1983

In vitro steroid biosynthesis by the adrenal tissue of prairie deermice (Peromyscus maniculatus Bairdi) from laboratory populations

Richard Paul Tucci

College of William & Mary - Arts & Sciences

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IN VITRO STEROID BIOSYNTHESIS BY THE ADRENAL TISSUE
OF PRAIRIE DEERMICE (PEROMYSCUS MANICULATUS BAIRDI)
FROM LABORATORY POPULATIONS

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
Richard Paul Tucci
1983
APPROVAL SHEET

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

Master of Arts

Author

Approved, April 1983

Eric L. Bradley

G. Richard Terman

Robert E. L. Black
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ABSTRACT

The production rates of the major steroid hormones produced by in vitro adrenal incubation were determined in control and reproductively inhibited prairie deer mice (Peromyscus maniculatus bairdii). Population males had significantly lighter mean seminal vesicle, testis, adrenal, and body weights when compared with control males. Population females had significantly lighter mean uterine, ovarian, adrenal, and body weights when compared with control females.

Paper chromatographic separation and radiographic scanning of some adrenal incubates yielded three major radioactive peaks, in addition to the peak of progesterone that served as the carbon source during incubation. The R_p values and color development after treatment with 17x phosphoric acid solution suggest that these three peaks contain the hormones corticosterone, deoxycorticosterone, and testosterone respectively.

In the second part of the experiment, adrenal incubates from control and population, male and female deer mice were separated by thin-layer chromatography, and the carbon energy of the eluted hormones was measured to determine their production rates. The mean corticosterone produced from the incubated adrenals of control males tended to be higher than that produced by population males, but this difference did not exist when expressed in terms of adrenal weight. There was no significant difference in the rate of production of deoxycorticosterone or testosterone between control and population male deer mice.

The absolute amount of deoxycorticosterone produced by the adrenal glands of population females was significantly lower than control females. This difference was not significant when expressed in terms of the weight of adrenal tissue. There was no difference seen in the amount of corticosterone produced between the two female groups. Population females did produce significantly higher levels of adrenal testosterone than control females, and this difference remained significant after expressing in relative terms for adrenal and body weight. In addition, ovarian weight was negatively correlated to the amount of adrenal testosterone produced in population females, but not in control females.

Several possible mechanisms of reproductive inhibition in male and female deer mice are discussed in view of previous and current findings.
IN VITRO STEROID BIOSYNTHESIS BY THE ADRENAL TISSUE OF PRAIRIE DEERMICE (PEROMYSCUS MANICULATUS BAIRDI) FROM LABORATORY POPULATIONS
INTRODUCTION

In some species of small mammals, an increase in the density of the population is associated with reproductive inhibition. Christian (1950; 1971; 1975) and Christian, Lloyd, and Davis (1965) developed a theory that an increase in population density causes intrinsic behavioral and endocrine responses that result in the regulation and limitation of population growth via the release of the hormone adrenocorticotrophin (ACTH) from the anterior pituitary. This mechanism was thought to act on the adrenal glands to cause the synthesis and release of steroid hormones that would result in reproductive inhibition. They also suggested that ACTH may alternatively act directly on the gonads to cause inhibition by an unspecified mechanism.

The release of ACTH is under the control of corticotrophin releasing factor (CRF) from the hypothalamus, which is released in greater amounts under stressful conditions compared with the moderate circadian fluctuations in the non-stressed state. Negative feedback inhibition of ACTH release occurs with elevated blood glucocorticoid concentration acting on either the hypothalamus or the pituitary (Turner and Bagnara, 1976). The release of ACTH is reported to be triphasic to chronic stimuli in rats. First there is a rapid increase of ACTH to high levels (Sato, 1975). The rapid increase is followed by a slow return to basal levels and then, in the event of prolonged stress, there is another rise to sustained high levels that remains
significant after four hours (Dallman and Jones, 1973). Cook, et al. (1973), using ether stress in rats for 30 seconds, 2.5 minutes, or continuously, found that in all groups the concentration of corticosterone was not different during the first 40 minutes, but ACTH levels were different. Thus, the magnitude of the ACTH concentration depends not only on the type, but also on the duration of stress. Dallman and Jones (1973) have suggested that because there was no graded response of corticosterone levels in proportion to increasing amounts of ACTH, the adrenal must respond maximally to even the lowest amount of ACTH released in response to stimulation. They also reported that corticosterone sensitizes the system so that a second stimulus provokes a faster response. Finally, another negative feedback loop controlling glucocorticoid production directly from the circulating glucocorticoid concentration on the adrenal gland was postulated by Loose, et al. (1980) after finding glucocorticoid binding sites in the adrenal cytosol of mice, rats, and bovine sources.

In some species of small mammals it has been shown that adrenocortical function increases with increased population density. Adrenal weight was found to increase with density in house mice (Mus musculus) (Christian, 1956; Bronson and Eleftheriou, 1963), lemmings (Lemmus trimucronatus) (Andrews, 1970; Andrews et al., 1975b; Andrews and Belknap, 1979), and voles (Microtus pennsylvanicus) (To and Tamarin, 1977). However, in Peromyscus maniculatus adrenal weight does not reflect an increase in population density (Bronson and Eleftheriou, 1963; Terman, 1969; Sung, Bradley, and Terman, 1977; Coppes, 1980; Peebles, 1981). In Mus, adrenocortical function and morphology were shown to be inversely related to social rank. When house mice were
grouped and injected with a beef serum antigen, the dominant animals were found to have a higher antibody titer than subordinates (Vessey, 1964). Social rank also showed an inverse relationship to adrenal weight and plasma corticosterone concentration. This finding was thought to be due to an increased ACTH secretion causing an increased glucocorticoid concentration in subordinate house mice (Louch and Higginbotham, 1967). Southwick (1964) using Peromyscus leucopus found that an increase in population density did not produce an increase in adrenal weight if the populations were socially "compatible", but if the animals fought, the adrenals of subordinate mice were significantly larger. Using low levels of eosinophils as an indication of high glucocorticoid levels, Southwick (1964) also found that when male P. leucopus were placed into empty colony pens, eosinophil numbers decreased by 70% within four hours, but returned to normal by 72 hours. However when male P. leucopus were placed in colony pens containing a resident pair, there was a 92% decline in eosinophils that remained significantly lowered for five days.

