>
<th>Adrenal tumors (months)</th>
<th>Per cent pituitary tumors</th>
<th>No. mammary tumors</th>
<th>Uterus changes‡</th>
<th>No. lung tumors</th>
<th>No. tumor types</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE-DBA</td>
<td>♀</td>
<td>25</td>
<td>1515 ± 1458§</td>
<td>5</td>
<td>6</td>
<td>50-60</td>
<td>0</td>
<td>CGH, AD, AH</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>DE-DBA</td>
<td>♀</td>
<td>25</td>
<td>2397 ± 23922</td>
<td>5</td>
<td>12</td>
<td>100</td>
<td>2</td>
<td>normal</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>CE-DBA</td>
<td>♂</td>
<td>27</td>
<td>1845 ± 1802</td>
<td>4</td>
<td>12</td>
<td>88</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DE-DBA</td>
<td>♂</td>
<td>15</td>
<td>4.45 ± 1.29</td>
<td>10</td>
<td>22</td>
<td>100</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

* Symbol for castrate.
† Measured in c. mm.
‡ CGH = Cystic glandular hyperplasia, AD = adenomyosis, AH = adenomatous hyperplasia.
§ ± = standard deviation.

secrete excess or atypical hormones which react on the pituitaries and they then become abnormal" (5). The histological sequence of events appears to be the same in both sets of hybrid gonadectomized mice. The pituitary tumors occurred only after the adrenal tumors were well established (Table 3).

The hyperestrogenic syndrome found in CE-DBA intact and gonadectomized females may interfere with the occurrence of the pituitary basophil tumors, since the incidence is lower in the hyperestrogenic gonadectomized females than in gonadectomized males or in the DE-DBA gonadectomized mice.

The genetic aspects of the problem are as complex as the endocrine. The adrenal gland of a gonadectomized animal responds similarly histo-

appear that in these hybrids, without gonads during their entire postnatal life span, the response is different because of factors either present or absent on the Y chromosome.

The two hybrid groups reported here are more closely related than other hybrids studied previously. Their responses show that certain characteristics fixed now in strain DE are unlike those in strain CE. Hybridization of both DE and CE with another strain, DBA, makes these differences apparent.

**SUMMARY**

Hybrids of strain DE/Wy × strain DBA/2Wy were studied to determine their response to gonadectomy. Neonatally gonadectomized and in-
tact animals, of both sexes, were autopsied at bi-
monthly intervals up to 24–30 months of age. Histological analyses of endocrine and accessory reproductive organs were made and results compared with those of strain CE/Wy × strain DBA/2Wy hybrids.

Comparison of these two groups of hybrids shows that (a) hyperestrinism was present only in CE-DBA intact and gonadectomized females, (b) adrenal cortical carcinomas developed after 6 months of age in CE-DBA and after 12 months in DE-DBA gonadectomized females, while in CE-

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>CE-DBA</th>
<th>DE-DBA</th>
<th>CE-DBA</th>
<th>DE-DBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 days—2 months</td>
<td>pituitary produces adrenal stimulating substance?</td>
<td>pituitary produces adrenal stimulating substance?</td>
<td>pituitary produces adrenal stimulating substance?</td>
<td>pituitary produces adrenal stimulating substance?</td>
</tr>
<tr>
<td>3</td>
<td>nodular hyperplasia develops in adrenal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>accessory reproductive organs mature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>adrenal carcinoma develops from hyperplasia</td>
<td>nodular hyperplasia develops in adrenal</td>
<td>accessory reproductive organs mature</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>CGH* appears in uteri</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>accessory reproductive organs mature</td>
<td></td>
<td>nodular hyperplasia develops in adrenal</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>adrenal carcinoma develops from hyperplasia</td>
<td>adrenal carcinoma develops from hyperplasia</td>
<td>accessory reproductive organs mature</td>
<td></td>
</tr>
<tr>
<td>15–16</td>
<td>AD* and AH* develop in uterus, pituitary tumors occur—50 per cent</td>
<td>pituitary tumors occur—100 per cent</td>
<td>pituitary tumors occur—88 per cent</td>
<td></td>
</tr>
<tr>
<td>17–18</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td></td>
<td></td>
<td>adrenal carcinoma develops from hyperplasia; pituitary tumors occur—100 per cent</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>animals die of carcinoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td></td>
<td>animals die of carcinoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>animals killed</td>
<td>animals killed</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* CGH = cystic glandular hyperplasia; AD = adenomyosis; AH = adenomatous hyperplasia.
ACKNOWLEDGMENTS
The authors wish to thank Dr. K. P. Hummel, Dr. M. N. Runner, and Dr. G. W. Woolley for their advice. They wish also to thank Mrs. Bessie Graves for technical assistance.

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