1990

Adrenal Histology and Reproductive Function in Peromyscus maniculatus bairdii and Peromyscus leucopus noveborocensis

Peggy Alicia Rouleau
College of William & Mary - Arts & Sciences

ADRENAL HISTOLOGY AND REPRODUCTIVE FUNCTION IN *PEROMYSCUS MANICULATUS BAIRDII* AND *PEROMYSCUS LEUCOPUS NOVEBORACENSIS*

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

Peggy Alicia Rouleau

1990

APPROVAL SHEET

This thesis is submitted in partial fulfillment of
the requirements for the degree of
Master of Arts

Author

Approved, July 1990

Eric L. Bradley, Ph. D.

Robert E. L. Black, Ph. D.

Joseph L. Scott, Ph. D.

C. Richard Terman, Ph. D.

DEDICATION

For Alice Selma Ellingsen

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

Many thanks are extended to my thesis advisor, Dr. Eric Bradley, whose guidance and assistance have been instrumental in the completion of this study. I also thank Dr. Joseph Scott for his considerable assistance with histology, Dr. C. Richard Terman for his advice and support at the Laboratory of Endocrinology and Population Ecology, and Dr. Robert Black for reviewing the manuscript. Thanks are also offered to Dr. Lawrence L. Wiseman for his general support and guidance and to Jewell Thomas, for her hours of patience and technical assistance. Finally, I would like to thank Jeffrey, for years of support and friendship.

LIST OF TABLES

LIST OF TABLES (continued)

LIST OF FIGURES

ABSTRACT

Reproductively inhibited P. maniculatus and P. leucopus have been shown to have elevated levels of the adrenal steroid, corticosterone, but these same animals exhibit no gravimetric evidence of adrenal hyperfunction. This study examined the adrenal histology of reproductively proven and inhibited mice of both species. Measurements were taken or calculated of the absolute and relative zonal areas of the adrenal, as well as measurements of the body weights, absolute and relative reproductive organ, and adrenal weights of proven and inhibited animals.

Body weights, absolute and relative reproductive organ weights, and absolute adrenal weights were significantly larger in proven animals than in reproductively inhibited animals the same age. There was no difference in relative adrenal weight between proven and inhibited animals. The histology revealed a larger zona fasciculata and zona reticularis in reproductively proven animals, providing no evidence for adrenal hyperfunction in reproductively inhibited animals.

ADRENAL HISTOLOGY AND REPRODUCTIVE FUNCTION IN *PEROMYSCUS MANICULATUS BAIRDII* AND *PEROMYSCUS LEUCOPUS NOVEBORACENSIS*

INTRODUCTION

The adrenal gland can be divided into four areas, each responsible for the secretion of a different hormone. The outermost zona glomerulosa, secretes aldosterone, regulating sodium and potassium excretion. The innermost medulla releases the neurotransmitters epinephrine and norepinephrine, which stimulate the sympathetic nervous system. The middle zones, zona fasciculata and zona reticularis, along with the zona glomerulosa, make up the adrenal cortex. Under the influence of the pituitary, the fasciculata and reticularis secrete glucocorticoids.

The glucocorticoids, such as cortisol and/or corticosterone, are involved in the regulation of food metabolism, inflammation and stress. They are responsible for gluconeogenesis, the conversion of amino acids and fats into carbohydrate. This is important for converting the body's stored reserves into energy. Glucocorticoids are well known for their anti-inflammatory effects and suppression of the immune response. They also mediate the responses characteristic of adaptations to chronic stress.

Selye (1946), developed a theory of adaptation to chronic stress called the General Adaptation Syndrome which

maps the reactions to stressful stimuli over time. The first stage, or Alarm Reaction, involves increased secretion of glucocorticoids, and correspondingly, an enlargement of the adrenal gland with a hypertrophy of that part of the adrenal gland responsible for the production of glucocorticoids. In the Resistance Stage, the prolonged increased secretion of glucocorticoids allows the animal to 'adapt' to the stressful stimuli. Finally, in the Exhaustion Stage, the animal cannot function normally in the continued presence of the stressful stimuli and eventually dies.

The effects of glucocorticoids combine to generally cope with a stressful situation of short duration. Their effect on metabolism may enable an animal faced with a stressful situation to reduce food intake. Amino acids, liberated by the catabolism of body stores, not only provide energy, but also constitute a source of amino acids for tissue repair, if an injury should occur.

Stress has been suggested as a density dependent force in wild populations of animals. There is ample evidence that, when certain natural populations of animals such as lemmings (Chitty, 1961), voles (Andrews and Belknap, 1979), and mice (Andrews, 1970; Christian, 1971) become crowded, reproduction is suppressed. One hypothesis concerning a mechanism regulating population size is that increased crowding and social contact result in stress (Christian,

1963; Christian and Davis, 1964) and that it is this stress that triggers hyperactivity of the hypothalamic-pituitary-adrenocortical system, which, in turn, alters gonadotrophic secretions. It is this cascade of hormonal changes that would result in a suppression of growth and inhibition of reproduction.

The role of the adrenal cortex and corticosterone has been studied in relation to population regulation. Increased adrenocortical function (measured by increased adrenal weights) with increased population size has been observed by numerous investigators (Christian, 1956; Varon and Christian, 1963; Hull et al., 1974; Purushotham, et al., 1978). Increased adrenocortical function, as measured by an increase in the plasma corticosteroid concentrations has also been observed in response to crowding in mice (Varon and Christian, 1963; Sung et al., 1977; Bradley and Terman, 1981a). Adrenocorticotrophic hormone (ACTH), the pituitary regulator of adrenal glucocorticoid function, has also been shown to be elevated in stressed animals (Cook et al., 1973) and to cause increases in plasma corticosterone and adrenal weight in the guinea pig (Fajer, 1963). Several researchers have also demonstrated that exogenous administration of ACTH may serve to reproductively inhibit males from several species of rodents (Pasley and Christian, 1971; Collu et al., 1979).

Laboratory populations of prairie deermice (Peromyscus

maniculatus bairdii) will regulate their numbers even when surplus food and water are provided (Terman, 1965, 1969). This control is achieved by cessation of reproduction or failure of young to survive, or both. Light microscopy studies from reproductively inhibited P. maniculatus have revealed no gravimetric or histological evidence of adrenal hyperfunction (Bradley and Terman, 1981a). Similar, animals however, have significantly elevated levels of the adrenocortical hormone, corticosterone (Sung et al., 1977). White-footed mice (Peromyscus leucopus noveboracensis) also show an ability to regulate their numbers in laboratory populations through similar mechanisms as deermice (Wolfe, 1981). Recent studies have indicated that there is an elevated serum corticosterone concentration in reproductively inhibited males but not females (Ransone, 1988).

A preliminary survey of selected adrenals of P. maniculatus has suggested that there may be some differences in the histology of the reproductively inhibited animals (Bradley and Terman, 1981a). The present study examined the differences in adrenal histology between reproductively proven and reproductively inhibited assembled population animals of both P. leucopus and P. maniculatus. Because hypertrophy of the adrenal gland may not be readily apparent by the weight of the gland, the area of the zones of the adrenal gland was measured to determine if adrenal hypertrophy might be explained by a relative hypertrophy of

the cortical zones responsible for secreting corticosterone (zona fasciculata and zona reticularis).

MATERIALS AND METHODS

ANIMAL MAINTENANCE

The animals used in this study were prairie deermice (*Peromyscus maniculatus bairdii*) and white-footed mice (*Peromyscus leucopus noveboracensis*) obtained from separate outbred laboratory colonies. The two species were kept entirely separate from each other in two different rooms.

CONTROL ANIMALS

P. maniculatus

Male deermice (age 59-66 days) were paired with nonsibling females (age 59-67 days) and kept on one side of a two chambered, wire topped, opaque plastic cage (12 cm X 26 cm X 14 cm). Food and water were continuously available pine shavings, approximately 3 cm deep, were used as bedding. After two weeks, animals were examined for signs of reproduction. Animals were checked daily for pregnancy at 21 days after pairing, the natural gestation period. If a pregnancy was noted, the pair was separated with one on each side of the two-chambered cage. The female was checked every day after that until a litter was born, at which time,

the litter was removed. If a pregnancy was missed, and young were born before the pair could be separated, the pair was separated within 24 hours of the birth of the litter and the litter was removed.

Twelve pairs were sacrificed at 121-131 days of age, an average of 25 days since the birth of the litter (14-39 days). No pregnant females were used for the study. These proven animals were anesthetized with diethyl ether at approximately one hour before the onset of the dark period in order to collect the blood at a time when ACTH would be at its peak (Retiene et al., 1968; Matsuyama et al., 1971). Only one animal of each pair was sacrificed daily to avoid any induced stress reaction in the other.

P. leucopus

Control white-footed mice were established and sacrificed in the same way as the deermice. The animals were 29-53 days old at pairing. Sixteen pairs were sacrificed at 121-130 days of age, an average of 37 days (24-47 days) after the birth of the litter. As with P. maniculatus, no pregnant females were used.

REPRODUCTIVELY INHIBITED ANIMALS FROM ASSEMBLED POPULATIONS

P. maniculatus

Two populations of 30 animals were assembled with 15 males and 15 females (ages 22-33 days) in each population.

No same sex siblings and no more than one opposite sex sibling for each animal were used in any given population. Animals were toe-clipped for identification.

Populations were maintained in circular, aluminum enclosures, 150 cm in diameter and approximately 70 cm in height. Each population was provided with 5 one quart plastic containers for nest boxes and pine shavings for bedding. Food and water were continuously available. The populations were maintained in a room where the light cycle was 14 hours of bright fluorescent light and 10 hours of darkness. Animals were captured and examined every two weeks for signs of reproductive development (scrotal or non-scrotal testes and perforate or an imperforate vagina). No evidence of reproduction occurred in either of the populations.