Bronson (1963) and Bronson and Eleftheriou (1963; 1964) found that exposure of male C57 mice and Peromyscus maniculatus to trained fighter mice or cold caused an increased adrenal weight and ascorbic acid depletion. When C57 mice were maintained at increased densities, similar responses were seen. However, crowding did not provoke these changes in Peromyscus maniculatus. Bronson (1965) found that 8 days of 1 minute/day exposure to trained C57 fighter mice resulted in significant increases in adrenal weight and pituitary ACTH in mice of the same species. Plasma corticosterone was elevated regardless of length of exposure. However, adrenal corticosterone levels, when
adjusted for changes in adrenal weight, showed no significant difference. Actual physical contact was not necessary to evoke this response once a contact had occurred, either in C57 mice (Bronson and Eleftheriou, 1965), or in SASTO mice (Archer, 1969).

In many species, reproductive function is known to decrease with an increase in population density. This is seen in male house mice (Christian, Lloyd and Davis, 1965; Brain and Nowell, 1971a; Christian, 1971; Lloyd, 1971; Gartner, Reznik-Schuller and Reznik, 1973; McKinney and Desjardin, 1973), and female house mice (Christian, 1960; Brain and Nowell, 1971b). Bronson, Stetson and Stiff (1973), using male CF-1 mice, showed progressive decreases in seminal vesicle weight and circulating FSH and LH in isolated controls, animals separated by a barrier that prevented only physical contact, dominants, and subordinates. Adrenal weight and ACTH concentration showed a progressive increase in the same hierarchy. Female house mice when placed in mixed sex groups, show increased uterine fetal mortality and total loss of reproductive function (Lloyd and Christian, 1969). When reproductively inhibited female house mice were removed from mixed sex groups, reproduction remained suppressed and inadequate lactation caused permanent stunting of offspring, which continued into the second generation (Christian and LeMunyan, 1958).

Exposure to several kinds of stress, both chronic and acute, has been shown to result in reproductive inhibition (Ajika et al., 1972; Gartner, Reznik-Schuller and Reznik, 1973; Buckingham and Hodges, 1974; Bassett and Cairncross, 1975; Blake, 1975; Gray et al., 1977; DuRuisseau et al., 1978; Tache et al., 1978). Eucker and Riegle (1972) found that chronic restraint stress decreased the number of fertilized
eggs that implant in female rats. ACTH administration also caused reproductive inhibition in house mice (Christian, 1964a; 1964b; Christian, Lloyd and Davis, 1965), rats (Ogle, 1977; Cohen and Mann, 1979; Kapil, Chowdburg and Swarup, 1979), voles (Pasley and Christian, 1971), P. leucopus (Pasley and Christian, 1972), and P. maniculatus (Ogle, 1974). Yang, Yang and Lin (1969) found that ACTH administration caused a marked inhibition of implantation and fetal development in rats, but did not alter the rate of tubal transport or development of ova.

Laboratory populations of Peromyscus maniculatus bairdi, when provided with excess food and water, will control growth at widely variable numerical levels (Terman, 1965). The control of growth is either by the failure of young to survive, or more commonly by the reproductive inhibition of the young (Terman, 1973a). The weights of the reproductive organs in both sexes are significantly reduced when compared with controls (Terman, 1969). When the reproductively inhibited animals are removed from the population and paired for 10 days with an animal of the opposite sex, 56% of the females produce successful litters within 28 days (Terman, 1973b). This indicates that endocrine systems and reproductive organs did not become permanently refractory to circulating gonadotrophins (Terman, 1973b). Terman (1980) used three experimental pairings of P. maniculatus between 130 and 150 days old with siblings of the opposite sex on no-contact cages. The three groups were paired within a population enclosure, paired outside the enclosure but in the same room, and freely ranging within a population enclosure. Mean body, testes, seminal vesicle, ovary and uterus weights averaged progressively larger from the animals that were free ranging,
to those paired within the enclosure, to those paired outside the
enclosure. Also, only 3% of the free ranging females became pregnant,
compared to 21% caged within and 48% caged outside the enclosure.
This study indicates that the prime determinant of inhibited reproduction
is physical contact.

Serum corticosterone levels were found to be significantly
higher in reproductively inhibited male and female deermice (P. maniculatus),
even though the absolute adrenal weights were significantly lower when
compared with controls (Sung, Bradley and Terman, 1977). In a follow-
up study, Bradley and Terman (1981a) again found a significantly higher
serum corticosterone level in population animals, and smaller adrenal
glands. However, the relative adrenal weight was larger in both sexes
of population animals. The smaller adrenal size was a result of
decreased cortical and medullary areas. This paradox of increased
serum corticosterone in the presence of smaller adrenals is still
unresolved. Differences in the binding of corticosterone to plasma
proteins, or in the breakdown and excretion of corticosterone may
play a role. Another factor that may cause elevated serum corticosterone
levels in inhibited deermice when compared with controls is an
increased rate of adrenal secretion in population animals. Adrenal
incubation studies to determine hormone production rates have been
done on a variety of animals including rats (Milewich and Axelrod,
1972; McCarthy, Green and Sohal, 1976; Goverde, Pesman and Benraad,
1980; Goddard et al., 1980; Sibley et al., 1980), hamsters (Andrews,
1968c), snowshoe hares (Fevold and Drummond, 1976; Feist, 1980),
lemmings (Andrews, 1968a; 1968b; 1970; Andrews et al., 1968; 1975b;
Andrews and Belknap, 1979), voles (Andrews, 1970), house mice
(Varon, Touchstone and Christian, 1966; Andrews, 1970), and deermice (Andrews et al., 1975a; Andrews and Belknap, 1979).

The present study examines the in vitro production rates of several adrenal steroid hormones from the adrenal glands of male and female, control and population deermice (P. maniculatus bairdi). The initial part of this study employs paper chromatography and radiographic scanning to investigate if there are any differences in the major steroid hormones produced, that may be causing the reproductive inhibition seen in both sexes of population deermice. In the second part of this work, the major steroids are separated by thin-layer chromatography, and their individual production rates are determined. This will determine if the elevated serum corticosterone levels, seen in male and female population deermice, are due to an increased adrenal production. The production rates of the other major hormones produced will be quantified to see if they contribute to the inhibition of reproduction.
MATERIALS AND METHODS

Animals

All of the animals were maintained on a 14 hour light (four 40W fluorescent tubes from 2200-1200 hrs):10 hour dark regimen. Water and food (Wayne Laboratory Animal Diet) were supplied ad libitum. The yearly temperature variation in the animal rooms was kept between 21°C and 30°C. The daily temperature fluctuation was approximately ±2°C.

