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A Study of the Metabolic Clearance Rate and Protein Binding of Corticosterone in Reproductively Inhibited Male Peromyscus maniculatus bairdi

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A STUDY OF THE METABOLIC CLEARANCE RATE AND PROTEIN BINDING OF CORTICOSTERONE IN REPRODUCTIVELY INHIBITED MALE *PEROMYSCUS MANICULATUS BAIRDI*

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Dedication

I would like to dedicate this thesis to John T. Black, for without his support and encouragement I would have been unable to complete this project.

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ABSTRACT

This study explored possible causes of the elevated serum corticosterone concentrations found in reproductively inhibited Peromyscus maniculatus bairdi or prairie deermice.

Differences in metabolic clearance rates between isolated male control deermice and reproductively inhibited population mice were explored. It was found that although there is no significant difference in the mean metabolic clearance rate values between reproductively inhibited and isolated male deermice, a significant correlation was found between small reproductive organ weights and lower rates of metabolic clearance.

Comparisons in the amount of biologically active corticosterone (free from protein binding) were made between the two groups of mice. Although the percentage of biologically active corticosterone is lower in reproductively inhibited animals, the actual concentration is higher.

This suggests that although protein binding may account for some of the high levels of corticosterone in reproductively inhibited animals, it is not the whole answer. It may be that in drastically inhibited deermice, as exhibited by very small gonad and accessory organ weights, metabolic clearance rate depression may also be involved in elevating serum corticosterone concentrations.

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Introduction

If laboratory populations of the prairie deermouse (Peromyscus maniculatus bairdi) are held in a confined space, they will eventually stop growing in number. Some of the young that are born into this population context do not survive, but more commonly, the fertility of the majority of the animals is sharply reduced and finally no more young are born or those born into the population do not survive (Terman 1965). The actual density at which growth ceases varies between populations and does not seem to be dependent on population density, per se.

About 90 % of the animals born into the population before growth ceases are reproductively inhibited; their body and reproductive organ weights are lower than those of similarly aged animals born outside the population setting (Terman, 1973). Many studies have been performed to determine the nature of the inhibition process. Among other things, it has been shown that serum thyroid hormone concentrations are lower and serum corticosterone concentrations are higher in inhibited population mice than in non-inhibited mice (Bradley and Terman, 1981c; Pitman and Bradley, 1984).

Some animals, such as Mus musculus, also show signs of

reproductive inhibition with increasing population density. Studies of Mus have shown that the adrenal glands of inhibited mice are significantly larger than those of reproductively capable mice (Christian, 1950, 1955, 1963; Christian and Davis, 1964). Adrenocortical activity is also high in these inhibited mice (Andrews et al., 1975; McCarthy et al., 1976). This suggests that in reproductively inhibited animals the adrenal hypertrophy and the increased activity of the adrenal gland may be due to high levels of ACTH. In support of this notion, injections of ACTH in reproductively capable Mus have resulted in reproductive inhibition (Christian, 1964).

Although there is a relation between increased serum corticosterone and increased population density in prairie deermice no relation has been found between adrenal weight and population density (Bronson and Eleftheriou, 1963; Terman, 1969; Sung et al., 1977; Bradley and Terman, 1981c). Furthermore, the plasma ACTH concentration of inhibited animals was not significantly elevated compared with non-inhibited mice (Coppes and Bradley, 1983). This suggests that although exogenous ACTH does cause adrenal hypertrophy (Coppes and Bradley, 1984) and reproductive inhibition (Pasley and Christian, 1972), naturally high levels of ACTH may not occur and be responsible for reproductive inhibition or the increased serum corticosterone concentration in inhibited prairie deermice.

Recent studies showing lower thyroid hormone concentration, smaller body weight and reproductive organ sizes, suggest that the inhibited animals are hypothyroid Peebles, et al., 1984; Pitman and Bradley, 1984). Since neither an increased level of ACTH nor adrenal hypertrophy can be demonstrated in these mice, it is possible that hypothyroidism might cause a decrease in the metabolic clearance rate of corticosterone, increasing the circulating levels of the hormone. Alternatively, a high level of corticosterone binding globulin (CBG) might also contribute to a high concentration of total serum corticosterone by increasing the amount of protective protein binding. High levels of CBG could lead to a lower concentration of circulating biologically active "free" hormone. Hence, the actual amount of free (biologically active) corticosterone available to the inhibited animals might actually be the same as that in a reproductively capable animal if there were a sufficient change in the degree of protien binding.

The purpose of this study was to assess the paradox of high serum corticosterone concentration in animals that do not possess hypertrophied adrenals. Both the metabolic clearance rate of corticosterone and the actual level of free (biologically active) corticosterone were investigated.

Materials and Methods

Experimental Animals

Controls: The animals used in this experiment were prairie deermice (Peromyscus maniculatus bairdi) derived from an outbred laboratory colony. Control mice were weaned at 21 days of age and then maintained in same-sex sibling cages until at least one week prior to sampling. At this time they were moved into the same room as the population mice and were housed individually. Although these males may be more accurately described as isolated males, the terms control(s) and isolated male(s) were used interchangeably.

Population Inhibited: These animals were taken from three laboratory populations that were started with four reproductively proven, non-sib pairs of mice. Any young born into the population during the first 21 days were removed, ensuring that all young that remained in the population were conceived in the population. Population enclosures were 1.5 meters in diameter. The floor was covered with wood chips and six plastic nest boxes were provided as needed.

All mice were provided with an excess of food and water. The lights were set on a 14 hour light, 10 hour

dark cycle with light on at 0830 EDT. Room temperature was maintained at between 21 and 30 C and there were 5-10 air changes per hour in the room.

Selection of Experimental Animals: Control and population males were between 105 and 172 days of age. On the morning of sampling all of the animals in a population were collected and individually examined until a male that was both aged 105 -172 days and was never observed to have had scrotal testess was located. Depending on the experiment (Protein Binding Study), additional male animals were selected. All population animals not selected were then returned to the enclosure.

Tissue Collection

Collection and Storage of Blood: Population animals were inspected at 1000 hours the morning of sampling and selected animals were placed in a cage containing food, water, and soiled bedding from the population. The cage was put back in the population enclosure by 1100 hours. Control animals that were to be sampled were also handled so that they would be similarly disturbed.

At 1400 hours when the corticosterone concentration was shown to be stable in rats (Matsuyama et al., 1972), sampling was begun. Each animal was anesthetized with diethyl ether, the abdominal cavity was exposed and the renal artery was clipped. Blood was removed within 2

minutes of first contact from the abdominal cavity using a 1 ml plastic syringe. The clotted blood was centrifuged to separate blood cells from serum. The serum was removed, placed in a glass test tube,covered with parafilm and frozen at below -25 C.

The animals were weighed and the abdominal and thoracic cavities were fully exposed. The animals were placed in buffered 10% formalin. The testes, seminal vesicles and adrenal glands were later removed, cleaned of surrounding tissue and weighed to the nearest 0.1 mg.

Measurement of Metabolic Clearance Rate

The animals for this experiment were weighed and anesthetized with amobarbital sodium (0.12 mg/g of body weight at a 25 mg/ml concentration). A jugular cannula was prepared by fitting PE 10 intramedic tubing (Clay Adams) over the tip of the needle of a 100 ul Hamilton syringe filled with heparin (100 U/ml, Sigma). Ten microliters of sodium heparin (Sigma) was injected into the cannula and the cannula was sized to contain 10 ul (about 12 cm long).

