Further Studies on the Pathogenesis of Uterine Lesions in DBA × CE and Reciprocal Hybrid Mice*

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It has been observed repeatedly in mice bearing adrenal cortical neoplasms consequent to early ovariectomy that the accessory organs of reproduction show both gross and microscopic evidence of hormonal stimulation—in reciprocal crosses between C57BL, DBA, CE, A, and C3H (2); in DBA (4); in C3H × A hybrids (10); in CE (11). In several of the studies cited above, cystic glandular hyperplasia and other histologic abnormalities were noted in the uteri. Similar observations have been reported in nonovariectomized NH mice in which adrenal cortical tumors arise spontaneously (5). Considerable evidence has accumulated which indicates that the growth and differentiation of the accessory reproductive organs in these animals are due to the secretion of estrogen (3, 4, 5, 11) and androgen (6, 11) by the neoplastic adrenal tissue.

A more recent study of the pathogenesis of the uterine lesions in DBA × CE and reciprocal hybrid mice has revealed the presence of hyperestrinism associated with the production of considerable amounts of progesterone and/or androgen (1). This hormonal imbalance manifests itself at 6–8 months of age and persists throughout life in both ovariectomized and nonovariectomized virgin animals. Because the findings were essentially the same in both intact and ovariectomized mice, the adrenal cortex was presumed to be the source of the steroid sex hormones, whether cortical tumors were present or not. The evidence for this assumption, however, was presumptive. The present experiment, therefore, was designed to determine more conclusively the sites of steroid sex hormone production in intact mice before and after the onset of the endocrinopathy as indicated by the development of uterine glandular hyperplasia and adenomyosis.

MATERIALS AND METHODS

Twenty-nine F_1 female mice, born and housed at the Jackson Laboratory throughout the experiment, were obtained from DBA/2Wy × CE/Wy and reciprocal matings. They were segregated from males at the time of weaning and received no treatment until the present study was begun. The animals were maintained on a diet of Purina Fox Chow and tap water, except in the case of the adrenalectomized mice noted below.

When the mice were from 8 to 24 months of age, the left uterine horn was removed surgically to determine whether lesions were present and the extent to which they had progressed. Nine to 13 days later, eighteen of the animals were bilaterally adrenalectomized, and the remaining eleven were ovariectomized. Sixteen to 27 days were allowed for the effects of adrenalectomy or ovariectomy on the cytology of the vaginal mucosa to become established. During this period the adrenalectomized mice were maintained on 1 per cent NaCl in their drinking water.

At the termination of the experiment the animals were sacrificed and examined grossly to ascertain the complete removal of the glands in question. In the adrenalectomized mice a search was made for the presence of accessory adrenal tissue. The remainder of the reproductive tract was fixed for several days in Vandergrift's fluid.[1] Longitudinal paraffin sections of the uterus and vagina, 6 μ in thickness, were prepared and stained with azure eosinate, buffered at pH 6.2 according to the method of Lillie and Pasternack (9).

The cytology of the vaginal epithelium was studied microscopically in order to ascertain the

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[1] Vandergrift's fluid: 95 per cent ethyl alcohol, 80 ml.; 40 per cent formalin, 12 ml.; glacial acetic acid, 4.5 ml.; picric acid, 4 gm.; urea, 0.5 gm.; and mercuric chloride, 0.2 gm.

activity of endogenous steroid sex hormones at the time of autopsy. The following cytologic patterns, established in the rat by Freud (7) and subsequently applied to the mouse (8), were used:

Type I (epithelium two to three cells thick. The long axes of cells in the most superficial layer tend to be perpendicular to the surface of the epithelium). Found in infantile, lactating, and ovariectomized animals.

Type II (epithelium five or more cells thick, without mucification or cornification).— Found in diestrus and proestrus and in early stages of estrogen treatment in the castrate.

Type III (epithelium, five or more cells thick, with mucification of one or more of the superficial layers of cells).— Found in pregnancy and pseudopregnancy and in the castrate treated with both estrogen and progesterone or androgen.

