The Pathogenesis of Uterine Lesions in Virgin Mice and in Gonadectomized Mice Bearing Adrenal Cortical and Pituitary Tumors

NICHOLAS P. CHRISTY,† MARGARET M. DICKIE, WILLIAM B. ATKINSON,‡ AND GEORGE W. WOOLLEY

(Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.)

Earlier papers from this laboratory have reported that, in certain strains of mice which develop adrenal cortical hyperplasia or cortical neoplasms following early gonadectomy, the accessory reproductive organs show evidence of hormonal stimulation. In the DBA strain, adrenal cortical hyperplasia appears between 2 and 6 months of age and is followed by growth of the uterus, vagina, and mammary glands at 7—12 months (6). In females of the CE strain, carcinomas develop in the areas of adrenal cortical hyperplasia in 100 per cent of the animals by 6—12 months of age, and growth of the accessory reproductive organs begins about the eighth month (24, 25). Transplantation of adrenal tumors into gonadectomized animals is followed by uterine growth. This would indicate that the abnormal adrenal cortex is the source of the steroid hormones which stimulate the reproductive tract (23, 28). A more recent study of various F₁ hybrids has shown the occurrence of nodular hyperplasia which progresses to carcinoma of the adrenal cortex following early gonadectomy (4). These adrenal changes are followed first by growth of the accessory reproductive organs and later by the appearance of basophile nodules of the anterior pituitary.

It has been observed that the uteri of gonadectomized females of the DBA and CE strains frequently show histologic abnormalities (26) which are also found in gonadectomized DBA × CE reciprocal hybrid females. Similar observations were made by Smith (17), who noted cystic and other changes in the uteri of C3H and C3H × A hybrid gonadectomized females with adrenal cortical hyperplasia, adenomas, and adenocarcinomas. We have found uterine abnormalities not only in gonadectomized females with structural changes in the adrenal cortex but also in intact virgin females.

The present study is designed to elucidate the pathogenesis of the uterine lesions in both intact virgin and gonadectomized females of the F₁ cross between DBA female and CE male and the reciprocal cross. The findings and the pathological and endocrinological conclusions drawn from them are to serve as a basis for histochemical studies on similar mice.

MATERIALS AND METHODS

A total of 101 mice was used in this study. Fifty-one animals were of the DBA female × CE male F₁ cross. The remaining 50 mice were the offspring of the reciprocal F₁ cross between the parent strains. Approximately one-half of each group of hybrids was gonadectomized from 1 to 8 days after birth. The intact females were isolated from males at weaning and were kept as virgin controls. The animals in both the experimental and control groups received no further treatment. They were maintained at a temperature of 70° F. with a diet of Purina Fox Chow and water ad libitum.

Virgin and castrate mice were sacrificed at intervals ranging from 15 days to 26 months of age.

† Visiting Junior Investigator at the Jackson Laboratory in 1948 under the sponsorship of grants administered by Dr. Earl T. Engle of the College of Physicians and Surgeons, Columbia University, New York, N.Y.; and in 1949 as third-year elective in partial fulfillment of the requirements for the degree of Doctor of Medicine in the Columbia University College of Physicians and Surgeons.

‡ Visiting Investigator at the Jackson Laboratory in 1948, aided by a grant from the Jane Coffin Childs Memorial Fund in support of investigations on hyperplastic and malignant uterine tissues in women in relation to their endocrine state, made jointly to the Departments of Anatomy and Obstetrics and Gynecology in the Columbia University College of Physicians and Surgeons.

Received for publication December 11, 1950.

G. W. Woolley, unpublished data.
and were immediately examined at autopsy. No attempt was made to sacrifice the animals at any particular stage of the estrous cycle. In each case the genital tract, the adrenals, pituitary, and submaxillary glands, etc. (and the ovaries in the intact virgins), were removed and fixed in modified Tellyesnizky's fluid (115 ml of 95 per cent alcohol, 35.7 ml of distilled water, 15 ml of formalin, and 7.5 ml of glacial acetic acid). Tissue specimens were imbedded in paraffin at 56°C. Sections were cut at 8 μ and were stained with Mayer's hematoxylin and aqueous eosin.

