SOME PHYSIOLOGICAL CHARACTERISTICS OF WILD, CAGED-STRESSED, AND SHOCK-COMATOSE GRAY SQUIRRELS

by

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Thesis submitted to the Graduate Faculty of the

Virginia Polytechnic Institute

in candidacy for the degree of

MASTER OF SCIENCE

in

Wildlife Biology

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July, 1966

Blacksburg, Virginia

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ACKNOWLEDGEMENTS

I sincerely wish to thank the following members of my graduate committee: Dr. H. S. Mosby, Dr. Alan G. Heath, and Dr. J. C. Osborne. Their continual encouragement and useful advice were an invaluable contribution to my study.

Special thanks is extended to Dr. Alan G. Heath for his helpful discussions and various laboratory items which he made available for this project and to Dr. Roy L. Kirkpatrick for his timely criticisms and suggestions.

I would also like to extend my genuine appreciation to the other staff members and graduate students for their guidance and aid with the numerous problems encountered during this study. They are: Mr. George W. Cornwell, who provided sources of literature, as well as, helpful recommendations; Professor Thomas C. Evans, Dr. Clyde Y. Kramer and Mr. Anthony Springall, for their assistance with the statistical analysis of these data; Mr. T. J. Harshbarger for his auspicious comments; Dr. Robert M. Smibert for his helpful direction in the glycogen analysis technique; and Mrs. Amelia Walton for her histological work on the comatose specimens.

Special gratitude is extended to the Virginia Commission of Game and Inland Fisheries, as well as, to the Virginia Agricultural Experiment Station for supporting my research; to the Department of Veterinary Science for permitting use of their hematological equipment;

to the Department of Biology for making various laboratory equipment available; and to the Virginia Cooperative Wildlife Unit for the use of vehicles and field equipment.

I value the interest shown and encouragement given to me by Dr. John F. Hosner and the staff members of the Department of Forestry and Wildlife.

To Mrs. Trudy Weatherholtz I wish to acknowledge my hearty appreciation for typing this thesis.

Special thanks go to my wife for providing financial assistance and possessing a great deal of faith in me and my chosen vocation.

I offer my deepest appreciation to all the others, who helped me complete this study, by offering timely suggestions and invaluable encouragement.

INTRODUCTION

Because of the gray squirrel's (Sciurus carolinensis) remarkable adaptability to changes in habitat, it has become one of the most sought after small game species in Virginia. Yet for all this popularity there has been little advancement in methods of efficient management and utilization of this valuable wildlife crop. Thus, with less available habitat and ever increasing hunting pressure, future management programs may be concerned with development of higher squirrel densities. However, before management can be effective, knowledge of the species' physiology is necessary.

The gray squirrel has been shown to be susceptible to confinement stress by developing shock during live-trapping operations at this university (Guthrie, 1965). Those confined-stressed individuals that go into the comatose state exhibit typical symptoms of hypoglycemic shock. Recently a number of workers have shown that the rate of non-hunting mortality, due to intraspecific stress, may increase with higher population densities in certain species (Christian and Davis, 1964). It is possible that the squirrel's susceptibility to confinement shock is an indication of severe intraspecific stress within the population. This factor, if proven, would definitely affect future squirrel management practices.

As a sequel to a previous study on fear stress in the gray squirrel, this project was concerned with the establishment of some of the normal and abnormal physiological characteristics of the squirrel not previously determined and to add additional data in areas where results were variable. Consequently, under future management programs researchers will have a number of standard values which will permit more meaningful investigations on the influence of intraspecific stress.

LITERATURE REVIEW

Until recently very little was known about the normal physiology of the gray squirrel (Sciurus carolinensis). However, Guthrie (1965) measured a number of physiological characteristics in his work on fear-stress, including packed-cell-volume, adrenal weights, blood glucose levels, hemoglobin concentration, and plasma corticosterone. He documented a number of changes from normal under conditions of confinement stress. However, further evaluation of the normal characteristics and relative changes in these values under stress is necessary to ascertain the effects of various population densities on the physiology of the gray squirrel.

The suspicion that various social phenomena are involved in controlling densities has prompted research on the mechanisms that could regulate population growth in a density-dependent manner. However, before any physiological changes due to stress can be discussed, some knowledge of normal physiology must be available. Differences in blood values have been found to be related to species, age, body size, and various other factors. Sealander (1964) found lower hematocrit values and hemoglobin concentrations during the summer in cotton rats (Sigmodon hispidus), harvest mice (Reithrodontomys fulvescens), and deer mice (Peromyscus leucopus, P. maniculatus, and P. boylii) and thought these changes might be correlated with either an increase in body weight or variations in the ambient temperature of the environment. The packed-cell-volumes of 31% for raccoons (Procyon lotor) and 39% for skunks (*Mephitis mephitis*) are very similar as was pointed out by Youatt *et al*. (1961). Guthrie (1965) found the average blood glucose level in the gray squirrel to be 110.5 mg/100 ml while the mean liver glycogen concentration was 15.20 mg per gram. The adrenal glands in the muskrat (*Ondatra zibethicus*) were found to reach a minor peak weight in April and a major peak in October by Beer and Meyer (1951). Thus it is imperative that a number of influencing factors be kept in mind when studying either normal or abnormal physiology.

Early investigators attempted to explain the cause of drastic population declines characteristic of certain species, as a result of intraspecific stress. However, recent trends have been toward seeking to understand and to describe the various interactions, inherent to these cyclic fluctuations rather than finding a major cause. Green and Larson (1938) were among the first investigators to surmise that certain factors intrinsic to a population were involved in its regulation. They noted apparent shock symptoms occurring in snowshoe hare (*Lepus americanus*) populations, and suggested that this was responsible for the periodic declines in population numbers. This syndrome was characterized by hypoglycemic convulsions, liver degeneration, and eventually death. The conclusions of Green et al. were later disputed by Chitty (1959).

Selye's (1946) treatise on stress illustrates various changes in the normal physiological characteristics due to systemic stressors in the environment. Selye (1950) mentions "any systemic stress (viz. one affecting large portions of the body), elicits an essentially similar syndrome with general manifestations." This syndrome, known as the "General-Adaptation-Syndrome," aids animals in adapting to various conditions, thus it is necessary for the perpetuation of life. It includes an Alarm Reaction, Stage of Resistance, and a Stage of Exhaustion. The Alarm Reaction consists of a shock phase in which a condition of suddenly developing intense systemic stress occurs; and a counter shock phase which is characterized by defense phenomena against shock such as adrenal cortex enlargement, hyperglycemia, etc. The Stage of Resistance represents a reaction or adaptation to a particular stressor with a subsequent decrease in resistance to other stimuli. When adaptation to a particular stimulus can no longer be maintained, there is a final systemic breakdown which is termed the Stage of Exhaustion. Since his original work on stress, a great deal more has been investigated and published by Selye (1951), Selye and Horava (1952, 1953), and Selye and Heuser (1954, 1956).

Compared to the effects of other systemic stressors, Selye (1950) mentions that the influence of nervous stimuli upon the gastric mucosa is very prominent, and is possibly regulated through the vagus nerve. Fear stress may induce hemoconcentration and hypotension which are probably the results of an initial adrenalin discharge, adrenergic vasoconstriction and, consequently, an increase in capillary permeability in the tissues which are deprived of blood. Turner (1960) mentions that the adaptive mechanisms which are called into operation during exposure to various stressors may cause disease through maladaptation.

Intra-specific stress is known to affect the normal physiology of various animals, and there is evidence which indicates it may be a density limiting factor in squirrel populations as well. It was suggested by Christian (1950) that adrenopituitary exhaustion caused by the intrinsic stress of overpopulation, adverse climatic conditions, and demands of spring breeding were the basic causes of population declines. Recurrent declines in numbers of voles (Microtus agrestis) were described by Chitty (1957) and were characterized by gross splenic hypertrophy which occurred in dense breeding populations. Christian and Ratcliffe (1952) described epidemics of fatal seizures following the development of hypoglycemia in certain species of free wild animals when populations increased to peak levels. They postulated that shock disease corresponded to the exhaustion phase in Selye's (1950) syndrome of general adaptation. Frank (1957) theorized that it took several stress-producing elements to finally cause a population "crash." This took place when all intraspecific stress stimuli reached a critical point simultaneously, or when a new stress was superimposed on existing stimuli. This would agree with Guthrie's (1965) findings, that gray squirrel were extremely susceptible to the increased stress of trap confinement and, thus, exhibited symptoms of hypoglycemic shock.

Initially it was thought that the relative weight of the adrenal gland indicated various changes in adrenocortical activities. However, Christian and Davis (1964) noted that adrenal weights could only be considered strong presumptive evidence of changes in adrenocortical function until validation was obtained by direct functional study. Christian (1953) found that adrenal atrophy frequently occurred in captive, closely confined, mammals. It was noted by Marsh and Rasmussen (1960) that when Swiss mice were confined to shuttle-boxes for approximately 28 days, there was a subsequent gain in adrenal weight. Chitty (1961) found that although adrenal weights in male and female voles (Microtus agrestis) fluctuated from year to year, no consistent relationship with population trend was found, except that females from expanding populations had the heaviest adrenals. She also mentions that seasonal variations were apparent in both breeding and sexually immature animals. Thus, weight gain probably was not due solely to gonadal activity. Nichols (1950) described an initial hypertrophy of the adrenal glands in Norway rats (Rattus norvegicus) which were captured and held in cages. This he attributed to the elaboration of ACTH hormone from the pituitary causing an increase in adrenal cortex activity. Christian (1956) found male mice, of two freely growing populations, had 25% greater adrenal weights than isolated controls. Female weights were approximately 7% greater. The increase in adrenal weight was associated with hyperplasia and hypertrophy of the cortical zona fasciculata and delayed involutions of the x-zone.

The adrenal glands of wild and domesticated Norway rats (Rattus norvegicus) were found to be quite different. The adrenal cortex of the wild rat was larger and contained more lipid, aldehyde, ketonic carbonyl groups, and had a richer blood supply, according to Turner (1960). He also found that when the wild animals were tamed there was a decrease in adrenal weight. Christian (1955b) found that the rate and amount of adrenal weight change in response to density was greater in wild than albino mice. Chitty and Clarke (1963) thought that the occurrence of larger adrenals in field voles (Microtus agrestis), than in laboratory specimens was due to hostile interactions between members of actively breeding populations.

A number of studies suggest that reproductive function is depressed with increasing density of natural populations. At present it appears that failures in reproductive function due to intraspecific stress may account for population declines more frequently than shock in some species (Christian, 1960). Experimental results have indicated that a small stimulus which brings on an increased adaptive response with inhibition of reproduction in mature animals by affecting lactation, increasing litter mortality, and possibly depressing fertility. A greater amount of stimulus will increase intra-uterine mortality and may even totally suppress reproductive functions. Christian and Le Munyan (1958) found that female mice which were moderately stressed by population density produced young which were permanently affected by decreased lactation, while those mice more severly stressed failed to reproduce and a 100% resorption of embryos was evident.

Hoffman (1958) found that a decline in male fertility, as well as a decrease in the incidence of pregnancy was directly related to population density in *Microtus montanus*. There was a significant negative correlation between embryo counts and population density of the red-backed mouse (*Clethrionomys gapperi*) reported by Patric (1962). Christian and Davis (1964) found that increased population density either delayed or totally inhibited maturation in female and male mice, so in some populations no females reached normal sexual maturity. These investigators think that the responses to intraspecific stress cause an inhibition of gonadotrophin secretion, resulting in a decrease in birthrate or increase in infant mortality or both, until all increase in population through the production of young may cease.

