1983

The existence and nature of hypothyroidism in reproductively inhibited prairie deermice (Peromyscus maniculatus bairdi) from laboratory populations

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College of William & Mary - Arts & Sciences

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THE EXISTENCE AND NATURE OF HYPOTHYROIDISM
IN REPRODUCTIVELY INHIBITED 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
John M. Pitman, III
1983
This thesis is submitted in partial fulfillment of the requirements for the degree of Master of Arts.
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ACKNOWLEDGEMENTS

I would like to express my deep appreciation to my advisor Dr. Eric Bradley, whose continuous patience, guidance, courage, and sense of humor enabled the completion of this project. I am indebted to my parents, Dr. and Mrs. John Pitman, without whose love and support, nothing is possible. I would like to thank Joel Whitley and Tammy Smith at the Williamsburg Community Hospital Laboratory for their help in the acquisition and use of the RIA materials. I am indebted to Mrs. Pearl Bazacos for her invaluable aid and efforts in the preparation of the histological specimens. I would like to thank Dr. John Dunn and Mr. Carlos Valdenegro at the Department of Endocrinology, University of Virginia School of Medicine, for the testing of the NIADDK rat TSH RIA materials. I am indebted to Dr. Robert Black and Dr. Lawrence Wiseman for their reading of the manuscript and Ms. Susan Jackson and Mr. Larry Kirkland for their assistance in its preparation. Finally, I would like to thank Mrs. Jewel Thomas for her help in the preparation of the photomicrographs.
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ABSTRACT

This study had two objectives. The first was to expand upon an earlier study indicating that prairie deermice from laboratory populations had depressed serum thyroxine, (Peebles, 1981), values by examining serum thyroxine and triiodothyronine concentrations in control and population deermice at another time. The second was to demonstrate the nature of thyroid dysfunction in deermice by a morphometric analysis of thyroid histology.

Examination of mean serum thyroxine concentrations every two hours over a twenty four hour time period revealed a significant increase in mean serum thyroxine concentration of control females from 1000 to 1200 h, three hours after the dark to light transition point. Males had very similar trends but there were no statistically significant changes. The time of seven hours before the light to dark transition point (1400 h) + 30 minutes was chosen as a sampling time for the subsequent population/control comparison of serum thyroxine and triiodothyronine due to the stability of mean serum thyroxine concentration for both sexes during that time.

Population males and females had significantly lower mean serum thyroxine and triiodothyronine concentrations at 1400 h. The reproductive organ and body weights were significantly reduced in population animals signifying inhibited development.

Morphometric analysis of thyroid histology of population and control animals revealed a significantly decreased mean cell height and mean number of follicle cells in population males. Population females had a significantly increased follicular area, colloidal area and number of cells per follicle. These results indicated a different origin for hypothyroidism in population males compared with population females. Population male thyroids appeared to be undergoing decreased thyroid stimulating hormone (TSH) stimulation while population females appeared to have excess TSH stimulation and low thyroid hormone output, thus indicating probable secondary hypothyroidism in males and primary hypothyroidism in females.
THE EXISTENCE AND NATURE OF HYPOTHYROIDISM
IN REPRODUCTIVELY INHIBITED PRAIRIE DEERMICE
(Peromyscus maniculatus bairdi)
FROM LABORATORY POPULATIONS
INTRODUCTION

The growth of experimental populations of *Peromyscus maniculatus bairdi* is characterized by cessation of growth at widely divergent animal numbers and is controlled either by failure of young to survive or by cessation of reproduction (Terman 1969, 1973a, 1973b). Asymptote occurs despite the presence of unlimited food and water and is density related but not density dependent due to the extreme variability in the number of animals in asymptotic populations (Terman, 1969).

There are certain ubiquitous characteristics of these reproductively inhibited population animals. The testis and seminal vesicle weights of males born into the population are highly significantly ($P<0.001$) less than the corresponding reproductively capable control males of the same age (Terman, 1969, 1973a). Testis function is also greatly depressed as evidenced by diminished spermatogenesis (Bradley and Terman, 1981c; Peebles et al., 1983) and reduced testosterone secretion (Bradley and Terman, 1981a).

Ovarian and uterine weights from population females are also significantly less than the corresponding values from
control females (Terman, 1969, 1973a). Eighty to ninety percent of females born into the populations that survive to at least ninety days fail to produce young (Terman, 1973a). Ovarian histology shows significantly reduced numbers of corpora lutea and Type 8 follicles (Pederson and Peters, 1968) compared with reproductively capable controls (Bradley and Terman, 1981b).

Serum gonadotropin concentrations of reproductively capable prairie deermice are distinctly different from reproductively inhibited population animals. Serum follicle stimulating (FSH) hormone concentrations are elevated for population animals of both sexes and serum luteinizing hormone (LH) concentrations are reduced in population females (Bradley and Terman, 1981c). Serum corticosterone in population males and females is elevated (Sung et al., 1977; Bradley and Terman, 1981b), but serum adrenocorticotropic hormone (ACTH) appears unchanged (Coppes, 1980) and in vitro adrenal secretion rates of corticosterone are not statistically different between population and control deermice (Tucci, 1983).

Christian (1950, 1971a, 1971b, 1975, 1980) has proposed that crowding stress promotes elevated ACTH secretion in many rodent populations which, in turn, leads to elevated adrenal cortex secretion of corticosteroids. He has proposed that this stress induced stimulation of the pituitary-adrenal axis is the
primary cause of delayed maturation in rodents, possibly by a direct inhibitory effect on the gonads (Christian 1964a, 1964b, 1978).

In light of the conflicting results concerning the role of ACTH in reproductive inhibition in prairie deer mice, a previous study was performed to examine serum concentrations of thyroxine in population and control deer mice (Peebles et al., 1983). Mean serum thyroxine concentration was demonstrated to be significantly reduced (P<0.03) in population males and tended to be reduced (P<0.10) in population females compared with reproductively capable controls.

Virtually all postnatal growth and development is dependent upon thyroid hormone through stimulation of growth hormone activity and effects on target tissues intrinsic to thyroid hormones alone (Bernal and DeGroot, 1980; Norris, 1980; Ingbar and Woeber, 1981). In humans, hypothyroidism before puberty leads to a delay in the onset of puberty and anovulatory cycles (Bernal and DeGroot, 1980, Ingbar and Woeber, 1981). The metabolism of androgens and estrogens is influenced by thyroid hormones and is significantly affected by hypothyroidism. Androgen secretion is decreased and the conversion of testosterone to androsterone is diverted to an increase in etiocholanone formation (Inbar and Woeber, 1981). Testis and ovary histological examination shows degeneration
associated with hypothyroidism especially before the onset of puberty (Jones and Tracy, 1974; Dixit et al., 1976; Amin and El-Sheikh, 1977; Ingbar and Woeber, 1981). Thus, the body weight reduction and reproductive developmental deficiency seen in laboratory populations of Peromyscus could be better explained by an overall deficiency in thyroid hormone.

The two most active thyroid hormones are triiodothyronine and thyroxine. Triiodothyronine is the most biologically active of the two hormones (Chopra et al., 1973; Bernal and DeGroot, 1980; Ingbar and Woeber, 1981). One-third of all thyroxine gives rise to triiodothyronine by peripheral conversion. The remaining triiodothyronine is secreted directly by the thyroid gland (Bernal and DeGroot, 1980, Ingbar and Woeber, 1981). Factors that decrease the formation of triiodothyronine also inhibit the activity of thyroxine. Thus, most thyroxine activity originates from the formation of triiodothyronine (Ingbar and Woeber, 1981). However, thyroxine has some intrinsic activity as well (Chopra et al., 1973; Ingbar and Woeber, 1981). Triiodothyronine initiates its activity at the target tissue level by binding to nuclear receptors (Bantle et al., 1980). The release and synthesis of the two hormones from the thyroid gland is regulated by thyroid stimulating hormone (TSH) release from the anterior pituitary (Norris, 1980). Thyroid hormones are sequestered within the follicles of the thyroid gland after synthesis by binding to thyroglobulin,
proteins (Norris, 1980; Ingbar and Woeber, 1981). In addition to the large store of thyroid hormones within the thyroid gland, thyroid hormones are bound to thyroid binding globulin (TBG) and prealbumin in the plasma (Norris, 1980; Ingbar and Woeber, 1981).

Hypothyroidism has many causes, and these are often characterized as primary or secondary. Primary hypothyroidism is a product of the thyroid gland's inability to secrete sufficient thyroxine and, to a lesser extent, triiodothyronine. It is characterized by elevated serum TSH. Secondary hypothyroidism is due to insufficient TSH release by the anterior pituitary and is characterized by depressed serum TSH (Bernal and DeGroot, 1980). TSH has a profound effect on the appearance of the thyroid gland, so the origin of hypothyroidism can be determined from thyroid histology (Meissner, 1980; Ingbar and Woeber, 1981) as well as from serum TSH concentration (Werner and Ingbar, 1978).

ACTH and thyroid function are highly associated. Fortier et al., (1970), Bogoroch and Timiras (1951), and Brown-Grant et al., (1954) argue that a stress induced rise in ACTH diminishes pituitary secretion of TSH. Kuhl and Ziff (1951) demonstrated a decrease in protein bound iodine in response to large doses of ACTH and cortisone. Fredrickson (1951) demonstrated a significant drop in thyroid I-131 uptake in patients receiving
massive cortisone therapy. Thus, it could be argued that the hypothyroidism suggested by the earlier study (Peebles et al., 1983) is linked to the activity of the pituitary-adrenal axis.

This study was performed with three objectives in mind:

1) To examine serum thyroxine concentrations in reproductively capable control deer mice over a twenty four hour period to find a time at which mean serum thyroxine concentrations are stable for both sexes.

2) To compare serum thyroxine and triiodothyronine levels between reproductively inhibited population animals and reproductively capable controls at the time of maximum stability.

3) To examine thyroid histology in the animals of the previous experiment to attempt elucidate whether a possible hypothyroidism is primary or secondary in origin.
MATERIALS AND METHODS

Animal Maintenance:

The animals used in this study were prairie deermice (Peromyscus maniculatus bairdi) obtained from a laboratory colony in which sibling matings were not allowed (Terman and Sassaman, 1967; Terman, 1974). Colony animals were maintained on a diet of Charles River Laboratory Mouse Diet and tap water in excess of utilization. They were kept on a light cycle of 14 hours light (0700-2100h) and 10 hours dark. Illumination was provided by four 40W fluorescent tubes which provided 15 ft-c at the floor level (Bradley and Terman, 1981c). The colony control animals were kept in 12.5 x 27 x 14.5 cm plastic wire topped cages. Wood shavings were used as bedding and were changed every 14 days unless newborns were present. Yearly laboratory temperatures were maintained between 20 and 30 C. Ventilation was such that room air was replaced five to ten times per hour, depending upon temperature. The young were kept with their parents until weening, when they were placed in separate cages with their littermates of the same sex. They were removed from the colony room at 81 ±6 days of age and placed in the experimental rooms.
Founding of Populations

Experimental populations were founded in 1.5 M diameter by 0.75 M deep circular enclosures constructed of corrugated aluminum. The floor of each enclosure was covered with wood shavings. Nonsibling colony males and females of 90-120 days of age were paired in 12.5 x 27 x 14.5 cm plastic wire topped cages and given food and water ad libitum. The first litter born to a producing pair was removed five to ten days following parturition. Following the removal of the litters, only those pairs that produced young were used to found the populations. Four pairs of founding animals were toe clipped and placed into plastic nest boxes. The nest boxes and the enclosed animals were placed in the population enclosure and were left undisturbed for five to ten minutes before the animals were allowed to leave. The first litter born to each female within the population enclosure was removed to insure that all births within the population enclosure were a result of impregnations within the population context. Food and water were provided ad libitum. Populations were ordinarily checked at two to four week intervals (with none occurring more than six weeks apart). All pregnant, lactating, perforate, or imperforate females and all males with scrotal or non-scrotal testes were noted during these checks. All new surviving young were toe clipped no earlier than 21 days of age and their reproductive condition was monitored during subsequent checks. All populations were
at least 8 months old before any animals were sampled. A total of four populations were used.

