WATER BALANCE IN FOUR SPECIES
OF SCIURID MAMMALS

A THESIS
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA

By
Eugene B. Bakko

IN PARTIAL FULLFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

December, 1973

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to Dr. William D. Schmid of the Department of Zoology, University of Minnesota, for his guidance, assistance, and advice throughout the tenure of this study and in the preparation of the manuscript. Special thanks are extended to Dr. J. C. Underhill, Duane Nelson, Thomas Clifford, Thomas Rollings, and James Evestvedt for their assistance in various parts of the field work and to Mr. Robert Cunningham, Chief Naturalist, Theodore Rooseveldt National Park, for permission to live trap prairie dogs. The comments and constructive criticism of Drs. E. C. Birney, P. J. Regal, J. C. Underhill, and J. R. Tester were most helpful in preparing this manuscript. The technical assistance of Wallace Saatala and Lynn Steere was greatly appreciated. I also wish to acknowledge the use of the Itasca Biology Station facilities during part of the research. Finally, I am especially grateful to my wife, Lois, for her encouragement throughout the study and for her help in typing the manuscript. This study was supported in part by NSF Traineeships GB6158 and GZ1628.

TABLE OF CONTENTS

INTRODUCTION

The study of water balance in wild mammals has generated considerable information to comparative animal physiology and biochemistry only within recent years (B. Schmidt-Nielsen 1964, K. Schmidt-Nielsen 1964, Chew 1965, Schmidt-Nielsen and Kerr 1970). However, most of this information has been a result of laboratory studies on the effects of stressful conditions such as water deprivation and salt loading.

While some field data have been collected concerning habitat conditions with respect to water balance (Vorhies 1945, Schmidt-Nielsen and Schmidt-Nielsen 1950, Carpenter 1966) there have been few physiological data collected from organisms in their natural environment. MacMillen (1972) reported seasonal variations in total osmolality of urine in some cricetid and heteromyid rodents in their natural environment. Schmid (1972) presented evidence that the daily temperature cycle may impose greater dehydration stress upon small, homeothermic animals at night compared to animals with diurnal activities because of its influence upon ambient vapor pressure. Mullen (1970) used doubly labeled water (D_2O^{18}) to measure respiratory metabolism and water turnover rates in *Perognathus formosus* in natural conditions. Jameson and Mead (1969) measured seasonal changes in body weight, fat, and body water in *Spermophilus lateralis* and *Eutamias speciosus*.

There have, however, been no measurements made of urine and plasma concentrations and their solute components of mammals from natural populations throughout the year. Thus, the purpose of this study has been to collect data from wild populations of four species of sciurid rodents during different seasons of the year in an effort to learn how these animals react physiologically with respect to water balance. In addition, laboratory experiments under varying conditions were designed to gain further insight to the physiological limits of these animals.

Sciurid mammals were studied because of their convenient body size, general availability, and the fact that adequate quantities of urine and blood for all analyses could be obtained from individuals of the species studied. The four species studied were gray squirrels (*Sciurus carolinensis*), red squirrels (*Tamiasciurus hudsonicus*), black-tailed prairie dogs (*Cynomys ludovicianus*), and white-tailed prairie dogs (*Cynomys leucurus*).

Gray squirrels are closely associated with eastern hardwood forests, whereas red squirrels occupy a wide variety of sylvatic habitats within their range, both coniferous and deciduous (Hall and Kelson 1959). I therefore decided to work with these two species to see what role, if any, water economy played in the ability of the red squirrel to occupy a wide variety of habitats compared to the gray squirrel, or conversely, if water balance characteristics might be limiting to the distribution of gray squirrels.

Although both species of prairie dogs occur in the grassland biome, white-tailed prairie dogs are more inter-montane in their distribution (Hall and Kelson 1959). These inter-montane prairies are more subject to rain shadow effects from the Rocky Mountains and consequently receive less annual precipitation than do the more eastern grasslands occupied by black-tailed prairie dogs, where average annual precipitation is greater. Based on this habitat difference it might be predicted that white-tailed prairie dogs are physiologically better adapted to conserve water. However, one other factor complicates this situation. Several papers have reported that white-tailed prairie dogs probably hibernate because this species has not been observed above ground in winter (Stockard 1930, Tileston and Lechleitner 1966, Bakko and Brown 1967). It has also been generally concluded that black-tailed prairie dogs do not hibernate because this species has been observed above ground during all seasons (King 1955, Koford 1958, Smith 1958). Because hypothermia may be an adaptation to stressful conditions imposed by lack of water (Fisher and Manery 1967, Schmid 1972), the question arises as to whether the white-tailed prairie dog is better adapted for water conservation because it inhabits a more arid environment than the black-tailed prairie dog, or, is the black-tailed prairie dog, in fact, better adapted for conserving water, even though it lives in a more mesic environment because it cannot escape environmental rigors by means of hibernation. The foregoing questions provided a basis for studying these species.

Because of the obvious differences in habitat and behavior between tree squirrels and prairie dogs, a results and discussion section is written for each group. I plan to publish this information in two separate papers. However, a general discussion follows to consider the overall relationships and possible evolutionary significance of water balance physiology in all four species.

MATERIALS AND METHODS

Collection of squirrels was initiated in August, 1968, and terminated in November, 1971. A total of 143 gray squirrels was collected in Anoka, Chisago, Ramsey, and Winona counties, Minnesota; while 137 red squirrels were collected in Anoka, Clay, Chisago, and Clearwater counties, Minnesota. Prairie dogs were collected during June and July, 1969; March, June, and August, 1970; and September and November of 1971. Seventy-five black-tailed prairie dogs were collected in Billings and Bowman counties, North Dakota, and in Butte County, South Dakota. Sixty-seven white-tailed prairie dogs were collected in Albany County, Wyoming. Tree squirrels collected for field data were shot with a .22 caliber rifle or a .410 gauge shotgun. Prairie dogs were shot with a .22 caliber rifle. Most animals collected in the above manner were killed instantly.

Urine samples were taken by bladder puncture with a syringe. Blood was collected by heart puncture with a lightly heparinized syringe. Blood was centrifuged immediately with a specially machined centrifuge head mounted on a car heater fan motor using a 12-V car battery as a power source. Plasma was decanted and preserved in air tight polyethylene centrifuge tubes (0.4 ml, A. H. Thomas Co., Philadelphia, Pa.) as were urine samples. These samples were placed immediately on ice until they could be frozen (within 2-3 hours) for later laboratory analyses.

Body weight was measured to the nearest 0.1 grams. Kidneys were excised and preserved in AFA (10% formaldehyde, 10% glacial acetic acid, 30% alcohol, 50% distilled water) for later examination. Reproductive tracts of females and testes of males often were preserved in AFA for possible use in determination of age and general reproductive activity. Field notes included locality, habitat, date, and weather conditions at the time of collection. General condition of the animals was noted with special reference to reproductive condition.

All animals live trapped for experimental laboratory study were collected with open wire traps constructed for this study or with live traps manufactured by the National Live-trap Company. Prairie dogs were housed in 2.4 X 2.4 meter floor cages with Purina Rat Chow, sunflower seeds, and water available ad libitum. Because of their availability, squirrels were trapped just prior to experimentation and held in metabolic cages for several days of adjustment before data were collected. Stainless steel cages (20 X 25 X 16.5 cm, Acme Metal Products Co., Chicago) were used to obtain daily quantitative measurements of food and water consumption and of urine and feces production. Body weight was measured daily. The cages provided for separation of urine and feces. Urine was collected under mineral oil to prevent evaporation. Spillage of drinking water by the animals was collected separately under oil and not allowed to contaminate urine samples. Pulverized, air-dried Purina Rat Chow was the food source in experiments. Urine samples to be analyzed for constituents were collected just after urination and then frozen in air tight (polyethylene) vials for later determinations. All metabolic cage experiments were conducted at $22^{\circ}C$ under either constant lighting or a 16:8 light-dark cycle. Two experimental procedures were conducted on laboratory animals in metabolic cages: 1) with food and water *ad libitum*, 2) with food *ad libitum* only.

Red squirrels were live trapped in July, 1969, in an attempt to collect a field urine sample from each, then release the animal and follow the same procedure after one month. Because red squirrels were somewhat hyperactive when the trap was approached, it was assumed that each animal had urinated before or while the trap was first handled. Consequently, the traps were placed on tin foil and kept under observation until urination occurred (2-3 hours). Urine samples were collected immediately and each animal released at the point of capture. A similar live trapping procedure was conducted on gray squirrels to collect urine samples in January and February of 1970. However, the animals were usually brought back to the laboratory to wait for urination to occur because this would often take a day or more, in contrast to red squirrels. Only fresh voided urine was analyzed.

Urine (1:9 dilution) and blood plasma (undiluted) were analyzed for total osmoconcentration by the freezing-point depression method on an Osmette osmometer (Model 2007, Precision Systems, Waltham, Massachusetts). Urea and ammonia concentrations were determined by the Conway boric acid-HCl microdiffusion technique as modified by Obrink (1955). Sodium and potassium levels of urine (1:400 dilution) and blood plasma (1:200 dilution) were measured on a flame photometer (Model 143, Instrumentation Laboratory, Inc.). Chloride was presumed to be present in substantial ammounts and approximately equal to potassium and sodium concentrations combined.

Relative medullary thickness (rmt) of kidneys was determined according to Sperber (1944) by the following formula: $rmt = 10(r)(TXHXL)^{-.33}$. This required measurements of 1) gross dimensions of the kidney, thickness (T), height (H), and length (L); and 2) the radial extension (r) of the medulla. The medulla radius was exposed by making a mid-sagittal cut through the kidney with a razor blade such that the maximum area of the medulla from the cortical-medullary boundary to the tip of the renal papilla was visible. Several measurements from the renal papilla tip to various locations on the cortical-medullary boundary were averaged to obtain the r value in Sperber's formula. Measurements were made to the nearest 0.1mm with dial calipers. Representative kidneys of each species were prepared histologically and examined microscopically to verify the cortical-medullary boundary. Relative medullary thickness was averaged for each pair of kidneys to obtain one value for each animal.

Statistical procedures involved the use of Student's t test to evaluate differences between means. All differences discussed in this paper were significant at the $P \leq 0.05$ level unless stated otherwise. Confidence intervals for each mean were calculated as $\bar{X} \pm T_{0.975}S.E.$ Variances of compared means were tested for similarity by an F-test at $P \leq 0.025$. If variances were different, a weighted t value was computed by the method of Cochran and Cox (1964) to conduct the test.

TREE SQUIRRELS

Results

Urine.--Table 1 shows annual and monthly precipitation as well as a 30 year average for the Itasca State Park area (coniferous habitat) and the Minneapolis-St. Paul area (mixed hardwood habitat) where most red and gray squirrels, respectively, were collected. Most precipitation occurs during the summer months with the June average being greater than other months for both localities. Snow usually accummulates from late November or early December and persists until mid- or late March. The winter season is slightly longer at Itasca than at Minneapolis.

