

CORRELATIONS BETWEEN SERUM CORTICOSTERONE LEVEL
AND REPRODUCTIVE FITNESS IN LABORATORY
POPULATIONS OF THE WHITE-FOOTED MOUSE
(PEROMYSCUS LEUCOPUS NOVEBORACENSIS)

A Thesis
Presented to
The Faculty of the Department of Biology of
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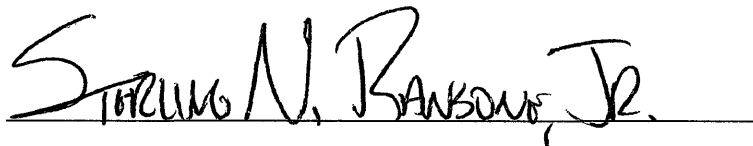
In Partial Fulfillment
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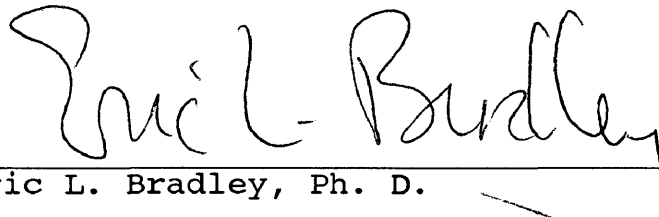
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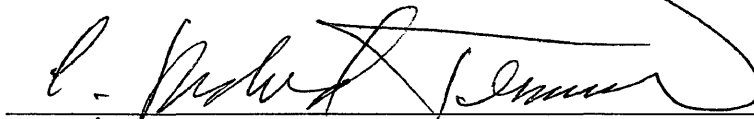


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ABSTRACT

The role of adrenal function in the reproductive inhibition process of laboratory populations of the white-footed mouse, (Peromyscus leucopus noveboracensis), was examined. Measurements were taken of body weights, reproductive organ weights, and paired adrenal weights. Serum concentrations of the adrenal steroid, corticosterone, were determined via radioimmunoassay. All data from reproductively inhibited animals were compared to values from laboratory reared control animals.

Data showed that paired testis, seminal vesicle, paired ovary, and uterus weights were significantly less in population animals than in their control counterparts. Paired adrenal weights showed no difference between control and population animals in either males or females. Population males showed a significant elevation in serum corticosterone concentrations over control levels. Population females showed no increase in serum corticosterone levels above control levels.

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INTRODUCTION

Densities in wild populations of some small mammals often fluctuate widely. This phenomenon is one which has been seen repeatedly, especially among microtine rodents such as lemmings (Elton, 1942; Curry-Lindahl, 1962; Christian, 1971; Krebs et al., 1973) and voles (Elton, 1942; Christian, 1971). Also, house mice (Evans, 1949; Pearson, 1963) and norway rats (Davis, 1953; Calhoun, 1962) exhibit outbursts of population size, though to a lesser degree. Indeed, outbreaks in rodent populations were recorded in biblical times, in the writings of Aristotle, and throughout the pages of European history (Krebs, et al. 1973).

The deermouse, Peromyscus maniculatus, rarely exhibits population outbreaks (Blair, 1940), and demonstrates much lower density fluctuations over a longer time than the animals mentioned above (Terman, 1966). A closely related animal, the white-footed mouse, Peromyscus leucopus, also shows a low average range of fluctuation, though the range is typically covered over a shorter time span (Terman, 1966). These two species show an intrinsic ability to regulate their population sizes to avoid

exceedingly high numbers, and stop growth well under the physical carrying capacities of their environments (Terman, 1966).

Suggested mechanisms for this intrinsic control of population size are a high mortality of young before reaching reproductive age and a slowing of reproductive rate for the overall population (Terman, 1965, 1966). In laboratory settings, populations of these animals have been shown to reach zero net population growth (asymptote), even when excess amounts of food and water are available (Terman, 1969). Cessation of reproduction in previously fertile adults is often seen, and young animals born into these populations frequently exhibit drastically smaller reproductive structures when compared to reproductively functional animals of the same age and weight (Terman, 1969). The same findings are also true in young animals from growing laboratory populations (Albertson, et al., 1975; Sung, et. al, 1977; Bradley and Terman, 1981a, 1981b, 1981c; Coppes and Bradley, 1984; Peebles et al., 1984; Pitman and Bradley, 1984; Kirkland and Bradley, 1986). The mechanism of this inhibition of reproductive development is a subject which has created much interest.

Christian (1950, 1971, 1975, 1980) patterned his endocrine based theory of population control after Selye's

General Adaptation Syndrome (for review, 1946), which describes an adrenal based physiological response to external stressors. It has been suggested that unseen forces due to crowding were acting on these animals which elicited an hypophysial-adrenal stress response. This response could involve an increase in serum levels of ACTH, glucocorticoids, or other adrenal steroids, ultimately resulting in reproductive inhibition.

Adrenal based physiological responses to stress have been demonstrated in a number of studies. In these investigations, either increased adrenal mass or increased serum glucocorticoid concentration has been positively correlated with population density in house mice (Christian 1955a, 1955b, 1956), lemmings (Andrews and Belknap, 1979), and voles (To and Tamarin, 1977).

In P. maniculatus bairdii, the prairie deer mouse, adrenocortical hyperfunction has been noted in inhibited population animals from growing laboratory populations, when compared with reproductively functional adults. Highly elevated serum concentrations of their predominant glucocorticoid, corticosterone, was readily apparent. However, no concurrent hypertrophy of the adrenal gland was observed (Sung, et al., 1977; Bradley and Terman, 1981a). (For reviews of various responses, see Andrews, 1970; Brain, 1971; Christian 1971, 1975, 1980; Terman

1980, 1987).

ACTH, the hypophysial regulator of adrenal function (Yeasting, 1986), is known to be elevated in stressed animals (Cook, et al, 1973), and is shown to cause adrenal hypertrophy P. maniculatus when administered exoginously (Coppes and Bradley, 1984). ACTH administration has also been shown to cause reproductive inhibition in males of various species (Christian et al., 1965; Paisley and Christian, 1972; Schaison, et al., 1978; Collu, et al., 1979). However, Coppes and Bradley (1984), did not show any differences in serum levels of ACTH between control and reproductively inhibited laboratory population animals.

The lack of hypertrophy of the adrenal glands in inhibited P. maniculatus , as well as the inhibited animals' lack of ACTH elevation, tends to rule out increased serum levels of ACTH as the primary cause of the inhibition process for that species.