Three experimental populations were founded by four non-sibling pairs, the female of each being pregnant. The animals were placed into a 1.5m diameter corrugated aluminum enclosure with wood chips as a floor covering. None of the young from the first litters remained part of the population. Animals were checked biweekly for reproductive condition (Terman, 1969; 1973).

Control animals were reared in same sex sibling groups from weaning at 21 days until 90 ± 7 days. The animals were paired with non-siblings of the opposite sex in 12cm X 26cm X 14cm metal cages, but were separated by a 2cm X 2cm square wire mesh barrier that prevented physical contact, but allowed visual, auditory and olfactory communication (Albertson, Bradley and Terman, 1975).

Incubation and Extraction

Animals were removed from their cages or enclosures and rapidly decapitated within one minute of initial disturbance. All animals
(control and population) were 120 ± 7 days old at the time of sampling. The populations were all in their growth phase when animals were removed for sampling. All samples were taken one hour before the onset of the dark cycle (at 1100 hours). The adrenal glands were rapidly removed, freed of fat, weighed to the nearest milligram, and transferred to a cold 25 ml Erlenmeyer flask containing an incubation medium of 0.2% glucose in 5 ml Krebs-Ringer bicarbonate buffer (KRBB), 200 mg bovine serum albumin (BSA), 1 mg crude collagenase (Sigma) and 430,000 cpm progesterone-4-C\(^{14}\) (equal to 0.1976 uCi; specific activity = 56mCi/mM, Amersham). The adrenals were cut into approximately 10 pieces and the tissue incubated in a Dubnoff metabolic incubator at 100 oscillations/minute in an atmosphere of 95% O\(_2\), 5% CO\(_2\), and 37\(^\circ\)C for one hour (Goverde, Pesman and Benraad, 1980). The bodies of the animals were weighed and placed in a 10% buffered formalin solution for at least 72 hours. The testes, seminal vesicles, ovaries, and uteri were dissected, cleaned of surrounding tissue and weighed on a Cahn electrobalance interfaced with a Wang programmable calculator.

Following incubation the medium was transferred to 15 ml centrifuge tubes containing 2 ml ethyl acetate. The tubes were extracted by vortexing for 1 minute and then centrifuging at 2000 rpm for 2 minutes. The supernatant was pipetted to clean centrifuge tubes and the extraction procedure repeated. The supernatants were pooled and frozen at -70\(^\circ\)C until assayed.

Upon removal from the freezer, the supernatant was decanted to a clean 20 ml scintillation vial leaving behind any frozen aqueous lipid interface. Known amounts (6000-7000 cpm) of 1,2,6,7-\(\text{H}^3\)-corticosterone (specific activity = 105 Ci/mM, Amersham) was added to
the extract to determine loss and to serve as a chromatographic marker. All radioactive materials were purified by TLC prior to use.

**Paper Chromatography**

The extract volume of some incubates was reduced to dryness under nitrogen and resuspended in 100 ul absolute ethanol. This was streaked onto a 55cm X 4cm Whatman No. 1 paper chromatography strip on a band not greater than 1cm wide. The vial was washed with 100 ul ethanol and this was streaked onto the same chromatography strip. The strips containing the incubation extracts, along with others containing 100 ug individual non-radioactive standards (corticosterone, deoxycorticosterone, testosterone, progesterone) and a mixture of all these standards, were hung in a descending chromatography tank containing a paper curtain.

A chromatography system of cyclohexane:dioxane:methanol:distilled water (100:75:50:25) was prepared in a separatory funnel. The stationary phase was placed on the floor of the tank, and the system was equilibrated for 20 hours. The mobile phase was then added and the descending system was developed for 12 hours at a temperature of 22 ± 2°C. The strips were removed, the front was marked, and the strips were hung to dry.

Those strips containing the radioactive incubation medium were scanned on a 4π scanner (Tracerlab) for radioactive peaks. The major peaks were identified and their $R_f$ values were calculated. $R_f$ values were also calculated on another set of chromatograms run in the same manner, containing the incubation extracts and standards. These chromatograms were placed in 17% phosphoric acid and dried for
20 minutes at 100°C, in order to visualize the location of the various steroids.

**TLC for Purification and Separation of Incubates**

The following experiment was performed on a separate set of incubates. Incubation and extraction procedures were the same as they were for paper chromatography. In addition to the previously added \(^3\)H-corticosterone, known amounts (9000-12,000 cpm) of 1,2,6,7-\(^3\)H-testosterone (specific activity = 109 Ci/mM, Amersham) and (8000-10,000 cpm) deoxy-1,2-(n)-\(^3\)H-corticosterone (specific activity = 55 Ci/mM, Amersham) were added to the lipid-free extract. Fifty ug of authentic corticosterone, deoxycorticosterone (DOC), testosterone, and progesterone (Sigma) were also added. This volume was reduced to dryness under nitrogen, and then resuspended in 100 ul ethanol.

The radioactive extracts, non-radioactive samples of authentic corticosterone, DOC, testosterone, and progesterone, and a mixture of all four non-radioactive standards were streaked in bands not greater than 1.5 cm onto separate 1.2 cm lanes of a silica gel TLC plate. The plates were developed in a cyclohexane:ethyl acetate:hexanes (45:45:10) system for one hour. The U.V. absorbing areas were marked, their \(R_f\) values were calculated, and the areas were removed by scraping and aspiration into Pasteur pipettes packed with glass wool. The steroids were eluted with ethanol:methanol (1:1 v/v) into clean, labelled scintillation vials and evaporated to dryness under nitrogen. Ten ml of Biosolve (Beckman) was added and the vials were counted for both \(^3\)H and \(^{14}\)C energy for 100 minutes or 1% error on a Beckman LS 3133T liquid scintillation counter (narrow tritium and carbon isosets).
The numbers were corrected for spillover and loss. Comparison of body, testis, ovary and adrenal weights, and steroid production was made by calculating the mean ± standard error. The means were compared by the Student's t-test.

TLC for Reproducibility

In order to estimate the variability in the TLC procedure, the following experiment was performed. Adrenal glands from 5 control male animals, sampled as before, were combined in a 125 ml Erlenmeyer flask containing 30 ml KRBG, 1200 mg BSA, 6 mg crude collagenase, and 1.1856 uCi progesterone 4-C\textsuperscript{14}. All capsules were sliced and the glands were incubated as before. The incubate was divided into 5 ml aliquots and pipetted into 5 centrifuge tubes. Extraction proceeded as before.

