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Serum Corticosterone Concentrations in Reproductively Mature and Inhibited Deermice (Peromyscus maniculatus Bairdii) from Experimental Laboratory Populations

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SERUM CORTICOSTERONE CONCENTRATIONS IN REPRODUCTIVELY MATURE AND INHIBITED DEERMICE (*PEROMYSCUS MANICULATUS BAIRDII*) FROM EXPERIMENTAL LABORATORY POPULATIONS

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Abstract

Reproductive maturation and function are significantly inhibited in Peromyscus in freely growing laboratory populations supplied with surplus food and water. It has been suggested that such inhibition is due to undefined stresses promoting an increased adrenocorticotropin secretion which interferes in some way with the reproductive process. In the present study serum corticosterone concentrations were measured in laboratory control and population inhibited deermice to see if there was any correlation between adrenal activity and reproductive inhibition.

Males and females from two growing and one stable (asymptotic) population had significantly smaller reproductive organs (seminal vesicles, testes, uteri, ovaries) than controls maintained outside the population. Likewise the weights of the adrenal glands of population mice averaged smaller and in some comparisons significantly smaller than controls. The mean serum corticosterone concentrations, however, of mice in the two growing populations tended to be higher than control values and significantly higher for the asymptotic population. These data suggest that adrenal function may be associated with the reproductively inhibited condition, but not in a direct or dose response fashion and that adrenal hyperfunction in this species may not be reflected in adrenal hypertrophy.

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INTRODUCTION

Growth of experimental laboratory populations of prairie deermice (Peromyscus maniculatus bairdii) is controlled under conditions in which excess food and water are available. Generally speaking, cessation of growth occurs at widely different levels in populations maintained under identical conditions of the physical environment, and has been shown to be achieved by one of two means; either failure of young to survive or by cessation of reproduction (Terman 1965, 1968, 1969a, 1969b, 1973a, 1973b).

Previous work to define the physiological bases of population regulation has focused on measures of adrenal hypertrophy (Southwich & Bland, 1959; Christian, 1956; Louch, 1956; Bronson & Eleftheriou, 1963), reproductive organ atrophy (Christian, 1956, 1963, 1967; Thiessen & Rogers, 1961; Terman, 1969b), and decreased litter survival and diminished pregnancy and birth rates (Southwick, 1955; Christian, 1956, 1963; Thiessen & Rogers, 1961). Some of these observations led to the proposal that crowded animals were responding to some undefined "stress" by increased adrenocorticotrophic hormone (ACTH) secretion, and that it was ACTH that was responsible for the observed reproductive inhibition, either through a direct inhibition of gonadotrophin secretion or indirectly through increased corticoid production (Christian, 1963; Christian & Davis, 1964). Early support for this interpretation was derived

primarily from the correlation between adrenal hypertrophy and increased population density in Microtus and Mus, and the observation that high doses of ACTH inhibit sexual maturation of prepuberal female housemice (Mus musculus) (Christian, 1964a).

Adrenal hypertrophy, however, has not been detected in Peromyscus species even though drastic reproductive inhibition has been recorded in laboratory populations (Terman, 1969b; Christian, 1971; Albertson, 1974). Certainly, hyperfunction and hypertrophy need not be correlated in every case (Eechaute, 1962; Andrew, 1968) and more recent studies with Peromyscus indicate that ACTH may have an important role in reproductive inhibition in Peromyscus. Long term injections of high doses of ACTH inhibited reproductive function of both sexes (Christian, 1971; Pasley & Christian, 1972). Ogle (1974a, b) has shown that high dose levels of ACTH interfere with ovarian function and pregnancy in female Peromyscus in a pattern similar to that expected when LH secretion is deficient. These effects seem to be consistant with the proposal of Christian and Davis (1964) that ACTH may inhibit luteinizing hormone (LH) production and/or release.