Ten minutes after the injection of amobarbital the mouse was further anesthetized with Halothane U.S.P. inhalation until surgical anesthesia was attained. An incision was made in the neck, the jugular vein was exposed and cleaned of surrounding fat and tissue. Two strands of 4-0 surgical silk were passed under the jugular, one

directed cranially, one caudally. A loose overhand knot was tied in each suture. The lower end (caudal) of the jugular was clamped off with a pediatric hemostat and the cranial suture was tightened down. The area in between was nicked with scissors, the hemostat was released, and the cannula was passed into the jugular and slowly advanced past the clavicle so that it was positioned in the vena cava. Blood was drawn back to ensure that the cannula was patent, then 10 ul of heparin was injected to keep the vein open. The needle and syringe were exchanged and 20 ul of a known concentration of tritiated corticosterone (about 0.5 uCi = 1.65ng of tritiated corticosterone or 5 ul of tritiated corticosterone diluted with 15 ul 0.9% NaCl, New England Nuclear S.A. = 105.0 Ci/mMol) was injected. The cannula was flushed with heparin and a timer was started. A duplicate 20 ul aliquot of the tritiated corticosterone were placed into a scintillation vial to determine the number of counts injected.

At t = 19 min, blood was withdrawn through the cannula until whole blood just entered the syringe (i.e., more than a 10 ul volume). The heparinized syringe was removed and replaced with a tuberculin syringe. At t =20 min, about 25 ul of blood was drawn into the hub of a 27g 1/2" needle which was then placed to one side. The first syringe containing the heparin and blood was reattached to the cannula and enough additional heparin/saline solution was

returned to the animal to clear the cannula of blood. Twenty microliters of blood was then removed from the hub of the sample needle with an Oxford pipette and placed directly into a scintillation vial containing 10 ml of scintillation fluid (Biosolve EP, Beckman). This procedure was repeated at t = 30, 40, 50, and 60 minutes. Following the last sample the animal was euthanized with an overdose of Nembutal. The abdominal area was cut open, exposing the reproductive organs. The bladder was punctured with a 27g 1/2" needle and all of the urine was removed and placed in a scintillation vial. The animal was stored in 10% buffered formalin. Later the testes and seminal vesicles were removed, cleaned of fat and weighed to the nearest milligram.

The apparent volume of distribution (AVD), half-life (T 1/2), and metabolic clearance rate (MCR) of corticosterone were calculated according to a single compartment model by the method described by Bradley and Holmes (1971). The injected corticosterone was assumed to be evenly distributed at the time of administration (t = 0), and it was further assumed that no metabolism of corticosterone occured until after it was evenly distributed. By taking the log of the cpm of each sample and plotting it against the time the sample was taken the equation of a line could be calculated: $\log Y = bX + \log A$. Y is defined as the plasma concentration in cpm/ml, b

is the slope of the line, A is the plasma concentration at t = 0, and X is the time in minutes after injection of the tritiated corticosterone. The AVD is the volume in which the tritiated corticosterone is distributed. It is calculated by the equation:

$$AVD = \frac{\text{cpm injected}}{\text{cpm/ml at t=0}}$$

This gives an apparent volume of distribution in ml at t = 0. The biological half-life of corticosterone (T 1/2) is the time that it takes for one half of the labelled steroid to be removed from the circulation. It is found by the formula:

$$T\ 1/2 = \frac{\ln 2}{-b \ln 10}$$

The MCR is that volume of plasma that is cleared of corticosterone per unit time. It is calculated by the formula:

$$MCR = \frac{AVD\ 1n2}{T\ 1/2}$$

Serum Corticosterone Protein Binding and Radioimmunoassay of Corticosterone: The method used was the unmodified protocol described in the Endocrine Sciences Radioimmunoassay Procedure for Corticosterone antiserum No. B3-163. All glassware was rinsed with absolute ethanol, then with redistilled methanol, and allowed to air dry. All tubes used in the assay were 12 X 75 mm plastic, conical bottomed tubes (Scientific Products). A 0.05 M borate buffer was prepared by dissolving 4 g of boric acid in 1000 ml glass distilled water. The buffer was adjusted to pH 8.0 by adding 10N NaOH. Bovine serum albumin (10%) was prepared by dissolving 1 g of bovine serum albumin (Sigma) in 10 ml 0.05 borate buffer containing 0.1% sodium azide. Bovine gamma globulin (2.5%) was prepared by adding 250 mg bovine gamma globulin (Sigma) to 10 ml of normal saline containing 0.1% sodium azide. To make a borate buffer/BSA mixture, 2.5 ml of 10% bovine serum albumin was added to 97.5 ml of borate buffer.

A corticosterone standard of 1 mg of corticosterone/ml redistilled methanol was prepared. From this stock, serial dilutions of 1.0, 0.5, 0.25, 0.125, 0.0625, and 0.0312 ng

corticosterone/0.1 ml redistilled methanol were prepared in duplicate. The tubes were placed in 50 ml beakers containing 20 ml of water and the alcohol was evaporated to dryness at 45 C in a vacuum oven. Fifty microliters of borate buffer/BSA was added to each tube and to four additional tubes to determine the radioactivity bound to zero ng corticosterone (Bo). The tubes were vortex mixed and then allowed to stand for one hour.

Frozen Peromyscus standard serum (PSSer) from a pool was thawed and a serial dilution of 20, 10, 5, and 2.5 ul was prepared in borate buffer/BSA. Control and population experimental serum was thawed and 20 ul was taken from each sample and placed in a sample tube. Both the sample tubes and the PSSer pool dilution tubes were brought to a total volume of 400 ul with borate buffer/BSA. The tubes were heated for 30 min at 60 C in a water bath to denature the corticosteroid binding proteins. Following removal from the bath, duplicate 50 ul aliquots from each tube were removed and placed into assay tubes.

A saturated solution of ammonium sulfate was prepared by adding an excess of the reagent grade salt to glass distilled water. Saturation was confirmed by the presence of crystals after two hours. A dilute antiserum mixture was made by combining: 24.0 ml of borate buffer; 30 ul of tritiated corticosterone (equal to 1,200,000 dpm - 1,2,6,7 3H Corticosterone New England Nuclear S.A. = 105.0

Ci/mMol); 0.6ml 10% bovine serum albumin; 0.6 ml 2.5% bovine gamma globulin; and 0.3 ml stock antiserum (corticosterone Antiserum B3-163; Endocrine Sciences, CA).

Before adding the antiserum, 0.2 ml of the dilute antiserum mixture was added to each of six conical tubes containing 50 ul of borate buffer/BSA. Three of these tubes were designated "total count tubes" and three were designated "non-specific binding tubes".

The dilute antiserum was centrifuged for 5 minutes at 3000 rpm. Two hundred microliters of dilute antiserum was added to each of the sample tubes, the plasma dilution tubes, the standard curve tubes, and the four Bo tubes. Each tube was vortex mixed and incubated for 45 minutes at 37 C, then for two hours at room temperature, and finally at 4 C overnight (20 hrs).

Free steroid and antibody-bound steroid fractions were separated by adding 0.25 ml of the saturated ammonium solution to each tube (with the exception of the total count tubes). The tubes were vortex mixed and centrifuged for 10 minutes at 3000 rpm at 4 C. Three hundred microliters of supernatant was transferred from each tube to a counting vial containing 10 ml of scintillation fluid (Biosolve EP, Beckman). The vials were assayed for tritium in a liquid scintillation counter for 50 minutes or to a 3% error.

Total serum corticosterone was calculated by finding

the slope of a log-logit transformation of the standard curve. The equation of that line (Y = 0.55826X - 1.03921) was used to calculate total serum corticosterone for each sample in pg/2.5 ul and the values were multiplied by 0.04 to give ug/100 ml.