Type IV (epithelium five or more cells thick, with cornification of the more superficial layers).—Found in estrus and in later stages of estrogen treatment in the castrate.

As noted above, uterine biopsies were taken in all animals shortly before removal of the adrenals or ovaries to determine whether lesions had developed and, if present, to what extent. The extent of uterine change was classified on the basis of the morphologic patterns of the endometrial glands previously described in detail (1). Uteri containing only an occasional gland showing cystic or adenomatous hyperplasia were designated as slightly pathologic; uteri in which a substantial minority of glands showed these morphologic abnormalities or in which an occasional adenomyotic gland was present were designated as moderately pathologic, uteri in which a majority of glands showed hyperplastic changes and/or in which more extensive adenomyosis was present were designated as markedly pathologic.

OBSERVATIONS

The effects of ovariectomy and adrenalectomy on the production of steroid sex hormones in virgin mice, as indicated by the type of vaginal cytology present 16–27 days postoperatively, are summarized in Tables 1 and 2. It can be readily seen that ovariectomy was followed by regression of the vaginal mucosa to the castrate type (Type I) in nine of eleven animals. After adrenalectomy, on the other hand, the vaginal mucosa showed cytologic evidence of continued hormonal stimulation (Types II, III, and IV) in all eighteen animals studied. The results were the same whether ovariectomy or adrenalectomy was performed before or after the onset of hyperestrinism, as indicated by the presence or absence of pathologic changes in the uterus.

The estimation of sex hormone activity on the basis of cytologic changes in the uteri themselves was not attempted, since preliminary study indicated that the results of such an investigation would be of questionable value in view of the extensive pathological changes present in many of the uteri.

TABLE 1

EFFECT OF OVARIECTOMY ON THE VAGINAL CYTOLOGY OF DBA × CE AND RECIPROCAL HYBRID MICE

Animal	Age (months)	Extent of uterine lesions 9–12 days before ovariectomy	Type of vaginal cytology 16–27 days after ovariectomy
14904	8	negligible	I
15014	8	slight	III
17705	8	negligible	I
17708	8	slight	I
12025	12	moderate	II
12827	12	slight	I
8118	18	marked	I
8599	18	marked	I
9712	18	moderate	I
4269	24	marked	I
4947	24	moderate	I

TABLE 2

EFFECT OF ADRENALECTOMY ON THE VAGINAL CYTOLOGY OF DBA × CE AND RECIPROCAL HYBRID MICE

Animal	Age (months)	Extent of uterine lesions 9–12 days before adrenalectomy	Type of vaginal cytology 16–27 days after adrenalectomy
14902	8	negligible	II
14903	8	slight	III
15304	8	negligible	II
15444	8	slight	II
17509	8	negligible	IV
17060	8	negligible	III
17703*	8	negligible	II
17704	8	negligible	II
17709	8	slight	IV
17710	8	moderate	II
14518*	10	moderate	III
15016	10	slight	III
15017*	10	moderate	IV
12023	12	moderate	IV
12024*	12	marked	III
12182	12	slight	IV
8598	18	marked	III
4268	24	marked	II

* Accessory adrenal tissue found at autopsy.

DISCUSSION

It is evident from the results of the present experiments that in virgin DBA × CE and reciprocal hybrid mice the ovary is the principal site of estrogen production. This conclusion is supported by the observation that ovariectomy was followed by regression of the vaginal mucosa to the castrate type in the majority of animals studied, whereas in no instance did adrenalectomy produce this result.

The evidence concerning the sites of production of progesterone and androgen is somewhat less direct. The occurrence of one instance each of Type II and Type III vaginal mucosae in the

ovariectomized series (Table 1) indicates that in these particular animals the adrenal cortex was elaborating physiologically effective amounts of estrogen and androgen or progesterone. Since there was no evidence of hormonal activity in the remaining nine ovariectomized mice, however, it seems reasonable to assume that the ovary is the chief source of androgen and progesterone as well as estrogen. The possibility remains that the adrenal cortex may produce quantities of sex hormones which are generally insufficient to elicit a vaginal response in the ovariectomized animal but which might play an important role in supplementing the hormones produced by the ovaries in the intact mouse.