The present experiments were designed to examine serum corticosterone concentration and the weights of reproductive organs in deermice to determine if "stress" on CNS is a central factor in, or correlated with, a sensory cortex-

hypothalamic-hypophysial-adrenal or -gonadial mechanism in laboratory populations of prairie deermice. If high endogenous concentrations of ACTH are present in deermice, there is strong likelihood that a concomitant elevated glucocorticoid concentration should also be detected.

MATERIALS AND METHODS

Experimental Animals:

Control animals:

Adult male deermice were paired with non-sibling females of approximately the same age (79 to 113 days) in cages (12 cm X 26 cm X 14 cm) which allowed reception of visual, olfactory and auditory cues but prevented contact by means of a partition made of two layers of hardware cloth separated by 2 cm. Twenty pairs of control animals were used in the study. The range of ages at death was 230-270 days.

Population animals:

Three experimental populations were founded by four pairs of prairie deermice (60-100 days of age) from eight different litters with the female of each pair being pregnant. The first young born to each female were removed within 24 hours in order to eliminate the possibility of variable prenatal experiences. All subsequent litters remained as part of that population.

The populations were maintained for approximately 300 days prior to sampling in corrugated, galvanized steel enclosures (diameter 48.3 cm; floor area 1829.2 cm^2) provided with wood shavings. The populations varied in size and growth rate at the time of sampling. Population 14 had not

increased in numbers for 167 days and was comprised of 79 animals (47 females and 32 males). Population 12 and 4 were still growing when sampled. Population 12 contained 48 animals (16 females and 32 males) while population 4 contained 32 animals (14 females and 18 males) greater than twenty-one days of age.

Animal Maintenance:

All control and experimental animals were maintained in the same 15 X 15 ft. room which was brightly lighted by four 40-watt fluorescent bulbs from 0800 hours to 1930 hours, and were dimly illuminated with four 10-watt incandescent bulbs from 2000 hours to 0730 hours. Between each light change there was a period of 30 minutes of darkness. There were 5-10 complete air exchanges per hour in the room. Room temperature was maintained within a range of 21-30° C. The animals were provided with excess food (D&G Laboratory Diet) and tap water.

Blood and Organ Collection:

Mice, more than 90, and less than 350 days old, were taken as encountered at the rate of one male or female per day from each population. Only females known to be nulliparous were sampled.

Between 1400 to 1530 hours (4 to 5½ hours prior to the onset of the dark/dim period) an animal was rapidly anesthetized by ether inhalation. Blood was obtained by

venipuncture from the vena cava at the level of the renal vein within three minutes from the time of the first contact with the animal. The blood was allowed to clot and then centrifuged at 6800 g for 20 minutes; the serum was stored in plastic tubes at below -20° C until assayed. The whole animal was placed in a 10% formalin solution. Later, testes, seminal vesicles, and adrenals were removed from all males, and adrenals, ovaries, and uteri plus oviducts were removed from all females. All uteri were checked to insure there were no embryos or scars. The organs of each mouse were cleaned of extraneous tissue, blotted, and weighed twice to the nearest 0.1 milligram on an electrobalance (Cahn) interfaced with a Wang 700B programmable calculator.

Radiosteroid Assay:

Materials and reagents:

1,2-^{3}H corticosterone (New England Nuclear Corporation, Boston, Mass., specific activity=1.75 ug/o.25 mc) and nonradioactive crystalline corticosterone (Schwartz/Mann, Orangeburg, New York) were used in this study. A working concentration of 1 mg/ml was prepared every four weeks using absolute ethanol. All steroids were checked for purity by thin layer chromatography. Precoated thin-layer plates (Silica Gel, 60 F-254, E.M. Laboratories, Inc.) were washed three times by ascending chromatography with chloro-

form:ethanol (95:5) before use and stored in an air-tight container with anhydrous $CaSO_4$.

All glassware was soaked for 2 days in methanol, rinsed in methylene chloride and dried at 150° C prior to use. Florisil (activated magnesium silicate, 60/100 Mesh, J.T. Baker Chemical Company) was used as purchased.