Many workers have presented evidence that intraspecific stress plays an important role in population limitation, either through direct mortality or through blockage of the normal reproductive cycle. The idea is further strengthened when we realize that social interaction or intraspecific competition is the only known element common to all populations. Christian and Davis (1964) feel that this type of population regulation is manifested by the existence of an endocrine feedback mechanism in response to increases in the overall social pressure, which in turn is the result of increased numbers and aggressive behavior. Thus, it is imperative that we continue studies of these complex behavioral interactions so that future wildlife management programs may be adapted to provide wildlife population densities best adapted to our long-range recreational needs.

TECHNIQUES AND PROCEDURES

Experiments were designed to compare the normal measurements with similar data obtained from squirrels subjected to confinement stress for long periods as well as with any confined-shocked individuals that became available during the investigation. Therefore, data were obtained from three different groups: normal, confined-stressed, and confined-shocked animals. Normal squirrels were collected by shooting with a .22 caliber rifle or shotgun. The confined-stressed animals were captured in wooden box traps, sexed, aged, and transferred to holding cages where they were held with adequate food and water for a period of four to six months. Confined-shocked animals were brought to the laboratory in carrying cages and examined upon arrival. All experimental animals were taken from selected areas within a 5 mile radius of Blacksburg. A total of 11 normal, 21 confined-stressed, and 14 confined-shocked animals were sampled within a period of 10 months from September 15, 1965 to June 30, 1966.

Due to the variability of sample size and sample values, it was decided to use a Least Squares program available at the Virginia Polytechnic Institute, computing center, to facilitate analysis of these data. An F-Test was computed to determine whether any significant differences were evident among treatments, age groups, and sexes; and a t-Test was then used to test selected contrasts for significance.

Field Collections

All normal squirrels were processed in the field as rapidly as possible, due to the lability of many physiological measurements immediately after death. Promptly after shooting, an incision was made from the base of the tail to the lower jaw exposing both the peritoneal and thoracic cavity. Two to 5 ml of cardiac blood were collected from the right atrium in a heparinized 10 ml syringe fitted with a 20-gauge needle. A clinical thermometer was placed in the rectum for approximately 3 minutes for normal temperature determination. Two heparinized microhematocrit tubes were filled with whole blood and plugged at both ends to prevent evaporation until they could be centrifuged in the laboratory. A $ZnSO_4$ and $Ba(OH)_2$ solution was added to approximately 0.2 ml of heparinized blood to precipitate the protein. The plasma supernatant was then refrigerated until a blood glucose analysis could be made. One ml of heparinized blood was added to 10 ml of trichloroacetic acid to precipitate the protein and the supernatant was then refrigerated until blood lactic acid levels could be determined. A portion of the liver and the left adrenal gland was removed, wrapped in separate pieces of aluminum foil and immediately frozen in a solution of dry ice and acetone for determination of glycogen and adrenal corticosterone levels. The right adrenal gland was removed and fixed in 10% formalin and later weighed to the nearest 0.1 mg in the laboratory. The carcass was examined to determine the condition of the animal, then taken to the laboratory and weighed to obtain an estimate of the live weight, since some of the organs were removed and weighed separately.

Laboratory Collections

The confined-stressed squirrels were forced from the holding pen into a wire funnel and killed by a blow on the head. The liver and left adrenal gland were removed, wrapped in aluminum foil and placed in a freezer (-10°F) for analysis at a later date. All other specimens were collected and measured in exactly the same manner as the wild ones.

During the study any animals which exhibited symptoms of confinement shock were brought to the laboratory, stunned with a blow on the head, and subjected to the same analysis as that used on the confined-stressed animals. An electrocardiogram was recorded on one of the confined-shocked squirrels and analyzed.

Blood Cytology

An aliquot of heparinized blood was taken for packed-cell-volume measurement.

Hematocrit

Two heparinized microhematocrit tubes were used to measure the packed-cell-volumes (PCV) of each animal. Both tubes were centrifuged for 5 minutes in an International Clinical Centrifuge with a special microhematocrit attachment. PCV values were determined from measurements taken from the centrifuged microhematocrit tubes.

Blood Chemistry

The heparinized cardiac blood was transferred from the syringe to two 15 ml centrifuge tubes. In one tube the protein was precipitated from the heparinized blood by a $ZnSO_4$ and $Ba(OH)_2$ procedure described in detail in the instructions issued with the enzymatic Glucostat reagents used in the plasma glucose determination. The solution was centrifuged and the supernatant refrigerated at -10° C until the analysis for glucose could be conducted. One milliliter of blood in the second tube was mixed with 10 ml of trichloroacetic acid to precipitate the protein and centrifuged. The supernatant was treated in the same manner as before until the blood lactic acid concentration could be determined.

Plasma Glucose

Plasma glucose was determined using the colorimetric "Glucostat" method. The reagents and instructions for this method were supplied by the Worthington Biochemical Corporation of Freehold, New Jersey. Glucostat utilizes a coupled enzyme system for the specific, quantitative determination of beta-D-glucose in 0.2 ml of plasma. The optical densities for all samples and standards were determined using a Bausch and Lomb "Spectronic 20" spectrophotometer.

Plasma Lactic Acid

Plasma lactic acid was determined by the method of Barker and Summerson (1941), using 1 ml of protein-free blood filtrate. The glucose and other interfering materials of the filtrate were removed by the Van Slyke-Salkowski method of treatment with copper sulfate and calcium hydroxide. An aliquot of the resulting solution was heated with concentrated reagent-grade sulfuric acid to convert lactic acid to acetaldehyde, which is then determined colorimetrically by reaction with p-hydroxydiphenyl in the presence of copper ions. The optical densities of the samples and standards were then determined using a Bausch and Lomb "Spectronic 20" spectrophotometer.

Liver Glycogen

The concentration of liver glycogen was determined colorimetrically by the method of Montgomery (1957), using the phenol-sulfuric acid procedure. One gram of tissue was weighed to the nearest 0.1 mg on an Ainsworth "Right-a-Weigh" balance and digested in 2 ml of a hot, 30% solution of potassium hydroxide. The glycogen was precipitated with 1.2 volumes of 95% alcohol and centrifuged. The supernatant was poured off and the precipitate was diluted to a known volume. Color was developed in a phenol-sulfuric acid mixture. The optical densities were determined using a Bausch and Lomb "Spectronic 20" spectrophotometer.

Adrenal Weight

The right adrenal gland was fixed in 10% formalin and later weighed to the nearest 0.1 mg on an Ainsworth "Right-a-Weigh" balance.

Rectal Temperature

Rectal temperature was taken immediately after death using a clinical thermometer.

Electrocardiogram

An electrocardiogram was recorded from one confined-shocked animal by a Grass Model 5D Polygraph, using three leads as follows: right foreleg and left foreleg (lead I), right foreleg and left hind leg (lead II), and left foreleg and left hind leg (lead III).

Histology

Tissues from several vital organs were taken from the confined-shocked group, soon after the animal died or after sacrifice, and were placed in 10% buffered neutral formalin. Tissue sections were prepared and examined at the veterinary pathology laboratory.

RESULTS

Tables 1 and 2 summarize the average biological data for adult gray squirrels according to treatment groups. The averages for immature squirrels are shown in Tables 3 and 4. Original data for this study may be found in Appendix Tables I and II. Figs. 1-11 represent graphically the means, standard deviations, and ranges for the physiological characters investigated in this study. The mean is represented by a horizontal line. The standard deviation about the mean is indicated by a rectangle, while the range is represented by the length of the vertical line.

All data were tested in a least squares program at the Virginia Polytechnic Institute computing center for any significant difference due to treatment, sex, or age.

Adrenal Weight

Statistical analysis indicated that there were differences in adrenal gland weights at the 99% level of confidence between the confined-shocked squirrels and the confined-stressed and normal groups. However, no significant difference was evident between normal and confined-stressed animals. No significant differences due to either sex or age or an interaction among sex, age, and treatment type were found.

All adrenal weights are expressed in milligrams per 100 grams of body weight. The average relative adrenal weights were 12.62 mg/100 g

Table 1. Average biological values for adult male gray squirrels collected near Blacksburg during the period from September 15, 1965 to June 30, 1966

	Treatments								
	Normal			Confined-stressed			Confined-shocked		
	no.	avg	SE	no.	avg	SE	no.	avg	SE
Body weight (grams)	3	503.20	--	4	524.40+	--	3	404.50	--
Relative adrenal weight (mg/100g)	3	12.22	0.86	4	11.37	1.14	3	20.36	1.32
Hematocrit (percent)	3	41.00	1.68	4	49.30	0.69	3	45.90	4.26
Plasma glucose (mg/100ml)	3	115.63	17.55	4	81.55	18.72	3	6.42	0.42
Plasma lactic acid (mg/100ml)	3	54.83	2.71	4	77.55	6.55	3	25.36	11.23
Liver glycogen (mg/g)	3	26.35	7.90	-	--*	--	-	--*	--
Rectal temperature (F)	3	101.50	1.04	4	99.50	1.90	3	80.50	1.32

+ Applies to weight at end of 4-6 months holding period.
* Specimens of liver lost due to breakdown of deep freeze in which they were stored.

Table 2. Average biological values for adult female gray squirrels collected near Blacksburg during the period from September 15, 1965 to June 30, 1966

	Treatments								
	Normal			Confined-stressed			Confined-shocked		
	no.	avg	SE	no.	avg	SE	no.	avg	SE
Body weight (grams)	3	497.30	--	7	497.10+	--	4	426.10	--
Relative adrenal weight (mg/100g)	3	12.54	0.87	7	10.90	0.71	4	18.30	2.86
Hematocrit (percent)	3	35.90	1.02	7	49.94	1.40	4	49.83	6.51
Plasma glucose (mg/100ml)	3	95.75	12.28	7	106.26	18.66	4	12.38	2.29
Plasma lactic acid (mg/100ml)	3	57.33	11.15	7	100.74	9.23	4	8.53	3.50
Liver glycogen (mg/g)	3	15.31	4.76	-	--*	--	-	--*	--
Rectal temperature (F)	3	98.80	0.73	7	101.40	0.71	4	77.30	2.84

+ Applies to weight at end of 4-6 months holding period.
* Specimens of liver lost due to breakdown of deep freeze in which they were stored.

Table 3. Average biological values for immature male gray squirrels collected near Blacksburg during the period from September 15, 1965 to June 30, 1966

	Treatments								
	Normal			Confined-stressed			Confined-shocked		
	no.	avg	SE	no.	avg	SE	no.	avg	SE
Body weight (grams)	3	267.50	--	4	481.40+	--	2	335.40	--
Relative adrenal weight (mg/100g)	3	11.86	0.51	4	10.38	0.74	2	15.47	4.72
Hematocrit (percent)	3	36.19	3.28	4	45.83	2.93	2	47.00	14.04
Plasma glucose (mg/100ml)	3	76.87	4.71	4	124.13	23.18	2	12.81	11.19
Plasma lactic acid (mg/100ml)	3	73.49	14.40	4	111.97	11.10	2	9.54	5.11
Liver glycogen (mg/g)	3	4.31	1.42	-	--*	--	-	--*	--
Rectal temperature (F)	3	99.70	0.19	4	101.00	0.41	2	77.80	3.76

+ Applies to weight at end of 4-6 months holding period.
* Specimens of liver lost due to breakdown of deep freeze in which they were stored.