Criteria used for evidence of steroid activity.—In addition to the histopathological studies described below, sections of the uterus, vagina, and submaxillary glands were examined for evidence of response to steroid activity. In the uterus, the morphological criteria defined by Hooker (9) were used to identify the response to estrogenic and progesterone-like substances. According to Hooker, under estrogenic influence mitotic figures are seen in the epithelium, the epithelial cells increase in height, and their cytoplasm becomes granular. The endometrial stroma becomes edematous and infiltrated with polymorphonuclear leukocytes, and the stromal nuclei become fusiform and pyknotic. Grossly, the organ is hyperemic and distended. Under progesterone influence the epithelial cells are slightly taller than those of the castrate uterus, and their cytoplasm is clear. In the stroma the nuclei enlarge and become rounded, vesicular, and granular, with a prominent nucleolus. There is no edema or leukocytic infiltration. Under the influence of both hormones both types of change are seen concurrently. It should be noted here that, according to Hooker and Forbes who used special technics, desoxycorticosterone and testosterone administered systemically may produce endometrial nuclear changes indistinguishable from those produced by progesterone (10). Prolonged estrogenic stimulation of the uterus results in endometrial fibrosis and hyalinization—changes which are also phenomena of aging (16). Androgenic stimulation is manifested in a thick, fibrous stroma and in endometrial nuclei intermediate between the castrate and progesterone-like conditions.

The vagina was examined for evidence of cyclic stimulation. Specific hormonal effects were judged by the criteria used by Atkinson and Kamell in an earlier study (1). The action of estrogen alone is indicated by the proliferation of the vaginal epithelium with subsequent cornification of the superficial layers. On the other hand, proliferation followed by mucification of the superficial layers is evidence of progesterone activity either concurrent with or following estrogen stimulation. Mucification may also be the result of androgenic stimulation (12).

The submaxillary gland reflects hormone stimulation grossly and microscopically. Under estrogen influence the gland is small and dark red grossly; microscopically, the terminal tubules are lined by low columnar cells with eosinophilic cytoplasm and vesicular nuclei in a central or apical position. Androgenic influence makes the gland large and pale, while in the terminal tubules the epithelial cells are tall columnar, with decreased cytoplasmic eosinophilia and with flattened nuclei in a very basal position (5, 13).

Observations

Gross Observations on the Uteri

Virgin Females

Grossly, the uteri of the virgin mice remained small in both F₁ groups until about 2 months of age, when post-puberal enlargement took place. Adult size and appearance were attained by 2 months in the DBA female X CE male series and by 4 months in the reciprocal group. The size of the organ in both groups remained within normal limits until 7—8 months of age. At this time the uterine horns began to increase in diameter. In the older age groups (15 months and older), a size up to 2—3 times that seen in early sexual maturity was attained. In most cases the horns were uniformly hypertrophied. In some, however, the enlargement was nodular in character. The hypertrophy and vascularity persisted until 26 months of age (the oldest animal in the series).

Gonadectomized Females

In the gonadectomized mice the uterus remained small until 5 months of age. By 6 months however, although the uterus was still small, there was evidence of beginning stimulation in the increased blood supply. By the eighth month, the uterus had enlarged to equal the size of the 8-month organ of the intact hybrid. This increase in size appeared after the development of adrenal cortical carcinoma. Adult size of the uterus persisted until 24 months (the oldest animal used), but the very marked hypertrophy seen in the intact virgin mice was not found.

Microscopic Observations on the Uteri Correlated with Adrenal Cortical Changes and with the Vaginal “Cycle”

Virgin DBA Female X CE Male F₁ Hybrids

One to 8 months.—The uteri of 1-month-old animals were small and undeveloped. In the two animals studied, there was no evidence of estrogenic stimulation of the uterus. Both lacked stromal...
edema or hyperemia, and both had very few uterine glands. Clear evidence of estrogenic effect was seen at the age of 2 months. The 2-month uteri were adult in size. Several glands were present, and the surface (lumenal) and glandular epithelia showed estrogenic change (tall columnar cells, cytoplasmic granularity, folding, mitoses). The stroma showed mixed effects—round, vesicular, granular nuclei (i.e., progesterone-like changes) in the presence of edema and leukocytic infiltration (estrogenic changes). This was not unexpected, since, as mentioned above, no attempt was made to obtain specimens at a definite stage in the estrous cycle.

