The Roles of the Pituitary-Adrenal Axis in the Reproductive Physiology of Male Prairie Deermice (Peromyscus maniculatus bairdi)

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THE ROLE OF THE PITUITARY-ADRENAL AXIS
IN THE REPRODUCTIVE PHYSIOLOGY OF MALE PRAIRIE DEERMICE
(PEROMYSCUS MANICULATUS BAIRDII)

A Thesis
Presented to
The Faculty of the Department of Biology
The College of William and Mary in Virginia

In Partial Fulfillment
Of the Requirements for the Degree of
Master of Arts

by
John Charles Coppes, Jr.
1980
This thesis is submitted in partial fulfillment of the requirements for the degree of Master of Arts.

Author

Approved, November 1980

Eric L. Bradley

C. Richard Terman

Robert E. L. Black
TABLE OF CONTENTS

ACKNOWLEDGEMENTS ........................................ iv
LIST OF TABLES ............................................... v
LIST OF FIGURES ........................................... vi
ABSTRACT .................................................. vii
INTRODUCTION ............................................. 2
MATERIALS AND METHODS .................................. 7
RESULTS ..................................................... 15
DISCUSSION ............................................... 33
APPENDIX .................................................. 51
BIBLIOGRAPHY ............................................. 53
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I am grateful for the gifts of Cosyntropin from Organon, Inc. and Metopirone from the Ciba-Geigy Corporation. Some of the materials used in this study were purchased with funds from NIH research grant HD 08906 from the National Institute of Child Health and Human Development.
LIST OF TABLES

Table                                                                 Page
1. COMPARISON OF BODY WEIGHT, ADRENALS, SEMINAL VESICLES, AND TESTES OF CONTROL AND POPULATION MICE .......................... 20
2. EFFECTS OF ACTH ON BODY WEIGHT, ADRENALS, SPLEEN, TESTES, AND SEMINAL VESICLES .................... 21
3. EFFECTS OF DEXAMETHASONE ON BODY WEIGHT, ADRENALS, SPLEEN, TESTES, AND SEMINAL VESICLES .................. 22
4. EFFECTS OF METYRAPONE ON BODY WEIGHT, ADRENALS, SPLEEN, TESTES, AND SEMINAL VESICLES ..................... 23
5. FRACTIONS OF MITOCHONDRIA AND LIPID DROPLETS IN THE ADRENAL CORTEX .................................. 24
6. PLASMA ACTH LEVELS ......................................................... 25
7. PEARSON PRODUCT-MOMENT CORRELATIONS: PLASMA ACTH VERSUS SELECTED GRAVIMETRIC PARAMETERS..... 26
8. PEARSON PRODUCT-MOMENT CORRELATIONS: ADRENAL WEIGHT VERSUS SEMINAL VESICLE AND TESTIS WEIGHTS ......................................................... 27
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>ZONA FASCICULATA OF CONTROL MALE PRAIRIE DEERMOUSE</td>
<td>29</td>
</tr>
<tr>
<td>2.</td>
<td>ZONA FASCICULATA OF ACTH-TREATED MOUSE</td>
<td>29</td>
</tr>
<tr>
<td>3.</td>
<td>ZONA FASCICULATA OF REPRODUCTIVELY INHIBITED POPULATION MALE</td>
<td>30</td>
</tr>
<tr>
<td>4.</td>
<td>ZONA FASCICULATA OF REPRODUCTIVELY INHIBITED POPULATION MALE: MITOCHONDRIAL WHORL</td>
<td>30</td>
</tr>
<tr>
<td>5.</td>
<td>ZONA FASCICULATA OF A RELATIVELY MATURE MALE FROM A POPULATION</td>
<td>30</td>
</tr>
<tr>
<td>6.</td>
<td>ACTH STANDARD CURVE</td>
<td>31</td>
</tr>
<tr>
<td>7.</td>
<td>ACTH LEVELS</td>
<td>32</td>
</tr>
</tbody>
</table>
ABSTRACT

The relationship between the pituitary-adrenal system and reproductive function in male prairie deermice (Pomyscus maniculatus bairdii) was examined. Three experiments were performed. In Experiment I, injections of ACTH or metyrapone caused significant decreases of testis and seminal vesicle weights. Dexamethasone did not affect testis weight, but caused a steady (although not significant) decrease of seminal vesicle weight with increasing dose. Adrenal ultrastructure of control, population, and ACTH-treated deermice was examined in Experiment II. Reproductively inhibited population animals had a different morphology than controls. However, the observed changes in the adrenals of population animals were unlike those seen with ACTH treatment. Measurement of plasma ACTH by radioimmunoassay in Experiment III showed no significant difference between control and population animals. Evidence obtained from these experiments suggests that although both ACTH and the glucocorticoids are probably capable of inhibiting reproduction in males, ACTH is not involved in the inhibition of reproduction seen in male deermice from my laboratory populations.
THE ROLE OF THE PITUITARY-ADRENAL AXIS
IN THE REPRODUCTIVE PHYSIOLOGY OF MALE PRAIRIE DEERMICE
(PEROMYSCUS MANICULATUS BAIRDII)
INTRODUCTION

Inhibition of reproductive function is often observed in small mammals from high density populations (for reviews on the subject, see Andrews, 1977, 1979; Brain, 1971; Christian, 1971, 1975, 1980; Christian, Lloyd, and Davis, 1965; Thiessen and Rodgers, 1961). Christian based his theory of population regulation on Selye's concept of the general adaptation syndrome (Selye, 1946). Christian proposed that factors associated with high population density (such as limited resources and increased social interactions) cause stress. Stress causes the release of corticotrophin (ACTH) from the anterior pituitary and the subsequent stimulation of steroid secretion by the adrenal cortex. Stress may inhibit reproduction via ACTH, the glucocorticoids, or other adrenal steroids (Christian, 1950, 1971, 1975, 1980; Christian, Lloyd, and Davis, 1965).

It is impossible to measure basal levels of ACTH and glucocorticoids in animals from natural populations, because the stress of capture and handling would induce a rapid rise in the blood levels of these hormones. However, there is substantial indirect evidence that changes in pituitary-adrenal function are associated with increasing population density. Several studies of males from natural and laboratory populations have shown a positive correlation between
adrenal weight and population density in various species of mice (Andrews and Belknap, 1979; Bronson and Eleftheriou, 1963; Christian, 1955a, 1955b, 1956; Purushotham, Mohano Rao, and Rajabai, 1978), voles (Tanaka, 1962; To and Tamarin, 1977), and lemmings (Andrews, 1970; Andrews and Belknap, 1979; Andrews et al., 1975). However, some investigators have observed no relationship between adrenal weight and population density in male deermice (Bronson and Eleftheriou, 1963; Sung, Bradley, and Terman, 1977; Terman, 1969) and voles (Chitty, 1961; To and Tamarin, 1977). In vitro incubations of adrenals from crowded laboratory animals (McCarthy, Green, and Sohal, 1976) or animals from high density natural populations (Andrews, 1970; Andrews and Belknap, 1979; Andrews et al., 1975) indicate that adrenal secretion of glucocorticoids increases with increasing population density. Animals from high density natural populations have a high incidence of renal pathology (Andrews and Belknap, 1979; Christian, Lloyd, and Davis, 1965), which is one of the effects of high levels of ACTH (Christian, 1967; Pasley and Christian, 1971). Low levels of circulating eosinophils, used as an indicator of high glucocorticoid levels, were found in voles from high density populations (Louch, 1956). Adrenal ascorbic acid depletion was associated with increasing population density in C57BL/10 mice (Bronson and Eleftheriou, 1963) and spiny field mice (Purushotham, Mohano Rao, and Rajabai, 1978).

Chronic stress (Collu, Tache, and Ducharme, 1979; Du Ruisseau et al., 1978; Tache et al., 1978, 1980) or ACTH ad-
ministration (Baker et al., 1950; Beitins et al., 1973; Bullock and New, 1971; Christian, Lloyd, and Davis, 1965; Doerr and Pirke, 1975; Forest, 1978, Irvine et al., 1974; Kapil, Chowdhury, and Swarup, 1979; Kim, De La Torre, and Diczfalusy, 1978; Magrini et al., 1978; Pasley and Christian, 1971, 1972; Rivarola et al., 1966; Saez et al., 1977; Schaison, Durand, and Mowszowicz, 1978) causes an inhibition of reproduction in males of various species. The mechanisms involved are not completely understood at the present time. It appears that both ACTH and the glucocorticoids may act directly to affect male reproduction (see discussion).

