The Effect of Hypophysectomy on the Development of Adrenal Tumors in C3H Mice*

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Adrenal cortical adenomas occur after gonadectomy in several inbred strains of mice (13, 14). Pituitary control of the adrenal cortex suggests that adenomatous changes in the cortex may also depend on pituitary hormonal influence. The present experiment deals with the effect of hypophysectomy on the development of postcastration adrenal changes in C3H mice. Since it was necessary to have pair-fed controls, some effects of partial inanition on the adrenals and other organs, as compared to the effects of hypophysectomy, were also observed.

Casas et al. (1) found that 33 and 50 per cent caloric restriction decreased but did not prevent adrenal cortical hyperplasia in ovariectomized C3H mice. Woolley (12) reported that castrated DBA mice did not respond well to hypophysectomy, but over a short period of time they did not develop nodular hyperplasia of the adrenals.

METHODS

C3H strain mice were used; they were 3 months old at operation. Castration and hypophysectomy were performed in 25 females and ten males, which were given Purina Fox Chow and water ad libitum. Castration and sham hypophysectomy, including removal of the bone plate, were carried out in equal numbers of animals, which were fed such daily weighed portions of fox chow that each animal matched one of the hypophysectomized animals in body weight. The average daily intake of hypophysectomized mice was 1.6 gm. The controls, which were much more active, required 1.9 gm. to maintain the same weight. Intact mice of the same age, given free access to food, consumed 4 gm./day.

Hypophysectomized animals and their controls were sacrificed at fairly evenly spaced intervals from 56 to 400 days after operation. Evidence of completeness of hypophysectomy was observed by the continuing loss of body weight in all animals during the experiment, absence of pituitary fragments at post mortem (using a dissection microscope), and absence of recognizable pituitary tissue in serial sections of the decalcified sellar region in five representative animals.

Body weights were obtained weekly, and vaginal smears on 3 consecutive days per month. At post mortem the principal organs were weighed. Serial sections at 5 μ were made of all adrenals.

Comparable observations were made on three to six mice of each sex in the following groups: (a) intact, 3–18 months of age; (b) hypophysectomized but not castrated; (c) castrated, sham-hypophysectomized, and fed ad libitum.

RESULTS

The most striking observation was the complete absence of large, pale-staining cells ("type B," Woolley and Little (14)) in the adrenal cortices of castrated-hypophysectomized mice and the presence of nests of these cells forming nodular hyperplasia, or adenomas, in all but one of the controls (Table 1). The one control mouse without hyperplasia was sacrificed 57 days after operation. Groups of small, darkly staining ("type A") cells, lying just under the capsule and sometimes penetrating into the middle of the cortex, were always present in castrated-hypophysectomized female mice, and in most males. According to Woolley and Chute (13), the criterion for the prevention of adrenal changes is the nonappearance of type B cells.

The histological changes in the adrenals of the control mice were similar to those described by Casas et al. (1) in castrated C3H mice which were restricted to 67 per cent or less of their normal caloric intake. In the present instance, the caloric restriction was a little over 50 per cent. The added operation of sham-hypophysectomy had no detectable permanent effect on the mice, either in regard to general health or the state of the adrenals. When such animals were given unlimited food they grew to normal size and weight.

In the control mice, after 2 months from the time of operation, there were small whitish or yellow opacities grossly visible just under the capsules of both adrenals. Microscopically, globular collections of the lipid-containing type B cells arose first in the outer part of the cortex in association with type A cells. Later the whole thickness of the cortex was involved, usually in two or three separate places in each cross section, and nodules of the abnormal cells bulged on the surface of the gland and into the medulla (Figs. 1, 2, 4, and 7).
In the castrated, sham-hypophysectomized controls without dietary restriction, the adenomatous process in the adrenal was similar in kind but more extensive than in the animals under food restriction (Figs. 1 and 7, compared to Figs. 2 and 4). No carcinomas were found.

Groups of type A cells were seen in the outer cortex of normal female C3H mice at 6 months of age. These cells also occurred in hypophysectomized, noncastrated mice, in which the adrenal cortex was apparently indistinguishable histologically from that found in hypophysectomized-castrated animals. In both these groups, a concentration of smaller, darkly stained cells in the innermost zone of the cortex was conspicuous, while it was absent or much less prominent in the controls, as may be seen in the figures. This area is usually referred to as the X zone.