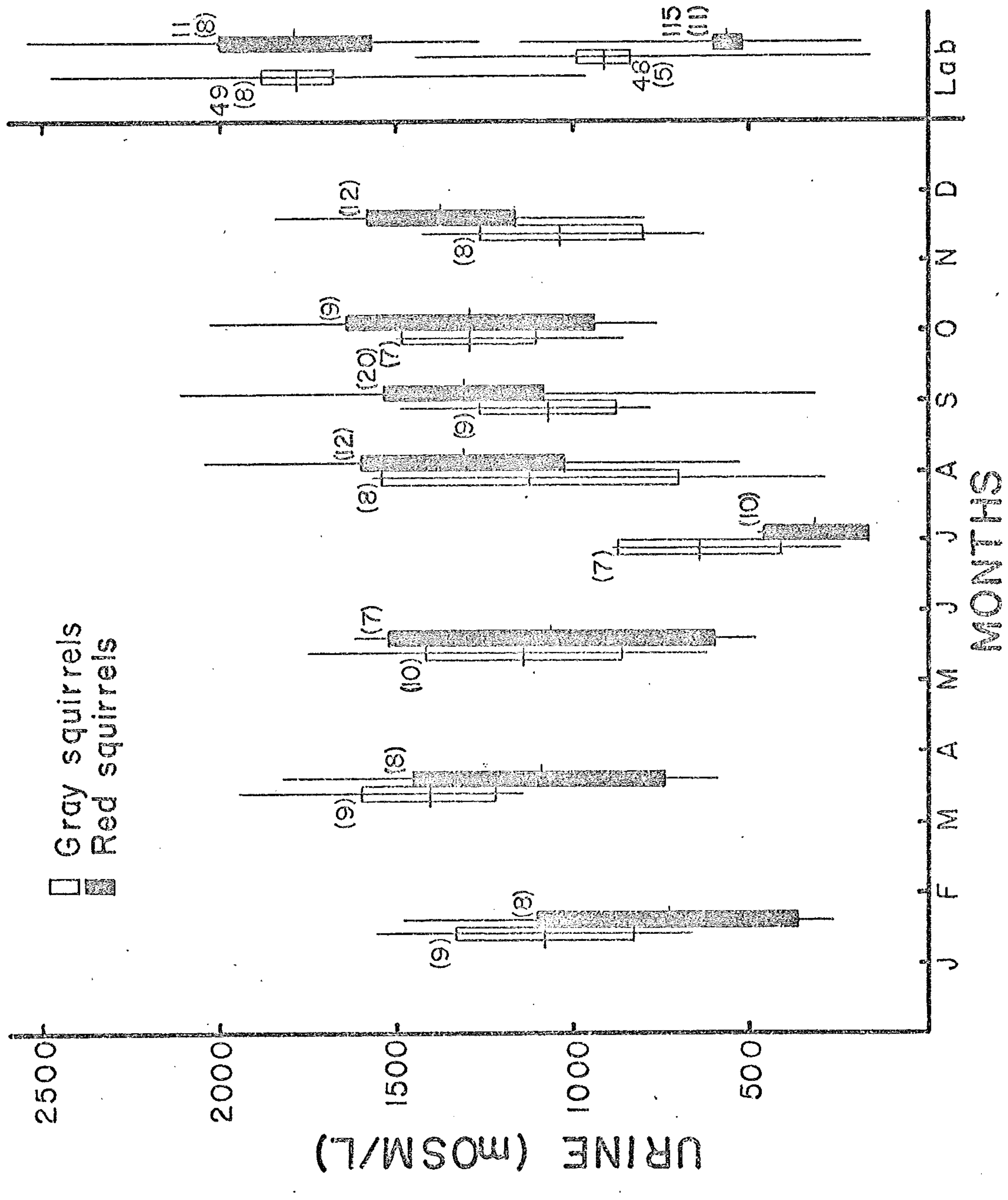
Urine concentrations of July samples for both gray and red squirrels were lower than any other month (Fig. 1). The July urine concentration for red squirrels was lower than that for gray squirrels. The July sample for red squirrels was collected near Itasca Park in 1969 and the July sample for gray squirrels was collected near St. Paul in 1970.

The January-February urine sample for gray squirrels was less concentrated than the March-April collection for that species. Although a similar trend was evident for red squirrels for the same periods, the difference between the two samples was not significant. There was a difference between the November-December collection and the January-February collection for red squirrels. Maximum urine concentration of dehydrated laboratory animals was greater than any of the monthly field collections.

TABLE 1. Annual and monthly precipitation in cm for Itasca State Park and Minneapolis-St. Paul, Minnesota, for the period 1931-1960 and for the years 1968, 1969, 1970 and 1971. (U. S. Dept. Commerce. Environmental Science Services Administration. Minnesota Climatological Data, vols. 74-77: no. 13.)

	Itasca State Park					Minneapolis-St. Paul				
Month	1931-1960	1968	1969	1970	1971	1931-1960	1968	1969	1970	1971
J	1.9	2.2	6.8	0.7	2.8	1.8	1.8	5.2	1.2	3.1
F	1.6	0.5	2.2	0.9	1.9	2.0	0.3	0.8	0.4	4.4
M	3.1	4.1	0.6	2.3	2.5	3.9	4.8	2.3	5.2	3.1
A	5.9	11.9	3.9	8.0	4.9	4.7	7.5	3.9	7.2	2.8
M	8.5	5.8	6.9	4.8	5.8	8.1	9.5	5.0	12.1	8.0
J	10.6	15.7	5.6	10.0	10.9	10.2	17.2	7.4	3.2	8.9
J	9.1	10.6	16.0	2.7	9.0	8.3	16.4	7.5	9.3	10.0
A	8.9	6.7	3.8	0.8	3.2	8.1	1.9	2.5	5.6	4.5
S	5.3	9.8	9.4	9.5	11.9	6.2	15.7	1.2	8.1	6.9
O	3.8	7.4	10.6	11.4	16.9	4.0	14.3	6.4	12.6	14.4
N	3.3	1.3	2.5	2.7	3.0	3.6	1.4	1.4	9.7	6.8
D	2.1	6.8	3.2	4.0	1.0	2.2	5.6	5.2	1.1	1.8
Total	64.1	82.9	71.6	57.8	73.8	62.9	96.3	49.0	75.7	74.8

FIGURE 1. Field and laboratory urine osmolalities for gray and red squirrels. Vertical lines represent ranges; horizontal lines represent means ($\bar{X}$); rectangles enclose the intervals $\bar{X} \pm T_{0.975}S.E.$ The numbers in parentheses represent the number of animals. For laboratory data, upper samples represent dehydrated animals and lower samples represent hydrated animals. Numbers outside the parentheses represent the number of measurements from the animals invloved.


URINE (mOSM/L)
2500
2000
1500
1000
500
Gray squirrels
Red squirrels
(9)
(8)
(9)
(8)
(10)
(7)
(7)
(10)
(8)
(12)
(9)
(20)
(7)
(9)
(8)
(12)
49
(8)
11
(8)
46
(5)
115
(11)
J
F
M
A
M
J
J
A
S
O
N
D
Lab
MONTHS

Urine samples from 13 live trapped red squirrels in July, 1969, showed a urine concentration of 2074.9 ± 272.4 mOsm (range = 990-2704 mOsm). This compared to 309.0 ± 147.1 mOsm (range = 170-832 mOsm) for 10 red squirrels shot during the same month of that year in the same location (Fig. 1). Urine concentration of eight dehydrated laboratory animals was 1787.2 ± 213.5 mOsm (range = 1272-2550 mOsm). The difference between urine from the live trapped animals and the dehydrated animals was not significant although the mean and the highest individual concentration for the live trapped animals was greater than for dehydrated animals.

Urine osmolality of nine live trapped gray squirrels in January-February, 1970, was 1458.9 ± 161.5 mOsm (range = 1223-1837 mOsm). This compared with 1082.4 ± 254.7 mOsm (range = 668-1558 mOsm) for nine gray squirrels shot during the same period. Eight dehydrated gray squirrels produced a urine concentration of 1783.3 ± 98.7 mOsm (range = 965-2475 mOsm). Consequently, all data for field animals reported in this study were taken from shot rather than live trapped animals.

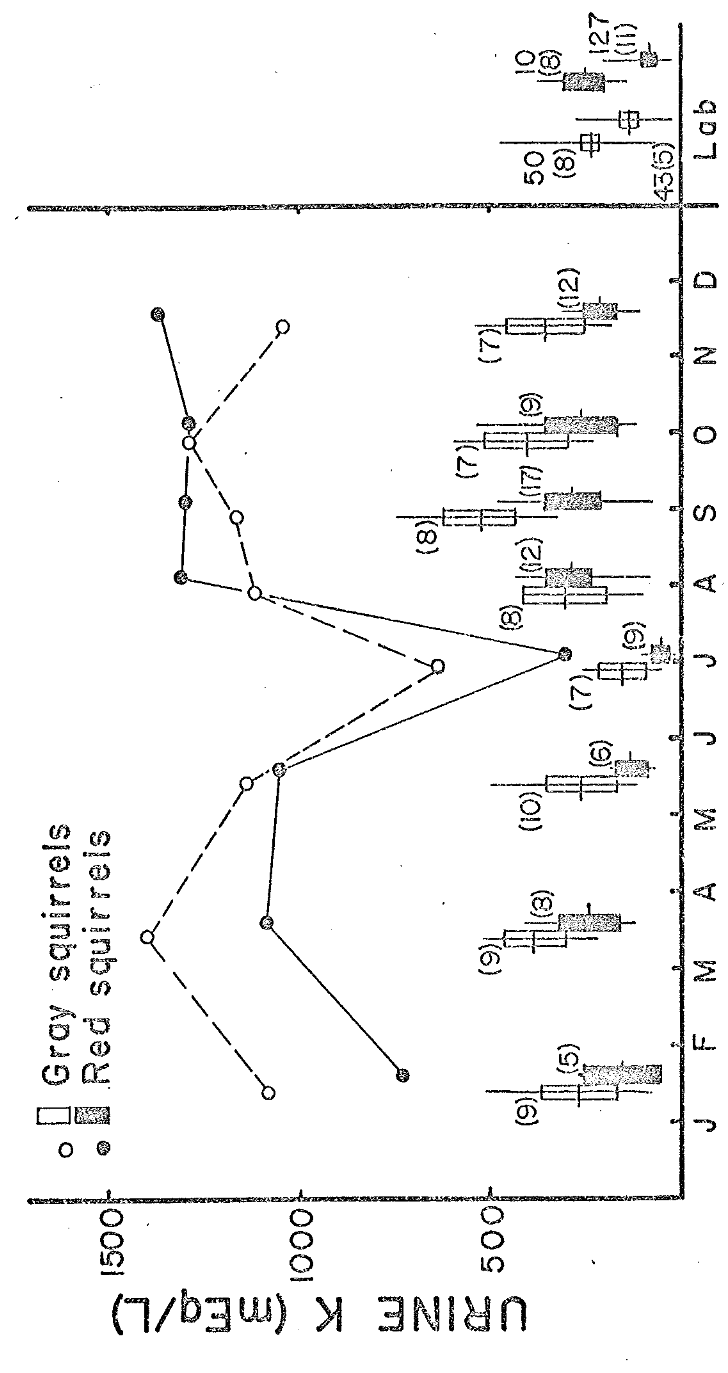
Potassium and urea were the major solute constituents present in urine collected from field animals. Figure 2 illustrates the influence of potassium on total urine osmolality. Potassium concentration was generally higher for gray squirrels than for red squirrels. Only the January-February and the August samples for gray squirrels were not significantly greater than the samples for red squirrels for the same periods. The July potassium sample for each species was lower than all the other field samples for each

Urine samples from 13 live trapped red squirrels in July, 1969, showed a urine concentration of 2074.9 ± 272.4 mOsm (range = 990-2704 mOsm). This compared to 309.0 ± 147.1 mOsm (range = 170-832 mOsm) for 10 red squirrels shot during the same month of that year in the same location (Fig. 1). Urine concentration of eight dehydrated laboratory animals was 1787.2 ± 213.5 mOsm (range = 1272-2550 mOsm). The difference between urine from the live trapped animals and the dehydrated animals was not significant although the mean and the highest individual concentration for the live trapped animals was greater than for dehydrated animals.

Urine osmolality of nine live trapped gray squirrels in January-February, 1970, was 1458.9 ± 161.5 mOsm (range = 1223-1837 mOsm). This compared with 1082.4 ± 254.7 mOsm (range = 668-1558 mOsm) for nine gray squirrels shot during the same period. Eight dehydrated gray squirrels produced a urine concentration of 1783.3 ± 98.7 mOsm (range = 965-2475 mOsm). Consequently, all data for field animals reported in this study were taken from shot rather than live trapped animals.

Potassium and urea were the major solute constituents present in urine collected from field animals. Figure 2 illustrates the influence of potassium on total urine osmolality. Potassium concentration was generally higher for gray squirrels than for red squirrels. Only the January-February and the August samples for gray squirrels were not significantly greater than the samples for red squirrels for the same periods. The July potassium sample for each species was lower than all the other field samples for each

FIGURE 2. Field urine potassium concentrations compared to laboratory urine potassium and to field urine osmolalities. Open circles connected by a dotted line represent mean ($\bar{X}$) urine osmolality for gray squirrels. Closed circles connected by a solid line represent mean ($\bar{X}$) urine osmolality for red squirrels. Vertical lines represent ranges; horizontal lines represent means ($\bar{X}$); rectangles enclose the intervals $\bar{X} \pm T_{0.975}$S.E. Numbers in parentheses represent the number of animals. For laboratory data, upper samples represent dehydrated animals and lower samples represent hydrated animals. Numbers outside the parentheses represent the number of measurements from the animals involved.