**Tissue Collection**

Blood samples were collected from the dorsal abdominal aorta by laparotomy during ether anaesthesia. All animals were sampled within three minutes of initial contact, although one report indicates that acute stress does not affect triiodothyronine levels for about 60 minutes after initial disturbance and had no effect on serum thyroxine levels, (Dohler et al., 1977). Blood samples were refrigerated at 3°C immediately after collection. Sera were obtained by centrifugation (Beckman Microfuge) at 9000 x g for four minutes, within two hours of sample collection and no sooner than 15 minutes after collection. Sera were stored at below -30°C until assayed.

The animals were placed in buffered formalin for at least 30 days and no more than 200 days until dissection. The testes, seminal vesicles, ovaries, uteri, and surrounding tissues were then removed and stored in buffered formalin for at least 96 hours prior to fine dissection of excess tissue and subsequent weighing. The organs were blotted dry with paper towels and the weights were measured on a Cahn DTL electrobalance interfaced with a Wang 700 programable
EXPERIMENT ONE: CIRCADIAN VARIATION OF SERUM THYROXINE

There is some evidence that thyroxine concentrations follow a circadian rhythm in rats (Jordan, 1980; Ottenweller 1982). In a previous study (Peebles et al., 1983) deer mouse serum thyroxine concentrations were measured during an eight hour period around the light to dark transition and so it was important to examine the changes in serum thyroxine over an entire 24 hour period. Control animals at 145 ±5 days of age that had been previously housed with their littermates were placed individually into 12.5 x 27 x 14.5 cm plastic, wiretop cages as nonsibling bisexual pairs with one female in one compartment of the cage and one male in the other. These animals were maintained on the same light cycle as the colony animals. In order to stimulate and synchronize estrus among females the pairs were switched with their mates between their compartments daily for four days. The animals were sampled two to seven days after the last switch. Six animals of each sex were sacrificed and sera collected at two hour +30 minutes intervals starting at 0000 hours. Only one animal from each pair was sampled in a given 24 hour period, and the animal in the adjoining compartment was sacrificed within 48 hours. The sex and animal to be sampled were chosen at random. The total time span of Experiment I was kept under 60 days to control for
seasonal variation on serum thyroxine concentrations. The mean serum thyroxine concentration was determined for each time period and differences between each sex and time period were also determined.

EXPERIMENT II: SERUM THYROXINE AND TRIIODOTHYRONINE IN REPRODUCTIVELY INHIBITED ANIMALS

This study was done to examine the difference between mean serum thyroxine and triiodothyronine concentrations, reproductive organ and body weights in reproductively inhibited population animals and control animals. The control animals were maintained as nonsibling bisexual pairs placed in wiremesh "no-contact cages". These 12 x 26 x 24 cm cages were divided by a two cm thick partition that allowed the exchange of visual, auditory and olfactory cues between the pair but permitted no physical contact (Albertson et al., 1975). Mice were paired at 104 ±4 days of age and sampled after 20 ±2 days in the no-contact cage. They were maintained on the same light cycle as the colony animals and animal maintainence was the same prior to isolation as in Experiment I. All control animals in this experiment were kept in the same experimental rooms as the population mice to insure uniform experimental effects. All animals were sampled at 1400 h ±30 min. This time was selected on the basis of the results of Experiment I, which indicated that mean serum thyroxine levels did not change significantly
in either sex from two hours before, to two hours after, 1400 h.

Population animals were sacrificed at 125 ±4 days of age, at 1400 h ±30 min. A 40W UV fluorescent tube was used to identify the animals to be sampled in the population that had been marked 24-120 hours earlier with a non-toxic UV sensitive dye (Raytech Industries). No more than two animals were sampled from one population at one time, and in most cases, only one was sampled in a single 24 hour period. Population females were selected on the basis of a history of continuously demonstrating non-perforate vaginae as well as a history of no pregnancy. Population males were selected on the basis of a history of showing non-scrotal testes throughout their lifespans. Sera were collected as previously described and stored frozen at -30 C until assayed. Sacrificed animals were placed in 10% buffered formalin until Experiment II was completed. Reproductive organs were removed, cleaned of fat, and weighed as previously described. The body weights were determined immediately after sacrifice.

**Thyroxine and Triiodothyronine Radioimmunoassay:**

Serum thyroxine was measured using an equilibrium, competitive binding technique prescribed by the T4 RIA (PEG) Diagnostic kit sold by Abbott Laboratories (Chicago, Illinois).
The Abbott T4 RIA (PEG) kit uses I-125 labelled thyroxine and rabbit thyroxine antiserum with sodium azide as a preservative. The rabbit antiserum contains 8-anilino-1-napthalenesulfonic acid (ANS) for extraction of thyroxine from its binding protein. An earlier experiment (Peebles et al., 1983) had demonstrated that some population females had thyroxine concentrations of less than 1 ug/dl and that most animals averaged around 3 mg/dl and none were above 12 mg/dl. Therefore, the 24 mg/dl standard from the kit was not used and the 3 mg/dl standard was serially diluted with the 0 mg/dl standard to produce additional standards of 1.5, 0.75, and 0.375 mg/dl. The subsequent standard curve remained linear using these standards.

A pooled standard Peromyscus serum was produced from serum samples from adult, colony animals (age 90-250 days) and was used in serial dilution to demonstrate parallel binding between the Peromyscus serum and the human standards supplied with the kit.

I-125 labelled thyroxine and thyroxine antisera were pipetted simultaneously into 12 x 70 mm borosilicate glass tubes containing 25 ul of standard or unknown using an automatic pipette (Micromedic Systems Model 25004, Horsham, Pa.). Standards were run in duplicate and unknowns were also run in duplicate unless sample size was insufficient. The
mixtures were incubated at room temperature (22-25 C) for one hour after each tube was vortex mixed for two to four seconds. The time of addition of antisera and labelled thyroxine was kept under 10 minutes between addition to the first and the last tube of each run. Free and bound fractions were separated following the addition of polyethylene glycol (PEG) and centrifugation at room temperature for 15 minutes at 1203xg.

Supernates were decanted and the inverted tubes were drained for 5 minutes after which the tube lips were blotted. One ml of glass distilled water was added to provide uniform pellet geometry. The net radioactivity of the I-125-thyroxin-antisera complex was determined using a Biogamma II (Beckman) counter. The counting time used for each assay was such that at least 30000 counts were accumulated in the total count tubes (a 1% counting error). The counts for each tube were entered into an IBM 370-158 computer according to the protocol of the RIANAL 7 program (Duddleson, et. al., 1972).

Triiodothyronine (T3) was assayed using an Amerlex T3 RIA kit sold by Amersham, Inc. (Arlington Heights, Illinois). This method utilizes I-125 labelled triiodothyronine in BSA barbitone buffer and sheep T3 antibodies bound to polymer particles of a uniform diameter (Amerlex antibody suspension). Thiomersalate is used to separate T3 from its binding protein and is included in the labelled triiodothyronine mixture. The
reaction mixtures were added to 25 µl of the standards and unknowns using an Eppendorf 500 µl pipette. Peromyscus standard sera was serially diluted using the 0 ng/ml standard provided in the kit as diluent. The Amerlex antibody suspension was then added taking no more than ten minutes between the first and last tube of each run. Standards and unknowns were run in duplicate, whenever possible. The reaction mixtures were incubated at 37°C for 90 minutes in a hot water bath after vortex mixing each tube for three to seven seconds. The Antibody – polymer – triiodothyronine complexes were separated from unbound T3 by centrifugation at 2357 x g for 20 minutes at 25°C. The supernatant was quickly decanted by inverting the tubes, blotting the tube lips and immediately reverting to insure that the pellets were not distorted. One ml of glass distilled water was added to each tube. The net radioactivity of the I 125 triiodothyronine bound to the Amerlex particles was counted using a Beckman Biogamma II counter. Counting time was as described above.

The standards included in the Amerlex kit were not in serial dilutions. To make the standards compatible with the requirements of the RIANAL 7 program (Duddleson, et. al., 1972), the actual counts from the assay were log-logit transformed and a linear regression analysis was performed. The predicted counts for a serial dilution of a standard of 6 ng/ml were obtained from this analysis and appropriate tests
ng/ml were obtained from this analysis and appropriate tests for linearity were performed. These values and all assay results from unknowns and pooled standard sera were entered into the RIANAL 7 program (Duddleson, et. al., 1972)

EXPERIMENT III: MORPHOMETRIC ANALYSIS OF THYROID HISTOLOGY

The thyroid glands of all population and control mice from Experiment II which had been fixed in 10 percent buffered formalin were via a ventral longitudinal cut in the neck. The sternocleidomastoid, omohyoid, and sternohyoideus muscles were cut laterally at the level of the mandible and ribcage. The cricoid cartilage was cut through laterally and a second cut was made six to eight tracheal rings caudally. The trachea and attached thyroid was carefully removed. This tissue was replaced into 10 percent buffered formalin for 24 hours and then dehydrated in acetone and xylene. The tissues were then infiltrated with paraffin, (melting point 56 C) using an Autotechnicon tissue processor. Five-um tissue sections were cut starting at the cricoid cartilage at 100 um intervals and each was stained with hemotoxylin and eosin. The sections were examined and a rough estimate of follicle number was made. If at least 15 follicles were present, five to ten more five-um serial sections were made at that depth and stained with hemotoxylin and eosin. Evaluation of sections revealed no
remarkable necrotic tissue resulting from long-term or variable fixation times.

The slides were photographed through a Zeiss Photomicroscope II using Kodak Panatomic-X film. 8x10 prints were made on Agfa Rapitone P-1-3 paper with care taken to preserve the same enlargement factor for all negatives throughout the printing process.

For the purposes of estimating the follicular area, each follicle was assumed to be ellipsoid. The prints were then analyzed by direct measurement of the major and minor axis of all distinct follicles. For every thyroid gland studied, at least 15 distinct, measurable follicles had to be present or the print was not used in the study. The cell height was also measured for the four cells at the points where the axes were measured. The number of cell nuclei surrounding each follicle were then counted to determine cell number.

A Fortran 77 program was written to determine the area of each follicle, colloidal area, area of the follicular cells, mean cell height, average number of cells surrounding each follicle, and the number of nuclei per 100 square um of cellular area surrounding each follicle (Appendix 9).
Statistical Methods:

For Experiment I, all inter- and intrasex comparisons were made a posteriori by Duncan's Multiple Range test if variances were homogenous. Kruskall-Wallace test was done a posteriori if the variances were not homogenous.

For Experiments II and III, all comparisons between control and population data were done by Students t-test unless variances were heterogenous, in which case Mann-Whitney U test was used. All correlations were performed using Pearson's Product Moment coefficient. Linear regression analysis was performed to test for linearity of the triiodothyronine standard curve. Probability levels of less than 0.05 were considered significant.
RESULTS

EXPERIMENT I: CIRCADIAL VARIATION OF THYROXINE

Females had a significantly (P<0.05) lower mean serum thyroxine concentration than males at 1000 and 1800 h (Table 1). These times corresponded to three hours after the lights were turned on and three hours before they were turned off. The change in serum thyroxine concentration for males followed that of females closely. Among females, the two lowest mean thyroxine concentrations were recorded at 0800 h and 1800 h. Among males the two lowest serum thyroxine concentrations were at 0800 and 2200 h (Figure 1 and Table 1).

