Differences in Adrenal Responsiveness to Post-castrational Alteration as Evidenced by Transplanted Adrenal Tissue*

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INTRODUCTION

In inbred strains of mice the removal of the gonads is followed by one of three different types of histological response on the part of the adrenal cortex. In most strains, some months after gonadectomy typical hyperplastic changes in the adrenal cortex develop, and these altered cortices may then produce significant amounts of sex steroids (4, 5, 6, 12, 14). In some animals of these strains, this hyperplastic process will progress until a definite adenoma or even a carcinoma is formed. In the CE strain, however, rather soon after the areas of cortical hyperplasia appear, areas of carcinoma develop within them so that in essentially all these animals sex steroid-producing adrenal cortical carcinomas are well established within about 9 months after the animals have been castrated (15-19). In the subline of A mice maintained in this laboratory, on the other hand, the morphology of the adrenals is altered but little after the animals are gonadectomized (12). Occasionally, small areas of cortical hyperplasia may develop, but we have seen no evidence of sex hormone production by these rather infrequently occurring small areas of hyperplasia. From a rather extensive study of the post-castrational changes that occur in F1 hybrid mice resulting from the mating of different inbred strains of mice (9), it appeared that the type of adrenal alteration noted was inherited equally through male and female parents and that the tendency for the adrenals to become carcinomatous was dominant over that for hyperplasia to develop, but that both of these were dominant over the tendency for no marked cortical alteration to appear. Since no F2 or back-cross generations were studied, no idea of the number of genetic factors involved in this inheritance was obtained.

The purpose of the present study was to determine, if possible, whether these differences in adrenal response are due primarily to differences in the responsiveness of the genetically different end organs (adrenals), or primarily to differences in the alterations of the endocrine system produced by castration in the various strains of mice. In other words, is the lack of significant post-castrational adrenal alteration in A mice, for instance, the result of a nonresponsive end organ or is it due to a quantitatively and/or qualitatively inadequate change in the endocrine system of this strain of mice so that even a responsive adrenal cortex is not altered? It would seem that an answer to this question might be obtained if the cortical tissues of two different strains of mice were placed in the same environment and thus subjected to the "external" stimuli.

Since the maintenance of transplants of both normal and malignant tissue is dependent upon genetic factors (1, 2, 11), it is possible to transplant tissue from individuals of either parent inbred strain into F1 hybrid animals resulting from the mating of the two inbred strains. Thus, it should be possible to place adrenal tissue of two inbred strains of mice into the environment of castrated, adrenal-ectomized F1 hybrid animals and to observe the response of these genetically different adrenal tissues to the same stimulating factors. In such preparations, if the histological appearance of the transplanted adrenals simulated that seen in castrate animals of the donor strain, it would appear that the responsiveness of the end organ itself was the principal factor in determining the type of adrenal response noted following castration; while, if it simulated that seen in castrates of the recipient hybrid stock, differences in the anin-
mal's response to castration would appear to be of the greater importance.

**MATERIALS AND METHODS**

The various inbred strains and hybrid crosses of the mice employed in this study are listed in Table 1. For convenience of reference, the type of post-castrational adrenal alteration noted in the various groups is also listed, as is the general type of sex steroid produced by these altered adrenals.

At the time of weaning, when both donor and recipient mice were from 4 to 6 weeks of age, the adrenals were removed from the donor animals, the periadrenal fat was dissected away, and the glands were cut in two. These four half adrenals were then implanted high in the axillary tissue of a recipient animal that was then immediately gonadectomized and adrenalectomized. Although it has been well demonstrated that the adrenal cortex will regenerate from the glomerulosa immediately under the capsule (10, 13), it was found in preliminary studies that a greater number of successful "takes" occurred when the adrenals were split open than when intact adrenals were grafted. For the first 2 or 3 months following the surgical procedure the animals were maintained on a semi-synthetic diet in which the potassium salts of the salt mixture had been replaced by the corresponding sodium salts.1 This diet was kindly supplied by Dr. J. T. King. It was found that, although the mortality rate was not excessive, the animals were generally in good health and that, although hyperplastic changes occurred regularly in the grafted adrenals, the general physical status of the animals included in this study is of considerable importance. In preliminary experiments carried out to determine the best methods of transplantation, etc., inbred Z animals were used. It was found that, although the mortality rate was not excessive, the animals were generally in good health and that, although hyperplastic changes occurred regularly in the grafted adrenals, the sex steroid output by these hyperplastic grafts appeared to be low as judged by the histo-