At from 3 to 7 months the uteri of this series showed increasing steroid sex hormone stimulation with morphological features which correspond more or less well to the observed vaginal estrous cycle. There was stratification of surface epithelium, an increase in the number of glands, and notable stromal stimulation by estrogen, progesterone, desoxycorticosterone, or testosterone. The myometria showed thickening, hyperemia, and edema (Fig. 1). At 6 months there was endometrial fibrosis associated with stromal nuclei intermediate between the fusiform and vesicular types. The vagina showed the metestrous morphology (cornified layer being delaminated, WBC in epithelial layers). From 8 months on, the uteri were all much increased in diameter. The 8-month uterus (in late estrus from the vaginal picture) was the first to show distinct pathologic change. Here, associated with a stroma showing a progesterone-like reaction there was glandular hyperplasia of both cystic and adenomatous types. The glands were very numerous, small, and closely spaced. The uterine lumen was extremely wide, and there were deep foldings of the surface epithelium. Adenomatous change of the uterine gland, defined by Hertig and Sommers (8) as an “outpouching and pinching off” resulting in groups of small, close-packed glands, was seen in several areas where the glandular epithelium was so stratified and folded that within a single basement membrane there were several minute lumina instead of one (Fig. 2). The absence of fine connective tissue and of a basement membrane in the interior of these formations suggests the hyperplasia of a single glandular element rather than the confluence of several. Cystic glandular hyperplasia was seen in one or two glands that had undergone tenfold enlargement, with flattening and patchy stratification of the epithelium. The cystic glands contained small amounts of lightly stained eosinophilic material.

Nine months and beyond.—In the 9-month uterus (obtained at estrus), stromal nuclei showing a progesterone-like reaction were accompanied by endometrial edema. The surface epithelium showed estrogenic change with some stratification. In addition, adenomyosis (internal endometriosis), i.e., invasion of the myometrium by glands and endometrial stroma, appeared for the first time. This lesion was also seen in the 10-month uterus (vaginal diestrous) (Fig. 3). Stromal nuclei again showed a reaction of the progesterone-like type. Leukocytic infiltration was apparent, but the epithelium exhibited no estrogenic changes. At three points in the circular muscle layer endometrial glands and stroma could be seen penetrating through, even reaching into, the longitudinal muscle layer. The invasive glands and stroma were not structurally different from the rest of the endometrium. In one area where the invading gland was sectioned longitudinally, a branch of the somewhat distended lumen could be followed in successive sections out into the myometrium. The myometrium was thick, hyperemic, and edematous, whereas in the normal diestrous female, the uterine wall is “collapsed and anemic” (18).

An 11-month uterus with a proestrous vagina and the 12-month uterus with diestrous vagina exhibited stromal nuclei with a progesterone-like reaction and mild, cystic glandular hyperplasia. In the 12-month uterus intense myometrial hyperemia was present, despite the diestrous phase of the cycle. At 13 months, the uterus (diestrous vagina) showed the same type of myometrium. Several foci of ectopic endometrium were noted in both muscle layers. Cystic glands could be seen in the endometrium proper and in the ectopic foci. Uteri from 14 through 17 months all had vesicular stromal nuclei and cystic glandular changes, as well as adenomyosis. The myometria of all were edematous, the 15-month uterus being distinguished by the presence of muscular hyperplasia. Another 15-month specimen displayed marked stromal edema, myometrial hyperplasia, cystic and adenomatous hyperplasia, with adenomyosis. Similar changes were found in the 18-month uterus. The 19-month organ was very large and was noteworthy as the only specimen in this virgin series which showed no nuclei with a progesterone-like change in the stroma. Observation of vaginal sections revealed an estrus condition. The myometrium was hyperplastic. The endometrial changes were the most extreme thus far observed. These changes included conspicuous stromal edema and great increase in the number of epithelial elements. Mixed with the numerous minute glands there were greatly distended glands and some showing adenomatous change. This specimen (Fig. 4) closely resembled
the so-called "Swiss-cheese endometrium" of human females (21). Changes as extreme as the above were also observed in the 20-month and in one of the 22-month uteri. The remaining 22-month and the 23- and 25-month uteri showed less marked pathologic change than those immediately preceding chronologically, but there was no indication in the accessory reproductive organs of decreased steroid hormone stimulation, even in these aged animals.

**Virgin CE Female × DBA Male F1 Hybrids**

There were no important differences between the uteri of this and the reciprocal series of virgin hybrids. Although the 1- and 2-month animals showed vaginal cyclic activity, the uteri were small in size. The epithelium of the 2-month specimen had clear-cut evidence of estrogenic stimulation. Adult size was not reached until 4 months. From 5 to 7 months there was progressive increase in uterine size and glandular development, and at 7 months adenomyosis appeared. Cystic glandular hyperplasia first occurred at 5 months and adenomatous hyperplasia at 11 months. Large size, stroma showing a progesterone-like reaction, adenomyosis, and glandular hyperplasia persisted without diminution up to the age of 26 months. The uterus of a 22-month animal manifested perhaps the most extreme cystic hyperplasia of the series, in the presence of a lutein cyst of the ovary. From 23 to 26 months of age there was thickening and fibrosis of the myometrium. The 23-month specimen had overgrowth of collagenous connective tissue around stromal blood vessels.