To the lipid-free extract, 50 ug of non-radioactive corticosterone was added to serve as a U.V. absorbing marker. The material was reduced to dryness under nitrogen, resuspended in 100 ml ethanol, and streaked onto 5 separate 1.2 cm wide lanes of a 20cm X 10cm silica gel TLC plate. The centrifuge tubes were rinsed with 50 ul ethanol and this was also streaked onto the plate. The lanes at the ends of the plate were not used to avoid any edge effect during the chromatography.

The plate was placed into a TLC chamber containing the same system as before. Following chromatography for 1 hour, the corticosterone area was located under U.V. light, removed from the plate and eluted as before. After drying under nitrogen, 10 ml of Biosolve was added to the vial. The vials were counted in a scintillation counter, the numbers were corrected for spillover and loss, and the percent difference between the values was determined.
RESULTS

The radioactive scans of the paper chromatograms from the incubated adrenal glands for a representative single population male and female, and control male and female are shown in Figure 1. Three major radioactive peaks, in addition to the peak of progesterone $^{14}$C source, could be identified on the scans for all four classes of animals. The $R_f$ values calculated from these peaks were within the same range of $R_f$ values determined from the paper chromatography of individual non-radioactive samples of corticosterone, deoxycorticosterone, and testosterone, and a mixture of all three of these steroids (Table 1). Those authentic standards and adrenal incubation extracts that were paper chromatographed and the spots visualized by treatment with a 17% phosphoric acid solution, also produced the same range of $R_f$ values (Table 1).

Thin layer chromatography of the adrenal incubates from population male and female and control male and female animals, as well as authentic standards, all yielded identical $R_f$ values. The $R_f$ values were corticosterone, 0.054; deoxycorticosterone, 0.15; and testosterone, 0.19.

Population males were found to have significantly ($P<0.001$) smaller mean seminal vesicle, testis, and adrenal weights when compared with control males (Table 2). Body weight was also significantly ($P<0.01$) lighter in population males (Table 2). The mean corticosterone
produced (cpm) from the incubated adrenals of control males tended 
(P < 0.1) to be higher than that produced by population males (Table 3). 
The amount of corticosterone produced per milligram of adrenal tissue was not significantly different between the control and population male deermice. There was also no difference in the absolute or relative amounts of deoxycorticosterone or testosterone produced by adrenal incubation (Table 3).

Correlation analysis for several variables yielded some interesting results (Appendix A) for male deermice. Seminal vesicle weight was significantly (R < 0.01) and positively correlated with the amount of testosterone produced by the incubated adrenals in control males, but not in population males. Seminal vesicle weight, however, was significantly (R < 0.01) and positively correlated with the amount of deoxycorticosterone produced by the in vitro adrenal incubation in population males, and not in control males.

Female population deermice showed significantly (P < 0.001) lighter mean uterine, ovarian, and body weights when compared with their controls. Adrenal weights were also significantly (P < 0.01) lighter in the population female group (Table 4). The absolute amount of deoxycorticosterone produced by the adrenal glands of population females was significantly (P < 0.01) lower than control females. This difference was not significant when expressed in terms of the weight of adrenal tissue (deoxycorticosterone/mg adrenal weight) (Table 5). Population females also produced significantly (P < 0.01) higher levels of adrenal testosterone than control females, and this difference remained significant after expressing in relative terms for adrenal and body mass (P < 0.01 and P < 0.001 respectively). There was no difference seen
in the amount of corticosterone produced between the two female groups (Table 5).

Correlation analysis yielded a significant ($R < 0.05$) and positive correlation between adrenal weight and adrenal corticosterone produced in control females but not in population females. Control females showed a significant ($R < 0.05$) and negative correlation for the same parameters. Finally, ovarian weight was significantly ($R < 0.05$) and negatively correlated to the amount of in vitro adrenal testosterone produced in population females. Control females did not exhibit this same correlation (Appendix B).

The amount of corticosterone that was produced by the adrenal incubation of five pair of adrenal glands from male deermice, and divided into five separate aliquots is given in Table 6. The losses during the TLC process, and therefore the variability within the method, was found to be 14%.

The percent of the original progesterone $4^-{C^{14}}$ precursor that was converted during the incubation into corticosterone, deoxycorticosterone, and testosterone for male and female, population and control deermice is shown in Table 7. The amount (cpm) and percent of the original $4^-{C^{14}}$ precursor that remained unconverted is also presented in this table. The data show for all four classes of animals that less than 4% of the original 430,000 cpm added were associated with corticosterone, deoxycorticosterone and testosterone produced by adrenal incubation. Also, in all four classes of animals, at least 94% of the precursor material was recovered as the unutilized progesterone.
The radioactive scans of the paper chromatograms from the incubated adrenal gland for a representative P. maniculatus. O indicates the origin; numbers indicate the $R_f$ values of the peaks.

Figure 1a. Control male
Figure 1b. Population male
Figure 1c. Control female
Figure 1d. Population female
TABLE 1