Determination of Percent Free Corticosterone: Fifty microliters of tritiated corticosterone (14,000 cpm/100 ul, New England Nuclear S.A. = 105.0 Ci/mMol) was added to each conical bottom plastic tube. The solvent was evaporated to dryness in a vacuum oven. One hundred microliters of PSSer was added to each of four tubes. One hundred microliters of control or population plasma was added to each of the remaining tubes. In some instances where serum volume was inadequate, a lesser amount was added (see Discussion). The tubes were vortex mixed for three minutes and allowed to incubate overnight (20 hrs) at 4 C. They were then incubated for two hours at room temperature.

The free corticosterone was separated from that which was protein bound using the Amicon MP Ultrafiltration System. Nintey microliters of serum was placed into each ultrafiltration cell when sufficient volume was available. The cells were centrifuged at 3000 rpm for five minutes. Twenty-five microliters of the ultrafiltrate containing free corticosterone was removed and placed in a

scintillation vial containing 10 ml of Biosolve EP (Beckman) scintillation fluid. The vials were counted on a liquid scintillation counter to a 1% error or for 100 minutes.

The percent free corticosterone was found by dividing the number of counts per microliter in the ultrafiltrate by the number of counts per microliter in the original aliquot.

Statistics

The mean and standard error of the mean were calculated for all values. Comparisons were made by the student's t-test, except for the body weight of animals in the protein binding study and the seminal vesicle weight in the MCR study. In these cases the variances were heteroscedastic and the Mann-Whitney U-Test was used. A Pearson Product Moment Coefficient was used to determine if correlations existed between selected parameters. A probability of less than 0.05 was considered significant in all cases.

Results

Metabolic Clearance Rate Study

Population mean values for body weight, testes weight and seminal vesicle weight were all significantly lower than those of the control mice mice ($P < 0.015$, $P < 0.001$, $P < 0.0005$ respectively; Table 1).

A trend ($P < 0.058$) toward a larger mean value of tritium excreted in the urine was identified in males from populations versus the isolated controls (Table 2). However, the mean values for the AVD, T 1/2, and MCR of population males were not significantly different from the respective values for isolated control males (Table 2).

A significant linear correlation was demonstrated between increasing testes weights and the MCR when all of the population and control values were combined ($r = 0.567$, $P < 0.02$) and between these same parameters when only values from population animals were used ($r = 0.813$, $P < 0.008$). No significant correlations were observed if only values for isolated male controls were used.

Also, if values for all male isolate controls and population animals were combined for seminal vesicle weight versus MCR, they were found to be significantly correlated

($r = 0.493$, $P < 0.04$), but only a trend ($r = 0.615$, $P < 0.08$) was demonstrated with values for population males and no correlation was demonstrated with isolated controls. No other significant correlations were demonstrated.

Corticosterone Protein Binding Study

Mean body, seminal vesicle and testes weights of a second group of population males were significantly smaller than those of their respective control male mice ($P < 0.0028$, $P < 0.000$, $P < 0.000$, respectively). There were no significant differences between control and population absolute and relative adrenal weights (Table 3).

The mean value for percent free corticosterone of the isolated male deermice was significantly larger than the population mean value ($P < 0.0045$; Table 4). A comparison between control and population mean values shows that the population mean corticosterone concentration is significantly larger than the control ($P < 0.0034$; Table 4). The total free serum corticosterone concentration was found by multiplying the percent free corticosterone by the total serum corticosterone (in ug/100 ml). The mean concentration of the total free corticosterone in population inhibited males was significantly higher than the control mean value ($P < 0.0347$; Table 4).

Table 1

Mean Age, Body Weight, Testes Weight and Seminal Vesicle Weight of Control and Population Deermice in the Metabolic Clearance Rate Study

(Mean ± S.E.M.)

	Age (days)	Body Weight (g)	Testes Weight (mg)	Seminal Vesicle Weight (mg)
Control				
Males	128 ±7.7 (9)	18.06 ±0.738 (9)	221 ±17.1 (8)	156 ±23.8 (8)
Population				
Males	137 ±7.2 (9)	15.28* ±0.696 (9)	111** ±18.8 (9)	33*** ±6.2 (9)

* $P < 0.015$, ** $P < 0.001$, *** $P < 0.0005$, with respect to the corresponding control values

Table 2

Comparison of Apparent Volume of Distribution (AVD), Half-Life (T 1/2) and Metabolic Clearance Rate (MCR) of Corticosterone and Tritium Activity Recovered in Bladder Urine of Control and Population Deermice

(Mean ± S.E.M.)

	AVD (ml)	T 1/2 (min)	MCR (ml/min)	Tritium Activity in Bladder Urine (cpm recovered/cpm injected)
Control				
Males	13.00	22.51	0.3848	0.0467
(9)	±2.765	±2.886	±0.0530	±0.0007
Population				
Males	11.80	26.62	0.2926	0.0650*
(9)	±2.516	±3.108	±0.0589	±0.0005

* $P < 0.058$, with respect to the corresponding control value

Table 3

Mean Age, Body Weight, Testes Weight, Seminal Vesicle Weight and Absolute and Relative Adrenal Gland Weight of Control and Population Deermice in the Corticosterone and Protein Binding Study

(Mean ± S.E.M.)

	Age (days)	Body Weight (g)	Testes Weight (mg)	Seminal Vesicle Weight (mg)	Absolute Adrenal Weight (mg)	Relative Adrenal Weight (mg/g)
Control						
Males	142 ±2.0 (10)	19.2 ±1.04 (10)	305.3 ±24.95 (10)	203.5 ±24.12 (10)	3.49 ±0.375 (10)	0.181 ±0.019 (10)
Population						
Males	138 ±4.6 (10)	14.7* ±0.33 (10)	98.9** ±26.04 (10)	29.0** ±16.97 (8)	2.78 ±0.231 (10)	0.192 ±0.016 (10)

* $P < 0.0028$, ** $P < 0.000X$, with respect to the corresponding control value.

Table 4

Comparison of Total Serum Corticosterone, of the Percent Free of Binding, and the Total Free Corticosterone in Serum of Control and Population Deermice

(Mean ± S.E.M.)

	Total Serum Corticosterone (ug/100ml)	Percent Free Corticosterone (%)	Free Corticosterone (ug/100 ml)
Control			
Males	42.28	4.66	1.955
(10)	±7.650	±0.215	±0.3391
Population			
Males	92.46***	3.70**	3.458*
(8)	±12.864	±0.186	±0.0595

* $P < 0.0347$, ** $P < 0.0045$, *** $P < 0.00034$, with respect to the corresponding control value

Discussion

Earlier studies established that reproductively inhibited deermice had smaller body and reproductive organ weights than reproductively capable mice (Terman, 1969; Sung, 1977; Bradley and Terman, 1981 a,b,c). Deermice selected from laboratory populations in this study had significantly smaller body and reproductive organ weights than those of the control mice (c.f., Tables 1,3). This indicates that these animals were reproductively inhibited in a manner similar to that previously reported. The testes weights of population animals were somewhat larger than previously reported (Bradley and Terman, 1981 a,b,c). This is probably due to the fact that older animals were used in this present study, but it may also reflect the fact that some of the animals were further developed sexually than animals used previously.

In earlier studies the adrenal gland weight of reproductively inhibited deermice has been reported to be either smaller (Sung et al., 1977; Bradley and Terman, 1981c), or not statistically different (Coppes et al., 1984; Kirkland et al., 1985) when compared with normal controls. In no case has the absolute adrenal weight of population deermice been found to be larger than that of

controls, which is characteristic of Mus and Rattus and would be expected if adrenal hypertrophy were the cause of the observed increase in serum corticosterone concentration. In this present study there was no statistical difference between the absolute or the relative adrenal gland weights of control versus population mice (Table 3), confirming again the apparent lack of adrenal hypertrophy in reproductively inhibited population animals.

