A PHYSIOLOGICAL STUDY OF THE COTTONTAIL RABBIT IN EASTERN KANSAS

A Thesis

Submitted to

the Division of Biological Sciences

Emporia State University, Emporia, Kansas

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

---

by

Johna K. Veatch

December, 1978

AN ABSTRACT OF THE THESIS OF

Johna K. Veatch for the Master of Science

in Environmental Biology presented on December, 1978

Title: A Physiological Study of the Cottontail Rabbit in Eastern Kansas

Abstract approved: Dwight L. Spencer

A physiological study of the cottontail rabbit was conducted on approximately 100 acres of Ross Natural History Reservation in Lyon County, Kansas, and in portions of Osage and Coffey counties. Two groups of rabbits were sampled: those that were live trapped on the Reservation and those that were shot in Lyon, Osage, and Coffey counties. Blood samples were removed from live trapped rabbits. Blood and liver samples were taken from shot rabbits and adrenals removed from shot rabbits and weighed.

Blood glucose samples for live trapped rabbits ranged from 121 to 254 mg/100 ml of sample. Blood glucose values for shot rabbits ranged from 110 to 278 mg/100 ml. Liver glycogen for shot rabbits ranged from 0.135 to 1.353 per cent glycogen by weight. Adrenal weights ranged from 0.007 to 0.044 g/100 g body weight. Statistical tests were made using the Student t test at the p=.05 level of significance to determine if

there were differences between the sexes, the seasons, or time the samples were collected for blood, liver, and adrenals.

Trapping results were recorded from 1 May 1975 to 1 May 1978. From 1 May 1976 to 1 May 1977, 157 rabbits were captured for a mean of 0.45 captures per 100 trap nights. Sixty-seven rabbits were captured from 1 May 1977 to 1 May 1978 for a mean of 0.24 captures per 100 trap nights. A peak in trapping success was noted in October and November.

A daily roadside survey was conducted from 1 May 1975 to 1 May 1978. From 1 May 1975 to 1 May 1976, 465 rabbits were observed with a mean of 1.43 rabbits per 100 miles. From 1 May 1976 to 1 May 1977, 1110 rabbits were observed for a mean of 2.71 rabbits per 100 miles. In the last period, 1 May 1977 to 1 May 1978, 598 rabbits were observed for a mean of 2.23 rabbits per 100 miles. An increase in sightings was noted in June and July.

Dwight L. Spencer

Approved for Major Department

Harold E. Durst

Approved for Graduate Council

ACKNOWLEDGEMENTS

I would like to thank Dean Blevins, Roger Ferguson, Dave Franz, Jim Hemmingway, Jeff Hemmingway, and Paul Milks for their assistance in gathering data. I would also like to thank the Kansas Fish and Game Commission for help in collecting specimens, and providing a vehicle. I am grateful to the Division of Biological Sciences of Emporia State University for providing financial support. I am indebted to Dr. Dwight Spencer, my major advisor, for his help and advice, and to Dr. Kay Smalley for her help and patience in developing the procedure. My special thanks go to Rhonda Baker not only for her help in the research but also for moral support through the period.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The cottontail rabbit, Sylvilagus floridanus (J. A. Allen), serves as a source of food and as a game animal in the United States (Kirkpatrick, 1950; Reilly and Dell, 1955; Tiemeier, 1955; Scott and Klimstra, 1955; Lord, 1963; Craighead and Craighead, 1969; Gipson and Sealander, 1976; Beason and Moore, 1977). Kansas hunters view the rabbit as an animal of economic importance, and they have become concerned about an apparent rabbit population decline from 1969 to the present. The Kansas Fish and Game Commission has set a daily bag limit of 10 rabbits per hunter, and it has been determined that an average daily bag of two rabbits per hunter and eight rabbits per hunter per season satisfies the hunters' demand for rabbits. However, from 1969 to the present there has been a reduction in the daily bag below two rabbits per hunter (Peabody, pers. comm.).

Field studies show that populations of animals, especially rodents, undergo periodic density fluctuations. The most common causes of population declines appear to be predation and disease. Hunters' questions concerning causes of the rabbit population decline served as a catalyst for the Fish and Game Commission to support a study of rabbit ecology in Lyon County, Kansas. It was hoped that a study of cottontail rabbits would provide some definite information about the cause(s) of periodic population declines and yield data that the Commission could use in improving the management program of Kansas cottontails.

A cottontail rabbit study was begun in 1974 by Hutton (1975) and Watt (1975) on 100 acres of Emporia State University's Ross Natural History Reservation. Studies were made to determine the home range and

mortality of the cottontail rabbit. Rabbits were live trapped on the Reservation and healthy, adult specimens weighing more than 650 grams were fitted with pulsating radio-transmission collars and released on the study area. The rabbits were located daily, using a hand-held receiver, and their positions were recorded. These data were then used to establish home ranges of cottontails on the Reservation (Clark, 1976). Upon the death of a collared rabbit a frequency change in the transmitter signal was used to locate the remains and the cause of death was determined. Gress (1976) and Baker (1977) continued the study and reported 49 per cent of observed rabbit mortalities were due to predation, 18 per cent to tularemia, and the remainder were due to a variety of causes.

Rabbit mortality studies by Gress (1976) and Baker (1977) failed to show a definite cause for the rabbit population decline. Following their studies a physiological abnormality in cottontails was considered as another possible cause for the population decline. Green and Larsen (1938) conducted a physiological study on snowshoe hares, Lepus americanus (J. A. Allen), in an effort to explain periodic fluctuations of the hare population. They reported on hares that died in captivity as well as those found dead or dying in the field. They found dead hares with remains intact with no evidence of predation. Likewise, Baker (1977) found 13 cottontail mortalities on the Reservation in which the remains were still intact and the cause of death could not be determined.

Green and Larsen (1938) reported finding wild snowshoe hares comatose or in convulsive states characterized by running motions of the

legs. The hares generally died within minutes after discovery. Upon death of the hares, Green and Larsen performed autopsies and blood chemistry analyses. The same procedures were performed on dead, captive hares. Results showed blood glucose levels of the hares were unusually low, and livers were in a hypotrophic state. They terms the snowshoe hare deaths resulting from hypoglycemia as "shock disease" and suggested that it was brought on by environmental or captive stress. This physiological study of the snowshoe hare by Green and Larsen (1938) prompted an inquiry as to whether cottontail rabbits in Lyon County were suffering from hypoglycemia which might be a cause of the unexplained mortalities noted by Baker (1977) as well as the decline in hunters' success reported by the Fish and Game Commission.

It is important that blood glucose in animals be maintained at certain concentrations because glucose is a major source of nourishment for the brain and heart. Blood glucose levels are regulated by carbohydrate metabolism in the liver (Turner and Bagnara, 1976). Ingested carbohydrates, following digestion and absorption, are converted into glycogen which is stored in the liver. Liver glycogen may also be formed from non-carbohydrate sources. Amino acids from ingested proteins and glycerol from ingested fats can both be converted into glucose-6-phosphate through the process of gluconeogenesis (Turner and Bagnara, 1976). Glucose-6-phosphate can then be converted to glycogen for storage or to glucose which is released into the blood, thereby increasing blood sugar level.

Several hormones affect blood glucose and liver glycogen levels. One is epinephrine which is released by the adrenal medulla upon

stimulation by the sympathetic nervous system. Other adrenal hormones affecting blood glucose levels are secreted by the adrenal cortex and are collectively referred to as glucocorticoids. Bush (1953) found major rabbit glucocorticoids to be hydrocortisone and corticosterone; they are secreted in a ratio of 0.05 parts to 1.0 parts, respectively.

Carbohydrate metabolism is markedly affected by stress which stimulates secretion of both epinephrine and glucocorticoids. Epinephrine has a short term effect on the liver during stress causing a portion of stored glycogen to be converted to glucose. The glucocorticoids act to conserve the animal's carbohydrate store by stimulating glucose and glycogen formation from non-carbohydrate stores such as amino acids, derived from body proteins, and glycerol, from fat deposits. Pituitary secretion of ACTH, adrenocorticotrophin, regulates adrenal secretion of glucocorticoids (Gorbman and Bern, 1962). In periods of stress, such as cold, heat, emotional stimuli, and reduction in blood glucose the pituitary will secrete ACTH thus activating adrenal glucocorticoid secretion (Lissak and Endroczi, 1965). Epinephrine also acts on the pituitary to increase ACTH secretion thus, indirectly, increasing glucocorticoids. If stores of glycogen are adequate these mechanisms serve to maintain blood glucose. However, if there is a shortage of glycogen, blood glucose may be used faster than it can be formed and hypoglycemia results. Hypoglycemia deprives the brain of its energy source and hence may lead to coma and death. Dead snowshoe hares studied by Green and Larsen (1938) appeared to have a liver malfunction which caused death due to exhaustion of glycogen stores, liver disease, and hypoglycemia.

Hypoglycemia has been used to explain fluctuations in mammal populations (Allen, 1954). Therefore, based on reports by Green and Larsen (1938) and Allen (1954), it was decided to test the hypothesis that hypoglycemia caused the rabbit population decline in Lyon County, Kansas. Two groups of animals were studied. One group was composed of rabbits shot in Lyon and contiguous counties of Osage and Coffey. The other group consisted of rabbits live trapped on the reservation. Blood samples from both groups were tested for blood glucose levels. Liver samples were removed from dead rabbits and analyzed for glycogen content. Adrenal glands were removed from dead rabbits and weighed with weight being considered an indicator of adrenal activity.

In conjunction with trapping and hunting, a roadside survey of rabbit sightings was conducted following predetermined routes. The survey was a continuation of a previous four-year study on rabbit population and mortality by Gress (1976) and Baker (1977).

The purpose of this study was to determine whether or not cottontail rabbits in Lyon County were suffering from hypoglycemia or conditions which might lead to hypoglycemia. In addition, statistical comparisons were made of trapping results and roadside observations of rabbits to determine if the population may have declined below levels of previous years.