COMPARISON OF THE RANGE OF Rf VALUES FROM PAPER CHROMATOGRAPHY OF AUTHENTIC STANDARDS AND RADIOACTIVE SCANS OF INCUBATES

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>AUTHENTIC STANDARDS</th>
<th>INCUBATES</th>
<th>COLOR AFTER 17% H3PO4 DEVELOPMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>CORTICOSTERONE (peak #1)</td>
<td>.29-.38</td>
<td>.32-.37</td>
<td>yellow-green</td>
</tr>
<tr>
<td>DEOXYCORTICOSTERONE (peak #2)</td>
<td>.62-.69</td>
<td>.63-.67</td>
<td>orange</td>
</tr>
<tr>
<td>TESTOSTERONE (peak #3)</td>
<td>.70-.75</td>
<td>.69-.74</td>
<td>red-purple</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>TREATMENTS</th>
<th>BODY WEIGHT (g)</th>
<th>ADRENAL WEIGHT (mg)</th>
<th>SEMINAL VESICLE WEIGHT (mg)</th>
<th>TESTIS WEIGHT (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL MALES (N=22)</td>
<td>16.7 ±0.54</td>
<td>4.09 ±0.12</td>
<td>147.6 ±14.3</td>
<td>297.5 ±17.2</td>
</tr>
<tr>
<td>POPULATION MALES (N=16)</td>
<td>14.4* ±0.44</td>
<td>2.99** ±0.15</td>
<td>14.1** ±5.8</td>
<td>89.3** ±12.3</td>
</tr>
</tbody>
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*P < 0.01; **P < 0.001
<table>
<thead>
<tr>
<th>TREATMENTS</th>
<th>CORTICO-STERONE</th>
<th>DOC</th>
<th>TESTOSTERONE</th>
<th>CORTICO./ ADRENAL WT. (cpm/mg)</th>
<th>CORTICO./ BODY WT. (cpm/g)</th>
<th>DOC./ ADRENAL WT. (cpm/mg)</th>
<th>DOC./ BODY WT. (cpm/g)</th>
<th>TESTO./ ADRENAL WT. (cpm/mg)</th>
<th>TESTO./ BODY WT. (cpm/g)</th>
<th>(N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL MALES</td>
<td>4515.8 ± 353.4</td>
<td>5372.4 ± 694.5</td>
<td>1846.4 ± 338.0</td>
<td>1133.1 ± 81.6</td>
<td>277.1 ± 24.5</td>
<td>1341.8 ± 199.0</td>
<td>314.9 ± 42.5</td>
<td>453.4 ± 82.4</td>
<td>108.8 ± 21.1</td>
<td>(22) (10) (10) (22) (22) (10) (10) (10) (10)</td>
</tr>
<tr>
<td>POPULATION MALES</td>
<td>3760.7* ± 257.8</td>
<td>4358.2 ± 590.2</td>
<td>1913.9 ± 323.5</td>
<td>1319.0 ± 107.9</td>
<td>253.0 ± 19.3</td>
<td>1474.7 ± 260.3</td>
<td>313.5 ± 40.8</td>
<td>678.7 ± 178.2</td>
<td>137.6 ± 22.3</td>
<td>(16) (10) (10) (16) (16) (10) (10) (10) (10)</td>
</tr>
</tbody>
</table>

* P<0.1
### TABLE 4

**COMPARISON OF BODY, ADRENAL, OVARY, AND UTERUS WEIGHTS IN CONTROL AND POPULATION FEMALES**

<table>
<thead>
<tr>
<th>TREATMENTS</th>
<th>BODY WEIGHT (g)</th>
<th>ADRENAL WEIGHT (mg)</th>
<th>OVARY WEIGHT (mg)</th>
<th>UTERUS WEIGHT (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL FEMALES (N=18)</td>
<td>17.0 ±0.49</td>
<td>4.1 ±0.29</td>
<td>17.6 ±1.65</td>
<td>44.1 ±10.5</td>
</tr>
<tr>
<td>POPULATION FEMALES (N=14)</td>
<td>12.9** ±0.38</td>
<td>3.08** ±0.21</td>
<td>5.05** ±0.69</td>
<td>8.05** ±1.1</td>
</tr>
</tbody>
</table>

**P < 0.01**
TABLE 5

ABSOLUTE AND RELATIVE ADRENAL PRODUCTION OF CORTICOSTERONE, DOC, AND TESTOSTERONE
IN CONTROL AND POPULATION FEMALES
(MEAN ± SEM)

<table>
<thead>
<tr>
<th>TREATMENTS</th>
<th>CORTICO-</th>
<th>DOC</th>
<th>TESTO-</th>
<th>CORTICO./</th>
<th>CORTICO./</th>
<th>DOC/</th>
<th>DOC/</th>
<th>TESTO./</th>
<th>TESTO./</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>STERONE</td>
<td></td>
<td>STERONE</td>
<td>ADRENAL WT.</td>
<td>BODY WT.</td>
<td>ADRENAL WT.</td>
<td>BODY WT.</td>
<td>ADRENAL WT.</td>
<td>BODY WT.</td>
</tr>
<tr>
<td>CONTROL</td>
<td>4124.5</td>
<td>8466.7</td>
<td>1901.5</td>
<td>994.8</td>
<td>243.4</td>
<td>2240.0</td>
<td>488.6</td>
<td>501.4</td>
<td>110.9</td>
</tr>
<tr>
<td>FEMALES</td>
<td>±621.2</td>
<td>±709.2</td>
<td>±208.0</td>
<td>±118.3</td>
<td>±36.8</td>
<td>±273.1</td>
<td>±34.9</td>
<td>±72.6</td>
<td>±10.5</td>
</tr>
<tr>
<td>POPULATION</td>
<td>3997.0</td>
<td>5786.1**</td>
<td>2555.2**</td>
<td>1413.2*</td>
<td>316.1</td>
<td>2101.8</td>
<td>435.5</td>
<td>898.4**</td>
<td>202.7**</td>
</tr>
<tr>
<td>FEMALES</td>
<td>±309.3</td>
<td>±514.0</td>
<td>±146.4</td>
<td>±171.9</td>
<td>±27.2</td>
<td>±31.2</td>
<td>±40.2</td>
<td>±81.0</td>
<td>±15.3</td>
</tr>
<tr>
<td>(N)</td>
<td>(14)</td>
<td>(13)</td>
<td>(14)</td>
<td>(14)</td>
<td>(14)</td>
<td>(13)</td>
<td>(13)</td>
<td>(14)</td>
<td>(14)</td>
</tr>
</tbody>
</table>

* P < 0.1
** P < 0.01
TABLE 6

THE AMOUNT OF CORTICOSTERONE (cpm) PRODUCED FROM PROGESTERONE 4- C\textsuperscript{14} BY THE ADRENAL INCUBATION
OF FIVE PAIR OF MALE DEERMICE ADRENAL GLANDS WHICH WERE DIVIDED INTO FIVE ALIQUOTS

<table>
<thead>
<tr>
<th>TUBE #</th>
<th>CORTICOSTERONE PRODUCED (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3645.7</td>
</tr>
<tr>
<td>2</td>
<td>3900.0</td>
</tr>
<tr>
<td>3</td>
<td>3590.7</td>
</tr>
<tr>
<td>4</td>
<td>3423.8</td>
</tr>
<tr>
<td>5</td>
<td>3979.8</td>
</tr>
</tbody>
</table>

Variability = \[ \frac{3979.8 - 3423.8}{3979.8} \] = 0.14% = 14%
TABLE 7

THE AMOUNT (cpm) AND PERCENT CONVERSION OF PROGESTERONE 4-C<sup>14</sup> PRECURSOR TO CORTICOSTERONE, DEOXYCORTICOSTERONE, AND TESTOSTERONE; AND UNCONVERTED PROGESTERONE RECOVERED IN CONTROL AND POPULATION MALES AND FEMALES