○ □ Gray squirrels
● ▨ Red squirrels
URINE K (mEq/L)
1500
1000
500
J
F
M
A
M
J
J
A
S
O
N
D
Lab
MONTHS
(9)
(5)
(9)
(8)
(10)
(6)
(7)
(9)
(8)
(12)
(8)
(17)
(7)
(9)
(7)
(12)
50
(8)
43(5)
10
(8)
127
(11)

respective species. The March-April potassium sample for gray squirrels was greater than either the January-February or the June samples for that species. Maximum potassium concentration from dehydrated laboratory animals of both species was exceeded by some of the field samples. However, only for gray squirrels was field potassium concentration significantly higher than those obtained under stressful laboratory conditions.

Urea concentration generally followed the same pattern as total urine concentration but perhaps not quite as closely as did potassium concentration (Fig. 3). For instance, the July urea sample for gray squirrels while lower than any other sample for that species, was not significantly lower than the March-April, September, or the November-December samples. Also, the difference between the July and the January-February sample for red squirrels was not significant. Urine urea concentration in red squirrels during September and November-December was higher than urea concentration in gray squirrels for those same periods. Urea concentration from dehydrated laboratory animals was greater than any of the field samples for both species.

Sodium concentration in urine from field animals of both species was low (Table 2). There was considerable variation within each collection period and consequently most of the samples were not significantly different from one another. However, sodium concentrations during late winter-early spring tended to be higher than for the rest of the year for both species. There was no difference between the two species for the annual mean sodium

FIGURE 3. Field urine urea concentrations compared to laboratory urine urea and to field urine osmolalities. Open circles connected by a dotted line represent mean ($\bar{X}$) urine osmolality for gray squirrels. Closed circles connected by a solid line represent mean ($\bar{X}$) urine osmolality for red squirrels. Vertical lines represent ranges; horizontal lines represent means ($\bar{X}$); rectangles enclose the intervals $\bar{X} \pm T_{0.975}$S.E. Numbers in parentheses represent the number of animals. For laboratory data, upper samples represent dehydrated animals and lower samples represent hydrated animals. Numbers outside the parentheses represent the number of measurements from the animals involved.

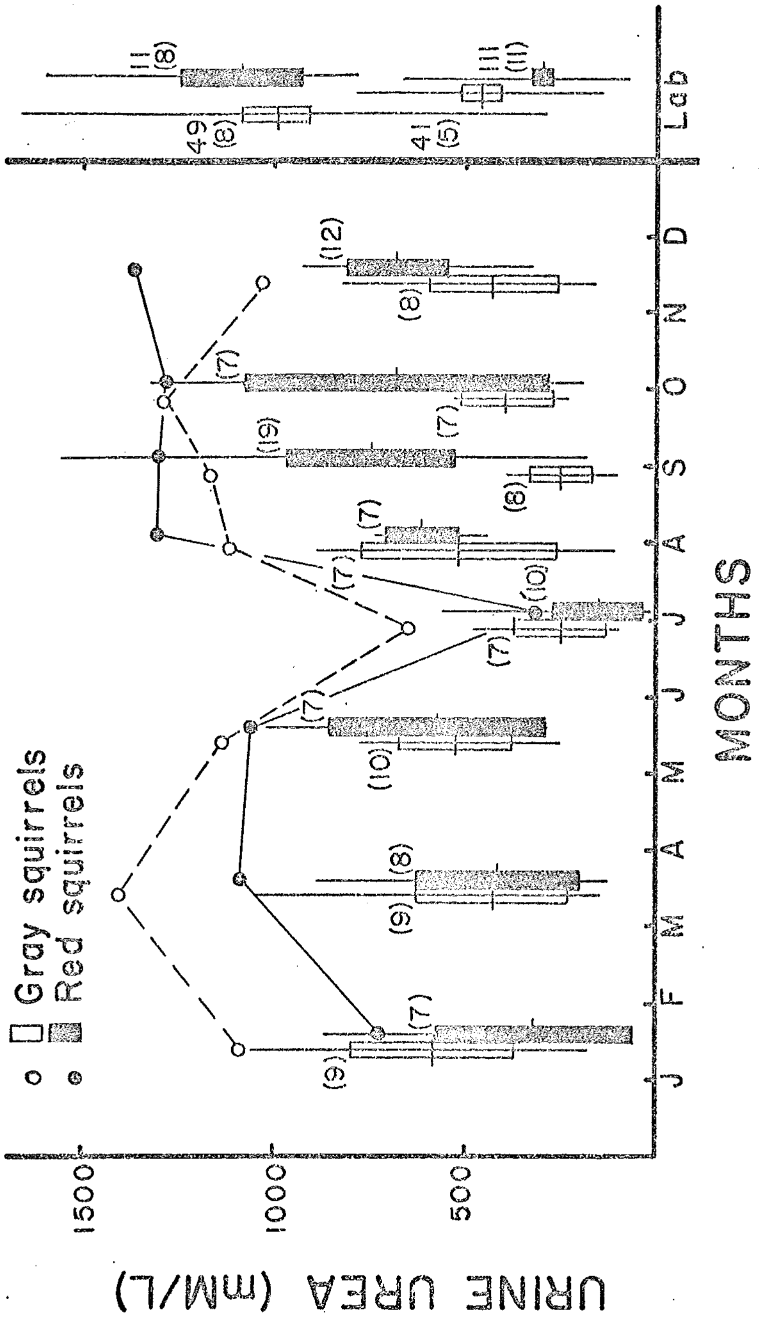

○ □ Gray squirrels
● ▨ Red squirrels
URINE UREA (mM/L)
1500
1000
500
J
F
M
A
M
J
J
A
S
O
N
D
Lab
MONTHS
(9)
(7)
(9)
(8)
(10)
(7)
(7)
(10)
(7)
(7)
(8)
(19)
(7)
(7)
(8)
(12)
49
(8)
41
(5)
11
(8)
11
(11)

TABLE 2. Field and laboratory urine sodium concentrations (mEq/L) in gray and red squirrels. Numbers in parentheses represent number of animals from which samples were taken.

	Gray squirrels			Red squirrels		
Period	$\bar{X} \pm (T_{0.975})(S.E.)$	N	range	$\bar{X} \pm (T_{0.975})(S.E.)$	N	range
J-F	4.1 ± 1.4	7	2.0 - 6.0	58.8 ± 67.2	4	9.0 -123.0
M-A	11.1 ± 3.0	8	5.5 - 16.5	11.6 ± 6.6	7	2.1 - 22.0
M-J	11.0 ± 7.7	9	3.2 - 38.0	6.6 ± 5.5	6	3.0 - 18.0
J	14.3 ± 11.7	4	8.0 - 26.0	3.4 ± 0.4	9	2.8 - 4.6
A	3.4 ± 2.9	5	2.1 - 8.2	6.6 ± 3.9	10	3.0 - 19.0
S	3.4 ± 0.9	13	2.0 - 8.1	3.7 ± 0.6	17	2.2 - 6.2
O	8.7 ± 8.5	7	3.0 - 31.0	3.8 ± 1.1	9	2.2 - 7.0
N-D	5.3 ± 5.2	4	1.9 - 9.0	6.3 ± 3.3	12	2.1 - 19.2
Average	7.6 ± 2.0	57	1.9 - 38.0	8.4 ± 3.8	74	2.1 -123.0
Lab w/ H_2O	92.0 ± 11.6	43(8)	22.0 -163.0	63.6 ± 6.0	127(11)	14.0 -194.0
Lab w/o H_2O	18.9 ± 8.4	50(5)	2.0 -172.0	13.5 ± 5.3	18(8)	2.0 - 42.0

concentration. Urine sodium concentration under laboratory conditions was higher than the field urine sodium concentrations.

Ammonia concentrations in urine were low (Table 3). Variability was high with little difference among months for either species. Urine concentrations of ammonia for red squirrels were generally higher than those of gray squirrels for most monthly periods although not all these differences were significant. The mean annual ammonia concentration for red squirrels was greater than for gray squirrels. Water deprived animals of both species demonstrated higher ammonia concentrations than field animals, with ammonia concentration in red squirrels being greater than for gray squirrels.

Plasma.--Osmolality of blood plasma from field samples was greater for gray squirrels than for red squirrels (Table 4). However, plasma osmolality of laboratory animals with water _ad libitum_ was higher for red squirrels. Plasma osmolality for both species under water deprivation was greatly increased over field plasma and plasma collected from hydrated animals. There was no difference between species when comparing plasma samples from dehydrated animals. No difference between plasma samples collected at different times of the year was observed for either species.

Sodium concentration in plasma samples from laboratory hydrated animals was greater than from field samples. Plasma sodium demonstrated a marked increase in dehydrated animals over hydrated animals. There were no differences between species in plasma sodium for field animals or for hydrated or dehydrated laboratory animals.

TABLE 3. Field and laboratory urine ammonia concentrations (mEq/L) in gray and red squirrels. Numbers in parentheses represent number of animals from which samples were taken.

	Gray squirrels			Red squirrels		
Period	$\bar{X} \pm (T_{0.975})$(S.E.)	N	range	$\bar{X} \pm (T_{0.975})$(S.E.)	N	range
J-F	60.0 ± 37.4	9	18-144	30.3 ± 41.3	6	10-114
M-A	22.4 ± 10.0	9	6- 52	73.5 ± 46.9	8	10-188
M-J	16.6 ± 6.9	10	0- 36	81.3 ± 53.4	6	23-140
J	36.7 ± 26.6	6	6- 62	40.5 ± 13.7	10	16- 77
A	22.0 ± 34.5	6	6- 88	50.7 ± 30.2	12	10- 44
S	18.9 ± 7.8	7	10- 34	49.1 ± 18.9	18	10-140
O	27.9 ± 18.1	7	6- 62	59.4 ± 45.4	7	16-160
N-D	28.0 ± 30.4	5	6- 64	75.5 ± 33.0	11	8-140
Average	29.3 ± 7.4	59	0-144	56.1 ± 10.0	78	8-188
Lab w/ H_2O	39.2 ± 6.8	39(8)	16-100	36.9 ± 4.0	111(11)	4- 98
Lab w/o H_2O	81.2 ± 12.8	49(5)	26-224	101.1 ± 16.1	16(8)	54-156

TABLE 4. Field and laboratory plasma concentrations in gray and red squirrels [$\bar{X} \pm (T_{0.975})(S.E.)$]. Numbers in parentheses represent number of animals from which samples were taken.

Species and condition	Total osmolality (mOsm/L)	Na^+ (mEq/L)	K^+ (mEq/L)	Urea (mM/L)
Gray squirrels				
Field	312.1 ± 4.1	140.2 ± 2.8	12.6 ± 1.0	5.07 ± 0.66
	N = 68	N = 72	N = 73	N = 76
	r = 252-360	r = 100-167	r = 4.4-27.0	r = 1.6-18.7
Lab w/ H_2O	301.3 ± 4.5	156.1 ± 6.0	3.41 ± 0.28	3.44 ± 0.68
	N = 6(3)	N = 7(3)	N = 7(3)	N = 7(3)
	r = 296-306	r = 145-164	r = 2.9-4.0	r = 2.8-5.8
Lab w/o H_2O	431.3 ± 55.2	197.0 ± 17.2	4.65 ± 0.82	11.35 ± 7.65
	N = 6(3)	N = 6(3)	N = 6(3)	N = 6(3)
	r = 364-510	r = 172-215	r = 3.4-5.4	r = 3.7-20.8

TABLE 4 (cont.). Field and laboratory plasma concentrations in gray and red squirrels $[\bar{X} \pm (T_{0.975})(S.E.)]$. Numbers in parentheses represent number of animals from which samples were taken.