MATERIALS AND METHODS

Collection Procedure

Approximately 20 single-door Tomahawk wire traps and approximately 70 conventional wooden box traps were set on 100 acres of the Ross Natural History Reservation to live trap rabbits. Trapping was conducted from May 1977 to April 1978 and traps were checked daily. In October 1977 the use of wire traps was discontinued due to exposure of trapped animals to adverse weather conditions and because the traps were nonfunctional when snow covered. Trapping with box traps was continued until the last of April 1978. Traps were left unbaited because no noticeable increase in trap success was reported by Gress (1976) in an experimental baiting program. The number of trap nights was determined by multiplying the number of traps by total number of nights they were open. Trapping success between the period 1 May 1976 to 1 May 1977 and the period 1 May 1977 to 1 May 1978 as well as the period 1 May 1975 to 1 May 1976 and the period 1 May 1977 to 1 May 1978 were compared statistically using the Student t test at $p=.05$ level of significance. The same statistical test was used to compare the number of trap nights during the period 1 May 1976 to 1 May 1977 with the period 1 May 1977 to 1 May 1978.

In conjunction with trapping, a daily roadside survey of cottontail rabbits was conducted. Routes were predetermined (Appendix A) and 1977-1978 was the fourth year of the survey. The period from 1 May 1976 to 1 May 1977 was compared statistically to the period 1 May 1977 to 1 May 1978 using the Student t test at $p=.05$ level of significance.

For the second portion of the research, rabbits were shot from 1 May 1977 to 1 May 1978 with most collections in October, December, February, and April. Liver and blood samples were removed in the field, then the body was transported to the lab for autopsy.

Blood Glucose

After reviewing several methods for glucose determination it was decided to use a colorimetric method on blood plasma as described by the Sigma Chemical Company (1974). Through personal communication with the company it was determined that this method of analysis for human blood could be used for rabbits. In the colorimetric method a sample of plasma was reacted with ortho-toluidine and glacial acetic acid (Dubowski, 1962). After heating the samples in a vigorously boiling water bath a colored solution resulted. Absorbance of this solution was read at 620-650 nanometers (nm) by a Baush and Lomb Spectrophotometer (Spec. 20).

Before blood samples were tested a glucose standard was diluted with various amounts of a 0.1 per cent solution of benzoic acid to give different glucose concentrations. Each glucose concentration was then treated with the o-toluidine method and absorbance read on a Spec. 20 to determine if the relationship between absorbance readings and glucose concentrations was linear. Preliminary results indicated the importance of mixing all solutions well, making sure the glucose was kept cold, and keeping the water bath boiling vigorously. When these precautions were observed the relationship between absorbance and concentration proved to be linear (Fig. 1). Since the relationship was linear the samples were compared directly to the standard, and mg/100 ml of glucose was calculated by the following equation:

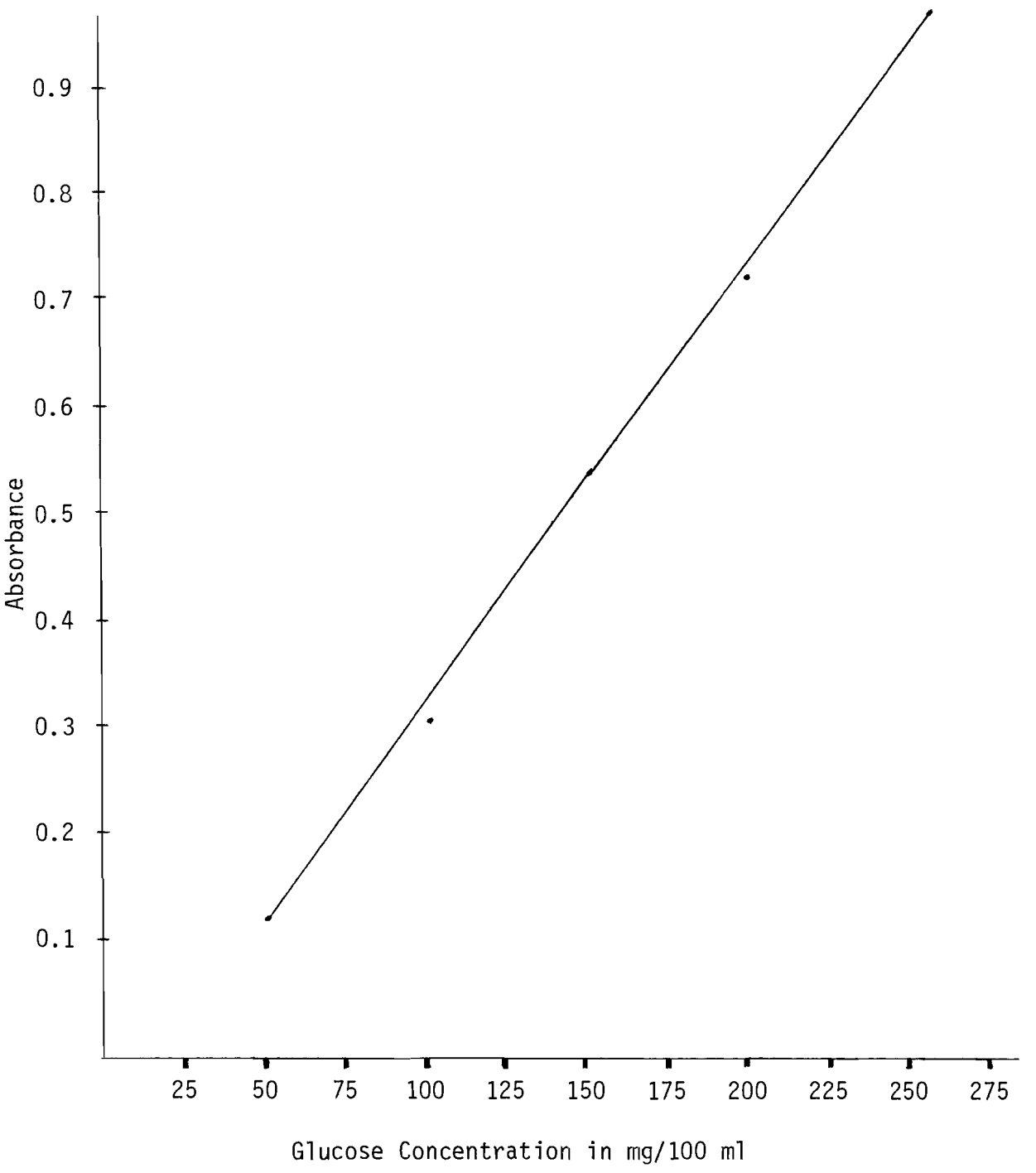

Figure 1. Linear relationship of various glucose concentrations to absorbance.

$$\frac{\text{Absorbance of test sample}}{\text{Absorbance of standard sample}} \times 100 = \text{mg/100 ml glucose}$$

After establishing the linear relationship the next step was to adapt the procedure to field work. It was necessary to find a proper method for collection and storage of blood samples. A blood sample was collected with a syringe from a domestic rabbit's ear as described by Hoar and Hickman (1967). After removal the blood sample was frozen. However, freezing resulted in hemolysis so it was discontinued as a method of storage.

Since blood could not be frozen for storage it was necessary to determine what happened to blood glucose measurements over a period of time if the blood was merely kept cool. A large sample was withdrawn from a domestic rabbit and aliquots were analyzed at one-hour intervals. The samples showed a sharp increase in glucose concentration from time zero to one hour after the sample was taken, followed by a decrease in concentration (Fig. 2). A second set of tests was run to check the apparent change in glucose concentrations between the first and second hour, and to determine if different glucose concentrations changed at the same rate. Three aliquots of a single blood sample were tested. The first aliquot contained .75 ml of 100 mg/100 ml glucose stock added to blood to increase the glucose concentration; the second was only blood; and the third had 0.50 ml of 0.9 per cent sodium chloride added to dilute the blood and decrease glucose concentration. Results of this study (Fig. 3) showed blood glucose concentrations did not change significantly (Student t test at $p=.05$ level of significance), when the samples were kept cool for two hours. Therefore, all subsequent field

collected samples were processed within two hours after collection.

Preliminary results indicated the following procedure was satisfactory to obtain a blood sample from a live rabbit: at least 0.2 ml of blood was withdrawn from the ear vein into a heparin-coated syringe, then transferred to a test tube coated with heparin and sodium fluoride (Meites and Bohman, 1963). A 23 gauge needle was substituted for the 26 gauge needle that came with the syringe as the smaller needle caused clotting of blood during withdrawal. The sample was then stored for no more than two hours in a cool place before it was spun down at 600 G for 10 minutes. A 0.1 ml sample of plasma was pipetted off because preliminary work showed that using whole blood or serum resulted in excessive hemolysis. All plasma samples were tested immediately after separation from the cells. From this point the analysis was performed as described by the Sigma Chemical Co. (1974).

The final procedure was to test wild rabbit blood. Two groups of rabbits were tested: those live trapped on the Ross Natural History Reservation, and those shot in Lyon, Osage, and Coffey counties. When rabbits were removed from live traps they were put in denim bags to facilitate transportation to the lab. Trapped rabbits were sexed, weighed, measured, ear-tagged, and their general physical condition was noted; then a blood sample was taken. It was found if a rabbit remained in the bag that was used for transporting it to the lab and only the ears were withdrawn from the bag, the rabbit remained quiet while a blood sample was taken. The ear was swabbed with xylene and held under an incandescent lamp which resulted in dilation of blood vessels. A blood sample was removed by the previously stated method.