### MALES

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>POPULATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm</td>
<td>% conversion</td>
</tr>
<tr>
<td>CORTICOSTERONE</td>
<td>4515.8</td>
<td>1.05</td>
</tr>
<tr>
<td>DEOXYCORTICOSTERONE</td>
<td>5372.4</td>
<td>1.25</td>
</tr>
<tr>
<td>TESTOSTERONE</td>
<td>1846.4</td>
<td>0.43</td>
</tr>
<tr>
<td>UNCONVERTED PROGESTERONE</td>
<td>413,313.0</td>
<td>96.1</td>
</tr>
</tbody>
</table>

### FEMALES

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>POPULATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm</td>
<td>% conversion</td>
</tr>
<tr>
<td>CORTICOSTERONE</td>
<td>4124.5</td>
<td>1.00</td>
</tr>
<tr>
<td>DEOXYCORTICOSTERONE</td>
<td>8466.7</td>
<td>1.97</td>
</tr>
<tr>
<td>TESTOSTERONE</td>
<td>1901.5</td>
<td>0.44</td>
</tr>
<tr>
<td>UNCONVERTED PROGESTERONE</td>
<td>403,701.0</td>
<td>93.9</td>
</tr>
</tbody>
</table>
DISCUSSION

The radioactive scans from the paper chromatographic separation of the adrenal incubates from all four classes of animals (population and control, male and female deer mice) used in this experiment show a similar pattern (Figure 1). A comparison of the position of the radioactive compounds in the scans, and the position of non-radioactive purified standards of corticosterone, deoxycorticosterone, and testosterone indicate a similar range of $R_f$ values. Further, the similar color patterns for the authentic materials and these radioactive peaks, seen after development in 17% phosphoric acid, supports the conclusion that the three peaks contain corticosterone, deoxycorticosterone, and testosterone (Table 1).

The thin-layer chromatography of the incubates and the non-radioactive purified standards also supports the identifications made from paper chromatography. Since authentic corticosterone, deoxycorticosterone, and testosterone were added to the incubates as U.V. absorbing markers, and only those areas that were U.V. sensitive were removed to be counted in the scintillation counter, these areas therefore contained the three hormones investigated in this study. The remainder of the silica gel area (the non-U.V. absorbing areas) was removed from the plate and counted in five of twenty separations. The amount of radioactivity found in this area never exceeded 10% of the combined radioactivity from the U.V. absorbing areas.
Consequently, at least 90% of the activity on the TLC plate was from the peaks associated with the hormones corticosterone, deoxycorticosterone, and testosterone. In this experiment, less than 4% of the original precursor progesterone that was put into the system was incorporated into corticosterone, deoxycorticosterone, and testosterone (Table 7). Also, at least 94% of the precursor was recovered as unutilized progesterone. Thus, the amount of radioactive material present during the incubation of the adrenal glands was not a limiting factor, and therefore the total amount of hormone produced is a function of the actual rates of production. The results shown in Table 7 also indicate that in all four classes of animals studied, at least 97% of the radioactivity put into the system, and separated by TLC, can be accounted for in the incorporation into corticosterone, deoxycorticosterone, testosterone, or left unincorporated and recovered as progesterone. Thus, the findings from both paper and thin-layer chromatography indicate that corticosterone, deoxycorticosterone, and testosterone represent the major adrenal secretory products produced under these conditions by in vitro incubation in P. maniculatus.

Terman (1969) found no significant difference in the body weights of male or female population deer mice when compared to their controls. In recent studies from our laboratory (Albertson, Bradley, and Terman, 1975; Sung, Bradley, and Terman, 1977; Copes, 1980; Bradley and Terman, 1981b; Peebles, 1981), the mean body weights of both male and female population animals were significantly lighter than their respective controls. Bradley and Terman (1981a) found population males to have lower mean body weights than controls, but population females only tended (P<0.1) toward lighter mean body weights. The body weight data
from the present study compare favorably with recent work indicating that both male and female deer mice from the populations had significantly (P<0.01, P<0.001 respectively) lighter mean body weights compared with control animals.

Highly significant reductions in the weights of reproductive organs of population deer mice of both sexes have been consistently reported (Terman, 1969; Sung, Bradley, and Terman, 1977; Coppes, 1980; Bradley and Terman, 1981a; 1981b; Peebles, 1981). In this study, the weights of paired testes and seminal vesicles in reproductively inhibited males, and uteri and ovaries in reproductively inhibited females were all significantly (P<0.001) lighter than the control animals. Thus, the conditions used in this experiment, while different from some of the previous studies, i.e. larger population enclosures, different light cycles, still resulted in the same marked reproductive inhibition.

Increases in density have been associated with increased in vitro adrenal function in several species. Slices of adrenal glands from house mice, that were stressed by increased density, and then incubated in vitro, show increased corticosteroid release (Varon, Touchstone, and Christian, 1966). McCarthy, Green, and Sohal (1978) found in rats, that a decrease in housing space from $110 \text{ cm}^2$/animal to $30 \text{ cm}^2$/animal resulted in an elevation of the $11\beta$-hydroxylation reaction. Andrews and Belknap (1979) found a positive correlation between population density in a natural population of deer mice, and the in vitro adrenal secretion of glucocorticoids. However, in the present study, adrenals incubated from population male deer mice tended (P<0.1) to have lower absolute corticosterone production rates than control males, but this difference disappeared when expressed relative to
adrenal mass. There was no difference seen in the amount of deoxycorticosterone produced by the incubated adrenal glands of male deermice (Table 3). Population females produced significantly ($P<0.01$) more deoxycorticosterone than control females, but this difference did not exist when expressed per unit weight of adrenal tissue.

Also, there was no difference in the rate of production of corticosterone by female deermice adrenal incubates. Control females, but not population females, showed a significant ($R<0.05$) and positive correlation between adrenal weight and the amount of corticosterone produced. This indicates that the adrenal glands of the control females show an increase in corticosterone production as weight increases; a process well documented in mice (Bronson, 1965; Louch and Higginbotham, 1967; Brain and Nowell, 1971a; 1971b; Christian, 1975). The adrenals of population females are refractory to an increase in adrenal corticosterone production.