Animals were rejected for inclusion in the study on the basis any one of the following criteria: 1. If the animal had escaped; 2. If the animal had been observed to be testis-scrotal or had a perforate vagina at any time; 3. If it was not within the age range of 120-136 days old during the time of sampling in that population; 4. If it had ever shown any sign of infection or injury; 5. If the sample size of at least 12 inhibited males and 12 inhibited females from both populations was completed before the animal was collected. Those animals to be sacrificed were tail-marked with a non-toxic, ultraviolet fluorescent dye (Blak-Ray Ink

A-946, Ultraviolet Products, Inc.) at least 24 hours in advance of when they were to be sampled. Approximately one hour before the onset of the dark period, the tail-marked animals were identified using a high intensity long wave ultraviolet light (Blak-Ray UVL-SC), quickly captured and anesthetized with diethyl ether. Care was taken to avoid disturbing the rest of the population and those tail-marked animals that were disturbed during the capture of another, were sacrificed on another day. Animals were approximately 120 days old at the time of sacrifice.

P. leucopus

Each of two populations of 30 animals of white-footed mice were assembled, maintained, and sacrificed in the same way as the populations of deermice. The only difference was that the animals were 29-58 days old at assembly.

TISSUE COLLECTION

The animal was anesthetized, the abdominal cavity was opened with a ventral abdominal incision, the left renal artery was cut and the blood was collected using a sterile 1 ml disposable syringe (without the needle). The blood was transferred to a 1.5 ml polypropylene microcentrifuge tube and allowed to clot for at least 5 minutes after which it

was centrifuged at 9000 x g to separate the cells from the serum. The serum was drawn off, frozen at -70° C, and stored for possible corticosterone assay.

After the blood was collected, the left and right adrenal glands were removed, the animal's body was weighed to the nearest 0.1 g, and was placed in a 10% buffered formaldehyde solution. Adrenal glands were dissected, placed in a 2% glutaraldehyde/1.5% paraformaldehyde solution and allowed to fix for 2-3 hours. The adrenals were then finely dissected, individually weighed to the nearest 0.1 mg, sliced in half along the minor axis and, prepared for light microscopy using a Historesin embedding kit for light microscopy (LKB-Bromma, Sweden) and an RMC 3189 Ultraprocessor. Only the left adrenal was prepared for light microscopy. The right adrenal was prepared for electron microscopy (which was not carried out as part of this experiment). Care was taken so that the cross sectional face of the adrenal half was oriented flush against the surface of the block that would be sectioned. Sectioning was done using a JB-4 Ultramicrotome and a hematoxylin and eosin stain was used to stain the sections. Right adrenals were quartered (P. maniculatus) or cut into eight sections (P. leucopus), postfixed in OsO_4, dehydrated in acetone, embedded in epoxy resin and held for a later study. Reproductive organs (testis, seminal vesicles, uterus and ovaries) were allowed to fix inside the body for

several weeks, after which time they were removed, cleaned of all fat, and weighed to the nearest 0.1 mg.

ADRENAL HISTOLOGY

Light Microscopy

Only the left adrenal was examined in this study. The area of adrenal zones were measured using the BioQuant System IV (R&M Biometrics, Inc., Nashville, Tn). From each animal, the largest adrenal cross-section was chosen based on a series of measurements of total area of several sections from each animal. Measurements taken or calculated were as follows: total adrenal area, zona glomerulosa area, adrenal medulla area, total cortex area and zona fasciculata plus zona reticularis area. A series of ten consecutive measurements was made for each cross section and the mean was calculated. All areas were measured in squared micrometer units which were later converted to mm^2 units and rounded off to the nearest 0.01 mm^2

STATISTICAL ANALYSIS

Data on any particular animal were used only if that animal had a value for every characteristic studied. For

example, if the adrenal glands of a particular animal were destroyed or lost before data could be read from them, the body weight and gonad weights were not included in this study. Results are given as the mean plus or minus the standard deviation. All comparisons between control and population animals were made with a two-way ANOVA and Tukey's test (SPSSX statistics program). Comparisons of selected parameters between populations were made with the Student's t-test. Differences were considered significant at $\underline{P} < 0.05$.

RESULTS

DIFFERENCES BETWEEN REPRODUCTIVELY INHIBITED ANIMALS FROM ASSEMBLED POPULATIONS

P. maniculatus

Some males selected from one of the assembled populations were more reproductively developed in terms of testis weight than males of the other population. Thus, there was a significant ($P < 0.01$) difference in the mean testis weight between the two groups. However, no other statistically significant differences were detected for any comparison in either sex. There was no reproduction or mortality in either population.

P. leucopus

There was no statistically significant difference between reproductively inhibited animals from the assembled populations of *P. leucopus* in any comparison for either sex. There was no reproduction or mortality in either population.

COMPARISONS BETWEEN PROVEN ANIMALS AND REPRODUCTIVELY INHIBITED ANIMALS OF THE SAME SEX AND SPECIES

BODY WEIGHT, ABSOLUTE AND RELATIVE GONAD AND ADRENAL WEIGHT

All animals were 118-136 days old at the time of death. Proven animals were significantly ($P < 0.05$) larger than reproductively inhibited animals with respect to body weight, absolute and relative reproductive organ weight, and absolute paired adrenal weight. It should be noted that some uterine scars were visible on uteri of proven animals. No significant differences were found between proven animals and reproductively inhibited animals in relative adrenal weight (cf. Tables 1, 2, 3, and 4).

ADRENAL HISTOLOGY

P. maniculatus proven males versus *P. maniculatus* inhibited males

Proven animals were significantly ($P < 0.05$) larger than reproductively inhibited animals with respect to total adrenal area, and absolute and relative zona fasciculata plus zona reticularis area. No significant differences were found in the absolute and relative areas of the total adrenal cortex, adrenal medulla, or zona glomerulosa (Table 5).

P. maniculatus proven females versus P. maniculatus inhibited females

No significant differences in adrenal histology were found between proven females and reproductively inhibited females (Table 6).

P. leucopus proven males versus P. leucopus inhibited males

The total adrenal area, absolute adrenal cortex area, absolute zona glomerulosa area, and absolute zona fasciculata plus zona reticularis area were found significantly ($P < 0.05$) larger in proven males than in reproductively inhibited males. No significant differences were found between proven and inhibited males with respect to the relative total adrenal cortex area, the absolute or relative adrenal medulla area, the relative zona glomerulosa area or, the relative zona fasciculata plus zona reticularis area (Table 7).

P. leucopus proven females versus P. leucopus inhibited females

The total adrenal area, absolute adrenal cortex area, relative zona glomerulosa area, and absolute zona fasciculata plus zona reticularis area were found significantly ($P < 0.05$) larger in proven females than in reproductively inhibited females. No significant

differences were found between proven and inhibited females with respect to the relative total adrenal cortex area, the absolute and relative adrenal medulla area, the absolute zona glomerulosa area or, the relative zona fasciculata plus zona reticularis area (Table 8).

COMPARISONS BETWEEN *P. maniculatus* AND *P. leucopus*

Proven males

P. leucopus proven males were significantly ($P < 0.05$) larger than *P. maniculatus* proven males with respect to body weight, absolute and relative adrenal weight, total adrenal area, absolute and relative cortex area, absolute and relative zona fasciculata plus reticularis area, and absolute zona glomerulosa area. *P. maniculatus* proven males were significantly ($P < 0.05$) larger than *P. leucopus* proven males with respect to relative testis weight, and relative medulla and zona glomerulosa area. No significant differences were found between the two species' proven males in the absolute and relative seminal vesicle weight, absolute testis weight, or the adrenal medulla area (cf. Tables 9 and 10.)

Reproductively Inhibited Males

P. leucopus inhibited males were significantly ($P < 0.05$) larger than *P. maniculatus* inhibited males with respect to body weight, absolute and relative adrenal weight, total adrenal area, absolute and relative cortex area, absolute and relative zona fasciculata plus reticularis area, and absolute zona glomerulosa area. *P. maniculatus* inhibited males were significantly ($P < 0.05$) larger than *P. leucopus* inhibited males with respect to relative medulla and zona glomerulosa area. No significant differences were found between the two species' population males in the absolute and relative seminal vesicle weight, absolute and relative testis weight, or in the absolute medulla area (cf. Tables 11 and 12).

Proven Females

P. leucopus proven females were significantly ($P < 0.05$) larger than *P. maniculatus* proven females with respect to absolute and relative uterus and adrenal weight, total adrenal area, absolute and relative cortex and zona fasciculata plus reticularis area, and absolute zona glomerulosa area. *P. maniculatus* proven females were significantly larger than *P. leucopus* proven females with respect to absolute and relative ovary weight, and relative medulla and zona glomerulosa area. No significant differences were found between the two species' proven

females in body weight or absolute medulla area (cf. Tables 13 and 14).

Reproductively Inhibited Females

P. leucopus inhibited females were significantly ($P < 0.05$) larger than P. maniculatus inhibited females with respect to body weight, absolute and relative adrenal weight, total adrenal area, absolute and relative cortex area, absolute and relative zona fasciculata plus reticularis area, and absolute zona glomerulosa area. P. maniculatus inhibited females were significantly ($P < 0.05$) larger than P. leucopus inhibited females with respect to relative medulla and zona glomerulosa area. No significant differences were found between the two species' inhibited females with respect to the absolute and relative ovary and uterus weight, or the absolute medulla area (cf. Tables 15 and 16).

LEFT AND PAIRED ADRENAL WEIGHT

In previous studies, the weight of the left and right adrenal has been considered as a paired unit and are reported as such in this study. However, the left and right adrenal were weighed separately, giving the opportunity to determine if the left and right adrenal glands are similar in weight. The mean weight of all the left adrenal glands

of P. maniculatus was 1.3 mg (± 0.30), the mean paired adrenal weight of all P. maniculatus was 2.4 mg (± 0.64). Mean left adrenal weight of P. leucopus was 4.8 mg (± 0.69). Mean paired adrenal weight of P. leucopus was 9.6 mg (± 1.56). A table of these results is included in Appendix I and Appendix J.

Table 1. Age, Body Weight, Absolute and Relative (to Body Weight) mean Seminal Vesicle, Testis, and Adrenal Weights in Proven and Inhibited *P. maniculatus* males. Values are mean ± standard deviation.

Treatments	Age (days)	Body Weight (g)	Paired Seminal Vesicle Weight		Paired Testis Weight		Paired Adrenal Weight	
			Absolute (mg)	Relative (mg/g)	Absolute (mg)	Relative (mg/g)	Absolute (mg)	Relative (mg/g)
Proven Males n=12	127 ± 2.2	18.8 ± 3.16	169.8 ± 51.00	9.19 ± 2.715	337.0 ± 58.00	18.1 ± 3.03	3.2 ± 0.77	0.17 ± 0.053
Inhibited Males n=13	122 ± 1.3	12.8 * ± 0.91	1.6 * ± 1.24	0.13 * ± 0.114	38.3 * ± 22.78	3.1 * ± 1.96	1.7 * ± 0.77	0.14 ± 0.060

* $P < 0.05$

Table 2. Age, Body Weight, Absolute and Relative (to Body Weight) mean Ovary, Uterus, and Adrenal Weights in Proven and Inhibited P. maniculatus females. Values are mean ± standard deviation.