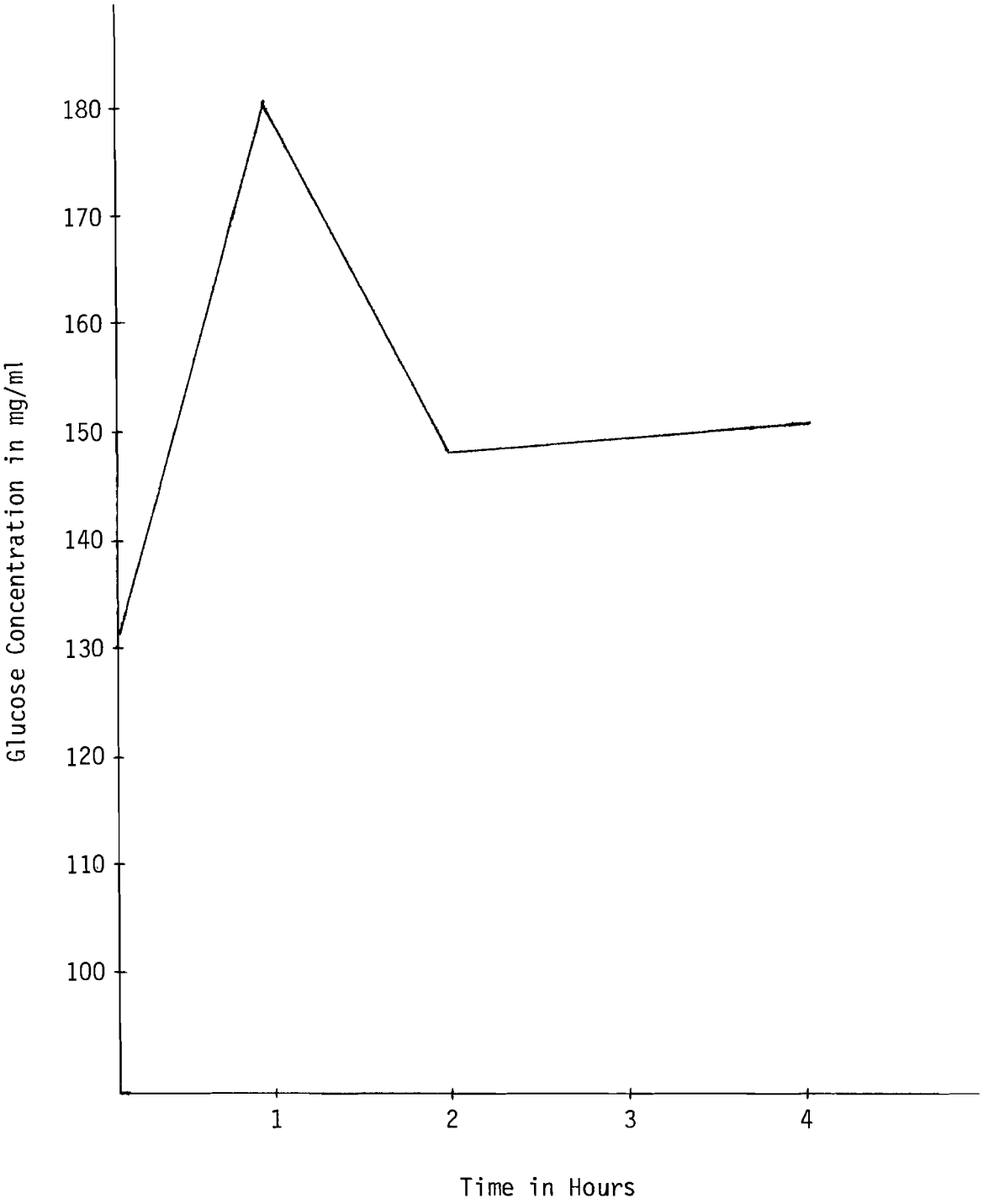

Figure 2. Change in glucose concentration with time measured at one-hour intervals for four hours.

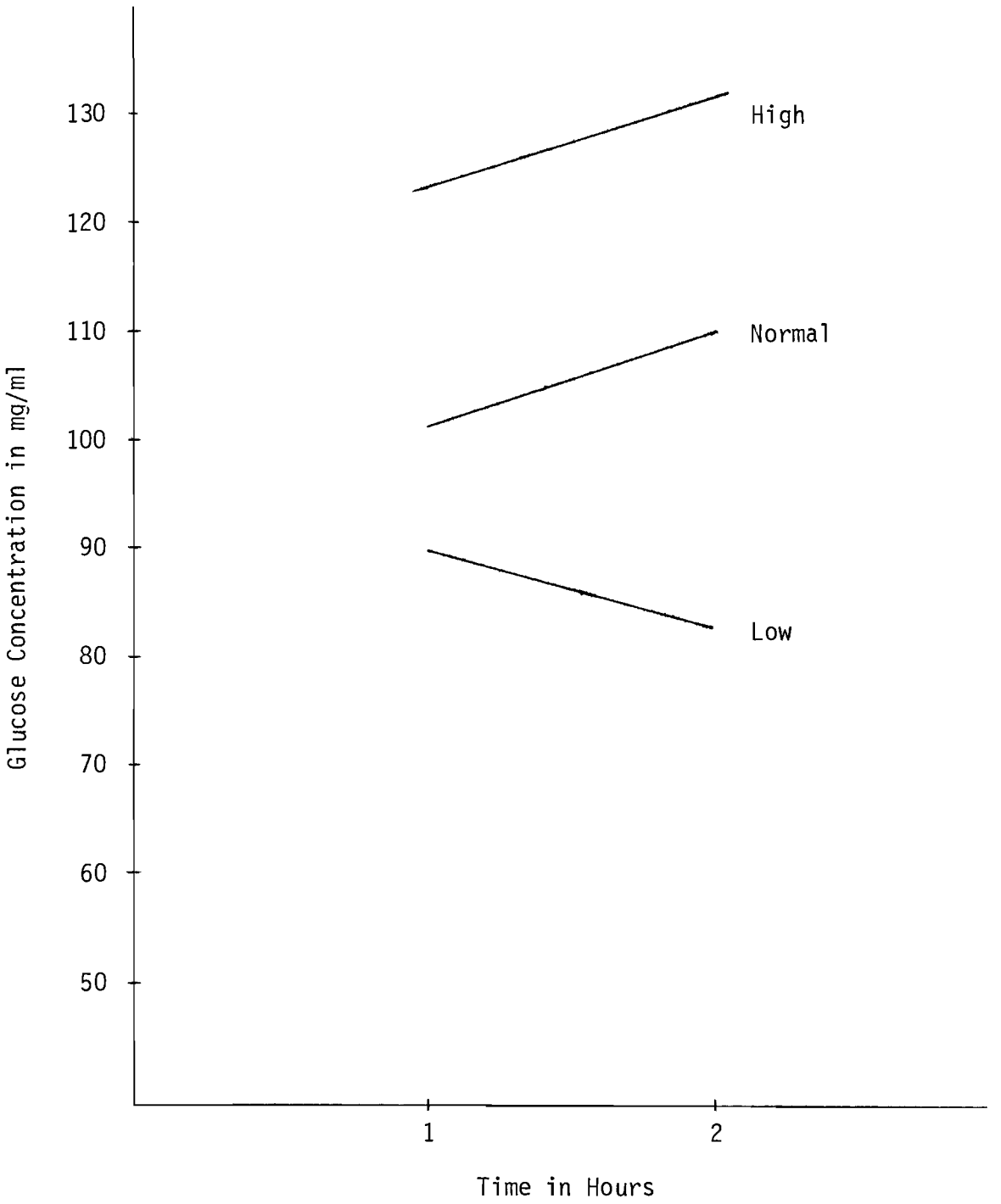

Figure 3. Effect of storage time on three concentrations of glucose.

On rabbits that were shot an attempt was made to withdraw blood from a major vessel such as the inferior vena cava, but often there was a large amount of blood in the body cavity which obscured the vessels. When this occurred, to avoid getting blood mixed with body fluids, the sample was usually taken from the heart. At least 0.2 ml was withdrawn into a heparin-coated syringe then treated as indicated above for live trapped rabbits.

Blood glucose data were statistically analyzed in three groups. Seasonal differences in blood glucose values were compared as were differences in blood glucose values between rabbits shot in the morning and those shot in the evening. The third comparison of blood glucose values was between male and female rabbits.

Liver Glycogen

Since blood glucose levels were variable in preliminary tests it was decided to test liver samples for stored glycogen. The method for liver glycogen determination was adapted from Montgomery (1956) with modifications from Good et. al. (1933). Various glycogen concentrations were tested with the procedure to determine the relationship between concentration of glycogen and absorption reading on a Spec. 20. A standard curve was constructed from the relationship and used to determine the glycogen concentration in the liver samples.

The final method of analysis was as follows:

1). a sample of liver was placed in a pre-weighed vial that contained potassium hydroxide and refrigerated,

2). for analysis, the sample was heated to facilitate liver digestion as evidenced by a clear sample,

3). next 5.0 ml of 95 per cent ethanol was added to the sample,

4). this was heated until boiling commenced, then removed and cooled,

5). the solution was centrifuged for 10 minutes at 600 G and the supernatant was poured off,

6). the remaining precipitate was redissolved in 10 ml distilled water,

7). then 1.0 ml of redissolved precipitate was removed and diluted with 9.0 ml distilled water,

8). a 2.0 ml sample was withdrawn and placed in a small test tube,

9). a blank was constructed using 2.0 ml distilled water,

10). at least 0.1 ml redistilled phenol was added to both the blank and the glycogen sample,

11). then 5.0 ml of concentrated sulfuric acid was added to both the blank and the sample with a 5.0 second delivery time,

12). the solutions sat for 30 minutes while the reaction went to completion,

13). sample absorbence was read at 490 nm.

Before tests were begun the potassium hydroxide and liver sample was reweighed to determine the amount of liver used. If the liver sample in the vial was much more than 0.5 to 0.6 g a second dilution was made by adding 9.0 ml distilled water to 1.0 ml redissolved precipitate and distilled water. This insured that the glycogen concentration in the sample would be low enough to be read by the Spec. 20. Each absorption reading on the Spec. 20 was compared to the standard curve and mg glycogen per ml of sample was extrapolated. Glycogen concentrations of each sample

were multiplied by the dilution factor, which was usually 100, to obtain the actual liver glycogen concentration.