There was no difference observed in the production rate of the two major glucocorticoids per unit mass of adrenal tissue in either male or female $P.\ maniculatus$ population inhibited animals compared with reproductively capable controls. The difference in this finding, and those in a similar study of a natural population of deermice (Andrews and Belknap, 1979) may be due to the trauma of capture that occurs when a natural population is used. Even though the animals from the natural population were brought to the laboratory and held for three days before being sacrificed, the effect of handling and artificial surroundings may still have caused an increased release of glucocorticoids from their incubated adrenals.
Work has been done on the question of whether reproductive suppression is due to the direct effect of ACTH, or indirectly through the glucocorticoids. Pasley and Christian (1972) found that ACTH administration caused decreased testis and seminal vesicle weights in adrenalectomized Peromyscus leucopus. However, in a similar experiment using exogenous ACTH administration Saez et al. (1977) found no reduction of plasma testosterone in adrenalectomized rats. Schaison, Durand, and Mowszowicz (1978) showed that treatment with dexamethasone, a synthetic glucocorticoid, and ACTH caused a decrease in plasma testosterone in man, but metyrapone treatments, which cause elevated ACTH levels while blocking cortisol production, did not change the concentration of testosterone. High levels of exogenous glucocorticoids have been found to cause decreased plasma testosterone (Doerr and Pirke, 1975) and decreased testosterone secretion (Magrini et al., 1978; Welsh, McGraw, and Johnson, 1979). Other reports on other species have shown a decreased level of plasma testosterone in the hyperglucocorticoid state, without lower plasma LH (Beitins et al., 1973; Irvine et al., 1974; McKenna et al., 1979). Sung, Bradley, and Terman (1977) and Bradley and Terman (1981a) found higher levels of serum corticosterone in reproductively inhibited P. maniculatus without a concomitant increase in adrenal weight. Coppes (1980) found that ACTH administration caused adrenal hypertrophy in male deer mice, but he also found no significant difference in plasma ACTH levels between population and control male deer mice. Also, the adrenal ultrastructure of population male deer mice further suggested the lack of elevated ACTH levels in population male deer mice. Bradley and Terman (1981c) found significantly lower concentrations of
testosterone in population male deermice, that were presumed to have elevated levels of serum corticosterone. In another experiment, (Bradley and Terman, 1981b) serum LH concentrations were not significantly different among male deermice. Since ACTH levels appear not to be elevated in population male deermice, it is possible that the reproductive inhibition seen in male deermice may be due to a high serum corticosterone concentration, and not due to direct inhibition by ACTH. However, since the production of adrenal glucocorticoids measured in this study appear not to be elevated in population males, some other cause for the high serum corticosterone concentrations found in male P. maniculatus may exist.

Peebles (1981) found that reproductively inhibited male deermice had a significantly lower mean serum thyroxine concentration than control males, and inhibited females tended to also have reduced thyroxine levels. If these levels reflect hypothyroidism, that condition may contribute to the elevated corticosterone levels seen in population deermice, since low thyroxine levels are known to reduce corticosterone catabolism and excretion (Brown, Englert, and Wallach, 1958; Peterson, 1958; Miller et al., 1970). It has been suggested however that low thyroxine levels may be a result of increased glucocorticoid levels, and not the cause (Otsuki, Dakoda, and Baba, 1973; Pameater and Hedge, 1980).

Another factor that may allow increased serum corticosterone levels without an increased rate of adrenal production is the presence of elevated corticosteroid binding globulin (CBG) levels. Serum testosterone levels from inhibited male deermice have been found to be significantly lower than controls (Bradley and Terman, 1981c).
Following castration in male rats, CBG activity is increased (Gala and Westphall, 1965), and in hypogonadal men, the elevated CBG production is inhibited by exogenous testosterone (Kley et al., 1973). The present study shows a significant ($P<0.01$) positive correlation in control males between seminal vesicle weight and adrenal testosterone produced in vitro. However, the seminal vesicles of reproductively inhibited males appear to be refractory to a similar adrenal testosterone concentration (Appendix A); presumably because of the lower testicular androgen production. Thus, in the hypogonal state of the population male deermice, CBG levels may be elevated. Sandberg and Slaunwhite (1963) found that corticosterone bound to CBG is protected from liver catabolism, and therefore CBG elevation could be contributing to the higher serum corticosterone levels found in male reproductively inhibited deermice. It is apparent that further work needs to be done to determine the cause(s) of the elevated circulating serum corticosterone concentration seen in population male deermice. Measurement of CBG activity and the liver catabolism of corticosterone needs to be investigated.

While there was no difference in the production rate of deoxycorticosterone by the adrenal incubation in male deermice, deoxycorticosterone was significantly ($P<0.01$) and positively correlated with seminal vesicle weight in population males, but not in controls (Appendix A). This could indicate that deoxycorticosterone may play a role in the regulation of reproduction in population males via some other mechanism.

Another important finding in the present study is the significantly ($P<0.01$) elevated adrenal production of testosterone by reproductively inhibited female deermice. This difference remained significant
(P < 0.01) when expressed per unit weight of adrenal and body tissue. In addition, the significant (R < 0.05) negative correlation between the amount of testosterone produced by adrenal incubation and ovarian weight seen in the inhibited females, but not in control females, suggests the presence of an important control mechanism. Adrenal androgens, as well as estrogens and progestins, may act as negative feedback inhibitors of gonadotrophin secretion at the level of the pituitary (Duckett, Varon, and Christian, 1963; Varon and Christian, 1963; Christian and Davis, 1964; Christian, Lloyd, and Davis, 1965; Andrews et al., 1972; Andrews, 1977). Immobilization stress abolishes the pulsatile release of LH (Blake, 1975). Bradley and Terman (1981b) found the mean serum LH concentration of population female deermice to be significantly lower than control females, due most likely to the failure of these females to undergo an LH surge. Also, there was no difference in the number of Type 6 and 7 follicles, or in the number of animals having these follicles among population and control females. There were significantly fewer Type 8 follicles found in population females. In view of all this, it appears that ovarian inhibition seen in population female deermice occurs at a late stage in follicular development. Rivarola et al., (1966) and Hajjar, Hill, and Samaan (1975) found that exogenous ACTH increases adrenal androgen production. Circulating ACTH and testosterone concentration have not been investigated in female P. maniculatus. However, with the current finding that the adrenal production rate of testosterone is elevated in population females, it is interesting to speculate that the cause of reproductive inhibition in female deermice is an elevated serum
concentration of testosterone from an adrenal source negatively inhibiting the release of LH, causing a failure of ovulation.