Table 4. Average biological values for immature female gray squirrels collected near Blacksburg during the period from September 15, 1965 to June 30, 1966

	Treatments								
	Normal			Confined-stressed			Confined-shocked		
	no.	avg	SE	no.	avg.	SE	no.	avg	SE
Body weight (grams)	2	315.50	--	6	451.12+	--	5	301.90	--
Relative adrenal weight (mg/100g)	2	13.48	1.00	6	12.28	1.39	5	16.10	1.38
Hematocrit (percent)	2	32.90	6.72	6	51.95	2.11	5	45.44	1.83
Plasma glucose (mg/100ml)	2	140.65	9.06	6	86.39	14.16	5	19.59	9.68
Plasma lactic acid (mg/100ml)	2	77.95	35.60	6	94.49	8.92	5	10.36	2.12
Liver glycogen (mg/g)	2	2.96	1.57	-	--*	--	-	--*	--
Rectal temperature (F)	2	99.50	0.50	6	101.70	0.60	5	80.60	2.26

+ Applies to weight at end of 4-6 months holding period.
* Specimens of liver lost due to breakdown of deep freeze in which they were stored.

for the normal group, 11.23 mg/100 g for confined-stressed animals, and 17.56 mg/100 g for the confined-shocked squirrels. Adult male squirrels from the confined treatment groups had heavier adrenals than the females. However, the immature female adrenal weights were greater than the males for all treatment groups. The confined-stressed animals had lower average adrenal weights than the normal animals, while the confined-shocked group had much higher average adrenal weights than either the normal or confined-stressed groups. These data are illustrated graphically in Figs. 1 and 2 and the original data are presented in Appendix Table I.

<u>Hematocrit</u>

The group average values for erythrocyte packed-cell-volume (PCV) in adult squirrels were: normal males, 41%; normal females, 35.9%; confined-stressed males, 49.3%; confined-stressed females, 49.94%; confined-shocked males, 45.9%; and confined-shocked females, 49.83%.

The average PCV's for immature gray squirrels were as follows: normal males, 36.19%; normal females, 32.9%; confined-stressed males, 45.83%; confined-stressed females, 51.95%; confined-shocked males, 47%; and confined-shocked females, 45.44%.

The confined-stressed group had higher average values than the confined-shocked or normal groups. The confined-shocked animals had significantly higher values than the normal group at the 99% level of confidence. The statistical analysis demonstrated significant differences at the 90% level of confidence, between confined-stressed

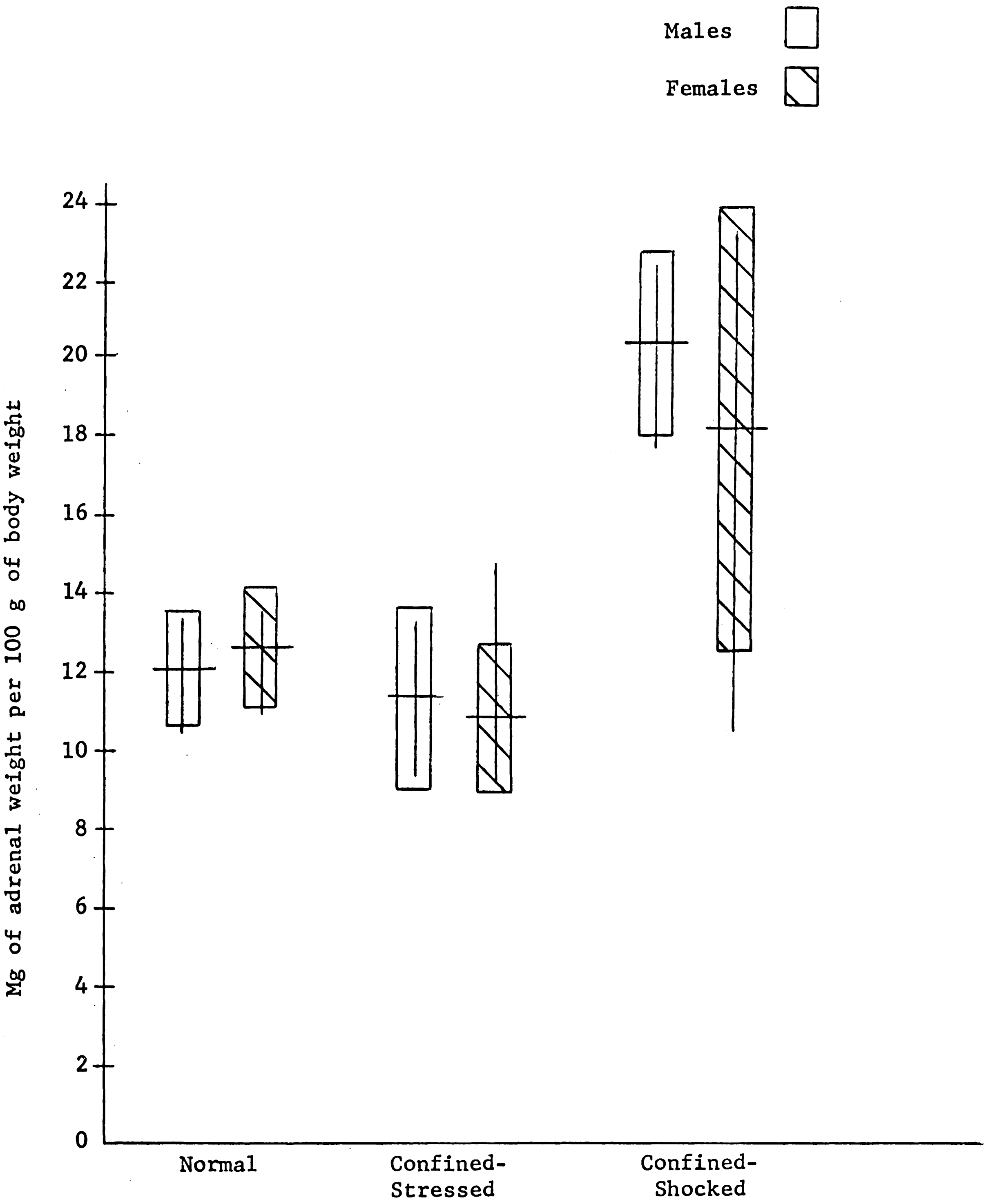

Fig. 1. Relative weight of adult right adrenal after fixation. Horizontal line = mean; rectangle = standard deviation; vertical line = range.

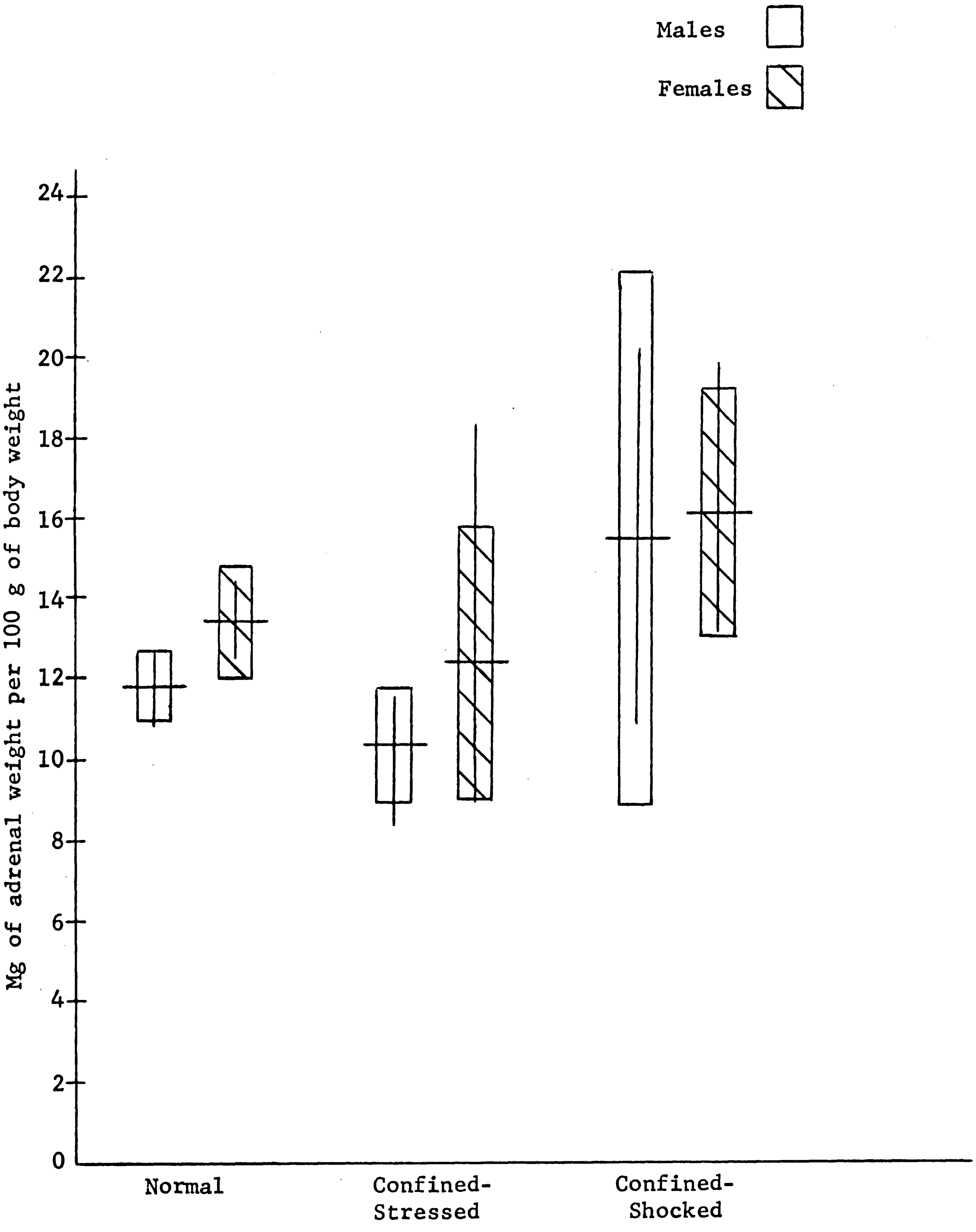

Fig. 2. Relative weight of immature right adrenal after fixation. Horizontal line = mean; rectangle = standard deviation; vertical line = range.

and confined-shocked groups. The normal males had higher average values than the normal females, however, this was not evident in the other two groups. There were no significant differences found due to sex or age in this study. These differences are illustrated graphically by Figs. 3 and 4. See Appendix Table I for original data.

Plasma Glucose

There was a highly significant difference at the 99% level of confidence in plasma glucose between confined-shocked, and the confined-stressed and normal groups. The average values for adult squirrels were: normal males, 115.63 mg/100 ml; normal females, 95.75 mg/100 ml; confined-stressed males, 81.55 mg/100 ml; confined-stressed females, 106.26 mg/100 ml; confined-shocked males, 6.42 mg/100 ml; and confined-shocked females, 12.38 mg/100 ml.

The immature gray squirrels had average plasma glucose values as follows: normal males, 76.87 mg/100 ml; normal females 140.65 mg/100 ml; confined-stressed males, 124.13 mg/100 ml; confined-stressed females, 86.39 mg/100 ml; confined-shocked males, 12.81 mg/100 ml; and confined-shocked females, 19.59 mg/100 ml.

The confined-shocked specimens exhibited acute hypoglycemia. The confined-stressed animals had slightly lower average values than the normal squirrels. There were no significant differences in the average values due to sex or age. However, a significant difference at the 95% level of confidence was evident due to the interaction among the treatments, sexes, and ages. See Figs. 5 and 6 and Appendix Table II.