In the field the liver sample was taken as soon as possible after the rabbit was shot. A few minutes delay in collection allowed enzymes to break down liver glycogen, thus decreasing stored glycogen levels (Cori, 1932). Placement of liver samples in potassium hydroxide arrested enzymatic breakdown. All samples were stored approximately one week under refrigeration, then analyzed. Differences in liver glycogen values were compared for different seasons, for males versus females, and rabbits shot in the mornings versus those shot in the evenings.

Adrenal Glands

Evidence of increased adrenal gland activity can be seen by noting an increase in gland weight or an increase in size of the zones that secrete the glucocorticoids (Christian and Ratcliff, 1952; Fickess, 1963; Barrett, 1964; Zarrow, 1964; Welch and Welch, 1969; Christian, 1975). For this study only adrenal weights were measured.

After each rabbit was shot liver and blood samples were taken. The rabbit was then transported to the lab where an autopsy was performed. Both adrenals were removed and, after excess fat was cleaned from them, they were weighed on an analytical balance. The rabbit was also weighed, measured, and sexed, and its general condition was noted. Internal and external parasites and breeding condition were also noted.

Statistical tests of significance were made on comparisons of rabbit adrenal weights by grouping the data in three different ways. Adrenal weights of male and female rabbits were compared and rabbits shot in the mornings were compared to those shot in the evenings. The

final statistical comparison of adrenal weights was made between rabbits shot in the various seasons to determine if significant differences existed.

The Student t test at $p=.05$ level of significance was used to determine if there were significant differences between males and females, between rabbits shot in the morning and those shot in the evening, and between seasons for blood glucose, liver glycogen, and adrenal weights. A correlation was made to determine whether or not there were relationships between some of the values obtained. Blood glucose levels were compared to liver glycogen and adrenal weights, and liver glycogen values were compared to adrenal weights.

RESULTS

Blood Glucose Analysis

During trapping and hunting phases of the study, 33 rabbits were sampled. Six rabbits were live trapped and blood samples were collected; the remaining 27 were shot and blood and liver samples were removed.

Table I shows seasonal means and ranges of rabbit blood glucose values. Male rabbits' blood glucose values ranged from 100 mg/100 ml of blood to 278 mg/100 ml and the mean was 169.3 mg/100 ml. Female rabbits' blood glucose values ranged from 121 mg/100 ml to 235 mg/100 ml and the mean was 166.6 mg/100 ml. Times of day rabbits were shot were also compared. Rabbits shot in the mornings had a blood glucose value range of 121 mg/100 ml to 278 mg/100 ml and a mean of 154.4 mg/100 ml. Rabbits shot in the evenings had a blood glucose value range of 110/mg/100 ml to 236 mg/100 ml and a mean of 171.8 mg/100 ml.

Results showed four shot rabbits had higher blood glucose values than other shot rabbits, and one shot rabbit exhibited a lower blood glucose than the others. Two trapped rabbits had a higher blood glucose than the others and one had a lower blood glucose level. This rabbit was first captured on 30 October 1977 and recaptured every second day until its death on 5 November 1977. During this time the rabbit exhibited a reduction in weight and blood glucose level.

Table I also shows that the blood glucose levels for the summer shot rabbits varied little though they were higher than values for the winter. Winter blood glucose values had a wider range than either summer or spring values, and winter and spring values were lower than summer values.

Table I. Seasonal values for number of rabbits per month, blood glucose ranges and blood glucose means for cottontail rabbits.

Season	Month of Collection	Number of Rabbits	Range of Blood Glucose mg/100 ml	Mean Glucose mg/100 ml
Summer	May	2	234-236	235
	July	2	209-211	210
Winter	December	14	110-278	61.3
	February	5	126-200	146.6
Spring	April	2	117-188	152.5
Trapped Rabbits		6	121-254	171.3

There is a diurnal rhythm in corticoid secretion with the level at its highest point in the morning then falling off in the evening (Lissak and Endroczi, 1965). This would directly affect blood glucose level causing an increased morning level and a decreased evening level. Therefore, blood glucose levels for rabbits shot in the morning were compared statistically to rabbits shot in the evening.

Liver Glycogen Analysis

During the course of the research it was discovered that a procedural step for liver glycogen analysis had been omitted. After the liver was added to potassium hydroxide the sample was allowed to sit under refrigeration one week, then it was removed and ethanol was added. The next step was to heat the mixture to precipitate the glycogen. The error was that after the liver and potassium hydroxide sample was removed from refrigeration the sample should have been heated before ethanol was added, thus insuring the total glycogen digestion. Two liver samples were analyzed by the revised method, and it was found that the proper method showed 1.5 to 2.0 times more glycogen than the original incorrect procedure. Recorded results were reported using the incorrect procedure and may in actuality have 1.5 to 2.0 times more glycogen than shown.

Table II shows seasonal distribution of liver glycogen values in per cent glycogen per sample. The range of glycogen for each season as well as the mean per cent glycogen for all rabbits shot during a season is shown. The mean glycogen per cent for shot male rabbits was 0.580 with a range of 0.211 to 1.25 per cent. Shot female rabbits had a mean per cent glycogen of 0.396 and a range of 0.135 to 1.353 per cent. The range of per cent glycogen for morning kills was 0.20 to 1.258 per cent

Table II. Number of rabbits sampled per month, and seasonal ranges and means of liver glycogen for cottontails.

Season	Month of Collection	Number of Rabbits	Range of Per Cent Glycogen (W/W)	Mean Per Cent Glycogen
Summer	July	3	.143-0.332	.222
Fall	October	1	1.353	1.353
Winter	December	9	.211-1.258	.603
	February	4	.183-0.561	.328
Spring	April	2	.135-0.256	.195

with a mean of 0.602; the range of per cent glycogen for evening kills was 0.135 to 1.353 per cent with a mean of 0.368. It appears as though a diurnal variation of liver glycogen exists.

Four liver glycogen samples were excluded from the data because they were not collected immediately after death. Delayed collection allows glycogen to be broken down so that liver glycogen found during analysis is approximately 0.33 per cent of glycogen found in the other samples. It is important to collect liver samples immediately after death due to rapid breakdown of the glycogen (Cori, 1932).

Adrenal Glands

Table III lists total body weights and adrenal weights of shot rabbits. Adrenal weights are comparable to values found by Wodzick and Rober (1960) and Bailey and Schroeder (1967) in their cottontail population studies. All adrenal weights were converted to grams per 100 grams body weight for comparison purposes.

Male adrenal weights ranged from 0.009 g/100 g body weight to 0.034 g/100 g with a mean of 0.018 g/100 g. Female adrenals ranged from 0.007 g/100 g to 0.044 g/100 g and the mean was 0.017 g/100 g.

An adrenal weight comparison was made between morning and evening killed rabbits. Morning killed rabbits had an adrenal weight range of 0.009 g/100 g to 0.020 g/100 g with a mean of 0.015 g/100 g. Evening killed rabbits had an adrenal weight range of 0.007 g/100 g to 0.034 g/100 g with a mean of 0.019 g/100 g.

Table IV compares seasonal differences in adrenal weights of shot rabbits. Adrenal weights decreased from summer to winter. Seasonal differences in adrenal weights found in this study agree with studies

Table III. Body weights and adrenal weights of cottontail rabbits.

Rabbit Number	Body Weight in Grams	Adrenal Weight in Grams
1	1073.0	.36
2	1030.0	.32
3	780.2	.34
5	509.0	.13
6	1249.7	.24
7	490.2	.08
9	1220.3	.21
12	1105.2	.15
15	967.0	.16
16	1097.6	.22
17	1097.3	.16
18	1129.0	.18
19	1295.1	.13
20	1170.8	.18
21	1136.4	.18
24	1143.5	.12
26	1209.0	.09
27	1033.5	.16
28	1088.0	.17
29	993.6	.99
31	960.0	.12
32	1200.0	.21
33	1258.0	.17

Table IV. Seasonal comparison of cottontail rabbit adrenal weights.

Season	Month of Collection	Number of Rabbits	Range of Adrenal Weight g/100 g	Mean Adrenal Weight in g.
Summer	May	2	.031-.034	.033
	July	2	.019-.044	.029
Fall	September	1	.016	.016
	October	1	.017	.017
Winter	December	12	.007-.020	.013
	February	4	.012-.019	.016
Spring	April	2	.014-.018	.016

by Beer and Mayer (1951), Pederson (1960), and Fickess (1963) who reported an adrenal weight increase during spring and summer corresponding to the breeding season and an adrenal weight decrease in winter during the non-breeding season.

Live Trapping

From 1 May 1975 to 1 May 1978 continuous trapping had been conducted on the Ross Natural History Reservation. Traps were closed only during brief vacation periods, while traps were relocated, and when traps were snow covered. Table V summarizes trapping results from 1 May 1975 to 1 May 1978. During the period 1 May 1976 to 1 May 1977, 157 rabbits were captured and the mean was 5.44 captures per 100 trap nights. From 1 May 1977 to 1 May 1978, 67 rabbits were captured and the mean was 2.9 captures per 100 trap nights. It appears there was a significant decrease in the total number of rabbits trapped as well as the number of captures per 100 trap nights.

Baker (1977) reported only one month, April, when no rabbit was trapped. However, data collected for the past year showed three months, January, March, and April of 1978 when no rabbit was trapped. Watt (1975), Gress (1976), and Baker (1977) reported a definite trapping success peak in October and a high level of success in November. Results of this study agree with the previous studies for there was an increase in the number of rabbits trapped in October and a higher number trapped in November than in other months studied.

The number of traps was not kept constant through the period because wire traps were removed in October to avoid exposure of the animals to adverse weather. Also, not all traps were functional in the

Table V. Trapping record of cottontail rabbits for the period 1 May 1975 to 1 May 1978. C/100 equals captures per 100 trap nights (after Gress, 1976).