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**TABLE 1**

**INBRED STRAINS AND HYBRID CROSSES EMPLOYED**

<table>
<thead>
<tr>
<th>Stock</th>
<th>Histology of post-castrational adrenal change</th>
<th>Types of sex hormones produced by altered adrenals</th>
<th>In Vivo</th>
<th>In Vivo</th>
<th>In Vivo</th>
<th>In Vivo</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>Very minimal hyperplasia</td>
<td>Feminizing</td>
<td>CE</td>
<td>CE</td>
<td>CE</td>
<td>CE</td>
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<tr>
<td>Z</td>
<td>Carcinoma</td>
<td>Feminizing and masculinizing</td>
<td>AZF</td>
<td>ACEF</td>
<td>ZCEF</td>
<td>ZCEF</td>
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<tr>
<td>A</td>
<td>Carcinoma</td>
<td>Feminizing and masculinizing</td>
<td>ZCE</td>
<td>ZCE</td>
<td>ZCE</td>
<td>ZCE</td>
</tr>
</tbody>
</table>

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**RESULTS**

Since it has been shown that caloric restriction will somewhat delay the appearance of post-castrational adrenal cortical hyperplasia and will inhibit the production of sex steroids by histologically hyperplastic adrenals (9), the general physical status of the animals included in this study is of considerable importance. In preliminary experiments carried out to determine the best methods of transplantation, etc., inbred Z animals were used. It was found that, although the mortality rate was not excessive, the animals were generally in good health and that, although hyperplastic changes occurred regularly in the grafted adrenals, the sex steroid output by these hyperplastic grafts appeared to be low as judged by the histological appearance of the secondary sex organs. When hybrid animals were employed, however, the mortality rate in the immediate postoperative period and again after the animals were transferred to the Fox Chow diet was low (the combined mortality was about 15 per cent), and the general health of the animals was almost uniformly excellent. This is attested by the fact that in the animals included in this report, no example of well developed adrenal alteration was found in an animal in which good evidence of sex steroid produc-

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1 This diet was kindly supplied by Dr. J. T. King.
tion was not also present. In two groups of animals, AZF\textsubscript{1} females bearing AZF\textsubscript{1} female adrenal grafts and AZF\textsubscript{1} females transplanted with Z male adrenals, the general health of many of the animals appeared somewhat below normal, however, and in these groups the degree of stimulation of the secondary sex organs was somewhat less than would be expected on the basis of the degree of histological alteration noted in the adrenal grafts.

Studies carried out in several inbred strains of mice and their F\textsubscript{1} hybrids had indicated that the latent period between castration and the appearance of a partially cornified vaginal epithelium as indicated by vaginal smears varied from strain to strain but was rather constant in individuals of a given strain or F\textsubscript{1} hybrid cross (9). It had originally been hoped that this aspect of the problem could also be studied in these animals bearing grafted adrenals. The vaginal smear data accumulated, however, indicated that in general the period between operation and the appearance of a sub-estrous smear is greatly prolonged in the case of the grafted adrenals and sufficiently variable as to preclude meaningful analysis. Whereas sub-estrous vaginal smears appeared in animals of the hybrid crosses studied here in from 4 to 8 months after castration, in the animals bearing adrenal grafts, relatively few showed sub-estrous smears before 10 months following operation, and many did not exhibit vaginal evidence of estrogen stimulation until from 14 to 16 months after operation.