Preparation of Corticosteroid Binding Globulin (CBG) Solution:

Plasma was obtained from a 63 kg male Irish Wolfhound by heparinized syringe and centrifuged for 1 hour at 5900 g. A 0.6 ml aliquot of the plasma was diluted to 50 ml with distilled water and dialyzed for 6 hours in seamless cellulose tubing, (molecular weight retention of 8-12,000, Union Carbide) against 250 ml of Tyrode Ringers solution maintained at 40° C. Just prior to use, 0.6 μCi of ^{3}H-corticosterone was evaporated to dryness in a 250 ml beaker. The CBG solution was added to the beaker and allowed to equilibrate at 6° C for at least an hour.

Competitive Protein Binding Assay:

The assay employed was a modification of the method of Murphy (1967). A 0.1 ml aliquot of the duplicate serum samples was added to 13 x 100 mm test tube which contained a known amount (approximately 2000 dpm) of ^{3}H-corticosterone as a tracer. The duplicate samples were equilibrated with

the tracer and then were extracted with 3 ml of methylene chloride for three minutes on the vortex mixer at medium speed. A 1 ml aliquot of distilled water was added and each tube was vortexed again. The aqueous layer was removed by aspiration and the extract evaporated to a low volume in a stream of air at room temperature. Tubes with methylene chloride blanks and water method blanks were treated the same as plasma samples.

The extracts were dissolved in 2 x 0.1 ml ethanol: methylene chloride (1:1) and spotted on 20 X 20 cm thin-layer plates divided into 9 lanes. One-hundredth of a mg of corticosterone in absolute ethanol was spotted on the two side and middle lanes to provide standard reference markers. After an 80 minute development, the corticosterone marker spots were located under a UV lamp, and an area of each sample lane corresponding to a distance of 1 cm above and below the center of the marker spot was scrapped off. The loosened silica gel was sucked into a disposable Pasteur pipet which had been tightly plugged with glass wool and previously washed with methanol and methylene chloride. The corticosterone was eluted with 2 X 1 ml ethanol followed by 1 ml of methylene chloride. The eluates were evaporated to dryness, and redissolved in 2 ml ethanol and 1 ml methylene chloride on a shaker in a 6° C cold room for 5 hours. After shaking, 500 µl of each sample was

transferred to liquid scintillation counting vials for recovery estimations. The remainder of each sample was evaporated to dryness in a stream of air. One ml of the CBG solution was added to each of the sample and standard tubes. Working standard solutions of non-radioactive corticosterone used in the assay included 0, 0.25, 0.50, 0.75, 1.00, 1.50, 2.00, 3.00, and 5.00 ng. All standards and samples were run in duplicate. The tubes were placed vertically in racks in a horizontal shaker after a brief vortexing and incubated for 10 minutes at 45° C. Following this, the tubes were placed in a cold room at 6° C, and shaken 10 more hours. A known amount (8.22 ± 0.12 mg; n=10) of Florisil was added to each tube at 30 second intervals with a calibrated spoon. The tubes were shaken 12 hours and then allowed to stand for 2 hours. Five hundred µl of the supernate from each sample were transferred to a counting vial and 10 ml of Bray's Solution were added. The vials were counted for 40 minutes or at least 4000 counts in a Nuclear Chicago Series 720 Liquid Scintillation Counter. A standard curve was constructed by plotting duplicate values for the corticosterone standards versus the dpm of corticosterone bound to protein. Nanograms of corticosterone in the samples were read directly from the curve. All values were corrected for losses by their respective recovery estimates. Final values were expressed as µg cor-

ticosterone per/100 ml plasma.

Statistical Analysis

Bartlett's test revealed significant heterogeneity of variance between mean values for body weight and age in both males and females, and for adrenal, uterus and ovary weights in females. Therefore, comparisons between groups with the above parameters were made using the non-parametric Mann-Whitney U Test, the remainder of the parameters did not show heterogeneity of variance and were compared using the Student's *t* test.