Naloxone, an opiate receptor antagonist, has been shown to reverse ACTH induced reproductive inhibition in white-footed mice (Yasukawa, et al., 1978). This suggests a possible role for endorphins in the inhibition process. Pro-opiomelanocortin, a substance found in the vertebrate pituitary and hypothalamus, is a precursor to both beta-endorphin and ACTH, as well as several other peptide

hormones (Hadley, 1984). It is possible that the adrenal cortex in reproductively inhibited animals is being stimulated by an ACTH-like opiate fragment of this substance, resulting in hypersecretion of glucocorticoid, and resultant inhibition of the reproductive development process.

Hypothyroidism has been exhibited in inhibited P. maniculatus from laboratory populations (Peebles et al., 1984; Pitman and Bradley, 1984) and also in inhibited laboratory populations of P. leucopus (Peden, 1988). Pitman and Bradley (1984) suggested that elevated concentrations of serum corticosterone could, in the manner described by Kuhl and Ziff (1952) lead to reduced serum thyroid hormone concentrations through a lowering of thyrotropin secretion. Otsuki, et al. (1973) showed suppression of thyroid stimulating hormone (TSH) due to a reduction in thyrotropin releasing factor (TRF) caused by long term glucocorticoid treatment in humans. This, in turn, leads to a lowering of thyroid hormone secretion and any resultant metabolic effects. Therefore, elevation of serum glucocorticoid concentrations in inhibited animals could be causing the observed reduction in serum thyroxin levels.

Alternatively, Bradley and Terman (1981a) suggested that lowered serum levels of thyroid hormones could alter

the metabolic breakdown of adrenocorticoids and corticosteroid binding globulins (CBG). Miller et al. (1970) showed reduced levels of corticosteroid excretion in severely hypothyroid humans.

Serum testosterone levels have also been shown to be lower in inhibited P. maniculatus males than in their control counterparts (Bradley and Terman, 1981b). In the rat, elevated testosterone levels have been shown to suppress CBG binding ability (Gala and Westphal, 1965) and increase the breakdown rate of glucocorticoids by the liver (Troop, 1959). It can be safely assumed that the converse is also true.

With the decreased levels of both thyroid hormone (Peebles, et al., 1984; Pitman and Bradley, 1984) and testosterone (Bradley and Terman, 1981b) reported for inhibited P. maniculatus, additional CBG could be available to sequester adrenocorticoids in the bloodstream, adrenocorticoids would be broken down at a lowered rate, and the resultant total serum concentration of the corticosteroid would be elevated. This is another possible explanation for the mechanism of increased glucocorticoids found in P. maniculatus.

In laboratory pairs of P. leucopus, Haigh (1987) has shown that approximately 98% of female P. leucopus do not reproduce when in the presence of an adult female. This

is comparable to the 90% figure found by Terman (1969, 1973) for P. maniculatus in laboratory populations. P. leucopus has also been shown to undergo reproductive inhibition in laboratory populations (Wolfe, 1982).

Determination of basal glucocorticoid levels in wild P. leucopus would be difficult or impossible to determine due to increased adrenal secretions caused by the stresses of capture and handling. Therefore, laboratory populations of P. leucopus were used in this study.

The objective of this study was to assess the nature of adrenal function in the reproductive inhibition process in laboratory populations of P. leucopus. Through gravimetric analysis of reproductive structures and through the assay of serum concentrations of corticosterone, the study seeks to see if the paradox of adrenal hyperfunction with lack of hypertrophy is limited to P. maniculatus or if it is exhibited in other related species.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

All animals used in this study were White-footed mice (Peromyscus leucopus noveboracensis) obtained from an outbred laboratory colony.

Colony Establishment: Thirty-five wild-trapped animals were subjected to a two week quarantine period, then mated to opposite sexed P. leucopus derived from an existing outbred colony. Animal pairs were kept in one side of a two-chambered, wire-topped, opaque plastic enclosure, 12.8 X 27.8 X 14.5 c.m. on a side. Pine shavings, approximately 2-3 cm. deep, were used as bedding. Food (Prolab Rat, Mouse, Hamster 3000, Agway, Inc., Syracuse, NY) and tap water were available continuously. Animals were inspected at two week intervals and bedding was changed at that time. All pregnancies were noted and pregnant animals were checked daily thereafter for births. After one month, a second, marked female obtained from the colony was added to those pairs which had not produced young in order to stimulate

reproductive activity. When possible, sibling females were paired together and at all times at least one wild animal was present.

All young produced were weaned at twenty-one days of age and placed in cages with same-sex sibs. At 60 ± 3 days of age, these F1 animals were mated with opposite sex F1's which did not share common ancestors in the previous three generations. Only those animals produced from wild/colony pairings were used. The first thirty pair which proved to be reproductively functional were used to found ten populations. Subsequent pairs were assigned colony status.

Control Maintenance: F1 colony pair were used to produce the F2 control generation and used to produce an F2 control generation for comparison with the F2 population animals. Colony pregnancy checks were made every week and shavings were changed every two weeks. Pregnant animals were observed daily and the dates of birth of their pups were noted. At seven days after birth of the litter, the family unit was transferred to fresh bedding. The week delay was necessary to avoid infant mortality. At twenty one days, the young were weaned and the parents were moved to new bedding and cages. Each pup was placed by itself on one side of a two-chambered

plastic box. In the other chamber, an opposite sex non-sibling animal was placed. Each member of a "pair" was within ± 2 days of age of the other. Neither animal was able to see or touch the other.

At two weeks after weaning (35 days of age), each animal was switched to the opposite compartment of their nest box. The bedding was not changed, thus allowing contact between the animal and its partner's urine and fecal material, while avoiding bodily contact. This regimen was followed again at 49, and 63 days of age. At 70 days the animal was sacrificed and tissue was collected in the manner described below. If the ages of the pair were within one day of each other, the pair was sacrificed when the older animal reached 70 days. If the age discrepancy was two days, each was sacrificed on its seventieth day of age, thus allowing the younger animal two days to recover from the disturbance of its cage. One male, however, was sacrificed at 68 days due to extenuating circumstances.

Population Founding and Maintenance: Ten experimental populations were founded, each using three F1 pairs. The female of each pair was either pregnant or had produced one litter at the time of founding. Females which had given birth were separated from their partners

before the birth of the litter in order to avoid possible insemination at the post-partum estrus. Litters were not placed in the populations with their mothers and the first litters of females which were pregnant at the time of founding were removed at one week if they survived to that point. This was done to remove any discrepancies in prenatal environment of the population's F2 generation. The youngest ages at founding for any population ranged between 83 and 93 days of age for population number one. The oldest ages at founding for a population were between 127 and 161 days of age for population ten.