A rather careful study was made of the extra-adrenal cortical tissue, since, if these deposits underwent hyperplastic or carcinomatous changes and produced significant amounts of sex steroids, interpretation of the function of the grafted adrenals would be made more difficult. Of the 128 animals autopsied (100 females and 28 males) and included in this study, adrenal rests were identified along the course of the mesovarii or the ureters, but, owing to the large amount of abdominal fat, it was not possible to inspect these areas as closely as might be desired. In one animal which was excluded from the study, the suprarenal vein terminated in a small mass of hyperplastic cortical tissue, and it was assumed that this represented an instance of incomplete adrenalectomy rather than extra-adrenal cortical tissue. Multiple deposits of "rest" tissue were not infrequently encountered. The rest tissue of 38 of the animals (44 individual rests) was sectioned serially for study. Histologically, these rests were found to be composed of rather normal appearing cortical cells which usually had no tendency to be arranged in a zonal pattern. Not infrequently a portion of the rest was composed of an eosinophilic staining hyaline material which appeared to be a residual of degenerated cortical cells. There was usually no definite adrenal capsule surrounding these nests of cortical cells. Only seven of the rests studied microscopically varied from the above pattern. Three showed an area with a definite capsule and a tendency for a zonal arrangement below the segment of capsule, and in two of these rests there was a definite proliferation of the small subcapsular cells. Two other rests had large areas of subcapsular proliferation with a fair number of large, foamy cells (Type B cells of Woolley) interspersed, which were suggestive of early cortical hyperplasia, and in one other rest a definite small nodule of typical cortical hyperplasia was found. The production of sex steroids by this latter rest could not be determined, since the animal also carried a grafted adrenal that showed extensive cortical hyperplasia. The seventh atypical rest had a medium-sized cluster of medullary cells to one side of the cortical tissue. Since the cortical tissue in this rest showed no tendency for a zonal arrangement and the medullary tissue lay to one side of the cortical tissue, it was felt that this represented extra-adrenal tissue rather than a remnant of an incompletely excised adrenal gland.

A summarization of the histological changes found in the adrenals grafted to female animals and the histological evidence of sex steroid production by these grafts is given in Tables 2-4. From an examination of these data, it is apparent that the histology of the adrenal grafts as well as the production of histologically detectable amounts of estrogenic hormones by them simulate rather well the situation seen in ovariectomized animals of the donor strains (Table 1). Of 27 pairs of A strain adrenals transplanted to AZF\textsubscript{1} or ACE\textsubscript{1} hybrid females, only two became significantly hyperplastic and produced effective amounts of estrogen. This is in sharp contrast to the 42 grafts from Z, AZF\textsubscript{1}, CE, and ACE\textsubscript{1} donors transplanted to AZF\textsubscript{1} or ACE\textsubscript{1} hybrid females, for 26 of these showed marked histological alterations accompanied by the production of significant amounts of estrogen. As mentioned earlier, the adrenals of oophorectomized A strain female mice occasionally show some small areas of hyperplasia, but these apparently do not produce detectable amounts of sex steroids. It is of some interest, then, that in the
ACEF₁ female in which the grafted A adrenal produced significant amounts of estrogen, only one of the four adrenal fragments had undergone hyperplastic change, while the other three retained their normal histological pattern. Also in the AZF₁ animal in which the A adrenal graft had become hyperplastic, about one-half of the graft recovered at autopsy was composed of normal cortical tissue. By contrast, the usual picture seen in the grafted Z or AZF₁ adrenals was that of a much more extensive cortical hyperplasia with less residual normal cortex. This would suggest that even in the two instances of hyperplasia of grafted A strain adrenals the degree of anatomical alteration found was somewhat less than that seen in the hyperplastic grafts from Z or AZF₁ animals.

It should be emphasized that the hybrid animals bearing grafted A strain adrenals appeared to be in as good health as did those bearing the other types of grafts and that, in most instances, significant amounts of rather normal appearing cortical tissue were recovered at autopsy. The histology of the cortical tissue found in these adrenals, 12–17 months after transplantation, was not significantly different from that described by other authors in rats (10, 18). The variation from graft to graft was considerable. In some, large clumps of cortical cells were seen which appeared to be somewhat larger than normal and showed only a slight tendency to possess the typical zonal arrangement seen in in situ adrenals (Fig. 1). In other grafts, however, the architecture of the normal adrenal had been reconstructed rather well (Fig. 2). There were all variations between these two extremes in the grafts found in different animals and even between different fragments of the grafts in the same animal. There frequently was residual evidence of the initial degeneration that has been described as occurring immediately after transplantation (10). This usually consisted of areas of hyaline material which sometimes contained “crystal clefts” or, even rarely, areas of calcification. Although the majority of all the animals included in this study had grafts that contained goodly amounts of either normal or altered cortical tissue, a few of the animals that survived more than a year were found to have only very small amounts of residual adrenal graft tissue, but in most of these, the function of the small graft was apparently being augmented by the animal’s own “rest” tissue.