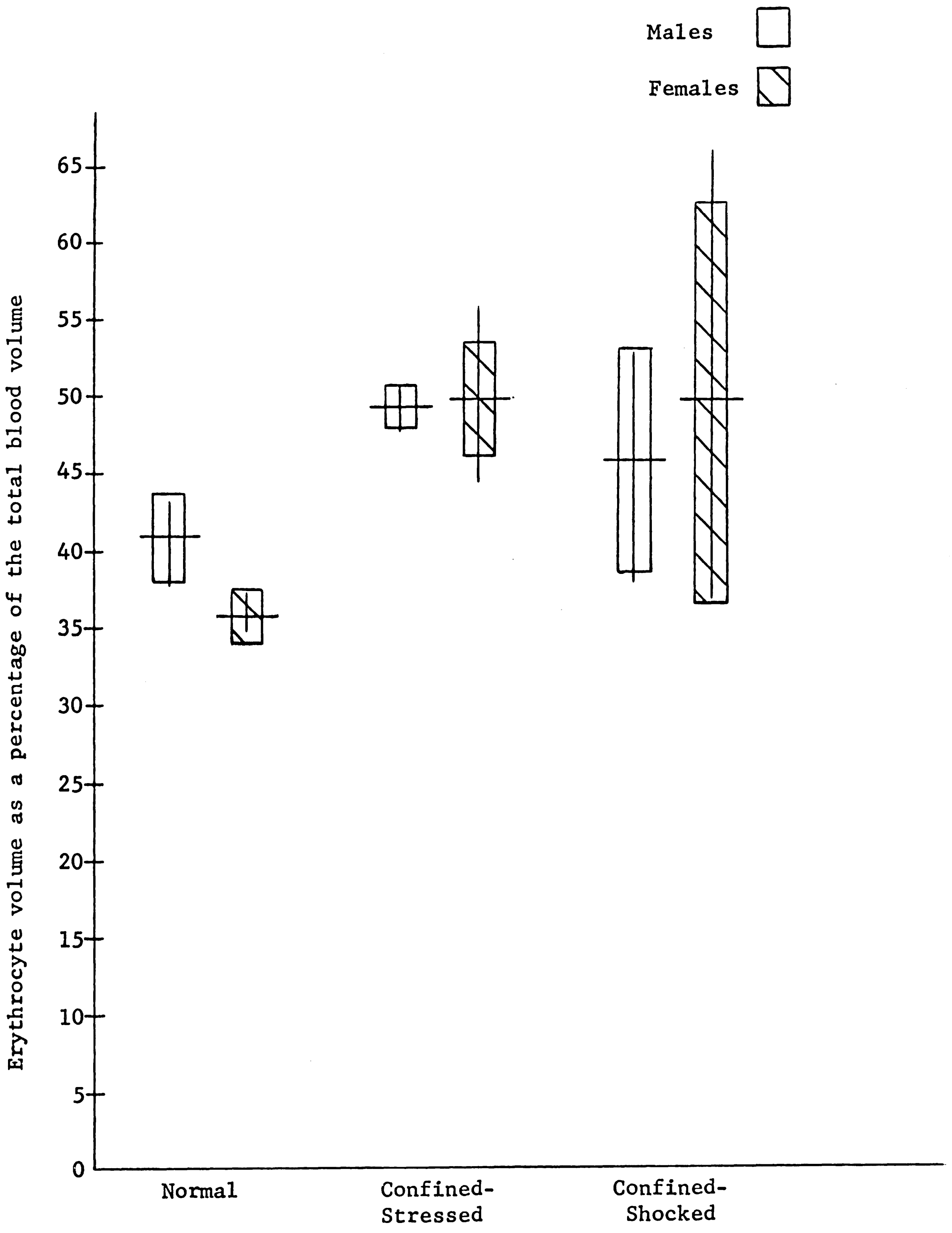

Fig. 3. Adult packed erythrocyte volume. Horizontal line = mean; rectangle = standard deviation; vertical line = range.

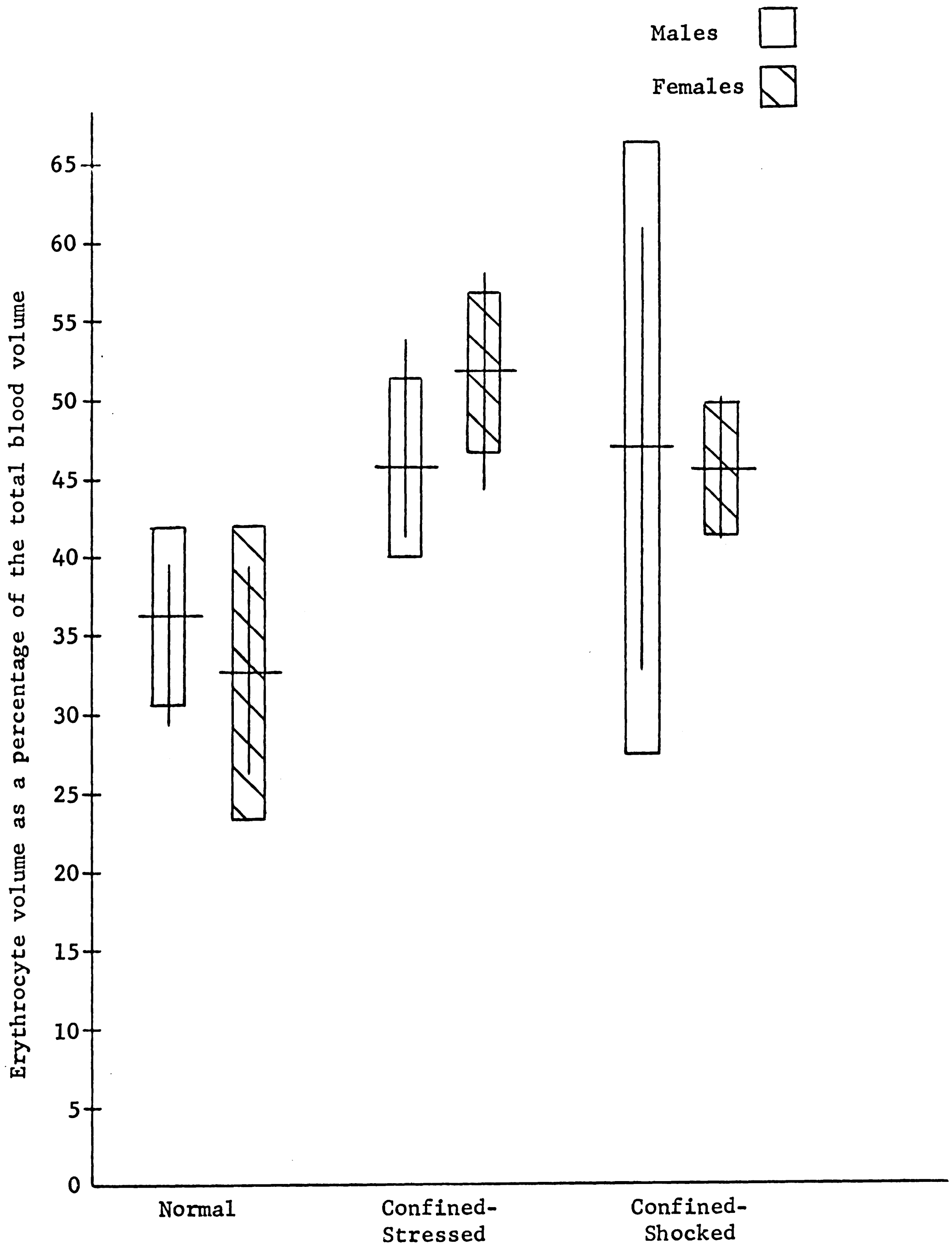

Fig. 4. Immature packed erythrocyte volume. Horizontal line = mean; rectangle = standard deviation; vertical line = range.

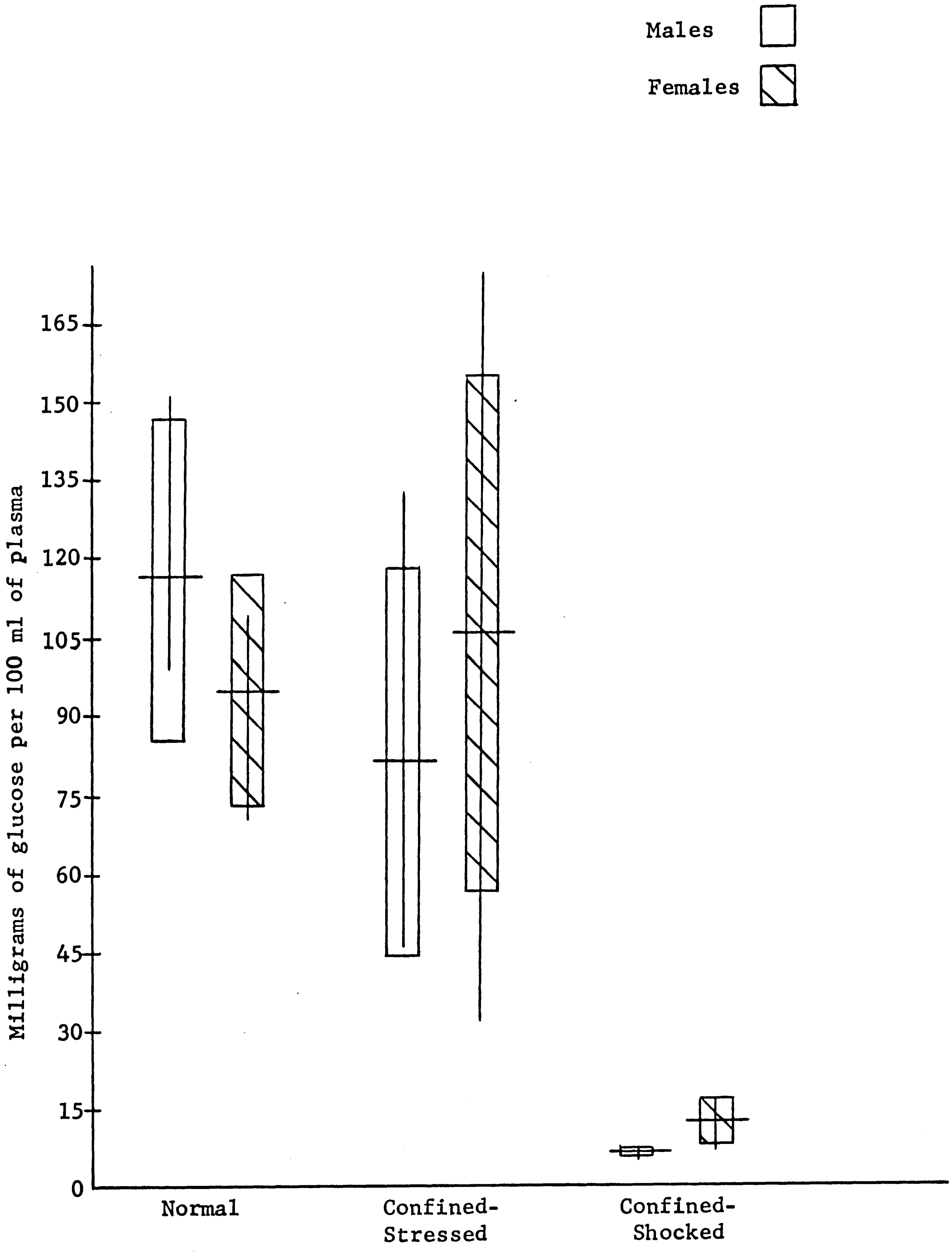

Fig. 5. Adult plasma glucose concentration. Horizontal line = mean; rectangle = standard deviation; vertical line = range.