**Gonadectomized DBA Female × CE Male F1 Hybrids**

Changes due to progesterone-like stimulation (testosterone or desoxycorticosterone) appeared earlier in these gonadectomized animals than did estrogenic changes. In a 15-day animal, the uterus was small with thin muscle layers and presented the castrate appearance. No evidence of either estrogenic or progesterone stimulation could be found in the 1-month uterus. The 4-, 5-, and 6-month uteri had the castrate morphology, but the nuclei in the endometrial stroma were round and vesicular (Fig. 5). By 7 months the uterus began to show some enlargement, and, although there was as yet no neoplastic change in the hyperplastic nodules of the adrenal cortex, the vagina was in proestrus. (The vaginas of the younger animals in this series had all been unstimulated.) At this stage of development (7 months) the muscle was thin, stromal nuclei vesicular, and lumen wide. In addition to vaginal activity, other evidence of estrogenic effect which may have contributed to the uterine enlargement were the presence of many small uterine glands, tall granular epithelium, epithelial folding with some stratification, and small cysts.

Definite pathologic change was first seen at 8 months. It is worth emphasizing that this is the same age at which glandular hyperplasia first developed in the intact virgins. The vaginal phase was late estrus. The uterus was large and had a well developed myometrium. The nuclei of the endometrial stroma were vesicular, but leukocytic infiltration of the stroma indicated some estrogenic influence. There was also considerable collagenous connective tissue in the stroma, a condition consistently found up to 21 months of age. The epithelium reflected predominantly estrogenic stimulation. The lumen was wide. The surface and glandular epithelium was very tall and granular. Although the glands were relatively few in number, cystic and adenomatous changes were seen.

The uteri of the 9-month animals, while similar to those at 8 months, had thicker muscle layers. These 9-month animals were the first to develop adrenal cortical carcinoma. All animals from 10 months on had bilateral adrenal cortical carcinomas. The 10- and 12-month uteri were similar to the above, with more pronounced cystic hyperplasia. The 14-, 15-, and 17-month uteri continued to show the same changes—stromal fibrosis, cystic and adenomatous hyperplasia, with the added feature of adenomyosis (Fig. 6). It is noteworthy that adenomyosis appeared first in these ovariec-tomized animals at 14 months while it was first demonstrable at 9 months in the corresponding virgin series.

Many of the gonadectomized animals showed pituitary basophile tumors after the age of 17 months (4). From 17 to 21 months the uteri showed, in addition to all the above changes, a peculiar increase in collagenous connective tissue around small vessels and lymphatics in the endometrium (Fig. 8). An increase in collagenous fibers, both diffusely distributed and localized about small vessels was noted in the myometrium. Finally, in two cases (18- and 19-month animals), a displacement of the nuclei of surface and glandular epithelium toward the apices of the cells was observed (Fig. 9). This phenomenon has been reported previously in the uteri of aged CE strain mice (26). There was no evidence in the uteri to indicate decreased hormonal activity with advancing age.

**Gonadectomized CE Female × DBA Male F1 Hybrids**

Reciprocal differences in the two series of gonadectomized females were not striking. As in the reciprocal series of castrate females, evidence
of some hormonal influence other than estrogenic was seen before estrogenic stimulation occurred. There was no uterine or vaginal enlargement in the 15-day and 1-month animals. The 4-month uterus showed the castrate picture. The uterus at 5 months had epithelium of the castrate type, but the stromal nuclei were vesicular. At 6 months, such vesicular stromal nuclei were accompanied by estrogen-stimulated epithelium, and the vagina had four to five epithelial layers with some superficial mucification. This 6-month animal exhibited an adrenal neoplasm. From 7 months on, "estrous" vagina was associated with an adult-sized uterus which had stromal fibrosis—a change found consistently throughout the series until 24 months of age. In the 7- and 8-month specimens, high degrees of estrogenic stimulation of the epithelial elements and edema and leukocytic infiltration of the stroma could be seen. Cystic glandular hyperplasia first made its appearance at 9 months, together with adenomatous hyperplasia. It will be remembered that these lesions first occurred at 8 months of age in the intact hybrids of this cross. All animals from 9 to 24 months had adrenal cortical carcinomas which were usually bilateral. From 10 to 15 months, high estrogen levels are expressed by the stromal and epithelial morphology. In the 12-month uterus some hyaline change could be observed in the connective tissue of the stroma. This might indicate prolonged, high-level estrogenic stimulation, according to Loeb et al. (16). The 15-month uterus had the most advanced cystic hyperplasia.

