A Comparison of the X-Zone of the Adrenal Cortex in Two Inbred Strains of Mice*

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Recent work by Woolley, Fekete, and Little (6, 8) has demonstrated that in certain inbred strains of mice striking changes in the adrenal cortex follow early castration. The female mice of the dilute brown (Dba) strain, castrated at birth, showed areas of nodular hyperplasia in the adrenal cortex, after one year involving and partly replacing all three zones of the cortex. These mice also showed marked development of the mammary glands. It was suggested that the action of the hyperplastic adrenals stimulated the development of the mammary glands. It was further shown that the adrenal hyperplasia occurred in two strains of mice with a high incidence of spontaneous mammary tumors (Dba and C3H) and was absent in the C57 black strain which has an exceedingly low incidence of spontaneous mammary carcinoma.

The differences between the castrated females of the Dba and C57 strains suggested the possibility that differences might be detected in the adrenals of intact females of the two strains. A comparison of the adrenal glands of two strains of mice varying in their incidence of mammary tumors (Dba and C3H) and was absent in the C57 black strain which has an exceedingly low incidence of spontaneous mammary carcinoma.

The x-zone of the adrenal cortex in the mouse. The x-zone is present in both sexes before puberty but regresses rapidly in the male at this time. In the female it persists for a variable period and eventually regresses, often with widespread vacuolization. The original descriptions indicated that there might be considerable differences in the structure and relationships of the x-zone in different strains of mice. The Danforth strain used by Howard showed an x-zone which was considerably more prominent than that of most other strains and was accompanied by very extensive vacuolization in the process of regression. The x-zone of the adrenal of mice of this strain averages 55.2 per cent of the width of the cortex as compared to an average width of less than 15 per cent of the cortex in 10 mice of the Dba strain in females between 45 and 55 days old (5).

The object of this paper is to report a difference between the x-zone of the adrenal in the intact female and castrated mice of both sexes of the Dba and of the C57 black strains, and to describe the x-zone of the hybrid females of a cross between the two strains.

MATERIALS AND METHODS

Two strains of inbred mice were used, the JAX Dba which has a high incidence of mammary tumors and the C57 black strain which has a very low incidence. Both strains have been extensively inbred and for the purposes of this work may be considered genetically uniform. Care was taken to keep the mice under as nearly identical conditions as possible. Factors which would affect the condition and structure of the x-zone, i.e. infection, starvation, pregnancy, etc., were guarded against.

In the castrated series, operations were performed at 1 day, 21 days, and 40 days of age. Mice castrated at 1 day were anesthetized by chilling. The older animals were anesthetized by intraperitoneal injection of nembutal. At the time the mice were autopsied the condition of the genital system was noted to determine whether castration had been successful.

While studying the early stages of adrenal changes following castration, we noted a histological difference between a high tumor strain and a low tumor strain. The appearance and persistence of the x-zone of the adrenal in one strain of mice differed from those of the other strain.

The x-zone, as first described by Howard (4) and confirmed by Deanesly (3), is a transitory juxtamedullary element of the adrenal cortex of the mouse. The x-zone is present in both sexes before puberty but regresses rapidly in the male at this time. In the female it persists for a variable period and eventually regresses, often with widespread vacuolization. The original descriptions indicated that there might be considerable differences in the structure and relationships of the x-zone in different strains of mice. The Danforth strain used by Howard showed an x-zone which was considerably more prominent than that of most other strains and was accompanied by very extensive vacuolization in the process of regression. The x-zone of the adrenal of mice of this strain averages 55.2 per cent of the width of the cortex as compared to an average width of less than 15 per cent of the cortex in 10 mice of the Dba strain in females between 45 and 55 days old (5).

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The left adrenal was used for histological examination. As soon as possible after the death of an animal tissues were fixed in Bouin’s or Tellynsicky’s fluid. The glands were imbedded in paraffin and serial sections were made. Adequate differentiation of the x-zone was obtained by routine staining with hematoxylin and eosin.
Measurements were made of the cortex and x-zone with a calibrated ocular micrometer. Sections were selected for measurement which showed the maximum area of medulla. In this way a section near the center of the gland was used.