Each population was founded in a metal enclosure which consisted of a circular stainless steel base with a diameter of 1.5 meters and aluminum siding which was at least 68 c.m. or higher. Each enclosure was lined with dry, clean pine shavings for bedding and each contained eight 0.90 liter plastic nest boxes for shelter. Food and tap water were provided ad libitum. Each population enclosure was in one of three rooms, each with approximately 5 square meters of floor space. The light cycle consisted of 14 hours bright light (from four 40 Watt fluorescent tubes between 0700 and 2100h E.S.T.) and 10 hours complete darkness. The room temperature was regulated at 23 ± 3 C and air was exchanged 5 - 8 times per hour.

Population inspections were performed at two week intervals after the day of founding. Each consisted of identifying an animal by its toe-clip and assessing its reproductive state. Male animals were noted as having either scrotal or non-scrotal testes while female animals were evaluated for vaginal perforation, apparent pregnancy, and the size and development of the mammary glands. Additional comments regarding the health of the animal were also often made. Any births during the previous two week period were noted and the day of birth for surviving young was estimated. All young were toe-clipped during the next population check. No simultaneous births between producing females occurred, so litter and maternal identifications were seldom difficult.

Selection of Population Animals: When a given litter reached 68 days of age, an additional population inspection was performed. The first male and female captured from that litter which had never had scrotal testes or a perforate vagina, respectively, were marked on their tail with a non-toxic ultraviolet fluorescing dye (Blak-Ray Ink A-946, Ultraviolet Products, Inc. San Gabriel, CA.) to facilitate identification. These two animals were sampled two days later, at seventy days of age. Sampling took place between 1845h and 1915h. This

time was chosen to bracket the peak in ACTH concentration one hour before the onset of the dark period (Retiene, et al, 1968; Matsuyama, et al, 1971). Effort was made to avoid disturbing the population during the interim period between marking and sampling to allow any stress reaction caused by the population check to subside. A high intensity long wave ultra-violet light (Blak-Ray UVL-56) was used to illuminate the dye at time of sampling. The animal was identified then quickly captured and anesthetized using diethyl ether.

TISSUE SAMPLING

A ventral abdominal incision was made and the left renal artery was cut near the dorsal aorta. Blood was allowed to pool in the abdominal cavity and was removed using a sterile 1 ml plastic tuberculin syringe without needle (Scientific Products). All blood was collected within two minutes of initial disturbance of the population. After sampling, the blood was placed in individual 1.5 ml polypropylene microcentrifuge tubes (Scientific Products), allowed to clot for at least two minutes, and centrifuged in a Beckman Microfuge (Palo Alto, Ca) at 9000 x g for two minutes, to separate the serum from the cells. The serum was drawn off and frozen at below -20 C and then transferred after no more than

sixty days to a -70 C freezer until analyzed.

The body was weighed to the nearest 0.1g using an Ohaus (Florham Park, NJ) Dial-O-Gram 2610g balance. Both adrenal glands were removed, the reproductive apparatus was grossly dissected out and the body was preserved in a 10 % buffered formaldehyde solution. Adrenal glands were placed in a 2.5% glutaraldehyde/ 2% formaldehyde pre-fixative overnight and then transferred to a 10% buffered formaldehyde solution. Reproductive structures were placed in 10% buffered formaldehyde directly. All adrenal glands and reproductive structures were allowed to fix for at least thirty days prior to removal of fat and fine dissection. Paired adrenal glands were weighed to 0.01 mg using a Sartorius type 2404 balance. Paired testis, seminal vesicles, paired ovaries, and uterus were all weighed to 0.1 mg using a Sartorius type 1801 electronic scale interfaced with a Commodore 64 computer.

RADIOIMMUNOASSAY (RIA) PROCEDURE FOR CORTICOSTERONE

Corticosterone antisera (B3-163, lot 76) was purchased from Endocrine Sciences (Tarzana, California). 1,2,6,7, tritiated corticosterone (TRK 56, batch 52) was purchased from Amersham International, Amersham, U. K.

Validation studies were performed prior to the actual experimental assay. Antibody was diluted in duplicate at

1:50, 1:100, 1:200, 1:400, 1:800, and 1:1600 levels with 10,000 counts per minute per tube after reconstituting the lyophilized sample according to its specifications. At this number of counts, thirty percent non-binding of the total counts added was reached at a 1:85 dilution which mirrored the specifications given by the manufacturer.

On the basis of this successful check, 10,000 counts per tube was judged to be a good level of activity for each 1:85 dilution of antibody. Validation of the experimental method was performed using standard serum pools from female P. leucopus, female P. maniculatus, and stressed female P. leucopus. Each pool was assayed at 1, 0.5, 0.25, and 0.125 times the natural concentration. When comparing the assay values for each serial dilution against the expected values and against values for the standard curve, all pools diluted linearly, with no significant differences in the slope of any curve.

Four separate assays were run, each having a random distribution of male or female and population or control sera. Standards for each run were assayed in triplicate by adding 0, 0.0625, 0.125, 0.25, 0.5, and 1.0 ng of authentic corticosterone (Schwartz-Mann, Orangeburg, NY) diluted with redistilled methanol. Duplicate standards were also run at the 0.625, 1.25, and 2.50 ng level. All standards were placed in 12 X 75 mm polystyrene conical

bottom culture tubes (Scientific Products). The methanol was then evaporated using nitrogen and each standard was brought to a constant volume with 70 ul of borate buffer (0.05 M, pH 8.0).

The collected serum samples, along with samples from a pool of standard female P. leucopus sera were prepared by diluting 20 ul of sera with 180 ul borate buffer (0.05 M, 8.0 pH) in 12 X 75 polystyrene tubes. After brief vortex mixing, 20 ul aliquots were transferred to duplicate 12 X 75 tubes and extracted according to the method of Sheldon and Coppinger (1977) by adding 50 ul of Subtilisin, Carlsburg (No. P-5380, Sigma Chem. Co., St. Louis, MO) which was diluted to 8 units of activity per sample in 0.05 M borate buffer. Each sample was incubated in a bath for one hour at 37 C and then the enzyme was inactivated by incubation in a water bath at 89 C for 3.5 minutes. The tubes were then quickly cooled under running tap water.