Species and condition	Total osmolality (mOsm/L)	Na^+ (mEq/L)	K^+ (mEq/L)	Urea (mM/L)
Red squirrels				
Field	303.3 ± 3.3	142.8 ± 3.9	12.0 ± 0.8	6.25 ± 0.61
	N = 43	N = 85	N = 85	N = 51
	r = 277-324	r = 105-228	r = 5.2-24.0	r = 1.8-11.0
Lab w/ H_2O	308.3 ± 3.8	156.6 ± 3.8	3.69 ± 0.53	6.08 ± 0.52
	N = 12(4)	N = 18(4)	N = 17(4)	N = 18(4)
	r = 299-316	r = 134-168	r = 2.4-5.8	r = 4.4-8.6
Lab w/o H_2O	430.8 ± 46.8	199.1 ± 19.1	5.93 ± 1.70	28.66 ± 19.78
	N = 8(5)	N = 9(5)	N = 9(5)	N = 8(5)
	r = 336-516	r = 171-257	r = 3.6-11.0	r = 7.3-66.9

Potassium concentration was substantially higher in plasma from animals collected in the field than in either hydrated or dehydrated laboratory animals. Dehydrated animals of both species demonstrated significantly higher plasma potassium concentrations than were found in hydrated animals. There were no interspecific differences in plasma potassium between field animals, hydrated animals, or dehydrated animals.

Plasma urea concentrations in red squirrels from field and hydrated laboratory conditions were greater than in gray squirrels under the same conditions. Urea was also higher in plasma from dehydrated red squirrels than in plasma from dehydrated gray squirrels, but this difference was not significant. Urea was higher in plasma from dehydrated red squirrels than either field or hydrated animals of that species. Plasma urea from dehydrated gray squirrels was greater than from hydrated gray squirrels but was not different from field collected gray squirrels.

Kidneys.--The countercurrent multiplier hypothesis, which employs the loops of Henle as an exchange system to create increased osmotic concentration in the kidney from cortex to papilla has become widely accepted with little doubt about its validity (Ullrich, Kramer, and Boylan 1961). An extensive morphological study by Sperber (1944) demonstrated that mammals of dry habitats possessed kidneys with longer renal papilla than species from more mesic or hydric habitats. Since then, other studies have shown that the length of the renal papilla is related to habitat and the ability of the animal to concentrate urine (Vimtrup and Schmidt-Nielsen 1952, Schmidt-Nielsen and O'Dell 1961, MacMillen and Lee 1969, Schmid 1972). Calculation of Sperber's relative medullary thickness was applied to red and gray squirrel kidneys to determine if morphological differences exist. (Table 5).

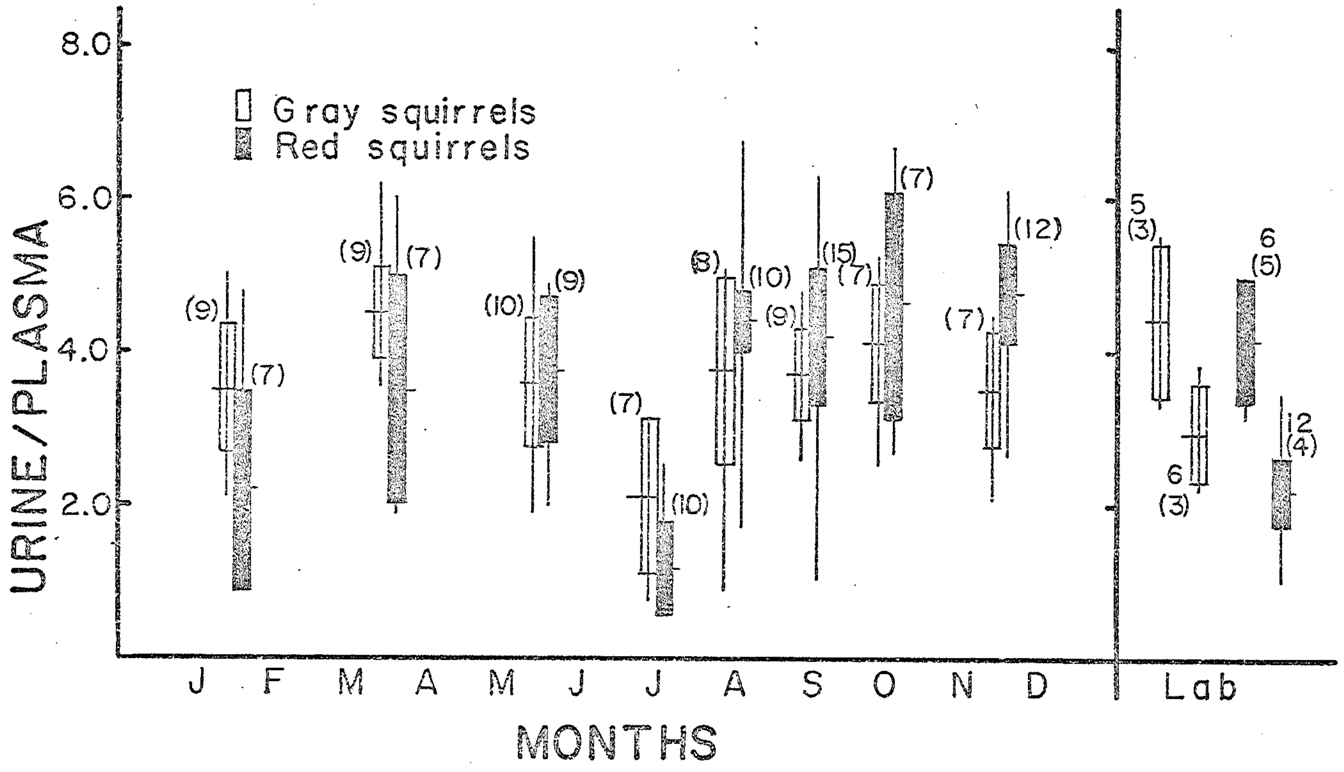
Red squirrels had the greatest relative medullary thickness when comparing kidneys from all animals of each species. However, if kidneys from red squirrels are separated into groups according to the habitat in which individuals were collected, intraspecific differences are observed. The overall mean for relative medullary thickness in red squirrels was less than the sample from coniferous and mixed hardwood habitats and was greater than the sample from river bottoms. The coniferous-mixed hardwood group was separated and tested for differences between samples collected in coniferous habitats and those collected in mixed hardwoods. No difference was detected. Relative medullary thickness for red squirrels from river bottoms was not significantly different from rmt for gray squirrels. There was no difference in rmt between juveniles, subadults, or adults for either species. Histological maturity of kidneys occurs in four weeks in rats (Baxter and Yoffey 1948). Similar kidney development apparently occurs in gray and red squirrels.

Urine osmolality compared to plasma osmolality as a urine/plasma ratio (U/P) is an indication of the ability of the kidney to concentrate urine. These ratios are shown in Figure 4. Because there was little variability in plasma concentration in either species (Table 4), seasonal trends in U/P were similar to seasonal trends in total urine concentration (Fig. 1). Maximum U/P values

TABLE 5. Relative medullary thickness of kidneys from gray and red squirrels.

	Gray squirrels	Red squirrels		
	All (N = 120)	All (N = 102)	Coniferous & mixed hardwood (N = 75)	River bottom (N = 27)
$\bar{X} \pm (T_{o.975})(S.E.)$	5.15 ± 0.08	5.79 ± 0.12	5.95 ± 0.12	5.32 ± 0.23
Range	(4.16 - 6.34)	(4.70 - 7.65)	(4.79 - 7.33)	(4.70 - 7.65)

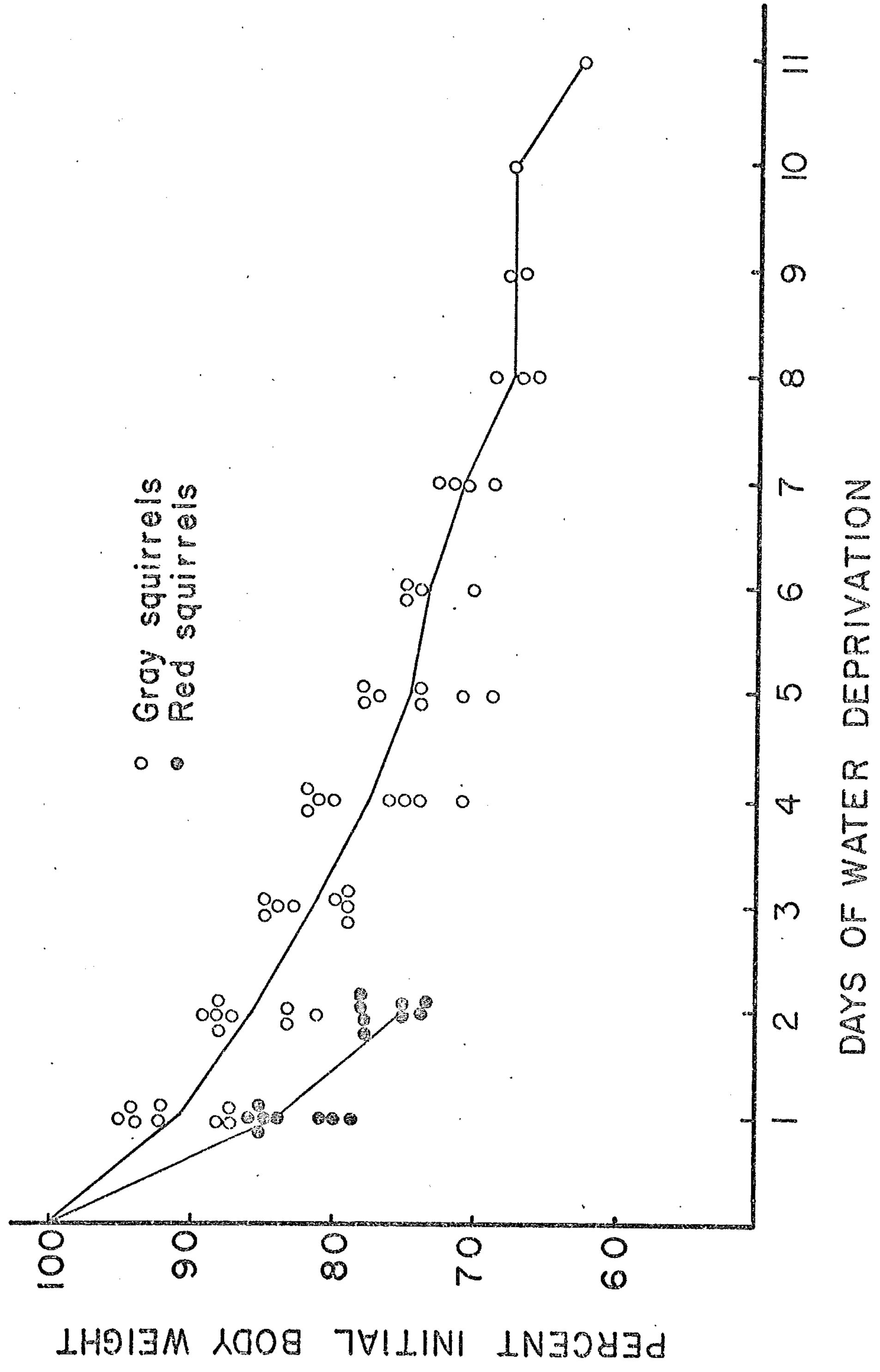
FIGURE 4. Field and laboratory urine/plasma ratios for gray and red squirrels. Vertical lines represent ranges; horizontal lines represent means ($\bar{X}$); rectangles enclose the intervals $\bar{X} \pm T_{0.975}S.E.$ Numbers in parentheses represent the number of animals. For laboratory data, upper samples represent dehydrated animals and lower samples represent hydrated animals. Numbers outside the parentheses represent the number of measurements from the animals involved.


Gray squirrels
Red squirrels
URINE/PLASMA
8.0
6.0
4.0
2.0
(9)
(7)
(9)
(7)
(10)
(9)
(7)
(10)
(8)
(10)
(9)
(15)
(7)
(7)
(7)
(12)
5
(3)
6
(3)
6
(5)
12
(4)
J
F
M
A
M
J
J
A
S
O
N
D
Lab
MONTHS

obtained in the laboratory under water deprivation were not different than maximum U/P values for either species collected in the field.