RESULTS

No significant differences were observed between control males and control females with respect to age, body weight, absolute or relative adrenal weight or serum corticosterone (cf Table 1 and 2). Similarly, no significant differences were observed between males and females in population 14 with respect to age, body weight, relative adrenal weight or serum corticosterone. However, the absolute adrenal weight of the females ($p < 0.05$) tended to be smaller than the males (Table 1 and 2).

Only two females in population 12 and only three females in population 4 were concomitantly nulliparous and within the age limit previously established for sampling. Consequently, these females were omitted from any comparative study. Thus, only females within population 14 were compared with their corresponding female controls. These comparisons indicated that the population 14 females were significantly ($p < 0.001$) older and had significantly lighter body weight, adrenal, ovary and uterine weights ($p < 0.02$, $p < 0.001$, $p < 0.01$, $p < 0.001$, respectively) and had more than twice the mean corticosterone concentration ($p < 0.02$) when compared with the corresponding values for their female controls (Table 1).

Since it was possible to sample 10 males in each of the populations within appropriate age range, comparisons

could be made between the several populations and the controls. Population 14 and 12 males were not significantly different in age from their corresponding male controls, whereas, population 4 males were significantly ($p < 0.001$) younger. Also, the mean age of population 14 was significantly older ($p < 0.01$) than population 4, but only tended ($p < 0.1$) to be older than population 12. The mean age of males of population 12 was significantly ($p < 0.002$) greater than population 4 males (cf Table 2 and 3).

When comparisons of body weight were made it was shown that there was no significant difference between population 14 and control males. But population 12 and 4 males were both significantly ($p < 0.01$) lighter than control males. The body weights of males within the three populations did not differ significantly (cf Tables 1 and 3).

The mean testes and seminal vesicle weights of the males of population 14, 12 and 4 were significantly ($p < 0.002$) lighter than control males but did not differ significantly among themselves (cf Table 2, 5 and 6).

When the means of the absolute adrenal weights were compared, those of the control males tended to be heavier than the males in population 12 ($p < 0.05$) and 14 ($p < 0.1$), and not significantly different from population 4, while the population members did not differ significantly among themselves. There were no significant differences between

the mean relative adrenal weights of the males of any of the populations and of the controls. (cf Table 2 and 4)

The mean serum corticosterone concentration in population 14 males was more than double the corresponding value for control males. This increase was the only value in population animals that was significantly ($p < 0.01$) higher than controls although all populations had higher mean values than controls. Also, population 14 was significantly higher than both population 12 and 4 ($p < 0.02$ and 0.05 respectively) and population 12 did not differ significantly from population 4 (cf Table 2 and 7).

No significant correlations could be demonstrated between serum corticosterone concentration versus age, body weight, adrenal weight or gonad weight; nor between adrenal versus gonad weight, for either sex in control or population animals.

DISCUSSION

There were no significant differences between sexually mature (230-270 day old) control males and females with respect to body weight, adrenal weight and serum corticosterone concentration. An attempt was made to use control animals with a mean age approximating the mean age of all of the population animals. Because the age range in controls was restricted (230-270 days) compared with the great range of ages of population animals (90-350 days), the variance between the groups was significant and significant mean age differences were demonstrated in some nonparametic comparisons. However, we attach little physiological significance to these differences because the population animals were all older than the minimum age for reproduction and were well under the 750 day age at which Peromyscus have been previously shown to be reproductively active (Terman, 1973a, 1976). Thus, there is confidence that the population animals were neither too old nor too young to have been reproductively functional if they had been removed from the population context.

The control females had significantly heavier adrenal, ovary and uterine weights than the nulliparous females in asymptotic population 14. It is very surprising that the value of mean serum corticosterone concentration of the con-

trol group was less than half that of population 14. The reproductively inhibited population females had significantly smaller yet hyperfunctional adrenals compared with the controls.

Comparison of the males from the asymptotic population 14 and the controls showed a result very similar to that of the females. Mean testes and mean seminal vesicle weights of population 14 males were significantly lighter than controls but corticosterone concentration was more than twice the corresponding value for control males (cf Table 2) even though their adrenal gland weights averaged smaller, though not significantly smaller, than those of the controls.

