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Effects of Metyrapone on Reproductive Organs of House Mice (38533)

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(Introduced by T. I. Koike)

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Suppression of reproductive function by exogenous ACTH has been produced in intact and adrenalectomized immature female mice, and intact mature female house mice (*Mus musculus*) (1). ACTH treatment also inhibits reproductive maturation in male and female voles (*Microtus*) (2), and intact adrenalectomized wild white-footed mice (*Peromyscus*) of both sexes (3). The present study was undertaken to test whether endogenous ACTH secretion stimulated by Metyrapone, a specific 11β-hydroxylase inhibitor, would induce inhibition of reproductive function in house mice similar to that seen with exogenous ACTH administration.

Materials and Methods. An outbred colony of house mice maintained in our laboratory were used in this study. Mice were weaned at at 21 days of age, caged in sexually separate groups of 3–4 per cage in a room with controlled illumination and temperature (14 hr of light/24 hr, 78 F), and fed Rockland mouse pellets in excess of consumption. In one experiment, mice received daily intraperitoneal (ip) injections of either metyrapone (Metopirone ditartrate, CIBA, 100 mg/kg in saline) or 0.9% saline from 30 to 60 days of age. In a second experiment, 90-day old mice were injected daily (ip) either with metyrapone (100 mg/kg) or saline (0.9%) for 21 days. Injections were administered between 8 and 10 AM and in a volume of 0.1 ml. Mice were killed 24 hr after the last injection, weighed, disemboweled and placed in 10% neutral buffered formalin. Organs were weighed wet after fixation. Tissues were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylineosin. Three sections taken at different levels were examined from each ovary, and the numbers of primary, developing and atretic follicles and corpora lutea were counted. Treatment means were compared using t test and probability levels of 0.05 or less were considered statistically significant.

Results. Daily injections of metyrapone in immature female mice significantly influenced weights of adrenal glands, ovaries and uteri (Table I). Compared to controls, adrenal glands were 14% lighter in metyrapone-treated females, while weights of ovaries and uteri were reduced 33% and 40%, respectively. In contrast to ovaries from controls of the same age (Fig. 1), ovaries of metyrapone-treated mice contained few large follicles and tertiary follicles present were usually atretic (Fig. 2). Moreover, the incidence of corpora lutea was reduced significantly by 82% in metyrapone-treated mice (Table I). Uteri in metyrapone-treated females were underdeveloped with dense endometria and little glandular development. Thus, metyrapone increased follicular atresia, and prevented ovulation and lutenization with corresponding uterine histological changes.

Body weights and seminal vesicle weights were reduced significantly in young male mice treated with metyrapone (Table I). Seminal vesicle weights were 49% lighter than in controls, while body weights were reduced 14%.

Among adult animals, mean adrenal weights between control (11.0 ± 0.3; $n = 12$) and metyrapone-treated (9.5 ± 0.2; $n = 12$) females differed significantly ($P < 0.01$). This 14% decrease in adrenal gland weight from adult females was the only effect of prolonged treatment with metyrapone among adult animals of both sexes.

Discussion. The present data demonstrate that treatment with metyrapone may inhibit development of reproductive organs in immature house mice of both sexes.

Metyrapone inhibits formation of major corticosteroids by blocking 11β-hydroxylation and induces a compensatory increase in ACTH production (4). Furthermore, ACTH stimulation in the absence of 11β-hydroxyla-

TABLE 1. MEAN (±SE) BODY AND ORGAN WEIGHTS FROM IMMATURE MICE RECEIVING SALINE OR METYRAPONE (100 mg/kg) ONCE DAILY FOR 30 DAYS. SIGNIFICANCE OF MEAN DIFFERENCES WAS DETERMINED BY STUDENT'S *t* TEST