In the studies employing ZCEF₁ female recipients (Table 4) it is also evident that the histology of the post-castrational adrenal alteration was similar to that seen in the donor stock, for all five of the altered adrenals from donor Z animals showed only hyperplasia which was indistinguishable from that seen in the Z adrenals grafted to AZF₁ recipients (Figs. 4 and 5). On the other hand, in four of the six adrenals from F₁ donors and in all three of the adrenals from CE donors that showed significant post-castrational change, typical cortical carcinomas had developed (Fig. 6). It is probable that the sex steroids produced by the altered adrenal grafts materially affect the endocrine system of the recipient animals and thus alter the endocrine environment in which the grafts reside. It is also possible that the effect upon the host’s endocrine system of grafted Z adrenals might differ from that of the grafted CE or ZCEF₁ adrenals and that such differences might be responsible for the development of carcinomas in

### TABLE 2

<table>
<thead>
<tr>
<th>Donor stock</th>
<th>No. animals</th>
<th>Genital tract</th>
<th>Histology of transplant</th>
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<tr>
<td>A</td>
<td>12</td>
<td>1 stimulated</td>
<td>1 hyperplastic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11 atrophic</td>
<td>10 normal; 1 not autopsied</td>
</tr>
<tr>
<td>F₁</td>
<td>10</td>
<td>6 stimulated</td>
<td>6 hyperplastic</td>
</tr>
<tr>
<td></td>
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<td>2 minimal hyperplasia; 2 normal</td>
</tr>
<tr>
<td>Z</td>
<td>14</td>
<td>12 stimulated</td>
<td>12 hyperplastic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 atrophic</td>
<td>1 minimal hyperplasia; 1 normal (small)</td>
</tr>
<tr>
<td>Z</td>
<td>11</td>
<td>7 stimulated</td>
<td>7 hyperplastic</td>
</tr>
<tr>
<td>Male</td>
<td>4 atrophic</td>
<td>2 minimal hyperplasia; 2 normal</td>
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### TABLE 3

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<tr>
<th>Donor stock</th>
<th>No. animals</th>
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<th>Histology of transplant</th>
</tr>
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<td>15</td>
<td>1 stimulate</td>
<td>1 hyperplastic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14 atrophic</td>
<td>10 normal; 1 minimal hyperplasia; 3 not autopsied</td>
</tr>
<tr>
<td>F₁</td>
<td>12</td>
<td>6 stimulated</td>
<td>5 carcinomas; 1 hyperplastic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 atrophic</td>
<td>2 minimal hyperplasia; 4 normal</td>
</tr>
<tr>
<td>CE</td>
<td>6</td>
<td>2 stimulated</td>
<td>1 carcinomas; 1 hyperplastic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 atrophic</td>
<td>3 normal; 1 not autopsied</td>
</tr>
</tbody>
</table>

### TABLE 4

<table>
<thead>
<tr>
<th>Donor stock</th>
<th>No. animals</th>
<th>Genital tract</th>
<th>Histology of transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z</td>
<td>8</td>
<td>6 stimulated</td>
<td>5 hyperplastic; 1 not autopsied</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 atrophic</td>
<td>1 minimal hyperplasia; 1 normal</td>
</tr>
<tr>
<td>F₁</td>
<td>8</td>
<td>6 stimulated</td>
<td>4 carcinomas; 2 hyperplastic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 atrophic</td>
<td>2 minimal hyperplasia; 3 normal</td>
</tr>
<tr>
<td>CE</td>
<td>6</td>
<td>4 stimulated</td>
<td>3 carcinomas; 1 not autopsied</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 atrophic</td>
<td>1 minimal hyperplasia; 1 normal</td>
</tr>
<tr>
<td>Z and F₁</td>
<td>5</td>
<td>5 stimulated</td>
<td>F₁; 4 carcinomas; 1 hyperplastic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Z; 4 hyperplastic; 1 scar</td>
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the CE and ZCEF₁ grafts, while the Z grafts remain hyperplastic. To preclude this possibility, a small number of adrenalectomized, ovariectomized ZCEF₁ females received a single Z adrenal graft in the right axilla and a single ZCEF₁ adrenal graft in the left. Since the two types of adrenal tissue were now in the same animal, it seems safe to assume that they were being subjected to the same stimuli throughout the entire experiment. Due to an unfortunate circumstance, only five such animals were available for final study. Although all the animals showed subestrous smears before they were a year of age, they were not autopsied until they were sixteen months of age in order to assure a complete development of the histological alteration in the adrenals. As can be seen in Table 4, four of the five F₁ hybrid adrenals were carcinomatous, while four of the five Z adrenals showed only cortical hyperplasia. It would appear, then, that the histological type of adrenal alteration noted after castration is also a reflection of the responsiveness of the adrenal tissue itself.