Comparisons between control males and those from the freely growing populations 12 and 4 revealed somewhat different responses from those noted above for asymptotic population 14. Although the testes and seminal vesicles weights of males from controls averaged significantly larger than those of the populations, other differences were not significant. Further, while population 12 males had smaller adrenals than controls ($p < 0.05$), the values of mean relative adrenal weight and mean serum corticosterone concentration were not significantly different from controls.

Comparisons among populations revealed that asymptotic population 14 males had significantly higher corticosterone levels than non-asymptotic population 12 or 4, while the latter two populations did not differ among themselves.

We fully recognize the labile nature of plasma corticosterone concentration with respect to changing environmental stimuli. But we feel that rapidly sampling each animal without any prior disturbance permitted us to obtain an accurate estimation of the resting value of corticosterone. Further, the control animals which were sampled similarly to the population animals constitute a valid point of comparison for whatever the effects of sampling *per se* might have been.

There is strong evidence from rats and some other laboratory species that corticosterone is significantly elevated in both acute and chronic "stress" (Friedman & Ader, 1968; Ganjam *et al.*, 1972; Zimmerman *et al.*, 1972: Cook *et al.*, 1973; Dallman & Jones, 1974; Riegle, 1974; Barlow *et al.*, 1975). There is also clear evidence that an increase in ACTH always precedes an increase in corticosterone concentration in the rat (Cook *et al.*, 1973). If it is assumed that corticosterone levels in *Peromyscus* are a reflection of ACTH levels, then the possibility is very strong that there were high titers of ACTH in animals born into the asymptotic population 14. Definitive evidence for this must involve simultaneous assay of ACTH in *Peromyscus* plasma. However, the small blood volume of the deermouse makes repeated sampling from the single animals, or simultaneous assay for several hormones, impossible. If one

tentatively accepts the possibility that the mean corticosterone concentration of the deermice studied could represent an elevated ACTH level, it then becomes harder to dismiss ACTH as a potential mediator of the depressed reproductive function displayed by Peromyscus populations. Also, there is preliminary evidence that the serum LH concentration in reproductively inhibited males born into asymptotic populations is significantly lower than in control animals (Hirsch et al., unpublished). When these results are extended to this present work there is reason to speculate that the serum ACTH level may be high concomitantly with a low serum LH peak in asymptotic population males. Other work has indicated that chronic stress delayed puberty and reduced fertility in female C57/B16 mice (Paris et al., 1974).

An interesting but unexplained finding of this study is that only animals from population 14 had a significantly higher serum corticosterone level than controls and while population 12 and 4 had higher mean values they were not significantly so. Population 14 males also had significantly higher serum corticosterone than other population males. The reason for the difference between populations is not clear. But we doknow that population 14 had ceased growing approximately six months before the sampling while the other two populations were still growing. Further,

population 14 was approximately twice as large as the others. Whether the cessation of growth and density are the two factors that account for these differences in corticosterone levels between populations remains to be demonstrated. Previous work has shown that population density *per se* is not reflected in differential body weights, adrenal or reproductive organ weights (Terman, 1973b).

It is important to note that no significant correlations could be demonstrated between adrenal and gonad weights, nor between adrenal function (in terms of serum corticosterone) and the weights of the adrenals or gonads. Thus, corticosterone concentration *per se* cannot be directly related to the weight of the gonads within any population or control group. This is not to say that corticosterone concentration is unrelated to reproductive activity. In fact, there was a trend toward increasing mean serum corticosterone concentrations from the low values in the presumably reproductive-capable control animals, through the freely growing population 12 and 4 males to the asymptotic population 14 males and females. Thus, in these populations, adrenal corticoid hyperfunction appeared to be associated with reproductive inhibition but not in a direct or dose response relationship.

TABLE 1

AGE, BODY WEIGHT, ADRENAL WEIGHT, OVARY WEIGHT, UTERUS WEIGHT AND SERUM CORTICOSTERONE CONCENTRATIONS FOR CONTROL AND POPULATION FEMALES. (Mean ± S.E.)