Month	Trap Nights	Total Captures	C/100
May 1975	1,153	4	0.35
June	1,879	5	0.27
July	2,134	10	0.47
August	2,292	14	0.61
September	2,393	62	2.63
October	3,010	100	3.63
November	3,503	65	1.83
December	2,874	16	0.56
January 1976	3,238	14	0.43
February	2,987	7	0.23
March	2,874	3	0.10
April	2,727	4	0.15
May	3,001	6	0.20
June	2,908	7	0.24
July	2,992	11	0.37
August	2,986	7	0.23
September	2,882	15	0.52
October	3,005	48	1.60
November	2,910	44	1.51
December	2,595	10	0.39

Table V. Continued.

Month	Trap Nights	Total Captures	C/100
January 1977	2,125	5	0.24
February	2,685	3	0.11
March	3,006	1	0.03
April	2,390	0	0.00
May	2,970	3	0.11
June	2,213	3	0.14
July	2,903	4	0.13
August	2,635	3	0.11
September	2,128	11	0.52
October	2,310	23	0.99
November	2,332	15	0.64
December	1,432	2	0.14
January	1,967	0	0.00
February	871	1	0.12
March	1,340	0	0.00
April	1,139	0	0.00
Total	88,780	526	$\bar{x}$ = 0.54

winter, especially during deep snow. This may have introduced bias in the data.

Roadside Survey

Roadside counts of cottontail rabbits have been used as indicators of relative rabbit abundance. Population fluctuations have been indicated by seasonal and yearly variation in rabbit abundance as viewed in roadside counts (Voris, 1957; Lord, 1961, 1963; Collins, 1967). In conjunction with a radio-telemetry project on the Reservation a daily roadside survey was begun on 1 June 1975. Two routes were alternately driven by researchers (Appendix A). The sites at which rabbits were observed were marked and total number of rabbits sighted per day was recorded. Table VI shows results of the survey from 1 June 1975 to 1 May 1978. From 1 May 1975 to 1 May 1976, 465 rabbits were observed and the mean was 1.43 rabbits per 100 miles. The next period from 1 May 1976 to 1 May 1977 had 1110 sightings and the mean was 2.71 rabbits per 100 miles. In the last period, 1 May 1977 to 1 May 1978, 598 rabbits were sighted and the mean was 2.23 rabbits per 100 miles. It was noted that rabbit sightings increased during summer, especially in June and July, then decreased in winter.

Table VI. Monthly summary of cottontail rabbit roadside survey for the period 1 June 1975 to 1 May 1978.

Month	Miles Traveled	Rabbits Sighted	Rabbits/ 100 Miles
June 1975	241	101	41.9
July	345	219	63.5
August	355	89	25.1
September	343	6	1.8
October	297	4	1.4
November	298	7	2.3
December	241	3	1.2
January 1976	298	13	4.4
February	287	1	0.3
March	253	4	1.6
April	309	18	5.8
May	364	25	6.9
June	349	340	97.4
July	379	475	125.3
August	337	193	57.3
September	303	20	6.6
October	349	16	4.6
November	362	6	1.7
December	326	5	1.5

Table VI. Continued.

Month	Miles Traveled	Rabbits Sighted	Rabbits/ 100 Miles
January 1977	325	9	2.8
February	326	2	0.6
March	350	9	2.6
April	314	10	3.2
May	370	68	18.4
June	299	219	73.2
July	232	168	52.2
August	231	23	9.9
September	184	2	1.1
October	219	8	3.7
November	172	3	1.7
December	115	3	2.6
January 1978	310	19	6.1
February	207	49	23.7
March	172	22	12.8
April	173	14	8.1
Total	10,035	2173	$\bar{x}$ = 17.75

DISCUSSION

Blood Glucose Analysis

As stated previously, carbohydrate metabolism is one source of liver glycogen (Anthony and Kolthoff, 1971). Liver glycogen can be converted to glucose and mobilized into the blood as a source of energy especially for the brain and heart. Muscle tissue has its own glycogen store and other tissues can also obtain energy from protein and fat catabolism.

During stress the brain, heart, and muscular systems need increased energy supplies. Stress stimulates the hypothalamus which stimulates the pituitary to secrete ACTH. ACTH activates the secretion of glucocorticoids from the adrenal cortex; at the same time the sympathetic nervous system triggers the release of epinephrine from the adrenal medulla. Epinephrine will aid in conversion of glycogen to glucose. Glucocorticoids act on extracellular amino acids and fat converting them to glycogen and glucose through gluconeogenesis (Guyton, 1976). Glucocorticoids also stimulate liver synthesis of glucose from pyruvate and lactate which is released by active muscle tissue into the blood and carried to the liver. The net result of all these glucocorticoid actions is an increase in stored glycogen and blood glucose.

Generally this system works as described. However, low blood glucose may results when all the glycogen has been mobilized or if something is wrong with carbohydrate metabolism or adrenal hormone secretion (Guyton, 1976).

It should be kept in mind that the blood glucose level is under the influence of many factors and exhibits a wide variation. Stress is not

the only variable that lowers glucose level. After periods of feeding, increased carbohydrate absorption increases blood glucose (Romsom and Leveille, 1974); this triggers release of insulin from the pancreas which in turn lowers blood glucose by storing glucose as liver glycogen (Guyton, 1976). As the body utilizes existing blood glucose the level becomes lower, and the pancreas begins glucagon secretion which converts liver glycogen to glucose. Thus, the blood glucose level may vary widely depending on the length of time since the last meal.

Thyroxine and triiodothyronine also affect blood glucose level. They increase cellular uptake of glucose, enhance glycolysis, and enhance gluconeogenesis ultimately leading to an increased blood glucose level (Guyton, 1976). Release of thyroxine and triiodothyronine by the thyroid gland is affected by such stimuli as cold or trauma. It has been shown that exposure of rats to cold increases thyroid stimulating hormone, TSH, secretion by the pituitary thus increasing thyroid hormone level. The end result of this increase would be an increased blood glucose level.

Growth hormone affects cellular metabolism in three ways: decreased utilization of glucose for energy; enhancement of glycogen deposition in cells; and decreased cellular uptake of glucose (Guyton, 1976). Growth hormone may increase fatty acid utilization for energy and decrease glucose utilization by the cells. Initially, cellular uptake of glucose increases after administration of growth hormone and this will lower blood glucose levels. Cells will eventually become saturated with glycogen and glucose uptake will diminish. The end result will be an increase in the blood glucose level. Growth hormone concentration will

increase after depletion of body proteins or carbohydrates. Hypoglycemia as well as poor protein nutrition also triggers growth hormone secretion by the pituitary.

Exercise is another factor influencing blood glucose. Exercise increases epinephrine secretion which will increase liver and muscle glycogenolysis thus increasing blood glucose (Anthony and Kolthoff, 1971). All of the above mentioned factors influence blood glucose levels and cannot be controlled in wild animals. Therefore, any one or a combination of these factors may account for observed variability.

Green and Larsen (1938, 1939) studying snowshoe hares, Lepus americanus (J. A. Allen), in Minnesota and Wisconsin described the rapid deaths of hares they observed in wild and captive populations. Upon death of a hare they performed an autopsy and blood chemistry analysis. Blood glucose values obtained were separated into four ranges. The number of hares in each range was separated by whether they appeared normal or showed convulsions or a comatose state. Mean blood glucose levels were calculated for normal and hypoglycemic rabbits according to the four ranges. Forty-nine hares fell in the range of 0.00 mg/100 ml of blood glucose to 25 mg/100 ml and showed convulsions or were comatose. Mean blood glucose level was 9.7 mg/100 ml and no hare that acted normally was in this range. The next range was 26 to 60 mg/100 ml and 16 hares in this range were comatose or convulsive. Mean blood glucose value for the 16 hares was 41.4 mg/100 ml. Again, no hare that Green and Larsen observed acting normally had a blood glucose level in this range. The third range was 61 to 160 mg/100 ml and seven hares exhibiting convulsions or coma fell in this range. The mean glucose value for the seven

was 92.0 mg/100 ml. Forty hares that acted normally exhibited a blood glucose in this range and the mean value for the 40 was 126.0 mg/100 ml. The last range was 161 mg/100 ml and above. Four hares in this range exhibited convulsions or coma and the mean glucose value for the four was 217.0 mg/100 ml. Green and Larsen observed eight normal-acting hares that had blood glucose values in this range. The mean blood glucose for the eight was 187.0 mg/100 ml. From the data Green and Larsen (1938) considered hares with blood glucose values of approximately 61 mg/100 ml and lower to be hypoglycemic. Blood glucose values for normal-acting hares ranged from 61 to 160 mg/100 ml with the mean being 126.9 mg/100 ml. In more recent showshoe hare studies by Keith et. al. (1968) blood glucose level for normal-acting hares was found to be 137 mg/ 100 ml while hares exhibiting coma or convulsions had a mean blood glucose value of 55 mg/100 ml.

When blood glucose values collected in this study were ranked according to categories established by Green and Larsen (1938) no rabbit exhibited convulsions or coma and all blood glucose values were in the range of values for the normal-acting hares. I could not find literature pertaining to cottontail blood glucose values, and it is possible that there is enough difference between glucose values for cottontails and snowshoe hares that data reported by Green and Larsen (1938) are not valid for this study.

In considering the data in this study four shot rabbits showed a blood glucose value approximately 1.5 times higher than other shot rabbits. Two trapped rabbits also had blood glucose values approximately 1.5 times higher than the others. An attempt was made to handle all shot rabbits in the same manner as well as handling all trapped

rabbits the same. However, of the four shot rabbits exhibiting high blood glucose all of the samples were not taken immediately upon death and not all rabbits were dead. Therefore, not being dead could have resulted in increased epineprine release due to stress which in turn could explain the high blood glucose. The high values for trapped rabbits were probably caused by any factor or factors previously discussed; therefore, they were considered individual variations.