Decreased LH levels have also been associated with increased glucocorticoid levels (Luton et al., 1977; Welsh, McCraw, and Johnson, 1979). Further, these lower LH levels may be due to a decreased pituitary responsiveness to LH-RH (Boccozzi, et al., 1975; Sakakura, Takebe, and Nakagawa, 1975; Luton et al., 1977). Serum corticosterone levels are elevated in population female deermice (Sung, Bradley, and Terman, 1977; Bradley and Terman, 1981a). Absolute adrenal deoxycorticosterone production is elevated in control females, even though this difference disappears after expression relative to adrenal mass (Table 5). Deoxycorticosterone production was found to be significantly (R<0.05) and negatively correlated with uterine weight in population females, but significantly (R<0.01) and positively correlated in control females (Appendix B). Consequently, the more adrenal deoxycorticosterone produced in population female deermice, the lower the uterine weight. However in control females, increased deoxycorticosterone production resulted in increased uterine weight. Because of this marked difference in the effect of deoxycorticosterone on the uterine weight of control and population deermice, it is possible that deoxycorticosterone may play a role in the reproductive inhibition seen in population females. The serum concentration of deoxycorticosterone needs to be investigated in order to see if deoxycorticosterone is adding to the already elevated corticosterone levels found in population females. Also, further study on the levels of ACTH and testosterone in female deermice needs to be done to determine if inhibition is due to an as yet undetermined elevated ACTH level, or to the elevated glucocorticoid pool.
CONCLUSIONS

Paper chromatography and radioactive scanning procedures indicate that adrenal glands of population and control male and female deermice produce steroid hormones that are not significantly different in character. The major adrenal secretory products are chromatographically associated with corticosterone, deoxycorticosterone, and testosterone.

The in vitro adrenal production rates of corticosterone, deoxycorticosterone, and testosterone were not significantly different between control and population males. Thus, something other than an increased adrenal glucocorticoid production rate is probably involved in maintaining the elevated serum corticosterone levels found in male P. maniculatus. However, population females had an increased adrenal incubation production rate of testosterone compared to control females. Also, testosterone production was significantly negatively correlated with ovarian weight in population females, but not in control females. Corticosterone and deoxycorticosterone production rates were not significantly different between female population and control deermice, even though corticosterone levels have previously (Sung, Bradley, and Terman, 1977; Bradley and Terman, 1981a) been found to be elevated in female population animals. The elevated glucocorticoid pool is probably contributing to the reproductive inhibition seen in male and female deermice. However,
in reproductively inhibited female deer mice, it is possible that an additional mechanism involving ACTH which has yet to be investigated and adrenal testosterone exists. Elevated ACTH levels may promote an increase in the production of adrenal testosterone. This increased production may elevate serum testosterone concentrations, resulting in a negative inhibition of LH release, and causing a failure of ovulation.
### APPENDIX A

#### CORRELATION ANALYSIS OF AMOUNT OF PRODUCED STEROIDS AND ADRENAL, SEMINAL VESICLE, AND TESTIS WEIGHTS IN CONTROL AND POPULATION MALES

<table>
<thead>
<tr>
<th></th>
<th>CONTROL CORRELATION COEFFICIENT</th>
<th>POPULATION CORRELATION COEFFICIENT</th>
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<tbody>
<tr>
<td>ADRENAL WT. VS. CORTICO. PROD.</td>
<td>0.355</td>
<td>0.122</td>
</tr>
<tr>
<td>SEM. VES. WT. VS. CORTICO. PROD.</td>
<td>0.155</td>
<td>-0.165</td>
</tr>
<tr>
<td>TESTIS WT. VS. CORTICO. PROD.</td>
<td>-0.114</td>
<td>-0.060</td>
</tr>
<tr>
<td>ADRENAL WT. VS. DOC PROD.</td>
<td>-0.324</td>
<td>-0.379</td>
</tr>
<tr>
<td>SEM. VES. WT. VS. DOC PROD.</td>
<td>0.186</td>
<td>0.636*</td>
</tr>
<tr>
<td>TESTIS WT. VS. DOC PROD.</td>
<td>0.002</td>
<td>0.258</td>
</tr>
<tr>
<td>ADRENAL WT. VS. TESTO. PROD.</td>
<td>-0.077</td>
<td>-0.564</td>
</tr>
<tr>
<td>SEM. VES. WT. VS. TESTO. PROD.</td>
<td>0.690*</td>
<td>0.319</td>
</tr>
<tr>
<td>TESTIS WT. VS. TESTO. PROD.</td>
<td>0.095</td>
<td>0.524</td>
</tr>
</tbody>
</table>

* = significant correlation
APPENDIX B

CORRELATION ANALYSIS OF AMOUNT OF PRODUCED STEROIDS AND ADRENAL, OVARY, AND UTERUS WEIGHTS IN CONTROL AND POPULATION FEMALES

<table>
<thead>
<tr>
<th></th>
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<th>POPULATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CORRELATION</td>
<td>CORRELATION</td>
</tr>
<tr>
<td></td>
<td>COEFFICIENT</td>
<td>COEFFICIENT</td>
</tr>
<tr>
<td>ADRENAL WT. VS. CORTICO. PROD.</td>
<td>0.580*</td>
<td>-0.240</td>
</tr>
<tr>
<td>OVARY WT. VS. CORTICO. PROD.</td>
<td>-0.389</td>
<td>-0.420</td>
</tr>
<tr>
<td>UTERUS WT. VS. CORTICO. PROD.</td>
<td>-0.104</td>
<td>-0.467</td>
</tr>
<tr>
<td>ADRENAL WT. VS. DOC PROD.</td>
<td>-0.185</td>
<td>-0.429</td>
</tr>
<tr>
<td>OVARY WT. VS. DOC PROD.</td>
<td>-0.041</td>
<td>-0.386</td>
</tr>
<tr>
<td>UTERUS WT. VS. DOC PROD.</td>
<td>0.754*</td>
<td>-0.620*</td>
</tr>
<tr>
<td>ADRENAL WT. VS. TESTO. PROD.</td>
<td>-0.187</td>
<td>-0.078</td>
</tr>
<tr>
<td>OVARY WT. VS. TESTO. PROD.</td>
<td>-0.208</td>
<td>-0.506*</td>
</tr>
<tr>
<td>UTERUS WT. VS. TESTO. PROD.</td>
<td>-0.008</td>
<td>-0.196</td>
</tr>
</tbody>
</table>

* = significant correlation


VITA

RICHARD PAUL TUCCI