Condition	Age (day)	Body Wt. (g)	Adrenal Weight		Ovary Wt. (mg)	Uterus Wt. (mg)	Serum Corticosterone (µg/100 ml)
			Absolute (mg)	Relative (mg/g b.w.)			
Control n=20	253.2 ±2.49	20.12 ±0.94	2.52 ±0.13	0.129 ±0.008	21.57 ±3.67	51.02 ±4.99	6.14 ±0.90
Population 14 n=19	294.3 ±10.11	15.28 ±1.42	1.85 ±0.08	0.122 ±0.007	7.10 ±0.69	10.33 ±1.92	13.17 ±1.87

TABLE 2

AGE, BODY WEIGHT, ADRENAL WEIGHT, TESTIS WEIGHT, SEMINAL VESICLE WEIGHT AND SERUM CORTICOSTERONE CONCENTRATIONS FOR CONTROL AND POPULATION MALES. (Mean ± S.E.)

Condition	Age (day)	Body Wt. (g)	Adrenal Weight		Testis Wt. (mg)	Seminal Vesicles (mg)	Serum Corticosterone (µg/100 ml)
			Absolute (mg)	Relative (mg/g b.w.)			
Control	240.8	20.10	2.72	0.136	377.35	210.90	5.36
n=20	±12.85	±0.60	±0.17	±0.010	±15.33	±15.17	±1.12
Population	277.9	20.3	2.20	0.117	209.88	71.43	13.48
14	±20.97	±2.11	±0.13	±0.013	±42.69	±21.38	±2.29
n=9							
Population	203.6	15.5	2.04	0.135	188.10	88.12	5.51
12	±30.22	±0.49	±0.09	±0.007	±18.71	±21.91	±1.12
n=10							
Population	119.9	16.6	2.27	0.140	187.40	56.03	7.29
4	±6.23	±0.85	±0.18	±0.008	±21.84	±14.54	±1.47
n=10							

TABLE 3

COMPARISONS OF AGE AND BODY WEIGHT BETWEEN CONTROL MALES AND MALES IN POPULATIONS 14, 12 AND 4.

	Control	Population 14	Population 12	Population 4
Control	-----	C<14	C>12	C>4
	-----	N.S.	N.S.	0.001
Population 14	C < 14	-----	14>12	14>4
	N.S.	-----	0.1	0.01
Population 12	C>12	14>12	-----	12>4
	0.001	N.S.	-----	0.02
Population 4	C>4	14>4	12<4	-----
	0.01	N.S.	N.S.	-----

COMPARISONS OF BODY WEIGHT

COMPARISONS OF AGE

TABLE 4

COMPARISONS OF ABSOLUTE AND RELATIVE ADRENAL WEIGHTS BETWEEN CONTROL MALE AND MALES IN POPULATION 14, 12 AND 4.

	Control	Population 14	Population 12	Population 4
Control	-----	C>14	C>12	C>4
	-----	0.1	0.5	N.S.
Population 14	C >14	-----	14>12	14<4
	N.S.	-----	N.S.	N.S.
Population 12	C>12	14<12	-----	12<4
	N.S.	N.S.	-----	N.S.
Population 4	C<4	14<4	12<4	-----
	N.S.	N.S.	N.S.	-----

TABLE 5

COMPARISONS OF ABSOLUTE AND RELATIVE TESTIS WEIGHT BETWEEN CONTROL MALES AND MALES IN POPULATIONS 14, 12 AND 4.

COMPARISONS OF RELATIVE TESTIS WEIGHTS

COMPARISONS OF ABSOLUTE TESTIS WEIGHTS

	Control	Population 14	Population 12	Population 4
Control	-----	C>14	C>12	C>4
	-----	0.002	0.001	0.001
Population 14	C>14	-----	14>12	14>4
	0.001	-----	N.S.	N.S.
Population 12	C>12	14<12	-----	12>4
	0.001	N.S.	-----	N.S.
Population 4	C>4	14<4	12>4	-----
	0.001	N.S.	N.S.	-----

TABLE 6

COMPARISONS OF ABSOLUTE AND RELATIVE SEMINAL VESICLES BETWEEN CONTROL MALES AND MALES IN POPULATIONS 14, 12 AND 4.