The present observations have rendered untenable the earlier hypothesis concerning the role of the adrenal cortex in the pathogenesis of uterine lesions in the virgin DBA × CE and reciprocal hybrid mouse (1). It can no longer be assumed that the adrenal cortex is the principal source of steroid sex hormones in the intact animal which does not show morphologic evidence of cortical dysfunction. It now seems apparent that the hormonal imbalance leading to the development of uterine lesions is mediated through the ovaries in the intact virgin mouse and through the neoplastic adrenal tissue in the castrate. In each case, the ultimate mechanism which initiates the chain of events leading to the steroid hormone imbalance and subsequent abnormal uterine growth remains obscure.

SUMMARY

The effects of ovariectomy or adrenalectomy on the production of steroid sex hormones, as indicated by the cytologic changes in the vaginal mucosa, were determined in a series of 29 virgin DBA × CE and reciprocal hybrid mice before and after the appearance of the uterine lesions which develop spontaneously in these animals at 6–8 months of age. Ovariectomy was followed by regression of the vaginal mucosa to the castrate type in nine of eleven animals, whereas after adrenalectomy there was evidence of hormonal activity in all eighteen animals examined. The results were the same both before and after the onset of pathologic changes in the uteri. These findings indicate that the ovaries are the immediate source of the steroid hormone imbalance responsible for the development of the uterine lesions.

REFERENCES

1. CHRISTY, N. P.; DICKIE, M. M.; ATKINSON, W. B.; and WOOLLEY, G. W. The Pathogenesis of Uterine Lesions in Virgin Mice and in Gonadectomized Mice Bearing Adrenal Cortical and Pituitary Tumors. Cancer Research, **11**:413–22, 1951.
2. DICKIE, M. M., and WOOLLEY, G. W. Spontaneous Basophilic Tumors of the Pituitary Glands in Gonadectomized Mice. Cancer Research, **9**:372–84, 1949.
3. DORFMAN, R. I., and GARDNER, W. U. Metabolism of Steroid Hormones. The Excretion of Estrogenic Material by Ovariectomized Mice Bearing Adrenal Tumors. Endocrinology, **34**:421–23, 1944.
4. FEKETE, E.; WOOLLEY, G. W.; and LITTLE, C. C. Histological Changes Following Ovariectomy in Mice. I. DBA High Tumor Strain. J. Exper. Med., **74**:1–8, 1941.
5. FRANTZ, M. J.; KIRSCHBAUM, A.; and CASAS, C. Endocrine Interrelationships and Spontaneous Tumors of the Adrenal Cortex in NH Mice. Proc. Soc. Exper. Biol. & Med., **66**:645–46, 1947.
6. FRANTZ, M. J., and KIRSCHBAUM, A. Sex Hormone Secretion by Tumors of the Adrenal Cortex of Mice. Cancer Research, **9**:257–66, 1949.
7. FREUD, J. Genital Accessories of Female Rats; Amoestrus and Oestrus Inhibition. Act. Brev. Neerland., **8**:127–30, 1938.
8. KAMELL, S. A., and ATKINSON, W. B. Effects of Ovarian Hormones on Certain Cytoplasmic Reactions in the Vaginal Epithelium of the Mouse. Proc. Soc. Exper. Biol. & Med., **68**:537–40, 1948.
9. LILLIE, R. D., and PASTERNACK, J. G. Romanowsky Staining of Tissues with Buffered Solutions. Arch. Path., **14**:515–16, 1932.
10. SMITH, F. W. The Relationship of the Inherited Hormonal Influence to the Production of Adrenal Cortical Tumors by Castration. Cancer Research, **8**:432–33, 1948.
11. WOOLLEY, G. W., and LITTLE, C. C. The Incidence of Adrenal Cortical Carcinoma in Gonadectomized Female Mice of the Extreme Dilution Strain. II. Observations on the Accessory Sex Organs. Cancer Research, **5**:203–10, 1945.