Natural populations of prairie deermice (Peromyscus maniculatus bairdi) maintain a relatively stable population density (Terman, 1966). Our laboratory has been studying the physiological and behavioral mechanisms by which laboratory populations of prairie deermice limit their size. Populations provided with excess food and water stop growing after some time. The control of population growth is achieved by the failure of litters to survive and/or the failure of surviving litters to mature sexually. Animals of both sexes from asymptotic populations have significantly lower reproductive organ weights than controls. Different populations reach asymptote at different levels, indicating that the observed changes are not directly related to population density (Terman, 1965, 1969, 1973).

Sung, Bradley, and Terman (1977) found that reproductively inhibited deermice from laboratory populations had higher levels of serum corticosterone than controls. However, the
absolute adrenal weights of the population males were lighter than those of controls. There was no difference observed in the relative adrenal weights. In a subsequent study, Bradley and Terman (1980a) found significantly higher serum corticosterone levels in population mice and trends toward lighter absolute adrenals ($P < 0.1$) and heavier relative adrenals ($P < 0.1$). In the latter study, it was also demonstrated that the smaller adrenal size was due to a reduction of both the cortex and medulla. The apparent paradox of population animals having smaller adrenals but highly elevated levels of corticosterone has not yet been resolved. The adrenal cortices of population animals may have a higher secretion rate of corticosterone due to increased levels of plasma ACTH. Alternatively, there may be changes in the binding of glucocorticoids to plasma proteins or alterations in corticoid catabolism and excretion which would allow high corticosterone concentrations despite low corticosterone secretion rates.

The purpose of the present study was to examine the role of the pituitary-adrenal axis in the reproductive physiology of male prairie deermice. Three experiments were performed:

Experiment I---Male deermice were injected with ACTH, dexamethasone, or metyrapone so that the effects of the various components of the pituitary-adrenal axis on reproduction could be separately analyzed.

Experiment II---The ultrastructure of the adrenal cortex of male mice was examined by transmission electron
microscopy for evidence of changes in the secretory activity of adrenals from reproductively inhibited population animals.

Experiment III--Plasma levels of ACTH in population animals and controls were measured by radioimmunoassay (RIA).
MATERIALS AND METHODS

ANIMAL MAINTENANCE

The animals used in this study were prairie deermice (Peromyscus maniculatus bairdi) obtained from an outbred laboratory colony.

In Experiment I, male mice were individually caged in 12.5 x 27 x 14.5 cm plastic cages at weaning.

The control animals used in Experiments II and III were kept with siblings of the same sex after weaning. At 90 ± 4 days of age, non-sibling bisexual pairs were caged in 12 x 26 x 14 cm "no-contact" cages (Albertson, Bradley, and Terman, 1975). These cages consisted of two compartments with a double layer of hardware cloth separating the male and female, but allowing visual, olfactory, and auditory cues to be exchanged.

Population animals were obtained from laboratory populations founded by four males and four pregnant females from eight different litters. The first litter of each female was removed to eliminate any possible prenatal effects occurring before the population was established. The populations were maintained in circular pens (1.5 m diameter) provided with pine shavings for bedding and eight plastic nest boxes for shelter (Terman, 1969, 1973).

For all three experiments, the light cycle consisted of 7.
14 hours bright light (four 40 W fluorescent tubes from 2200-1200h): ½ hour darkness: 9 hours dim light (four 15 W incandescent bulbs from 1230-2130h): ½ hour darkness. Temperature was maintained at 21-31 °C. Tap water and food (D & G Laboratory Diet) were provided in excess of utilization.

**BLOOD AND ORGAN COLLECTION**

In Experiments II and III, male mice 120-140 days of age were sampled one hour before the onset of the dark period (1100h), which corresponds to the peak of the plasma ACTH circadian rhythm in rats (Matsuyama, Rhumann-Wennhold, and Nelson, 1971; Retiene et al., 1968). Care was taken not to disturb the animals on the morning of sampling. Population mice of the appropriate age were labelled with a non-toxic ultraviolet-sensitive dye (Raytech Industries) applied at least two days before sacrifice. At the time of sampling, a 40 W long-wave UV lamp was turned on to identify the marked mice.

The mice used in Experiment II were stunned with a sharp blow to the head and decapitated within 20 seconds of the initial disturbance. Both adrenals were removed via ventral abdominal incision, immediately placed in 2% glutaraldehyde in 0.1 M phosphate buffer (pH=7.4), and cut into small pieces. The adrenals were removed within two minutes of the initial disturbance of the animal.

Two methods were used to collect blood in Experiment III. In one group of mice, the animals were decapitated as described above. Trunk blood (100-200 μl) was collected in plastic
weighing boats containing 25 U heparin. The other group of mice was sampled by laparotomy. These animals were rapidly anesthetized with ether, the abdomen was opened, and the abdominal aorta and inferior vena cava were cut at the level of the kidneys. Blood (200-400 μl) was collected in plastic syringes containing 25 U heparin. Samples were obtained within two minutes. The heparinized blood was transferred to plastic centrifuge tubes and centrifuged at 11,000 x g for 2.5 minutes. Fifty or 100 μl aliquots of plasma were transferred to polystyrene tubes and frozen within ten minutes of sampling. Plasma was stored at -70 °C until it was assayed.

After sampling, the animals were weighed to the nearest 0.1 g and fixed in 10% buffered formalin. After fixation, testes (Experiments I, II, and III), seminal vesicles (Experiments I, II, and III), adrenals (Experiments I and III), and spleens (Experiment I) were removed, cleaned of extraneous tissue, and weighed twice on a Cahn electrobalance interfaced with a Wang programmable calculator. Spleens were examined in Experiment I because spleen weight is inversely related to levels of circulating glucocorticoids (Andersson and Muntzing, 1971; Gotjamanos, 1970).

**EXPERIMENT I: PHARMACOLOGY OF ACTH, DEXAMETHASONE, AND METYRAPONE**

Male mice received daily subcutaneous injections of a test substance for 30 days beginning at 30 days of age. The animals were sacrificed 24 hours after the last injection.
The volume of the injected preparation (0.08-0.10 ml) was adjusted to the average body weight of males for a given age so that the dose administered/g body weight was approximately constant throughout the treatment.

There were ten treatment groups: vehicle control; 2.5, 25, or 250 U ACTH (Acthar, Armour) kg; 5, 50, 500 μg dexamethasone sodium phosphate (Decadron Phosphate, Merck, Sharp & Dohme)/kg; 1, 10, or 100 mg metyrapone (Metopirone, Ciba)/kg.

The highest dose of ACTH was a commercial preparation (H.P. Acthar Gel, Armour). All other preparations were made up in 5% gelatin. Phenol (0.5%) was added as a preservative to the vehicle control, ACTH, and dexamethasone preparations. Fresh metyrapone preparations were made on a weekly basis without phenol because the addition of phenol caused a marked turbidity of the solution.

**EXPERIMENT II: ADRENAL ULTRASTRUCTURE**

The adrenal glands of 5 control, 5 population, and 2 ACTH-treated (4 U/day for seven days) male mice were examined. The adrenals were fixed for two hours with 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. After several buffer rinses, the tissue was postfixed with 1% OsO₄ in the same buffer for two hours. The material was rinsed with 50% acetone, stained overnight en bloc with 2% uranyl acetate in 70% acetone, and dehydrated through a graded acetone series. Luft's Epon 812 was used for infiltration and embedding. Embedded material was polymerized for three days at 60 °C.
Blocks of tissue were oriented so that the zona fasciculata could be easily located. Silver to silver-gold sections were cut with diamond (Du Pont) or glass knives on a Porter-Blum MT-2B ultramicrotome. Sections were placed on formvar-coated slot grids, stained with Sato's lead for 60 seconds, and observed on a Zeiss EM 9S-2 transmission electron microscope.

The relative fractions of mitochondria and lipid droplets in each treatment group were evaluated. Eight cells of the zona fasciculata were randomly selected from each of the three groups. The cross-sectional areas of mitochondria and lipid droplets were determined for a 3 x 3 μm nucleus-free area of each cell using a modification of the method described by Weibel (1969).

EXPERIMENT III: PLASMA ACTH

Four groups of mice were sampled by decapitation: control, population, ether-stressed (two minutes of ether inhalation), and dexamethasone-suppressed (400 μg/day for seven days). Additional groups of controls and population animals were sampled via laparotomy. A standard pool of ether-stressed plasma for use as an internal standard was obtained by subjecting eight mature male mice to 4-5 minutes of ether inhalation and collecting the blood via laparotomy.

Plasma ACTH was measured by radioimmunoassay (RIA). The procedure was a modification of that described by Berson and Yalow (1968) using unextracted plasma. All solutions were made in the same diluent buffer (0.05 M PO₄, 0.25% bovine
serum albumin, 0.5% 2-mercaptoethanol, 400 KIU aprotinin/ml, 1% benzyl alcohol, pH 7.5). Mercaptoethanol and aprotinin were added to prevent incubation damage of ACTH. Benzyl alcohol served as a preservative.

Two hundred and fifty μl of diluent buffer was added to 50 μl of unknown plasma in a 12 x 75 mm polystyrene tube, followed by the addition of 100 μl of rabbit anti-human ACTH serum (Wellcome, lot # K7653) diluted 1:20,000, and 100 μl of diluent buffer containing 1-2 pg 125I-ACTH (Immuno Nuclear Corporation, lot # 26320). The 125I-ACTH was purified immediately before use on a 1 x 15 cm Sephadex G-25 fine column. Tubes used for producing the standard curve were treated in a similar fashion except that ACTH-free plasma (prepared by extracting the ACTH from female rat plasma with Amersham glass absorbent) was used instead of unknown plasma and 1-256 pg 1-24ACTH (Cosyntropin, Organon, lot # 34770) was added with the 250 μl of diluent buffer (see Appendix for details of standard curve). In addition, an aliquot from the standard pool of ether-stressed plasma was serially diluted and assayed. The dilutions of standard plasma were made with ACTH-free plasma so that the final concentration of plasma was equal for all dilutions. The volume of the first incubation mixture was 500 μl, with a plasma dilution of 1:10 and a final antibody dilution of 1:100,000. The assay tubes were vortexed, covered, and incubated for three days at 4 °C.

A second antibody was used to separate free from antibody-bound 125I-ACTH. One hundred μl of 20% goat anti-rabbit gamma globulin (Antibodies Incorporated, lot # 8MH15W and
100 μl of 5% normal rabbit serum were added to the first incubation mixture. The tubes were vortexed, covered, and incubated for an additional 24 hours at 4 °C. At the end of the final incubation, the tubes were centrifuged at 5000 x g for 20 minutes at 4 °C. The supernatant was discarded. The pellet containing antibody-bound \( {^{125}\text{I}}-\text{ACTH} \) was washed with 1.0 ml of cold phosphate buffer (0.05 M PO\(_4\), pH 7.5) and centrifuged for ten minutes at 5000 x g. The supernatant was discarded and the tubes containing the pellets were counted to 4000 counts (=3% error, 95% confidence limits) in a Beckman Biogamma II gamma counter.

All determinations for the standard curve and the serial dilution of the standard pool of ether-stressed Peromyscus plasma were made in duplicate. Unknown samples were assayed in duplicate when possible. All standards and unknowns were measured in a single assay. Unknown plasma samples obtained from the standard pool were randomly placed throughout the experiment so that the inter-assay variability of the assay could be determined.

**STATISTICAL METHODS**

The Student's t-Test was used to compare experimental groups if the F-Test showed a homogeneity of variances. If the variances were heterogeneous, comparisons were made with the Mann-Whitney U Test. Correlations were determined using the Pearson product-moment coefficient. RIA data was analyzed on an IBM 370 computer using the RIANAL program of Duddleson, Midgley and Niswender (1972). Probabilities less
than 0.05 were considered significant.
RESULTS

COMPARISON OF BODY WEIGHT AND ORGAN WEIGHTS OF CONTROL AND POPULATION ANIMALS

The control and population animals used in Experiments II and III were treated in an identical manner. Consequently, the data on the body weights and organ weights from the two experiments were combined (Table 1). There was no statistical difference in the absolute or relative organ weights of similarly treated groups. The body weights of the population animals of Experiment II, however, were slightly lighter than those of Experiment III (11.70 ± 0.45 g versus 13.33 ± 0.37 g, respectively; P < 0.05).

Male population mice had significantly lighter body weights, testis weights, and seminal vesicle weights than controls. There was no significant difference noted in the adrenal weights of population animals.

EXPERIMENT I: PHARMACOLOGY OF ACTH, DEXAMETHASONE, AND METYRAPONE

Injections of ACTH (Table 2) caused a dose-dependent increase in adrenal weight and a dose-dependent decrease in testis, seminal vesicle, and spleen weights. ACTH had no effects on body weight.

Dexamethasone (Table 3) had no apparent effect on body weight or testis weight. Decreases were noted in adrenal,
spleen, and seminal vesicle weights, but these changes were not significant except for the relative spleen weight at 500 μg dexamethasone/kg.

Mice treated with metyrapone (Table 4) showed significantly higher body weights with the highest dose used (100 mg/kg). There were no apparent changes in adrenal or spleen weights. Metyrapone caused decreases in testis and seminal vesicle weights, but not in a dose-dependent fashion. The greatest inhibition was seen at the lowest dose (1 mg/kg). Although the testis and seminal vesicle weights increased with higher doses, the relative weights of these organs still tended to be lighter than controls in those animals treated with 100 mg/kg (P < 0.1).

EXPERIMENT II: ADRENAL ULTRASTRUCTURE

All of the control animals examined had a similar ultrastructure of the zona fasciculata (Figure 1). Most of the mitochondria were spherical, but ovoid and elongated mitochondria were common. The cristae of the mitochondria usually appeared vesicular or saccular. However, this vesiculo-saccular appearance may be due to the plane of sectioning because longitudinal sections of tubular mitochondrial cristae were often seen. The number of lipid droplets in the cytoplasm varied from animal to animal. Small, dense lysosomes were common. The smooth endoplasmic reticulum (SER) was moderately well developed, sometimes appearing vacuolar. Large numbers of free ribosomes were present in the cytoplasm.

The zona fasciculata of the ACTH-treated mice had a mor-
phology similar to that of controls (Figure 2). The cells appeared somewhat larger and lipid droplets were more numerous.

Four of the five population mice examined had a distinctly different adrenal morphology than controls (Figure 3). The most obvious difference was a gross alteration of mitochondrial structure. Most of the mitochondria had irregular shapes, some showing disruption of their inner structure. Sometimes, bundles of tubular cristae were seen in the constrictions of dumbell-shaped mitochondria. Concentric whorls formed by complexes of elongated mitochondria were observed (Figure 4). There was a high degree of variability in the proportions and qualities of lipid droplets, lysosomes, and SER. No particular pattern was apparent for these characteristics.

One of the population animals had an adrenal ultrastructure similar to that of controls (Figure 5). Upon examination of organ weights, it was evident that this mouse was reproductively more developed than the other four population mice examined (testis weight=153 mg, compared with 52-84 mg for the other four mice; seminal vesicle weight=30 mg, compared with 2-6 mg for the others).

The relative fractions of mitochondria and lipid droplets in each of the treatment groups were estimated (Table 5). ACTH-treated mice had a smaller mitochondrial fraction than either control or population mice (P<0.05). There was a significant increase (P<0.01) in the volume of lipid droplets after ACTH administration.
EXPERIMENT III: PLASMA ACTH

The standard curve for the ACTH radioimmunossay is shown in Figure 6. The logit transformation of the standard curve was linear (see Appendix for statistics). The assay had a limit of detection (= 1 standard error of the buffer control) of 33 pg/ml plasma and a sensitivity (= 1 standard deviation of the assay) of 112 pg/ml. The dilutions of standard ether-stressed Peromyscus plasma are shown in the same figure. The logit transformation of the serial dilution was linear, but it was not parallel to the logit transformation of the standard curve (see Appendix). Unknown plasma samples were evaluated using the transformed standard curve. Some of the unknown samples had ACTH concentrations greater than the highest ACTH concentration used in the standard curve. Since the kinetics of antibody-antigen binding change as the concentration of the antigen increases, it is not possible to extrapolate the standard curve. Consequently, all values greater than 5120 pg/ml were assigned that value (see Figure 7). Aliquots from a pool of standard ether-stressed Peromyscus plasma measured 17 times throughout the assay gave a value of 1839 ± 53 pg/ml.

Results for plasma ACTH are given in Table 6. The distributions of ACTH values for each treatment group are shown in Figure 7. In animals sampled by decapitation, population animals had plasma ACTH levels twice as high as controls (3019 ± 811 versus 1351 ± 612 pg/ml; P < 0.2). Dexamethasone-suppressed (713 ± 180 pg/ml) and ether-stressed (1559 ± 808 pg/ml) mice were not significantly different than controls.
When mice were sampled via laparotomy, there was no significant difference in plasma ACTH concentrations between population animals and controls (1151 ± 530 versus 858 ± 258 pg/ml, respectively). There was no significant difference between the sampling time of population and control laparotomized mice (75 ± 8 versus 71 ± 3 seconds).

Pearson product-moment correlations (Table 7) revealed a significant negative correlation (P=0.007) between plasma ACTH levels and relative testis weights in control animals sampled by laparotomy. In addition, the relative seminal vesicle weights of controls tended (P=0.106) to be inversely related to ACTH concentrations. No such relationships were seen in population mice. There was a trend toward an inverse relationship between plasma ACTH and relative adrenal weight in control (P=0.066) and population (P=0.116) animals. There was a significant negative correlation (P=0.027) between plasma ACTH and adrenal weight if values for control and population animals were combined.

Relationships between adrenal and testis or seminal vesicle weight were also examined (Table 8). The relative seminal vesicles of controls increased in weight with the relative adrenals (P=0.019). There was a similar trend with relative testis weights (P=0.113). No correlations were noted between adrenal weight and testis or seminal vesicle weight in population mice.
### TABLE 1
COMPARISON OF BODY WEIGHT, ADRENALS, SEMINAL VESICLES, AND TESTES OF CONTROL AND POPULATION MICE
(MEAN + SEM)

<table>
<thead>
<tr>
<th></th>
<th>BODY WT (g)</th>
<th>ADRENALS</th>
<th>SEM. VES.</th>
<th>TESTES</th>
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<tr>
<td></td>
<td></td>
<td>ABS (mg)</td>
<td>REL (mg/g)</td>
<td>ABS (mg)</td>
</tr>
<tr>
<td>CONTROL</td>
<td>17.81</td>
<td>3.41</td>
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<td>157.2</td>
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<tr>
<td>MALES</td>
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<td>±0.24</td>
<td>±0.01</td>
<td>±11.6</td>
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<td></td>
<td>(23)</td>
<td>(18)</td>
<td>(18)</td>
<td>(23)</td>
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<td>POPULATION</td>
<td>12.93***</td>
<td>2.79</td>
<td>0.20</td>
<td>14.5**</td>
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<td>MALES</td>
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<td>±0.30</td>
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<td>±5.4</td>
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<td>(21)</td>
<td>(16)</td>
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<td>(21)</td>
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COMPARISON WITH CONTROLS:

** P<0.01  
*** P<0.001
TABLE 2
EFFECTS OF ACTH ON BODY WEIGHT, ADRENALS, SPLEEN, TESTES, AND SEMINAL VESICLES
(MEAN ± SEM)

<table>
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<tr>
<th>TREATMENT</th>
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<th>SPLEEN</th>
<th>TESTES</th>
<th>SEM. VES.</th>
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<td>0.199</td>
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<tr>
<td></td>
<td></td>
<td>±0.78</td>
<td>±0.36</td>
<td>±0.018</td>
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<td>±0.12</td>
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<tr>
<td>2.5</td>
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<tr>
<td></td>
<td></td>
<td>±0.37</td>
<td>±0.30</td>
<td>±0.018</td>
<td>±1.6</td>
<td>±0.11</td>
</tr>
<tr>
<td>25</td>
<td>8</td>
<td>16.00</td>
<td>5.07</td>
<td>0.322</td>
<td>15.0</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.78</td>
<td>±1.25</td>
<td>±0.086</td>
<td>±2.7</td>
<td>±0.17</td>
</tr>
<tr>
<td>250</td>
<td>9</td>
<td>17.06</td>
<td>18.82**</td>
<td>1.180**</td>
<td>9.5**</td>
<td>0.57**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±1.17</td>
<td>±2.48</td>
<td>±0.197</td>
<td>±1.0</td>
<td>±0.06</td>
</tr>
</tbody>
</table>

COMPARISON WITH CONTROLS:
  * P<0.05
  ** P<0.01
### Table 3

Effects of Dexamethasone on Body Weight, Adrenals, Spleen, Testes, and Seminal Vesicles

(Mean ± SEM)

<table>
<thead>
<tr>
<th>Treatment µg DEX/kg</th>
<th>N</th>
<th>Body WT (g)</th>
<th>Adrenals ABS (mg)</th>
<th>Adrenals REL (mg/g)</th>
<th>Spleen ABS (mg)</th>
<th>Spleen REL (mg/g)</th>
<th>Testes ABS (mg)</th>
<th>Testes REL (mg/g)</th>
<th>Sem. Ves. ABS (mg)</th>
<th>Sem. Ves. REL (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9</td>
<td>16.67</td>
<td>3.31</td>
<td>0.199</td>
<td>18.8</td>
<td>1.10</td>
<td>220.0</td>
<td>13.36</td>
<td>70.3</td>
<td>4.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.78</td>
<td>±0.36</td>
<td>±0.018</td>
<td>±2.9</td>
<td>±0.12</td>
<td>±9.8</td>
<td>±0.69</td>
<td>±8.4</td>
<td>±0.49</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>17.71</td>
<td>3.20</td>
<td>0.187</td>
<td>17.5</td>
<td>1.00</td>
<td>224.6</td>
<td>13.02</td>
<td>59.9</td>
<td>3.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±1.26</td>
<td>±0.37</td>
<td>±0.026</td>
<td>±1.5</td>
<td>±0.08</td>
<td>±18.4</td>
<td>±1.27</td>
<td>±10.1</td>
<td>±0.63</td>
</tr>
<tr>
<td>50</td>
<td>9</td>
<td>16.11</td>
<td>3.12</td>
<td>0.198</td>
<td>15.0</td>
<td>0.94</td>
<td>218.9</td>
<td>13.70</td>
<td>57.1</td>
<td>3.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.63</td>
<td>±0.23</td>
<td>±0.019</td>
<td>±1.0</td>
<td>±0.08</td>
<td>±12.4</td>
<td>±0.86</td>
<td>±8.6</td>
<td>±0.47</td>
</tr>
<tr>
<td>500</td>
<td>9</td>
<td>15.57</td>
<td>2.57</td>
<td>0.166</td>
<td>12.7</td>
<td>0.82*</td>
<td>230.3</td>
<td>14.86</td>
<td>53.0</td>
<td>3.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.47</td>
<td>±0.17</td>
<td>±0.011</td>
<td>±0.6</td>
<td>±0.05</td>
<td>±16.6</td>
<td>±1.13</td>
<td>±6.4</td>
<td>±0.44</td>
</tr>
</tbody>
</table>

Comparison with Controls:

* P<0.05
TABLE 4
EFFECTS OF METYRAPONE ON BODY WEIGHT, ADRENALS, SPLEEN, TESTES, AND SEMINAL VESICLES
(MEAN ± SEM)

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>N</th>
<th>BODY WT (g)</th>
<th>ADRENALS</th>
<th>SPLEEN</th>
<th>TESTES</th>
<th>SEM. VES.</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg MET/kg</td>
<td></td>
<td></td>
<td>ABS (mg)</td>
<td>REL (mg/g)</td>
<td>ABS (mg)</td>
<td>REL (mg/g)</td>
</tr>
<tr>
<td>0</td>
<td>9</td>
<td>16.67</td>
<td>3.31</td>
<td>0.199</td>
<td>18.8</td>
<td>1.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.78</td>
<td>±0.36</td>
<td>±0.018</td>
<td>±2.9</td>
<td>±0.12</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>16.87</td>
<td>2.94</td>
<td>0.177</td>
<td>17.3</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.53</td>
<td>±0.27</td>
<td>±0.018</td>
<td>±1.6</td>
<td>±0.07</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>17.80</td>
<td>3.32</td>
<td>0.190</td>
<td>19.5</td>
<td>1.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.55</td>
<td>±0.21</td>
<td>±0.016</td>
<td>±1.1</td>
<td>±0.08</td>
</tr>
<tr>
<td>100</td>
<td>10</td>
<td>18.97*</td>
<td>4.09</td>
<td>0.216</td>
<td>20.1</td>
<td>1.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.36</td>
<td>±0.27</td>
<td>±0.014</td>
<td>±1.7</td>
<td>±0.10</td>
</tr>
</tbody>
</table>

COMPARISON WITH CONTROLS:
* P<0.05
**TABLE 5**

**FRACTIONS OF MITOCHONDRIA AND LIPID DROPLETS IN THE ADRENAL CORTEX**

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th># CELLS EXAMINED</th>
<th># ANIMALS REPRESENTED</th>
<th>MITOCHONDRIA</th>
<th>LIPID DROPLETS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>8</td>
<td>5</td>
<td>0.423</td>
<td>0.041</td>
</tr>
<tr>
<td>ACTH-TREATED</td>
<td>8</td>
<td>2</td>
<td>0.315 *(a)</td>
<td>0.183 **(a)</td>
</tr>
<tr>
<td>POPULATION</td>
<td>8</td>
<td>4</td>
<td>0.425 *(b)</td>
<td>0.059 **(b)</td>
</tr>
</tbody>
</table>

* P<0.05  
** P<0.01  

(a) COMPARISON WITH CONTROLS  
(b) COMPARISON WITH ACTH-TREATED MICE
### TABLE 6

**PLASMA ACTH LEVELS (pg/ml)**

<table>
<thead>
<tr>
<th>SAMPLING METHOD</th>
<th>TREATMENT</th>
<th>MEAN ± SEM</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DECAPITATION:</strong></td>
<td>CONTROL</td>
<td>1351 ± 612</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>POPULATION</td>
<td>3019 ± 811</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>DEXAMETHASONE</td>
<td>713 ± 180</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>2 MIN ETHER STRESS</td>
<td>1559 ± 808</td>
<td>8</td>
</tr>
<tr>
<td><strong>LAPAROTOMY UNDER ETHER ANESTHESIA:</strong></td>
<td>CONTROL</td>
<td>858 ± 258</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>POPULATION</td>
<td>1151 ± 530</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>4-5 MIN ETHER STRESS</td>
<td>1839 ± 54</td>
<td>1</td>
</tr>
</tbody>
</table>

1 This value was obtained from a pool of 5 animals. Since individuals were measured in the other treatment groups, no statistical comparisons were made.

All comparisons with controls within the same sampling group were not significant.
TABLE 7
PEARSON PRODUCT-MOMENT CORRELATIONS:
PLASMA ACTH VERSUS SELECTED GRAVIMETRIC PARAMETERS

<table>
<thead>
<tr>
<th></th>
<th>CONTROLS (N=9)</th>
<th>POPULATION (N=8)</th>
<th>CONT. + POP. (N=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CORREL. COEFF.</td>
<td>PROB.</td>
<td>CORREL. COEFF.</td>
</tr>
<tr>
<td>ABSOLUTE ADRENALS</td>
<td>-0.121</td>
<td>0.756</td>
<td>-0.608</td>
</tr>
<tr>
<td>RELATIVE ADRENALS</td>
<td>-0.635</td>
<td>0.066</td>
<td>-0.599</td>
</tr>
<tr>
<td>ABSOLUTE SEMINAL VESICLES</td>
<td>-0.369</td>
<td>0.328</td>
<td>-0.297</td>
</tr>
<tr>
<td>RELATIVE SEMINAL VESICLES</td>
<td>-0.574</td>
<td>0.106</td>
<td>-0.285</td>
</tr>
<tr>
<td>ABSOLUTE TESTES</td>
<td>-0.394</td>
<td>0.294</td>
<td>-0.294</td>
</tr>
<tr>
<td>RELATIVE TESTES</td>
<td>-0.817</td>
<td>0.007</td>
<td>-0.276</td>
</tr>
</tbody>
</table>

(ACTH WAS ASSayed IN PLASMA SAMPLES OBTAINED VIA LAPAROTOMY)
<table>
<thead>
<tr>
<th></th>
<th>CONTROLS (N=18)</th>
<th>POPULATION (N=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CORREL.</td>
<td>PROB.</td>
</tr>
<tr>
<td>REL ADRENALS VS REL SEMINAL VESICLES</td>
<td>0.545</td>
<td>0.019</td>
</tr>
<tr>
<td>REL ADRENALS VS REL TESTES</td>
<td>0.386</td>
<td>0.113</td>
</tr>
<tr>
<td>ABS ADRENALS VS ABS SEMINAL VESICLES</td>
<td>0.526</td>
<td>0.025</td>
</tr>
<tr>
<td>ABS ADRENALS VS ABS TESTES</td>
<td>0.413</td>
<td>0.089</td>
</tr>
</tbody>
</table>
SYMBOLS USED IN FIGURES 1-5

L—lysosome
LD—lipid droplet
M—mitochondrion
Nu—nucleus
Figure 1. Zona fasciculata of control male prairie deer-mouse. Mitochondria (M) are mostly spherical or ovoid. The cristae of the mitochondria are tubular (some may appear vesicular or saccular due to the plane of sectioning). Lipid droplets (LD) are seen, usually about the same size as the mitochondria. Small, electron-dense lysosomes (L) were observed in most cells. The smooth endoplasmic reticulum (the light vesicles in the cytoplasm) was moderately well developed. x 30,000.

Figure 2. Zona fasciculata of ACTH-treated mouse (4 U/day for 7 days). The morphology is similar to that of controls, with the exception of lipid droplets being more numerous. x 30,000.
Figure 3. Zona fasciculata of reproductively inhibited population male. Note the abnormal shapes of the mitochondria and the vacuolated appearance of the cytoplasm. x 30,000.

Figure 4. Complex of several elongated mitochondria seen several times in population animals. x 30,000.

Figure 5. Zona fasciculata of a relatively mature male from a population. Note similarities with the control animal in Figure 1. x 30,000.
FIGURE 6
ACTH STANDARD CURVE

% PEROMYSCUS PLASMA

ether-stressed Peromyscus plasma

standard ACTH

\[ \frac{B}{B_0} \]

pg ACTH / ml PLASMA
FIGURE 7
ACTH LEVELS

plasma ACTH pg/ml

CONT. POP. DEX. 2 MIN. CONT. POP. STD. ETHER POOL

DECAPITATION LAPAROTOMY
DISCUSSION

PHARMACOLOGY OF ACTH, DEXAMETHASONE, AND METYRAPONE

This experiment was a modification of the design used on human subjects by Schaison, Durand, and Mowszowicz (1978). ACTH, dexamethasone, and metyrapone each affect the pituitary-adrenal axis in a different fashion. By using these drugs, it is possible to selectively increase or decrease the circulating levels of ACTH and/or the glucocorticoids. The effects of these substances on experimental animals has been reported by various investigators, but often the doses used were unphysiologically high or the duration of treatment was short. This experiment was performed to determine the effects of chronic treatment with these drugs on testis and seminal vesicle weight over a wide range of dosages in Peromyscus.

ACTH treatment caused a dose-dependent inhibition of reproduction, indicated by decreased testis and seminal vesicle weights (Table 2). Since ACTH stimulates the adrenals to secrete glucocorticoids, the observed effects could be due to ACTH or corticosterone. These observations are in agreement with most published reports on the subject. ACTH administration has been found to cause decreased concentrations of plasma testosterone (Beitins et al., 1973; Doerr and Pirke, 1975; Forest, 1978; Rivarola et al., 1966; Saez et al., 1977; 33.
Schaison, Durand, and Mowszowicz, 1978), decreased testosterone production and secretion (Bullock and New, 1971; Kim, De La Torre, and Dizafalusy, 1978); Magrini et al., 1978), decreased testis and accessory sex organ weights (Baker et al., 1950; Christian, Lloyd, and Davis, 1965; Pasley and Christian, 1971, 1972), and disrupted testis histology (Kapil, Chowdhury, and Swarup, 1979). ACTH treatment in this experiment resulted in adrenal hypertrophy. Since male mice from populations in our laboratory have adrenal weights comparable to controls, it is plausible to assume that ACTH levels are not elevated in population mice. It should be noted, however, that adrenal hypertrophy is not always associated with hyperfunction of the cortex (Hull et al., 1976; Ogle, 1974).

Dexamethasone is a synthetic glucocorticoid. Treatment with dexamethasone would be expected to increase circulating levels of glucocorticoids while inhibiting ACTH secretion. Dexamethasone administration (5-500 µg/kg) had no significant effects on testis weights, but there was a steady (although nonsignificant) decrease in seminal vesicle weight with increasing dosage (Table 3). A larger sample size or a higher dose of dexamethasone would have probably produced a significant decrease in seminal vesicle weight. Saez et al., (1977) reported significantly lower plasma testosterone levels in rats treated with doses comparable to the highest dose used in this experiment. Other authors have found decreased plasma testosterone (Doerr and Pirke, 1975; McKenna,
et al., 1979; Schaison, Durand, and Mowszowicz, 1978; Smals, Kloppenborg, and Benraad, 1977; Welsh, McCraw, and Johnson, 1979), and decreased testosterone secretion (Kim, De La Torre, and Diczfalusy, 1978; Magrini et al., 1978) directly associated with high levels of glucocorticoids. These studies indicate that ACTH is not necessary for the inhibition of reproductive function caused by exogenous ACTH in intact animals.

Metyrapone inhibits glucocorticoid production by blocking 11β-hydroxylase and, to a lesser degree, the desmolase system (Carballeira, Cheng, and Fishman, 1974; Dominguez and Samuels, 1963; Ontjes, 1980). Treatment with metyrapone causes an increase in the plasma levels of ACTH as the result of decreased glucocorticoid concentrations (Liddle, 1972; Matsukura et al., 1971; Matsuyama, Ruhmann-Wennhold, and Nelson, 1971). In the present study, doses as low as 1 mg/kg significantly decreased seminal vesicle weight and caused a 15% (nonsignificant) reduction of testis weight (Table 4). Ten mg of metyrapone/kg caused a significant decrease of relative testis weights. At higher doses, the absolute testis and seminal vesicle weights tended to increase. The increases can probably be explained by the heavier body weights in the animals receiving higher doses, since the relative organ weights were similar for all three doses. Alternatively, the 11β-hydroxylase block may be causing increased adrenal androgen production by increasing the androgen precursor pool (Milewich and Axelrod, 1972; Schaison, Durand,
and Mowszowicz, 1978)—resulting in a stimulation of testis and seminal vesicle weights. Pasley (1974) and Pasley, McKinney, and Blue (1975) found that metyrapone caused lower seminal vesicle weights in voles and mice and concluded that ACTH may have been responsible for the observed effects. Metyrapone has also been shown to cause a degeneration of seminiferous tubules (Dixit and Lohiya, 1974) and to inhibit androgen secretion in hCG-stimulated testes (Yamashita et al., 1975). Schaison, Durand, and Mowszowicz (1978), in contrast, found no suppression of plasma testosterone levels in men treated with metyrapone. Any studies using metyrapone to study the effects of ACTH on reproduction must be viewed with caution. Carballeira, Fishman, and Durnhofer (1974) demonstrated that metyrapone directly inhibits the testosterone secretion of in vitro testis preparations. Whether or not this observation is applicable to in vivo studies at the doses employed in this experiment is not known.

This experiment demonstrated that ACTH administration causes an inhibition of reproductive function in male deermice. The observed effects of dexamethasone and metyrapone treatment suggest that both ACTH and the glucocorticoids may contribute to the reproductive dysfunction seen after ACTH treatment. The mechanisms involved in this dysfunction are not completely understood at the present time.

As discussed above, ACTH treatment causes a suppression
of reproductive processes in males. There have been conflicting reports on the ability of ACTH to act directly to inhibit reproduction. Results from some studies using metyrapone suggest that ACTH may inhibit reproductive function without glucocorticoid mediation, but a similar study found no changes in plasma testosterone after metyrapone administration (see above). Pasley and Christian (1972) reported that ACTH caused lower testis and seminal vesicle weights in adrenalectomized Peromyscus leucopus. However, Saez et al., (1977), observed no depression of plasma testosterone in similarly-treated rats. It has been suggested that ACTH may affect reproductive function centrally, by interacting with opiate receptors in the central nervous system (Yasukawa et al., 1978), or peripherally, by interfering with gonadotropin receptors in the testis (Beitins et al., 1973).

Several investigators have demonstrated that glucocorticoids may alter reproductive function (see above). It appears that different mechanisms may be involved. Smith, Johnson, and Weick (1971) found that cortisol acetate implanted in the medial basal hypothalamus caused impaired testis, seminal vesicle, and prostate development. Depressed levels of plasma LH are sometimes associated with high levels of circulating glucocorticoids (Liptrap and Raeside, 1968; Luton et al., 1977; Welsh, McCraw, and Johnson, 1979). These lower LH levels may be due to a decreased pituitary responsiveness to LH-RH (Boccuzzi et al.,
1975; Luton et al., 1977; Sakakura, Takebe, and Nakagawa, 1975). However, several investigators have reported suppressed plasma testosterone in hyperglucocorticoidism without lower plasma LH—indicating that the glucocorticoid effects need not be mediated by the pituitary (Beitins et al., 1973; Bradley and Terman, 1980b; Irvine et al., 1974; McKenna et al., 1979; Schaison et al., 1978; Smals, Kloppenborg, and Benraad, 1977).

Ballard et al., (1974) and Evain, Morera, and Saez (1976a) have demonstrated the presence of glucocorticoid receptors in the testis. Dexamethasone inhibits hCG-stimulated testicular testosterone synthesis in vivo and in vitro (Evain, Morera, and Saez, 1976b; Saez et al., 1977; Schaison, Durand, and Mowszowicz, 1978). Glucocorticoids interfere with cholesterol sidechain cleavage (Saez et al., 1977), testicular glucose utilization (Desjardins and Ewing, 1971), and testicular DNA and protein synthesis (Evain, Morera, and Saez, 1976b; Saez et al., 1977). Saez et al., also found fewer hCG binding sites in the testes of dexamethasone-treated rats.

Glucocorticoids may act peripherally to interfere with testosterone stimulation of target tissues. Tveeter and Aakvaag (1969) observed that corticosterone administration blocks $^3$H-testosterone uptake by the prostate and seminal vesicles. Cortisone has a suppressive effect on the testosterone-induced stimulation of the accessory sex organs (Tisell, 1972).
It should be noted that adrenal products other than the glucocorticoids may be involved in stress-induced suppression of reproduction. It has been suggested that adrenal androgens, estrogens, and progestins are elevated in stress and that they may act to inhibit gonadotrophin secretion via negative feedback on the pituitary (Andrews, 1977; Christian, Lloyd, and Davis, 1965; Duckett, Varon, and Christian, 1963; Varon and Christian, 1963).

ADRENAL ULTRASTRUCTURE

The ultrastructure of the adrenal cortex has been shown to vary from species to species and even differences between laboratories studying the same species have been reported (Idelman, 1970; Nussdorfer, Mazzocchi, and Meneghelli, 1978). To our knowledge, the adrenal cortex of Peromyscus has not been previously examined with the electron microscope. Studies on other species of mice have shown spherical mitochondria with tubular cristae in the zona fasciculata (Nickerson, 1975; Shelton and Jones, 1971; Zelander, 1957)—similar, but not identical, to those observed in this experiment. The mitochondria of the rat zona fasciculata usually have vesicular or vesiculotubular cristae (Idelman, 1970; Nishikawa, Murone, and Sato, 1963; Rhodin, 1971).

The volume of the mitochondrial compartment is generally positively correlated with the level of adrenocortical activity. This would be expected since several stages of
steroid biosynthesis take place in the mitochondria (Fawcett, Long, and Jones, 1969; Idelman, 1970; Nussdorfer, Mazzocchi, and Meneghelli, 1978). Raising plasma ACTH levels may cause an increase in the volume of the mitochondrial compartment by increasing the number and/or the volume of mitochondria (Buuck, Tharp, and Brumbaugh, 1976; Nickerson, 1975; Nishikawa, Murone, and Sato, 1963; Nussdorfer, Mazzocchi, and Rebonato, 1971; Nussdorfer et al., 1974; Rhodin, 1971). The volume of individual mitochondria may increase or decrease. The mitochondria remain mostly spherical or ovoid after ACTH stimulation (Nussdorfer, Mazzocchi, and Rebonato, 1971).

Lipid droplets are thought to contain cholesterol and other compounds which are involved in steroid synthesis (Idelman, 1970; Nussdorfer, Mazzocchi, and Meneghelli, 1978). There are conflicting reports on the fate of lipid droplets after stress or ACTH administration. The volume of the lipid compartment has been found to increase (Buuck, Tharp, and Brumbaugh, 1976; Molne, 1969; Nickerson, 1975; Nussdorfer, Mazzocchi, and Rebonato, 1971; Nussdorfer et al., 1974) or decrease (Buuck, Tharp, and Brumbaugh, 1976; Molne, 1969; Nishikawa, Murone, and Sato, 1963; Rhodin, 1971) when the adrenal cortex is stimulated. Buuck, Tharp, and Brumbaugh (1976) found more lipid with acute stress and less lipid after chronic stress, but Molne (1969) found the opposite—the lipid volume was decreased in acute stress, increased in chronic stress.
The smooth endoplasmic reticulum (SER) is involved in steroid synthesis (Fawcett, Long, and Jones, 1969; Idelman, 1970; Nussdorfer, Mazzocchi, and Meneghelli, 1978). It becomes more developed with stress or ACTH treatment (Buuck, Tharp, and Brumbaugh, 1976; Nickerson, 1975; Nishikawa, Murone, and Sato, 1963; Nussdorfer, Mazzocchi, and Rebonato, 1971; Nussdorfer et al., 1974) and less developed with hypophysectomy (Fujiata, 1972; Volk and Scarpelli, 1966) or glucocorticoid administration (Nussdorfer, 1970; Nussdorfer and Mazzocchi, 1970; Nussdorfer et al., 1975). According to Nussdorfer and Mazzocchi (1970), the development of the SER is the single most important criterion for determining the functional state of the adrenal.

It should be emphasized that the present study was primarily qualitative—the sample size was too small to perform a proper morphometric analysis. Estimates of the mitochondrial and lipid droplet densities (Table 5) revealed that the relative proportions of these structures are similar in control and population animals. ACTH-treated mice had a higher lipid fraction than controls or population mice. Surprisingly, the mice treated with ACTH had lower densities of mitochondria. This is probably due to the large increase in the number of lipid droplets. Development of the SER was variable within each of the treatment groups and no patterns between groups could be detected. The most striking difference between the adrenals of control and reproductively inhibited population mice was the irregular morphology of the mitochon-
It is interesting to note that the adrenal morphology of a relatively mature population mouse (Figure 5) was similar to that of control animals, suggesting a possible correlation between sexual development and the ultrastructure of the zona fasciculata.

Irregular mitochondria have been reported for ACTH-free adrenal cultures (Kahri, 1966; Nussdorfer et al., 1977) and hypofunctional adrenals in vivo (Fujiata, 1972; Nussdorfer et al., 1975; Sabatini, DeRobertis, and Bleichmar, 1962; Sharawy and Penney, 1973). However, the general cytological patterns were not identical to those seen in the inhibited population mice of this study. It is highly unlikely that the adrenals of population mice are hypofunctional, because of the extremely high levels of serum corticosterone present in these mice (Bradley and Terman, 1980a).

The only conclusions that can be made from the observations noted in this experiment are that the adrenal cortices of reproductively inhibited population mice are unlike those of their sexually mature controls and that the observed changes are unlike those seen after ACTH stimulation in this study or in the literature. It is possible, however, that ACTH is acting in conjunction with other substances to change the adrenal ultrastructure. The altered adrenal morphology seen in inhibited mice presumably represents a difference in the functional state of the adrenal cortex. Indeed, quantitative and qualitative changes occur in the adrenal cortex before and during puberty (Cutler et al., 1978; Ducharme
et al., 1976; Herrera-Justiniano et al., 1979). Castration causes an increase in the production of adrenal androgens (Bardin and Peterson, 1967; Kniewald, Zanisi, and Martini, 1971). Since reproductively inhibited male mice from our populations have decreased levels of serum testosterone (Bradley and Terman, 1980c), the changes in their adrenal ultrastructure may reflect increased adrenal androgen production. Hypogonadism effects other aspects of adrenal function, which could conceivably result in an altered adrenal morphology (see below). Mausle and Sherrer (1974) observed changes in the adrenal ultrastructure of rats after castration, but the morphology was unlike that seen in the reproductively inhibited mice examined in this study. In vitro incubation studies currently underway in our laboratory should provide valuable information concerning changes in adrenal secretion which might explain the morphological changes seen in the zona fasciculata.

PLASMA ACTH

Several observations support the contention that the ACTH radioimmunossay employed in this study was valid. Dilutions of standard ACTH and ether-stressed Peromyscus plasma gave appropriate dose response curves (Figure 6). Measurement of plasma ACTH after etherization and laparotomy (Table 6) gave values comparable to those reported in the literature for similarly stressed rats (Cook et al., 1973; Rees et al., 1971). Mice exposed to ether for 4-5 minutes
had ACTH levels more than two times higher than those sampled within 2 minutes, which would be expected since plasma ACTH levels increase for several minutes after the onset of ether stress (Cook et al., 1973). The logit transformation of serially diluted ether-stressed *Peromyscus* plasma was not parallel to the transformed standard curve. However, the slopes were similar (-2.38 for the standard curve versus -2.48 for the serial plasma dilution). The statistical difference between the two slopes is probably due to the different variances of the curves (see appendix). If the two curves are in fact not parallel, it is possible that substances in the *Peromyscus* plasma are interfering with the assay. The difference in slopes could also be due to the differences between the ACTH used as a standard and *Peromyscus* ACTH. The standard was a synthetic peptide containing the first 24 N-terminal amino acids of natural ACTH. *Peromyscus* ACTH is probably a 39 amino acid peptide similar to other mammalian species studied (Ramachandran, 1973). If the anti-human ACTH antibody used in the assay was not exclusively specific for the N-terminal portion of the ACTH molecule, the binding kinetics of synthetic 1-24 ACTH and *Peromyscus* 1-39 ACTH could be different. If the slopes of the two curves are different, absolute values for plasma ACTH in the unknown samples cannot be determined. However, the data are useful for making relative comparisons.

Values obtained for plasma ACTH in unstressed deer mice sampled by decapitation are suspiciously high (Table 6).
Basal ACTH levels of approximately 20-110 pg/ml plasma have been reported for rats (Cook et al., 1973; Marton et al., 1978; Matsuyama, Ruhmann-Wennhold, and Nelson, 1971; Rees et al., 1971), in contrast to a mean of 1351 pg/ml in this study. Decapitated controls should have lower plasma ACTH concentrations than etherized-laparotomized mice, but this was not the case (Table 6). If the ACTH assay was indeed valid, then the high ACTH levels observed in decapitated mice must be due to some aspect of the sampling technique. The mice were stunned with a sharp blow to the head before decapitation. The trauma may have caused a massive release of ACTH from the pituitary. Alternatively, substances entering the plasma sample from the stomach or the respiratory tract may interfere with the assay causing false values to be obtained.

Since the ACTH values obtained from decapitated mice appear to be unreasonable, the higher levels in population mice must be noted with caution. However, it is probably justifiable to compare the ACTH concentrations of controls to those of population mice if samples obtained from etherized-laparotomized mice are used. With this sampling technique, there was no significant difference between the plasma ACTH levels of mature control animals and reproductively inhibited population animals (Table 6). It must be emphasized that these values do not represent basal levels of ACTH, because plasma ACTH rises rapidly after etherization (Cook et al., 1973; Rees et al., 1971).
The animals used in this study were sampled one hour before the onset of the dark period. At this time of day, most of the mice were inactive. Several authors have postulated that social interaction may cause increased pituitary secretion of ACTH in subordinate individuals (Brain, 1971; Bronson, 1979; Christian, 1971; Davis and Christian, 1957; Louch and Higginbotham, 1967). It is possible that plasma ACTH levels are elevated in subordinate mice during the periods of the day when the mice are interacting with one another. It would be useful for any subsequent studies to examine plasma ACTH at different times of the photoperiod.

It is interesting to note that there was a negative correlation between plasma ACTH and reproductive function in controls, but not in population animals (Table 7). This observation suggests that ACTH is capable of inhibiting reproduction in male deermice, but that it is not involved in the inhibition of reproduction seen in population mice.

The observed relationships between adrenal and testis and seminal vesicle weights (Table 8) are consistent with Christian's studies on Peromyscus showing that testosterone causes adrenal hypertrophy (1964) and that there is a positive correlation between adrenal and testis weight (1975).

Sung, Bradley, and Terman (1977) and Bradley and Terman (1980a) have shown that high levels of serum corticosterone are present in reproductively inhibited prairie deermice from laboratory populations. The experimental conditions were similar to those of the present study, so it is
surprising that ACTH levels are not concomitantly elevated in population mice. However, the results observed for plasma ACTH are consistent with the observations in our laboratory showing that male deermice from populations do not have heavier adrenals than controls. If ACTH is not elevated, then other factors must be responsible for the higher levels of corticosterone. One possibility is an extra-adrenal source of corticosterone, such as the testis (Subhas and Michaels, 1979). Alternatively, substances other than ACTH may be influencing adrenal corticosterone production, or there may be alterations in glucocorticoid metabolism, excretion, or binding to plasma proteins.

Prolactin (Colby, 1979; Lis, Gilardeau, and Chretien, 1973) and growth hormone (Colby, Caffrey, and Kitay, 1973; Kramer, Greiner, and Colby, 1977) have been shown to augment the ACTH-induced stimulation of adrenal corticosterone. Both of these hormones may act by decreasing the activity of adrenal 5α-reductase (Colby, Caffrey, and Kitay, 1973; Colby, 1979; Witorsch and Kitay, 1972). Growth hormone may also enhance cholesterol sidechain cleavage (Kramer, Greiner, and Colby, 1977).

The pineal gland has been shown to influence the hypothalamo-pituitary-adrenal axis (Cardinali, 1974; Collu and Fraschini, 1972). Pinealectomy causes increased secretion of corticosterone (Kinson, Wahid, and Singer, 1967; Nir et al., 1971). This observation is probably at least in part due to increased levels of pituitary ACTH (Kitay, 1963a).
Preliminary studies in our laboratory indicate that reproductively inhibited population deermice may possibly be hypothyroid (David Peebles and Eric Bradley, personal communication). Hypothyroidism may contribute to the elevation of plasma glucocorticoids by decreasing the catabolism and excretion of these steroids (Brown, Englert, and Wallach, 1958; Miller et al., 1970; Peterson, 1958). It should be noted, however, that hypothyroidism may be caused by elevated plasma glucocorticoids (Otsuki, Dakoda, and Baba, 1973; Pamenter and Hedge, 1980; Wilber and Utiger, 1969).

Gonadal steroids affect many extra-pituitary factors involved in the control of circulating glucocorticoid levels. This is of particular relevance to the present study because Bradley and Terman (1980c) found significantly lower levels of serum testosterone in inhibited male mice from populations. The influence of sex steroids on adrenal function and glucocorticoid catabolism appears to vary from species to species. Since little work has been done with Peromyscus, it is only possible to speculate about some of the possible effects of testosterone in deermice.

Castrated male rats exhibit adrenal hypertrophy, but corticosterone secretion is decreased due to increased adrenal 5α-reductase activity. These effects are reversed by testosterone (Colby and Kitay, 1972; Kitay, Coyne, and Swygert, 1970; Malendowicz, 1976). Hamsters, in contrast, show a decrease in adrenal weight and lower plasma glucocorticoids after castration (Gaskin and Kitay, 1970, 1971).
Christian (1964) demonstrated that testosterone stimulates adrenal growth in *Peromyscus*.

Testosterone inhibits and castration stimulates the glucocorticoid catabolism of male rats (Colby, Gaskin, and Kitay, 1973; Kitay, 1963b; Troop, 1959; Yates *et al.*, 1961), but the opposite is true in hamsters (Colby, Gaskin, and Kitay, 1973; Gaskin and Kitay, 1970, 1971). The production of corticosteroid binding globulin (CBG) is inhibited by testosterone (Kley *et al.*, 1973) and stimulated after castration (Gala and Westphal, 1975). Since corticosterone bound to CBG is protected from liver catabolism (Sandberg and Slaunwhite, 1963), elevation of CBG levels in hypogonadal animals could contribute to higher concentrations of glucocorticoids in the circulation.

**CONCLUSION**

The literature and the data obtained in the present study indicate that exogeneous ACTH causes an inhibition of reproduction in males. Both ACTH and adrenocortical products may contribute to this inhibition. However, evidence from all three of the experiments performed in this study suggest that plasma ACTH levels are not elevated in reproductively inhibited male deermice from our laboratory populations: (1) exogenous ACTH caused adrenal hypertrophy, but the adrenal weights of control and population mice were comparable; (2) the ultrastructure of the zona fasciculata of inhibited population mice was different from that of con-
trols, but it did not resemble the characteristic morphology seen with ACTH treatment; (3) plasma ACTH levels measured by radioimmunossay were not significantly elevated in population mice. If plasma ACTH levels are indeed not elevated, then the inhibition of reproduction observed in male population deermice from our laboratory populations is not mediated by ACTH and other factors must be involved in maintaining the high serum corticosterone levels reported by Sung, Bradley, and Terman (1977) and Bradley and Terman (1980a). It is likely that corticosterone is causing, or at least contributing to, the impairment of reproductive function in these mice.
APPENDIX

STATISTICAL INFORMATION FROM THE RIANAL PROGRAM
FOR THE ACTH STANDARD CURVE

(NB: The following values for ACTH are expressed as pg/tube.
To convert to pg/ml, multiply x 20)

TOTAL COUNTS PER MINUTE  233 CPM  %SD  2.58
   RAW CPM  227  233  239

AVERAGE BACKGROUND  35 CPM  %TOTAL  15.0
   COR CPM  35  35  35

BUFFER CONTROL  62 CPM  %TOTAL  26.5 %SD 3.65
   COR CPM  59  62  64  V. RATIO  0.1668
   COR CPM  59  62  64  V. RATIO  0.1667

VARIANCE RATIOS FOR ASSAY REPLICATES
-0.0000  0.0001  0.0165  0.0196  0.0257  0.0274  0.0568  0.0577
  0.1224  0.1667  0.1668  0.2092  0.2176  0.2353  0.4285

MEDIAN VARIANCE RATIO  0.0568
VARIANCE RATIO OF B.C.  0.1668

STANDARD DOSE-RESPONSE CURVE

<table>
<thead>
<tr>
<th>DOSE</th>
<th>CURVE1</th>
<th>CURVE2</th>
<th>RANGE</th>
<th>MEAN</th>
<th>LOGIT</th>
<th>DIFF</th>
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<tr>
<td>256.0000 PG</td>
<td>29.2%</td>
<td>24.4%</td>
<td>4.8</td>
<td>26.8%</td>
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<td>128.0000 PG</td>
<td>43.8%</td>
<td>42.2%</td>
<td>1.5</td>
<td>43.0%</td>
<td>42.4%</td>
<td>-0.6</td>
</tr>
<tr>
<td>63.9999 PG</td>
<td>56.7%</td>
<td>58.4%</td>
<td>1.7</td>
<td>57.6%</td>
<td>60.1%</td>
<td>2.5</td>
</tr>
<tr>
<td>32.0000 PG</td>
<td>76.2%</td>
<td>77.9%</td>
<td>1.7</td>
<td>77.0%</td>
<td>75.5%</td>
<td>-1.5</td>
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<tr>
<td>16.0000 PG</td>
<td>89.1%</td>
<td>82.8%</td>
<td>6.4</td>
<td>86.0%</td>
<td>86.3%</td>
<td>0.4</td>
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<tr>
<td>8.0000 PG</td>
<td>92.4%</td>
<td>94.1%</td>
<td>1.8</td>
<td>93.3%</td>
<td>92.8%</td>
<td>-0.4</td>
</tr>
<tr>
<td>4.0000 PG</td>
<td>97.4%</td>
<td>100.6%</td>
<td>3.4</td>
<td>98.9%</td>
<td>96.4%</td>
<td>-2.6</td>
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<tr>
<td>2.0000 PG</td>
<td>98.9%</td>
<td>102.2%</td>
<td>3.4</td>
<td>100.6%</td>
<td>98.2%</td>
<td>-2.4</td>
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</table>

EST SLOPE  S.E. SLOPE
1  -2.368  0.000
10 -2.380  0.086
11 -2.380  0.086

51.
SLOPE OF STANDARD CURVE: -2.38
INTERCEPT OF STANDARD CURVE: -2.43
RESIDUAL VARIANCE: 0.0314
LAMBDA: -0.0132
OBSERVED F OF LINEARITY: 0.7288
SIGNIFICANCE LEVEL: 0.6402
STANDARD CURVE IS LINEAR

DOSES ON THEORETICAL CURVE

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<th>Dose</th>
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<tr>
<td>95.0%</td>
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<td>90.0%</td>
<td>11.3604 PG/TUBE</td>
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<td>85.0%</td>
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<td>80.0%</td>
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<td>70.0%</td>
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<td>50.0%</td>
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<td>30.0%</td>
<td>216.2041 PG/TUBE</td>
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<td>20.0%</td>
<td>363.2322 PG/TUBE</td>
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<tr>
<td>10.0%</td>
<td>798.3159 PG/TUBE</td>
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</table>

100% - 1 S.E. OF B.C. (LIMIT OF DETECTION) 1.6514 PG (98.5%)

100% - 1 S.D. OF ASSAY (ASSAY SENSITIVITY DERIVED FROM MED. VAR. RATIO) 5.5964 PG (94.9%)

100% - 2 S.D. OF B.C. 8.1514 PG (92.7%)

COEFFICIENT OF VARIATION - 80% 12.8960%
COEFFICIENT OF VARIATION - 20% 6.4612%

SERIAL DILUTION OF STANDARD POOL OF PEROMYSCUS PLASMA

<table>
<thead>
<tr>
<th>Dilution</th>
<th>CPM</th>
<th>%</th>
<th>ng</th>
<th>ng/ ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>50.0 UL/T</td>
<td>35 CPM</td>
<td>57%</td>
<td>0.073</td>
<td>1.47</td>
</tr>
<tr>
<td>50.0 UL/T</td>
<td>32 CPM</td>
<td>52%</td>
<td>0.089</td>
<td>1.77</td>
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<tr>
<td>25.0 UL/T</td>
<td>49 CPM</td>
<td>79%</td>
<td>0.026</td>
<td>1.03</td>
</tr>
<tr>
<td>25.0 UL/T</td>
<td>49 CPM</td>
<td>79%</td>
<td>0.026</td>
<td>1.03</td>
</tr>
<tr>
<td>12.5 UL/T</td>
<td>54 CPM</td>
<td>94%</td>
<td>0.007</td>
<td>530.22</td>
</tr>
<tr>
<td>12.5 UL/T</td>
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<td>94%</td>
<td>0.000</td>
<td>100.19</td>
</tr>
<tr>
<td>6.3 UL/T</td>
<td>58 CPM</td>
<td>97%</td>
<td>0.003</td>
<td>482.21</td>
</tr>
<tr>
<td>6.3 UL/T</td>
<td>64 CPM</td>
<td>104%</td>
<td>0.000</td>
<td>19.07</td>
</tr>
</tbody>
</table>

VARIANCE: 0.0008
SLOPE: -2.477
LAMBDA: -0.011
OBS F: 1.704
LIN SIG: 0.170
LINEAR?: YES
CRIT F: 4.35
OBS F: 24.45
PARALLEL?: NO


Bradley, E.L. and C.R. Terman. 1980c. Serum testosterone concentrations in male prairie deermice (Peromyscus maniculatus bairdii) from laboratory populations. Submitted for publication.


Dixit, V.P. and N.K. Lohiya. 1974. Some observations on the effect of metopirone (SU-4885 Ciba) on the testicular epithelium of the bat (Rhinopoma kinneari), gerbil (Meriones hurrianae), hedgehog (Hemiechinus auritus collaris) and mouse. Acta Anat. 89:240-250.


VITA

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