	No. of mice	Initial body wt (g)	Final body wt (g)	Adrenal wt (mg)	Testis/ovary wt (mg)	Seminal vesicle/ uterus wt (mg)	Corpora lutea/ovary	Thymus wt (mg)	Spleen wt (mg)
Males									
Saline	15	15.6 ± 0.7	23.0 ± 0.7	5.5 ± 0.3	175 ± 8	72 ± 9	—	39 ± 3	56 ± 4
Metyrapone	15	15.0 ± 0.4	19.8 ± 0.6**	5.8 ± 0.1	188 ± 5	37 ± 3***	—	32 ± 2	48 ± 4
Females									
Saline	15	12.9 ± 0.3	17.7 ± 0.6	9.3 ± 0.4	13.6 ± 1.6	57 ± 7.5	1.1 ± 0.3	44 ± 3	48 ± 3
Metyrapone	15	13.0 ± 0.3	16.8 ± 0.5	8.0 ± 0.4*	9.1 ± 1.1*	34 ± 0.5*	0.2 ± 0.2*	47 ± 3	51 ± 3

[a] Significance by Student's *t* test: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

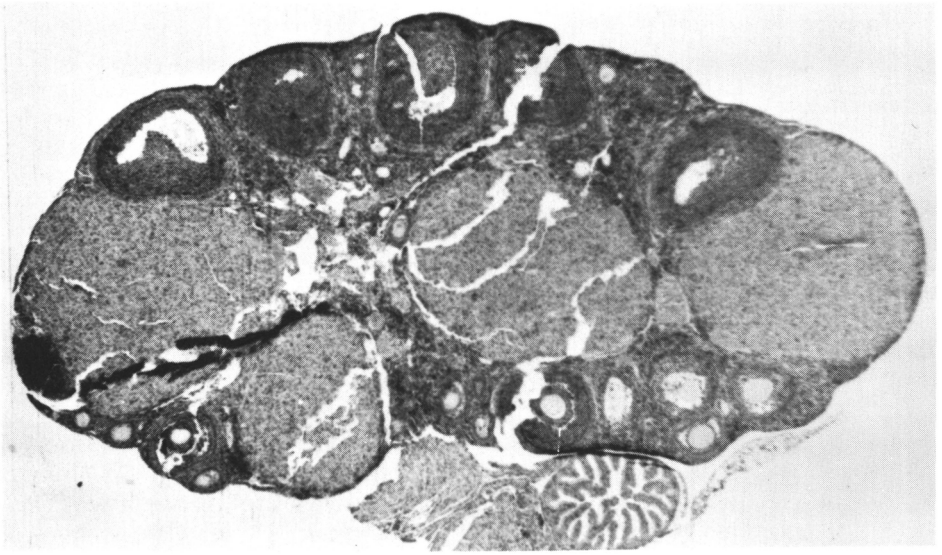

FIG. 1. Section of ovary from a house mouse injected daily with saline for 30 days, beginning at 30 days of age. Note presence of large corpora lutea. × 31.

tion appears to enhance adrenal androgen secretion (5, 6).

Our results with metyrapone are consistent with those of Christian *et al.* (1) who reported inhibition of reproductive maturation in several strains of mice following exogenous ACTH treatment. Moreover, the absence of significant testicular weight differences with metyrapone coincides with our earlier findings (7) where exogenous ACTH treatment had no effect on testes of maturing male house mice while seminal vesicle weight was significantly depressed. The cause of seminal vesicle weight depression after metyrapone treatment is not known, but one explanation may be that the inhibition of corticosteroid secretion by metyrapone prevents the normal augmentation by cortisol of testosterone effects on seminal vesicle growth (8). Another explanation may be that testicular androgen secretion is inhibited by metyrapone resulting in lower seminal vesicle weights.

Since low, nonvirilizing doses of weak androgens have been shown to inhibit gonadotrophin secretion and maturation in immature female mice (9), adrenal androgen suppression of gonadotrophin secretion via metyrapone seems to be a likely possibility in the present experiments.