Although all the evidence obtained from grafting the adrenals of female mice would indicate that end organ responsiveness per se plays the major role in determining the type of adrenal response noted in various strains of mice after oophorectomy, the situation in male mice remains of considerable interest. It had been found previously in certain strains of mice, e.g., Z strain, that, whereas oophorectomy is routinely followed by extensive hyperplastic changes in the adrenals with the production of goodly amounts of estrogic hormones, orchiectomy results in much less adrenal alteration and considerably less production of estrogen. In other stocks of animals, e.g., CE and ZCEF₁, on the other hand, both the degree of histological alteration noted and the production of sex steroids by these altered adrenals seem to be about the same in the two sexes. The question then arises whether this dissimilarity in the response of the adrenal in male and female Z animals is also due to differences in responsiveness of the end organ itself or to differences in the hormone alteration effected by gonadectomy in the two sexes.

Two experiments were set up to investigate this problem. In the first, a group of oophorectomized, adrenalectomized AZF₁ female animals was grafted with Z male adrenals. As can be seen in Table 9, seven of eleven of these male adrenal grafts became extensively hyperplastic and produced detectable amounts of estrogen. As mentioned earlier, for some reason the general health of this group of animals was somewhat inferior to that usually seen in the animals reported here, and the degree of stimulation of the secondary sex organs was correspondingly less than that seen in the healthier AZF₁ females bearing Z female adrenals; but it was approximately the same as that seen in the AZF₁ female recipients bearing AZF₁ female grafts (this latter group of animals tended also to be in somewhat poorer general health). In the second experiment, Z male adrenals were grafted into castrated, adrenalectomized ZCEF₁ male mice, and the results of this experiment are summarized in Table 5. In nine of the fourteen animals bearing Z male adrenal grafts and living to 18 months of age, the grafts showed extensive hyperplastic changes which were indistinguishable from those seen in the Z female adrenals grafted to ZCEF₁ female mice. The mammary glands of these animals showed extensive mammary gland development which was equal to that seen in the ZCEF₁ male animals bearing ZCEF₁ male adrenal grafts. It would thus appear that the Z male adrenal gland itself is not immediately evident, but,
on histological examination, considerable hyalination of these grafts was noted. The only major difference noted in the response of grafted adrenals to that seen in in situ adrenals was in the production of significant amounts of masculinizing hormones. The adrenal carcinomas that arise in gonadectomized CE mice, both male and female, frequently produce significant amounts of masculinizing as well as feminizing hormones (15–19). This is also true of the adrenal carcinomas developing in ACEF₁ and ZCEF₁ hybrid mice. In none of the animals bearing grafted CE, ACEF₁, or ZCEF₁ adrenals was there evidence of any significant masculinization from the histological examination of their salivary glands, kidneys, or seminal vesicles. The reason for the failure of these carcinomas developing in grafted adrenals to produce detectable amounts of androgenic hormones is not evident at the present time.

It is of some interest to record the presence of normal-appearing medullary tissue in these adrenal grafts. Although the adrenals were not prepared so that stains for chromaffin could be applied, since our primary interest was in the cortical changes, the appearance of the medullary cells is sufficiently characteristic in the routine hematoxylin-eosin preparation to allow easy identification. In 93 of the 127 adrenal grafts studied, histologically normal-appearing medullary cells were found. These cells were arranged in cords or clumps of varying sizes, and not infrequently where the morphology of the graft had not been too extensively altered either by transplantation or by post-castrational changes the location of these cells was similar to that seen in the in situ adrenals (Figs. 2 and 3). Whether this medullary tissue was functioning could not, of course, be determined by histological examination. However, it is of considerable interest that in most of these transplanted adrenals histologically normal medullary tissue was in evidence 12–17 months after the tissue had been transplanted, since there appears to be some species variation in the matter of the maintenance of grafted medullary tissue (for review see [7]). It is apparent, however, that in mice the medullary tissue need not be removed prior to the transplantation of cortical tissue and that significant amounts of medullary tissue will be maintained if the adrenals to be grafted are cut through to allow earlier vascularization of the medullary zone. In addition to the normal medullary tissue, areas of basophilic hyaline material were also frequently encountered which appeared to have resulted from a degeneration of medullary tissue.