Dilute antisera was prepared by diluting 1 part reconstituted antisera with 80 parts borate buffer (0.05 M, pH 8.0), 2 parts 10% bovine serum albumen (No. A-7888, Sigma; in borate buffer), and 2 parts 2.5% bovine gamma globulin (No. G-5009, Sigma; in normal saline). 10,000 counts per sample tritiated corticosterone were also added to the mixture. Each extracted serum sample

received 200 ul of the dilute antibody mixture, was parafilm, vortexed, and incubated at 4 C for 16 hours.

In each tube, the antigen-antibody complex, was precipitated using 250 ul of saturated ammonium sulfate. The tubes were covered with parafilm and centrifuged at 9,000 x g at 4 C for 20 minutes in a Sorvall RC-5B (Dupont, Inc., Newtown CT) centrifuge using an HS-4 head. Four hundred ul of the supernatant was pipetted into a plastic scintillation vial (Kimble) and 10 ml of Beckman Ready-Solv EP scintillation cocktail was added. Each vial was vortex mixed, and placed in a Beckman LS-3133T liquid scintillation counter, and counted to a 1% error. All repetitive pipetting in the study was done using a Micromedic Systems model 25004 high-speed automatic pipette.

STATISTICS

All hormone data collected were in counts per minute (cpm) and were then corrected to disintegrations per minute (dpm) with the external standards channels ratio value (ESCR). The sample dpm were converted to percent of total dpm added and then further transformed to a standard logit value to linearize the results. Unknown sample concentrations were calculated using the regression equation derived from the values obtained for the standard

curve of each individual run. Values were expressed in ng/ml of sera. As a measure of inter-assay variability, samples from a pool of female P. leucopus standard sera were run in each assay.

Statistics were run on the data using a model one one-way ANOVA. The resultant data showed heterogeneous variance, so the nonparametric Mann-Whitney U test was used. Additionally, the small sample size of this study, as well as the non-random selection of population animals, made nonparametric analysis seem appropriate. All tests run were contained in the SPSS-X statistics package (SPSS, Inc., Chicago Ill.) on a Prime model 9955 computer (Prime Computer, Inc., Framingham, Mass.). Standard curve regression lines were compared using the (BMDP) BMD1R multiple-regression program from Biomedical Computer Programs (University of California, Berkley) on an NAS 6660 computer. No significant differences were seen between the four runs (Figure 1). The limits of detection for each assay were calculated from the variation of the borate buffer control tubes. The mean limit was determined to be 0.46 pg per tube.

Statistics were performed on an animal's data only if values for every characteristic studied were available from that individual animal.

All data were reported as mean \pm the standard error

of the mean. In all cases, the probability was reported as significant if P was less than 0.05. All correlation data was analyzed using Spearman's nonparametric ranked correlation test.

RESULTS

Weight comparisons between males and females

Control males were significantly ($P < 0.001$) heavier than control females (Tables 1 and 2). No such difference was seen between the sexes in population animals (Tables 1 and 2). No significant difference was seen in absolute adrenal weight between males and females in either control and population situations (Tables 1 and 2). Control females did, however, exhibit a significantly ($P < 0.04$) heavier mean relative adrenal weight (65.0 ± 3.84 mg/100g body weight) than control males (53.7 ± 2.68 mg/100g). This difference was not seen between population females (59.5 ± 5.23 mg/100g) and population males (53.4 ± 4.18 mg/100g).

Weight differences between control and population animals

Population male mean body weight was significantly ($P < 0.001$) lighter than that for their their control counterparts (Table 1). No significant difference was seen between population and control females (Table 2). A

significant ($\underline{P} < 0.004$) negative correlation was exhibited between corticosterone concentrations and body weight in males when population and control data were combined (Appendix 1). In females no such correlation was evident (Appendix 1). When each sex was divided by experimental groups, no significant correlation was seen in any group (Tables 4 and 5).

Absolute adrenal weight was not significantly different between control and population animals in males (Table 1) or in females (Table 2). Relative adrenal weights were, likewise, not different. A significant ($\underline{P} < 0.03$) correlation existed between concentration of corticosterone and adrenal weight in control males, but in population males the relationship was random (Table 4). Comparisons using relative adrenal weights also reflected this. Females showed no correlation between adrenal weight and corticosterone concentration (Table 5).

In males, both paired testis weight and seminal vesicle weight were significantly (both $\underline{P} < 0.001$) lighter in the population animals than in controls (Table 1). A trend was seen in corticosterone concentrations and seminal vesicle weight ($\underline{P} < 0.07$) and negative relationships were seen in correlations between serum corticosterone concentration and testis weight ($\underline{P} < 0.12$) when population and control data was pooled (Appendix 1),

but when population and control values were separated, all hints at correlation disappeared. Correlations between paired testis and seminal vesicle weights in both control and population mice were significant ($\underline{P} < 0.02$ and $\underline{P} < 0.001$, respectively; Table 4).

In females, both paired ovary weight and uterus weight were significantly ($\underline{P} < 0.001$ for each) lighter in population animals than in control animals (Table 2). In control females, ovary weight was highly correlated with adrenal weight ($\underline{P} < 0.001$), yet in population females, no such correlation existed (Table 5).

Hormone differences between population and control animals

A significant ($\underline{P} < 0.04$) elevation was seen in the serum corticosterone concentrations of population males when compared to control males (Table 3). No significant difference was observed between the experimental groups of females (Table 3). Differences between the sexes in corticosterone concentration were not significant in either control or population animals (Table 3).

Table 1.- Body, testis, seminal vesicle, and adrenal weights in control and population males. Values are mean \pm SEM.

Treatments	Body weight (g)	Testis weight (mg)	Seminal vesicle weight (mg)	Adrenal weight (mg)
Control males (n = 19)	20.0 \pm 0.43	273.3 \pm 14.13	112.6 \pm 7.56	10.64 \pm 0.468
Population males (n = 10)	16.8 *** \pm 0.71	84.3 *** \pm 20.77	11.0 *** \pm 4.16	9.00 n.s. \pm 0.861

*** $P < 0.001$

Table 2.- Body, ovary, uterus, and adrenal weights in control and population females. Values are mean \pm SEM.

Treatments	Body weight (g)	Ovary weight (mg)	Uterus weight (mg)	Adrenal weight (mg)
Control females (n = 18)	17.7 \pm 0.61	11.6 \pm 0.95	49.5 \pm 5.36	11.39 \pm 0.678
Population females (n = 12)	16.6 n.s. \pm 0.68	4.5 *** \pm 0.66	11.8 *** \pm 2.58	9.78 n.s. \pm 0.832

*** $\underline{P} < 0.001$

Table 3.- Serum corticosterone concentrations (ng/ml) in control and population animals of both sexes. Values are mean \pm SEM.