Present at birth but appears between 10 to 20 days of age. In the males the x-zone parallels the development of the females until about 30 days. During the next 10 days a regression occurs in the x-zone of both strains so that by the 48th day the x-zone can no longer be recognized. In both strains the process of regression is unaccompanied by lipoid degeneration such as is found in the Dba females and the females of the Danforth strain described by Howard. Following the regression of the x-zone the adrenal cortex of the male mouse has reached its permanent pattern and undergoes no marked change until senile changes occur.

The measurements of female mice have been compared graphically in Fig. 1. The linear thickness of the x-zone is plotted against the age of the mouse.

The development and regression of the x-zone in the Dba and C57 black strains of mice follow the general descriptions of Howard (4) and Deanesley (3). In comparison to the variable appearance of the x-zones in random bred mice the x-zones in each

Observations

Measurements of 204 adrenal cortices have been made of mice of the following types:

I. Dba:—virgin females, 37; males, 22; castrated females, 13; castrated males, 11.
II. C57 black:—virgin females, 38; males, 17; castrated females, 15; castrated males, 5.
III. Hybrid (Dba x C57 black):—Dba ♀ x C57♂, 17; C57 ♀ x Dba ♂, 21.

The adrenals of normal males of the Dba and C57 black strains of mice possess x-zones which are essentially similar to the strain described by Howard (4). As in the females of these two strains, no x-zone is present at birth but appears between 10 to 20 days of age.
inbred strain presented a uniform and predictable appearance.

The most obvious difference between the x-zones of the two strains is the early reduction of the width of the x-zone of the virgin C57 black female beginning at about 70 days. At 100 days it can no longer be identified as a distinctive region of the cortex. The virgin female of the Dba strain possesses an x-zone which persists for over twice as long. The x-zone of this strain does not begin to show a terminal regression until 190 to 200 days and it may persist at 210 days or slightly longer.

The x-zones of the two strains also differ in the degree of vacuolization. The vacuolization which played such a large part in the degeneration of the x-zone of the Danforth strain of mice, described by Howard, is found in the Dba mice but to a lesser extent. In the C57 black strain vacuolization does not take place in the x-zones of the adrenals of the virgin female. The degeneration proceeds entirely by a regression and shrinking of the cells, unaccompanied by fatty degeneration.

Castration of either male or female before puberty resulted in the persistence of the x-zone. The histological appearance and duration of the persistence in each case resembled the normal virgin female of the strain. The adrenal changes following castration reported by Woolley, Fekete, and Little (6) began as localized nodules of cortical cells in the glomerular region, quite distinct from x-zone material.

An unusual anomaly was noted in one castrated male of the Dba strain. This animal was castrated at 40 days of age and killed when 130 days old. In addition to the expected finding of a definite x-zone still prominent at this age, the adrenal had an accessory gland associated with it which showed the same differentiation into x-zone and remaining cortical tissue as found in the main gland. This accessory adrenal was entirely devoid of medullary elements.

Virgin females which were hybrids of the two strains showed x-zones more closely akin to the Dba parent type. The x-zone was still intact at 180 days of age and the hybrids showed the vacuolization found in the Dba strain. It was found that females which had C57 mothers had on the average a larger x-zone than those with Dba mothers. Although the numbers are small it was found that between the ages of 90 to 180 days the former group of hybrids had about a 50 per cent larger x-zone.

**Discussion**

The function of the x-zone is as yet unknown and therefore the differences observed between these strains of mice cannot at present be given any definite physiological significance. The described differences in the x-zone of strains whose adrenal glands show such a different reaction to castration may well, however, be more than a chance coincidence.

Cramer and Horning (2) have stated that the x-zone is to be associated with the medulla. The occurrence of typical x-zone cells in an accessory gland entirely devoid of medullary elements is evidence that the two types of tissue may exist separately.

The use of genetically uniform strains of mice will help to throw light on the nature and function of the x-zone. To ascertain the effect of experimental procedures it will be of advantage to have strains of mice whose x-zone shows distinguishable and reproducible characteristics.

**Summary**

1. A difference between the x-zone of virgin females of the Dba and C57 black strains of mice is reported. The x-zone of the Dba mice persists for over 200 days and its regression is accompanied by vacuolization. The x-zone of the C57 black mice on the other hand undergoes complete regression by 100 days without vacuolization.

2. The x-zones of the male and female mice castrated before puberty resemble those of the virgin females of the same strain.

3. The x-zone of the hybrid of the Dba and the C57 black strains resembles the Dba parent. Hybrids having the C57 black mother seem to show a larger x-zone than those having a Dba mother.

**References**


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