In the hyperplastic areas of the adrenal cortex shown in Figures 2 and 4, there were areas of "brown degeneration" near the medulla, where masses of pigmented material filled the cells and coalesced. Frantz and Kirschbaum (4) suggested that this process, which consists of deposition of a brown ceroid pigment, was a degenerative alteration associated with aging. They observed it particularly in steroid-secreting cells of the adrenal cortex, ovary, and testes.

There is no evidence that the hypophysectomized mice or the controls under food restriction in the present experiment produced a significant amount of estrogenic hormone. Vaginal smears and sections were invariably castrate in type. The controls which were not under food restriction showed continuous sub-estrous smears after 2–3 months from the time of operation, presumably due to estrogen secreted by the adrenal adenomas.

### TABLE 1

**BODY WEIGHT AND INCIDENCE OF NODULAR HYPERPLASIA OF THE ADRENAL CORTEX**

Mice of the C3H strain were used. The castrated, sham-hypophysectomized controls were pair-fed to the same body weight as that of the castrated, hypophysectomized mice.

<table>
<thead>
<tr>
<th></th>
<th>HYPOPHYSECTOMY</th>
<th></th>
<th>SHAM-HYPOPHYSECTOMY</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Female</strong></td>
<td>23 19.4 13.5</td>
<td>77–400</td>
<td>23 0</td>
<td>23 18.0 13.6</td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td>10 22.1 14.2</td>
<td>56–248</td>
<td>6 0</td>
<td>10 22.2 14.5</td>
</tr>
</tbody>
</table>

* Number of mice in which groups of small, darkly stained, type A cells were found in the adrenal cortex.
† Corresponding figure for nests of large, pale-staining, type B cells. These constitute the nodular hyperplasia or adenomas.

### TABLE 2

**ORGAN AND BODY WEIGHTS**

The data from nine castrated-hypophysectomized female mice, autopsied 150–400 days after operation, are compared to corresponding data from nine sham-hypophysectomized, castrated control animals, which were underfed to the same body weight.

<table>
<thead>
<tr>
<th>ORGAN</th>
<th>HYPOPHYSECTOMY</th>
<th>SHAM-HYPOPHYSECTOMY</th>
<th>P&lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean wt. S.D.</td>
<td>Mean wt. S.D.</td>
<td></td>
</tr>
<tr>
<td>Adrenal (mg.)</td>
<td>0.73 0.15</td>
<td>1.97 0.23</td>
<td>0.01</td>
</tr>
<tr>
<td>Kidney (mg.)</td>
<td>62 3.5</td>
<td>169 8.6</td>
<td>0.01</td>
</tr>
<tr>
<td>Liver (mg.)</td>
<td>463 51</td>
<td>754 151</td>
<td>0.01</td>
</tr>
<tr>
<td>Heart (mg.)</td>
<td>36 3.9</td>
<td>66 2.7</td>
<td>0.01</td>
</tr>
<tr>
<td>Thymus (mg.)</td>
<td>10.7 2.1</td>
<td>2.3 0.9</td>
<td>0.01</td>
</tr>
<tr>
<td>Uterus (mg.)</td>
<td>2.7 0.36</td>
<td>3.0 0.95</td>
<td></td>
</tr>
<tr>
<td>Spleen (mg.)</td>
<td>27 4.0</td>
<td>21 6.2</td>
<td></td>
</tr>
<tr>
<td>Body wt. (gm.)</td>
<td>At operation</td>
<td>19.1 18.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>At autopsy</td>
<td>13.1 13.5</td>
<td></td>
</tr>
</tbody>
</table>

The adrenal cortex showed very little atrophy in the underfed controls, compared to that occurring after hypophysectomy (see figures and Table 2), while the medulla was little changed in either group. The organ weights given in Table 2 indicate that there was a much more profound atrophy of the heart, liver, and kidneys in the hypophysectomized animals than in the controls fed to the same body weight. The thymus, on the other hand, and possibly the spleen, were heavier in the hypophysectomized mice. Histologically, the cells of the organs weighing less in the hypophysectomized mice appeared to be smaller, and contained less cytoplasm than in the controls. These data indicate significant activity of the pituitary during a state...
Photomicrographs of mid-sections of mouse adrenals. 
Hematoxylin and eosin stain. Mag. ×150.

Fig. 1.—Female, castrated, sham-hypophysectomized, full-fed, 164 days after operation. Nests of pale-staining type B cells extend across the entire cortex and protrude into the medulla. Normal cortex to the right. Nuclei of nodule are darker than those of medulla.

Fig. 2.—Female, castrated, sham-hypophysectomized, underfed, 214 days after operation. Groups of type B cells, mingled with type A cells, extend through the cortex. Near the medulla are areas of brown degeneration. Normal cortex on both sides.

Fig. 3.—Female, castrated-hypophysectomized, 157 days after operation. Atrophy of cortex. Type A cells present.

Fig. 4.—Male, castrated, sham-hypophysectomized, underfed, 213 days after operation; control for mouse in Figure 6. Groups of both A and B cells extend across the cortex. Normal cortex on both sides.

Fig. 5.—Female, castrated-hypophysectomized, 210 days after operation. Similar to Figure 3, but with more atrophy. The medulla remains unchanged. Underfed control shown in Figure 2.

Fig. 6.—Male, castrated-hypophysectomized, 230 days after operation. Atrophy of cortex with no type A or B cells.

Fig. 7.—Male, castrated, sham-hypophysectomized, full-fed, 146 days after operation. Nests of type B cells extend across the cortex. Only part of the extent of the nodule is cut in this section. Adjacent normal cells are compressed.
of partial starvation in which body weight was maintained at less than one-half of normal for mice of the same age and strain in this laboratory.

DISCUSSION

Spontaneous adrenal cortical tumors are rare in human beings and in most if not all other species. Hyperplastic adrenal changes appear after gonadectomy in mice, guinea pigs (11), and hamsters (5). The spontaneous tumors in humans, as well as those induced in mice and guinea pigs, are capable of secreting androgen or estrogen. The administration of adrenocorticotropic hormone may cause the adrenal to secrete sex hormones in the absence of the gonads (7, 10). Apparently, the adrenal cortex, having a close embryological association with the gonads, retains a latent capacity to assume some of the gonadal function when stimulated by the pituitary. Removal of the gonads may act to promote adrenal hyperplasia by removing an inhibitory effect of sex hormone on the pituitary, or by releasing to the adrenals the pituitary hormone normally absorbed by the gonads.

The absence of adenomatous changes in the adrenals of castrated and hypophysectomized mice and their occurrence in controls fed to the same body weight are evidence that pituitary secretion is necessary for development of these tumors.

It has been found that large doses of estrogen prevent pathologic changes in the adrenals of mice after castration (6, 15), but "physiological" doses of estrogen, estrogen plus progesterone, or testosterone (3) do not prevent adenoma formation, at least in gonadectomized NH strain mice. The production of adrenal cortical tumors in rats by injection of growth hormone (8), and in one mouse by injection of adrenocorticotropic hormone (2), is perhaps significant as to which pituitary hormones are involved, but the experiments were done in nonhypophysectomized animals.

A state of "pseudo-hypophysectomy" resulting from inanition has been described (9), and evidence of depressed pituitary function has often been observed during starvation. Statistically significant differences in the weights of heart, kidney, liver, adrenal, and thymus between hypophysectomized mice and control mice pair-fed to maintain equal weight prove that the pituitary gland exerts important physiological actions even in extreme caloric deficiency.

SUMMARY

1. The development of postcastration adrenal cortical nodular hyperplasia in male and female CSH mice is prevented by hypophysectomy.
2. Food restriction in castrated and sham-hypophysectomized mice, fed so as to maintain the same body weight as that of castrated-hypophysectomized animals, does not prevent nodular hyperplasia in the adrenal cortex, though it does prevent evidence of estrogen secretion from the adrenal.
3. Significant differences in organ weights, between hypophysectomized mice and underfed controls with the same body weight, indicate that some important pituitary functions persist in spite of severe dietary restriction.

REFERENCES

The Effect of Hypophysectomy on the Development of Adrenal Tumors in C3H Mice

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