From 14 to 24 months, many of the animals had basophile adenomas of the anterior pituitary (4). The myometria of the uteri of these animals showed increasing edema, smooth-muscle hyperplasia, and fibrosis, more pronounced with advancing age. Vescicular stromal nuclei and fibrosis of the endometrium persisted until the end of the series. Increased amounts of perivascular and perilymphatic collagenous connective tissue were first seen at 18 months and were present in about half of the 18- to 24-month uterus. Surface and glandular epithelium indicated undiminished estrogenic stimulation, even in the oldest animals. Adenomyosis was present in the 16-, 17-, 20-, 22-, 23-, and 24-month specimens (Fig. 7). (Note that this change first occurred at 7 months in the corresponding intact females.) Cystic hyperplasia and adenomatous hyperplasia were also seen up to 23 months of age. The 21-, 23-, and 24-month animals demonstrated the peculiar migration of epithelial nuclei from the basal to the apical portions of the cells, a change which first occurred at 18 months in the uterus of the reciprocal castrate series.

Evidence of Endocrine Dysfunction in Structures Other than the Uterus

The uterine changes in both control and gonadectomized female hybrids showed clear evidence of an altered internal environment with respect to endocrine secretions, an alteration detectable even in old animals. The other accessory reproductive structures also offered such evidence as did the endocrine glands. Details of vaginal, submaxillary, adrenal, and ovarian abnormalities will be reported later, and those of pituitary and mammary glands have already been described (4), so that only a few general remarks bearing on the endocrine factors involved in the genesis of the uterine lesions need be made here. The vagina, as has been indicated above, responds to steroid stimulation in both intact and castrate females. Following adrenal hyperplasia in the castrates, the vagina shows evidence of hormonal stimulation, but, unlike the uterus, it usually reverts to the castrate morphology in the older animals. The submaxillary gland, by its differential response, serves as an indicator of estrogen and androgen activity. In the control females, the submaxillary is predominantly female in type, with signs of low androgen activity appearing soon after sexual maturity. In the castrates, at a given age, androgenic activity is more pronounced and becomes quite extreme in some of the older animals. The androgenic effects follow the development of adrenal hyperplasia and precede the appearance of marked steroid stimulation in either the uterus or vagina.

In regard to the endocrine glands themselves, the ovary in the intact hybrids undergoes cyclic changes, paralleled by the vaginal cycle, for several months following sexual maturity. In older animals, degenerative changes appear—decreasing numbers of follicles, increasing amounts of lipochrome pigment, increasing numbers of hyalinized corpora lutea, calcification, and an occasional cyst in older mice. In spite of the progressive morphological regression of the ovaries, there is, as has been shown, no uterine evidence of decreased estrogenic activity with advancing age. The adrenal changes in the castrates are comparable to those described in one of the parent strains (CE) by Woolley and Little (24, 26). Changes in the adrenals of intact animals will be discussed in a forthcoming report. Most pertinent to the present investigation is the observation that in the castrate hybrids adrenal cortical hyperplasia always precedes morphological evidence of growth and marked steroid stimulation of the accessory reproductive structures. Previous studies have suggested that the basophile nodules of the pituitary appearing in the older gonadectomized animals
may have an endocrine function, an inference made on the basis of the “extreme alveolar development of the mammary glands” in these animals, a change which accompanies the pituitary tumors and never occurs in their absence (4). There is no clear-cut evidence in the uterus of such a direct anterior pituitary effect.

**DISCUSSION**

The results demonstrate the occurrence in hybrid mice of uterine lesions in the presence of endocrine secretions which differ either qualitatively or quantitatively from the normal. Smith has reported the prevalence of such lesions in gonadectomized mice bearing adrenal tumors (17). The present studies show that pathological endometrial changes may develop in intact animals without adrenal cortical carcinoma, as well as in the castrates which have cortical neoplasms. Adrenal cortical neoplasia per se is thus seen not to be the only or the essential factor in the pathogenesis of the endometrial abnormalities. Rather, the adrenal cortical tumors appear to be but one manifestation of a generalized alteration of the endocrine “milieu intérieur.” Moreover, the intact female need not be exempt from suspicion of adrenal dysfunction merely because of the absence of histopathologic evidence of carcinoma.

