Estrogenic Effects of Adrenal Tumors of Ovariectomized Mice*

W. U. Gardner, Ph.D.

(From the Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

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Certain tumors of the adrenal glands in man have been associated with evidences of masculinization. Precocious sexual development or intersexual conditions are associated with the development of adrenal tumors in young individuals (8). Androgenic substances have been obtained in large amounts from the urine of several patients with such tumors (1, 3, 5). More recently the urinary androgens of individuals with adrenal tumors have been extracted and chemically identified (3, 5). An increased amount of estrogen, presumably estrone, was eliminated in the urine of one patient with an adrenal tumor (1).

Few tumors of the adrenal glands of experimental animals have been described, especially tumors demonstrating stimulation of the genital tissues. Masculinization accompanied adrenal adenomas in old male guinea pigs which were castrated shortly after birth (13). Mammary tumors have appeared in old female mice of the JAX Dba strain which had been ovariectomized immediately after birth (17, 18). These mice showed tumorous changes of the adrenal glands which were associated with mammary proliferation. These observations have been extended more recently to include mice of one other strain (19).

During the last few years a number of tumorous conditions of the adrenal glands have been observed in our laboratory in ovariectomized mice receiving intravaginal instillation of benzpyrene dissolved in oil, in untreated ovariectomized mice, or in mice receiving estrogens for prolonged periods. The adrenal tumors in the untreated ovariectomized mice and the associated evidence of physiological activity will be described.

Materials and Methods

Fifteen mice were ovariectomized when 43 to 65 days of age. The ovaries, ovarian capsules, uterine tubes, and upper part of the uterine horns were removed. The mice were from the third and fourth generations of the NH strain, developed by Dr. L. C. Strong by hybridizing the JK and CBAN strains. The mice of the parental strains showed a very low incidence of spontaneous mammary tumors. Three of the test animals had received one injection of a substance tested for possible estrogenic activity and found to be inactive. They were fed Purina Fox Chow and water. All of the animals were autopsied shortly after death or were killed when death was considered to be imminent. The genital tissues, adrenal and mammary glands, and skeletal tissues were examined and prepared for histological examination.

Observations

The mice of the early generations of inbreeding were not homogenous for coat color or, presumably, for other characters. The castrated mice attained weights of over 40 gm. and were obese until they became quite old. Two mice developed mammary tumors and 2 had lymphatic leukemia. At autopsy the accessory genital tissues and mammary glands were well developed in the first animal examined and both adrenals were enlarged to several times the usual size (No. 9, Table I).

The adrenal glands or tumors of the mice varied in gross appearance at the time of autopsy, depending in part on their size and, as determined later, their microscopic structure. The 3 largest tumors (Nos. 10, 23, and 25, Table I) were reddish and approximately two-thirds as large as the kidneys, weighing from 146 to 158 mgm. Their external peritoneal surfaces were smooth and they were apparently quite loosely attached to the perirenal adipose tissue. Small brownish or red spots were seen in one of the tumors. Adrenal tissue was not detected on gross examination of the larger tumors. Small tumors which appeared in one or both adrenals of 6 mice were irregularly shaped and adrenal cortical tissue was still recognizable (Figs. 1 and 2). The tumors of several animals in this group weighed 30 to 50 mgm. Areas of pale reddish tissue of variable sizes appeared at one or more points of the enlarged glands and brownish and light yellowish adrenal tissue could be identified at other points. The glands had irregular surfaces and were pear-shaped or irregular rather than rounded or oval as the normal adrenal gland or the largest tumors. The smaller tumors of the adrenal
glands of 2 mice were not identified definitely before microscopic examination (Fig. 3). The adrenal glands presenting such tumors were usually spotted with pinkish, brown, and yellowish patches, had a slightly irregular surface, and were enlarged. Tumors arose in both adrenals of at least 7 mice although one tumor was usually larger than the other. When the tumors were unilaterally they appeared on either side although 2 of the 3 largest were on the right.

The larger tumors were composed of cords of small, densely staining cells or follicle-like structures lined by a stratified epithelium. The cords of cells or follicles were surrounded by delicate connective tissue septa (Figs. 4 and 5). Peripherally the cords contained cords of small, hyperchromatic cells and large cells with a vacuolated or clear cytoplasm. The follicle-like cavities were filled with either a coagulated material or with blood. The smaller cells resembled those in the glomerulosa of a hyperplastic adrenal cortex. Cell boundaries were usually indefinite, in this respect resembling the granulosa cells of the ovarian follicles. Hyperplasia was not uniform throughout the tumors. Some fields observed at high magnification showed numerous mitotic figures while few dividing cells were found in other regions. Fragments of normal cortical tissue were detected at one or more comparatively limited areas at the periphery of the larger tumors. Although a definite capsule was not present, invasion by the larger tumors of the surrounding tissue was limited. The tumors of moderate size (Figs. 1 and 2) were more definitely circumscribed although they had destroyed the greater part of the normal adrenal tissues and had greatly enlarged the adrenal structure. The smaller tumors were more or less circumscribed in the cortical tissues as well as by the surrounding tissue. In the smaller tumors, cords of large vacuolated cells intermingled with the peripheral cords and islands of the smaller hyperchromatic tumor cells. Occasionally small masses of brownish cellular material (brown degeneration) also intermingled with the hyperchromatic cells (Figs. 3 and 4).

The number of small tumors was insufficient to permit a definite determination of their site of origin. The smaller lesions (Fig. 3) involved the entire thickness of the adrenal cortex, and although their cells resembled those of the glomerulosa, which may be quite thick in mice, the present observations merely indicate a glomerular origin. Around some of the adrenal glands containing the smaller tumors nodular areas were observed which apparently had penetrated the adrenal capsule and pushed out into the perirenal fat. These nodules contained irregular cords of cells apparently undergoing fatty degeneration with loss of cellular detail. This might indicate regression of the smaller tumors which formerly contained a preponderance of large vacuolated cells.