The metabolic clearance rate (MCR) of corticosterone was determined from the biological half-life (T 1/2) and the apparent volume of distribution (AVD) of the injection of a single dose of tritiated corticosterone. This method is similar to that used in several other studies of metabolic clearance (Tait et al., 1961; Herbst et al., 1967; Bradley and Holmes, 1971; Holmes et al., 1972; Sabourea and Boissin, 1980; Kovacs and Peczely, 1983). Studies comparing a single injection of tritiated corticosterone with constant tritium infusion techniques, indicate that there are no significant differences in the results obtained by either method (Tait et al., 1969; Saboureau and Boissin, 1980).

The AVD of population mice was smaller then that of controls which may have been due to the smaller body size of inhibited animals. Also the T 1/2 of corticosterone in population mice was somewhat larger than that of control mice (Table 2). However, neither of these tendencies were

statistically significant. It is therefore not surprising that the ratio of the AVD ln 2 over the T 1/2, which is equal to the MCR, was also not statistically significantly different. Indeed, if the mean MCR for both population and control animals is adjusted for body weight the apparent differences are further reduced.

However, despite the lack of difference between the mean value for each of the two groups, interesting and important differences are demonstrated when individual testes weights and the respective MCR values are compared for each animal. There is a significant positive correlation ($P < 0.02$) if values for both control and population inhibited animals are used. This significant ($P < 0.008$) correlation also persists if only values from population inhibited animals are used, but, no such relationship exists if only values from reproductively capable animals are used. Similar significant ($P < 0.04$) correlations between seminal vesicle weight and MCR values for control plus population inhibited mice further support the conclusion that the most drastically inhibited deermice have the lowest MCR.

The mean value for the tritium activity recovered in the bladder urine was 39 % higher in population mice compared with the isolated males (Table 2). This may be because more corticosterone is excreted via the biliary route in control mice. Variations of about 25 % in biliary

and fecal corticosterone excretion are known to occur in other species following hypophysectomy or ACTH treatment (Bradley and Holmes, 1971). Another possibility for apparently increased urinary excretion of the tritium dose may be that control mice have more receptors for corticosterone than population mice. This would give the appearance of an increased excretion rate in inhibited animals, when in reality there is simply less corticosterone bound to the "down-regulated" cells in the inhibited population animals and so more corticosterone is available for both metabolite production and rapid urinary secretion. No correlations were demonstrated between absolute urine tritium or tritium concentration and MCR.

Total serum corticosterone concentration of population mice was twice as large as that of control mice. This agrees well with the results of Sung et al. (1977) and Bradley and Terman (1981c). However, the actual values for both control and population animals are much higher than those previously recorded. Although high, these values are not unreasonable. Several studies have shown similar corticosterone concentrations in response to acute stress in rats (Kaneko, et al., 1980, Benwell and Balfour, 1982; Dobrakovova et al., 1982). The animals in this study were acutely stressed during and immediately prior to sampling due to the sampling design that caused them to be removed from the population enclosure or the home cage three to

four hours prior to sampling. Reynolds et al. (1980) has shown that in rats the pulsatile corticosterone response to stress occurs in less than 3-5 minutes and so all of these animals should have had elevated corticosterone. Indeed, in isolated male control mice, the order of sampling is well correlated with increasing serum corticosterone concentration.

The MPS-1 Amicon micropartition system used to determine the percent of corticosterone that is not bound to plasma proteins has been previously investigated in our laboratory (Huyen Van Cao, unpublished Honors Thesis, 1984). The system was found to compare favorably with other methods, such as those using dextran coated charcoal, Florisil in Peromyscus (Cao, 1984) and equilibrium dialysis in humans (MacMahon et al., 1983). In the present study it was sometimes necessary to use an aliquot of plasma smaller than 100 ul because the amount of plasma obtained from the individual mouse was too small. The earlier study (Cao, 1984) also compared the ultracentrifugation of varying amounts (80, 100, 120 ul) of plasma and found no significant difference in the binding estimates.

The percentage of corticosterone free of protein binding was significantly lower ($P < 0.0045$) in population mice compared with controls Table 4). When the actual concentration of free (biologically active) serum corticosterone was calculated there was a greater

concentration of biologically active corticosterone in population mice. Although the biologically active corticosterone concentration of population mice is higher than that of controls, a large part of the total serum corticosterone is not available for metabolism by the liver because it is protected by binding to protein. The higher concentration of biologically active corticosterone may be partially due to the tendency toward reduction in metabolic clearance rate found in reproductively inhibited mice, but it cannot account for all the difference.

Even though the absolute mean MCR value for all inhibited deermice is a non-significant value 25 % lower than for all control mice, there is still a significant ($P < 0.02$, $P < 0.04$, respectively) positive correlation between the smallest testes and seminal vesicle weights and reduced MCR. This indicates that the most inhibited animals have the lowest MCR. The population animals used in this study were not uniformly "inhibited". There is a wide range of testes weights and the mean body and reproductive organ weights are higher than those previously reported for reproductively inhibited population deermice. While some of these differences may be explained by the age of these animals as compared with those of earlier studies, it is possible that some of the animals were also not as inhibited. Also, control animals used in this study were not as well developed as reproductively proven males. In

contrast to many previous studies, the control male deermice were never exposed to female deermice and their gonad and seminal vesicle weights were reduced from compared with those of previous studies. (Testes weight: 221 mg; seminal vesicle weight: 111mg; compared with testes weight: 314.2; seminal vesicle weight: 144.9; Bradley and Terman, 1981b). Taking these factors into consideration, it is quite reasonable that the metabolic clearance rate is lower in reproductively inhibited animals, especially those animals that have small testes and seminal vesicle weights.

In a study of Japanese quail, Kovacs and Peczely (1980) demonstrated that the MCR of corticosterone decreased with increasing hypothyroidism. Hypothryoidism could be the primary cause of the metabolic clearance rate reduction in those reproductively inhibited population deermice that have a drastic reduction in gonad and sexual accessory weights. Peebles and Bradley (1984) demonstrated a significant decrease in serum thyroxine concentration and Pitman and Bradley (1984) demonstrated significantly lower concentrations of both serum thyroxine and triiodothyronine in reproductively inhibited populaton deermice as compared with reproductively capable controls. A decrease in these hormones could slow the metabolism of corticosterone in the liver. Hypothyroidism could also explain other changes in the inhibited deermouse such as smaller body size and

reproductive organ weight and development (Amin and El-Shiekh, 1977; Kalland et al., 1978).

Since the discovery of a specific corticosterone binding protein (transcortin or corticosterone binding globulin - CBG) much attention has been focused on the function of transcortin (Daughaday, 1958 a, b; Upton and Bundy, 1958; Bush, 1957; Slaunwhite and Sandberg, 1959). Subsequent studies established that corticosterone bound to transcortin (or other proteins such as albumin) was "biologically inactive". That is, it was not available for use by the body - it was not metabolized by the liver and could not be used by the cells (Slaunwhite and Sandberg, 1963; Slaunwhite, Lockie, Back and Sandberg, 1962). The corticosterone free of protein binding or "biologically active" corticosterone was a small percentage of the total serum corticosterone concentration (3-5%), as was shown in the present study. An increase in total serum hormone concentration should cause an increase in the amount of corticosterone bound to transcortin and the other binding proteins, so that the ratio of free to bound remains somewhat the same to preserve the biologically effective level of the hormone at a fairly constant level. This has been demonstrated to be the case in pregnant women. The level of glucocorticoids in the serum rises during pregnancy, but an increase in the amount of binding occurs concomittantly, leaving the amount of circulating

biologically active hormone the same (Slaunwhite and Sandberg, 1958; MacMahon et al., 1983).

If the blood volume is assumed to be 10% of body weight, then the absolute mass of free serum corticosterone of inhibited mice in this study was only about 35 % higher than that of the control mice, as compared with a 200 % increase in the total concentration (without any adjustment). This, coupled with the probable decrease in MCR by at least the same magnitude in the more drastically inhibited animals from the population could easily explain how there could be an apparent 2-fold increase in total serum corticosterone concentration without hypertrophied adrenal glands. In fact, the MCR of the population animal with the lowest testes and seminal vesicle weights is 75 % lower than the mean MCR value for the population inhibited animals as a group.

Higher concentrations of biologically active corticosterone could have caused a reduction in thyroid hormone production (Wilbur and Utriger, 1969; Otsuki, Dokada and Baba, 1973; Parmenter and Hedge, 1980). Elevated serum glucocorticoids have also been related to reproductive inhibition. Studies have shown that they inhibit testosterone production in mice and humans (Gartner, Reznik-Schuller and Reznik, 1973; Doerr and Pirke, 1976; Schiason, Durand and Mowszowicz, 1978) and prolactin production in rats (Collu, Tache and Ducharme,

1979).

Although strongly suggestive, the results of this study are not conclusive. Further research on both MCR and protein binding is indicated. MCR studies should be done with both male and female animals, and the animals should be standardized for the extent of reproductive inhibition, i.e. reproductively proven control animals versus population animals that are inhibited as evidenced by drastically reduced gonads and body weight. Corticosterone concentrations for population inhibited and control mice need to be established for a particular non-stressed context. Three different estimates of corticosterone concentration have been obtained in both population and control mice in three studies done in this lab. These differing results may be due to different times of day and sampling criteria. Protein binding levels should be repeated with animals that are less stressed than these were to establish that the short-term binding ratio remains the same regardless of the level of stress.

Appendix 1

Number, Age, Body, Testes, Seminal Vesicle Weight, and Apparent Volume of Distribution (AVD), Half-Life (T 1/2) and Metabolic Clearance Rate (MCR) of Corticosterone in Control and Population Deermice

	Number	Age (Days)	Body Weight (g)	Testes Weight (mg)	Seminal Vesicle Weight (mg)	AVD (ml)	T 1/2 (min)	MCR (ml/min)
Control Males	5236	120	19.53	266	230	14.89	19.40	.5322
	5315	140	16.21	229	194	3.93	13.35	.2040
	5295	120	21.32	238	79	9.21	20.12	.3173
	5261	105	14.63	149	61	17.22	35.13	.3398
	5278	180	18.65	242	129	4.10	17.74	.1602
	5282	105	15.81	205	247	14.94	16.79	.6170
	5261	140	18.11	155	134	11.38	24.47	.3222
	5276	120	17.80	N/A	N/A	10.04	17.16	.4056
	5349	120	20.46	282	172	31.29	38.42	.5646
Population Males	220	120	14.34	136	54	13.29	30.86	.2986
	111	135	18.14	112	39	5.56	20.36	.1893
	142	160	13.22	41	4	1.30	12.39	.0730
	130	150	12.76	94	23	9.81	27.51	.2470
	321	165	18.46	168	38	24.25	28.08	.5986
	329	150	13.96	10	5	4.26	16.68	.1771
	342	135	16.96	184	53	20.36	25.19	.5603
	160	105	14.52	114	37	11.58	41.82	.1920
	347	110	15.17	137	43	15.74	36.69	.2974

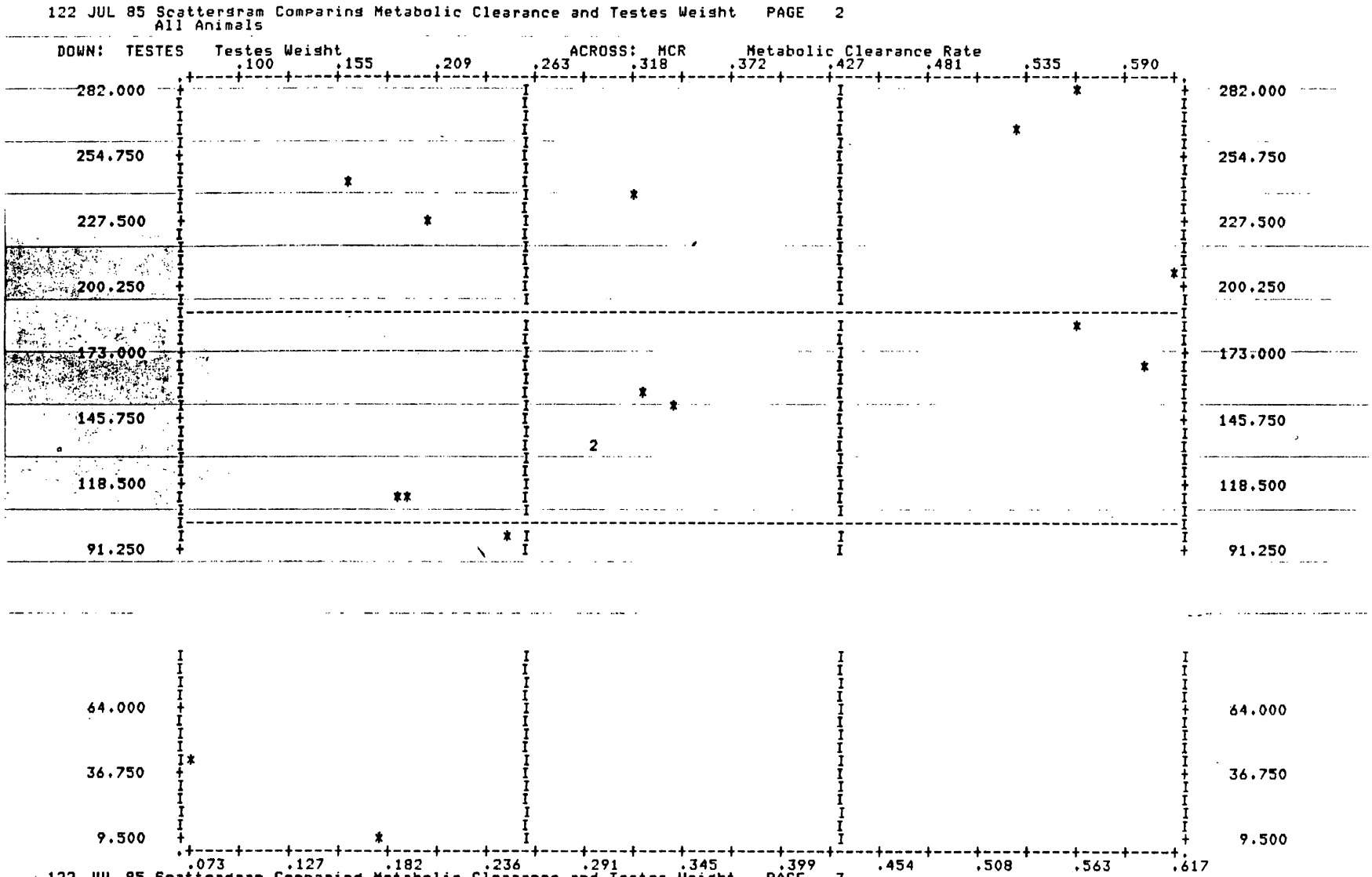
Appendix 2

Number, Age, Body, Testes, Seminal Vesicle, Absolute and Relative Adrenal Weights and Total Serum, Percent Free and Total Free Corticosterone in Control and Population Deermice

	Number	Age	Body Weight	Testes Weight	Seminal Vesicle Weight	Abs. Adrenal Weight	Rel. Adrenal Weight	Total Serum Corticosterone	% Free	Tot. Free
Control Males	5319-1	138	18.2	292.4	278.2	4.00	.220	19.59	4.07	0.80
	5319-2	138	18.7	404.6	236.2	4.46	.239	81.67	4.05	3.31
	5242	143	20.4	323.8	231.6	3.03	.149	4.09	5.03	0.21
	5236	143	16.0	324.0	186.4	2.53	.158	14.52	3.93	0.57
	5249	143	24.8	288.4	150.0	4.62	.186	40.00	5.52	2.21
	5236-1	143	20.3	403.2	322.2	4.47	.220	63.40	4.99	3.16
	5328	138	23.1	313.2	219.6	3.32	.144	40.93	5.39	2.21
	5255-1	134	13.7	123.2	40.4	1.04	.076	50.36	4.16	2.09
	5642	143	16.6	260.4	177.2	4.70	.283	63.40	4.00	2.54
	5255	143	19.8	320.0	193.4	2.73	.138	44.88	5.49	2.46
Population Males	180	120	14.2	26.2	3.3	3.98	.280	N/A	N/A	N/A
	365	142	14.6	16.2	1.4	2.90	.199	166.7	3.09	5.15
	362	142	13.7	48.0	4.6	1.96	.143	123.6	3.22	3.98
	179	120	16.1	238.0	56.2	1.88	.117	126.5	3.87	4.90
	173	135	15.0	144.6	19.2	3.70	.247	102.8	4.64	4.77
	171	135	16.3	137.4	N/A	2.96	.182	96.0	3.35	3.22
	360	142	13.8	40.0	4.3	2.96	.214	62.0	3.71	2.30
	351	172	15.3	208.6	138.8	2.08	.136	18.3	4.21	0.77
	363	142	13.0	39.2	N/A	2.28	.175	94.8	3.49	3.27
	164	135	14.7	30.4	4.2	3.26	.222	N/A	N/A	N/A

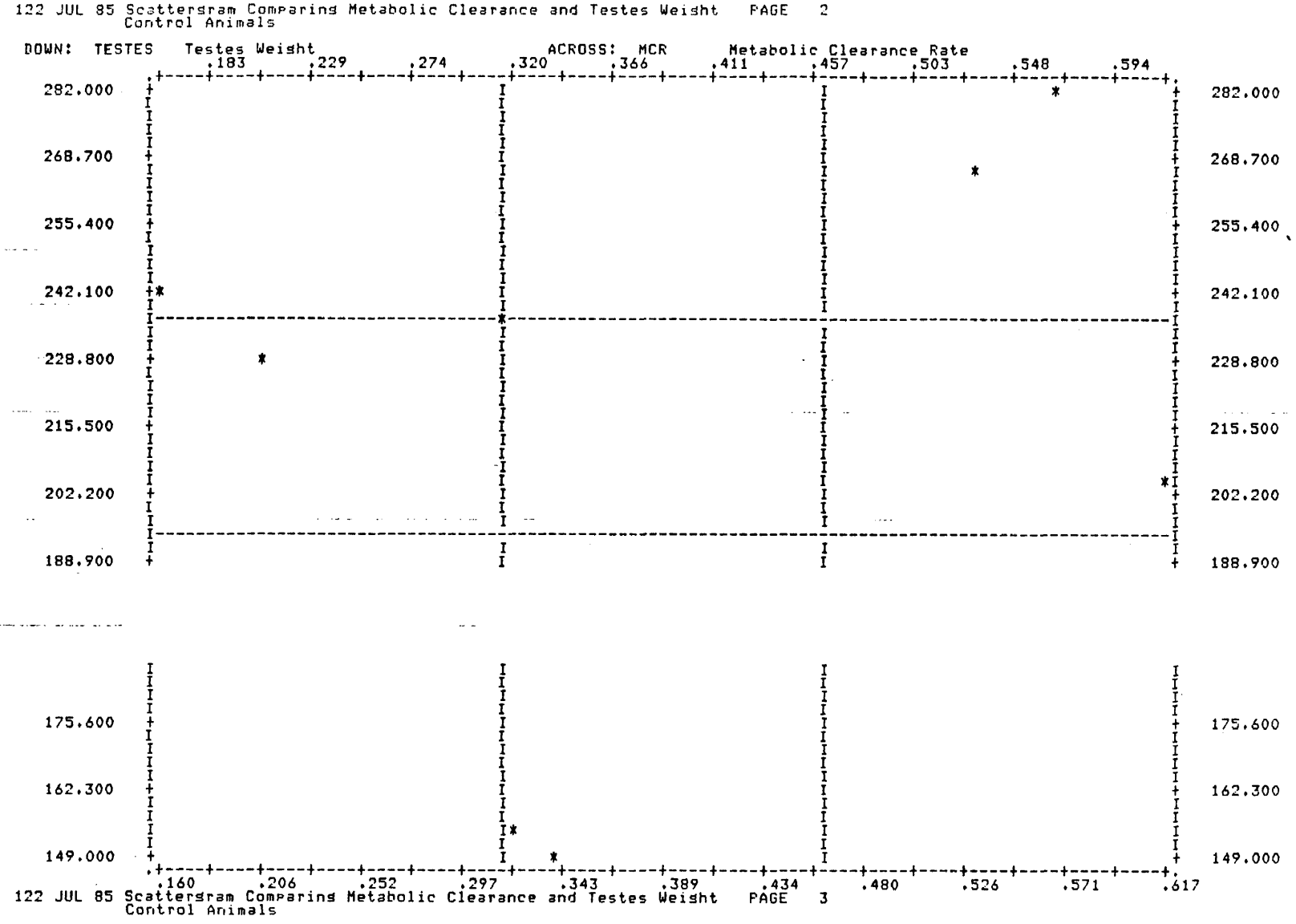
Appendix 3

Scattergram Comparing Metabolic Clearance and Testes Weight in All Animals



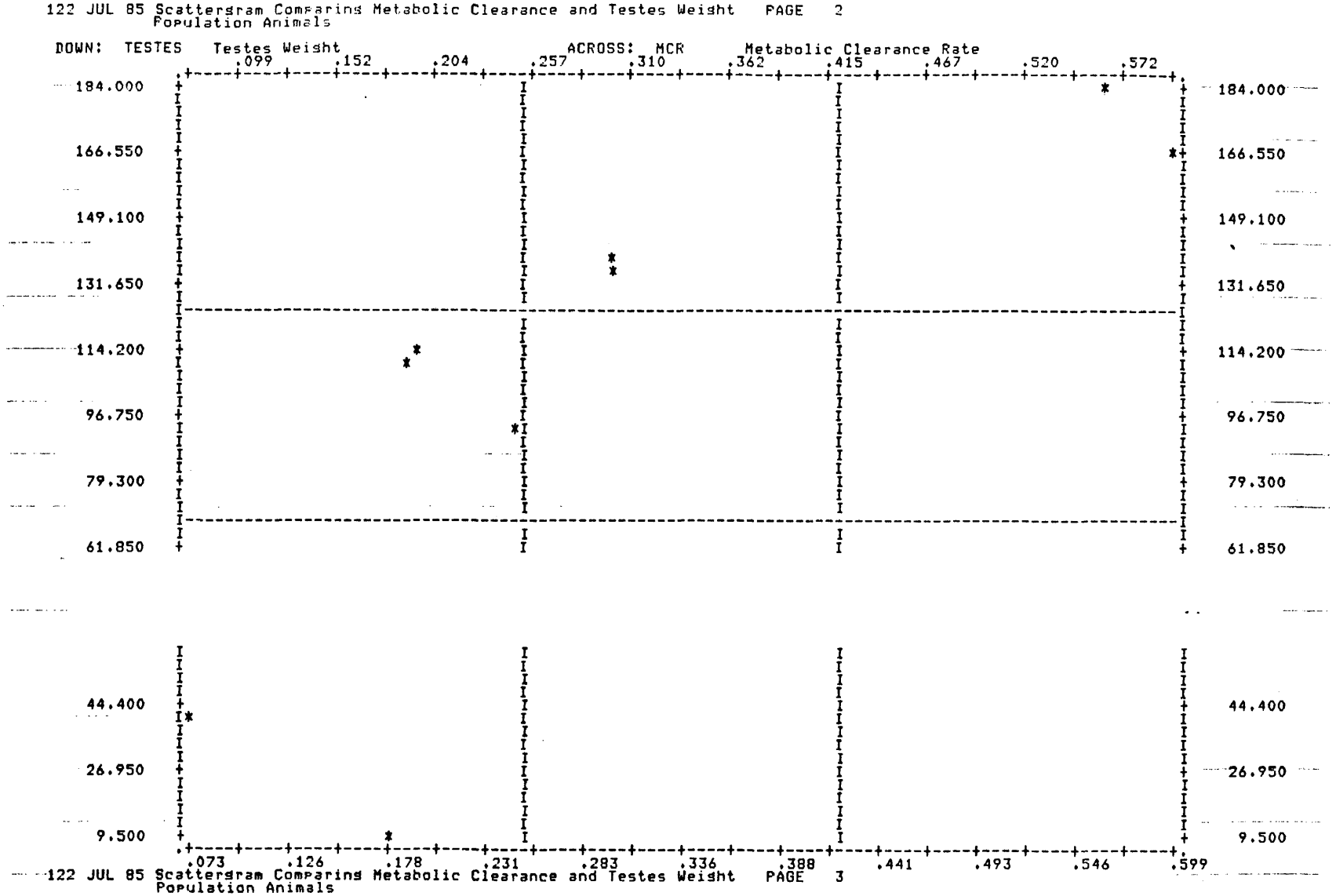
Appendix 4

Scattergram Comparing Metabolic Clearance and Testes Weight in Control Animals



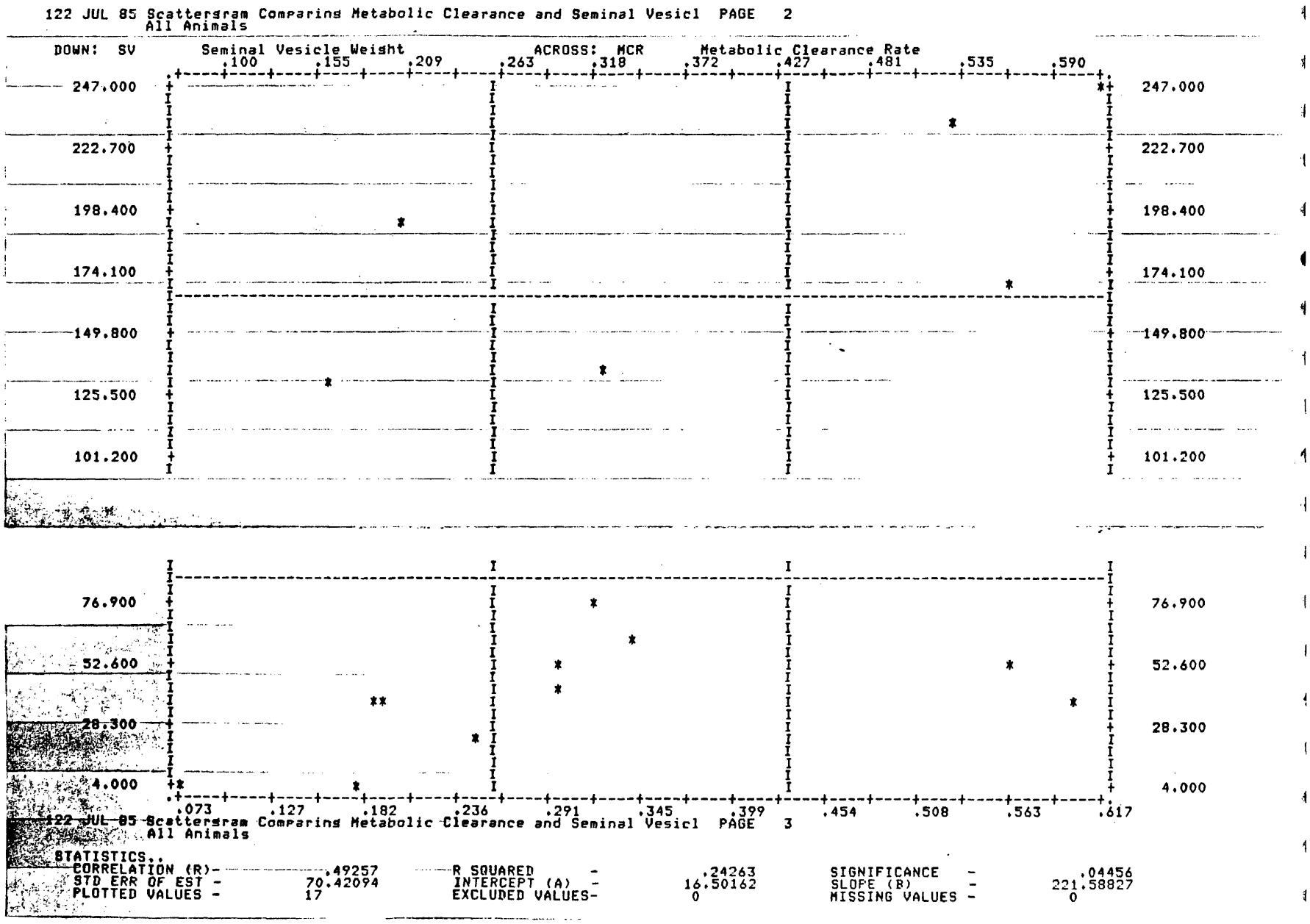
Appendix 5

Scattergram Comparing Metabolic Clearance and Testes Weight in Population Animals