	Males	Females
Control	145.3 \pm 14.41 (n = 19)	189.1 \pm 19.25 (n = 18)
Population	201.9 \pm 16.05 * (n = 10)	168.5 \pm 16.35 n.s. (n = 12)

* $\underline{P} < 0.05$

Table 4.- Spearman's ranked correlation coefficients and P values for control and population males.

	Testis weight	Seminal vesicle weight	Adrenal weight	Serum corticosterone concentration
CONTROL MALES				
Body weight	$r = 0.3993$ $\underline{P} < 0.090$	0.2922 0.225	-0.0246 0.920	-0.2735 0.257
Testis weight		0.5456 0.016	0.2071 0.395	0.2062 0.397
Seminal vesicle wt.			0.0351 0.887	0.0351 0.887
Adrenal weight			0.4697 0.042	0.4697 0.042
POPULATION MALES				
Body weight	$r = 0.4182$ $\underline{P} < 0.2289$	0.4667 0.174	0.5152 0.128	-0.3333 0.347
Testis weight		0.9173 0.001	0.2242 0.533	-0.0424 0.907
Seminal vesicle wt.			0.2971 0.405	-0.1152 0.751
Adrenal weight			0.0667 0.855	0.0667 0.855

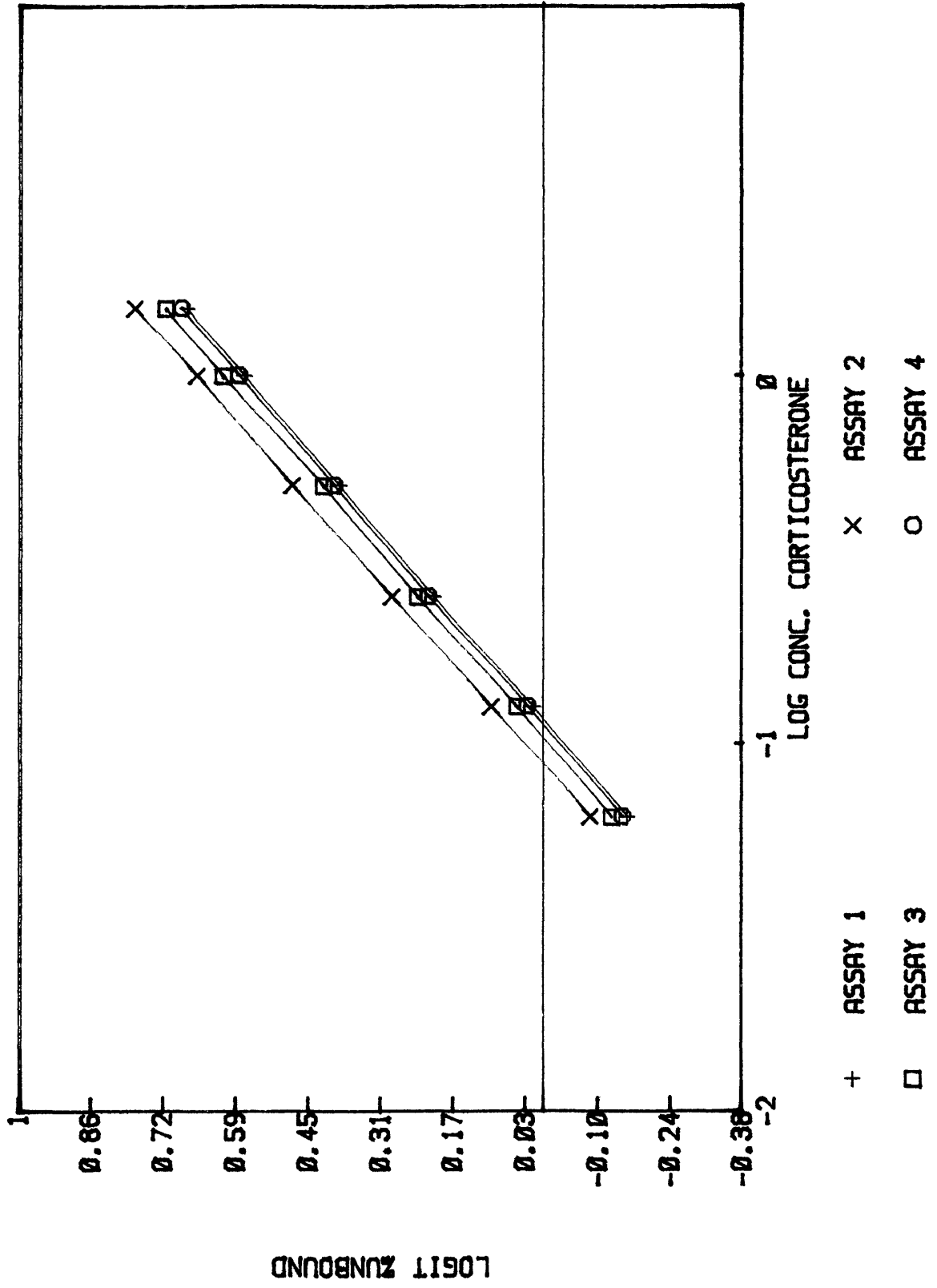
Table 5.-Spearman's ranked correlation coefficients and P values for control and population females.

	Ovary weight	Uterus weight	Adrenal weight	Serum corticosterone concentration
CONTROL FEMALES				
Body weight	$r = 0.2838$ $\underline{P} < 0.254$	0.2301 0.358	0.1982 0.430	0.1269 0.616
Ovary weight		0.3478 0.157	0.7806 0.001	-0.3932 0.106
Uterus weight			0.2808 0.259	-0.4861 0.041
Adrenal weight				0.3841 0.116
POPULATION FEMALES				
Body weight	$r = 0.3147$ $\underline{P} < 0.319$	0.0315 0.923	0.1888 0.557	-0.2937 0.354
Ovary weight		0.6305 0.028	0.0350 0.914	-0.0629 0.846
Uterus weight			-0.0946 0.770	-0.3222 0.307
Adrenal weight				-0.5524 0.063

Figure 1.

Standard Curves Derrived from the
Corticosterone Radioimmunoassay

FIGURE 1



DISCUSSION

All P. leucopus used in this study were 69 \pm 1 days of age, 8 to 10 days older than the sixty day or less minimum age for reproduction reported for P. maniculatus by Terman (1987). The body weights of both the males and females in this study were comparable to reported normal, adult weights. Corpora hemorrhagica were found in several control females, indicating ovulation. Therefore, it is believed that the seventy day old experimental animals were valid representatives of adult P. leucopus.