Among females there was a significant (P<0.05) rise in concentration at 1000 h to a high point at 1200 h. Among males there was a marked but non-significant rise in serum thyroxine at 1000 h coming to a peak at 1200 h, thus mimicking the inflection and maximum seen in females. After reaching the maximum at 1200 hours, the mean serum thyroxine concentrations for females steadily declined through 1800 hrs followed by a steady rise through 0000 hrs. With the exception of a very slight upward inflection at 1600 hours, males showed a similar downward trend in mean serum thyroxine concentration through
2200 hours with an upward inflection at 0000 hours.

Since the mean serum thyroxine concentration variations of males followed that of females so closely for the period from 1200 h through 1800 h, it was determined that a sampling time for Experiment II should be selected at the center of those times, 1400 h. This period was also equidistant between the time at which the lights were turned on and the time when they were turned off, 0700 and 2100, respectively.

**EXPERIMENT II: POPULATION VERSUS CONTROL SERUM

THYROXINE AND TRIIODOTHYRONINE CONCENTRATIONS

In Experiment II, serum triiodothyronine and thyroxine levels were compared between reproductively inhibited population-derived animals and reproductively capable controls. In addition, attempts were made to determine if correlations existed between thyroid hormone concentrations and the reproductive organ weights and body weights of the experimental animals.

The mean serum thyroxine concentrations were highly significantly (P<0.001) reduced in population animals compared with the respective control animals of both sexes (Tables 2 and 3). The mean serum triiodothyronine concentrations were highly significantly (P<0.001) reduced in population males compared
with control males (Table 3), and significantly lower ($P<0.014$) in population females compared with control females (Table 2). The mean body weight of population males was significantly less ($P<0.004$) than control males. The mean paired testis and seminal vesicle weights of population males were highly significantly less ($P<0.001$) than control males (Table 3). The mean body weight, uterine weight, and ovary weight of population females were highly significantly less ($P<0.001$) than control females.

**Correlation Analysis:** Pearson product-moment correlations were carried out with all males taken as a single group, control males alone, and population males alone. Unless otherwise noted, all significant correlations mentioned are positive correlations. Among all males combined, serum thyroxine and triiodothyronine showed highly significant ($P<0.001$) positive correlation with paired testis weight and seminal vesicle weight. Serum triiodothyronine showed highly significant ($P<0.001$) correlation with serum thyroxine and significant correlation ($P<0.017$) with body weight (Appendix 1).

Among control males, the mean serum thyroxine concentration showed very significant ($P<0.007$) correlation with triiodothyronine. Among population males, no significant
correlations were noted between serum thyroxine and triiodothyronine and any of the gravimetric determinations. In addition, serum thyroxine and triiodothyronine showed no significant correlation with each other among population males (Appendix 1).

Among all females taken together, the mean serum thyroxine and triiodothyronine concentrations showed highly significant (P<0.001) correlations with ovary weight and serum thyroxine concentration was very significantly correlated (P<0.003) with body weight. Serum triiodothyronine was significantly correlated (P<0.04) with serum thyroxine level and body weight (Appendix 2).

Among control females, only serum triiodothyronine was significantly correlated (P<0.03) with ovary weight. No significant correlations were observed among population females for Experiment II (Appendix 2).
EXPERIMENT III: MORPHOMETRIC ANALYSIS OF THYROID HISTOLOGY

The thyroid glands taken from the control and population animals from Experiment II were used for this study. Population females showed very significantly (P<0.003) increased total follicular area and colloidal area when compared with control females. The number of follicle cells was significantly (P<0.05) increased in population females as compared with control females (Table 4).

Population males showed significantly (P<0.012) decreased cell height and a significantly (P<0.047) decreased number of follicle cells compared with control males. An intersex comparison revealed no significant differences between control females and males across all of the thyroid histological parameters measured. Population females had a very significantly (P<0.009) increased follicular area, colloidal area, cellular area, and number of follicle cells compared with population males. In addition, population females had a very significantly (P<0.009) reduced number of nuclei per 100 square um of follicular cellular area, indicating increased cytoplasmic area.

Among all males used in Experiment III, serum thyroxine concentration was very significantly (P<0.01) correlated with
cellular area and cell height. Serum thyroxine was significantly (P<0.041) negatively correlated with number of cells per 100 square um of follicular cellular area (Appendix 5).

Among control males the mean serum thyroxine concentration showed significant (P<0.022) correlation with cell height. Cell height was significantly (P<0.048) negatively correlated with body weight and seminal vesicle weight (Appendix 3). Among population males only the number of cells per 100 square um of follicular cellular area was significantly (P<0.048) correlated with serum triiodothyronine (Appendix 4).

Among all females used in Experiment III, the mean number of cells per follicle showed a significant (P<0.035) negative correlation with ovary weight (Appendix 8). Among control females, the mean number of cells per follicle showed a significant (P<0.039) negative correlation with serum triiodothyronine concentration (Appendix 6). Among population females, the number of cells per follicle was significantly correlated with body weight, ovary weight, uterus weight, and serum triiodothyronine (Appendix 7).
TABLE 1

Serum Thyroxine Concentrations (over 24 Hours)
(Lights on at 0700 h, off at 2100 h).
Values are mean ± SEM.

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>0000</th>
<th>0200</th>
<th>0400</th>
<th>0600</th>
<th>0800</th>
<th>1000</th>
<th>1200</th>
<th>1400</th>
<th>1600</th>
<th>1800</th>
<th>2000</th>
<th>2200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males (n=6)</td>
<td>3.5 ±0.57</td>
<td>3.2 ±0.67</td>
<td>4.4 ±0.84</td>
<td>4.1 ±0.52</td>
<td>3.2 ±0.38</td>
<td>4.7 ±0.48</td>
<td>4.7 ±1.27</td>
<td>4.0 ±0.94</td>
<td>4.0 ±0.18</td>
<td>3.8 ±0.52</td>
<td>3.4 ±0.18</td>
<td>3.2 ±0.40</td>
</tr>
<tr>
<td>Females (n=6)</td>
<td>2.8 ±0.22</td>
<td>2.4 ±0.34</td>
<td>3.1 ±0.55</td>
<td>2.7 ±0.31</td>
<td>2.0 ±0.30</td>
<td>3.1* ±0.45</td>
<td>3.5 ±0.25</td>
<td>3.4 ±0.18</td>
<td>2.8 ±0.56</td>
<td>2.0* ±0.31</td>
<td>2.7 ±0.20</td>
<td>2.7 ±0.36</td>
</tr>
</tbody>
</table>

*P<0.050 with respect to the corresponding males.
TABLE 2

Serum Thyroxine and Serum Triiodothyronine Concentrations, Ovary, Uterus, and Body Weights in Control and Population Females (Mean ± SEM).

<table>
<thead>
<tr>
<th>Animal Condition</th>
<th>N</th>
<th>Serum Thyroxine (µg/100 ml)</th>
<th>Serum Triiodothyronine (ng/ml)</th>
<th>Ovary Weight (mg)</th>
<th>Uterus Weight (mg)</th>
<th>Body Weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Females</td>
<td>23</td>
<td>4.0</td>
<td>0.78</td>
<td>18.5</td>
<td>34.3</td>
<td>17.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.30</td>
<td>±0.037</td>
<td>±2.23</td>
<td>±2.88</td>
<td>±0.34</td>
</tr>
<tr>
<td>Population Females</td>
<td>23</td>
<td>2.6***</td>
<td>0.64**</td>
<td>3.5***</td>
<td>10.2***</td>
<td>13.3***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.19</td>
<td>±0.164</td>
<td>±0.32</td>
<td>±3.72</td>
<td>±0.30</td>
</tr>
</tbody>
</table>

**P<0.015 and ***P<0.001 with respect to the corresponding control value.
TABLE 3

Serum Thyroxine and Serum Triiodothyronine Concentrations, Testes, Seminal Vesicles, and Body Weights in Control and Population Males.
(Mean ± SEM)

<table>
<thead>
<tr>
<th>Animal Condition</th>
<th>N</th>
<th>Serum Thyroxine (ug/100 ml)</th>
<th>Serum Triiodothyronine (ng/ml)</th>
<th>Testes Weight (mg)</th>
<th>Seminal Vesicles Weight (mg)</th>
<th>Body Weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Males</td>
<td>22</td>
<td>5.4</td>
<td>0.90</td>
<td>298.6</td>
<td>164.1</td>
<td>17.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.35</td>
<td>±0.038</td>
<td>±18.11</td>
<td>±14.12</td>
<td>±0.63</td>
</tr>
<tr>
<td>Population Males</td>
<td>19</td>
<td>3.1***</td>
<td>0.56***</td>
<td>69.6***</td>
<td>30.3***</td>
<td>15.1**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.21</td>
<td>±0.044</td>
<td>±11.27</td>
<td>±6.95</td>
<td>±0.47</td>
</tr>
</tbody>
</table>

**P<0.004 and ***P<0.001 with respect to the corresponding control value.
TABLE 4

Morphometric Analysis of Thyroid Histology in Females
(Mean ± SEM)

<table>
<thead>
<tr>
<th>Animal Condition</th>
<th>N</th>
<th>Follicular Area (square um)</th>
<th>Colloidal Area (square um)</th>
<th>Area of Follicle Cells (square um)</th>
<th>Cell Height (um)</th>
<th>Number of Follicle Cells</th>
<th>Number of Nuclei per 100 Square um Follicle Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Females</td>
<td>14</td>
<td>670.4 ± 35.29</td>
<td>408.0 ± 21.95</td>
<td>266.2 ± 17.08</td>
<td>4.6 ± 0.20</td>
<td>15.2 ± 0.65</td>
<td>11.5 ± 0.01</td>
</tr>
<tr>
<td>Population Females</td>
<td>12</td>
<td>1046.6** ± 116.37</td>
<td>769.8** ± 124.82</td>
<td>315.9 ± 25.23</td>
<td>4.4 ± 0.13</td>
<td>17.4* ± 0.87</td>
<td>11.1 ± 0.05</td>
</tr>
</tbody>
</table>

**P<0.010 and *P<0.050 with respect to the corresponding controls.
TABLE 5

Morphometric Analysis of Thyroid Histology in Males.
(Mean + SEM)

<table>
<thead>
<tr>
<th>Animal Condition</th>
<th>N</th>
<th>Follicular Area (um2)</th>
<th>Colloidal Area (um2)</th>
<th>Area of Follicle Cells (um2)</th>
<th>Cell Height (um)</th>
<th>Number of Follicle Cells</th>
<th>Number of Nuclei per 100 Square um of Follicle Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
<td>608.84</td>
<td>353.47</td>
<td>255.37</td>
<td>4.94</td>
<td>15.71</td>
<td>12.64</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td>+90.51</td>
<td>+21.95</td>
<td>+18.50</td>
<td>+0.18</td>
<td>+0.49</td>
<td>+0.80</td>
</tr>
<tr>
<td>Population</td>
<td>18</td>
<td>559.96</td>
<td>341.67</td>
<td>216.33</td>
<td>4.41*</td>
<td>14.17*</td>
<td>13.30</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td>+71.71</td>
<td>+55.48</td>
<td>+17.13</td>
<td>+0.11</td>
<td>+0.57</td>
<td>+0.60</td>
</tr>
</tbody>
</table>

*P<0.050 with respect to the corresponding controls.
Figure 1: 24 Hour Variation in Mean Serum Thyroxine Concentration in Control Males and Females of Experiment I.
THYROXINE DAILY VARIATION

LEGEND: SEX  —— M  ——— F
Figure 2: Photomicrograph of Control Male

Thyroid Cross-section (Magnification 770 diameters).
Figure 3: Photomicrograph of Population Male Thyroid Cross-section (Magnification 770 diameters).
Figure 4: Photomicrograph of Control Female Thyroid Cross-section (Magnification 770 diameters).
Figure 5: Photomicrograph of Population Female Thyroid
Cross-section (Magnification 770 diameters).
DISCUSSION

Experiment I was performed to demonstrate a daily variation in total serum thyroxine among reproductively capable males and females and to find a time when male and female serum thyroxine concentrations are similar and reasonably stable. Among the females tested, there was a significant (P<0.05) increase in total serum thyroxine from 1000 to 1200 h, reaching zenith at 1200 h (Lights on at 0700 h, off at 2100 h). Among males there were no statistically significant differences between any two times, but the trends established were so similar to those seen in females, a larger sample size would likely have yielded statistically significant changes at the same times of day (Table 1 and Figure 1).