We shall attempt to show how endocrine factors operate in the development of the uterine changes and, in so doing, to throw some light on the nature of the altered endocrine status of these animals. Chart 1 is a diagrammatic representation of the occurrence of some of these uterine changes.

**Cystic Glandular Hyperplasia**

The most prominent uterine change observed is cystic glandular hyperplasia. There is a great deal of experimental and clinical evidence pointing to hyperestrinism as the most important factor in the development of this condition (21). A simple excess of estrogenic hormone is not the essential agent, but, rather, continuity of estrogenic stimulation without the periods of rest provided in the normal menstrual or estrous cycle. The experiments of Burch, Zondek, and others, cited in Taylor’s excellent review (21), show that the typical picture of cystic hyperplasia can be produced by estrogen injection. But the work of Lipschütz, in which partial ovariectomy in guinea pigs produced ovari-

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**Chart 1**—Diagrammatic representation of cross sections of uteri at critical periods during life. The four groups of females—virgin DBA X CE F1, gonadectomized DBA X CE F1, virgin CE X DBA F1, and gonadectomized CE X DBA F1—are compared.
an hypertrophy with absent or incomplete corpus luteum formation and consequent endometrial hyperplasia, clearly demonstrates that the lesion is more nearly related to disturbed rather than simple excessive ovarian function (14, 15). Clinical evidence of hyperestrinism in association with endometrial hyperplasia has been shown by several workers (91) in the form of increased excretion of estrogen in the urine.

In these experimental animals, intact and gonadectomized, the presence of estrogen is indicated by vaginal cornification, by growth of the uterus and mammary glands, and by clear-cut estrogenic changes in the cytology of the uterine epithelium and stroma. There is also evidence, some of it inferential, that estrogen secretion in our animals is both excessive and prolonged. Dorfman and Gardner, working with NIH strain female mice bearing adrenal tumors (9), demonstrated a fourfold increase of urinary estrogen excretion in their animals. Such determinations have not been made on the DBA × CE hybrids, but we may assume a similar hyperestrinism on the basis of several histopathologic findings. First, the mice show uterine overgrowth, most marked in the intact females, and myometrial hyperemia and edema frequently occur in the face of a diestrous vaginal condition. Second, many of the uteri show extreme epithelial and stromal hyperplasia, clearly demonstrates that the lesion is more nearly related to disturbed rather than simple excessive ovarian function (14, 15). Clinical evidence of hyperestrinism in association with endometrial hyperplasia has been shown by several workers (91) in the form of increased excretion of estrogen in the urine.

Third, endometrial fibrosis (and hyaline change in some cases) is constantly present in intact and gonadectomized females after the age of 8 months. This phenomenon has been produced in mice by the administration of estrogen (16). Burack et al. (2) associate deposition of collagenous connective tissue in the endometrium, in the myometrium, and about the intra-uterine arteries, as well as increase in uterine size, with long-continued, unopposed activity of estrogen in rats. This conclusion was based on the finding that the above changes are more extensive in virgin than in breeding animals. We have made similar observations (unpublished) on DBA and CE female mice. In these animals, the parent strains of our experimental hybrids, changes like those found in the hybrids (and in Burack’s rats), are seen more often and in more severe forms in gonadectomized females (with adrenal cortical hyperplasia or carcinoma) and in virgin females (without extensive adrenal pathologic change) than in the animals that have borne several litters. Fourth, in support of the idea that the hyperestrinism in our hybrids is prolonged as well as excessive, it should be noted again that hyperplastic and other uterine changes persist into old age.

There is ample evidence that the source of estrogenic hormone in gonadectomized mice with adrenal cortical hyperplasia or neoplasms is the abnormal adrenal cortex (4, 6, 17, 23, 25, 26, 28). That adrenal cortical estrogen is probably the factor responsible for the excessive endometrial stimulation which results in hyperplasia in the intact as well as the castrate animal is strongly suggested by the onset of the endometrial hyperplasia at very nearly the same age in both intact and castrate females. The ovary is apparently not necessary as an estrogen source for the development of the lesion.