At least one animal was sampled in seven of the ten populations. Of the animals which reached seventy days of age, 92.9% were reproductively inhibited as evidenced by never having had scrotal testes or a perforated vagina. When the young born into populations were divided by sex, 29 of 33 males (87.9%) and 36 of 37 females (97.4%) were judged to be reproductively inhibited. This is approximately the same as the 98% inhibited figure reported for female P. leucopus in laboratory pairs reported by Haigh (1987). The 92.9% overall inhibition is comparable to the 90% figure for laboratory populations of P. maniculatus reported by Terman (1969, 1973). In each

available population litter, those animals which met sampling criteria but were not sampled were either removed to a separate study at 71 days of age or were left in the population.

Male population animals were sampled from six separate populations; females were taken from seven populations. Possible inter-population differences were tested using the nonparametric Kruskal-Wallis one-way analysis of variance. No significant differences were noted across populations in any of the variables measured for either sex.

Weight comparisons between males and females

Control males were significantly ($P < 0.001$) heavier than control females (Tables 1 and 2). This sex-based discrepancy in weights has also been seen in P. maniculatus (Terman, 1969; Gardner and Terman, 1970; Bradley and Terman (1981a, 1981c). However, Sung, et al. (1977), showed no significant weight differences between the sexes in much older deermice. The present data seem to correspond with the hypothesis of Bradley and Terman (1981a) that sex-based body weight differences are greatest in the normally developing young adult control animals. No difference in body weight was seen between

the sexes in population animals (Tables 1 and 2).

Body and Organ Weight Comparisons Between Control and Population Animals:

Population male mean body weight was significantly ($P < 0.001$) less than male controls. No significant difference in body weight was seen between females. These results are similar to those reported by Bradley and Terman (1981a) for P. maniculatus. This suggests that for female P. leucopus, the reproductive inhibition process is not merely a reduction in the overall rate of growth for the animal.

A significant ($P < 0.004$) negative correlation was exhibited between corticosterone concentrations and mean body weight in all males taken as one group, yet in all females no such correlation was evident. When each sex was divided according to experimental group, no significant correlation was seen in any group. The difference in body weights between population males and control males, explains the correlation in the grouped data.

In population males, both paired testis weight and seminal vesicle weight were significantly (both $P < 0.001$) reduced compared with controls. This greatly expands

Wolfe's (1982) report of slight reproductive inhibition of young in P. leucopus from laboratory populations.

A trend was seen in correlations between serum corticosterone concentration and seminal vesicle weight ($P < 0.07$) when the data from control and population males were pooled, but when population and control weights were separated, all hints of correlation disappeared. Similarly, negative relationships were seen between serum corticosterone concentration and testis weight ($P < 0.19$). Correlations between testis and seminal vesicle weights in both control and population mice were significant ($P < 0.02$ and $P < 0.001$, respectively). This suggests that a linkage exists in the growth and development of the reproductive structures in P. leucopus.

In population females, both paired ovary weight and uterus weight were significantly (both $P < 0.001$) reduced when compared to control animals. Control female ovary weight was highly correlated with adrenal weight ($P < 0.001$), yet in population animals, no such correlation existed. Again, this suggests a link between development of reproductive structures.

Absolute adrenal weight was not significantly different between control and population males (Table 1) or control and population females (Table 2). A trend ($P < 0.10$) seen in males and a tendency ($P < 0.123$) in females

indicated that the control paired adrenal weights tended to be heavier than the population paired adrenal weights. Relative adrenal weights were, likewise, not different, but in this case no trends were evident between groups in either the males or the females.

These findings are similar to those from studies which showed no adrenal hypertrophy in P. maniculatus from either growing or asymptotic laboratory populations (Bronson and Eleftheriou, 1963; Terman, 1966; Sung, et al. 1977; Bradley and Terman, 1981a). Similar results were also found in crowded natural populations of P. maniculatus (using relative weights, McKeever, 1964), and voles (Chitty, 1961). Both Sung et al. (1977) and Bradley and Terman (1981a) showed significantly smaller adrenal glands in inhibited animals and increased serum corticosterone concentration, much like the findings of this study.

These results are different from findings in some other species of small rodents. Density dependent adrenal hypertrophy has been reported in albino Mus of the NMRI strain (Christian, 1955a, 1955b, 1956), in C57BL/105 Mus (Bronson and Eleftheriou, 1963), and in spiny field mice (Purushotham, et al., 1978). In a correlative study using two widely divergent species from high density populations, Andrews and Belknap (1979) showed a much

stronger correlation between adrenal weight and density in lemmings ($r = .90$) than was exhibited in deer mice ($r = .45$).

Direct comparisons of gravimetric data from laboratory studies and field studies may not always be appropriate because seasonal fluctuations in adrenal weight have been observed in P. maniculatus (McKeever, 1964; Andrews, et al., 1975), and in voles (Chitty, 1961). In several laboratory studies, (Sung, et al., 1977; Bradley and Terman, 1981a; Coppes and Bradley, 1984; Kirkland and Bradley, 1986), P. maniculatus adrenal weights were, on average, three to four times lighter (average values: control males, 2.95 mg; control females, 2.81 mg; population males, 2.48 mg; population females, 2.42 mg)

than the adrenal weights found in P. leucopus in this study. A comparative histological evaluation of the adrenal tissues from both species may explain this discrepancy in mean adrenal weight.

A significant ($P < 0.05$) correlation existed between the serum concentration of corticosterone and adrenal weight in control male P. leucopus in this study, but in population males the relationship was random. Comparisons using relative adrenal weights were also random. Females showed no significant correlation between adrenal weight

and corticosterone concentration.

Corticosterone antibody

The validation study done prior to assay of experimental sera confirmed that corticosterone (4-pregnen-11beta,21-diol-3,20-dione) was the substance being measured. The B3-163 antisera (Endocrine Sciences, Tarzana, CA) used was highly specific, and much less cross-reactive than that used by Bradley and Terman (1981a) in their P. maniculatus serum radioimmunoassay. The two major cross-reactive compounds for B3-163 are deoxycorticosterone (DOC) and 5-beta-pregnanedione, with reported cross-reactivities of 4% and 1%, respectively. All other steroid compounds reported were less than 1% crossreactive. DOC is an adrenal steroid and a precursor to corticosterone, so if any were present in the sample, 4% would be measured as corticosterone. This crossreaction is potentially of some concern, because the possibility of differential DOC product concentration between population and control animals has not been investigated.