	Control	Population 14	Population 12	Population 4
Control	-----	C>14	C>12	C>4
	-----	0.001	0.001	0.001
Population 14	C>14	-----	14<12	14>4
	0.001	-----	N.S.	N.S.
Population 12	C>12	14<12	-----	12>4
	0.001	N.S.	-----	N.S.
Population 4	C>4	14<4	12>4	-----
	0.001	N.S.	N.S.	-----

COMPARISONS OF RELATIVE SEMINAL VESICLES WEIGHTS

COMPARISONS OF ABSOLUTE SEMINAL VESICLES WEIGHTS

TABLE 7

COMPARISONS OF SERUM CORTICOSTERONE CONCENTRATION BETWEEN CONTROL MALES AND MALES IN POPULATIONS 14, 12 AND 4.

	Control	Population 14	Population 12	Population 4
Control	-----	C<14	C<12	C<4
	-----	0.01	N.S.	N.S.
Population 14	-----	-----	14>12	14>4
	-----	-----	0.02	0.05
Population 12	-----	-----	-----	12<4
	-----	-----	-----	N.S.
Population 4	-----	-----	-----	-----
	-----	-----	-----	-----

BIBLIOGRAPHY

BIBLIOGRAPHY

Albertson, B.D., Bradley, E.L., and Terman, C.R.,(1975). Plasma progesterone concentrations in prairie deer-mice (Peromyscus maniculatus bairdii) from experimental laboratory populations. J. Reprod. & Fertil. 42: 407-417.

Andrew, R.V.,(1968). Effect of exogenous ACTH on precursor utilization in adrenal steroidogenesis. Endocrinology 83: 1387-1389.

Barlow, S.M., Morrison, P.J.,and Sullivan, F.M.,(1975). Effects of acute and chronic stress on plasma corticosterone levels in the pregnant and non-pregnant mouse. J. Endocr. 66: 93-99.

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

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

Christian, J.J.,(1955b).Effect of population size on the adrenal gland 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. Ecology 37: 258-273.

Christian, J.J., (1963). Endocrine adaptive mechanisms and the physiological regulation of population growth. In Physiological Mammology, ed. W.V. Mayer and R.G. VanGelder (New York: Academic Press), 189-353.

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

Christian, J.J., (1964a). Effect of chronic ACTH treatment on maturation of intact female mice. Endocrinology 74: 669-679.

Christian, J.J., (1964b). Action of ACTH in intact and corticoid-maintained adrenal-ectomized female mice with emphasis on the reproductive tract. Endocrinology 75: 653-669.

Christian, J.J., Lloyd, J.A. and Davis, D.E., (1965). The role of endocrines in the self regulation of mammalian populations. Rec. prog. Hormone Res. 21: 501-578.

Christian, J.J., (1967). Effect of population size on the adrenal glands and reproductive organs of male mice in populations of fixed size. In Hormones and Behavior, ed. R.E. Whalen (Princeton, N.J.: D. VanNostrand Co.), 23-36.

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

Cook, D.M., Kendall, J.W., Greer, M.A., and Kramer, R.M., (1973). The effect of acute or chronic ether stress on plasma ACTH concentration in the rat. Endocrinology

93: 1019-1024.

Dallman, M.F. and Jones, M.T., (1973). Corticosteroid feedback control of ACTH secretion: effect of stress-induced corticosterone secretion on subsequent stress responses in the rat. Endocrinology 92: 1367-1375.

Friedman, S.B. and Ader, R., (1968). Adrenocortical response to novelty and noxious stimulation. Neuro-endocrinology 2: 209-212.