An earlier study on the chronobiological variation of serum thyroxine concentration in deermice covered only an eight hour period (Peebles et al., 1983) and, thus, did not elucidate the daily rhythm. Although the light changes occurred at times different from this study (lights off 1900 h, on at 0500 h; versus lights off at 2100 h, on at 0700 h, respectively) the photoperiod was the same. Therefore, it is probably more important to examine the changes in mean serum thyroxine
concentration in terms of time with respect to the light changes. There are some notable similarities over the eight hour time period studied by Peebles et al., (1983) and in this present study. The thyroxine concentration nadir for females occurred three hours before the lights went off and nadir for males occurred one hour after the lights went off (Peebles et al., 1983). In this study, the second lowest value for mean serum thyroxine concentration for females was determined to be three hours before the lights went off. Nadir for males occurred one hour after the light to dark transition (Table 1, Figure 1). The nadir for males was the same for both studies. Also, in both studies, nadir for males occurred after nadir for females. In the earlier study, the dark period was fairly stable for the four hours measured (Peebles et al., 1983). In the present study, the dark period was also stable for the first five hours. In fact, there was only one notable difference between the two studies. There was a time in the previous study in which females had a higher mean serum thyroxine concentration than males, but the results of this study showed females to be consistently lower than males in mean serum thyroxine concentration over all times evaluated.

The previous study (Peebles et al., 1983) used a sampling time located one to two hours before the precipitous changes in mean serum thyroxine demonstrated to be associated with the light to dark transition point. The results of this study
indicate 1400h, seven hours before the light to dark transition point, to be a more stable time for sampling.

The literature concerning the existence of circadian variation in serum thyroxine concentration is very contradictory. Several reports (Fukuda et al., 1975; Rookh et al., 1979) failed to find any circadian variation in serum thyroxine in rats and one report found it in males only (Tonooka et al., 1980). Others have demonstrated the existence of a circadian rhythm for serum thyroxine in rats of both sexes combined (Jordan et al., 1980) and in females only (Ottenweller et al., 1982). Part of the confusion concerning thyroxine diurnal variation in rats may be realated to the strain used. Wong et al. (1983) found circadian variation of thyroxine in BH/Ztm male rats but failed to find any distinct twenty four hour periodicity among SD rats. Thyroxine apparently undergoes circadian variation in man (Decostre et al., 1971; O'connor et al., 1974; Balsam et al., 1975; Lucke et al., 1977; Chan et al., 1978). Since TSH stimulates thyroxine synthesis and release, it would seem logical to find a similar inconsistency regarding TSH circadian variation. Paradoxically, thyrotropin (TSH) has been well documented as having a circadian variation in rats (Ducommun et al., 1966; Leppaluoto et al., 1974; Fukuda et al., 1975; Phelps et al., 1973; Rookh et al., 1979; Jordan et al., 1980; Tonooka et al., 1980; Ottenweller, 1982; Wong et al., 1983) and man (Lemarchand-Beraud et al., 1969;
Jordan et al., (1980) used a different experimental photoperiod (twelve hours light, twelve hours dark) than this study (Fourteen hours light, ten hours dark) thus comparisons are difficult to make. However, some characteristics of the thyroxine circadian rhythms he demonstrated in rats are similar. Serum thyroxine nadir occurred during the lights on phase, zenith during the lights off phase (Jordan et al., 1980). In this study, nadir occurred during the lights on phase for both sexes and zenith occurred during lights off for males. Zenith for females occurred one hour after the dark to light transition point in the present study. Jordan et al., (1980) demonstrated a precipitous decline in mean serum thyroxine after nadir, which was demonstrated only for females in the present study (Figure 1, Table 1). One probable reason for the discrepancy in results is that the Jordan et al., (1980) study pooled both sexes which possibly masked the separate results for females demonstrated in this study. Another discrepancy was the general observation that the results of the published study appeared to be phase shifted seven hours earlier, but the different photoperiod used makes the importance difficult to evaluate. Nonetheless, the results of this study demonstrate some twenty four hour variation in mean serum thyroxine
concentration in reproductively capable females and similar trends in males. The similarities with previous studies as well as between the two sexes suggest that Experiment I was successful in characterizing the chronobiological variation in mean serum thyroxine in deermice and that it is similar in some respects to the circadian variation demonstrated in rats in previous work (Jordan et al., 1980).

Experiment II examined the mean serum concentration of triiodothyronine and thyroxine among reproductively inhibited population males and females compared with male and female controls of the same age at a more stable sampling time than the Peebles et al. (1983) study. Also, both hormones produced and secreted by the thyroid, (thyroxine and triiodothyronine), were studied. It was felt these measurements would give a more accurate picture of the thyroid status of reproductively inhibited population deermice compared with reproductively capable controls.

A previous study demonstrated a significant decrease (P<0.03) in serum thyroxine concentration among male population deermice compared with controls and a trend (P<0.10) towards less serum thyroxine among population females (Peebles et al., 1983). The results of this study demonstrate a highly significant (P<0.001) reduction in serum thyroxine and triiodothyronine in population males and in serum thyroxine in
population females. There was also a significant (P<0.015) reduction in serum triiodothyronine in population females (Table 2). One possibility for the greater difference in mean serum thyroxine between population and control female deermice observed in this study is the difference in sampling time. It is less likely that sampling conditions and animal ages had any influence since both were very similar to the previous study (Peebles et al., 1983).

The measurement of serum triiodothyronine represent the first determination of the serum levels of this hormone in deermice and gives further weight to the notion that the population animals of both sexes are hypothyroid. Triiodothyronine is considered to be the functional hormone and thyroxine the prohormone in most cases. A low serum triiodothyronine could result in a hypothyroid state at the target tissue level. Harris et al., (1978) demonstrated depressed serum thyroxine and triiodothyronine levels in hypothyroid rats. Serum thyroxine is considered to be a better indicator of thyroid function since depressed levels of serum triiodothyronine can be seen in functionally euthyroid conditions (Ingbar and Woeber, 1981). Often, states of acute illness are associated with lowered serum triiodothyronine and normal serum thyroxine (Bermudez et al., 1975; Ingbar and Werner, 1981).
The results of Experiment II strongly suggested a hypofunctional thyroid status among population deermice and it was felt that the primary versus secondary nature of the hypothyroid state should be determined. Serum TSH concentration is the best indicator of secondary versus primary hypothyroidism (Ingbar and Woeber, 1981). However, extensive testing demonstrated a lack of cross reactivity of deermouse TSH with available anti-TSH antibodies in several rat and human RIA kits. Hence, a morphometric analysis of thyroid histology was used as the next best indicator of primary versus secondary hypothyroidism, and as another means of confirming hypothyroidism in population deermice. Since the free thyroid hormone indices were not determined, histological confirmation of a hypothyroid state would argue against the possibility that low serum thyroid binding globulin would produce the low total thyroxine and triiodothyronine observed in this study in what would be a functionally euthyroid animal.

Experiment III offers histological evidence that the reproductively inhibited deermice of both sexes are experiencing significant thyroid dysfunction. The mean follicular cell height and the number of follicle cells of population males are significantly (P<0.012 and P<0.05, respectively) reduced compared with reproductively capable control males (Table 5, Figures 2 and 3). Population male thyroid glands appear to be receiving less TSH stimulation than male controls and are thus
classed secondary hypothyroid. The understimulated thyroid gland of population males would secrete less triiodothyronine and thyroxine leading to the reduced serum concentrations of these hormones observed in Experiment II. Thyroid follicular cell height and numbers positively reflect the amount of TSH stimulation of the gland (Lissitsky et al., 1971; Winand and Kohn, 1975; Wollman and Brietman, 1976; Wadaleux et al., 1978; Meissner, 1980; Ingbar and Woeber, 1981; Valente et al., 1983). Thyroid follicular cell growth may be completely dependent upon, as opposed to only a partial reflection of, TSH stimulation (Ambesi-Impiombato et al., 1980; Nitsch and Wollman, 1980). As would be expected from a cell height dependence upon TSH stimulation leading to thyroxine and triiodothyronine secretion, mean cell height showed significant positive correlation with both thyroid hormones for all males combined (Appendix 5).

It is also possible that the thyroid glands of males are refractory to TSH stimulation instead of experiencing reduced serum TSH concentrations. Beamer et al., (1981) reported an autosomal recessive mutation in mice that causes primary hypothyroidism indicated by elevated serum TSH and reduced serum thyroxine concentrations. However, the morphometric analysis of the thyroids indicated the glands were hypoplastic, showing reduced follicular size and cell height. They concluded that the animals were primary hypothyroid due to
thyroid gland refractoriness to TSH. The results of Experiment III do not rule out this possibility, but in consideration of the genetically heterogenous nature of deermice used in this study, (sytematically out-bred) it seems unlikely that any single genetic defect would produce the histology seen for population males in this study. Further, the mean follicle cell height was positively correlated with thyroxine concentration for both population and control males (P<0.10 and P<0.022 respectively). If the thyroid glands of poulation males were refractory to TSH stimulation, cell height should not vary with thyroxine output, but should instead remain low and variable. Thus, although these histological data do not permit an uncategorical statement that population males have diminished serum TSH, it is most probable.

The results of Experiment III for population females are entirely different than those of population males. The increased mean follicular area, colloidal area, and number of follicle cells (Table 4, Figures 4 and 5) correspond to an increased storage of colloid material among population female deermice compared with controls. This would correspond to elevated serum TSH coupled with an inability to synthesize sufficient thyroid hormones (Denef et al, 1980). In many respects the histology of population female thyroid resembles a goiterous condition, which is not necessarily indicative of hypothyroidism. The inability to synthesize thyroxine leading
to goiter produces an "exhaustion atrophy" that produces enlarged follicles distended with colloid (Meissner, 1980). In many instances, severe goiters are found in clinically euthyroid subjects (DeVisscher et al., 1961; Choufoer et al., 1965; Patel et al., 1973; Pharoah et al., 1973; Ermans, 1980). Euthyroid goiter is characterized by depressed serum thyroxine, elevated serum TSH and normal or elevated serum triiodothyronine. This is true for humans (Choufoer et al., 1963; Adams et al., 1968; Kochupillai et al., 1973; Patel et al., 1973; Pharoah et al., 1973; Thilly et al., 1973; Vagenakis et al., 1973; Chopra et al., 1975; Refetoff et al., 1976; Goslings et al., 1977) and rats (Santisteban et al., 1980). Determination of serum triiodothyronine concentration differentiates between hypothyroid and euthyroid goiter. Hypothyroidism in humans with goiter is indicated by low serum triiodothyronine (Goslings et al., 1977). Euthyroid goiter can be produced in rats by feeding them a low iodine diet but serum triiodothyronine concentration is normal in this case. When the same rats are given 1% sodium perchlorate to produce severe hypothyroidism, serum triiodothyronine concentrations decline (Santisteban et al., 1982).