One shot rabbit showed a low blood glucose value. This rabbit was shot in the winter; therefore reduced food intake might explain the low blood glucose level. It is also possible that there was a physiological malfunction in the system that lowered the blood glucose. Blood samples taken from one trapped rabbit showed a decreased glucose level. This rabbit was first captured on 30 October 1977 and recaptured every second day until its death on 5 November 1977. During this time the rabbit exhibited a reduction in weight and blood glucose level. Repeated confinement in traps of rabbits decreases time spent in obtaining food (Fitch, 1947; Bailey, 1968). A reduction in food intake probably lowered the formation of liver glycogen from ingested carbohydrates. Glucocorticoids would have been utilizing protein and fat stores to maintain the liver glycogen at the expense of other tissues as indicated by a weight reduction. Additional trap stress may have depleted the liver glycogen resulting from gluconeogenesis. Thus it is possible that the rabbit's final time in the trap caused enough stress that it developed hypoglycemic shock from reduced glycogen stores.

After collection of the data, statistical analyses were made of three sets of data. Individual blood glucose values were statistically

analyzed by comparing rabbits shot in the spring-summer season to rabbits shot in winter. No significant differences by season were found. Blood glucose values between the sexes were compared statistically and no significant difference was found. There is a diurnal rhythm in corticoid secretion with the level at its highest point in the morning then falling off in the evening (Lissak and Endroczi, 1965). This would directly affect blood glucose level, causing a high morning level and a lower evening level. In this study the blood glucose levels for rabbits shot in the morning were compared statistically to rabbits shot in the evening and there was no significant difference between blood glucose levels for the two time periods.

Liver Glycogen Analysis

Glycogen deposition in the liver results from conversion of blood glucose to glycogen or by glucocorticoid conversion of protein and fats. Like blood glucose, the glycogen level is affected by a variety of factors such as food intake, stress, thyroid hormones, growth hormone, insulin, and others. Low liver glycogen levels may indicate a deficiency resulting from inadequate food supply, adrenal insufficiency, or liver disease. Delay in collection also affects liver glycogen because glycogenolysis occurs rapidly upon death.

Green and Larsen (1938) classified hares into three different categories according to per cent glycogen in liver samples. The categories were normal, pre-shock, and shock. They found the greatest number of hares in the normal category with a glycogen range of 1.23 per cent to 11.96 per cent and a mean of 5.56 per cent. These hares did not exhibit characteristic hypoglycemic symptoms of convulsions or coma.

The second category was pre-shock with a glycogen range from 0.05 per cent to 0.96 per cent and a mean of 0.32 per cent. Hares in this category appeared normal when discovered in the field but after handling or being placed in captivity they developed hypoglycemic convulsions ending in death.

The last category established by Green and Larsen (1938) was shock. Hares in this category were either found in the field in a convulsive state or they exhibited convulsions in captivity. Range of per cent glycogen was 0.02 per cent to 0.10 per cent with a mean of 0.08 per cent glycogen.

Recorded liver glycogen values for this study ranged from 0.122 to 1.353 per cent. All values were recorded as per cent of body weight. If data recorded in this study were classified according to Green and Larsen's categories, only three rabbits that had glycogen levels above 1.0 per cent could be considered normal. The remaining rabbits would be classified as pre-shock and shock. Correction of glycogen levels adjusted to twice as much glycogen still placed 14 rabbits in the pre-shock category. According to Green and Larsen (1938), such pre-shock rabbits should have had enough stored liver glycogen to maintain existence during non-stress. However, it is possible that had rabbits been handled or placed in captivity there would not have been enough glycogen for conversion to glucose so the blood glucose level would have decreased and hypoglycemia would have resulted. In previous years of the cottontail study trapped rabbits had been reported dying while being handled in the lab and no cause of death could be determined. Unfortunately, this did not occur during the past year so blood or liver samples could

not be taken to determine if the rabbits were in pre-shock and died from hypoglycemia.

Green and Larsen (1938) considered liver disease to be one explanation for low liver glycogen. They described the hypoglycemic hare's liver as hypotrophic and dark in color. Only one rabbit collected in this study appeared to have a diseased liver and it was firm in texture and yellow in color. The last two rabbits collected showed what may have been tularemia lesions in an early stage of development. The remaining livers appeared normal.

Statistical comparison of liver glycogen levels between sexes, by season, and between morning and evening shot rabbits showed no significant differences. Since livers sampled in this study were apparently not diseased, it is rather inconclusive why liver glycogen levels were low. It is possible that the rabbits had inadequate carbohydrate metabolism or adrenal insufficiency which caused low glycogen stores.

Adrenal Glands

Adrenal glands have frequently been studied as stress indicators because they are easily accessible. Increased activity of the pituitary and sympathetic nervous system causes increased adrenal size and increased secretions. Thus, adrenal gland size is an indirect indicator of increased pituitary activity (Barrett, 1964; Bailey and Schroeder, 1967). Studies have shown that in rodent populations increased social interaction, fighting, or increased population densities cause adrenal glands to increase in size and weight, especially in subordinate animals (Christian, 1960; Christian et. al., 1960; Chitty, 1961; Bronson and Eleftheriou, 1963; Christian and Davis, 1966; Welch and

Welch, 1969; Holst, 1972; Windberg and Keith, 1976). Enlargement results in increased epinephrine and glucocorticoid secretion. Adrenal secretions also play a role in an animal's adaptation to environmental changes and captivity (Olsen, 1973).

Table III lists adrenal weights in grams per 100 grams body weight for rabbits collected. Statistical comparisons were made between adrenal weights of male and female rabbits that were shot. Adrenal glands of some female rodents are heavier than male adrenals especially during "sexually active" periods (Christian, 1953; Christian and Davis, 1966). Increased estrogen secretion during sexually active periods stimulates adrenocorticoid secretion thus causing increased adrenal weight. Pederson (1960) and Fickess (1963) reported that adrenal glands of male rabbits were heavier than female adrenals. In this study statistical differences between the adrenal weights of male and female rabbits were not significant. There were no significant adrenal weight differences between rabbits shot in the morning and those shot in the evening. However, statistical differences existed in seasonal adrenal weights. Adrenal weights are heavier in the spring-summer season corresponding to the breeding season than they are in the winter (Beer and Mayer, 1951; Pederson, 1960; Fickess, 1963).

Live Trapping

Table V summarizes trapping success from the period 1 May 1975 to 1 May 1978. During the past two-year period there was a decline from 157 to 65 total rabbits trapped. However, there was no significant difference between the numbers of rabbits trapped per 100 trap nights for the two trapping periods. There was a significant difference in the

number of captures per 100 trap nights between 1975 to 1976 and 1977 to 1978. Thus, there may have been a population decline on the Reservation.

Since there was no significant difference between captures in 1976 to 1977 and 1977 to 1978 the total number of trap nights for the two periods was analyzed. There proved to be a significant difference. The fact that this variable was not kept constant between the two periods may have introduced bias to the data. It is possible that had the number of trap nights been kept constant a significant drop in the total captures would have been recorded.

In previous studies as well as in this one, a peak in trapping success was noted in October and November with a decline in January through March. Studies by Huber (1962), Eberhardt et. al. (1963), and Chapman and Tretheway (1972) explained fall peaks in trapping success as the result of juveniles, four to five months of age, being more susceptible than adults to trapping and the per cent of this age group being greater in the fall than at other times of the year. Bailey (1969) reported an October trapping success peak and a low in March which he attributed to a decline in trappability. Results of this study coincided with Bailey's (1969) findings. He also found snow decreased trapping success. Snow could account for no rabbit being trapped in January and only one in February of this study because during this time there was a persistent snow cover.

Roadside Survey

Results of the roadside survey are summarized in Table VI. Roadside observations are influenced by weather, time of day, and seasonal variations in activity (Alkon, 1965; Payne and Provost, 1967).

Statistical comparisons were made between the number of rabbits observed from 1 May 1976 to 1 May 1977 and the number of rabbits observed from 1 May 1977 to 1 May 1978. No significant difference was found. Because of the absence of significant difference in number of rabbits sighted in the two one-year periods it cannot be stated that there was a cottontail population decline. However, Lord (1961) found more rabbits were observed during spotlight censuses than early morning censuses. In previous years the time the survey was conducted did not change with change in day length. If more rabbits were active before sunrise than later they would not have been included in the 1976 to 1977 count because the survey was not conducted before sunrise. During the past year an effort was made to conduct the survey before sunrise. It is possible that more rabbits were observed before sunrise in the last year than would have been observed had the survey been conducted later in the morning. Therefore, if surveys for the two years had been conducted at the same time of day there might have been a significant difference in the number of rabbits sighted. For this reason the data reported do not rule out the possibility that there was a rabbit population decline.

For a total picture of what is happening in the rabbit population, blood glucose level, amount of stored liver glycogen, and adrenal weights are considered together because they are interrelated. As stated previously, liver glycogen results from carbohydrate metabolism and is a source of energy for the brain and heart (Turner and Bagnara, 1976). Muscles have their own glycogen stores but these stores cannot be converted directly to free glucose. Glucocorticoids secreted by the adrenal cortex aid in converting pyruvate and lactate from muscle glycogen to

glucose which may be stored as glycogen or enter the blood as glucose. Glucocorticoids also increase liver glycogen and blood glucose by utilization of protein and fat through gluconeogenesis.

During stress, liver glycogen is converted to glucose and moved into the blood under the influence of epinephrine from the adrenal medulla. The pituitary secretes ACTH which stimulates adrenal glucocorticoid secretion (Long et. al., 1940). Increased glucocorticoid levels convert protein and fat to glucose and glycogen thus conserving liver glycogen stores and at the same time helping to increase blood glucose. Conditions such as inadequate food supply, adverse weather conditions, or social interactions stress the rabbits and cause epinephrine and glucocorticoid secretion (Fickess, 1963).

Increased stimulation of the adrenal by the pituitary will lead to an increase in size or weight of the adrenal gland. This will in turn result in increased secretions of glucocorticoids and epinephrine. Thus an increase in size or weight of the gland is a measure of its activity. Greater amounts of adrenal hormones will mobilize stored glycogen as well as protein and fat to increase the blood glucose. Therefore, with high adrenal weight blood glucose should be high and liver glycogen may be normal or low.