Adenomatous Hyperplasia

The relation of adenomatous hyperplasia of the endometrium to hyperestrinism is not so clearly indicated by experimental work. Hertig and Sommers (8) question whether the lesion is due to excessive estrogen or to abnormal pituitary-ovarian or pituitary-adrenal relationships. In a 16-month-old diethylstilbestrol-treated CE female reported by Woolley and Little (27), adenomatous hyperplasia was seen in association with beginning adenocarcinoma of the endometrium, which suggests that this type of hyperplasia may be precancerous in the mouse as it apparently is in the human (8). It is noteworthy that in none of the DBA × CE hybrid uteri were there changes indicative of carcinomatous transformation in foci of adenomatous hyperplasia.

Adenomyosis

Adenomyosis appears to be related to estrogenic stimulation. Taylor states that in women the lesion is found only when the ovaries are active (21). Lipshütz reported adenomyosis in his partially ovariectomized guinea pigs which showed cystic hyperplasia (15). In our experimental series, adenomyosis appeared later in the castrate than in the virgin uteri. Why this should be is not entirely clear, unless it is an expression of a quantitative difference in estrogenic activity in the two groups.

Hypothetical Endocrine Mechanisms in the Production of Endometrial Lesions

The principal uterine lesions in DBA × CE hybrid mice appear to be related, at least in part, to prolonged high levels of estrogen, of which the main source is presumably the adrenal cortex, whether morphologically neoplastic or not. However, as has been indicated, there is also evidence of other hormonal activity. The stromal nuclei of the uterus show changes which may be proges- terone-like reactions (9) or secondary to testosterone or desoxycorticosterone stimulation (10). Endometrial fibrosis which is so constant a feature here may also be produced, in rats at least, by ad-
ministration of testosterone (12). Vaginal mucification, seen in our old castrate hybrids, may be an androgenic effect (12), a fact which is emphasized by the prominent androgenic changes in the submaxillary glands of these animals. What role androgens, adrenal cortical steroids, and progesterone may play in association with estrogens in the etiology of endometrial hyperplasia and adenomyosis is obscure. However, in gonadectomized CE females, in which adrenal cortical tumor formation is suppressed by implantation of pellets of potent estrogenic hormones, cystic glandular hyperplasia has not been seen and adenomyosis and adenomatous hyperplasia only rarely. The inference is that in such CE animals and perhaps in our hybrids a mixture of several steroids acting synergistically and in improper balance, rather than a single predominant hormone, is required for the production of the uterine picture seen in the present series. How much of the pathologic change is due to intrinsic uterine tissue differences and how much to altered endocrine environment are questions as yet unanswered.

Pituitary-adrenal and pituitary-ovarian relationships.—The uterine cytology is similar in the castrate and intact females. This implies a rough similarity in the pattern of steroid secretion in the two groups, with more androgenic predominance in the older castrates, as seen in the submaxillary morphology. Because the suspected common endocrine denominators in the two groups are the adrenal cortex and the pituitary, since comparable uterine lesions (cystic hyperplasia) appear at approximately the same age in castrate and intact alike, we may assume a corresponding similarity in hypophyseal and adrenal cortical dysfunction on a physiologic level in the two groups. What is the origin of the dysfunction? Genetic factors must play a part, in view of the incidence in the parent strains DBA and CE, of very similar endometrial hyperplastic phenomena which as in their hybrid offspring are only one manifestation of widespread endocrine abnormality. Closer to this discussion is the hypothesis of Dickie and Woolley (4) who postulate the anterior pituitary as the origin of the stimulus for the adrenal cortical change in DBA × CE and other hybrids. The suggested mechanism of the pituitary-adrenal relationship is that in the castrate, which has both adrenal and pituitary tumors, the fundamental change may occur first in the pituitary, even though morphological pituitary change does not develop until after structural change of the adrenal. The pituitary may thus react on the adrenal, the resultant adrenal secretions then reacting on the pituitary in turn, so that structural hypophyseal changes are not apparent until after the adrenal cortex becomes neoplastic (4).

We must go one step further with this hypothesis to explain the uterine evidence in the intact virgins of endocrine dysfunction, so similar to that seen in the castrate animals, despite the absence in the virgins of pituitary or adrenal tumors. The assumption is made that in the intact female hybrid the ovarian steroids inhibit the action of the anterior pituitary sufficiently to prevent the extreme hypophyseal activity (and basophile change) seen in the castrate, but not sufficiently to prevent physiological over-stimulation of the adrenal cortex which, in response, secretes an excess of steroids with consequent uterine maldevelopment, without itself becoming carcinomatous. We do not attempt to answer definitively Kepler’s question as to whether the adrenal cortex or the pituitary basophile cells are primarily the cause of this experimental type of Cushing’s syndrome (11), but we wish to point out that there is suggestive evidence from our uterine studies that the pituitary and adrenal may both be physiologically hyperactive without showing marked structural change.