Body weight under water deprivation.--Figure 5 shows percent body weight loss during water deprivation. There were no overlapping values during the first two days for the two species. All red squirrels survived two days but none survived longer. All gray squirrels survived at least four days and none survived longer than 11 days. Mean body weight of red squirrels at time of death from dehydration was 74.4 ± 3.1% of initial body weight which was 207.0 ± 10.2 grams. Mean body weight of gray squirrels at time of death from dehydration was 70.4 ± 4.9% of initial body weight which was 673.3 ± 91.9 grams. Intraperitoneal body fat was present in some animals of both species after death from dehydration stress.

FIGURE 5. Response of body weight to water deprivation in gray and red squirrels. Circles represent data for individual animals; lines connect mean ($\bar{X}$) body weight for each day for each species.


○ Gray squirrels
● Red squirrels
PERCENT INITIAL BODY WEIGHT
100
90
80
70
60
DAYS OF WATER DEPRIVATION
1
2
3
4
5
6
7
8
9
10
11

Discussion

Many rodents get enough water in their food and only occasionally drink free water (Davis and Golley 1963). C. Smith (1968) reported that free water was absent on most of the territories in which he studied red squirrels, and was seldon drunk when present. He stated that commonly eaten foods such as mushrooms (90-95% water), fungi infected bark (54% water), Arnica, blueberries, and raspberries probably supplied most of the squirrel's water during his study. M. Smith (1968) suggested that mushrooms probably supplied the major portion of the water requirement in red squirrels during the summer in Alaska because no open water was available. McKeever (1964) found that fungi comprised 56% of the annual diet of the pine squirrel (Tamiasciurus douglasi) in northeastern California. In the same study fungi comprised 91% of the diet during the month of June. However, Layne (1954a) observed wild red squirrels drinking from puddles and noted that captive animals required drinking water. He presumed that wild red squirrels needed water for drinking. Finley (1969) reported red squirrel middens were located in moist areas and out of sunlight so cones would not dry out and release seeds. Occasionally these middens were partially under water in the spring. Brown and Yeager (1945) reported tracks of gray and fox squirrels at the edge of surface water in central Illinois. They stated that water may not be a requirement of fox squirrels but seems to be necessary for gray squirrels.

Pearson (1969) found a correlation between the availability of dew and the above ground activity in Microtus californicus.

The extent to which gray and red squirrels may use dew as a possible water source warrants further investigation.

In the present study, no squirrels of either species were observed drinking water in natural conditions and most animals were collected at least 200-800 meters from surface water. It was assumed, therefore, that most water requirements for squirrels collected in this study were fulfilled by preformed water in the food during non-winter months.

Seasonal trends in urine osmolality indicated that gray and red squirrels were not maximally stressed for water balance although a few individuals may have been stressed during spring and fall (Fig. 1). This was when highest average and highest individual maximum urine concentrations were observed. At this time of year, water probably would be most limited because water content in food would be lower than during summer months and snow would not be available for consumption. Annual precipitation at both locations was above average during three out of the four years of study (Table 1). Although no measurements were made of mast production, field observation indicated a good mast crop for each of these years. Both species of squirrels were abundant. The fact that some individuals, primarily among red squirrels, had urine concentrations greater than the average urine concentration from animals under dehydration stress (1787 mOsm for red squirrels and 1783 mOsm for gray squirrels) may indicate that a sizeable proportion of the population may experience dehydration stress during years of below average precipitation. Because intraperitoneal body fat was present in both species at time of death from dehydration indicates that neither could survive solely on metabolic water.

Access to water in the form of snow during the winter may have been a factor in reducing urine osmolality at that time. Urine osmolality for gray squirrels in the January-February sample was lower than that in the March-April sample. Also, January-February urine osmolality for red squirrels was lower than the November-December urine sample. Klugh (1927) reported that red squirrels frequently ate snow during the winter.

The significant drop in urine osmolality during July for both species (Fig. 1) was probably indicative of the water content of the food at that time. Layne (1954a) found that fleshy fruits occurred in 61.1% of red squirrel stomachs during July. He found that freshly killed squirrels had a distinct odor of berries. Their stomachs were stained dark purple and the seeds occurred throughout the digestive tract. Similar conditions were observed in red squirrels collected during July in this study. Layne also found that fleshy fruits occurred in only 23.1% of red squirrel stomachs in August while mast occurred in 73.1% of the stomachs during that month. Brown and Yeager (1945) reported dewberries and raspberries being consumed by gray squirrels in June and July and blackberries and gooseberries were eaten in July and August. They also reported fungi taken in maximum quantities in July and August. Nixon _et al_. (1968) reported that blackberries comprised 52.0% by volume and fungi comprised 22.6% by volume of the gray and fox squirrel diets during July in southeast Ohio. Blackberries were not found in gray squirrel stomachs during June or August and fungi occurred only in trace amounts in August in the same study while mast (hickory nuts, beech nuts and acorns) increased from 5.7% of the gray squirrel diet in July to 76.4% of their diet in August.

Above normal precipitation in July, 1969, at Itasca (16.0 cm) and in July, 1970, at Minneapolis-St. Paul (9.3 cm) when the July samples for red and gray squirrels, respectively, were collected, may have influenced urine concentration for those periods (Table 1). However, normal rainfall during June is greater than in July for both areas. Consequently, most succulent food sources (i. e., berries, fungi, etc.) are available shortly afterwards in maximum quantities. Also, October precipitation for all years of sampling was above normal for both areas and urine concentrations for both species was as great then as any other time of year. This further substantiates that water obtained in natural conditions during non-winter months was probably derived from food.

Gray squirrel urine concentration was greater than that of red squirrel urine in July and red squirrel urine concentration was greater than that of gray squirrel urine in November-December. Concentration of urine did not differ between the species at other collection periods. This was probably indicative of the fact that their respecitve diets were similar with respect to the water content of the food due to the similar precipitation patterns at the two areas. However, urine urea trends indicated that red squirrel diet from September to December may have been higher in protein content than gray squirrels as evidenced by greater urine urea concentration in red squirrel urine at that time (Fig. 3). Gray squirrel urine potassium was greater than red squirrel urine potassium except during January-February and August (Fig. 2). This probably resulted from dietary differences in potassium concentration. Potassium availability, particularly to gray squirrels, must have been greater in natural foods than in the Purina Chow used in the laboratory because urine potassium from field animals was greater than from laboratory animals. Even if urine sodium concentration (Table 2) is added to potassium concentration (Fig. 2) for laboratory animals with or without water, the combined electrolyte concentration is still lower than the potassium concentration found in gray squirrels for the March-April, September, October, and November-December periods.

Urine sodium concentration (Table 2) constituted a small part of the total urine concentration and probably represented traces of animal material in the diet of both gray squirrels (Goodrum 1940, Nixon _et al_. 1968) and red squirrels (Klugh 1927, Layne 1954a, C. Smith 1968). Urine sodium concentration in both species tended to be higher during late winter and spring although variation within those samples was high and not all differences between them and ones collected later in the year were significant. Nixon _et al_. (1968) reported a higher frequency of occurrence of animal material in gray and fox squirrel stomachs from March to July than for the rest of the year. Layne (1954a) reported animal material in red squirrel stomachs only during June and July. Several studies have correlated increased consumption of animal material with reproductive activity in female squirrels (Goodrum 1940, Bakken 1952, C. Smith 1968). In the present study, of 12 gray squirrels and 12 red squirrels that had urine sodium concentrations above 10 mEq/L, four (33.3%) gray squirrels were reproductively active females (enlarged uterus, pregnant, or lactating) and six (50%) red squirrels were reproductively active females. All animals of both species with urine sodium concentration over 10 mEq/L were adults except for one juvenile red squirrel. Of a total of seven gray squirrel females in reproductive condition, four (57.1%) had urine sodium above 10 mEq/L. Of 13 red squirrel females in reproductive condition, six (46.2%) had urine sodium above 10 mEq/L. During the same periods (February through July) 6/14 (42.9%) male gray squirrels and 4/14 (28.6%) male red squirrels had high urine sodium concentrations. Urine sodium data from the present study do not refute this idea. However, further investigation is needed to clarify the relationships of ingested animal material to sodium excretion in small rodent herbivores.

Plasma potassium concentration for field animals was similar for both species (Fig. 2), but these values were higher than expected after review of other mammalian plasma. Human potassium concentration in plasma is 4.2 (3.1-5.5) mEq/L (Davson and Eggleton 1968). Wilber and Robinson (1958) reported potassium concentration of 10.4 mEq/L in white-tailed deer. They assumed this high value was due to shock from shooting. Trauma in animals may result in a sodium-potassium shift in plasma during which time sodium falls and potassium rises (Friedman and Friedman 1963). Because field animals collected in this study were shot, this may explain why plasma sodium was lower and plasma potassium was higher, respectively, than sodium and potassium in plasma from hydrated laboratory animals. However, potassium is in high concentration in plants and sodium is in low concentration in most plants (Ovington 1962). This also may have been a factor responsible for the high plasma potassium and urine potassium concentrations observed in field animals. Likewise, the fact that Purina Chow used in the laboratory studies was higher in sodium and lower in potassium than food consumed in natural conditions (Schmid unpublished) may have been responsible for the lower plasma and urine potassium concentrations observed in laboratory animals.

The other apparent difference in plasma between the two species in the field was in urea concentration. Plasma urea overall was highest for red squirrels. For parts of the year, urine urea was greater in red squirrels but it was never higher in gray squirrels. This may have been a reflection of the higher plasma urea observed in red squirrels. No differences were observed in seasonal trends for plasma urea between gray or red squirrels.

The high plasma osmolalities observed in dehydrated animals of both species were due primarily to an increase in plasma sodium. The Purina Chow used in the laboratory had a substantially higher sodium content than natural food and therefore may have been partly or largely responsible for the observed response.

Urine/plasma ratios for osmolality throughout the year for both species (Fig. 4) followed a pattern very similar to urine concentration (Fig. 1). This was because plasma concentration had little variation throughout the year, i. e., an indication that kidneys maintained homeostasis of blood. The U/P ratios for each species were neither different from each other when comparing dehydrated laboratory animals nor for each of the collecting periods except for November-December when U/P was greater for red squirrels than for gray squirrels. Both species of squirrels produced comparable urine concentrations in the field and under negative water balance, yet the relative medullary thickness (Table 5) of gray squirrels was less than for red squirrels. This apparent incongruity may be explained in part by the higher plasma concentration in gray squirrels. Also, the slightly higher (but not significant) urine osmolality from trapped red squirrels in July, 1969, compared to dehydrated laboratory animals, indicated that maximum urine concentrations in red squirrels may not have been observed in the laboratory for this species. Irving et al. (1955) reported a metabolic rate 80% greater than would be predicted by its size for red squirrels. They stated that red squirrels would not remain quiet enough in metabolic chambers to show their resting metabolism. A similar hyperactivity of red squirrels in metabolic chambers was noted in this study. In addition, the rapid weight loss during dehydration (Fig. 6) supports the premise that hyperactivity may have interferred with the kidney's ability to concentrate urine to the maximum degree in captive red squirrels.

Within its range the red squirrel occupies a wide variety of sylvan habitats, both coniferous and deciduous (Klugh 1927, Hall and

Kelson 1959). In Minnesota the red squirrel is found in all arboreal habitats (Gunderson and Beer 1953). The distribution of the gray squirrel, however, is closely associated with the eastern hardwood forests (Hall and Kelson 1959). The wide variety of habitats in which red squirrels are found suggests that possible physiological variations occur between different populations within the species. For example, red squirrels produce two litters per year in eastern North America (Hamilton 1939, Layne 1954a) and usually produce one litter per year in western North America (Hatt 1943, C. Smith 1968, Kemp and Keith 1970, Dolbeer 1973). Therefore, Dolbeer (1973) suggested that red squirrels have shifted toward "k strategists" rather than "r strategists" in the mountain regions where the snow free period is shorter than in the eastern part of its range. Kemp and Keith (1970) reported an anticipatory rise in reproduction for red squirrels in Alberta, Canada, immediately before a medium or heavy white spruce cone crop. This maximized the red squirrel's ability to utilize a fluctuating food supply.