Although the combined results of Experiments II and III suggest population females have hypothyroid goiter indicated by increased follicle area and colloidal area and depressed serum triiodothyronine, goitrous thyroid glands are highly
thyrotropin stimulated and generally display an increase in cell height in humans (Meissner, 1980). The cell height was not significantly different between population and control female deermice (Table 4, Figures 4 and 5). However, there is some evidence that the nature of primary hypothyroid goiter in rodents is different than that of humans. Colloid rich goiters formed in iodine deficient mice and rats without a follicular cell hyperplastic phase and cell height remained unchanged even after additional thyrotropin stimulation (Gerber, et al, 1981). The results of Experiment III suggest that the thyroid gland of population females is experiencing greater thyrotropin stimulation than controls and is unable to secrete thyroxine and triiodothyronine to the same extent as control females. Therefore, it appears likely that population female prairie deermice have primary hypothyroidism.

The gravimetric determinations of body weight and reproductive organ weights have historically been the best indicators of reproductive inhibition in prairie deermice. (Terman, 1969, 1973a; Bradley and Terman, 1981c). The mean reproductive organ weights were highly significantly (P<0.001) reduced among population animals of both sexes when compared with controls (Tables 2 and 3) in this study as well. The mean body weights of population males and females were also significantly reduced (P<0.004 and P<0.001, respectively). Terman (1969), did not find a significant reduction in mean
body weight. Later studies found significant reduction of mean population deer mouse body weight (Bradley and Terman, 1981a,b,c). The gravimetric determinations made in this study indicate results similar to those of the more recent studies.

Of all previously studied hormones in prairie deer mice, the present finding of significantly decreased thyroid hormones most directly explains both the reduced reproductive organ and body weights. Hypothyroidism has been clearly linked to a drop in growth rate during postnatal development (Samel, 1968; Bakke et al., 1970; Amin and El-Sheikh, 1977; Ganong, 1979). Pituitary growth hormone secretion declines following thyroidectomy in rats (Contopolous et al., 1958 Solomon and Greep, 1958; Kikuyama et al., 1974; Hervas et al., 1975; Coirio et al., 1979; Bernal and DeGroot, 1980; Wong et al., 1980; Santisteban et al., 1982) and domestic fowl (Decuypere and Scanes, 1983; Harvey, 1983). Further, thyroidectomy reduces the synthesis of growth hormone as evidenced by diminished uptake of tritium labelled amino acids into growth hormone (Augustine and Hymer, 1978). There is also an increase in epidermal growth factor activity with increased thyroid hormone in neonatal mice (Hoath et al, 1983). There is some evidence that thyroid hormone has a direct effect on growth at the target tissue level (Ganong, 1979; Bernal and DeGroot, 1980; Norris, 1980) but it is not certain that this is due to a positive effect of thyroid hormones on growth hormone
receptors (Chernausek et al., 1982). Thus, it is possible that the reduced body and reproductive weights are a product of reduced serum growth hormone, epidermal growth factor, and/or a diminished direct growth effect produced by hypothyroidism.

Thyroid hormones also have direct, distinct effects on the reproductive organs that would effect their size and function independent of the general growth effects mentioned above. Thyroidectomy of adult female rats produces a decrease in ovarian weight (Leathem, 1958; Contopolous and Koneff, 1963; Evans et al., 1964; Gaunt et al., 1970; Hagino, 1971; Jones and Tracy, 1974; Hendrich et al., 1976; Norris, 1980). Adult female rats that have been hemigonadectomized show a compensatory hypertrophy of the remaining ovary which is decreased in a hypothyroid condition (Saiduddin, 1972). Finally, exogenous thyroxine administration increases ovarian weight in adult rats (Hendrich et al., 1976). In addition to influencing ovarian weight in adults, thyroid hormones are involved in ovarian development leading to puberty. (Kikuyama, et al., 1973; Dixit et al., 1976). Thus, hypothyroidism leads to reduced ovarian weight either by regression in adults or failed development in neonates. Since the effect is produced in adults, it is probably a direct effect, independent of growth hormone or epidermal growth factor activity.

Thyroid hormone has effects on testis weights as well.
The testis weights of population males in this study and many previous studies on prairie deer mice are drastically reduced (Table 2). Although thyroid hormone has a profound effect on testis size, it is developmental in nature. Testis weight increases linearly with increasing body weight in rats (Samel, 1968). When immature male rats are thyroidectomized, testis weights are significantly smaller than intact controls (Masquood and Reineke, 1950; Vilchez-Martinez, 1973; Kalland et al., 1978). Hypothyroidism in humans beginning at prepubescent ages is characterized by sexual immaturity and retarded growth and development (Ingbar and Woeber, 1981). Population males of any age are arrested at early stages of development and are comparable to 25 to 35 day old control males (Bradley and Terman, 1981c). The above evidence and the diminished body weights argue for hypothyroidism beginning at an early age in males. If the retarded growth is due to reduced growth hormone activity, the effect would have to be exerted at an early age to produce the body weights and retarded development seen among population males.

In fact, the body weights and other indices argue for prepubertal hypothyroidism in population females as well, who are infertile and appear to have never reached puberty. Female rats thyroidectomized as late as 24 days of age (Puberty occurs around 30 days) have significantly delayed ovulation. When adult female rats are thyroidectomized, often there is no loss
in fertility (Bruce and Sloviter, 1957). There is also no loss in fertility in ewes rendered hypothyroid by immunization to thyrotropin releasing hormone (Fraser and McNeily, 1982). Thus, the retarded growth and sexual development observed in population females is likely to be due to the maturational delay effects of prepubertal hypothyroidism in population females.

Serum thyroxine undergoes dramatic increases and several peaks in early postnatal development followed by a decline to adult values (Samel, 1968; Vigoroux, 1976). There is a distinct possibility that these peaks have been attenuated in population prairie deer mice which leads to a halt in development. Serum thyroxine and triiodothyronine concentrations did not show a positive correlation with testis weight among either population or control males (Appendix 1). This is indicative of a threshold for thyroid stimulation of testicular development. Without sufficient thyroid stimulation, testis fail to develop, and with sufficient thyroid stimulation, testicular development proceeds normally, relatively independent of serum thyroid hormone concentration. Serum triiodothyronine concentration showed significant positive correlation with ovary weight among control females but not population females (Appendix 2). Since thyroid hormone had a direct effect on ovary weight independent of age, this is expected. The lack of positive correlation among population
females would again indicate failed development.

Thyroid hormones have many effects on gonad function in addition to growth and development. Past studies in this laboratory have examined testis and ovary histology which are an excellent reflection of gonad function. Bradley and Terman (1981c) demonstrated that few corpora lutea or Type eight follicles (Pederson and Peters, 1968) develop in population female ovaries. The numbers of Type six and Type seven follicles were not significantly different. Terman (1973a) reported an increase in atretic follicles among population female deermice. These results are indicative of a failure of population female deermice to ovulate. Hypothyroidism has a marked effect on ovarian histology. The ovaries of thyroidectomized female gerbils had ovaries that contained very few corpora lutea and many atretic follicles, most of which were arrested in late Graffian phase (Dixit et al., 1976). Hemicastrate thyroidectomized female rats also have a marked decrease in the number of corpora lutea in the remaining ovary (Jones and Tracy, 1973). The decreased corpora lutea is reflected in the prolonged estrus seen in adult thyroidectomized guinea pigs (Hoar et al., 1956) rats (Krohn and White, 1950; Bakke et al., 1970) and mice (Bruce and Sloviter, 1957). In terms of ovary weight and numbers of corpora lutea, female population deermice are similar to the hypothyroid rodents discussed above.
The testicular histology of population males is also suggestive of neonatal hypothyroidism leading to failed testis maturation. Bradley and Terman (1981a) and Peebles et al. (1983) reported decreased seminiferous tubule lumen diameters and markedly fewer spermatids in population male testis leading to obvious infertility. Male rats thyroidectomized as adults show no loss in fertility (Bruce and Sloviter, 1957), whereas, rats rendered hypothyroid before weaning have destructive changes in the lumen of the seminiferous tubules and spermatogenic cells (Amin and El-Shiekh, 1977).

If reduced serum concentrations of thyroxine were the cause of the testicular histology seen in population prairie deer mice, exogenous thyroxine should restore spermatogenesis. Peebles, Painter and Bradley (1983) demonstrated a return of spermatogenesis to population males given exogenous thyroxine by injection and feeding, respectively, but not to the level of control males of the same age. It would seem that although low thyroid hormone titers could explain the arrested development of male testis, the thyroxine injections should restore full testicular function if hypothyroidism was the cause. TSH appears to be involved in spermatogenesis. Hutson and Stocco (1981) report that TSH has very similar Sertoli cell stimulatory activity to FSH. Since the histological data for population males implicated a possibility of secondary hypothyroidism and thus low TSH, it is possible that reduced
spermatogenesis is a product of diminished TSH as well as thyroxine and triiodothyronine. Administration of thyroxine combined with TSH could possibly restore normal spermatogenesis.

Testosterone secretion is another part of testicular function that is affected by hypothyroidism. Bradley and Terman, (1981c) found a lowered serum testosterone concentration among population male deer mice compared with control males and a positive correlation between seminal vesicle weight and testosterone concentration among control males. Thyroidectomy causes a decrease in basal testosterone level (Boutrand et al, 1979). It is possible that the reduced seminal vesicle weights are produced by decreased serum testosterone mediated by hypothyroidism. Increasing testosterone concentration increases responsiveness of TSH to TRH (Christianson et al., 1981). This effect could further augment a thyroid mediated mechanism of reproductive inhibition. A reduced TSH responsiveness to TRH due to decreased serum testosterone would amplify the possible secondary hypothyroidism indicated by results of Experiment III.

In addition to direct effects on the gonads, the pituitary control of the gonads via the gonadotropins are altered by different thyroid states. Serum follicle stimulating hormone
(FSH) levels are elevated in population males and female deermice. Serum luteinizing hormone (LH) levels are significantly reduced in population females and not significantly different among control and population males, which demonstrated that the reproductive inhibition seen in population animals is not due to the lack of proper FSH stimulation in either sex (Bradley and Terman, 1981c). The low serum LH was considered to be due to the lack of ovarian LH surges among population females. The effect of hypothyroidism on FSH and LH is very controversial. When sexually mature adult male rats are thyroidectomized, serum LH and FSH decrease (Bruni et al., 1975; Amin and El-Shiekh, 1977; Wong, 1980) or are unchanged (Kalland et al., 1978). Pituitary LH and FSH have also been shown to decrease in hypothyroidism (Contopolous et al., 1958). The result of decreased serum and pituitary LH and FSH is obtained in a thyroidectomized male rat with intact, mature gonads. Therefore, the experimental condition is one of normal, or more likely, elevated testicular products such as testosterone and inhibin (Norris, 1980). When thyroidectomized male rats are subsequently gonadectomized, a condition where testosterone and other testicular products are low or absent is produced. This condition is more comparable to the condition of reproductive inhibition in population deermice. Adult male rats gonadectomized after thyroidectomy have increased titers of serum LH and FSH (Contopolous et al., 1958; Bruni et al., 1975) and pituitary FSH and LH (Contopolous
et al., 1958). Thyroxine and triiodothyronine inhibit the production of gonadotropins by isolated rat pituitary cells in culture (Khar et al., 1979). Thus, without the stimulation of testosterone and other testicular products, the pituitary produces increased levels of gonadotropins in hypothyroid rats.