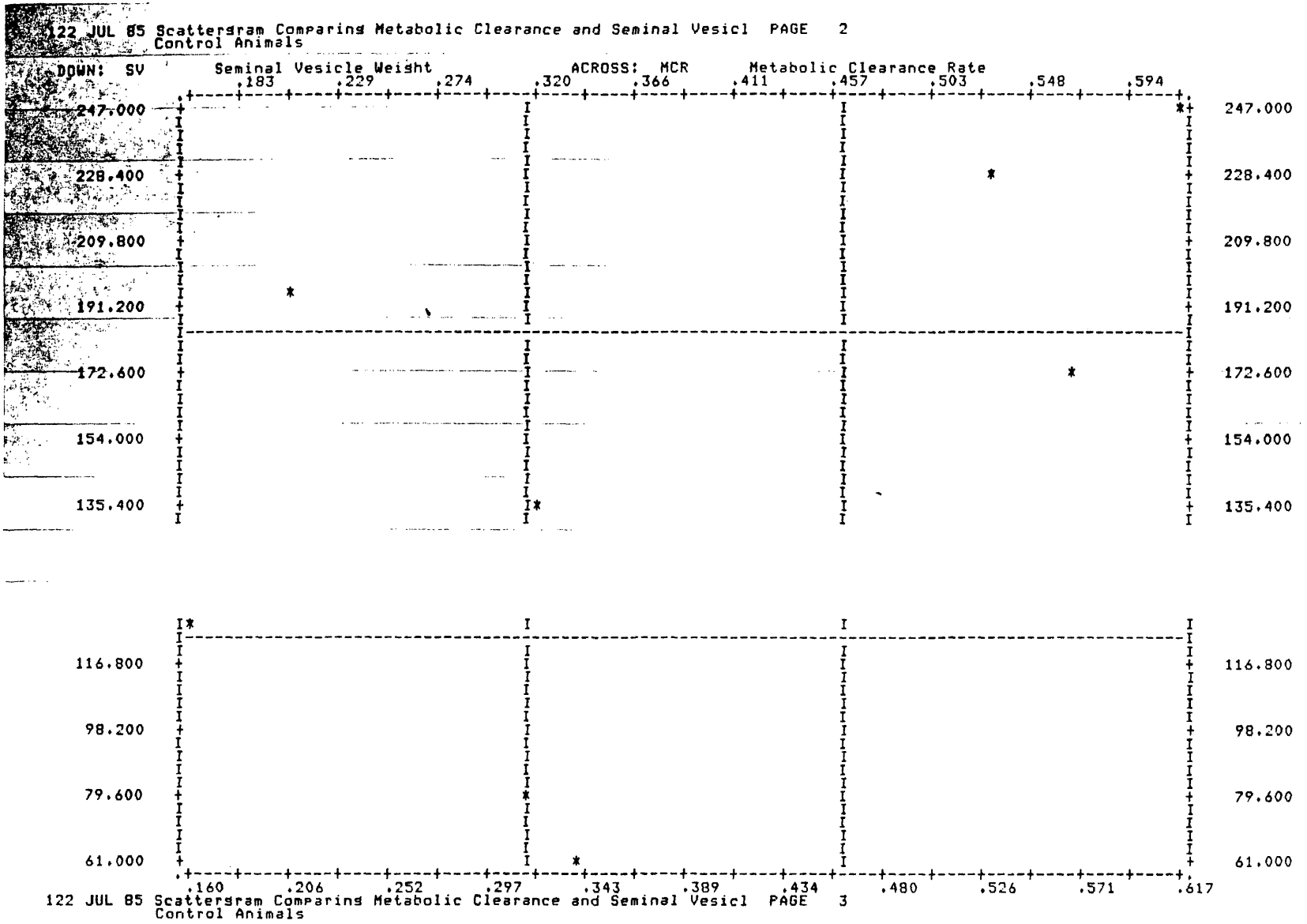
Appendix 6

Scattergram Comparing Metabolic Clearance and Seminal Vesicle Weight in All Animals



Appendix 7

Scattergram Comparing Metabolic Clearance and Seminal Vesicle Weight in Control Animals

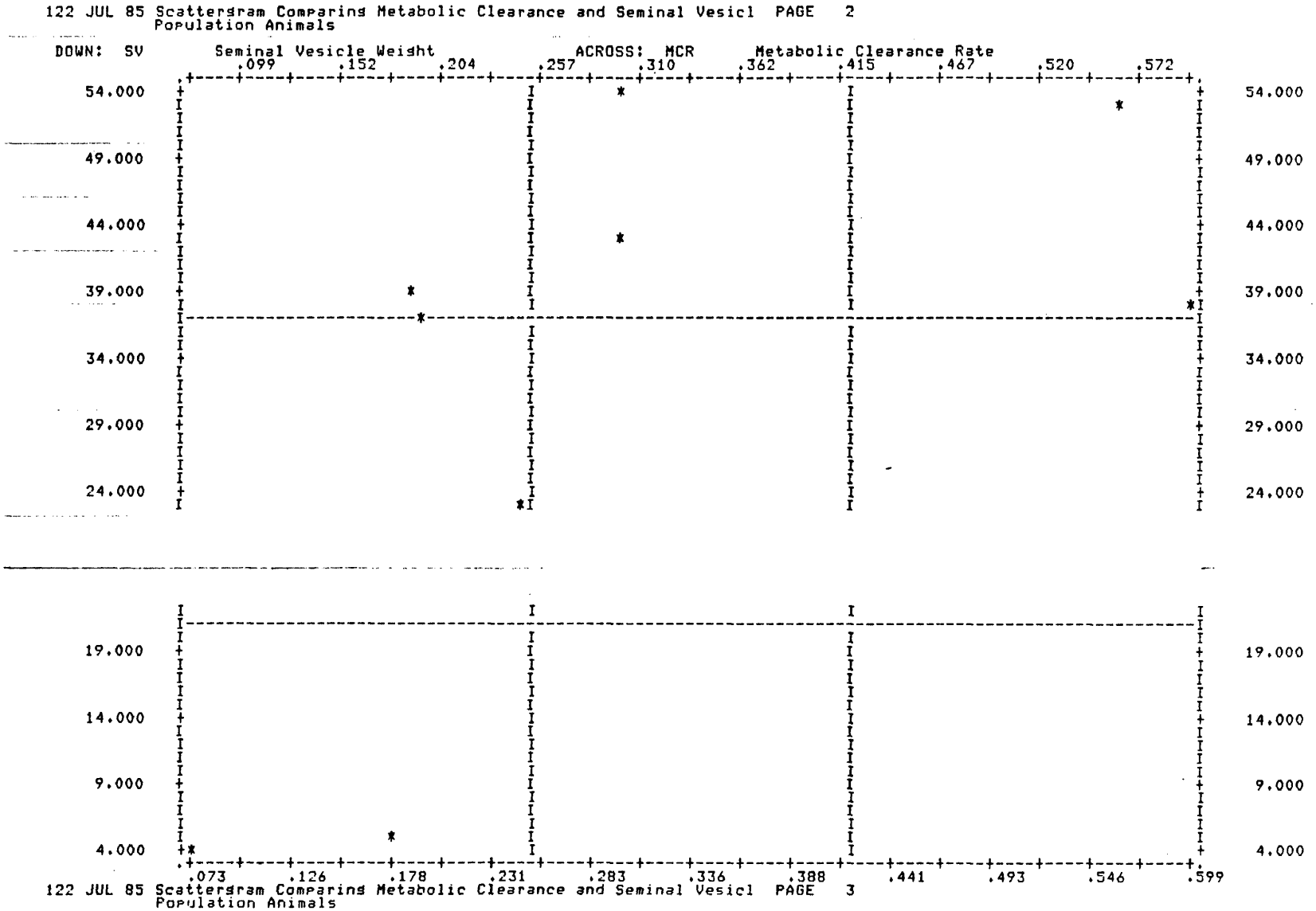


STATISTICS..

CORRELATION (R)-	.56207	R SQUARED -	.31592	SIGNIFICANCE -	.14705
STD ERR OF EST -	60.03059	INTERCEPT (A) -	70.62453	SLOPE (B) -	222.58324
PLOTTED VALUES -	8	EXCLUDED VALUES-	0	MISSING VALUES -	0

Appendix 8

Scattergram Comparing Metabolic Clearance and Seminal Vesicle Weight in Population Animals



STATISTICS..

CORRELATION (R) -	.61485	R SQUARED -	.37804	SIGNIFICANCE -	.07805
STD ERR OF EST -	15.61116	INTERCEPT (A) -	14.03732	SLOPE (B) -	64.42999
PLOTTED VALUES -	9	EXCLUDED VALUES-	0	MISSING VALUES -	0

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