Considering the wide variety of habitats in which red squirrels are found and the wide variety of foods they can utilize (Klugh 1927, Layne 1954a) it is probable that they would also have to adapt in some way to water availability, assuming free drinking water was not relied upon. Consequently, a difference in relative medullary thickness between the populations from different habitats may be expected. Whether red squirrels collected along river bottoms in this study actually used free drinking water to an appreciable extent was not ascertained. However, because of drainage to these areas, water content of food would probably be greater than in upland woods, especially during dry periods. Other aspects such as increased relative humidity in the more mesic regions may also be a factor in the lower relative medullary thickness observed in red squirrel kidneys by decreasing respiratory water loss. No obvious difference in urine concentration was noted between red squirrels collected along river bottoms and those collected in upland woods. Therefore it may be that because animal populations in the upland regions are probably exposed to drought conditions more frequently, increased kidney efficiency has resulted to a greater degree than in animals found in the more continuously mesic river bottom areas. The greater relative medullary thickness in red squirrels was probably a function of relative body size rather than environment because larger surface area to volume ratio would put more emphasis on water conservation than would occur in gray squirrels, which are about 2 1/2 times as large. The difference in relative medullary thickness observed between red squirrels collected in different habitats was not observed in gray squirrels. This suggests a physiological adaptation to water balance in different populations of red squirrels in their respective habitats.

This situation poses an interesting question, mainly, is the observed difference in relative medullary thickness between different wild populations of red squirrels due to genetic differences or is it environmentally induced? Blount and Blount (1968) reported an increase in the relative length of the renal papilla of kidneys in _Mus musculus_ treated with NaCl as drinking fluid, by hydropenia, or by antidiuretic hormone injection. They concluded that any condition or treatment which brings about a requirement for conservation of water results in a relative lengthening of the renal papilla. Whether relative renal papilla length in red squirrel kidneys can or cannot change within individuals has not been demonstrated.

If red squirrel renal papilla length cannot change within individuals to the degree that would be necessary to account for the relative medullary thickness differences reported in this study, then genetic considerations must be made. Selander (1970) reported heterogeneity in allele frequencies among samples of _Mus musculus_ from farms in the same region and even within single barns. He attributed the cause of this fine-scale genetic subdivision to territorial behavior of family groups. The relative importance of gene flow, natural selection, and breeding units with respect to relative medullary thickness is not known for this species.

PRAIRIE DOGS

Results

Urine.--The environment around Laramie, Wyoming, where all white-tailed prairie dogs were collected, is more arid than that around Bowman, North Dakota, where most black-tailed prairie dogs were collected (Table 6). The biggest difference in precipitation between the two areas occurs in May and June, when Bowman gets an average of 6.7 cm more rainfall than Laramie for the two month period. During other seasons, precipitation differences between the two areas is not nearly as appreciable although Bowman usually receives slightly more. Precipitation was above normal at Bowman and slightly below normal at Laramie during the three years of this study.

Seasonal trends in urine osmolality demonstrated that urine was least concentrated in June for white-tailed prairie dogs and in July for black-tailed prairie dogs (Fig. 6). However, the June sample for white-tailed prairie dogs was not significantly lower than the March or July samples for that species. Although urine osmolality in black-tailed prairie dogs was lower than for white-tailed prairie dogs during March and June, these differences were not significant. Both species showed an increase in urine osmolality in August and September. This increase was most dramatic for black-tailed prairie dogs. Urine osmolality for black-tailed prairie dogs in September was slightly higher than for white-tailed prairie dogs for that month but the difference was not significant. Urine osmolality of black-tailed prairie dogs under

TABLE 6. Annual and monthly precipitation in cm for Bowman, North Dakota, and Laramie, Wyoming, for the period 1931-1952 and for the years 1969, 1970, and 1971.

	Bowman, North Dakota				Laramie, Wyoming			
Month	1931-1952[a]	1969[b]	1970[b]	1971[b]	1931-1952[c]	1969[d]	1970[d]	1971[d]
J	1.2	1.2	0.6	1.6	1.0	1.1	0.2	0.8
F	0.9	0.6	0.2	0.6	0.9	2.9	0.5	3.5
M	2.1	0.7	0.4	0.9	1.9	0.5	3.6	2.2
A	3.6	3.5	4.7	5.8	3.8	1.7	0.8	2.4
M	5.6	3.3	12.4	3.8	3.8	1.8	0.7	5.3
J	8.6	15.2	9.8	14.0	3.7	7.7	5.0	0.7
J	4.9	11.0	1.9	1.3	4.3	1.9	1.3	2.3
A	4.3	1.1	7.6	0.4	3.0	1.1	1.4	5.2
S	3.0	0.6	3.4	1.6	2.1	3.2	3.9	2.7
O	2.4	1.5	1.6	6.5	1.8	5.2	3.8	0.2
N	1.0	0.1	3.2	0.9	1.1	1.1	2.8	0.7
D	0.8	1.5	1.0	0.4	1.1	0.4	0.7	0.2
Total	38.6	40.9	49.3	43.6	28.6	28.5	24.8	25.9

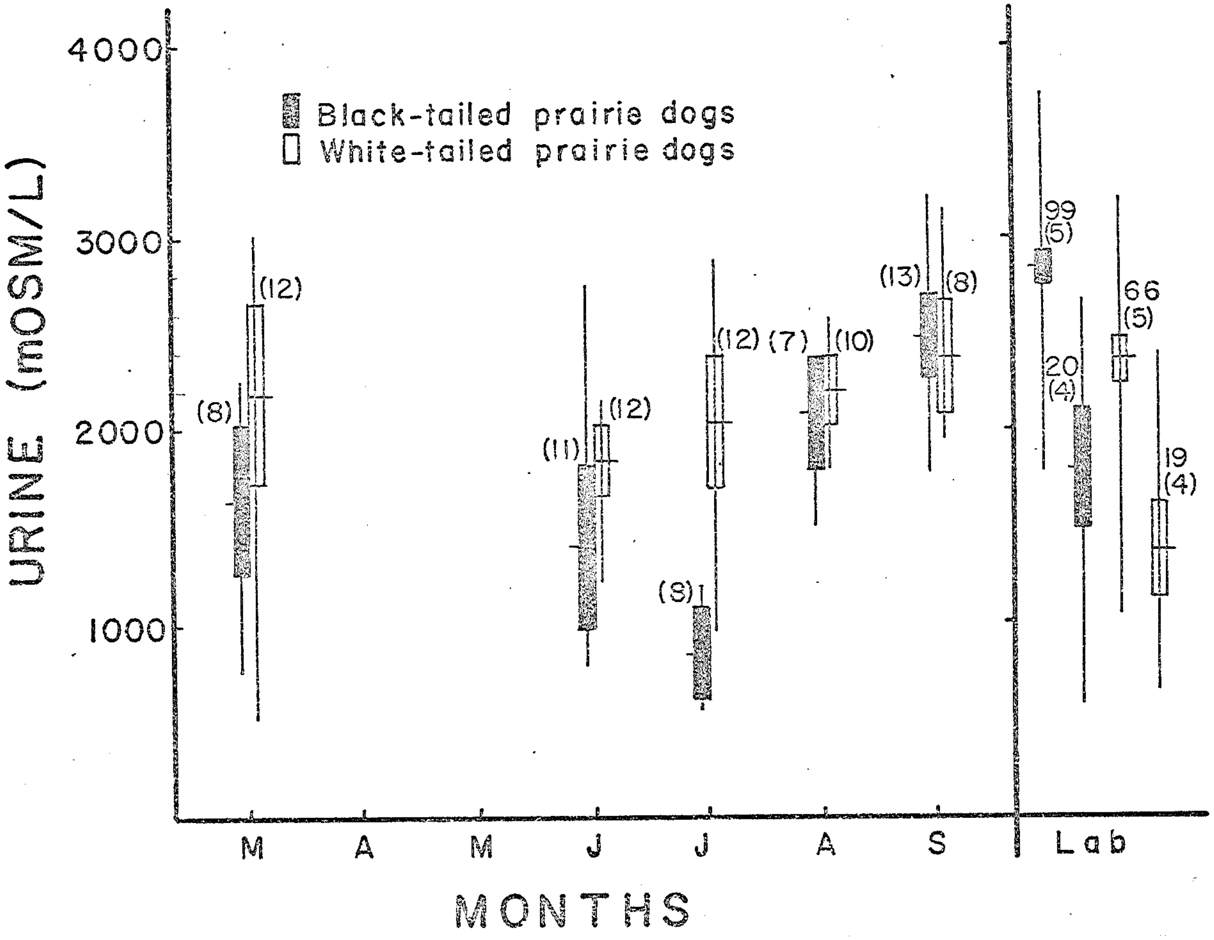
[a] U. S. Dept. of Comm. Weather Bureau. Climatic summary of the U. S., 1931-1952, for North Dakota.

[b] U. S. Dept. of Comm. Environmental Science Services Administration. North Dakota Climatological Data, Vols. 78-80: no. 13.

[c] U. S. Dept. of Comm. Weather Bureau. Climatic summary of the U. S., 1931-1952, for Wyoming.

[d] U. S. Dept. of Comm. Environmental Science Services Administration. Wyoming Climatological Data, vols. 78-80: no. 13.

FIGURE 6. Field and laboratory urine osmolalities for black-tailed and white-tailed prairie dogs. Vertical lines represent ranges; horizontal lines represent means ($\bar{X}$); rectangles enclose the interval $\bar{X} \pm T_{0.975}$S.E. Numbers in parentheses represent the number of animals. For laboratory data, upper samples represent dehydrated animals and lower samples represent hydrated animals. Numbers outside parentheses represent the number of measurements from the animals involved.


URINE (mOSM/L)
4000
3000
2000
1000
Black-tailed prairie dogs
White-tailed prairie dogs
(8)
(12)
(11)
(12)
(S)
(12)
(7)
(10)
(13)
(8)
99
(5)
20
(4)
66
(5)
19
(4)
M
A
M
J
J
A
S
Lab
MONTHS

laboratory dehydration stress was greater than any of the field collections. However, only the June urine sample from white-tailed prairie dogs was lower than the urine collected from that species under dehydration stress.

Seasonal trends in urine potassium concentration for white-tailed prairie dogs coincided closely to the pattern of total urine concentration (Fig. 7). However, the differences between samples were more pronounced. For instance, June urine potassium was lower than any other period of collection. August and September potassium concentrations were above any other sample for white-tailed prairie dogs. Urine potassium concentrations in black-tailed prairie dogs also correlated closely with total urine concentration trends for that species except during March. Potassium concentration for that month was not different from the June or July potassium concentrations for black-tailed prairie dogs. July urine potassium was lower than for June in black-tailed prairie dogs. Urine potassium concentration from dehydrated animals was as low or lower than most field samples. There was no difference in urine potassium between hydrated and dehydrated black-tailed prairie dogs while dehydrated white-tailed prairie dogs had lower urine potassium than hydrated animals.

Urine urea concentration in black-tailed prairie dogs correlated with total urine concentration for March, June, and July but remained relatively low in August and September when urine concentration increased sharply (Fig. 8). Urea concentration in white-tailed prairie dogs, on the other hand, remained quite constant with no

FIGURE 7. Field urine potassium concentrations of black-tailed and white-tailed prairie dogs compared to laboratory urine potassium and to field urine osmolalities. Open circles connected by a dotted line represent mean ($\bar{X}$) urine osmolality for white-tailed prairie dogs. Closed circles connected by a solid line represent mean ($\bar{X}$) urine osmolality for black-tailed prairie dogs. Vertical lines represent ranges; horizontal lines represent means ($\bar{X}$); rectangles enclose the interval $\bar{X} \pm T_{0.975}$S.E. Numbers in parentheses represent the number of animals. For laboratory data, the left sample for each species represents dehydrated animals and the right sample represents hydrated animals. Numbers outside parentheses represent the number of measurements from the animals involved.