A significant question is why the testis of population males remain insensitive to continued elevated FSH stimulation while serum LH concentrations are not significantly different from control males. One possibility is halted development of the testicular response to LH stimulation (Odell et al., 1974). Serum FSH concentrations in prepubertal males are higher than in mature male rats (Goldman et al., 1971; Swerdloff et al., 1971; Ojeda and Ramirez, 1972; Miyachi et al., 1975). Proper FSH stimulation is necessary for proper testicular response to LH (Odell et al., 1971, 1973), and it is possible that population males are halted developmentally in a prepubertal stage with elevated FSH and unresponsive testis. Additionally, it is possible that the typical pituitary response of increased LH to decreased testosterone (Norris, 1980) has failed to develop due to a hypothyroid effect on pituitary development. It is also possible that the pituitary LH response to thyroid hormones is different from that of FSH. Schneider et al (1979) demonstrated a decrease in serum FSH in hyperthyroid rats but no significant change in serum LH. It should be mentioned that Bradley and Terman (1981c) found slightly elevated serum LH in
population males, though not at a statistically significant level. This could be due to depressed serum thyroid hormones leading to less LH than FSH responsiveness.

Bradley and Terman (1981c) demonstrated lower serum LH levels and elevated serum FSH concentrations among population females. They postulated that the lower LH levels are due to a loss of LH surges. Serum LH and FSH increase in adult female rats, like males, after thyroidectomy and gonadectomy (Bruni et al., 1975; Freeman et al., 1975; LaRochelle and Freeman, 1975). The pulsatile discharge of LH remains unaffected by thyroidectomy but maximum and minimum LH levels are two to three times higher than euthyroid gonadectomized females (Freeman et al., 1975). Conversely, Dunn et al., (1976) demonstrated a loss of the rhythmicity of gonadotropin release after thyroidectomy in rats. The diminished LH levels could be due to a differential effect of hypothyroidism on the pituitary as discussed above for males.

It is of interest to note that a state of hyperprolactinemia produces elevated serum FSH and depressed serum LH in female rats (Wuttke, et al., 1980). Preliminary experiments in this laboratory (Kirkland, pers. comm.) have demonstrated no significant difference between population and control deer mouse serum prolactin concentrations. However, the link between thyroid hormone and prolactin is an area of
intense current study and this may yet prove to be an important aspect of reproductive inhibition.

As discussed earlier (see Introduction) much previous work on reproductive inhibition in rodents in this laboratory and others has centered around the involvement of the hypophysea-adrenal axis. Changes in the hypophyseal-adrenal axis often produce changes in the hypophyseal-thyroid axis and visa versa. Thus, a question that should be addressed is whether the thyroid changes demonstrated in this study are a cause of, or a product of, possible adrenal changes demonstrated in previous studies of deer mice (Sung et al., 1977; Coppes, 1980; Bradley and Terman, 1981b; Tucci, 1983). Elevated corticosterone (Sung et al., 1977; Bradley and Terman, 1981b) could lead to hypothyroidism in population deer mice. Increased serum corticosterone causes a decrease in serum thyroxine and triiodothyronine (Kuhl and Ziff, 1951; Williams et al., 1975). This effect is probably due to an inhibition of thyrotropin secretion (Levin and Daughaday, 1955; Guillemin, 1968; Retiene et al., 1968; Wilber and Utinger, 1969; Nicoloff et al., 1970; Faglia et al., 1973; Otsuki et al., 1973; Tolis et al., 1974; Van Cauter et al., 1974; Re et al., 1976; Pamenter and Hedge, 1980) and the effect is amplified in hypothyroid humans (Tolis et al., 1974). Serum TSH increases during glucocorticoid insufficiency as well (Shimiza et al., 1975). This effect of diminished TSH
secretion occurs at the suprahypophyseal level (Wilber and Utinger, 1969) probably by decreasing TSH responsiveness to TRH (Pamenter and Hedge, 1980). Population males appear to have diminished TSH secretion and would fit this model of elevated corticosterone leading to hypothyroidism. Population females, on the other hand appear to have elevated TSH and would not fit this model.

Thyroid hormones generally affect the pituitary-adrenal axis by altering the clearance of adrenocorticoids; corticosterone binding globulin (CBG) level; and adrenal responsiveness to ACTH. In hypothyroid patients, secretion of 17-hydroxy-corticosteroids decreases (Brown, et al., 1957). Increased serum thyroxine increases secretion of 17,21-dihydroxy-20-ketosteroids (Levin and Daughaday, 1955; Peterson, 1957; Ingbar and Woeber, 1981) and cortisol (Melby et al., 1960). Hypothyroidism produces decreased utilization of corticosteroids (Zarrow et al., 1957; McCarthy et al., 1959) Also, Alger and Bocabella (1968) reported no loss in thyroid activity following adrenalectomy. In hypothyroid rat pups treated with subnormal (0.1 ug/g) doses of thyroxine, CBG increased and remained elevated for the twenty days the experiment lasted (D'Agostino and Henning, 1982). Hypothyroidism promoted a reduction in ACTH responsiveness to provocative tests in humans while corticosterone responsiveness was normal (Minozzi et al., 1973). Adrenal glands of
thyroidectomized rats have increased sensitivity to ACTH stimulation (Steinetz and Beach, 1962). It is possible that these effects of thyroid hormones on CBG, corticoid secretion, and adrenal responsiveness are responsible for the seemingly contradictory demonstration of elevated corticosterone, but no significant increase in ACTH concentration in population deermice (Sung et al., 1977; Coppes, 1980; Bradley and Terman, 1981b).

In addition to the hormones secreted by the thyroid gland, TSH itself exerts an inhibitory effect on serum corticosterone concentration. Euthyroid patients injected with TRH have depressed plasma corticosterone concentrations (Anderson et al., 1971; Ducobu et al., 1976). When TSH secretion is experimentally reduced by microwave radiation, serum corticosterone rise (Shin-Tsu et al., 1981). It is of interest to note that Bradley and Terman (1981b) showed population males to have an almost three-fold increase in serum corticosterone, whereas population females exhibited only a 50 percent increase over control values. Considering the elevated TSH levels for population females and depressed levels for population males suggested by this study, a direct TSH inhibitory effect on serum corticosterone would produce a similar result if other factors acted to raise serum corticosterone independently as well to produce the overall elevation.
Thyroid hormones have profound effects on the adrenals themselves. Although past studies of prairie deermice have yielded inconsistent results, two recent studies using control caging arrangements similar to this study as well as similar population designs have demonstrated a significant reduction in absolute adrenal weight for population males, but not females. In addition, relative adrenal weights of both sexes were significantly increased compared with controls (Bradley and Terman, 1981a and 1981b). Adrenal histology of population males showed a decreased cross sectional area, cortex width, and combined zona reticularis and fasciculata area. Population females did not show the same changes. Instead, they showed a significantly increased cortex width. Adrenal weight decreases following thyroidectomy (Peterson, 1959; Gaunt et al., 1970). Thyroidectomy produces atrophic changes in adrenal cortex histology (Johansson and Jonsson, 1971). Coppes (1980) demonstrated a highly marked difference in mitochondrial appearance among population males compared with controls. The existence of thyroid hormone receptors in mitochondria is well established (Sterling et al., 1978; Tata, 1975). Thus, a lack of thyroid hormone stimulation could have been responsible for the profound morphological changes seen in population male deermouse mitochondria. Although the atrophic changes present in population males can be explained by a direct effect of hypothyroidism, the lack of change in female population deermouse adrenal histology and the increased relative adrenal
weights cannot. This may again be linked to the differences in TSH in population males and females suggested by this study.

One important aspect of the thyroid condition of reproductively inhibited deermice is the degree of the observed hypothyroidism. It has been previously demonstrated that reproductive inhibition in population deermice is reversible. Terman (1973b) has shown that 75 percent of population deermice that are at least 100 days old eventually mature and reproduce if placed with reproductively mature mates. Juvenile hypothyroidism in humans is usually a cause for permanent neurological impairment (Werner and Ingbar, 1978; Bernal and DeGroot, 1980; Ingbar and Woeber, 1981). Thus, it may be that the degree and or timing of the thyroid depression is not similar between the two species.

Anorexia nervosa is one human condition that resembles the state of reproductive inhibition in deermice. Anorexia nervosa is characterized by below normal serum thyroxine, triiodothyronine, TSH, LH, and FSH concentrations with ovulation being invariably absent and body weights 70 to 80 percent of normal (Miyai et al., 1975; Moshang et al., 1975; Beaumont et al., 1976; Croxson and Ibbertson, 1977; Boyer, 1978 Moore and Mills, 1978). During subsequent weight gain in anorexics, these hormones increase to supranormal values and then eventually return to normal (Moore and Mills, 1978). The
similarity between the anorexic human and reproductively inhibited deermouse is at least threefold: first, anorexia nervosa is a hypogonadal state linked to a hypothyroid state; second, it is a state from which full reproductive recovery can take place; third, it is a syndrome presenting a spectrum of nearly similar endocrinological changes, insofar as have been investigated to date. The only major difference between anorexia nervosa and reproductive inhibition of deermice that is presently known is the elevated FSH values seen in population deermice. This difference could be due to a less marked effect on gonadotropins in reproductive inhibition or to some developmental role played by thyroxine and triiodothyronine and FSH production separate from the general hypofunctional changes inherent in anorexia nervosa. Further research in this area and establishing links with hypothyroidism and past hormonal studies, especially serum gonadotropins and the pituitary-adrenal axis are obviously warranted.

SUMMARY

1). Peromyscus maniculatus bairdi females have a statistically significant daily variation in mean serum thyroxine concentration and males display a similar trend.

2). Total serum thyroxine and triiodothyronine are
significantly lower in male and female reproductively inhibited population animals compared with reproductively capable controls.

3). Thyroid histology of male and female population prairie deermice is significantly different from that of control males and females, suggesting functional hypothyroidism in both cases.

4). Thyroid histology of male population deermice is different from that of female population animals. In males, the data suggests that TSH stimulation is insufficient due to some undetermined secondary hypothyroidism or refractorines to TSH stimulation. In females, insufficient thyroxine and triiodothyronine production was observed due to primary hypothyroidism of unknown origin.
APPENDIX 1

Pearson Product-Moment Correlations: Mean Serum Thyroxine and Triiodothyronine Concentrations Versus Selected Gravimetric Determinations, Thyroxine or Triiodothyronine Concentration Among Males.

Values are given as Correlation Coefficient (Probability)

<table>
<thead>
<tr>
<th></th>
<th>Body Weight (gm)</th>
<th>Testis Weight (mg)</th>
<th>Seminal Vesicle Weight (mg)</th>
<th>Serum Thyroxine Concentration (ug/dl)</th>
<th>Serum Triiodothyronine Concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Males (n=22)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Thyroxine</td>
<td>-0.14 (0.27)</td>
<td>0.13 (0.27)</td>
<td>-0.03 (0.45)</td>
<td>--</td>
<td>0.51** (0.007)</td>
</tr>
<tr>
<td>Serum Triiodothyronine</td>
<td>(0.33)</td>
<td>(0.35)</td>
<td>(0.29)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Population Males (n=16)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Thyroxine</td>
<td>-0.03 (0.45)</td>
<td>-0.09 (0.36)</td>
<td>-0.10 (0.34)</td>
<td>--</td>
<td>0.15 (0.30)</td>
</tr>
<tr>
<td>Serum Triiodothyronine</td>
<td>(0.26)</td>
<td>(0.42)</td>
<td>(0.40)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>All Males (n=38)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Thyroxine</td>
<td>0.22 (0.08)</td>
<td>0.59*** (0.000)</td>
<td>0.51*** (0.000)</td>
<td>--</td>
<td>0.67*** (0.000)</td>
</tr>
<tr>
<td>Serum Triiodothyronine</td>
<td>(0.017)</td>
<td>(0.000)</td>
<td>(0.000)</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

*p<0.050,**p<0.010,***p<0.001
## APPENDIX 2

### Pearson Product-Moment Correlations: Mean Serum Thyroxine and Triiodothyronine Concentrations Versus Selected Gravimetric Determinations, Thyroxine or Triiodothyronine Concentration Among Females.