Serum corticosterone differences between population and control animals

Serum corticosterone concentrations in population

males were significantly ($P < 0.04$) higher than in the control males. This is similar to the report by Sung, et al. (1977) and Bradley and Terman (1981a) in P. maniculatus, though some differences were noted. Basal concentrations in the control P. leucopus males were over twice as high as the mean level (68.8 ng/ml) reported for P. maniculatus males by Bradley and Terman (1981a). This difference is thought to be real because a two-fold difference was also measured between the two species in the protocol validation done as part of this study. In the population setting, the serum corticosterone concentration in male P. leucopus sera were much lower than the mean level (348.3 ng/ml) for P. maniculatus reported by Bradley and Terman (1981a).

In females, no significant differences were seen in serum corticosterone levels between population and controls. In contrast, Sung, et al. (1977) and Bradley and Terman (1981a) reported elevated serum corticosterone levels in population female P. maniculatus. In control females, P. leucopus serum corticosterone concentrations were approximately the same as those reported for P. maniculatus (178.3 ng/ml) by Bradley and Terman (1981a). In population females, P. leucopus sera was roughly three times lower in corticosterone concentration than that reported for P. maniculatus (326.3 ng/ml) by Bradley and

Terman (1981a).

Control females tended ($P < 0.09$) to have higher serum levels than control males. No significant difference was seen between sexes in the population animals. Bradley and Terman (1981a) reported a significant difference ($P < 0.001$) between sexes in control P. maniculatus with females being higher. Sung, et al., however, did not show any significant differences between sexes in their study. Kitay et al. (1971) showed an increase in corticosterone levels in female rats during proestrus. It may be that cyclic variation among control females in this study elevated the mean serum corticosterone concentration reported.

The higher serum corticosterone concentrations found in male population animals could indicate a greater sensitivity to population conditions than that exhibited by females. Incidental observation of the populations suggested a greater level of aggressiveness in males than was seen in females. Typically one, and occasionally two, of the founding males in each population would be found dead as a result of fighting, within the first two weeks after population establishment. Females were seldom observed fighting, and no founding females were dead at the end of the study. Wolfe (1982), also reported high founding male mortality and suggested an inverse

relationship between aggression and reproduction in P. leucopus.

Conclusions

Although P. leucopus serum corticosterone concentrations are about two times the reported corticosterone levels of P. maniculatus, the three to five fold increase in serum corticosterone concentration of reproductively inhibited population P. maniculatus is not observed in P. leucopus. Inhibited male P. leucopus from laboratory populations are significantly increased, but are only 1.3 times as high as control males. Population females show no increase in serum corticosterone concentration. This is in conjunction with significantly larger adrenal glands in P. leucopus. Control P. maniculatus adrenal weights are 1/4 to 1/3 the size of P. leucopus adrenal weights but in neither species is there an increase in adrenal weight in inhibited population animals -- indeed, in P. maniculatus the absolute and relative adrenal weights are decreased and in P. leucopus there is no significant difference. Thus, the stress induced, hypophysial-adrenal based theory of population control (Christian, 1950, 1971, 1975, 1980; Christian et al., 1965) seems to have even less support in P. leucopus than in P. maniculatus.

APPENDIX 1.

Spearman's ranked nonparametric correlation coefficients and P values for experimental males and females; control and population values combined.

Males		Testis weight	Seminal vesicle weight	Adrenal weight	Serum corticosterone concentration
Body weight	$r = 0.6766$ $\underline{P} = 0.001$	0.6581 0.001	0.3243 0.086	-0.5324 0.003	
Testis weight		0.8670 0.001	0.3759 0.044	-0.2513 0.189	
Seminal vesicle			0.3759 0.044	-0.3423 0.069	
Adrenal weight				0.0793 0.682	
Females		Ovary weight	Uterus weight	Adrenal weight	Serum corticosterone concentration
Body weight	$r = 0.2915$ $\underline{P} = 0.118$	0.1906 0.313	0.2822 0.131	-0.0358 0.851	
Ovary weight		0.7830 0.001	0.5240 0.003	-0.1115 0.558	
Uterus weight			0.2949 0.114	-0.2071 0.272	
Adrenal weight				-0.3518 0.057	

APPENDIX 2.

BASIC program for the computation of per-tube corticosterone values

```

10 DEFINE FILE #1='DPMS',ASC
20 C=0
30 T=0
40 T1=0
50 INPUT "PLEASE ENTER AVERAGE ADJUSTED TOTAL DPMS > ": B1
60 PRINT
70 PRINT " # ESCR %EFF CPMS DPMS %UNbd LOGIT CONC B "
80 PRINT " "
90 PRINT
100 FOR I=1 TO 46
110 READ A,B
120 C=C+1
130 T=T+A
140 P=1.05*A
150 P1=P-.299
160 T1=T1+P1
170 D=B/P1
181 B2=D/B1
192 U=1-B2
200 U2=B2/U
210 IF U2<0 THEN DO
220 U2=1
230 DO END
240 L1=LOG(U2)
250 L2=LOG(10)
260 L=L1/L2
270 F=L-.66309
280 Y=F/.62907
290 Z=10^Y

```


APPENDIX 3.

SPSS-X file for computing descriptive statistics

```
TITLE Master statistics file
SUBTITLE Peromyscus leucopus noveboracensis
SET WIDTH=80
DATA LIST FREE/ TRT SEX BOD ADR RADR TEST SEMV OV UT CONB ID
VARIABLE LABELS TRT 'TREATMENT'
SEX 'SEX'
BOD 'BODY WT.'
ADR 'ADRENAL WT.'
RADR 'REL. AD. WT.'
TEST 'TESTIS WT.'
SEMV 'SEM. VES. WT.'
OV 'OVARY WT.'
UT 'UTERUS WT.'
CONB 'CONCENTRATION B'
ID 'IDENTIFICATION'
VALUE LABELS TRT 1 'CONTROL'
                2 'POPULATION'
VALUE LABELS SEX 1 'MALE'
                2 'FEMALE'
SPLIT FILES BY <<>>
<<test to be run>>
<<options, etc. >>
BEGIN DATA
```