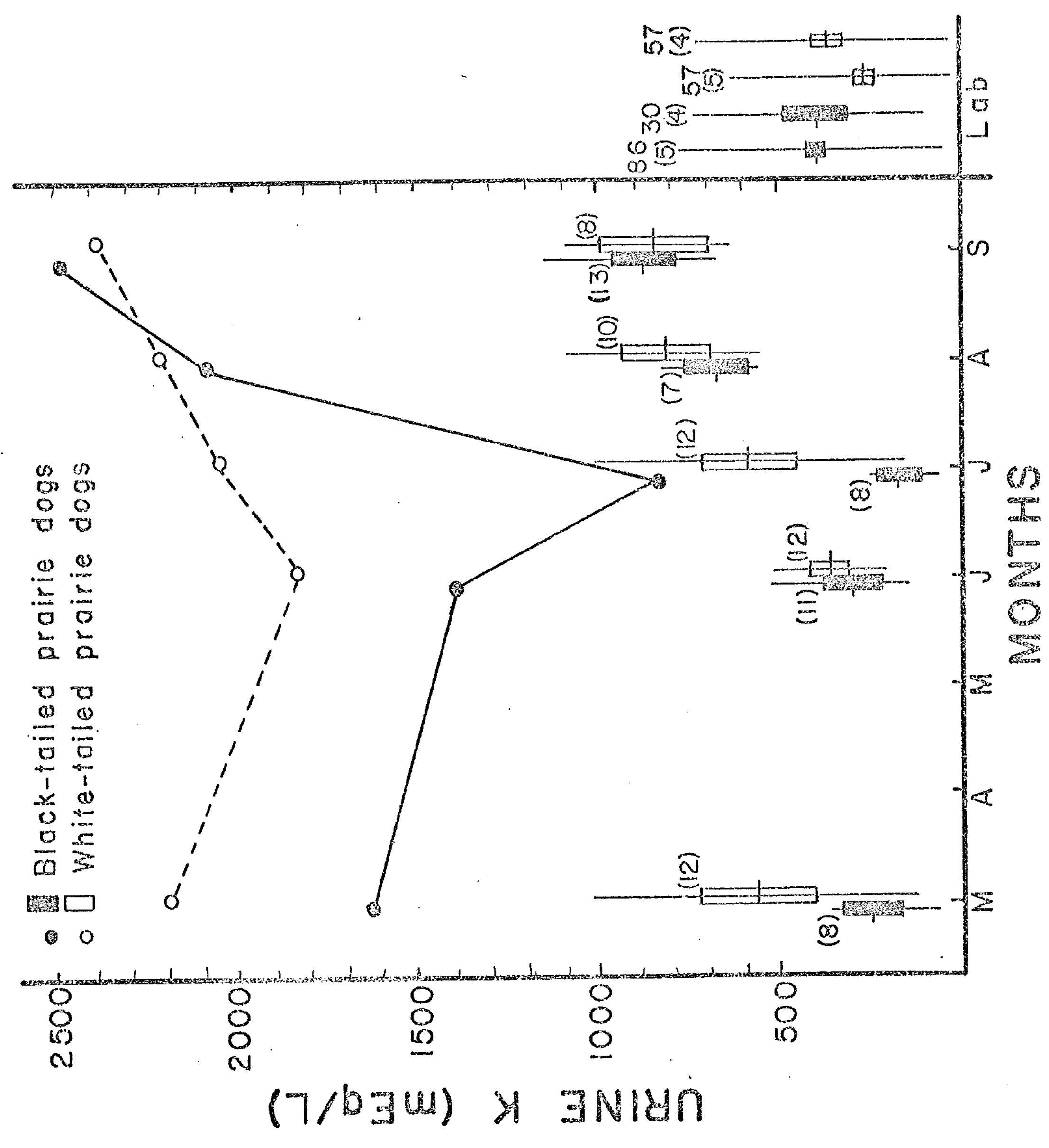
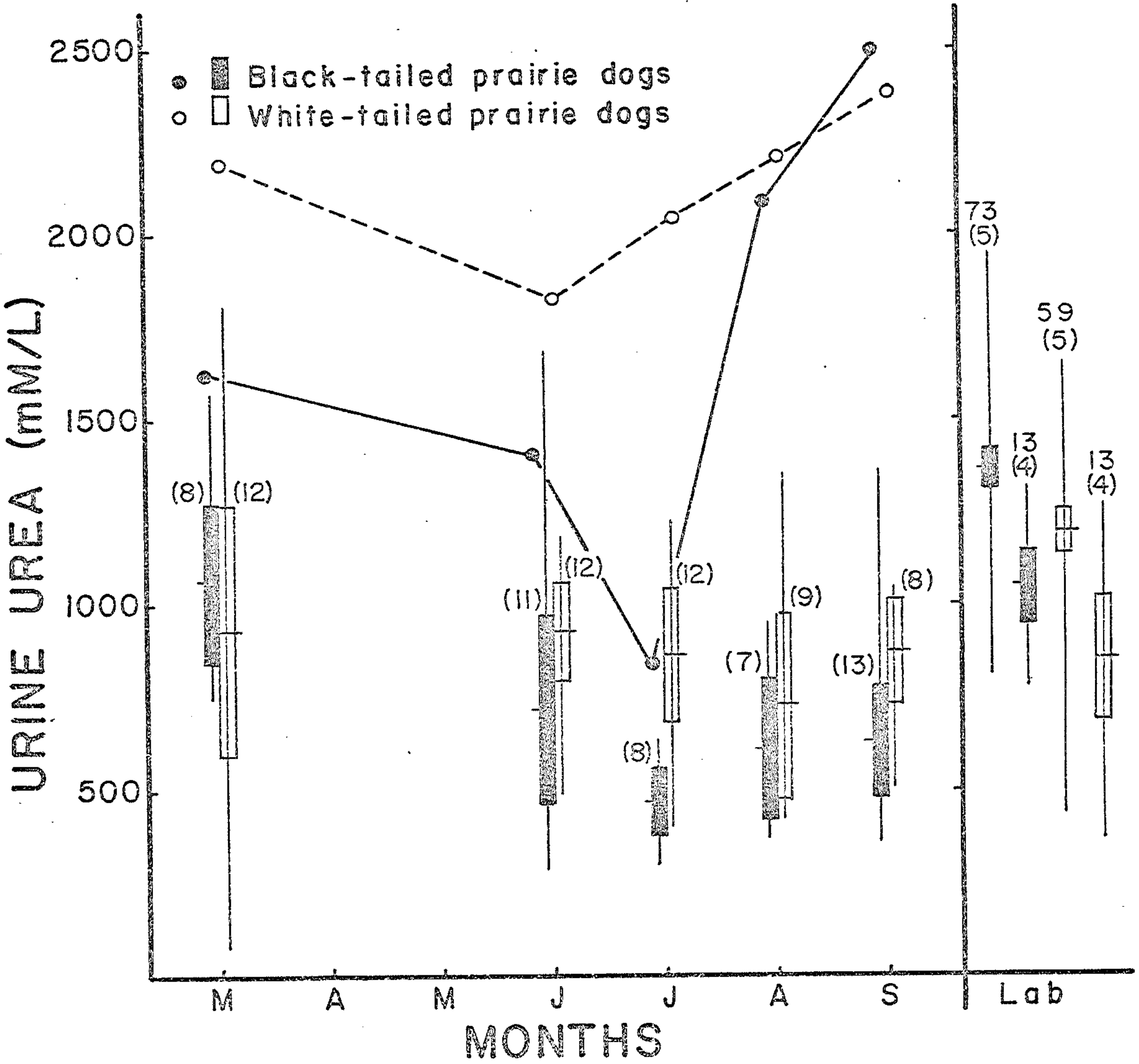

Black-tailed prairie dogs
White-tailed prairie dogs
URINE K (mEq/L)
2500
2000
1500
1000
500
MONTHS
M
A
M
J
J
A
S
Lab
(8)
(12)
(11)
(12)
(8)
(12)
(7)
(10)
(13)
(8)
86
(5)
30
(4)
57
(5)
57
(4)

FIGURE 8. Field urine urea concentrations of black-tailed and white-tailed prairie dogs compared to laboratory urine urea and to field urine osmolalities. Open circles connected by a dotted line represent mean ($\bar{X}$) urine osmolality for white-tailed prairie dogs. Closed circles connected by a solid line represent mean ($\bar{X}$) urine osmolality for black-tailed prairie dogs. Vertical lines represent ranges; horizontal lines represent means ($\bar{X}$); rectangles enclose the interval $\bar{X} \pm T_{0.975}$S.E. Numbers in parentheses represent the number of animals. For laboratory data, upper samples represent dehydrated animals and lower samples represent hydrated animals. Numbers outside parentheses represent the number of measurements from the animals involved.

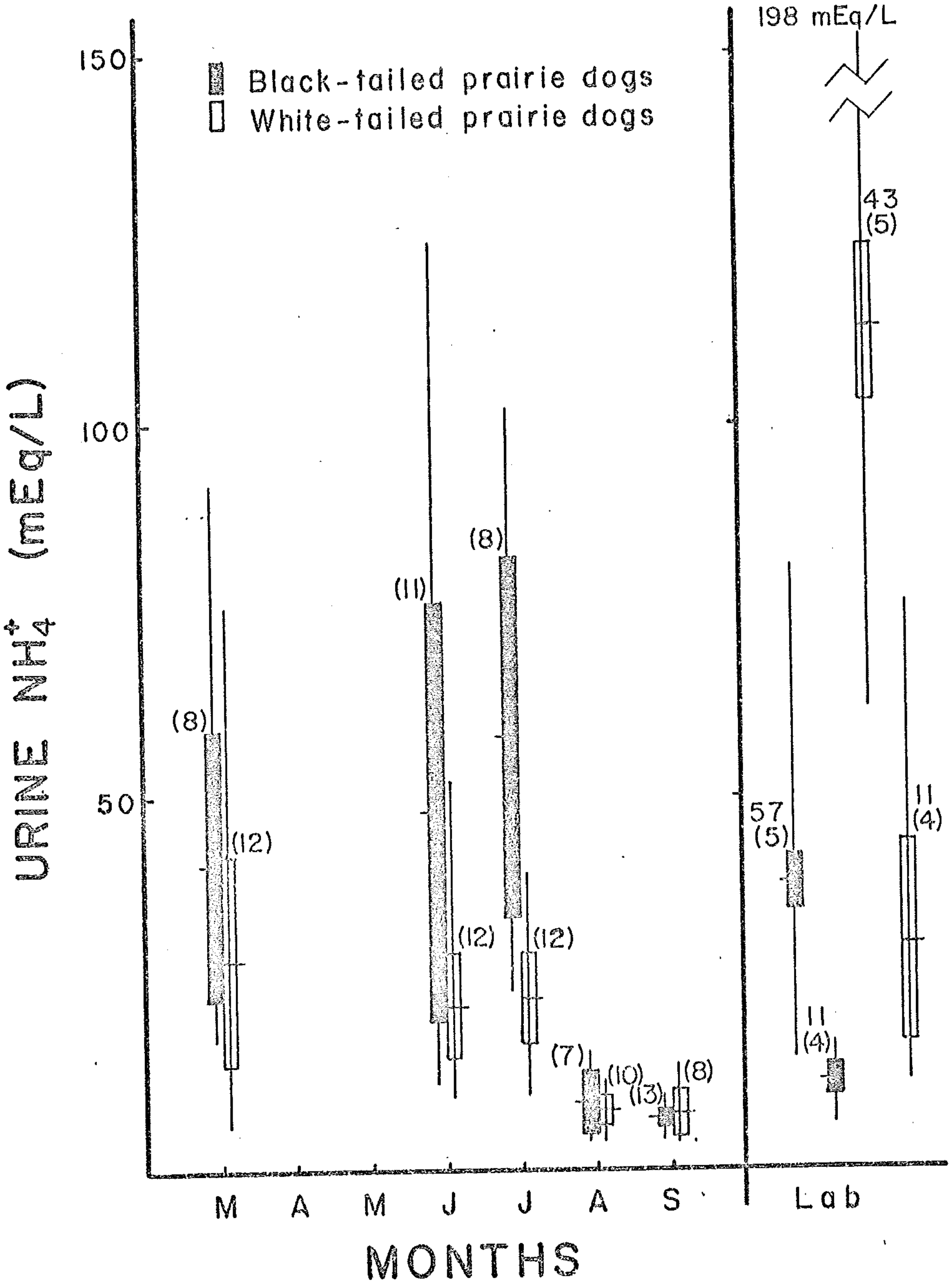

● ▨ Black-tailed prairie dogs
○ ☐ White-tailed prairie dogs
URINE UREA (mM/L)
2500
2000
1500
1000
500
MONTHS
M
A
M
J
J
A
S
Lab
(8)
(12)
(11)
(12)
(8)
(12)
(7)
(9)
(13)
(8)
73
(5)
13
(4)
59
(5)
13
(4)

differences observed between any samples and no obvious correlation with urine osmolality. Urine urea from dehydrated black-tailed prairie dogs was greater in concentration than any field sample. Urea concentration from dehydrated white-tailed prairie dogs was not greater than the March sample but was greater than the other field samples for that species.