Some factors affect the balance of adrenal secretions to liver glycogen amount, and adrenal secretions to blood glucose concentration. Adrenal insufficiency results in a lack of epinephrine and glucocorticoids causing a low blood glucose and low liver glycogen (Chitty, 1960; Fickess, 1963). The final result of adrenal insufficiency is hypoglycemic shock and death. If the liver is damaged or diseased little

stored glycogen is available to raise blood glucose rapidly during stress and hypoglycemia can result. Green and Larsen (1938) attributed low blood glucose and hypoglycemic shock they found to liver disease. In their study low glycogen stores could not respond to epinephrine increase during stress.

In this study live trapped rabbits did not show low blood glucose levels. High levels that were found may have been due to excitement, food consumption, exercise, or other factors. One trapped rabbit showed a blood glucose level reduction from the first to the second time it was trapped. This probably resulted from inadequate time the rabbit spent eating due to time spent in a trap. Food reduction probably lowered liver glycogen and thus lowered blood glucose.

The majority of shot rabbits had high blood glucose levels. However, using categories established by Green and Larsen (1938), 14 rabbits shot in this study had lower liver glycogen stores than normal. It has been found by Barrett et. al. (1960) that rats exposed to stress such as attack or change in ambient temperature lose most of their liver glycogen but maintain or raise blood glucose. It may be possible that rabbits were capable of maintaining blood glucose levels by mobilization of liver glycogen thus lowering the glycogen level.

A rabbit shot the last of December exhibited a low blood glucose value, 110 mg/100 ml, in relation to other blood glucose values. The liver glycogen found in this rabbit placed it in Green and Larsen's (1938) pre-shock category, yet glycogen level was not much lower than glycogen levels found in rabbits with high blood glucose levels. Adrenal weight relative to body weight was lower for this rabbit than adrenal

weights of other shot rabbits. The rabbit could have been experiencing adrenal insufficiency as indicated by lower adrenal weight and it may have been on the verge of hypoglycemia.

One other shot rabbit exhibited a lower blood glucose level than other shot rabbits. This was a pregnant female and its blood glucose was 117 mg/100 ml. The low blood glucose was contrary to results of Lissak and Endroczi (1965) who reported adrenocortical secretion increases during pregnancy thus producing blood glucose levels higher than normal. Christian and Davis (1966) also reported increased estrogen production in pregnancy increases glucocorticoids thus increasing blood glucose level. Low blood glucose and low adrenal weight of the pregnant rabbit could have been due to individual variation because the preceding rabbit, also pregnant, exhibited an elevated blood glucose value. Actual weights of the pregnant rabbit's adrenals were higher than non-pregnant winter adrenal weights but not as high as non-pregnant summer weights. Since pregnancy alters blood glucose levels, the last two rabbits were not used in statistical analyses because they were atypical.

The recorded data show that summer blood glucose levels are fairly consistent and higher than some winter values. Summer is also the time when adrenal glands exhibited a weight increase. This supports the contention that increased adrenal secretion increased blood glucose. Thus increased adrenal size due to the breeding season influenced the blood glucose levels.

Determination of relationships between rabbit blood glucose levels, liver glycogen amount, and adrenal weights were analyzed by the correlation test. Blood glucose and liver glycogen had a correlation of 0.3.

Blood glucose and adrenal weight had a correlation of 0.4; liver glycogen and adrenal weight had 0.0 correlation. Since the first two correlations were closer to zero than to one, there was little correlation among the groups.

After reviewing data presented by Green and Larsen (1938), Chitty (1959) concluded that high death rates among juveniles cause snowshoe hare population declines. Chitty disagreed with the findings of Green and Larsen (1938) and stated that their results were based on inadequate data. The juvenile decline was not considered by Green and Larsen (1938). Chitty also believed that deaths in captivity resulted from overcrowding although some physiological alteration may have occurred. Shock disease was recognizable only in dead animals, and the symptoms were non-specific. There was no relationship between abnormalities of captive hares and change in density of wild populations (Chitty, 1959). It appears that Chitty believed that hypoglycemia may have been evident upon snowshoe hare death but it may not have been the cause of death.

Since all blood glucose and liver glycogen values in this study were in the range of the normal-acting hares and in view of the fact that no significant difference was found between sexes or time or day rabbits were collected for either blood glucose or liver glycogen, and significant differences were found only between the seasons for adrenal weights, it does not appear as though hypoglycemia was evident in the rabbit population studied. Since the data were variable and showed little correlation no conclusion can be drawn. It appears as though Chitty's (1959) observations may be applicable to this study, and since the cottontails were not crowded or placed in captivity hypoglycemia did

not occur.

In conclusion, statistical analyses of samples taken from 33 rabbits showed no significant differences for blood glucose level and liver glycogen between sexes, time the rabbits were shot or season in which rabbits were shot. Adrenal weights showed no significant differences between sexes, or time of day the rabbits were shot. However, there was a significant difference in adrenal gland weights between summer and winter seasons. Adrenals weighed more in summer, during the breeding season, and less in winter. There was a significant difference in trapping success between 1975-1976 and 1977-1978. There was no significant difference in roadside observations between 1976-1977 and 1977-1978.

It does not appear that hypoglycemia occurred to an appreciable degree in the study population or that it was a contributing factor in a cottontail rabbit population decline. It cannot be stated that a population decline in fact did occur.

SUMMARY

1). A blood glucose study of the cottontail rabbit was conducted on approximately 100 acres of Ross Natural History Reservation in Lyon County, Kansas, and in portions of Osage and Coffey counties. A vehicle and a special hunting permit were provided by the Kansas Fish and Game Commission, and additional financial support was provided by the Division of Biological Sciences of Emporia State University. Data were gathered from 1 May 1977 to 1 May 1978.

2). Two groups of rabbits were studied. Blood samples were removed from six rabbits that were live trapped on the Reservation. Blood samples, liver samples, and adrenal glands were removed from 27 rabbits shot in Lyon, Osage, and Coffey counties. Tests were conducted to determine proper sampling and storage of blood and liver samples.

3). Sixty-seven captures were reported during the 12-month period from 1 May 1977 to 1 May 1978. Six blood samples were removed from live trapped rabbits. Trapping success was greatest in fall months, peaking in October and November.

4). Twenty-seven rabbits were shot in Lyon, Osage, and Coffey counties. Four were shot in May to July, two were shot in September and October, 19 were shot in December and February, and two were shot in April.

5). After shooting a rabbit, investigators removed blood and liver samples in the field. The body was transported back to the lab and adrenal glands were removed and weighed. Adrenal weight was used as an indicator of activity. The rabbits were weighed, measured, sexed, breeding condition was noted, and internal and external parasites were

noted.

6). Blood samples from live trapped and shot rabbits were analyzed for glucose concentration in mg/100 ml of sample. Liver was analyzed for glycogen concentration, reported as per cent glycogen.

7). Blood glucose concentrations, liver glycogen percentages, and adrenal weights were compared statistically to determine if there were differences between sexes, between time of day the samples were collected, and between seasons. The Student t test at p=.05 level of significance was used to determine if significant differences existed.

8). Blood glucose values for trapped rabbits ranged from 121 to 254 mg/100 ml. There was no significant difference between seasons. Blood glucose for shot rabbits ranged from 110 to 278 mg/100 ml and there was no significant difference between the seasons. No significant difference existed between sexes or between times of day the samples were collected.

9). Liver glycogen for shot rabbits ranged from 0.135 to 1.353 per cent glycogen. No significant difference existed between seasons, sexes, or times the samples were taken.

10). Adrenal weights of shot rabbits ranged from 0.007 to 0.044 g/100 g body weight. There was no significant difference between sexes or times of day samples were collected.

11). Adrenal weights of rabbits shot in the summer were higher than those shot in the winter. The mean adrenal weight for summer was 0.033 g/100 g, and the mean for winter was 0.015 g/100 g. A significant difference in adrenal weight existed between seasons. Heavier adrenal weights corresponded to the breeding season while the lower weights were

found during non-breeding season.

12). A correlation was made to determine whether or not there were relationships between some of the values obtained. Blood glucose levels were compared to liver glycogen and adrenal weights, and liver glycogen values were compared to adrenal weights. Blood glucose and liver glycogen had a correlation of 0.3. Blood glucose and adrenal weight had a correlation of 0.4. Liver glycogen and adrenal weight had a 0.0 correlation. Since the first two correlations were closer to zero than to one, there was little correlation among the groups.

13). No rabbit showed convulsions or coma. All blood glucose values were in the range established by Green and Larsen (1938) for normal-acting hares. All livers used appeared normal though the per cent glycogen may have been low. There was no significant difference between sexes, seasons, or times of collection for blood and liver samples, and there was little correlation between the groups. Therefore, it cannot be said that the rabbits were suffering from hypoglycemia.

14). From 1 May 1976 to 1 May 1977, 157 rabbits were captured for a mean of 0.45 captures per 100 trap nights. In the period 1 May 1977 to 1 May 1978, 67 rabbits were captured for a mean of 0.24 captures per 100 trap nights. Using the Student t test at $p=.05$ level of significance there was no significant difference in capture per 100 trap nights for the two periods.

15). A daily roadside survey was conducted from 1 May 1975 to 1 May 1978. From 1 May 1975 to 1 May 1976, 465 rabbits were observed with a mean of 1.43 rabbits per 100 miles. From 1 May 1976 to 1 May 1977, 1110 rabbits observations were recorded for a mean of 2.71 rabbits

per 100 miles. In the last period, 1 May 1977 to 1 May 1978, 598 rabbits were observed for a mean of 2.23 rabbits per 100 miles. Statistical comparisons were made for the periods using the Student t test at $p=.05$ level of significance and no significant difference was found.

16). Because of the lack of significant difference between roadside observation and trapping results, it cannot be said that there was a population decline.

LITERATURE CITED

LITERATURE CITED

Allen, D. L. 1954. Our wildlife legacy. Funk and Wagnalls.