Treatments	Age (days)	Body Weight (g)	Paired Ovary Weight Absolute (mg)	Paired Ovary Weight Relative (mg/g)	Uterus Weight[a] Absolute (mg)	Uterus Weight[a] Relative (mg/g)	Paired Adrenal Weight Absolute (mg)	Paired Adrenal Weight Relative (mg/g)
Proven Females n=12	126 ± 3.0	17.8 ± 3.17	18.9 ± 4.30	1.07 ± 0.209	64.2 ± 24.27	3.6 ± 1.30	2.9 ± 0.47	0.17 ± 0.044
Inhibited Females n=17	122 ± 2.2	12.4 * ± 1.16	2.8 * ± 1.03	0.23 * ± 0.085	5.4 * ± 1.62	0.43 * ± 0.115	1.9 * ± 0.67	0.15 ± 0.061

* $P < 0.05$

[a] includes uteri from proven females that contained uterine scars

Table 3. Age, Body Weight, Absolute and Relative (to Body Weight) mean Seminal Vesicle, Testis, and Adrenal Weights in Proven and Inhibited P. leucopus males. Values are mean ± standard deviation.

Treatments	Age (days)	Body Weight (g)	Paired Seminal Vesicle Weight Absolute (mg)	Paired Seminal Vesicle Weight Relative (mg/g)	Paired Testis Weight Absolute (mg)	Paired Testis Weight Relative (mg/g)	Paired Adrenal Weight Absolute (mg)	Paired Adrenal Weight Relative (mg/g)
Proven Males n=16	125 ± 2.9	21.9 ± 2.52	164.9 ± 53.87	7.54 ± 2.500	337.8 ± 74.00	15.5 ± 2.95	11.3 ± 4.01	0.51 ± 0.155
Inhibited Males n=14	126 ± 4.7	16.4 * ± 0.98	3.6 * 4.50	0.22 * ± 0.264	26.9 * ± 20.93	1.6 * ± 1.22	8.5 * ± 1.91	0.52 ± 0.126

* $P < 0.05$

Table 4. Age, Body Weight, Absolute and Relative (to Body Weight) mean Ovary, Uterus, and Adrenal Weights in Proven and Inhibited P. leucopus females. Values are mean ± standard deviation.

Treatments	Age (days)	Body Weight (g)	Paired Ovary Weight Absolute (mg)	Paired Ovary Weight Relative (mg/g)	Uterus Weight[a] Absolute (mg)	Uterus Weight[a] Relative (mg/g)	Paired Adrenal Weight Absolute (mg)	Paired Adrenal Weight Relative (mg/g)
Proven Females n=15	123 ± 1.8	19.9 ± 2.34	15.7 ± 3.25	0.79 ± 0.161	95.3 ± 35.06	4.80 ± 1.839	11.0 ± 3.08	0.56 ± 0.201
Inhibited Females n=14	123 ± 3.7	16.2 * ± 1.63	2.7 * ± 0.90	0.17 * ± 0.054	6.7 * ± 1.99	0.42 * ± 0.123	7.7 * ± 2.07	0.48 ± 0.115

* $P < 0.05$

[a] includes uteri from proven females that contained uterine scars

Table 5. Mean Adrenal Area, Mean Absolute and Relative (to total area) Cortex, Medulla, Zona Glomerulosa and, Zona Fasciculata plus Reticularis Area in Proven and Inhibited P. maniculatus males. Values are mean ± standard deviation.

Treatments	Total Area (mm^2)	Adrenal Cortex Absolute (mm^2)	Adrenal Cortex Relative	Adrenal Medulla Absolute (mm^2)	Adrenal Medulla Relative	Zona Glomerulosa Absolute (mm^2)	Zona Glomerulosa Relative	Zona Fasciculata + Reticularis Absolute (mm^2)	Zona Fasciculata + Reticularis Relative
Proven Males n=12	1.07 ± 0.290	0.82 ± 0.293	0.75 ± 0.054	0.23 ± 0.049	0.23 ± 0.064	0.13 ± 0.041	0.12 ± 0.032	0.72 ± 0.51*	0.66 ± 0.079
Inhibited Males n=13	0.85 * ± 0.156	0.63 ± 0.141	0.73 ± 0.058	0.23 ± 0.046	0.27 ± 0.058	0.12 ± 0.016	0.14 ± 0.019	0.51 * ± 0.128	0.59 * ± 0.059

* $P < 0.05$

Table 6. Mean Adrenal Area, Mean Absolute and Relative (to total area) Cortex, Medulla, Zona Glomerulosa and, Zona Fasciculata plus Reticularis Area in Proven and Inhibited P. maniculatus females. Values are mean ± standard deviation.

Treatments	Total Area (mm^2)	Adrenal Cortex Absolute (mm^2)	Adrenal Cortex Relative	Adrenal Medulla Absolute (mm^2)	Adrenal Medulla Relative	Zona Glomerulosa Absolute (mm^2)	Zona Glomerulosa Relative	Zona Fasciculata + Reticularis Absolute (mm^2)	Zona Fasciculata + Reticularis Relative
Proven Females n=12	0.88 ± 0.128	0.61 ± 0.126	0.69 ± 0.045	0.27 ± 0.033	0.31 ± 0.045	0.12 ± 0.018	0.14 ± 0.017	0.49 ± 0.113	0.55 ± 0.047
Inhibited Females n=17	0.87 ± 0.164	0.64 ± 0.159	0.72 ± 0.057	0.24 ± 0.036	0.28 ± 0.058	0.12 ± 0.023	0.14 ± 0.012	0.51 ± 0.140	0.58 ± 0.059

No significant differences were found.

Table 7. Mean Adrenal Area, Mean Absolute and Relative (to total area) Cortex, Medulla, Zona Glomerulosa and, Zona Fasciculata plus Reticularis Area in Proven and Inhibited *P. leucopus* males. Values are mean ± standard deviation.

Treatments	Total Area (mm^2)	Adrenal Cortex Absolute (mm^2)	Adrenal Cortex Relative	Adrenal Medulla Absolute (mm^2)	Adrenal Medulla Relative	Zona Glomerulosa Absolute (mm^2)	Zona Glomerulosa Relative	Zona Fasciculata + Reticularis Absolute (mm^2)	Zona Fasciculata + Reticularis Relative
Proven Males n=16	2.54 ± 0.394	2.32 ± 0.392	0.91 ± 0.016	0.22 ± 0.029	0.09 ± 0.016	0.20 ± 0.029	0.08 ± 0.009	2.12 ± 0.373	0.83 ± 0.023
Inhibited Males n=14	2.12 * ± 0.364	1.89 * ± 0.355	0.89 ± 0.022	0.24 ± 0.043	0.11 ± 0.022	0.17 * ± 0.025	0.08 ± 0.010	1.71 * ± 0.340	0.80 ± 0.028

* $P < 0.05$

Table 8. Mean Adrenal Area, Mean Absolute and Relative (to total area) Cortex, Medulla, Zona Glomerulosa and, Zona Fasciculata plus Reticularis Area in Proven and Inhibited P. leucopus females. Values are mean ± standard deviation.

Treatments	Total Area (mm^2)	Adrenal Cortex Absolute (mm^2)	Adrenal Cortex Relative	Adrenal Medulla Absolute (mm^2)	Adrenal Medulla Relative	Zona Glomerulosa Absolute (mm^2)	Zona Glomerulosa Relative	Zona Fasciculata + Reticularis Absolute (mm^2)	Zona Fasciculata + Reticularis Relative
Proven Females n=15	2.38 ± 0.500	2.15 ± 0.492	0.90 ± 0.033	0.23 ± 0.064	0.10 ± 0.03	0.18 ± 0.044	0.08 ± 0.012	1.97 ± 0.464	0.83 ± 0.038
Inhibited Females n=14	2.03 * ± 0.323	1.81 * ± 0.320	0.89 0.027	0.21 ± 0.048	0.11 ± 0.028	0.19 ± 0.018	0.10 * ± 0.014	1.62 * ± 0.313	0.80 ± 0.036

* $P < 0.05$

Table 9. Age, Body Weight, Absolute and Relative (to Body Weight) mean Seminal Vesicle, Testis, and Adrenal Weights in Proven *P. maniculatus* and *P. leucopus* males. Values are mean ± standard deviation.

Treatment	Age (days)	Body Weight (g)	Paired Seminal Vesicle Weight		Paired Testis Weight		Paired Adrenal Weight	
			Absolute (mg)	Relative (mg/g)	Absolute (mg)	Relative (mg/g)	Absolute (mg)	Relative (mg/g)
Pmb								
Proven Males n=12	127 ± 2.2	18.8 ± 3.16	169.8 ± 51.00	9.19 ± 2.715	337.0 ± 58.00	18.1 ± 3.03	3.2 ± 0.77	0.17 ± 0.053
Pln								
Proven Males n=16	125 ± 2.9	21.9 * ± 2.52	164.9 ± 53.87	7.54 ± 2.500	337.8 ± 74.00	15.5 * ± 2.95	11.3 * ± 4.01	0.51 * ± 0.155

* $P < 0.05$

Table 10. Mean Adrenal Area, Mean Absolute and Relative (to total area) Cortex, Medulla, Zona Glomerulosa and, Zona Fasciculata plus Reticularis Area in Proven *P. maniculatus* and *P. leucopus* males. Values are mean ± standard deviation.