1	2	17.90	.00921	51.453	0	.00305	0	.0077	1.48627	720
1	1	19.65	.00760	38.677	.1321	0	.0268	0	.22385	760
1	1	17.85	.01217	68.179	.2463	0	.0802	0	.5195	699
1	2	17.10	.01113	65.088	0	.01515	0	.0655	.6127	725
1	1	20.40	.01451	71.127	.2013	0	.0732	0	1.16215	699
1	2	20.00	.01178	58.900	0	.01485	0	.0817	.1038	727
1	1	24.00	.01058	44.083	.3014	0	.1248	0	.5958	699.1
1	2	19.70	.01114	56.548	0	.0081	0	.0538	.79645	727.1
1	1	17.90	.01352	75.531	.2192	0	.1293	0	.6675	703
1	1	18.20	.01254	68.901	.31645	0	.1116	0	1.2101	736
1	2	17.00	.01248	73.412	0	.0131	0	.0306	.4295	703
1	1	20.20	.01247	61.733	.3391	0	.1216	0	.82995	736.1
1	2	17.70	.01792	101.243	0	.0139	0	.0643	.84265	703.1
1	1	20.90	.01007	48.182	.3732	0	.1195	0	.95125	736.2
1	2	14.80	.00902	60.946	0	.0083	0	.0494	.69155	651
1	1	17.70	.01058	59.774	.2483	0	.0934	0	1.0733	651
1	2	25.70	.01513	58.872	0	.0161	0	.0460	1.5506	736
1	1	19.50	.01277	65.487	.36535	0	.15705	0	.97227	737
1	2	14.00	.00878	62.714	0	.0071	0	.02295	1.2792	651.1
1	1	23.60	.01236	52.373	.2938	0	.1259	0	.5663	660
1	2	18.20	.00940	51.648	0	.0120	0	.0621	1.1677	701
1	1	18.80	.00777	41.329	.25105	0	.1035	0	.7869	701
1	2	16.10	.00735	45.652	0	.0065	0	.0294	1.65837	660
1	2	19.00	.00972	51.158	0	.0115	0	.0764	.9930	660.1
1	1	19.85	.00887	44.685	.2180	0	.1232	0	.2403	748
1	1	18.20	.00772	42.418	.2385	0	.1218	0	1.16215	748.1
1	2	16.70	.01559	93.353	0	.0203	0	.02985	.94525	750
1	1	20.70	.01002	48.406	.25505	0	.0978	0	.57445	750
1	2	19.25	.00777	40.364	0	.0109	0	.0270	1.26145	748
1	1	19.60	.00967	49.337	.2671	0	.09775	0	.6506	748.2
1	2	16.40	.01178	71.829	0	.0112	0	.05865	.69585	750.1
1	1	23.10	.00907	39.264	.3439	0	.1351	0	.2435	758
1	2	15.30	.01278	83.529	0	.0136	0	.0372	.56685	710
1	1	19.10	.00937	49.058	.2580	0	.1108	0	.49625	747
1	2	17.60	.01412	80.227	0	.0129	0	.05335	1.05937	758

1	1	20.10	.01048	52.139	.3247	0	.18535	0	.8805	758.1
1	2	15.80	.00990	62.658	0	.0106	0	.0944	.8779	747
2	2	18.20	.01000	54.945	0	.00725	0	.0091	.97385	207
2	1	17.60	.01215	69.034	.0396	0	.0037	0	1.3182	115
2	2	14.10	.01258	89.220	0	.0090	0	.0198	.9768	314
2	1	14.10	.00590	41.844	.0362	0	.0018	0	.83115	609
2	2	17.20	.00772	44.884	0	.0041	0	.0091	.72605	608
2	1	17.50	.00993	56.743	.20415	0	.0242	0	1.2013	307
2	2	19.50	.01290	66.154	0	.0058	0	.01035	.3576	312
2	1	19.10	.00578	30.262	.0575	0	.0040	0	.93365	613
2	2	14.30	.00677	47.343	0	.0015	0	.00365	1.29745	612
2	1	19.60	.01062	54.184	.1074	0	.0137	0	.4717	121
2	2	19.60	.01394	71.122	0	.0032	0	.0055	.46895	120
2	1	14.60	.00884	60.548	.0523	0	.0025	0	1.16063	713
2	2	12.80	.00912	71.250	0	.0028	0	.0045	1.0480	712
2	1	16.80	.01003	59.702	.0254	0	.0016	0	1.05905	811
2	2	20.00	.00864	43.200	0	.0046	0	.0062	1.14295	808
2	2	16.40	.00721	43.963	0	.0063	0	.0207	1.05305	614
2	2	16.25	.00687	42.277	0	.0036	0	.0103	.73795	910
2	1	13.30	.00740	55.639	.0768	0	.0116	0	1.28795	915
2	2	15.60	.00735	47.115	0	.0040	0	.0345	.6514	914
2	1	16.10	.00586	36.398	.0462	0	.00485	0	.92355	815
2	1	19.30	.01345	69.689	.19785	0	.0425	0	.9055	317
2	2	15.50	.01431	92.323	0	.0014	0	.0085	.67785	316

END DATA

FINISH

APPENDIX 4.

(BMDP) BMD1R multiple regression program used for
the comparison of regression lines between runs

```
//#REGR JOB (0000,WBIG,1,4),SNR,NOTIFY=WBIGSNR
/*ROUTE PRINT U4
// EXEC BIMED,PROG=BMDPIR
//FT08F001 DD DSN=WBIGSNR.REGRESS.PROG.DATA,DISP=SHR
PROBLEM TITLE='DOSE RESPONSE EXAMPLE'./
INPUT UNIT = 8.
    VARIABLE = 3.
    FORMAT = '(F1.0,1X,F5.4,1X,F6.4)'./
VARIABLE BLANKS = MISSING.
    NAMES = GROUP,DOSE,RESPONSE.
    GROUPING = GROUP./
TRANSF DOSE = LOG(DOSE)./
GROUP CODE(1) = 1,2,3,4. NAME(1) = CURVE1,CURVE2,CURVE3,CURVE4./
PLOT RESIDUALS./
REGRESSION TITLE = 'DOSE RESPONSE'.
    DEPEND = 3.
    INDEPEND = 2./

END/
FINISH/
/*
//
```

APPENDIX 5.

Regression Equations from the
Corticosterone Radioimmunoassay

Assay number	Regression Equation
1	$y = (.60717 \pm .02319) x + (.57020 \pm .01540)$
2	$y = (.62907 \pm .02052) x + (.66390 \pm .01247)$
3	$y = (.61611 \pm .03853) x + (.60796 \pm .02739)$
4	$y = (.61396 \pm .02838) x + (.57530 \pm .02838)$

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