Alkon, P. U. 1965. Some effects of weather and other influences on summer roadside counts of cottontails. N. Y. Fish and Game J. Vol. 12. No. 2.

Anthony, C. P., and N. J. Kolthoff. 1971. Textbook of anatomy and physiology. C. V. Mosby Co., St. Louis.

Bailey, J., and R. E. Schroeder. 1967. Weights of left and right adrenal glands in cottontails. J. Mamm. Vol. 48. No. 3.

Bailey, J. A. 1968. A weight-length relationship for evaluating physical condition of cottontails. J. Wildl. Mgmt. Vol. 32. No. 4.

_____. 1969. Trap responses of wild cottontails. J. Wildl. Mgmt. Vol. 33. No. 1.

Baker, R. J. 1975. Some aspects of rabbit ecology in Lyon Co. M. S. Thesis, Emporia State University.

Barrett, S. A., J. C. Eaton, and H. M. McCallum. 1960. Physiological effect of "social stress" in wild rats. Liver glycogen and blood glucose. J. Psychosomatic Res. Vol. 4.

Barrett, S. A. 1964. Social stress. Viewpoints in Biology. Vol. 3.

Beason, S. L., and R. A. Moore. 1977. Bobcat food habit response to a change in prey abundance. Southwestern Nat. Vol. 21. No. 4.

Beer, J. R., and R. K. Mayer. 1951. Seasonal changes in the endocrine organs and behavior patterns of the muskrat. J. Mamm. Vol. 32.

Bronson, F. H., and B. E. Eleftheriou. 1963. Adrenal responses to crowding in Peromyscus and C57 BL/10J mice. Physiol. Zoo. Vol. 36. No. 2.

Bush, I. E. 1953. Ciba found. Colloquium Endocrinology. Vol. 7.

Chapman, J. A., and D. C. Tretheway. 1972. Factors affecting trap response of introduced eastern cottontail rabbits. J. Wildl. Mgmt. Vol. 36. No. 4.

Chitty, H. 1959. A note on shock disease. Ecology. Vol. 40.

Chitty, H. 1960. Population process in the vole and their relevance to general theory. Can. Jour. Zool. Vol. 58.

Chitty, H. 1961. Variations in the weight of the adrenal glands of the field vole Microtus agrestis. J. Endocrin. Vol. 22.

Christian, J. J., A. B. Christian, and H. L. Ratcliffe. 1952. Shock disease in captive wild mammals. Am. J. Path. Vol. 28.

Christian, J. J. 1953. The relation of adrenal weight to body weight in mammals. Science. Vol. 117.

Christian, J. J. 1960. Endocrine adaptive mechanism and the physiological regulation of the population growth. Naval Med. Research Institute Lect. and Rev. Ser. Vol. 60. No. 2.

Christian, J. J., V. Flyger, and D. E. Davis. 1960. Factors in mass mortality of a herd of Sika deer, Cervus nippon. Chesapeake Science. Vo. 1. No. 2.

Christian, J. J., and D. E. Davis. 1966. Adrenal glands in female voles Microtus pennsylvanicus, as related to reproduction and population size. J. Mamm. Vol. 47. No. 1.

Christian, J. J. 1975. Hormonal control of population growth. Hormonal correlates of behavior. 1. B. E. Eleftheriou and R. L. Sprott, ed.

Clark, R. J. 1976. Cottontail rabbit ranges and movements in eastern Kansas. M. S. Thesis, Emporia Kansas State College.

Collins, J. O. 1967. Population trends by random road counts. P. R. Job Completion Report Proj. No. W-29-R-15.

Cori, G. T. 1932. Carbohydrate changes during anaerobiosis of mammalian muscle. J. Biol. Chem. Vol. 96.

Craighead, J. J., and F. C. Craighead, Jr. 1969. Hawks, Owls, and Wildlife. Dover Publications, Inc., N. Y.

Debowski, K. M. 1962. An o-Toluidine method for body-fluid glucose determination. Clinc. Chem. Vol. 8. No. 3.

Eberhardt, L., T. J. Peterle, and R. Schofield. 1963. Problems in a rabbit population study. Wild. Monogr. Vol. 10.

Fickess, D. R. 1963. Seasonal sudanophilic variation in the cottontail, Sylvilagus floridanus. Ph.D. Dissertation, Univ. Mo.

Fitch, H. S. 1947. Ecology of the cottontail rabbit (Sylvilagus auduboni) population in central California. California Fish and Game. Vol. 33.

Gipson, P. S., and J. A. Sealander. 1976. Changing food habits of wild Canis in Arkansas with emphasis on coyote hybrids and feral dogs. Am. Midl. Nat. Vol. 95. No. 1.

Green, R. G., and C. L. Larsen. 1938. A description of shock disease in the snowshoe hare. Am. J. Hygiene. Vol. 28.

_____. 1939. Shock disease as the cause of the periodic decimation of the snowshoe hare. Vol. 30-P.

Gress, R. J. 1976. Cottontail rabbit mortality in Lyon Co., Kansas. M. S. Thesis, Emporia Kansas State College.

Good, C. A., H. Kramer, and M. Somogyi. 1933. The determination of glycogen. J. Biol. Chem. Vol. 100.

Gorbman, A., and H. A. Bern. 1962. Comparative Endocrinology. Wiley, N. Y.

Guyton, A. C. 1976. Textbook of medical physiology. W. B. Saunders Co. Philadelphia.

Hoar, W. S., and C. P. Hickman, Jr. 1967. General and comparative physiology in laboratory companion. Prentice-Hall, Inc., N. J.

Holst, D. 1972. Renal failure as the cause of death in Tupia belangeri exposed to persistent social stress. J. Compar. Physiol.

Huber, J. J. 1962. Trap response of confined cottontail populations. J. Wildl. Mgmt. Vol. 26. No. 2.

Hutton, T. A. 1975. Establishment and testing of a radiotelemetry system. M. S. Thesis, Emporia State Univ.

Keith, L. B., E. C. Meslow, and O. J. Rongstad. 1968. Techniques for snowshoe hare population studies. J. Wldl. Mgmt. Vol. 32. No. 4.

Kirkpatrick, C. M. 1950. Crow predation upon nestling cottontails. J. Mamm. Vol. 31. No. 3.

Lissak, K., and E. Endroczi. 1965. The neuroendocrine control of adaptation. Pergamon Press, N. Y.

Long, C. N. H., B. Katzin, and E. G. Fry. 1940. The adrenal cortex and carbohydrate metabolism. Endocrinology. Vol. 26. No. 2.

Lord, R. D., Jr. 1961. Mortality rates of cottontail rabbits. J. Wildl. Mgmt. Vol. 25. No. 1.

_____. 1963. The cottontail rabbit in Illinois. 111. Dept. of Conserv. Techn. Bull. No. 3.

Meites, S., and N. Bohman. 1963. In vitro stabilization of blood glucose with water. Clinic. Chem. Vol. 9. No. 5.

Montgomery, R. 1957. Determination of glycogen. Archives of Biochem. and Biophysics. Vol. 67. No. 2.

Olsen, D. E. 1973. Adrenocortical responses to captivity in Microtus penn. J. Mamm. Vol. 511.

Payne, R. L., and Provost. 1967. The effects of some atmospheric variables on roadside activity in the cottontail rabbit. Proc. 21st. Ann. Conf. SE Assoc. Game and Fish Comms.

Peabody, W. C. 1977. Personal communication on harvest, abundance, and predation of cottontails. Big Game Project Leader, Kansas Forestry, Fish and Game Commission.

Pederson, V. C. 1960. Adrenal and spleen weight variation in the rabbit. M. S. Thesis. Dept. Zoo. Univ. of Missouri.

Reilly, J. R., and J. Dell. 1955. Diseases and parasites of the cottontail rabbit. N. Y. State Conserv. Vol. 9.

Romsom, D. R., and G. A. Leveille. 1974. Effect of meal frequency and diet composition on glucose tolerance in the rat. Vol. 104.

Scott, T. G., and W. D. Klimstra. 1955. Red foxes and a declining prey population. Monogr. Ser. No. 1, Southern Ill. Univ., Carbondale.

Sigma Chemical Co. 1974. A quantitative procedure for determining glucose using Ortho-Toluidine reagent. Technical Bulletin. Vol. 635.

Teimeier, O. W. 1955. Winter foods of Kansas coyotes. Trans. Kans. Acad. of Sci. Vol. 58. No. 2.

Turner, C. D., and J. T. Bagnara. 1976. General Endocrinology. W. B. Saunders Co. Philadelphia.

Voris, J. C. 1956. Factors influencing the summer roadside count of the cottontail rabbit (Sylvilagus floridanus mearnsi) in southcentral Iowa. M. S. Thesis, Iowa State College.

Watt, C. C. 1975. Cottontail rabbit ranges and mortality in eastern Kansas. M. S. Thesis, Emporia Kansas State College.

Welch, B. L., and A. S. Welch. 1969. Sustained effects of brief daily stress upon brain and adrenal catecholamines and adrenal, spleen, and heart weights of mice. Proc. of the Nat. Acad. of Sci.

Windberg, L. A., and L. B. Keith. 1956. Snowshoe hare population responses to artificial high densities. J. Mamm. Vol. 57. No. 3.

Wodzicki, K., and H. S. Roberts. 1960. Influence of population density on the adrenal and body weights of the wild rabbit, Oryctolagus cuniculus L. in New Zealand. J. of Sci. Vol. 3.

Zarros, M. X., J. M. Yochim, and J. L. McCarthy. 1964. Experimental endocrinology, a source book of basic techniques. Academic Press, N. Y.

APPENDIX A

Roadside Observation

Data Sheet

ROADSIDE OBSERVATION DATA SHEETS

ROUTE #1: ROUTE #2:

DATE:

TIME LEFT:

OBSERVERS:

WEATHER CONDITIONS:

TEMP.:

PRECP.:

RABBIT SIGHTING=1

COYOTE SIGHTING=2

DEER SIGHTING=3

COMMENTS: