Neoplasms of the Adrenal Cortex in Noncastrate Mice*

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Neoplasms of the adrenal cortex have appeared following gonadectomy in mice of both sexes of certain strains (2, 3, 10-17), and there were indications that such adenomas secreted estrogenic hormone.

Although the incidence of cortical adenomas was high in gonadectomized mice of these strains, similar tumors in noncastrate animals are apparently very rare (1, 8). Consequently, it was of interest to the authors that in the Minnesota line of the NH strain of mice 1 adenomas appeared spontaneously in a majority of female mice over 12 months of age. Although true carcinomas have not been observed thus far in females of this stock, two malignant adrenal cortical tumors have appeared in male animals, one in an intact NH mouse almost 2 years of age, a second in an F1 hybrid male (NH × Strong A) which had received whole body irradiation with x-rays. These tumors were approximately one-third the size of the kidney. Cortical neoplasms also appeared in mice of other stocks which have been irradiated with x-rays. This subject will be discussed in a future communication.

MATERIALS AND METHODS

Zenker-fixed adrenal glands of untreated NH male and female mice of different ages were sectioned serially at 4 microns, every fifth section being mounted. Sections were stained with hematoxylin and counterstained with either eosin or Mallory's basic fuchsin. All female mice had been bred. The reproductive tracts were examined grossly and microscopically for evidence of the effects of secretion of sex hormones by the abnormal adrenal cortices.

RESULTS

NH female mice.—The adrenal glands of 14 female mice that were killed after one year of age were sectioned serially at 4 microns, every fifth section being mounted. Sections were stained with hematoxylin and counterstained with either eosin or Mallory's basic fuchsin. All female mice had been bred. The reproductive tracts were examined grossly and microscopically for evidence of the effects of secretion of sex hormones by the abnormal adrenal cortices.

merely nodular and only slightly enlarged, if at all, whereas in other cases the gland was from 2 to 4 times normal size. The glands of female NH mice were in general larger and whiter than those of males. In order to demonstrate adenomas histologically, serial sections of the adrenal glands were required. If an area of subcapsular hyperplasia invaded almost the entire thickness of the adrenal cortex, and "clear cells" as well as fibroblast-like elements were present, then the development was classified as an adenoma (Fig. 3). In several instances most of the adrenal cortex was replaced by adenomatous tissue (Figs. 6 and 10) although grossly the gland was but slightly enlarged.

Hyperestrinism was definitely demonstrated in one case. This was in a mouse 18 months of age. In spite of the obvious secretory inactivity of the ovary (Fig. 13), the endometrium was hypertrophic (Fig. 11) and the vaginal epithelium (Fig. 12) showed evidence of estrogenic stimulation. Fig. 10 indicates the appearance of an adrenal gland of this animal; most of the cortex was transformed into adenomatous tissue. The latter was histologically similar to the cortical alterations which have been described in castrate mice (14). Immediately beneath the capsule were basophilic fibroblast-like elements, the subcapsular "type A" (12) cells (Fig. 2). Among these there developed rosettes of the "clear" or "type B" cells (Fig. 4). Some spontaneous tumors were composed chiefly of subcapsular type A cells (Fig. 1), but in most instances rosettes of type B cells were found within the new growth.

Concerning the origin of the type A cells, it was the impression of the authors that they were derived from the capsule itself, rather than from cells of the zona glomerulosa. The type B cells represent differentiated type A elements. Regeneration of normal adrenal cortex from capsular elements has been described by several investigators (5, 9, 18).

NH male mice.—In 8 male mice over one year of age adenomas were not observed although subcapsular hyperplasia of type A cells (Fig. 5) was present. One spontaneous cortical tumor has been observed in a male mouse and this was larger (5 mm. in diameter) than any of the tumors that appeared in

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1 This stock was obtained in the 8th inbred generation from Dr. L. C. Strong of the Yale University School of Medicine.
females. The tumor was composed of cords of undifferentiated cells which resembled type A cells to a greater degree than any other cortical cell (Fig. 8). The cytoplasm was more abundant, however, than in the type A cell of an adenoma, and there was a tendency towards the rosette formation which characterizes the "clear cells." Mitotic figures were present. Neither cortex nor medulla of the original adrenal gland could be found. Histologically this neoplasm might be classified as an adenoma rather than a carcinoma. It proved to be transplantable, and a growth 1 cm. in diameter was attained in the first transfer generation 6 weeks following transplantation. This tumor was similar in structure to the spontaneous cortical tumor reported in an intact female of the C strain (1).

Cortical carcinomas have been found in male mice irradiated with x-rays. One of these was in an F1 hybrid cross between strains NH and Strong A that had received a total of 1,000 r of x-rays more than a year preceding autopsy. Sections are shown in Figs. 9 and 14. This tumor proved to be transplantable in F1 hybrid mice of the same genetic constitution as the original host. This tumor and the neoplasm in the other NH male described above are classified as malignant on the basis of their microscopic structures. In neither host was there evidence of metastasis.

The germinal epithelium of both tumor-bearing male mice was spermatogenically active (Fig. 15). In the intertubular areas there were numerous "brown cells" which might suggest estrogenic stimulation by these tumors; injections of estrogenic hormone into male mice result in the appearance of similar brown cells of the testis (4). Seminal vesicles were atrophic and did not evidence any effects of androgenic secretion.

**DISCUSSION**

These observations indicate that in the NH stock of mice gonadectomy is not necessary for the development of cortical neoplasms of the adrenal. They occur spontaneously in intact mice of both sexes. Animals bearing such tumors have all been beyond 1 year of age. Gonadectomy in this stock accelerates the onset of adenoma formation (6). Other adenomas which have been described following ovariectomy in the NH stock were in general larger than the spontaneous tumors reported here (3). They were also reported in older animals, and all of such tumors gave evidence of secretion of estrogen.

In most instances there was no evidence of pronounced estrogenic stimulation of the female reproductive tract in animals bearing spontaneous cortical adenomas. Further study is necessary to determine whether involution of uterus and vagina is delayed in this stock as compared with others that do not develop cortical adenomas. In one case hyperestrinism was demonstrated, indicating that spontaneous adenomas of the adrenal cortex, as well as those induced by castration, may secrete estrogenic hormone. Additional observations will be required to determine whether steroid hormones other than the estrogenic are produced by these adenomas.

The cells of the cortical adenomas which have been associated with the secretion of estrogen are the type B "clear cells." These possess a cytoplasm with little affinity for either acid or basic dyes; sparse acidophilic granulation appears in many of the cells. Similar cells can be seen occasionally in the ovaries of old mice of the NH stock (Fig. 7). The authors have studied an x-ray-induced ovarian tumor that was actively secreting estrogen and possessed similar clear cells.

The tendency towards development of adrenal cortical tumors following gonadectomy has been found to be present in stocks of mice with striking susceptibility (appearance of cancer in a high percentage of virgin females) towards the development of mammary tumors (10). This susceptibility was manifested only in the presence of the milk agent when adrenal adenomas served as the source of estrogenic hormone.

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**FIGURES 1 TO 9**

Fig. 1.—Spontaneous tumor of the adrenal cortex composed almost entirely of basophilic type A cells. From female NH mouse 16 months of age. Mag. X 40.

Fig. 2.—Type A cells from a spontaneous adenoma occurring in a female NH mouse. Mag. X 250.

Fig. 3.—Spontaneous adenoma from adrenal cortex of female NH mouse. Note type A cells and rosettes of the type B "clear cells." Mag. X 160.

Fig. 4.—Type B "clear cells" from a spontaneous cortical adenoma. Mag. X 350.

Fig. 5.—Hyperplasia of type A cells beneath the capsule of the adenoma of a male NH mouse 14 months of age. Mag. X 160.

Fig. 6.—Section through the adrenal gland of a female NH mouse 17 months of age. Adenomatous tissue of the cortex is outlined in white; remaining cortical tissue normal. The cells which photograph black are "brown cells" stained with basic fuchsin. Mag. X 40.

Fig. 7.—Cells of ovary from an old NH female. Note resemblance of these cells to the type B "clear cells" in Fig. 4. Mag. X 250.

Fig. 8.—Section from cortical neoplasm occurring in an NH male 23 months of age. The tumor cells resemble type A cells, although the cytoplasm is more abundant. Grossly tumor was 5 mm. in diameter. Mag. X 250.

Fig. 9.—Section of carcinoma of the adrenal cortex appearing in an F1 hybrid male (strain NH crossed with Strong A strain). Mouse was 16 months of age and had been irradiated with 1000 r of x-rays in divided doses one year earlier. Mag. X 250.
in gonadectomized mice (7). The NH stock does not develop spontaneous mammary cancer (6), and reciprocal crosses have demonstrated that this stock lacks the milk agent (6). NH females might possess the "hormonal constitution" associated with pronounced susceptibility to mammary cancer development, but lack other factors necessary for the genesis of spontaneous mammary cancer.

Since adrenal cortical adenomas develop following castration, it might be inferred that the adenomas of the NH stock appear subsequent to the spontaneous cessation of gonadal activity. In the two cases of cortical tumor appearing in males, however, the germinal epithelium was active in the production of sperm (Fig. 15). These mice were not "physiologic castrates" from the standpoint of germ cell production, although the androgenic output of the testes was probably low as judged by the histology of the seminal vesicles. Androgenic secretion might have been counterbalanced, however, by the estrogen production of the tumors.

SUMMARY

In the NH stock of mice gonadectomy is not necessary to induce the development of neoplasms of the adrenal cortex. Adenomas of the adrenal cortex appeared spontaneously in 13 of 14 intact female mice over one year of age. In one instance the secretion of relatively large amounts of estrogenic hormone by such an adenoma was demonstrated. Cortical adenomas are probably relatively infrequent in male mice of this stock. Two histologically malignant adrenal cortical tumors found in male mice are described. The testes of both mice were spermatogenically active.

REFERENCES


DESCRIPTION OF FIGURES 10 TO 15

Fig. 10.—Cortical adenoma occurring spontaneously in an NH female mouse 18 months of age. Adenomatous tissue is outlined in white. Remainder of cortex normal. Mag. X 40.

Fig. 11.—Hypertrophic endometrium taken from same mouse whose adrenal is shown in Fig. 10. Cystic glands are indicative of hyperestrinism. Mag. X 90.

Fig. 12.—Vaginal epithelium from same mouse. Marked stratification of the squamous epithelium indicative of hyperestrinism. Mag. X 100.

Fig. 13.—Ovary of same mouse, tissues from which are shown in Figs. 10-12. Ovary composed of basophilic connective tissue cells and "brown cells," which are the large cells appearing light-grey in this photograph. Mag. X 40.

Fig. 14.—Section from carcinoma of the adrenal cortex appearing in F1 hybrid male (NH X Strong A) which had previously been irradiated with x-rays. Mag. X 500.

Fig. 15.—Section through a seminiferous tubule of the NH male bearing the adrenal carcinoma pictured in Fig. 14. Note the active germinal epithelium with mature sperm cells. Mag. X 300.
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