DISCUSSION

Many differences in the anatomy and function of the endocrine system have been described in intact and experimentally manipulated mice of various inbred strains. It seems very likely that many, if not most, of these differences are in some way controlled by genetic factors. In most instances it has been as yet impossible to determine to what extent such differences result from dissimilarities in the inherent reactivity of the target tissues to similar degrees of stimulation or from differences in the general endocrine constitution of the different strains of mice resulting in differences in the stimulation to which the end organs are subjected. This problem can be studied in certain instances by placing the genetically dissimilar end organs from two inbred strains of mice into the identical environment of F₁ hybrid individuals arising from the mating of the two inbred stocks under consideration. In this way, genetically dissimilar tissue would be subjected to the same stimuli, and, in the case of grafted endocrine glands, the hormones produced by the genetically different glands would be metabolized in the same manner and act upon genetically similar secondary sexual tissues of the host animals.

In the initial experiments employing this technique, the histology and function of the ovaries of Z and A strain mice were compared (8). It was found that the differences in normal histology seen in the ovaries of these two strains of mice appeared to be due mainly to differences in the stimuli, probably pituitary in origin, that are acting upon the ovaries, for when these histologically different ovaries were placed in the environment of gonadectomized AZF₁ hybrid females they became histologically similar. More specifically, the A strain ovaries were altered histologically so that they became very similar in appearance to the ovaries of Z or AFZ₁ hybrid animals. The function of these histologically altered A strain ovaries appeared, however, to have certain characteristics that differed from those seen in the grafted Z strain ovaries suggesting that certain aspects of ovarian function were inherent in the grafted ovarian tissue itself.

The experiments reported here, in which the post-castrational alterations noted in grafted adrenals were studied, indicate rather clearly, however, that in this situation both the type of abnormal histological pattern that results and the function of this altered adrenal tissue are largely a reflection of a genetically controlled responsiveness of the adrenal tissue per se. In all instances, the type of post-castrational adrenal response noted in the grafted adrenals was that found in the adrenals of
gonadectomized animals of the donor stock. Adrenals from A strain mice when transplanted to AZF₁ or ACEF₁ recipients tended to show no particular post-castrational alteration and Z strain adrenals, whether grafted to AZF₁ or ZCEF₁ recipients, showed only hyperplastic changes with no increased tendency for carcinoma formation when the adrenals were in the environment of gonadectomized, adrenalectomized ZCEF₁ recipients. Although somewhat larger numbers of animals would have been desirable in each of the experimental groups, by pooling certain groups, numbers with greater statistical significance can be obtained. Thus, eighteen Z adrenals transplanted to ZCEF₁ recipients showed extensive post-castrational alteration, and in all instances serial sections of these grafts showed only hyperplastic changes, while of the nineteen CE or ZCEF₁ adrenal grafts showing extensive changes fourteen were carcinomatous. Some indication of variations in the response of the endocrine systems of different strains of mice to gonadectomy was, however, obtained. Thus, Z male adrenals, when transplanted to adrenalectomized, gonadectomized AZF₁ females or ZCEF₁ males, did show extensive hyperplastic changes and did produce effective amounts of estrogen. This finding would suggest that the dissimilarity in degree of adrenal hyperplasia noted in gonadectomized female and male Z strain animals results from a difference in the stimuli acting upon the cortical tissues of the two sexes following the removal of the gonads. Also, there were two instances in which grafted A strain adrenals did become more hyperplastic than is usually the case in oophorectomized A strain mice, and in these instances detectable amounts of estrogen were produced. It would appear then, that although the lack of reactivity of the adrenal tissue itself is primarily responsible for the lack of post-castrational adrenal alteration in A strain mice, the stimuli that these adrenals are subjected to within gonadectomized A mice may also be somewhat less than those to which they are subjected when grafted to gonadectomized, adrenalectomized AZF₁ or ACEF₁ animals.