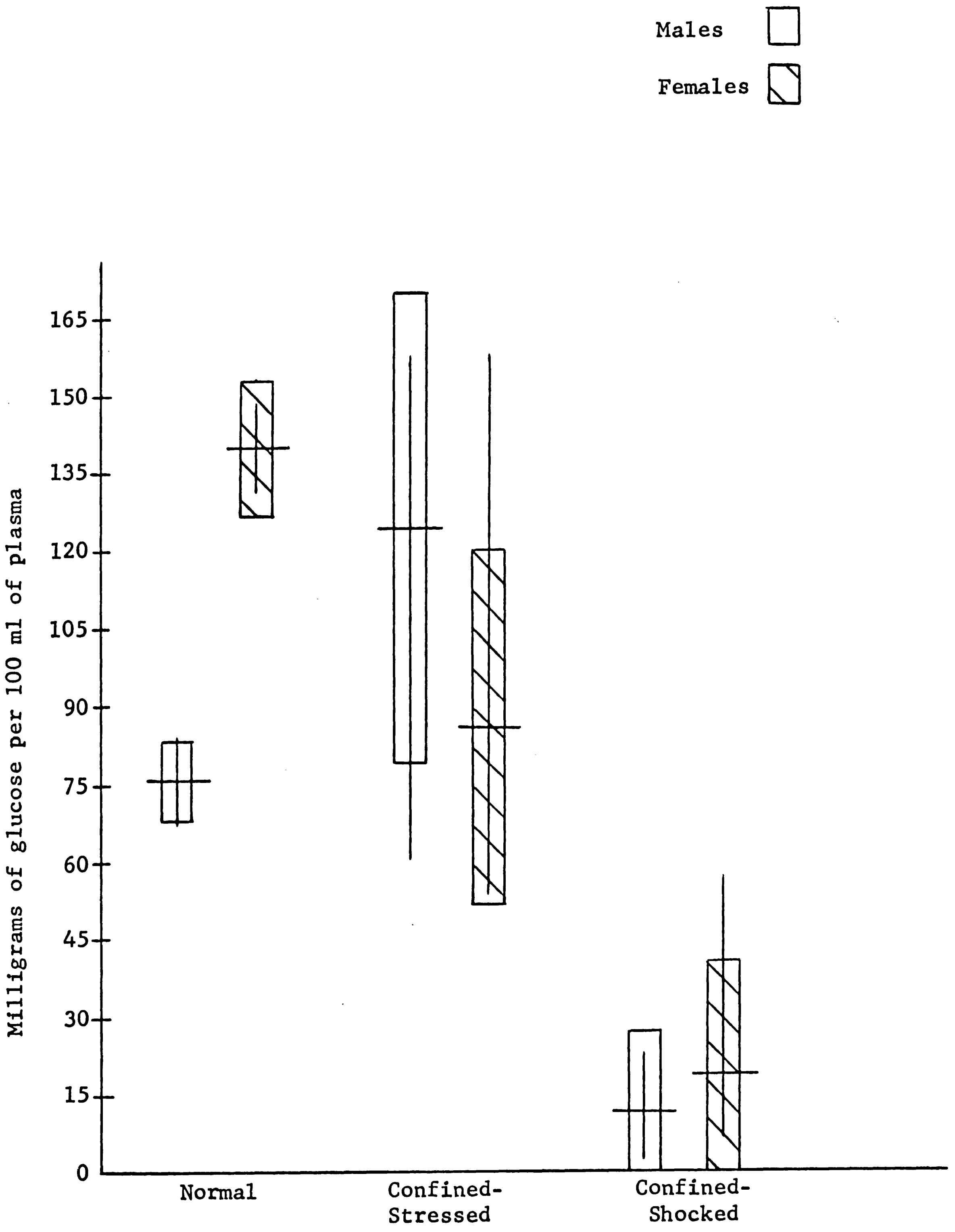

Fig. 6. Immature plasma glucose concentration. Horizontal line = mean; rectangle = standard deviation; vertical line = range.

Plasma Lactic Acid

The average lactic acid concentration was 56.08 mg/100 ml for the normal adults, 89.15 mg/100 ml for the confined-stressed adults, and 16.95 mg/100 ml for the confined-shocked adults.

Average values for the immature squirrels were: normal, 75.72 mg/100 ml; confined-stressed, 103.23 mg/100 ml; and confined-shocked, 9.95 mg/100 ml.

The confined-shocked group exhibited a hypolacticemic condition when compared to the normal and confined-stressed squirrels. The differences due to treatment were highly significant at the 99% confidence level. There were no significant differences evident in either sex or age. See Figs. 7 and 8 and Appendix Table II.

Liver Glycogen

Liver glycogen values were available for normal squirrels only. Due to an accidental disconnection of the deep freeze, the confined-stressed and confined-shocked liver specimens thawed and the subsequent glycogen analysis proved to be too variable to be of value.

The average values for normal gray squirrels were: adult male, 26.35 mg/g; adult female, 15.31 mg/g; immature male, 4.31 mg/g; and immature female 2.96 mg/g.

The adult squirrels were found to have significantly higher liver glycogen concentrations than the immatures at the 95% level of confidence when tested statistically.

Fig. 9 illustrates this age difference graphically and the original data may be found in Appendix Table II.

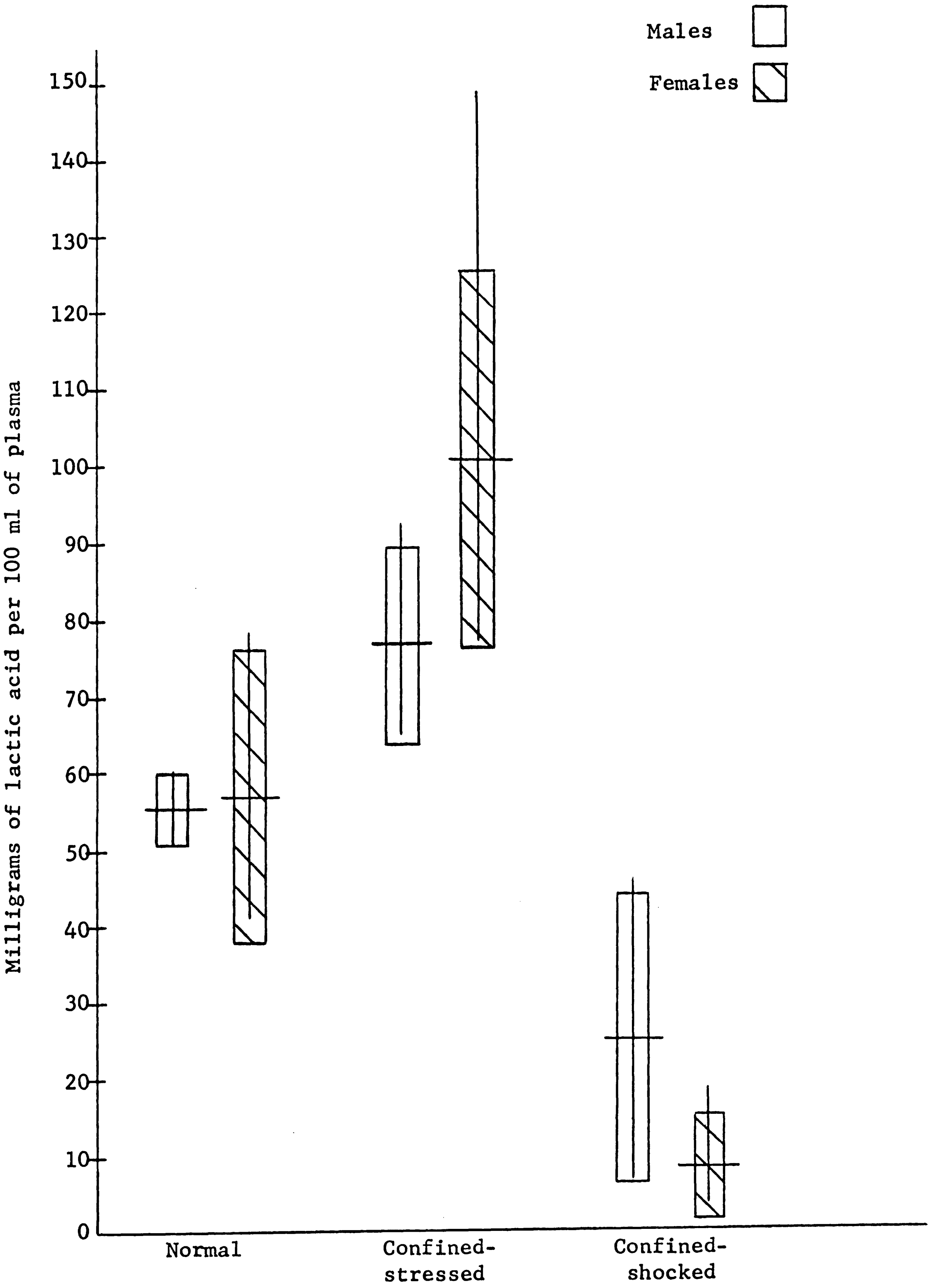

Fig. 7. Adult plasma lactic acid concentration. Horizontal line = mean; rectangle = standard deviation; vertical line = range.