The nature of the pituitary and adrenal hormones.—The final step in our hypothesis concerning the origin of the abnormal endocrine conditions in these hybrids has already been indicated by Dickie and Woolley—“the substance or substances...from the pituitary and adrenal glands seem to be of a gonadotrophic and gonadal nature respectively” (4). That the adrenal cortical steroids are gonadal in nature has been shown at length in the foregoing observations. The gonadotropic potentialities of the pituitary in this connection may be inferred from several pieces of evidence. Frantz et al. (7) have shown that gonadotropic hormones injected into adrenalectomized NH female mice fail to produce an ovarian response in terms of estrogenic effect on the vaginal smear. However, these workers were able to demonstrate vaginal evidence of estrogenic hormone secretion following gonadotrophin administration to gonadectomized NH females with adrenal cortical tumors.

Evidence from this laboratory that the pituitary stimulus to the adrenal may be gonadotropic is afforded by three findings. (a) Ovarian steroids, known to inhibit gonadotropic activity, appear to reduce the atypical adrenocorticotrophic effects by preventing the development of adrenal cortical carcinoma in the intact females of the series presented here. (b) Potent estrogenic and androgenic steroids, both of which inhibit gonadotropic action, have been shown to suppress the development of adrenal cortical neoplasms in CE gonadectomized females (22, 27). (c) Unpublished experi-
ments suggest that, although ACTH is not effective in stimulating hyperplastic DBA strain adrenal cortices to neoplasm-formation, FSH (and to a lesser extent LH) may be effective. (Intense study of these data has not yet been completed.)

These experimental results suggest that pituitary gonadotrophins acting on the adrenal cortex are the origin of hyperplastic changes in the uteri of DBA × CE hybrid mice. This idea is in accord with the speculations of Sommers et al. (19) and of Speert (20) who indicate that pituitary-adrenal factors may operate in the pathogenesis of endometrial hyperplasia and carcinomas.

SUMMARY

The pathogenesis of the uterine lesions in intact virgin and gonadectomized hybrid mice of the F₁ reciprocal crosses between the DBA and CE strains was studied in animals sacrificed from 15 days to 26 months of age. Cystic hyperplasia of the endometrial glands appeared in both the intact and gonadectomized mice at about 8 months of age. The cystic changes were frequently accompanied by adenomatous hyperplasia of the glandular epithelium. In many of the intact animals these conditions were seen concurrently with invasion of the myometrium by the endometrial glands and surrounding stroma (adenomyosis or myometrial endometriosis). In the gonadectomized group, however, adenomyosis occurred later—not before 14 months of age.

Adrenal cortical neoplasms developed in gonadectomized mice by 6–9 months of age. However, the occurrence of uterine lesions in the intact animals indicates that the morphologic changes in the adrenals of the castrates are not necessary per se to the development of the endometrial lesions.

The endocrine status of the intact and gonadectomized mice with respect to the activity of the steroid sex hormones was assayed on the basis of the well established cytological changes which are found in the uterus, vagina, and submaxillary glands. Microscopic study of these organs indicated the presence of an endocrine imbalance first manifested at about 6–8 months and persisting to extreme old age, in both the intact and gonadectomized animals. This imbalance was characterized most notably by the continuous, noncyclic, prolonged, and excessive production of estrogen. Since the phenomena are essentially the same in both the intact and gonadectomized mice, the adrenal cortex is presumed to be the source of the aberrant estrogenic hormone.

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FIG. 6.—Adenomyosis as well as cystic hyperplasia can be seen in 14-month gonadectomized DBA × CE F1 female WK328. ×25.

FIG. 7.—Adenomyosis and cystic hyperplasia found in the reciprocal group of females. This is a 17-month gonadectomized CE ♀ × DBA♂ F1 female WK397. ×25.

FIG. 8.—Perivascular connective tissue as seen in a virgin female of parent strain CE after 24 months. Same condition can be found in the reciprocal hybrid females. ×47.5.

FIG. 9.—Migration of nuclei of surface and glandular epithelium toward the lumen found in an 18-month gonadectomized DBA × CE F1 female WK401. ×850.
The Pathogenesis of Uterine Lesions in Virgin Mice and in Gonadectomized Mice Bearing Adrenal Cortical and Pituitary Tumors

Nicholas P. Christy, Margaret M. Dickie, William B. Atkinson, et al.

Cancer Res 1951;11:413-422.

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