Ganjam, V.K., Campbell, A.L., and Murphy, B.E.P., (1972). Changing patterns of circulating corticosteroids in rabbits following prolonged treatment with ACTH. Endocrinology 91: 607-611.

Hagino, N., Watanabe, M., and Goldgieher, J.W., (1969). Inhibition by adreno-corticotrophin-induced ovulation in immature female rats. Endocrinology 84: 308-314.

Hirsh, L., Bradley, E.L., and Terman, C.R.,(unpublished).

Lidicker, W.Z., (1965). Comparative study of density regulation in confined populations of four species of rodents. Res. Pop. Ecology 7: 57-72.

Louch, C.D., (1956). Adrenocortical activity in relation to density and dynamics of three confined populations of Microtus pennsylvanicus. Ecology 37: 701-713.

Murphy, B.E.P., (1967). Some studies of the protein-binding of steroids and their application to the routine micro- and ultramicromeasurement of various steroids

in body fluids by competitive protein binding radioassay. J. Clin. Endocrinol. 27: 973-990.

Nicholson, A.J., (1957). The self-adjustment of populations to change. Symp. Quant. Biol. 22: 153-173.

Ogle, T.F., (1974a). Effects of ACTH on organelle interrelationships in the corpus luteum of the pregnant deermouse. Cell Tiss. Res. 153: 195-209.

Ogle, T.F., (1974b). Effects of ACTH on ovarian histochemistry and maintenance of pregnancy in deermice. Biol. Reprod. 11: 288-296.

Paris, A.L. and Ramaley, J.A., (1974). Adrenal-gonadal relations and fertility: the effects of repeated stress upon the adrenal rhythm. Neuroendocrinology 15: 126-136.

Pasley, J.N. and Christian, J.J., (1972). The effect of ACTH, group caging, and adrenalectomy in Peromyscus leucopus with emphasis on suppression of reproductive function. Proc. Soc. Exp. Biol. Med. 139: 921-925.

Ramaley, J.A., (1974). Minireview: Adrenal-gonadal interactions at puberty. Life Sci. 14: 1623-1633.

Southwick, C.H., (1955a). The population dynamics of confined house mice supplied with unlimited food. Ecology 36: 212-225.

Southwick, C.H., (1955b). Regulatory mechanisms of house populations: Social behavior affecting litter survival. Ecology 36: 627-634.

Southwick, C.H. and Bland, V.P., (1959). Effect of population density on adrenal glands and reproductive organs of CFW mice. Am. J. Physiol. 197: 111-114.

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

Terman, C.R., (1968). Inhibition of reproductive maturation and function in laboratory populations of prairie deermice: A test of phermone influence. Ecology 49: 1169-1172.

Terman, C.R., (1969a). Pregnancy failure in female prairie deermice related to parity and social environment. Anim. Behav. 17: 104-108.

Terman, C.R., (1969b). Weights of selected organs of deermice (Peromyscus maniculatus bairdii) from asymptotic laboratory populations. J. Mammal. 50: 311-320.

Terman, C.R., (1973a). Reproductive inhibition in asymptotic populations of prairie deermice. J. Reprod. Fert., Suppl., 19: 457-461.

Terman, C.R., (1973b). Recovery of reproductive function by prairie deermice (Peromyscus maniculatus bairdii) from asymptotic populations. Anim. Behav. 21: 443-448.

Terman, C.R., (1976). Laboratory studies of population regulation in prairie deermice. Proc. Centennial Symp. Sci. & Res., Phila. Zoo. Soc., pp. 199-220.

Thiessen, D.D. and Rodgers, D.A., (1961). Population density and endocrine function. Psychol. Bull. 58: 441-451.

Velardo, J.T., (1957). Action of adrenocorticotropin on pregnancy and litter size in rats. Am. J. Physiol. 191: 319-322.

Zimmerman, E., Smyrl, R., and Critchlow, V., (1972). Suppression of pituitary-adrenal response to stress with physiological plasma levels of corticosterone in the female rat. Neuroendocrinology 10: 246-256.

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