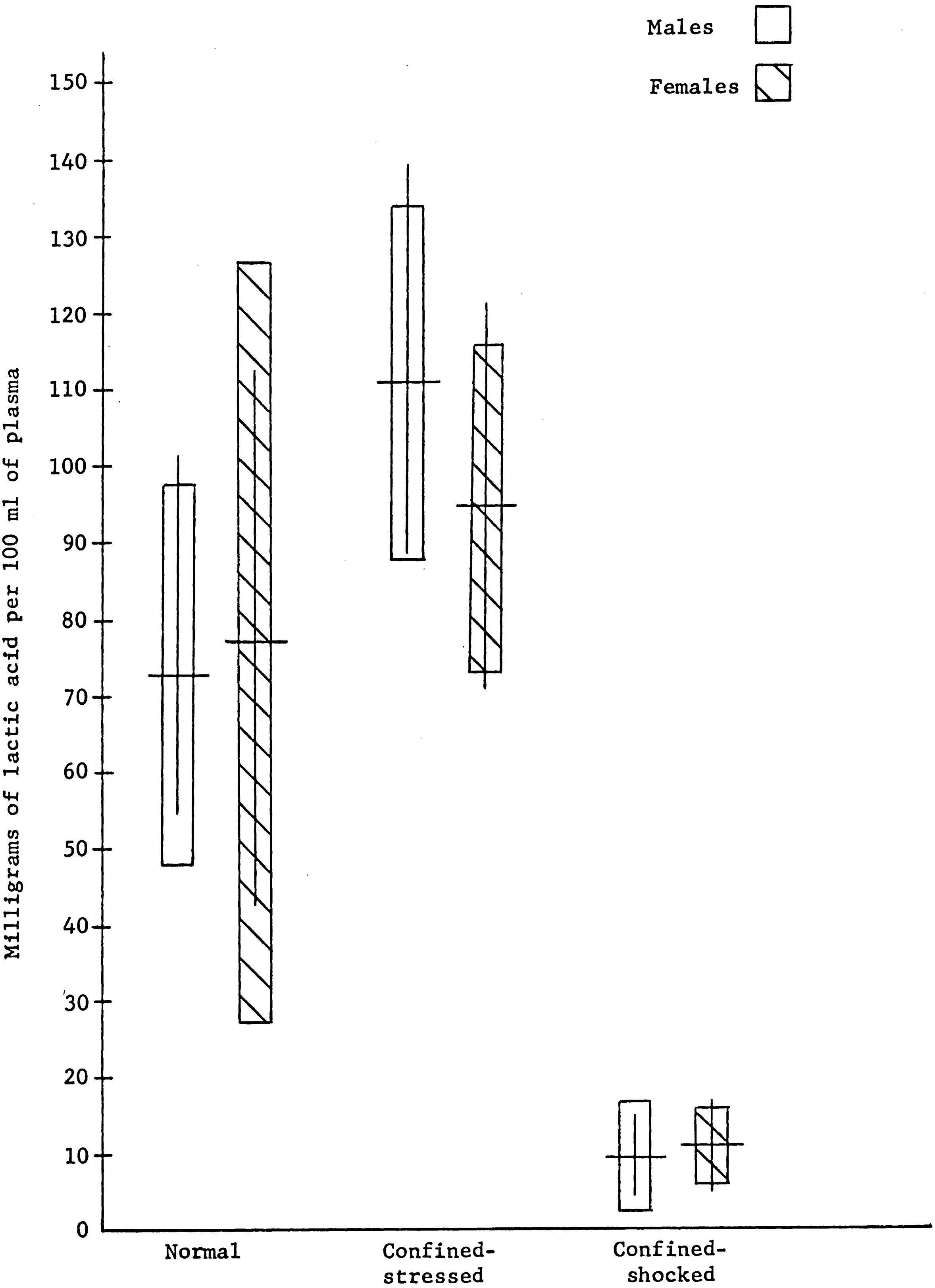

Fig. 8. Immature plasma lactic acid concentration. Horizontal line = mean; rectangle = standard deviation; vertical line = range.

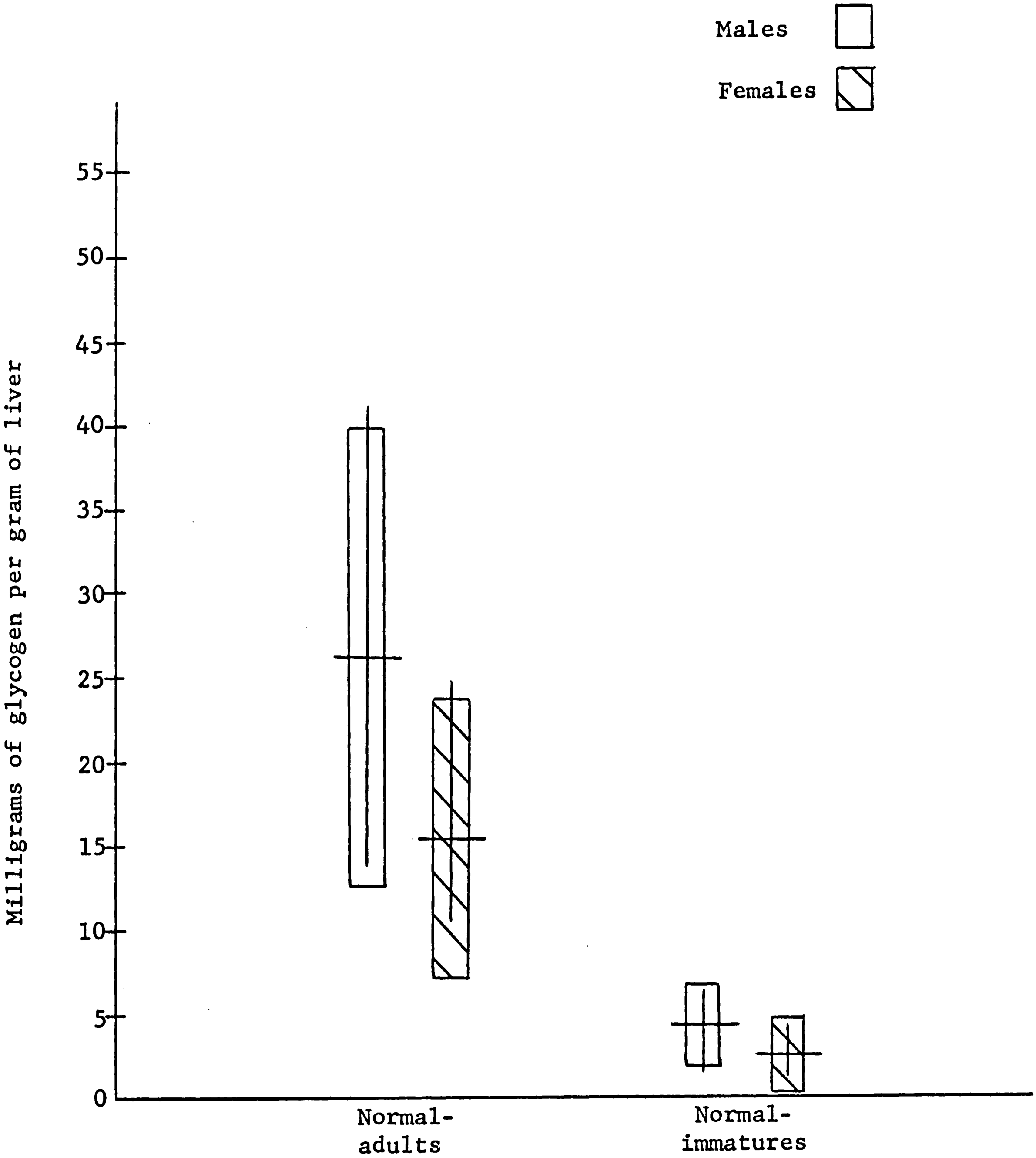

Fig. 9. Liver glycogen concentration. Horizontal line = mean; rectangle = standard deviation; vertical line = range.

Rectal Temperature

The average rectal temperatures were: 99.4° F for the normal group, 100.9° F for the confined-stressed group, and 79.1° F for the confined-shocked group.

When tested statistically it was found that the confined-shocked animals had significantly lower temperatures at the 99% level of confidence. However, there was no significant difference found due to sex or age. See Figs. 10 and 11 and Appendix Table I.

Electrocardiogram

An EKG was recorded on one confined-shocked squirrel. Its heart rate at the beginning of the test varied from 2 to 3 beats per second, or from 120 to 180 beats per minute. This became more erratic as the squirrel's condition deteriorated. After approximately 15-20 minutes and a number of convulsive periods the heart developed an arrhythmic condition and ventricular systole was eliminated completely from every other beat.

Histology

Intestinal coccidia and a few helminths were encountered in histological sections of the intestines. A parasite tentatively identified as _Capallaria_ sp. was noted in a branch of the pulmonary artery in one squirrel. A protozoan-like parasite was found in the parenchyma of the lung and in granulomatous reaction tissue around the kidney in one squirrel. Blood vascular shock-like lesions were present in the lungs and kidneys of 2 of 14 squirrels. Stomach ulcers and/or erosions in

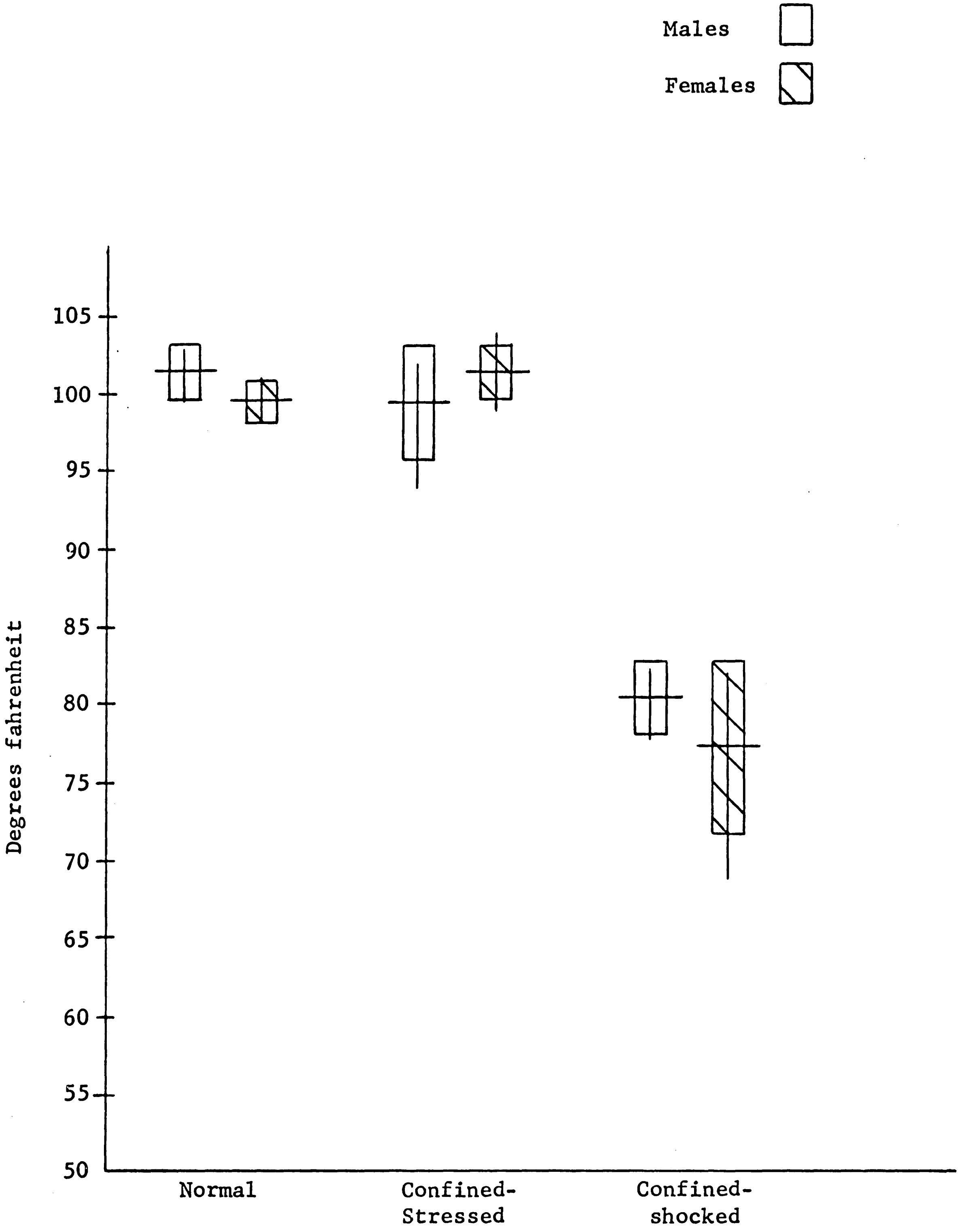

Fig. 10. Adult rectal temperature. Horizontal line = mean; rectangle = standard deviation; vertical line = range.