Values are given as Correlation Coefficient (Probability)

<table>
<thead>
<tr>
<th>Sexual Status</th>
<th>Body Weight (gm)</th>
<th>Ovary Weight (mg)</th>
<th>Uterus Weight (mg)</th>
<th>Serum Thyroxine Concentration (ug/dl)</th>
<th>Serum Triiodothyronine Concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control Females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=23</td>
<td>Serum</td>
<td>-0.07</td>
<td>0.17</td>
<td>-0.10</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Thyroxine</td>
<td>(0.38)</td>
<td>(0.22)</td>
<td>(0.32)</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>-0.09</td>
<td>0.40*</td>
<td>0.24</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Triiodothyronine</td>
<td>(0.48)</td>
<td>(0.028)</td>
<td>(0.24)</td>
<td>--</td>
</tr>
<tr>
<td><strong>Population Females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=23</td>
<td>Serum</td>
<td>0.14</td>
<td>0.06</td>
<td>-0.09</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Thyroxine</td>
<td>(0.26)</td>
<td>(0.39)</td>
<td>(0.34)</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>0.05</td>
<td>0.17</td>
<td>0.07</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Triiodothyronine</td>
<td>(0.41)</td>
<td>(0.24)</td>
<td>(0.39)</td>
<td>--</td>
</tr>
<tr>
<td><strong>All Females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=46</td>
<td>Serum</td>
<td>0.39**</td>
<td>0.45***</td>
<td>0.24</td>
<td>0.28*</td>
</tr>
<tr>
<td></td>
<td>Thyroxine</td>
<td>(0.003)</td>
<td>(0.001)</td>
<td>(0.052)</td>
<td>--</td>
</tr>
<tr>
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<td>Serum</td>
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<td>0.48***</td>
<td>0.24</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Triiodothyronine</td>
<td>(0.023)</td>
<td>(0.001)</td>
<td>(0.06)</td>
<td>--</td>
</tr>
</tbody>
</table>

*P<0.050, **P<.010, ***P<.001
## APPENDIX 3

Pearson Product-Moment Correlations: Thyroid Histology Determinations Versus Selected Gravimetric Determinations, Thyroxine or Triiodothyronine Concentration Among Control Males. Values are given as Correlation Coefficient (Probability)

<table>
<thead>
<tr>
<th>Control Males (n=14)</th>
<th>Body Weight</th>
<th>Testis Weight</th>
<th>Seminal Vesicle Weight</th>
<th>Serum Thyroxine Concentration</th>
<th>Serum Triiodothyronine Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pollicular Area</td>
<td>-0.07</td>
<td>0.06</td>
<td>-0.13</td>
<td>0.06</td>
<td>0.03</td>
</tr>
<tr>
<td>(0.40)</td>
<td>(0.42)</td>
<td>(0.33)</td>
<td>(0.42)</td>
<td>(0.46)</td>
<td></td>
</tr>
<tr>
<td>Colloidal Area</td>
<td>0.01</td>
<td>0.10</td>
<td>-0.06</td>
<td>-0.02</td>
<td>-0.01</td>
</tr>
<tr>
<td>(0.49)</td>
<td>(0.37)</td>
<td>(0.42)</td>
<td>(0.47)</td>
<td>(0.48)</td>
<td></td>
</tr>
<tr>
<td>Cellular Area</td>
<td>-0.38</td>
<td>-0.12</td>
<td>-0.41</td>
<td>0.41</td>
<td>0.22</td>
</tr>
<tr>
<td>(0.09)</td>
<td>(0.34)</td>
<td>(0.07)</td>
<td>(0.07)</td>
<td>(0.23)</td>
<td></td>
</tr>
<tr>
<td>Cell Height</td>
<td>-0.46*</td>
<td>-0.31</td>
<td>-0.47*</td>
<td>0.55*</td>
<td>0.35</td>
</tr>
<tr>
<td>(0.047)</td>
<td>(0.14)</td>
<td>(0.043)</td>
<td>(0.021)</td>
<td>(0.11)</td>
<td></td>
</tr>
<tr>
<td>Number of Cells</td>
<td>-0.22</td>
<td>-0.04</td>
<td>-0.27</td>
<td>0.13</td>
<td>0.10</td>
</tr>
<tr>
<td>(0.22)</td>
<td>(0.45)</td>
<td>(0.17)</td>
<td>(0.33)</td>
<td>(0.27)</td>
<td></td>
</tr>
<tr>
<td>Cells per 100 square</td>
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<td>0.05</td>
<td>0.25</td>
<td>-0.28</td>
<td>-0.01</td>
</tr>
<tr>
<td>um</td>
<td>(0.20)</td>
<td>(0.44)</td>
<td>(0.19)</td>
<td>(0.16)</td>
<td>(0.49)</td>
</tr>
</tbody>
</table>

*P<0.050
### APPENDIX 4

**Pearson Product-Moment Correlations: Thyroid Histology Determinations Versus Selected Gravimetric Determinations, Thyroxine or Triiodothyronine Concentration Among Population Males**

Values are given as

**Correlation Coefficient (Probability)**

<table>
<thead>
<tr>
<th>Population Males (n=18)</th>
<th>Body Weight</th>
<th>Testis Weight</th>
<th>Seminal Vesicle Weight</th>
<th>Serum Thyroxine Concentration</th>
<th>Serum Triiodothyronine Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular Area</td>
<td>-0.16</td>
<td>-0.13</td>
<td>-0.06</td>
<td>-0.01</td>
<td>-0.33</td>
</tr>
<tr>
<td></td>
<td>(0.26)</td>
<td>(0.30)</td>
<td>(0.41)</td>
<td>(0.49)</td>
<td>(0.12)</td>
</tr>
<tr>
<td>Colloidal Area</td>
<td>-0.18</td>
<td>-0.15</td>
<td>-0.06</td>
<td>-0.01</td>
<td>-0.32</td>
</tr>
<tr>
<td></td>
<td>(0.23)</td>
<td>(0.28)</td>
<td>(0.40)</td>
<td>(0.48)</td>
<td>(0.12)</td>
</tr>
<tr>
<td>Cellular Area</td>
<td>0.08</td>
<td>0.14</td>
<td>0.03</td>
<td>-0.06</td>
<td>-0.36</td>
</tr>
<tr>
<td></td>
<td>(0.38)</td>
<td>(0.37)</td>
<td>(0.45)</td>
<td>(0.41)</td>
<td>(0.10)</td>
</tr>
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<td>0.03</td>
<td>0.33</td>
<td>-0.24</td>
</tr>
<tr>
<td></td>
<td>(0.09)</td>
<td>(0.29)</td>
<td>(0.46)</td>
<td>(0.10)</td>
<td>(0.20)</td>
</tr>
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<td>0.04</td>
<td>-0.26</td>
<td>-0.31</td>
</tr>
<tr>
<td></td>
<td>(0.16)</td>
<td>(0.36)</td>
<td>(0.44)</td>
<td>(0.15)</td>
<td>(0.13)</td>
</tr>
<tr>
<td>Cells per 100 square um</td>
<td>-0.04</td>
<td>0.15</td>
<td>0.09</td>
<td>-0.20</td>
<td>0.45*</td>
</tr>
<tr>
<td></td>
<td>(0.49)</td>
<td>(0.28)</td>
<td>(0.36)</td>
<td>(0.22)</td>
<td>(0.047)</td>
</tr>
</tbody>
</table>

*P<0.050
**APPENDIX 5**

Pearson Product-Moment Correlations: Thyroid Histology Determinations Versus Selected Gravimetric Determinations, Thyroxine or Triiodothyronine Concentration Among All Males

Values are given as Correlation Coefficient (Probability)

<table>
<thead>
<tr>
<th>All Males (n=32)</th>
<th>Body Weight</th>
<th>Testis Weight</th>
<th>Seminal Vesicle Weight</th>
<th>Serum Thyroxine Concentration</th>
<th>Serum Triiodothyronine Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular Area</td>
<td>-0.05</td>
<td>0.06</td>
<td>0.02</td>
<td>0.15</td>
<td>-0.08</td>
</tr>
<tr>
<td></td>
<td>(0.38)</td>
<td>(0.37)</td>
<td>(0.45)</td>
<td>(0.35)</td>
<td>(0.21)</td>
</tr>
<tr>
<td>Colloidal Area</td>
<td>-0.05</td>
<td>0.02</td>
<td>-0.01</td>
<td>0.06</td>
<td>-0.12</td>
</tr>
<tr>
<td></td>
<td>(0.39)</td>
<td>(0.45)</td>
<td>(0.49)</td>
<td>(0.37)</td>
<td>(0.26)</td>
</tr>
<tr>
<td>Cellular Area</td>
<td>-0.05</td>
<td>0.18</td>
<td>0.12</td>
<td>0.42**</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>(0.39)</td>
<td>(0.16)</td>
<td>(0.25)</td>
<td>(0.009)</td>
<td>(0.30)</td>
</tr>
<tr>
<td>Cell Height</td>
<td>0.10</td>
<td>0.33*</td>
<td>0.26</td>
<td>0.49**</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>(0.30)</td>
<td>(0.034)</td>
<td>(0.07)</td>
<td>(0.002)</td>
<td>(0.02)</td>
</tr>
<tr>
<td>Number of Cells</td>
<td>-0.01</td>
<td>0.28</td>
<td>0.25</td>
<td>0.20</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>(0.49)</td>
<td>(0.06)</td>
<td>(0.08)</td>
<td>(0.14)</td>
<td>(0.23)</td>
</tr>
<tr>
<td>Cells per 100 square</td>
<td>0.05</td>
<td>-0.07</td>
<td>-0.03</td>
<td>-0.32*</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>(0.39)</td>
<td>(0.34)</td>
<td>(0.44)</td>
<td>(0.040)</td>
<td>(0.38)</td>
</tr>
</tbody>
</table>

*P<0.050, **P<0.01
### APPENDIX 6

**Pearson Product-Moment Correlations: Thyroid Histology Determinations Versus Selected Gravimetric Determinations, Thyroxine or Triiodothyronine Concentration Among Control Females.**

Values are given as Correlation Coefficient (Probability)

<table>
<thead>
<tr>
<th>Control Females (n=23)</th>
<th>Body Weight</th>
<th>Ovary Weight</th>
<th>Uterus Weight</th>
<th>Serum Thyroxine Concentration</th>
<th>Serum Triiodothyronine Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular Area</td>
<td>0.14 (0.31)</td>
<td>-0.07 (0.40)</td>
<td>0.14 (0.31)</td>
<td>-0.03 (0.35)</td>
<td>0.46 (0.45)</td>
</tr>
<tr>
<td>Colloidal Area</td>
<td>0.12 (0.34)</td>
<td>-0.09 (0.38)</td>
<td>0.22 (0.23)</td>
<td>0.21 (0.23)</td>
<td>0.08 (0.39)</td>
</tr>
<tr>
<td>Cellular Area</td>
<td>0.14 (0.31)</td>
<td>-0.03 (0.46)</td>
<td>0.01 (0.48)</td>
<td>-0.03 (0.45)</td>
<td>-0.17 (0.28)</td>
</tr>
<tr>
<td>Cell Height</td>
<td>0.01 (0.49)</td>
<td>0.04 (0.45)</td>
<td>-0.08 (0.40)</td>
<td>-0.05 (0.43)</td>
<td>-0.22 (0.23)</td>
</tr>
<tr>
<td>Number of Cells</td>
<td>0.18 (0.27)</td>
<td>-0.35 (0.11)</td>
<td>0.22 (0.23)</td>
<td>-0.35 (0.11)</td>
<td>-0.49* (0.038)</td>
</tr>
<tr>
<td>Cells per 100 square um</td>
<td>0.03 (0.46)</td>
<td>-0.27 (0.17)</td>
<td>0.19 (0.26)</td>
<td>-0.22 (0.23)</td>
<td>-0.16 (0.29)</td>
</tr>
</tbody>
</table>

*P<0.050
APPENDIX 7

Pearson Product-Moment Correlations: Thyroid Histology Determinations Versus Selected Gravimetric Determinations, Thyroxine or Triiodothyronine Concentration Among Population Females.

Values are given as Correlation Coefficient (Probability)

<table>
<thead>
<tr>
<th>Population Females (n=12)</th>
<th>Body Weight</th>
<th>Ovary Weight</th>
<th>Uterus Weight</th>
<th>Serum Thyroxine Concentration</th>
<th>Serum Triiodothyronine Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular Area</td>
<td>0.24</td>
<td>0.32</td>
<td>0.30</td>
<td>0.48</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>(0.22)</td>
<td>(0.15)</td>
<td>(0.17)</td>
<td>(0.06)</td>
<td>(0.17)</td>
</tr>
<tr>
<td>Colloidal Area</td>
<td>0.32</td>
<td>0.28</td>
<td>0.21</td>
<td>0.65*</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>(0.15)</td>
<td>(0.19)</td>
<td>(0.26)</td>
<td>(0.012)</td>
<td>(0.16)</td>
</tr>
<tr>
<td>Cellular Area</td>
<td>0.08</td>
<td>0.19</td>
<td>0.28</td>
<td>0.30</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>(0.41)</td>
<td>(0.28)</td>
<td>(0.19)</td>
<td>(0.17)</td>
<td>(0.32)</td>
</tr>
<tr>
<td>Cell Height</td>
<td>-0.31</td>
<td>-0.12</td>
<td>0.16</td>
<td>-0.26</td>
<td>-0.14</td>
</tr>
<tr>
<td></td>
<td>(0.16)</td>
<td>(0.35)</td>
<td>(0.31)</td>
<td>(0.21)</td>
<td>(0.37)</td>
</tr>
<tr>
<td>Number of Cells</td>
<td>0.50*</td>
<td>0.63*</td>
<td>0.52*</td>
<td>0.18</td>
<td>0.55*</td>
</tr>
<tr>
<td></td>
<td>(0.049)</td>
<td>(0.014)</td>
<td>(0.041)</td>
<td>(0.29)</td>
<td>(0.031)</td>
</tr>
<tr>
<td>Cells per 100 square mm</td>
<td>0.03</td>
<td>0.19</td>
<td>-0.01</td>
<td>-0.21</td>
<td>-0.04</td>
</tr>
<tr>
<td></td>
<td>(0.17)</td>
<td>(0.27)</td>
<td>(0.49)</td>
<td>(0.26)</td>
<td>(0.46)</td>
</tr>
</tbody>
</table>

*P<0.050
APPENDIX 8

Pearson Product-Moment Correlations: Thyroid Histology Determinations
Versus Selected Gravimetric Determinations, Thyroxine or
Triiodothyronine Concentration Among All Females.
Values are given as
Correlation Coefficient
(Probability)

<table>
<thead>
<tr>
<th>All Females (n=30)</th>
<th>Body Weight</th>
<th>Ovary Weight</th>
<th>Uterus Weight</th>
<th>Serum Thyroxine Concentration</th>
<th>Serum Triiodothyronine Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular Area</td>
<td>-0.34*</td>
<td>-0.35*</td>
<td>-0.09</td>
<td>-0.13</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>(0.043)</td>
<td>(0.040)</td>
<td>(0.33)</td>
<td>(0.26)</td>
<td>(0.47)</td>
</tr>
<tr>
<td>Colloidal Area</td>
<td>-0.31</td>
<td>-0.33*</td>
<td>-0.12</td>
<td>-0.06</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>(0.06)</td>
<td>(0.048)</td>
<td>(0.28)</td>
<td>(0.039)</td>
<td>(0.40)</td>
</tr>
<tr>
<td>Cellular Area</td>
<td>0.11</td>
<td>0.10</td>
<td>0.13</td>
<td>-0.10</td>
<td>-0.10</td>
</tr>
<tr>
<td></td>
<td>(0.18)</td>
<td>(0.16)</td>
<td>(0.49)</td>
<td>(0.31)</td>
<td>(0.36)</td>
</tr>
<tr>
<td>Cell Height</td>
<td>0.11</td>
<td>0.10</td>
<td>0.13</td>
<td>-0.04</td>
<td>-0.14</td>
</tr>
<tr>
<td></td>
<td>(0.26)</td>
<td>(0.31)</td>
<td>(0.26)</td>
<td>(0.43)</td>
<td>(0.27)</td>
</tr>
<tr>
<td>Number of Cells</td>
<td>-0.12</td>
<td>-0.36*</td>
<td>0.13</td>
<td>-0.32</td>
<td>-0.17</td>
</tr>
<tr>
<td></td>
<td>(0.28)</td>
<td>(0.034)</td>
<td>(0.26)</td>
<td>(0.06)</td>
<td>(0.22)</td>
</tr>
<tr>
<td>Cells per 100 square mm</td>
<td>0.16</td>
<td>0.09</td>
<td>0.11</td>
<td>-0.12</td>
<td>-0.09</td>
</tr>
<tr>
<td></td>
<td>(0.22)</td>
<td>(0.34)</td>
<td>(0.29)</td>
<td>(0.28)</td>
<td>(0.36)</td>
</tr>
</tbody>
</table>

*p<0.050
This program reads data from a file named "HISTDATA." The data is then processed assuming an ellipsoid shape for all follicles measured and places all tabulated results into a file named "HISTCOMPS."

REAL MAJ, MIN, MACH1, MACH2, MICH1, MICH2, TOTC, MCH, A1, A2, MMCH, CPF
REAL B1, B2, TAREA, INAREA, PI, CELLAREA, MTAREA, MCELLAREA, MINAREA
INTEGER FOLLNO
CHARACTER*6, ID
LOGICAL END, FOLLEND
PI=3.1415
END=.FALSE.
FOLLNO=0
MTAREA=0.0
MINAREA=0.0
MMCH=0.0
CPF=0.0
OPEN (UNIT=10, FILE='HISTCOMPS')
OPEN (UNIT=11, FILE='HISTDATA')
WHILE (.NOT. END) DO
READ (11,*) ID, MAJ, MIN, MACH1, MACH2, MICH1, MICH2, TOTC
IF (MAJ .GT. 98.0) THEN
CALL FOLLCOMP (FOLLNO, MTAREA, MINAREA, MCELLAREA, MMCH, CPF)
FOLLEND = .TRUE.
ELSEIF (MAJ .LE. 0.0) THEN
END = .TRUE.
WRITE (UNIT=10, FMT=11)' '
WRITE (UNIT=10, FMT=11)' '
WRITE(UNIT=10, FMT=10)' END OF DATA, RUN COMPLETE'
10 FORMAT(T25, A)
11 FORMAT(A)
ELSE
FOLLEND = .FALSE.
ENDIF
IF ((.NOT. FOLLEND) AND (.NOT. END)) THEN
MCH=(MACH1+MACH2+MICH1+MICH2)/4.0
A1=MAJ/2.0
A2=(MAJ-(MACH1+MACH2))/2.0
B1=MIN/2.0
B2=(MIN-(MICH1+MICH2))/2.0
TAREA=PI*A1*B1
INAREA=PI*A2*B2
CELLAREA=TAREA-INAREA
FOLLNO=FOLLNO+1
IF (FOLLNO .EQ. 1) THEN
WRITE (UNIT=10, FMT=1000)' ANIMAL'/' FOLLICLE'/' TOTAL' ,
+' COLLOIDAL'/' CELLULAR'/' MEAN'/' NUMBER'
WRITE (UNIT=10, FMT=1001)' ID'/' NUMBER'/' FOLLICULAR' ,
+ 'AREA', 'AREA', 'CELL', 'OF'
WRITE (UNIT=10, FMT=1002) 'AREA', 'HEIGHT', 'CELLS'
1000 FORMAT (T5, A, T14, A, T26, A, T38, A, T48, A, T59, A, T66, A)
1002 FORMAT (T27, A, T58, A, T66, A)
ENDIF
WRITE (UNIT=10, FMT=1003) ID, FOLLNO, TAREA, INAREA, CELLAREA, MC +, TOTC
MTAREA = MTAREA + TAREA
MINAREA = MINAREA + INAREA
MCELLAREA = MCELLAREA + CELLAREA
MMCH = MMCH + MCH
CPF = TOTC + CPF
FOLLEN = .TRUE.
ENDIF
ENDWHILE
CLOSE (UNIT=11)
CLOSE (UNIT=10)
END
SUBROUTINE FOLLCOMP (FOLLNO, MTAREA, MINAREA, MCELLAREA, MMCH, CPF)
REAL MTAREA, MINAREA, MCELLAREA, MMCH, GMTAREA, GMINAREA, GCELLAREA, GMC
H
REAL GCPF, MM2, CPF
INTEGER FOLLNO
GMTAREA = MTAREA / FOLLNO
GMINAREA = MINAREA / FOLLNO
GCELLAREA = MCELLAREA / FOLLNO
GMCH = MMCH / FOLLNO
GCPF = CPF / FOLLNO
MM2 = GCPF / GCELLAREA
WRITE (UNIT=10, FMT=1004)
1004 FORMAT (A)
WRITE (UNIT=10, FMT=1005) 'GRAND MEANS'
1005 FORMAT (T33, A)
WRITE (UNIT=10, FMT=1004)'
WRITE (UNIT=10, FMT=1006) 'MEAN', 'MEAN', 'MEAN', 'MEAN', 'NUMBER', + 'MEAN', 'MEAN'
WRITE (UNIT=10, FMT=1007) 'FOLLICULAR', 'COLLOIDAL', 'CELLULAR', + 'CELL', 'OF', 'CELLS', 'CELLS'
WRITE (UNIT=10, FMT=1008) 'AREA', 'AREA', 'AREA', 'HEIGHT', 'FOLLIC
L
+ES', 'PER', 'PER'
WRITE (UNIT=10, FMT=1009) 'READ', 'FOLLICLE', 'CELLmm2'
WRITE (UNIT=10, FMT=1011)'
WRITE (UNIT=10, FMT=1010) GMTAREA, GMINAREA, GCELLAREA, GMC, FOLLN +, GCPF, MM2
WRITE (UNIT=10, FMT=1011)'

WRITE (UNIT=10,FMT=1012)'******************************'
WRITE (UNIT=10,FMT=1011)' '
1006 FORMAT(T13,A,T26,A,T37,A,T47,A,T59,A,T68,A,T77,A)
1008 FORMAT(T13,A,T26,A,T37,A,T46,A,T57,A,T69,A,T78,A)
1009 FORMAT(T60,A,T66,A,T76,A)
+ ,F8.4)
1011 FORMAT(A)
1012 FORMAT(T25,A)
   FOLLNO=0
   MTAREA=0.0
   MINAREA=0.0
   MCELLAREA=0.0
   MMCH=0.0
   CPF=0.0
RETURN
END
BIBLIOGRAPHY


Wong, C-C., Dohler, K. D., vonzur Muhlen, A. 1980. Effects of tri-iodothyronine, thyroxine and isopropyl-di-iodothyronine on thyroid-stimulating hormone in serum and pituitary gland and on pituitary concentrations of prolactin, growth hormone, luteinizing hormone and follicle-stimulating hormone in hypothyroid rats. J. Endocr. 87:255.


VITA

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Born in Williamsburg, Virginia. August 11, 1957. Graduated from Walsingham Academy, June, 1975. Received B.A. degree in Biology and Psychology from Northwestern University, June, 1980. Entered the Biology Graduate Program of the College of William and Mary in Virginia. Awarded a Teaching Assistantship, June, 1982. Presently attending the School of Medicine at the University of Virginia. Also a candidate for the Master of Arts degree.