Treatments	Total Area (mm^2)	Adrenal Cortex Absolute (mm^2)	Adrenal Cortex Relative	Adrenal Medulla Absolute (mm^2)	Adrenal Medulla Relative	Zona Glomerulosa Absolute (mm^2)	Zona Glomerulosa Relative	Zona Fasciculata + Reticularis Absolute (mm^2)	Zona Fasciculata + Reticularis Relative
Pmb Proven Males n=12	1.07 ± 0.290	0.82 ± 0.293	0.75 ± 0.054	0.23 ± 0.049	0.23 ± 0.064	0.13 ± 0.041	0.12 ± 0.032	0.72 ± 0.299	0.66 ± 0.079
Pln Proven Males n=16	2.54 * ± 0.394	2.32 * ± 0.392	0.91 * ± 0.016	0.22 ± 0.029	0.09 * ± 0.016	0.20 * ± 0.029	0.08 * ± 0.009	2.12 * ± 0.373	0.83 * ± 0.023

* $P < 0.05$

Table 11. Age, Body Weight, Absolute and Relative (to Body Weight) mean Seminal Vesicle, Testis, and Adrenal Weights in Inhibited *P. maniculatus* and *P. leucopus* males. Values are mean ± standard deviation.

Treatments	Age (days)	Body Weight (g)	Paired Seminal Vesicle Weight		Paired Testis Weight		Paired Adrenal Weight	
			Absolute (mg)	Relative (mg/g)	Absolute (mg)	Relative (mg/g)	Absolute (mg)	Relative (mg/g)
Pmb Inhibited Males n=13	122 ± 1.3	12.8 ± 0.91	1.6 ± 1.24	0.13 ± 0.114	38.3 ± 22.78	3.1 ± 1.96	1.7 ± 0.77	0.14 ± 0.060
Pln Inhibited Males n=14	126 ± 4.7	16.4 * ± 0.98	3.6 ± 4.50	0.22 ± 0.264	26.9 ± 20.93	1.6 ± 1.22	8.5 * ± 1.91	0.52 * ± 0.126

* $P < 0.05$

Table 12. Mean Adrenal Area, Mean Absolute and Relative (to total area) Cortex, Medulla, Zona Glomerulosa and, Zona Fasciculata plus Reticularis Area in Inhibited *P. maniculatus* and *P. leucopus* males. Values are mean ± standard deviation.

Treatments	Total Area (mm^2)	Adrenal Cortex Absolute (mm^2)	Adrenal Cortex Relative	Adrenal Medulla Absolute (mm^2)	Adrenal Medulla Relative	Zona Glomerulosa Absolute (mm^2)	Zona Glomerulosa Relative	Zona Fasciculata + Reticularis Absolute (mm^2)	Zona Fasciculata + Reticularis Relative
Pmb Inhibited Males n=13	0.85 ± 0.156	0.63 ± 0.141	0.73 ± 0.058	0.23 ± 0.046	0.27 ± 0.058	0.12 ± 0.016	0.14 ± 0.019	0.51 ± 0.128	0.59 ± 0.059
Pln Inhibited Males n=14	2.12 * ± 0.364	1.89 * ± 0.355	0.89 * ± 0.022	0.24 ± 0.043	0.11 * ± 0.022	0.17 * ± 0.025	0.08 * ± 0.010	1.71 * ± 0.340	0.80 * ± 0.028

* $P < 0.05$

Table 13. Age, Body Weight, Absolute and Relative (to Body Weight) mean Ovary, Uterus, and Adrenal Weights in Proven P. maniculatus and P. leucopus females. Values are mean ± standard deviation.

Treatments	Age (days)	Body Weight (g)	Paired Ovary Weight Absolute (mg)	Paired Ovary Weight Relative (mg/g)	Uterus Weight[a] Absolute (mg)	Uterus Weight[a] Relative (mg/g)	Paired Adrenal Weight Absolute (mg)	Paired Adrenal Weight Relative (mg/g)
Pmb Proven Females n=12	126 ± 3.0	17.8 ± 3.17	18.9 ± 4.30	1.07 ± 0.209	64.2 ± 24.27	3.6 ± 1.30	2.9 ± 0.47	0.17 ± 0.044
Pln Proven Females n=15	123 ± 1.8	19.9 ± 2.34	15.7 * ± 3.25	0.79 * ± 0.161	95.3 * ± 35.06	4.8 * ± 1.839	11.0 * ± 3.08	0.56 * ± 0.201

* $P < 0.05$

[a] includes uteri from proven females that contained uterine scars

Table 14. Mean Adrenal Area, Mean Absolute and Relative (to total area) Cortex, Medulla, Zona Glomerulosa and, Zona Fasciculata plus Reticularis Area in Proven *P. maniculatus* and *P. leucopus* females. Values are mean ± standard deviation.

Treatments	Total Area (mm^2)	Adrenal Cortex Absolute (mm^2)	Adrenal Cortex Relative	Adrenal Medulla Absolute (mm^2)	Adrenal Medulla Relative	Zona Glomerulosa Absolute (mm^2)	Zona Glomerulosa Relative	Zona Fasciculata + Reticularis Absolute (mm^2)	Zona Fasciculata + Reticularis Relative
Pmb Proven Females n=12	0.88 ± 0.128	0.61 ± 0.126	0.69 ± 0.045	0.27 ± 0.033	0.31 ± 0.045	0.12 ± 0.018	0.14 ± 0.017	0.49 ± 0.113	0.55 ± 0.047
Pln Proven Females n=15	2.38 * ± 0.500	2.15 * ± 0.492	0.90 * ± 0.033	0.23 ± 0.064	0.10 * ± 0.033	0.18 * ± 0.044	0.08 * ± 0.012	1.97 * ± 0.464	0.83 * ± 0.038

* $P < 0.05$

Table 15. Age, Body Weight, Absolute and Relative (to Body Weight) mean Ovary, Uterus, and Adrenal Weights in Inhibited *P. maniculatus* and *P. leucopus* females. Values are mean ± standard deviation.

Treatments	Age (days)	Body Weight (g)	Paired Ovary Weight Absolute (mg)	Paired Ovary Weight Relative (mg/g)	Uterus Weight Absolute (mg)	Uterus Weight Relative (mg/g)	Paired Adrenal Weight Absolute (mg)	Paired Adrenal Weight Relative (mg/g)
Pmb Inhibited Females n=17	122 ± 2.2	12.4 ± 1.16	2.8 ± 1.03	0.23 ± 0.085	5.4 ± 1.62	0.43 ± 0.115	1.9 ± 0.67	0.15 ± 0.061
Pln Inhibited Females n=14	123 ± 3.7	16.2 * ± 1.63	2.7 ± 0.90	0.17 ± 0.054	6.7 ± 1.99	0.42 ± 0.123	7.7 * ± 2.07	0.48 * ± 0.115

* $P < 0.05$

Table 16. Mean Adrenal Area, Mean Absolute and Relative (to total area) Cortex, Medulla, Zona Glomerulosa and, Zona Fasciculata plus Reticularis Area in Inhibited P. maniculatus and P. leucopus females. Values are mean ± standard deviation.

Treatments	Total Area (mm^2)	Adrenal Cortex Absolute (mm^2)	Adrenal Cortex Relative	Adrenal Medulla Absolute (mm^2)	Adrenal Medulla Relative	Zona Glomerulosa Absolute (mm^2)	Zona Glomerulosa Relative	Zona Fasciculata + Reticularis Absolute (mm^2)	Zona Fasciculata + Reticularis Relative
Pmb Inhibited Females n=17	0.87 ± 0.164	0.64 ± 0.159	0.72 ± 0.057	0.24 ± 0.036	0.28 ± 0.058	0.12 ± 0.023	0.14 ± 0.012	0.51 ± 0.140	0.58 ± 0.059
Pln Inhibited Females n=14	2.03 * ± 0.323	1.81 * ± 0.320	0.89 * ± 0.027	0.21 ± 0.048	0.11 * ± 0.028	0.19 * ± 0.018	0.10 * ± 0.014	1.62 * ± 0.313	0.80 * ± 0.036

* $P < 0.05$

DISCUSSION

In the past, populations from this laboratory have been founded by several pairs of reproducing animals, and grown until they reach asymptote (Terman, 1965, 1969, 1973; Sung et al., 1977; Bradley and Terman, 1981a, 1981b, 1981c; and Coppes and Bradley, 1984 with P. maniculatus; Wolfe, 1981, and Ransone, 1988 with P. leucopus). Reproductive inhibition is achieved by the failure of litters to survive and/or the failure of surviving litters to mature sexually. In the present study, inhibited animals were produced by placing 15 males and 15 females together at roughly the same age (22-33 days for P. maniculatus, 29-53 days for P. leucopus). Although the inhibited animals from the present study were not produced in the same way as inhibited animals from the previous studies, they do exhibit physiological characteristics similar to reproductively inhibited animals from asymptotic populations, i.e. their body, reproductive organ, and adrenal weights are significantly ($P < 0.05$) smaller than that of proven or control animals of the same sex and species (cf. Tables 1-4).

It is important to note that the assembled population animals used in this study remained in a reproductively

inhibited state in the presence of cohorts of similar age. This is in contrast to the founded asymptotic populations in which reproductive inhibition is maintained in animals in the presence of one or more, usually older, reproductively proven males. The differences in social structure betweeen assembled and founded populations and how that structure relates to reproductive inhibition warrants further study.

Comparisons of P. maniculatus proven and inhibited males

Reproductively proven males were significantly ($P < 0.05$) larger than reproductively inhibited males with respect to body weight, absolute and relative reproductive organ weight, and absolute adrenal weight. No significant differences were found in the relative adrenal weight (See Table 1). Sung et al., (1977) had similar findings. Bradley and Terman (1981a) found that the adrenal weight of control males tended ($P < 0.1$) to be larger than that of population males but that the relative adrenal weight tended ($P < 0.1$) to be larger in population males, indicating a possible relative hypertrophy of the adrenal gland in population males, and prompting the present study. Coppes (1984) found no significant differences in the absolute adrenal weight between control and population males.

Proven P. maniculatus were found significantly ($P < 0.05$) larger than reproductively inhibited males with respect to the total adrenal area, and absolute and relative

zona fasciculata plus reticularis area (See Table 4). Bradley and Terman (1981a) observed this as well, but they also found that the medulla area was significantly ($P < 0.01$) smaller in population males and suggested that if the medulla was smaller in population males, the fasciculata and reticularis may be relatively larger in population males. This could account for the elevated levels of serum corticosterone that they and others (Sung et al., 1977) found in inhibited males. However, the present study found no significant difference in the medulla or zona glomerulosa area between proven and inhibited P. maniculatus males, but there is an increase in the zona fasciculata plus reticularis in proven males, indicating no hypertrophy of corticosterone-producing tissue in inhibited males.

Comparisons of P. maniculatus proven and inhibited females

P. maniculatus reproductively proven females were significantly ($P < 0.05$) larger than reproductively inhibited females with respect to body weight, absolute and relative reproductive organ weight, and absolute adrenal weight. No significant difference was found in the relative adrenal weight between proven and inhibited females (See Table 2). This same trend was observed by Sung et al. (1977). Bradley and Terman (1981a) however, found no significant difference in body weight or adrenal weight between control and population females. This may have been

due to the fact that they used non-parous females for control animals, whereas in the present study, parous animals were used. Sung et al., (1977), also used non-parous animals, but his control females were much older (230-270 days old versus 120-140 days old in the Bradley and Terman (1981a) study, and 121-131 days old in the present study). An increase in female adrenal weight with sexual maturation has been observed in rats (Christian, 1967) and Microtus (Christian and Davis, 1964, 1966), but there has been no previous indication of an increase in female adrenal weight with sexual maturation in P. maniculatus or P. leucopus (Christian, 1967). Bradley and Terman (1981a) also found that, like the males in their study, their population females had significantly ($P < 0.02$) larger relative adrenal weights when compared with control females. Bradley and Terman (1981a) also found significantly elevated ($P < 0.001$) levels of serum corticosterone in their population females when compared with control females.

The present study found no significant differences in any adrenal areas between proven and reproductively inhibited females (see Table 6). Bradley and Terman (1981a) observed no significant difference in the total adrenal or zona fasciculata plus reticularis area, but the medulla area tended ($P < 0.1$) to be smaller in population females than in control females, as it had been in the males of their study. It is difficult to comment on the

importance of the lack of differences found in this study between the proven and inhibited females of P. maniculatus when compared to P. maniculatus males, because P. leucopus females (See Table 8) do show differences in adrenal histology similar to those seen in P. leucopus males (See Table 7). This potentially disproves any general sex-based adrenal size difference related to Peromyscus. However, there may be differences in adrenal function related to reproductive condition between the two species that have yet to be determined.

Comparisons of P. leucopus proven and inhibited males

P. leucopus proven males were significantly ($P < 0.05$) larger than reproductively inhibited males with respect to body weight, absolute and relative reproductive organ weight, and absolute adrenal weight. No significant difference was found between proven and inhibited males in terms of the relative adrenal weight (See Table 3). This is similar to that seen in P. maniculatus males and females (cf. Tables 1 and 2). Ransone (1988) also found P. leucopus proven males significantly ($P < 0.001$) larger than population males with respect to body, and reproductive organ weight, but no significant difference in the absolute adrenal weight.

Adrenal histology revealed significantly ($P < 0.05$) larger total area, absolute cortex, zona glomerulosa, and

zona fasciculata plus reticularis area in reproductively proven males when compared with reproductively inhibited males (See Table 7). This is similar to the findings for P. maniculatus males of this study. The total area and absolute zona fasciculata plus reticularis area are significantly ($P < 0.05$) larger in proven males when compared with inhibited males of the same species. This suggests that, in reproductively inhibited males, there is no hypertrophy of the corticosterone producing tissue of the adrenal, nor is there a decrease in the medulla or zona glomerulosa tissue to accomodate an expanding zona fasciculata and reticularis, while maintaining a smaller total adrenal area. In comparing reproductively proven and inhibited males from this study, it is clear that proven males have a larger total adrenal area, resulting from an increase in the amount of zona fasciculata and reticularis, and zona glomerulosa to a lesser extent in P. leucopus males. It is also clear that the high levels of circulating corticosterone in population males found in other studies (Sung et al., 1977; Bradley and Terman, 1981a) may not necessarily be explained by a hypertrophy of the zona fasciculata and reticularis area.

Comparisons of P. leucopus proven and inhibited females

P. leucopus proven females were significantly ($P < 0.05$) larger than reproductively inhibited females with

respect to body weight, absolute and relative reproductive organ weight, and absolute adrenal weight. No significant difference was found between proven and inhibited females with respect to relative adrenal weight (See Table 4). Ransone (1988) found no significant difference in the body weight or adrenal weight between control and population animals, however; he used non-parous females that were only 70 days old as his control animals, while the present study used older, parous females. This may account for the larger body weight of the control-proven females in this study, and for the significantly ($\underline{P} < 0.05$) larger body weight found for reproductively proven versus reproductively inhibited females in this study, but lacking in Ransone's study. Reproductive organ weights from his study were, like those found in this study, significantly ($\underline{P} < 0.001$) larger in control females than in population females.

Adrenal histology revealed significantly ($\underline{P} < 0.05$) larger total adrenal area, absolute cortex area and absolute zona fasciculata plus reticularis area in P. leucopus proven females versus reproductively inhibited females (See Table 8). This suggests that, like the males from both species of Peromyscus in this study, the total adrenal area from the proven animal is larger as a result of an increase in the amount of zona fasciculata plus reticularis. It is not known why this same trend is not observed in P. maniculatus females, but it may be the result of some sex based

difference between the species with respect to adrenal function.

Summary of comparisons between proven and inhibited animals

The results of this study on the body weight and absolute and relative reproductive organ and adrenal gland weight show that proven animals have significantly ($\underline{P} < 0.05$) larger body weights than inhibited animals. They also have significantly ($\underline{P} < 0.05$) larger absolute and relative reproductive organ weights than reproductively inhibited animals of the same age. The adrenal glands of proven animals are also significantly ($\underline{P} < 0.05$) larger in absolute but not significantly different in relative weight, indicating that any increase in adrenal activity in the inhibited animals is not reflected by a relative increase in the weight of the gland.

Previous studies have indicated that strong correlations between adrenal secretion, adrenal weight, and animal weight can be demonstrated in lemmings (Andrews, 1968), voles (Andrews, 1970), and mice (Bronson and Eleftheriou, 1963; Purushotham, et al., 1978). Christian (1955a, b) found that increasing the population density produced a proportional increase in the weight of the adrenal gland along with a decrease in testicular and seminal vesicle weight of white mice. Christian (1963) also reviewed the subject of the effect of population density on

adrenal response and concluded that hypertrophy of adrenal glands following density increase is a widespread phenomenon in various species of rodents and that in house mice, increased adrenal weight with increased population density was attributable to a hypertrophy of the adrenal cortex (Christian, 1956).

Any elevation in serum corticosterone in reproductively inhibited animals selected from assembled populations of P. maniculatus and P. leucopus, cannot be explained by a hypertrophy of the zona fasciculata and reticularis or a decrease in the area of medulla and glomerulosa. In males of both species, and females of P. leucopus, proven animals have larger adrenal glands, due to larger absolute zona fasciculata and reticularis area. This is not consistent with other studies which have demonstrated a density dependent adrenal hypertrophy in Mus (Christian, 1955a, b, 1960). The results of this study indicates that there must be some other reason for the previously found elevation of serum corticosterone in reproductively inhibited animals of Peromyscus.

ACTH, an adenohypophysial regulator of corticosterone production, has been shown to increase the amount of zona fasciculata and reticularis area in Mus (Molne, 1969), but serum levels of ACTH have not been shown to be elevated in population animals of P. maniculatus (Coppes and Bradley, 1984). Bradley and Terman (1981a) suggested that increases

in plasma binding proteins for corticosterone might protect the hormone from metabolism or that if reductions in hepatic or other metabolism were to decrease the rate of corticosterone inactivation, there would be an increase in corticosterone in the circulation that would not necessarily be related to adrenal hypersecretion. Hypothyroidism, in term of reduced serum thyroid hormone secretion, has been demonstrated in population males of P. maniculatus (Peebles et al., 1984; Pitman and Bradley, 1984), and may actually have an effect on the metabolic clearance of corticosterone.

Electron microscopy of adrenocortical tissue may lend itself well to resolving the question of adrenal atrophy in reproductively inhibited animals of Peromyscus. Studies have shown that stimulation of the zones producing corticosterone can be measured by an increase in the volume of smooth endoplasmic reticulum and mitochondrial compartments of the zona fasciculata and zona reticularis (Mazzocchi et al., 1976; Nussdorfer et al., 1978). An ultrastructural study of the embedded adrenal glands from this study may provide answers to the question of adrenal hypertrophy that elude light microscopy studies.

DIFFERENCES BETWEEN *P. maniculatus* and *P. leucopus*
(See Tables 9-16)

P. leucopus animals were, with a few exceptions, significantly ($P < 0.05$) larger with respect to every measurement of weight and adrenal histology. Because of this, a discussion of similarities seems more appropriate. One interesting similarity between the species was that few significant differences were found in reproductive organ weight. Within the males, only the relative testis weight of *P. maniculatus* proven males was significantly larger ($P < 0.05$) than proven males of *P. leucopus* (cf. tables 9 and 11). Among the females, *P. maniculatus* proven females had significantly larger absolute and relative uterus weights and significantly smaller absolute and relative paired ovary weights than proven females of *P. leucopus* (cf. Tables 13 and 15). These difference may have been the result of the shorter time between parturition and sacrifice of *P. maniculatus* proven females (average of 25 days), than *P. leucopus* proven females (average of 35 days) which may have resulted in an increased uterine weights of *P. maniculatus*. This may also have been reflected in a lack of significant differences in the body weight between the two species' proven females. Significant differences in adrenal histology were the same in every group (cf. Tables 10, 12, 14, and 16). *P. leucopus* animals were significantly larger

than P. maniculatus animals ($P < 0.05$) with respect to everything except relative medulla area and relative glomerulosa area, which were significantly smaller in P. leucopus. One interesting similarity was that there were no significant differences in the absolute area of the adrenal medulla. This suggests that, regardless of the reproductive condition, the amount of medulla tissue is relatively constant in animals from both sexes of P. maniculatus and P. leucopus. Christian (1967) also found a striking difference in the larger adrenal size of P. leucopus when compared with P. maniculatus, but no significant differences in adrenal weight relative to body weight with respect to reproductive condition.

DIFFERENCES BETWEEN MALES AND FEMALES

(See Appendix A-H)

No significant differences were found between males and females of P. maniculatus with respect to body weight. This corresponds to the findings of Sung et al. (1977) in mice that were as old as 270 days. Several previous studies found that control males were significantly larger than control females (Bradley and Terman, 1981a, 1981b; Terman, 1969) and suggested that the sex based difference in body weight is greatest in the young adult control and may

diminish as the animal grew older. That hypothesis is not supported by the findings of this study for P. maniculatus. This may be the result of a lack of sufficient time after parturition for the females, which could have increased their mean body weight. However, P. leucopus proven males were significantly heavier than proven females (P. leucopus proven females experienced an average of ten days longer between parturition and sampling.). Ransone (1988) also observed a significantly ($P < 0.05$) larger body weights in control males versus control females of P. leucopus at 70 days of age. No significant differences were found in this study between the body weights of population males and females. There were no significant differences in the absolute or relative adrenal weights between the sexes. This is consistent with previous studies on P. maniculatus (Sung et al., 1977; Bradley and Terman, 1981a) and P. leucopus (Christian, 1967).

ADRENAL WEIGHT

(See Appendix I and J.)

The weights of left versus the paired adrenal glands was compared. Paired adrenal glands were found to be approximately twice the weight of the left adrenal. This result suggests that the left and right adrenal are

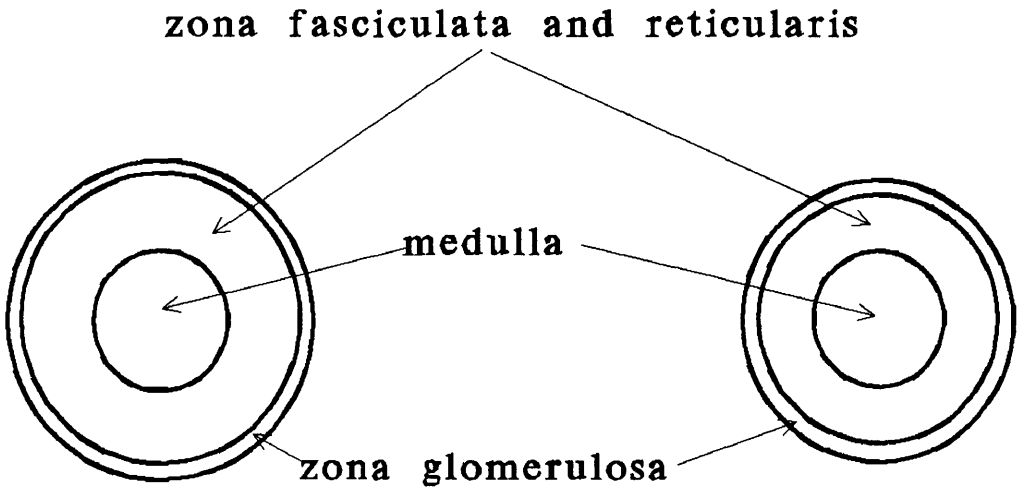
approximately the same weight.

CONCLUSION

The objective of this study was to examine the adrenal histology of P. maniculatus and P. leucopus to see if the elevated levels of serum corticosterone previously found in reproductively inhibited animals could be reflected by a relative increase in the zona fasciculata and zona reticularis. This study has revealed no such evidence of adrenal hypertrophy. On the contrary, except for P. maniculatus females, where the values were not different, the zona fasciculata and reticularis were significantly ($P < 0.05$) larger in proven animals than in inhibited animals. Determining the levels of serum corticosterone in the animals from this study will be vital in establishing a relationship between reproductive function, adrenal histology, and adrenal function.

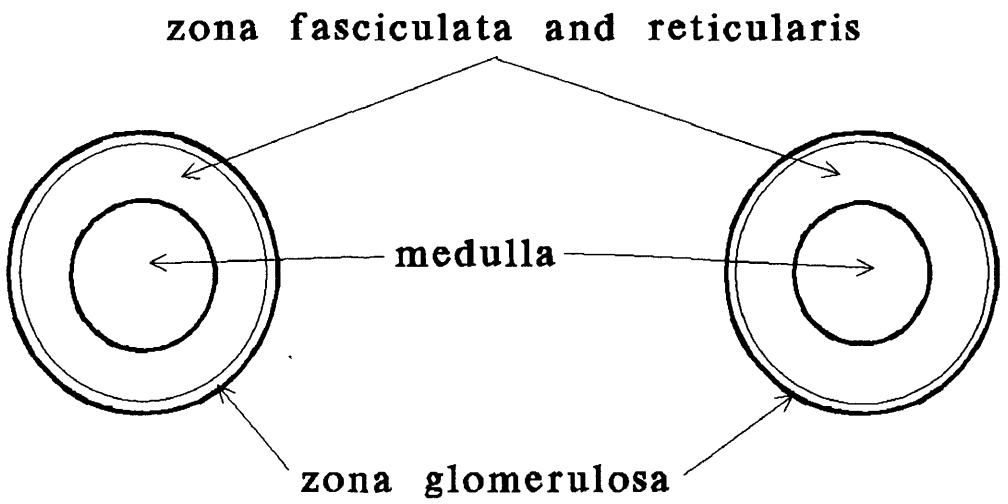
Previous ultrastructural studies with rodents have shown that the level of activity of the zona fasciculata and zona reticularis can be determined by examination of the smooth endoplasmic reticulum and mitochondria. Electron microscopy investigations of the right adrenal glands collected in this study may resolve the apparent paradox of adrenal function in P. maniculatus and P. leucopus.

Figure 1. Adrenal gland of *P. maniculatus* proven male versus inhibited male



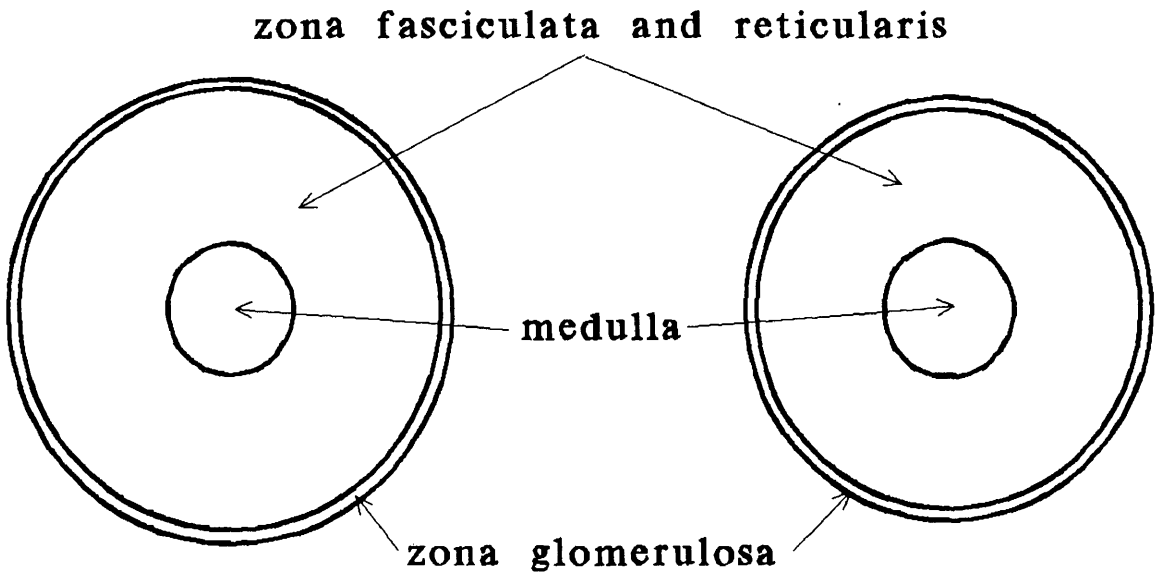
proven male inhibited male

Figure 2. Adrenal gland of *P. maniculatus* proven female versus inhibited female



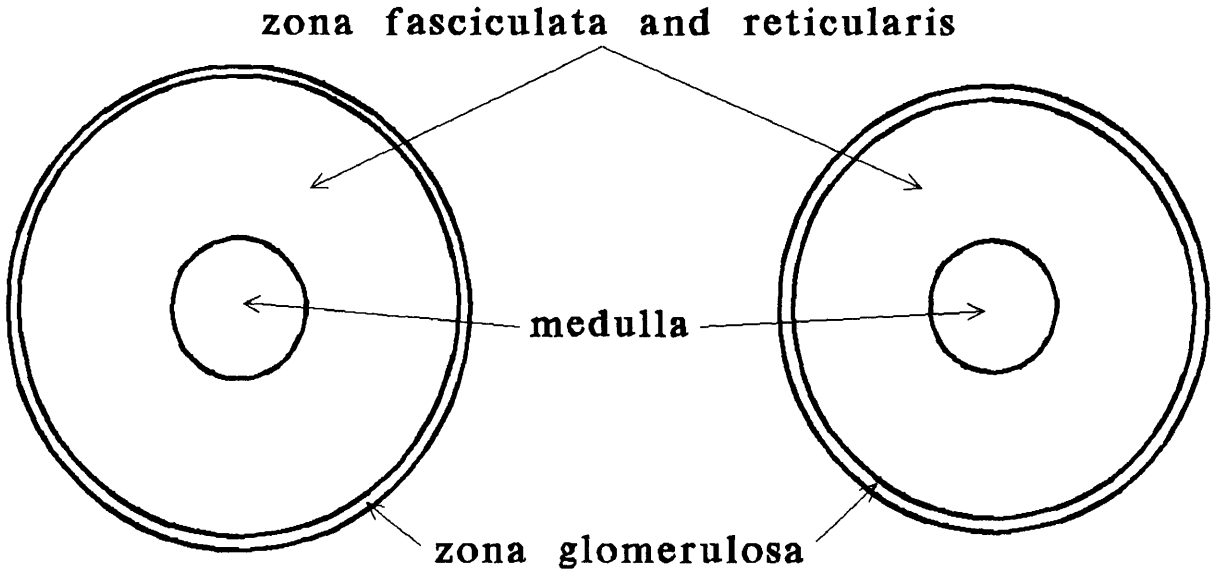
proven female inhibited female

Figure 3. Adrenal gland of *P. leucopus* proven female versus inhibited female



proven female inhibited female

Figure 4. Adrenal gland of *P. leucopus* proven male versus inhibited male



proven male inhibited male

APPENDIX A

Age, Body Weight, Absolute and Relative (to Body Weight) mean Adrenal Weights in Proven *P. maniculatus* males and females. Values are mean ± standard deviation.

Treatments	Age (days)	Body Weight (g)	Paired Adrenal Weight Absolute (mg)	Paired Adrenal Weight Relative (mg/g)
Proven Males n=12	127 ± 2.2	18.8 ± 3.16	3.2 ± 0.77	0.17 ± 0.053
Proven Females n=12	126 ± 3.0	17.8 ± 3.17	2.9 ± 0.47	0.17 ± 0.044

No significant differences were found.

APPENDIX B

Age, Body Weight, Absolute and Relative (to Body Weight) mean Adrenal Weights in Inhibited *P. maniculatus* males and females. Values are mean ± standard deviation.

Treatments	Age (days)	Body Weight (g)	Paired Adrenal Weight Absolute (mg)	Paired Adrenal Weight Relative (mg/g)
Inhibited Males n=13	122 ± 1.3	12.8 ± 0.91	1.7 ± 0.77	0.14 ± 0.060
Inhibited Females n=17	122 ± 2.2	12.4 ± 1.16	1.9 ± 0.67	0.15 ± 0.061

No significant differences were found

APPENDIX C

Mean Adrenal Area, Mean Absolute and Relative (to total area) Cortex, Medulla, Zona Glomerulosa and, Zona Fasciculata plus Reticularis Area in Proven P. maniculatus males and females. Values are mean ± standard deviation.

Treatments	Total Area (mm^2)	Adrenal Cortex Absolute (mm^2)	Adrenal Cortex Relative	Adrenal Medulla Absolute (mm^2)	Adrenal Medulla Relative	Zona Glomerulosa Absolute (mm^2)	Zona Glomerulosa Relative	Zona Fasciculata + Reticularis Absolute (mm^2)	Zona Fasciculata + Reticularis Relative
Pmb Proven Males n=12	1.07 ± 0.290	0.82 ± 0.293	0.75 ± 0.054	0.23 ± 0.049	0.23 ± 0.064	0.13 ± 0.041	0.12 ± 0.032	0.72 ± 0.299	0.66 ± 0.079
Pmb Proven Females n=12	0.88 ± 0.128	0.61 * ± 0.126	0.69 * ± 0.045	0.27 ± 0.033	0.31 * ± 0.045	0.12 ± 0.018	0.14 ± 0.017	0.49 * ± 0.113	0.55 * ± 0.047

* $P < 0.05$

APPENDIX D

Mean Adrenal Area, Mean Absolute and Relative (to total area) Cortex, Medulla, Zona Glomerulosa and, Zona Fasciculata plus Reticularis Area in Inhibited P. maniculatus males and females. Values are mean ± standard deviation.

Treatments	Total Area (mm^2)	Adrenal Cortex Absolute (mm^2)	Adrenal Cortex Relative	Adrenal Medulla Absolute (mm^2)	Adrenal Medulla Relative	Zona Glomerulosa Absolute (mm^2)	Zona Glomerulosa Relative	Zona Fasciculata + Reticularis Absolute (mm^2)	Zona Fasciculata + Reticularis Relative
Pmb Inhibited Males n=13	0.85 ± 0.156	0.63 ± 0.141	0.73 ± 0.058	0.23 ± 0.046	0.27 ± 0.058	0.12 ± 0.016	0.14 ± 0.019	0.51 ± 0.128	0.59 ± 0.059
Pmb Inhibited Females n=17	0.87 ± 0.164	0.64 ± 0.159	0.72 ± 0.057	0.24 ± 0.036	0.28 ± 0.058	0.12 ± 0.023	0.14 ± 0.012	0.51 ± 0.140	0.58 ± 0.059

No significant differences were found.

APPENDIX E

Age, Body Weight, Absolute and Relative (to Body Weight) mean Adrenal Weights in Proven *P. leucopus* males and females. Values are mean ± standard deviation.

Treatments	Age (days)	Body Weight (g)	Paired Adrenal Weight Absolute (mg)	Paired Adrenal Weight Relative (mg/g)
Proven Males n=16	125 ± 2.9	21.9 ± 2.52	11.3 ± 4.01	0.51 ± 0.155
Proven Females n=15	123 ± 1.8	19.9 * ± 2.34	11.0 ± 3.08	0.56 ± 0.201

* $P < 0.05$

APPENDIX F

Age, Body Weight, Absolute and Relative (to Body Weight) mean Adrenal Weights in Inhibited *P. leucopus* males and females. Values are mean ± standard deviation.

Treatments	Age (days)	Body Weight (g)	Paired Adrenal Weight Absolute (mg)	Paired Adrenal Weight Relative (mg/g)
Inhibited Males n=14	126 ± 4.7	16.4 ± 0.98	8.5 ± 1.91	0.52 ± 0.126
Inhibited Females n=14	123 ± 3.7	16.2 ± 1.63	7.7 ± 2.07	0.48 ± 0.115

No significant differences were found.

APPENDIX G

Mean Adrenal Area, Mean Absolute and Relative (to total area) Cortex, Medulla, Zona Glomerulosa and, Zona Fasciculata plus Reticularis Area in Proven *P. leucopus* males and females. Values are mean ± standard deviation.

Treatments	Total Area (mm^2)	Adrenal Cortex Absolute (mm^2)	Adrenal Cortex Relative	Adrenal Medulla Absolute (mm^2)	Adrenal Medulla Relative	Zona Glomerulosa Absolute (mm^2)	Zona Glomerulosa Relative	Zona Fasciculata + Reticularis Absolute (mm^2)	Zona Fasciculata + Reticularis Relative
Pln Proven Males n=16	2.54 ± 0.394	2.32 ± 0.392	0.91 ± 0.016	0.22 ± 0.029	0.09 ± 0.016	0.20 ± 0.029	0.08 ± 0.009	2.12 ± 0.373	0.83 ± 0.023
Pln Proven Females n=15	2.38 ± 0.500	2.15 ± 0.492	0.90 ± 0.033	0.23 ± 0.064	0.10 ± 0.033	0.18 * ± 0.044	0.08 ± 0.012	1.97 ± 0.464	0.83 ± 0.038

* $P < 0.05$

APPENDIX H

Mean Adrenal Area, Mean Absolute and Relative (to total area) Cortex, Medulla, Zona Glomerulosa and, Zona Fasciculata plus Reticularis Area in Inhibited *P. leucopus* males and females. Values are mean ± standard deviation.

Treatments	Total Area (mm^2)	Adrenal Cortex Absolute (mm^2)	Adrenal Cortex Relative	Adrenal Medulla Absolute (mm^2)	Adrenal Medulla Relative	Zona Glomerulosa Absolute (mm^2)	Zona Glomerulosa Relative	Zona Fasciculata + Reticularis Absolute (mm^2)	Zona Fasciculata + Reticularis Relative
Pln Inhibited Males n=14	2.12 ± 0.364	1.89 ± 0.355	0.89 ± 0.022	0.24 ± 0.043	0.11 ± 0.022	0.17 ± 0.025	0.08 ± 0.010	1.71 ± 0.340	0.80 ± 0.028
Pln Inhibited Females n=14	2.03 ± 0.323	1.81 ± 0.320	0.89 ± 0.027	0.21 ± 0.048	0.11 ± 0.028	0.19 ± 0.018	0.10 * ± 0.014	1.62 ± 0.313	0.80 ± 0.036

* $P < 0.05$

APPENDIX I

Weights of Left Adrenal Glands and Paired Adrenal Glands for *P. maniculatus*. Values are mean ± standard deviation.

Treatment	Left Adrenal Weight (mg)	Paired Adrenal Weight (mg)
Proven Male n=12	1.6 ± 0.54	3.2 ± 0.77
Inhibited Male n=13	0.9 ± 0.43	1.7 ± 0.77
Proven Female n=12	1.5 ± 0.21	2.9 ± 0.47
Inhibited Female n=17	1.0 ± 0.33	1.9 ± 0.67
All *Pmb* n=54	1.3 ± 0.30	2.4 ± 0.64

APPENDIX J

Weights of Left Adrenal Gland and Paired Adrenal Gland of *P. leucopus*. Values are mean ± standard deviation.

Treatment	Left Adrenal Weight (mg)	Paired Adrenal Weight (mg)
Proven Males n=16	5.6 ± 1.68	11.3 ± 4.01
Inhibited Males n=14	4.3 ± 1.04	8.5 ± 1.91
Proven Females n=15	5.4 ± 1.29	11.0 ± 3.08
Inhibited Females n=14	4.0 ± 1.12	7.7 ± 2.07
All *Pln* n=59	4.8 ± 0.69	9.6 ± 1.56

BIBLIOGRAPHY

Albertson, B.D., E.L. Bradley, and C.R. Terman. 1975. Plasma progesterone concentrations in prairie deermice (Peromyscus maniculatus bairdii) from experimental laboratory populations. J. Reprod. Fert. 42:407-414.

Andrews, R.V. 1968. Daily and seasonal variation in adrenal metabolism of the brown lemming. Physiol. Zool. 41:86-94.

Andrews, R.V. 1970. Effects of climate and social pressure on the adrenal response of lemmings, voles, and mice. Acta End. 65:639-644.

Andrews, R.V. 1977. Influence of the adrenal gland on gonadal function. Adv. Sex Hormone Res. 3:197-215.

Andrews, R.V. 1979. The physiology of crowding. Comp. Biochem. Physiol. 63A:1-6.

Andrews, R.V. and R.W. Belknap. 1979. Deer mouse and lemming adrenal and pathological responses in increases in animal numbers. Comp. Biochem. Physiol. 63A:15-18.

Blair, W.F. 1940. A study of prairie deer-mouse populations in southern MIchigan. Amer. Midl. Nat. 24:272-304.

Bradley, E.L., and C.R. Terman. 1981a. A comparison of the adrenal histology, reproductive condition and serum corticosterone concentrations of prairie deermice (Peromyscus maniculatus bairdii) in captivity. J. Mamm. 62:353-361.

Bradley, E.L., and C.R. Terman. 1981b. Serum testosterone concentrations in male prairie deermice (Peromyscus maniculatus bairdii). J. Mamm. 62:811-814.

Bradley, E.L., and C.R. Terman. 1981c. Studies on the nature of reproductive inhibition in animals from laboratory populations of prairie deermice (Peromyscus maniculatus bairdi): serum LH and FSH concentrations. Comp. Biochem. Physiol. 68A:563-570.

Brain, P.F. 1971. The physiology of population limitation in rodents. A review. Commun. Behav. Biol. 6:115-123.

Bronson, F.H., and B.E. Eleftheriou. 1963. Adrenal response to crowding in Peromyscus and C57BL/10J mice. Physiol. Zool. 36:161-166.

Chitty, H. 1961. Variations in the weight of the adrenal gland of the field vole, Microtus agrestis. J. End. 22:387-393.

Christian, J.J. 1950. The adreno-pituitary system and population cycles in mammals. J. Mamm. 31:247-259.

Christian, J.J. 1955a. Effects of population size on the weights of the reproductive organs of white mice. Am. J. Physiol. 181:477-480.

Christian, J.J. 1955b. Effects of population size on the adrenal glands and reproductive organs of male mice in populations of fixed size. Am. J. Physiol. 182:292-300.

Christian, J.J. 1956. Adrenal and reproductive responses to population size in mice from freely growing populations. Ecol. 37:258-273.

Christian, J.J. 1963. Endocrine adaptive mechanisms and the physiological regulation of population growth. In Physiological Mammology, W.V. Mayer and R.G. VanGelder, ed., Academic Press, N.Y., pp. 189-353

Christian, J.J. 1967. Adrenal weight in prairie deer mice (Peromyscus maniculatus bairdii) and white-footed mice (Peromyscus leucopus noveboracensis). J. Mammol. 48:598-605.

Christian, J.J. 1971. Population density and reproductive efficiency. Biol. Reprod. 4:248-294.

Christian, J.J. 1975. Hormonal control of population growth. In Hormonal Correlates of Behavior, vol. I, B.E. Eleftheriou and R.L. Sprott, ed., Plenum Publ. Corp., N.Y., pp. 205-274.

Christian, J.J. 1980. Endocrine factors in population regulation. In Biosocial Mechanisms of Population Regulation, M.N. Cohen, R.S. Malpass, and H.G. Klein, ed., Yale Univ Press, New Haven, Conn., pp. 55-571.

Christian, J.J. and D.E. Davis. 1964. Endocrines, behavior, and population. Science, 146:1550-1560.

Christian, J.J., J.A. Lloyd, and D.E. Davis. 1965. The role of endocrines in the self regulations of mammalian populations. Rec. Prog Hormone Res. 21:501-571.

Christian, J.J., and D.E. Davis. 1966. Adrenal glands in female voles (Microtus pennsylvanicus) as related to reproduction and population size. J. Mammol. 47:1-18.

Colby, H.D. 1979. Mechanism of action of prolactin on adrenocortical steroid secretion in hypophysectomized female rats. End. 104:1299-1303.

Colby, H.D. and J.I. Kitay. 1972. Effects of gonadal hormones on adrenocortical secretion of 5 alpha-reduced metabolites of corticosterone in the rat. End. 91:1523-1527.

Collu, R., Y. Tache, and J. Ducharme. 1979. Hormonal modifications induced by chronic stress in rats. J. Ster. Biochem. 11:989-1000.

Cook, D.M., J.W. Kendall, M.A. Greer, and R.M. Kramer. 1973. The affect of chronic stress on plasma ACTH concentrations in the rat. End. 93:1019-1024.

Coppes, J.C. and E.L. Bradley. 1984. Serum ACTH and adrenal histology in reproductively inhibited male prairie deermice (Peromyscus maniculatus bairdii). Comp. Biochem. Physiol. 78A,2:297-306.

Fajer, A.B. and M. Vogt. 1963. Adrenocortical secretion in the guinea-pig. J. Physiol. 169:373-385.

Hess, G.D. and G.D. Riegle. 1972. Effects of chronic ACTH stimulation on adrenocortical function in young and aged rats. Am. J. Physiol. 222:1458-1461.

Hull, E.M., C. Kastaniotis, G. L'Hommedieu, and J. Franz. 1974. Environmental enrichment and crowding: Behavioral and hormonal effects. Physiol. Beh. 17:735-741.

Idelman, S. 1970. Ultrastructure of the mammalian adrenal cortex. Intl. Rev. Cytol. 27: 181-281.

Kitay, J.I., M.D. Coyne, N.H. Swyzert, and K.A. Gaines. 1971. Effects of gonadal hormones and ACTH on the nature and rates of secretion of adrenocortical steroids by the rat. Endo. 89:565-570.

Krebs, C.J., M.S. Gaines, B.L. Keller, J.H. Myers, and R.H. Tamarin. 1973. Population Cycles in Small Rodents. Science. 179:36-41.

Lavoie, H.A. 1989. Reproductive and metabolic characteristics in Peromyscus leucopus noveboracensis from laboratory populations and females during release from reproductive inhibition. Masters Thesis, The College of William and Mary in Virginia, Williamsburg, Virginia.

Long, J.A., 1975. Zonation of the mammalian adrenal cortex. In Handbook of Physiology, Section 7: Endocrinology, Volume VI. Adrenal Gland, R.O. Greep, E.B. Astwood, H. Blaschko, A.D. Smith, S.R. Geiger, ed., Am. Physiol. Soc., Washington, D.C., pp. 13-24.

Matsuyama, H.A., Ruhmann-Wennhold, and D.H. Nelson. 1971 Radioimmunoassay of plasma ACTH in intact rats. Endo. 88:692-695.

Mazzocchi, G., C. Robba, P. Rigotta, A.S. Belloni, A.M. Gambino, and G.G. Nussdorfer. 1976. Experientia 32:244-246.

Molne, K. 1969. Histometric studies on the adrenal cortex of mice with spontaneous adrenocortical lipid depletion. A comparison with dexamethasone- and ACTH-treated C57/B1 mice. Acta Path. Microbiol. Scand. 75:51-63.

Nickerson, P.A. 1975. Quantitative study on the effect of an ACTH-producing pituitary tumor on the ultrastructure of the mouse adrenal gland. Am. J. Pathol. 80:295-316.

Nussdorfer, G.G. 1970. Effects of corticosteroid hormones on the smooth endoplasmic reticulum of rat adrenocortical cells. Z. Zellforsch Mik. Anat. 106:143-154.

Nussdorfer, G.G. 1983. Long-term effects of ACTH on rat adrenocortical cells: A coupled stereological and enzymological study. J. Steroid Biochem. 19:1753-1756.

Nussdorfer, G.G. 1986. Morphological-functional correlations in adrenocortical cells. Int. Rev. Cytol. 98:283-318.

Nussdorfer, G.G., G. Mazzocchi, and V. Meneghelli. 1978. Cytophysiology of the adrenal zona fasciculata. Intl. Rev. Cytol. 55:291-365.

Pasley, J.N. and J.J. Christian. 1971. The effect of ACTH, group caging, and adrenalectomy in Peromyscus leucopus with emphasis on suppression of reproductive function. End. Metab. 36:95-107.

Peebles, E.D., J.A. Painter, and E.L. Bradley. 1984. A possible role for the thyroid in reproductive inhibition in laboratory populations of the prairie deermouse (Peromyscus maniculatus). Comp. Biochem. Physiol. 77A,2:293-298.

Pitman, J.M. and E.L. Bradley. 1984. Hypothyroidism in reproductively inhibited prairie deermice (Peromyscus maniculatus bairdii) from laboratory populations. Biol. Reprod. 31:895-904.

Purushotham, K.R., A.M.K. Mohano Rao, and B.S. Rajabai. 1978. Adrenal response to crowding in the spiny field mouse, Mus platythrix (Bennett). Gen. Comp. End. 36:439-441.

Ransone, S.N. 1988. Correlations between serum corticosterone level and reproductive fitness in laboratory populations of the white-footed mouse (Peromyscus leucopus noveboracensis). Masters Thesis, The College of William and Mary in Virginia, Williamsburg, Virginia.

Retiene, K., E. Zimmerman, W.J. Schindler, J. Neuenschwander, and H.S. Lipscomb. 1968. A correlative study of endocrine rhythms in rats. Acta End. 57:615-622.

Rhodin, J.A.G. 1971. The ultrastructure of the adrenal cortex of the rat under normal and experimental conditions. J. Ultrastr. Res. 34:23-71.

Selye, H. 1946. The general adaptation syndrome and the diseases of adaptation. J. Clin. End. 6:117-230.

Southwick, C.H. 1964. Peromyscus leucopus: An interesting subject for studies of socially induced stress responses. Science 142:55-56.

Sung, K.P., E.L. Bradley, and C.R. Terman. 1977. Serum corticosterone concentrations in reproductively mature and inhibited deermice (Peromyscus maniculatus bairdii). J. Reprod. Fert. 49:201-206.

Terman, C.R. 1965. A study of population growth and control exhibited in the laboratory by prairie deermice. Ecol. 46:890-895.

Terman, C.R. 1969. Weights of selected organs of (Peromyscus maniculatus bairdii) from asymptotic populations. Anim. Behav. 21:443-448.

Terman, C.R. 1973. Reproductive inhibition in asymptotic populations of prairie deermice. J. Rep. Fert. Suppl. 19:4555-461.

Terman, C.R. 1980. Behavior and regulation of growth in laboratory populations of prairie deermice. In Biosocial Mechanisms of Population Regulation, M.N. Cohen, R.S. Malpass, and H.G. Klein, ed., Yale Univ Press, New Haven, Conn., pp. 23-36.

To, L.P. and R.H. Tamarin. 1977. The relation of population density and adrenal gland weight in cycling and noncycling voles (Microtus). Ecol. 58:928-934.

Varon, H.H. and J.J. Christian. 1963. Effects of adrenal androgens on immature female mice. End. 72:210-222.

Wolfe, C.C. 1981. Population growth, social behavior, and selected organ weights in laboratory populations of Peromyscus leucopus noveboracensis. Masters Thesis, The College of William and Mary in Virginia, Williamsburg, Virginia.

Yeasting, R.A. 1986. Selected morphological aspects of human suprarenal glands. In The Adrenal Gland, P.J. Mulrow, ed. Elsivier Sci. Pub. Co., Inc. Amsterdam.

VITA

Peggy Alicia Rouleau

Born in Duluth, Minnesota, July 25, 1964. Graduated from Denfeld High School, Duluth, Minnesota, June 1982. Received a B.S.in Biology from James Madison University, May 1987. Entered the graduate program of the College of William and Mary in Virginia. Served as a graduate teaching assistant 1987-1989. Currently a candidate for the Master of Arts degree in Biology.