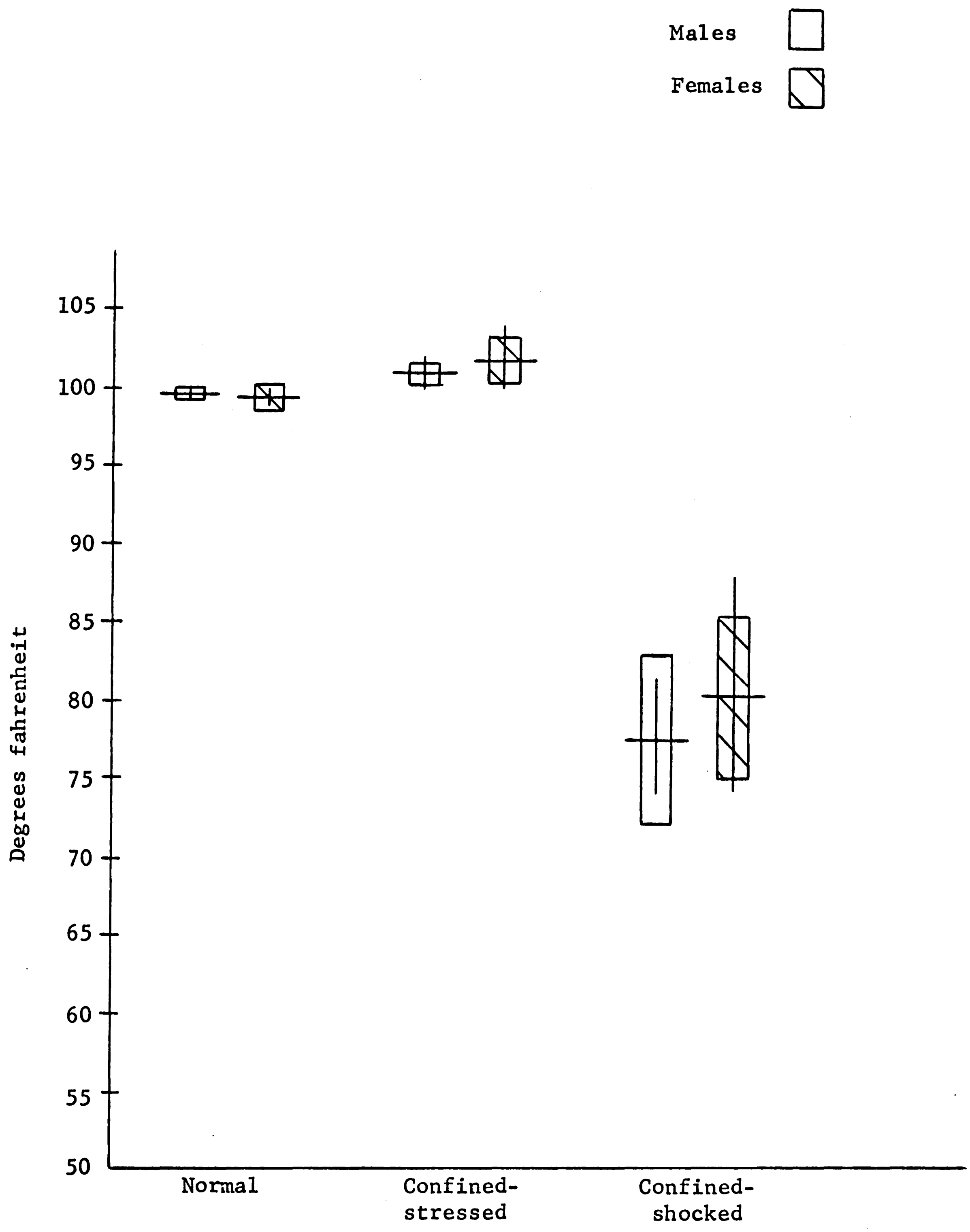

Fig. 11. Immature rectal temperature. Horizontal line = mean; rectangle = standard deviation; vertical line = range.

the pyloric region were noted in 2 of 14 squirrels. A few squirrels had minimal subcutaneous inflammatory reaction on the forehead which was apparently related to bruising on the cage.

DISCUSSION

These experiments were undertaken to study the effects of continued close confinement on the normal physiological characteristics of the gray squirrel. We were especially interested in the shock condition that is related to confinement-stress in these animals.

Some of the problems associated with this type of study are prevalent in many wildlife investigations. Small and variable sample sizes probably introduced the greatest amount of error into this investigation. Other sources of error included: (1) inability to determine the exact age of the sample specimens; (2) all confined-stressed and confined-shocked specimens were collected in the fall, while normal animals were sampled in the spring; (3) the inability to determine the exact length of time the confined-shocked animals had been in a comatose condition when first observed in the trap; and (4) a small amount of error was probably introduced by the slightly different techniques used in sampling the various treatment specimens.

Both adult and immature gray squirrels were sampled. Although there were no significant differences evident due to either sex or age in this study, slight dissimilarities did occur.

Adrenal Weight

The average value for the normal specimens was 12.62 mg/100 g, while the confined-stressed group had an average of 11.23 mg/100 g, and the confined-shocked animals averaged 17.56 mg/100 g.

Normal values in this study were slightly lower than those described by Guthrie (1965). However, this may be due to the different seasons in which these specimens were collected. Beer (1951) and Chitty (1961), both found relatively low adrenal weights in the spring increasing to a maximum weight during midsummer and fall in muskrats (*Ondatra zibethicus*) and voles (*Microtus agrestis*).

The confined-shocked animals had significantly larger adrenal weights than either the confined-stressed or normal squirrels. These weights from the comatose squirrels were somewhat higher than those described by Guthrie (1965). Weight increase may be due to hyperplasia and hypertrophy after increased stimulation by ACTH and thus indicate an enhancement of glucocorticoid secretion. However, as Christian and Davis (1964) suggest, this increase may also be due to the accumulation of lipids with the cessation of ACTH stimulation.

Adrenal weights of confined-stressed specimens were lower than either the normal or confined-shocked weights, a condition also reported by Guthrie (1965). He suggested that this may be a result of lipid depletion from the cortex due to increased steroid production and supported this statement by demonstrating a concurrent increase in plasma corticosteroid.

Chitty and Clarke (1963) found that female voles tended to have larger adrenal glands than males. Rogers and Richter (1948) discovered that female Norway rats (*Rattus norvegicus*) had considerably heavier adrenals than the males. In this investigation the adult males exhibited slightly larger adrenal weights than the adult females; however, immature females were found to have heavier adrenals than immature males. None of these weight differences were statistically significant, however.

Hematocrit

The average adult packed-cell-volume was 41% for normal males and 35.9% for normal females. These results were quite similar to those obtained by Guthrie (1965). However, there were smaller sex-related differences in PCV values for immature individuals, with 36.19% for normal males, and 32.9% for normal females. Anderson and Gee (1958) found some sex differences in mature dogs. According to Kitts *et al.* yearling beaver (*Castor canadensis*) had an average packed-cell-volume of 40.3%, while the adult value was 42.1%. The confined-stressed group had average values of 49.6% for adults and 48.9% for immatures. The average values for the confined-shocked group were somewhat lower at 47.9% for adults and 46.2% for the immature animals. Sealander (1964) mentions that hematocrit values in immatures are often significantly lower than in adults in some small mammals. Immature squirrels in this study seemed to have somewhat lower packed-cell-volumes; however, these differences were not significant. Gough and Kilgore (1964) and Sealander (1960; 1961) reported higher values for various species of *Peromyscus*, than were characteristic for normal squirrels in this study. Sealander (1964) mentions, however, that smaller species generally have higher blood values. The polycythemia which is evident in both confined-stressed and confined-shocked groups may be caused by hemoconcentration, or possibly by some other condition such as lowered blood oxygen tension. Whatever the cause, the higher the packed-cell-volume the more viscous the blood becomes, thus reducing blood flow in the peripheral vasculature. It is possible that this enhances the shock condition evident in some gray squirrels.

Plasma Glucose

The normal and confined-stressed groups had quite similar plasma glucose values. Normal adults averaged 95.7 mg/100 ml, while the confined-stressed adults had a mean of 93.9 mg/100 ml. These values were in agreement with the 98.8 mg/100 ml average reported by Stevenson et al. for the beaver (*Castor canadensis*). The average glucose values for immature squirrels were slightly higher than the adults. Normal immatures exhibited a mean value of 108.8 mg/100 ml, while the confined-stressed group was 105.3 mg/100 ml. Both adult and immature squirrels in the confined-shocked group exhibited extremely low plasma glucose levels. The adult average was lower with 9.40 mg/100 ml, while the immature mean was 16.2 mg/100 ml. The extreme hypoglycemia is undoubtedly the main cause of death in the comatose squirrels. Green *et al.* (1938) demonstrated extremely low blood sugar levels in the snowshoe hare (*Lepus americanus*), and described the typical convulsive symptoms of hypoglycemic shock. Christian and Ratcliffe (1952) also attributed a number of deaths in captive zoo animals to an acute hypoglycemic condition. Christian (1963), originally postulated that this was due to adrenocortical exhaustion; however, it is just as likely that the readily mobilizable sources of glycogen and glucose become exhausted with continued stimulation of the adrenal medulla and cortex.

Plasma Lactic Acid

Lactic acid levels varied greatly; however, they followed the same general pattern exhibited by plasma glucose concentrations. The confined-stressed animals had the highest mean values with 96.2 mg/100 ml, while the normal animals had a level of 65.9 mg/100 ml. Acute hypolacticemia characterized the confined-shocked animals, with a value of 13.45 mg/100 ml. Spector (1956) reported a blood lactic acid range from 5-21 mg/100 ml for the normal rat.

Liver Glycogen

Data on the average liver glycogen concentrations are available for the normal squirrels only. These values did compare favorably with the results obtained by Guthrie (1965) for his normal specimens. There was, however, a significant difference found between the immature and adult age groups. The average glycogen level in normal adults was 20.9 mg/g of liver tissue, while that of the immatures was 3.64 mg/g of liver tissue. Green and Larson (1938) described normal liver glycogen levels ranging from 1.23-11.96% in the snowshoe hare (*Lepus americanus*). They felt that the decrease in liver glycogen levels to a range of 0.02-0.18%, coupled with the decrease in plasma glucose levels, were the main causes of the shock disease manifested in their experimental animals.

Rectal Temperatures

The confined-shocked animals exhibited a significantly lower rectal temperature than the normal and confined-stressed animals. This helps to illustrate the serious breakdown in normal homeostatic mechanisms which takes place during the development of fatal shock. The average temperatures for the normal and confined-stressed group were almost the same (99.4^{o} and 100.9^{o} F), while the temperatures for the confined-shocked animals were about 20^{o} less than normal.

Histology

Squirrels trapped in this area were found to have light to moderate infections with coccidia and other internal parasites. These infections were probably contributory to the more severe shock observed in some animals. Bruising of the forehead on the cage with release of histamine from the damaged cells would likewise be an additive factor in shock. Whether the ulcers noted in 2 squirrels were directly related to the shock syndrome is not known.

SUMMARY

The confined-shocked animals had significantly ($p < 0.01$) higher adrenal weights than either the normal or confined-stressed squirrels.

The normal group had slightly higher adrenal weights than the confined-stressed group.

The normal adult squirrels had higher packed-cell-volumes than the normal immatures.

The packed-cell-volumes of the confined-stressed and confined-shocked squirrels were similar; however, the confined-stressed values were slightly higher.

Confined-shocked animals suffered from acute hypoglycemia, while the normal and confined-stressed values were approximately the same.

Lactic acid levels were severely depleted in the confined-shocked specimens. Confined-stressed animals had higher concentrations than normal squirrels.

There was a significant difference in normal squirrels' liver glycogen concentrations between age groups. The normal adults had a larger amount of liver glycogen per gram of liver tissue.

Rectal temperatures in the confined-shocked group were extremely low when compared to normal and confined-stressed groups.

Histological study of selected organs of the confined-shocked group revealed light to heavy helminth and coccidia infections.