The present results with metyrapone treatment, therefore, lend further support to the

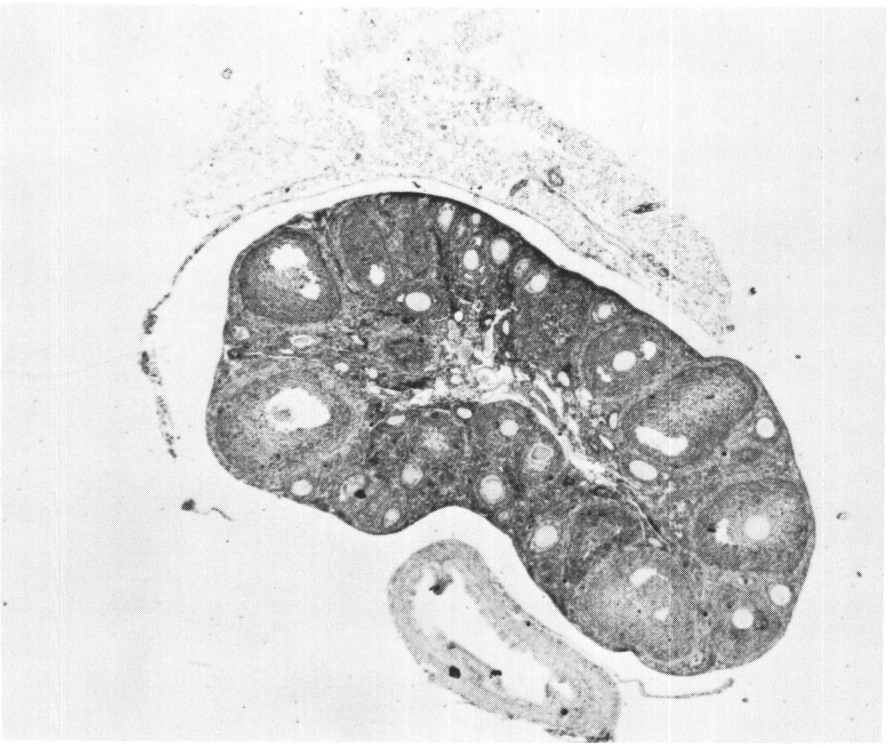

FIG. 2. Section of ovary from a house mouse injected daily with 100 mg/kg of metyrapone for 30 days, beginning at 30 days of age. Note absence of corpora lutea; compare with ovary of saline treated control (Fig. 1). × 31.

hypothesis that increased pituitary–adrenocortical function may impair reproductive function in mice. The above hypothesis provides a density-dependent negative feedback mechanism that is significant in the suppression of growth of natural and freely growing populations of small rodents.

Lowered ovarian weights in the metyrapone-treated mice were apparently associated with the decreased number of corpora lutea in these mice.

A decrease in thymic and splenic weights indicative of increased adrenocortical function (1) was not observed. The absence of thymic and splenic weight reduction after metyrapone treatment was apparently due to the blockade of corticoid release by metyrapone.

The reason for reduced adrenal weight by metyrapone treated females is not known but may be related to the involution of the adrenal cortex or to quantitative changes in the corticosteroids secreted. As discussed earlier, metyrapone enhances adrenal androgen production. Moreover, the size of the adrenal cortex in rodents decreases with androgen treatment (10, 11). It is possible therefore, that metyrapone induced an involution of the inner adrenal cortical areas which offset the ACTH-induced increase in the fasciculata–reticularis zones in females. Another possibility is that the decrease in corticosterone production produced by metyrapone is reflected in smaller adrenals in female mice since larger adrenals in female voles have been correlated with greater corticosterone production in females than in males (12).

Summary. The effects of chronic administration of metyrapone, a specific adrenal 11β-hydroxylase inhibitor, were examined on the reproductive organs of house mice. In one experiment, immature (30 days old) mice of both sexes received daily injections of either metyrapone (100 mg/kg) or saline ip for 30 days. In a second experiment, mature mice (90 days old) were treated in like manner either with metyrapone (100

mg/kg) or saline for 21 days. Twenty-four hours after the last injection, mice were killed, fixed in formalin, and organs were weighed and examined by light microscopy. There was significant impairment of ovarian and uterine development in the young female metyrapone-treated mice with the incidence of corpora lutea being reduced 82%. Seminal vesicle and body weights were significantly reduced in juvenile males. Among mature animals, a 14% decrease in adrenal gland weight from adult females was the only significant effect of prolonged treatment with metyrapone. These data support the hypothesis that increases in pituitary–adrenocortical function may impair development of reproductive organs in small rodents.

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