Careful examination at autopsy of the regional nodes and lungs failed to disclose metastatic tumors.

The accessory genital organs presented varying states of hypertrophy or regression closely paralleling the

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<th>Mouse No.</th>
<th>Age castrated, days</th>
<th>Age at death, days</th>
<th>Uterus</th>
<th>Interpubic ligaments, mm.</th>
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condition of the adrenal glands. The mammary glands were all developed more extensively than those usually observed in castrated mice. The mammary glands were especially well developed in the mice with both medium sized and larger adrenal tumors. The ducts were large, and many small branches and alveoli were present (Fig. 7). The glands of two mice contained localized alveolar nodules. The ducts contained concretion-like secretory products similar to those found in the glands of mice subjected to prolonged estrogenic treatment. Two mice had mammary adenocarcinomas.

The uteri usually resembled those of mice which had received variable amounts of estrogen for prolonged periods. Some were large and contained many distended and cystic glands (Fig. 6). One mouse died with pyometra. One mouse had small uterine horns and the stroma of the endometrium showed marked hyaline degeneration. All of the mice with the larger tumors had a thick stratified or cornified vaginal epithelium (Fig. 8). The cervices were greatly enlarged by a hypertrophy and mucoid-like transformation of the stroma.

The separation of the pubes at the symphysis by an interpubic ligament also indicated the influence of estrogenic hormone. The femurs of two of the mice contained numerous osseous spicules in the medullary cavities, and the diaphyseal walls were thickened.

**Discussion**

The relationship between the gonads and adrenal glands has been indicated in several ways. The clinical picture accompanying adrenal tumors may indicate abnormal stimulation by gonadal hormones. The administration of gonadal hormones and at least some of the adrenal hormones has revealed some overlapping of physiological activities (15). The injection of desoxycorticosterone results in the formation of a progestational endometrium in rabbits when preceded by estrogen and in the appearance of the copulatory response in guinea pigs (15). The above tissue or animal reactions were also induced by progesterone.

Desoxycorticosterone will also induce mammary growth in mice (16). On the other hand, several sex hormones have effects qualitatively similar to some adrenal hormones on salt metabolism (14). The gonadal hormones, especially the estrogenic, induce changes of the pituitary glands, which in some species are manifest both histologically and functionally (6).

The removal of the gonads increases the gonadotropic hormone in the hypophysis. The influence of chronically administered estrogens upon the degranulation of the chromophilic cells of the hypophysis, in rats and mice, also indicates a reciprocal hypophysial-gonadal relationship. Hypophysial tumors arise in such animals although other factors are probably also involved (8).

In mice of at least one strain interstitial cell tumors of the testis appear in estrogen-treated mice (7, 10), although prolonged estrogenic stimulation more typically leads to regression of both the glandular interstitial cells and the seminiferous epithelium.

In common with the influence of estrogens on the development of hypophysial and testicular tumors an explanation of the mechanism contributing to the proliferation of the adrenal glands with the formation of tumors following castration is not available. That they result from a hormonal disturbance or lack of balance is entirely probable but not especially informative.

The autonomy of the adrenal tumors has not been proved by transplantation into genetically related hosts in various physiological states. Some tumors, malignant in some respects, are capable of progressive growth in closely related hosts only when these hosts are properly adapted physiologically. Transplantation was not attempted in the present experiments because the heterozygous nature of the animals would probably have led to negative results even with tumors unquestionably malignant.

Adrenal tumors in ovariectomized mice have been uniformly associated with evidence of estrogenic stimulation of their hosts. In the present experiments the size of the tumor could be roughly correlated with the evidences of the high or low levels of estrogenic stimulation. That the endocrine activity was exerted by, or at least associated with these tumors, was in-
cated by the condition of all the accessory genital tissues and the skeletal tissues. The dissolution of the pubic symphysis, for example, was inhibited by adequate testosterone even when estrogens were administered. The cystic uteri, enlarged cervixes, well developed mammary glands containing intraductal concretions and nodules have not accompanied the injection of androgens.

The appearance of mammary adenocarcinomas in two of the mice and of lymphoid tumors in two other animals provides little significant material for interpretation since control animals maintained on the same diet are not available. On another diet, mammary or lymphoid tumors occurred rarely in NH mice of these particular generations. All hormones which have been capable of inducing and maintaining adequate mammary development in male mice from strains with an appreciable susceptibility to mammary tumors have been followed by mammary neoplasia. It seems possible that intrinsic hormones capable of eliciting mammary growth, whether produced by the hyperplastic or neoplastic adrenal tissue or the ovary, might result in mammary tumors in the proper animals. The point of major interest is not that mammary tumors appeared, but that adrenal neoplasia associated with evidences of estrogenic stimulation were acquired by ovariectomized mice, in this instance from a stock of presumably low susceptibility to mammary tumor.

These adrenal tumors were associated with evidences of estrogenic stimulation as determined by the condition of the uteri, pelves, and mammary glands.

REFERENCES


SUMMARY

Tumors of the adrenal glands arose in 13 of 15 ovariectomized mice of the early generations of the NH strain. The tumors weighed from 158 to 146 mgm. in 3 mice. The adrenal glands of 6 mice were two or more times the usual dimensions; in some animals they weighed 30 to 50 mgm. The tumors consisted of cords or, in one case, follicular structures of small hyperplastic cells. The smaller tumors showed some indication of cellular differentiation, since large vacuolated cells frequently intermingled with the cords or islands of smaller cells.


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