In general, the anatomic restoration and function of the adrenal tissue following transplantation appeared to be rather good throughout these experiments, and the subsequent development of the post-castrational alterations reproduced rather well that seen in in situ adrenals. A few differences, however, were evidenced. In the group of female mice studied, 69 received female adrenal grafts from strains of animals in which definite post-castration adrenal alterations occur. In 47, or 68 per cent of these, the adrenal grafts showed extensive histological alterations with estrogen production, whereas essentially 100 per cent of in situ adrenals in oophorectomized females of these donor stocks showed the change. The time that elapsed between the grafting of the adrenal tissue and the appearance of a subestrus vaginal smear was usually considerably longer than the latent period between oophorectomy and the appearance of vaginal subestrus in otherwise intact mice of the donor strains, and the variations from animal to animal in the length of this latent period were much greater in the case of the grafted adrenals. The “delaying” effect of transplantation is probably also reflected in the grafted CE, ACEF₁, and ZCEF₁ adrenals in that, of the 27 grafts showing definite post-castrational alteration, only twenty had become carcinomatous, whereas essentially all animals of the donor stocks would have cortical carcinomas by from 9 to 12 months after gonadectomy. Probably the only disturbing difference, however, was the failure of grafted CE, ACEF₁, and ZCEF₁ adrenals to produce significant amounts of masculinizing hormones.

The failure of development of cortical hyperplasia or cortical carcinoma in the majority of the adrenal rests of the recipient animals was very fortunate, for it made evaluation of the sex hormone production of the adrenal grafts much easier. That these deposits of cortical tissue do not generally undergo hyperplastic or carcinomatous changes in gonadectomized animals probably reflects their usual lack of a histologically distinct capsule and underlying glomerulosa in which these cortical changes appear to be initiated in the adrenal gland itself.

SUMMARY

By a series of experiments in which the adrenals of mice of inbred strains were transplanted into the common environment of adrenalectomized, gonadectomized F₁ hybrid animals, evidence has been obtained to indicate that the differences in the changes noted in the adrenals of different inbred strains of mice following gonadectomy are due mainly to differences in a responsiveness inherent in the adrenal tissue itself. When adrenals of A strain females (which show only minimal post-castrational changes) were transplanted to ovariectomized, adrenalectomized AZF₁ and ACEF₁ recipients, only 2 of 27 pairs became hyperplastic and produced detectable amounts of estrogen, whereas 26 of 42 grafts from donor Z, AZF₁, CE and ACEF₁ animals showed estrogen-producing hyperplastic or carcinomatous changes characteristic of the donor stock when transplanted to similar recipient animals. Similarly, Z adrenals, trans-
Fig. 1.—An adrenal graft from an A strain donor that has been carried in an AZF₁ hybrid recipient for 17 months. The graft is composed of clumps of somewhat enlarged cortical cells with only minimal tendency for a zonal arrangement. Mag. ×70.

Fig. 2.—An adrenal graft from an A strain donor that has been carried in an AZF₁ hybrid recipient for 17 months. The histology of this graft rather faithfully reproduces that of an in situ adrenal. Note the definite zoning and the clump of medullary cells surrounding the central vein. Mag. ×70.

Fig. 3.—A high-powered view of a portion of the medullary tissue of the graft shown in Figure 2. Mag. ×350.

Fig. 4.—A portion of an adrenal graft from a Z strain donor that has been carried in an AZF₁ recipient for 17 months. Much of the graft is composed of hyperplastic cortex with some residual normal appearing cortex. Mag. ×70.

Fig. 5.—A portion of an adrenal graft from a Z strain donor that has been carried in a ZCEF₁ hybrid recipient for 16 months. The histology of the altered cortex is that of typical cortical hyperplasia with no evidence of carcinoma formation. Although the histology is somewhat different from that of the graft pictured in Figure 4, such minor degrees of variation were also present from graft to graft in recipients of the same hybrid cross so that no consistent difference was evident between Z strain grafts carried in AZF₁ and ZCEF₁ recipients. Mag. ×70.

Fig. 6.—A small portion of an adrenal graft from a ZCEF₁ donor carried in a ZCEF₁ recipient for 15 months. Most of the graft is composed of typical cortical carcinoma with a few areas of normal and hyperplastic cortex remaining. Mag. ×70.
planted to ZCEF₁ hybrid animals, showed only hyperplastic changes, while CE or ZCEF₁ adrenals, when transplanted to genetically identical recipients, tended to become carcinomatous. Evidence was obtained, however, to indicate that the lesser tendency for the adrenals of orchiectomized Z male mice to show extensive hyperplastic changes is caused, most probably, by a difference in the response of the endocrine system of Z male and female animals following gonadectomy.

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