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Appendix Table I. Body weight, adrenal weight, hematocrit, and rectal temperature of gray squirrels collected at Blacksburg, Virginia, during the period of September 15, 1966 to June 30, 1966. N = normal, C = confined-stressed, and S = confined-shocked animals

Animal	Sex	Age	Body wt. (grams)	Relative wt. of right adrenal (mg/100g body wt.)	PCV (per cent)	Rectal temp. (Fahrenheit)
N - 6	M	A	459.00	10.37	37.75	99.50
N - 8	M	A	556.50	12.83	41.95	102.00
N - 10	M	A	494.00	13.46	43.30	103.00
Average			503.16	12.22	41.00	101.50
Std. Dev.			--	1.48	2.90	1.80
N - 5	F	A	489.00	10.80	34.89	100.00
N - 7	F	A	486.50	13.26	37.35	98.50
N - 9	F	A	516.50	13.55	35.40	101.00
Average			497.33	12.54	35.88	99.83
Std. Dev.			--	1.51	1.77	1.26
N - 3	M	I	247.50	12.20	29.53	99.50
N - 4	M	I	232.50	10.80	39.74	100.00
N - 11	M	I	322.50	12.59	39.30	99.50
Average			267.50	11.86	36.19	99.67
Std. Dev.			--	0.88	5.68	0.32
N - 1	F	I	365.00	14.47	39.60	99.00
N - 2	F	I	266.00	12.48	26.19	100.00
Average			315.50	13.48	32.90	99.50
Std. Dev.			--	1.41	9.48	0.71

Appendix Table I. Body weight, adrenal weight, hematocrit, and rectal temperature of gray squirrels collected at Blacksburg, Virginia, during the period of September 15, 1966 to June 30, 1966. N = normal, C = confined-stressed, and S = confined-shocked animals (continued)

Animal	Sex	Age	Body wt. (grams)	Relative wt. of right adrenal (mg/100g body wt.)	PCV (per cent)	Rectal temp. (Fahrenheit)
C - 2	M	A	519.00	13.39	47.90	102.00
C - 4	M	A	505.00	9.23	50.30	102.00
C - 5	M	A	553.50	13.26	48.40	94.00
C - 10	M	A	520.00	9.58	50.70	100.00
Average			524.38	11.37	49.33	99.50
Std. Dev.			--	2.27	1.38	3.79
C - 1	F	A	502.50	10.61	46.50	103.00
C - 3	F	A	592.50	10.25	52.50	99.50
C - 6	F	A	459.00	9.19	44.70	99.00
C - 7	F	A	529.00	11.66	48.60	102.00
C - 8	F	A	461.00	9.57	51.30	102.00
C - 9	F	A	485.50	10.01	55.70	104.00
C - 11	F	A	450.00	14.69	50.30	100.00
Average			497.10	10.85	49.94	101.36
Std. Dev.			--	1.87	3.72	1.89

Appendix Table I. Body weight, adrenal weight, hematocrit, and rectal temperature of gray squirrels collected at Blacksburg, Virginia, during the period of September 15, 1966 to June 30, 1966. N = normal, C = confined-stressed, and S = confined-shocked animals (continued)

Animal	Sex	Age	Body wt. (grams)	Relative wt. of right adrenal (mg/100g body wt.)	PCV (per cent)	Rectal temp. (Fahrenheit)
C - 13	M	I	495.00	11.39	41.10	100.00
C - 14	M	I	502.50	8.39	53.80	102.00
C - 18	M	I	460.50	10.16	46.60	101.00
C - 21	M	I	467.70	11.59	41.80	101.00
Average			481.43	10.38	45.83	101.00
Std. Dev.			--	1.47	5.85	0.82
C - 12	F	I	523.50	12.44	51.30	102.00
C - 15	F	I	402.50	18.46	57.60	104.00
C - 16	F	I	511.50	8.97	50.50	102.00
C - 17	F	I	491.50	9.48	44.50	102.00
C - 19	F	I	410.00	11.27	58.10	100.00
C - 20	F	I	367.70	13.05	49.70	100.00
Average			451.12	12.28	51.95	101.70
Std. Dev.			--	3.40	5.16	1.48

Appendix Table I. Body weight, adrenal weight, hematocrit, and rectal temperature of gray squirrels collected at Blacksburg, Virginia, during the period of September 15, 1966 to June 30, 1966. N = normal, C = confined-stressed, and S = confined-shocked animals (continued)

Animal	Sex	Age	Body wt. (grams)	Relative wt. of right adrenal (mg/100g body wt.)	PCV (per cent)	Rectal temp. (Fahrenheit)
S - 4	M	A	392.00	17.70	46.50	78.00
S - 5	M	A	378.50	20.93	38.30	81.00
S - 6	M	A	443.00	22.44	53.00	82.50
Average			404.50	20.36	45.93	80.50
Std. Dev.			--	2.29	7.37	2.29
S - 9	F	A	461.50	21.54	37.00	79.00
S - 10	F	A	471.50	23.46	54.30	79.00
S - 14	F	A	429.00	17.60	42.00	82.00
S - 18	F	A	342.50	10.54	66.00	69.00
Average			426.10	18.29	49.83	77.30
Std. Dev.			--	5.71	13.01	5.68
S - 8	M	I	250.80	20.18	33.00	74.00
S - 17	M	I	420.00	10.76	61.00	81.50
Average			335.40	15.47	47.00	77.80
Std. Dev.			--	6.66	19.80	5.30
S - 11	F	I	288.00	18.65	41.30	79.00
S - 12	F	I	314.00	13.15	46.60	74.00
S - 13	F	I	268.00	19.96	48.30	88.00
S - 15	F	I	248.50	13.36	41.00	80.00
S - 16	F	I	391.00	15.37	50.00	82.00
Average			301.90	16.10	45.44	80.60
Std. Dev.			--	3.09	4.10	5.07

Appendix Table II. Plasma glucose, plasma lactic acid, and liver glycogen concentrations of gray squirrels collected at Blacksburg, Virginia, during the period from September 15, 1965 to June 30, 1966. N = normal, C = confined-stressed, and S = confined-shocked animals

Animal	Sex	Age	Plasma glucose (mg/100ml)	Plasma lactic acid (mg/100ml)	Liver glycogen (mg/g)
N - 6	M	A	97.96	53.64	13.95
N - 8	M	A	98.25	60.05	24.10
N - 10	M	A	150.69	50.80	41.00
Average			115.63	54.83	26.35
Std. Dev.			30.36	4.68	13.66
N - 5	F	A	110.26	41.53	10.63
N - 7	F	A	71.25	51.58	24.80
N - 9	F	A	105.75	78.87	10.50
Average			95.75	57.33	15.31
Std. Dev.			21.25	19.29	8.25
N - 3	M	I	68.23	54.44	5.03
N - 4	M	I	84.30	101.72	1.60
N - 11	M	I	78.08	64.32	6.30
Average			76.87	73.49	4.31
Std. Dev.			8.15	24.91	2.46
N - 1	F	I	131.86	113.43	1.39
N - 2	F	I	149.43	42.46	4.53
Average			140.65	77.95	2.96
Std. Dev.			12.78	50.17	2.22

Appendix Table II. Plasma glucose, plasma lactic acid, and liver glycogen concentrations of gray squirrels collected at Blacksburg, Virginia, during the period from September 15, 1965 to June 30, 1966. N = normal, C = confined-stressed, and S = confined-shocked animals (continued)

Animal	Sex	Age	Plasma glucose (mg/100ml)	Plasma lactic acid (mg/100ml)	Liver glycogen (mg/g)
C - 2	M	A	78.65	84.43	--
C - 4	M	A	45.68	92.55	--
C - 5	M	A	133.80	65.46	--
C - 10	M	A	68.06	67.76	--
Average			81.55	77.55	--
Std. Dev.			37.44	13.09	--
C - 1	F	A	115.83	104.35	--
C - 3	F	A	73.98	149.50	--
C - 6	F	A	160.95	109.91	--
C - 7	F	A	174.61	81.88	--
C - 8	F	A	86.15	87.47	--
C - 9	F	A	32.55	77.58	--
C - 11	F	A	99.74	94.50	--
Average			106.26	100.74	--
Std. Dev.			49.46	24.43	--

Appendix Table II. Plasma glucose, plasma lactic acid, and liver glycogen concentrations of gray squirrels collected at Blacksburg, Virginia, during the period from September 15, 1965 to June 30, 1966. N = normal, C = confined-stressed, and S = confined-shocked animals (continued)

Animal	Sex	Age	Plasma glucose (mg/100ml)	Plasma lactic acid (mg/100ml)	Liver glycogen (mg/g)
C - 13	M	I	158.37	101.04	--
C - 14	M	I	60.32	88.48	--
C - 18	M	I	158.36	118.81	--
C - 21	M	I	119.48	139.56	--
Average			124.13	111.97	--
Std. Dev.			46.35	22.19	--
C - 12	F	I	53.95	72.65	--
C - 15	F	I	72.86	86.24	--
C - 16	F	I	76.72	120.61	--
C - 17	F	I	151.25	121.46	--
C - 19	F	I	66.94	70.96	--
C - 20	F	I	96.59	95.04	--
Average			86.39	94.49	--
Std. Dev.			34.69	21.85	--

Appendix Table II. Plasma glucose, plasma lactic acid, and liver glycogen concentrations of gray squirrels collected at Blacksburg, Virginia, during the period from September 15, 1965 to June 30, 1966. N = normal, C = confined-stressed, and S = confined-shocked animals (continued)

Animal	Sex	Age	Plasma glucose (mg/100ml)	Plasma lactic acid (mg/100ml)	Liver glycogen (mg/g)
S - 4	M	A	5.60	46.25	--
S - 5	M	A	6.94	22.03	--
S - 6	M	A	6.72	7.82	--
Average			6.42	25.36	--
Std. Dev.			0.72	19.43	--
S - 9	F	A	10.30	18.96	--
S - 10	F	A	14.35	5.57	--
S - 14	F	A	7.21	5.55	--
S - 18	F	A	17.65	4.05	--
Average			12.38	8.53	--
Std. Dev.			4.57	6.99	--
S - 8	M	I	1.65	14.63	--
S - 17	M	I	23.97	4.45	--
Average			12.81	9.54	--
Std. Dev.			15.78	7.20	--
S - 11	F	I	58.07	9.76	--
S - 12	F	I	11.38	13.69	--
S - 13	F	I	7.83	6.11	--
S - 15	F	I	13.71	5.68	--
S - 16	F	I	6.97	16.58	--
Average			19.59	10.36	--
Std. Dev.			21.68	4.75	--

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ABSTRACT

This project was undertaken to determine the effects of continued close confinement on the normal physiological characteristics of the gray squirrel. A total of 46 squirrels divided into three groups: normal, confined-stressed, and confined-shocked were sampled within a period of 10 months from September 15, 1965 to June 30, 1966.

The confined-shocked squirrels exhibited acute hypoglycemia, hypolacticemia, and hypothermia indicating a general breakdown in the overall homeostatic processes.

The adrenal glands from the confined-shocked individuals were approximately 6-8 mg heavier than either the normal or confined-stressed animals.

Packed-cell-volumes averaged from 31-36% higher than normal in both the confined-stressed and confined-shocked animals indicating a polycythemic condition which developed due to the increased stress of confinement.

Normal adult liver glycogen levels were approximately 17.2 mg/g of liver tissue higher than immature squirrels.

Death from shock could have been caused by the acute reduction of plasma glucose, plasma lactic acid, or body temperature or any combination of these three physiological characteristics. High PCV may have been an additive factor to the shock syndrome evident in this study. However, further intensive study must be conducted before we can contribute the observed shock losses to any specific physiological mechanism.