Ammonia concentration (Fig. 9) in urine from field samples was similar for both species, but differed in seasonal trend from urine osmolality (Fig. 6). There was a sharp drop in urine ammonia in August and September for both species (Fig. 9). Ammonia concentration was lower in white-tailed prairie dogs urine than in black-tailed prairie dogs for March, June, and July, but only the July samples were different from each other. Urine ammonia from dehydrated black-tailed prairie dogs was not greater than several of the field samples but white-tailed prairie dog urine ammonia from dehydrated animals was greater than any field sample.

Urine sodium concentrations from white-tailed prairie dogs were greater than urine sodium from black-tailed prairie dogs during March, July, and August (Table 7). It was also higher during June but the difference was not significant. The September urine sodium sample for black-tailed prairie dogs was skewed upward by one unusually high value (232 mEq/L). Urine sodium from hydrated and dehydrated laboratory animals of both species was much higher than from field samples. This was undoubtedly due to the high NaCL content of the Purina Chow supplied to the animals.

FIGURE 9. Field and laboratory urine ammonia concentrations of black-tailed and white-tailed prairie dogs. Vertical lines represent ranges; horizontal lines represent means ($\bar{X}$); rectangles enclose the interval $\bar{X} \pm T_{0.975}$S.E. Numbers in parentheses represent the number of animals. For laboratory data, upper samples represent dehydrated animals and lower samples represent hydrated animals. Numbers outside parentheses represent the number of measurements from the animals involved.



URINE NH_4^+ (mEq/L)
150
100
50
▨ Black-tailed prairie dogs
☐ White-tailed prairie dogs
198 mEq/L
(8)
(12)
(11)
(12)
(8)
(12)
(7)
(10)
(13)
(8)
57
(5)
11
(4)
43
(5)
11
(4)
M
A
M
J
J
A
S
Lab
MONTHS

TABLE 7. Field and laboratory urine sodium concentrations (mEq/L) from black-tailed and white-tailed prairie dogs. Numbers in parentheses represent number of animals from which samples were taken.

	Black-tailed prairie dogs			White-tailed prairie dogs		
Period	$\bar{X} \pm (T_{0.975})$(S.E.)	N	range	$\bar{X} \pm (T_{0.975})$(S.E.)	N	range
March	7.8 ± 5.5	8	4.2 - 24.0	14.6 ± 8.9	12	6.0- 58.0
June	8.7 ± 5.7	11	3.8- 33.4	12.1 ± 3.4	12	6.3- 26.4
July	4.5 ± 1.0	8	3.6- 7.5	9.5 ± 4.0	12	5.0- 26.1
August	8.3 ± 4.1	7	4.0- 17.0	17.7 ± 8.0	10	7.2- 38.3
September	52.1 ± 43.1	12	5.0-232.0	18.9 ± 14.0	7	6.1- 48.2
Lab w/ H_2O	161.0 ± 29.4	30(4)	30.1-284.0	147.3 ± 24.4	57(4)	11.0-364.0
Lab w/o H_2O	169.2 ± 24.8	86(5)	7.3-485.0	70.3 ± 70.4	57(5)	11.2-335.0

Plasma.--Although the mean plasma osmolality in field collected black-tailed prairie dogs was higher than for white-tailed prairie dogs, this difference was not significant (Table 8). There was also no difference between the two species when comparing plasma potassium or urea. However, sodium concentration in plasma of white-tailed prairie dogs was lower than in black-tailed prairie dogs. There was no significant difference among months in plasma osmolality, plasma sodium, or plasma potassium.

Both species reacted similarly to laboratory conditions in that there were no differences between the species for any of the plasma measurements made when the animals were provided with water *ad libitum*. The only difference detected in comparisons of dehydrated animals was higher plasma sodium in white-tailed prairie dogs. Total plasma concentration and plasma sodium was higher in dehydrated animals than in field animals for both species. However, plasma potassium and urea were higher in field animals than in either hydrated or dehydrated animals for both species. The difference between field and dehydrated plasma urea samples for black-tailed prairie dogs was not significant, however.

Although differences among months in total plasma osmolality, plasma sodium, or plasma potassium was not observed for either species, differences were observed in plasma urea from month to month (Fig. 10). Plasma urea in black-tailed prairie dogs followed the trend of urine osmolality (Fig. 6) with lowest concentrations occurring in July (Fig. 10). Plasma urea was higher in black-tailed prairie dogs during March, August, and September than in dehydrated

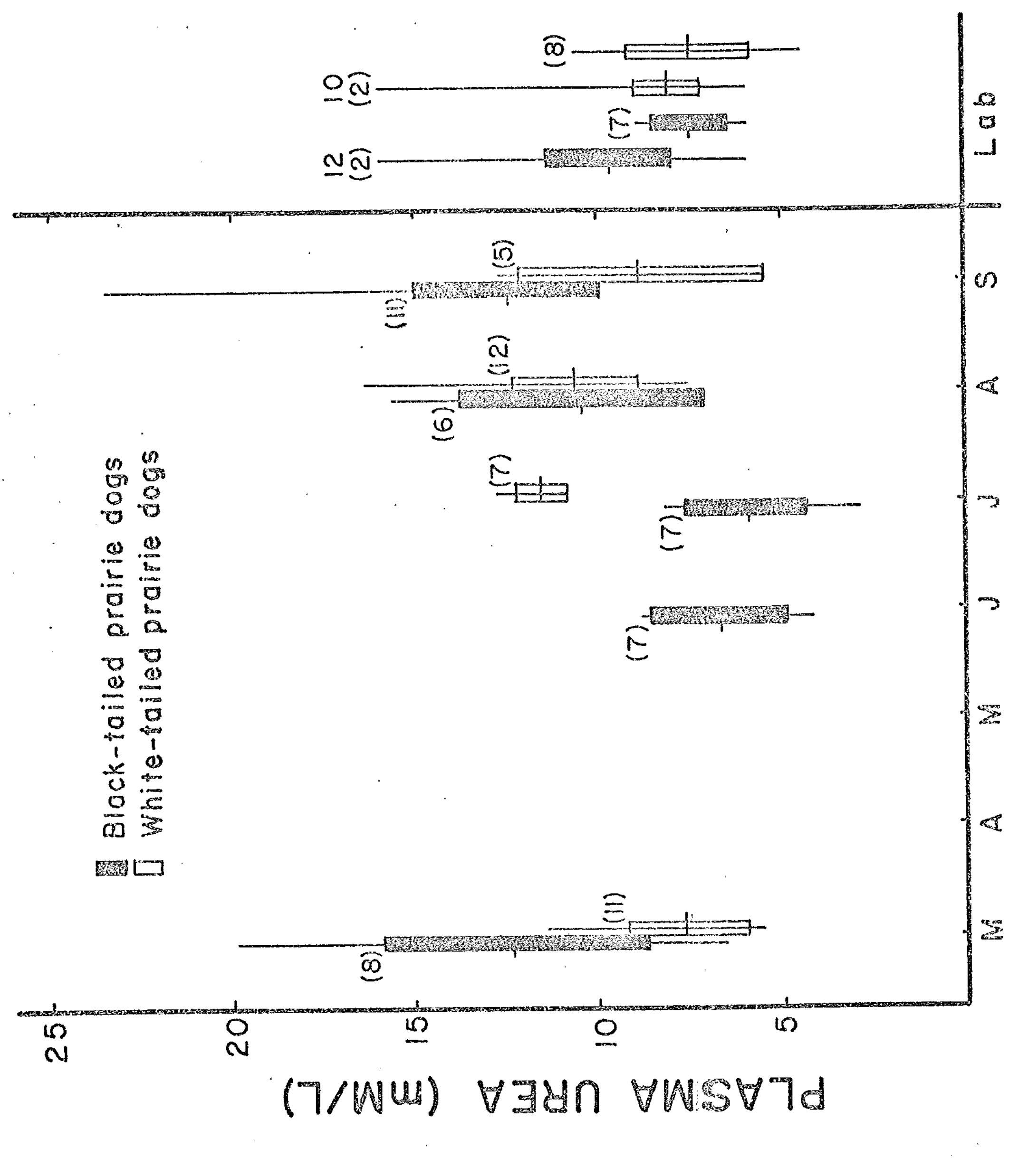
TABLE 8. Field and laboratory plasma concentrations in black-tailed and white-tailed prairie dogs [$\bar{X} \pm (T_{0.975})(S.E.)$]. Numbers in parentheses represent the number of animals from which the samples were taken.

Species and condition	Total osmolality (mOsm/L)	Na^+ (mEq/L)	K^+ (mEq/L)	Urea (mM/L)
Black-tailed prairie dogs				
Field	298.1 ± 3.0	135.4 ± 4.2	9.4 ± 0.8	10.5 ± 1.1
	N = 49	N = 52	N = 52	N = 51
	r = 274-317	r = 96-187	r = 5.0-18.2	r = 2.9-23.5
Lab w/ H_2O	306.9 ± 7.1	151.2 ± 7.8	3.2 ± 0.3	7.4 ± 1.0
	N = 8	N = 10	N = 10	N = 7
	r = 297-319	r = 134-169	r = 2.6-3.9	r = 5.9-8.8
Lab w/o H_2O	343.2 ± 15.0	156.2 ± 7.7	3.5 ± 0.3	9.6 ± 1.7
	N = 13(2)	N = 13(2)	N = 13(2)	N = 12(2)
	r = 318-412	r = 132-180	r = 2.6-4.6	r = 5.9-16.0

TABLE 8 (cont.). Field and laboratory plasma concentrations in black-tailed and white-tailed prairie dogs $[\bar{X} \pm (T_{0.975})(S.E.)]$. Numbers in parentheses represent the number of animals from which the samples were taken.

Species and condition	Total osmolality (mOsm/L)	Na^+ (mEq/L)	K^+ (mEq/L)	Urea (mM/L)
White-tailed prairie dogs				
Field	293.7 ± 4.1	120.1 ± 8.2	8.6 ± 0.9	9.6 ± 0.9
	N = 31	N = 34	N = 34	N = 35
	r = 265-310	r = 69-184	r = 5.3-17.3	r = 5.6-16.3
Lab w/ H_2O	308.0 ± 8.1	152.3 ± 10.2	3.3 ± 0.6	7.4 ± 1.7
	N = 6	N = 8	N = 8	N = 8
	r = 295-315	r = 125-163	r = 2.4-4.6	r = 4.4-10.5
Lab w/o H_2O	335.0 ± 6.8	169.2 ± 4.6	3.7 ± 0.3	8.0 ± 0.9
	N = 10(2)	N = 10(2)	N = 10(2)	N = 10(2)
	r = 318-347	r = 159-178	r = 3.0-4.2	r = 6.4-10.5

FIGURE 10. Field and laboratory plasma urea concentrations in black-tailed and white-tailed prairie dogs. Vertical lines represent ranges; horizontal lines represent means ($\bar{X}$); rectangles enclose the interval $\bar{X} \pm T_{0.975}$S.E. Numbers in parentheses represent the number of animals. For laboratory data, the left sample for each species represents dehydrated animals and the right sample represents hydrated animals. Numbers outside parentheses represent the number of measurements from the animals involved.


PLASMA UREA (mM/L)
25
20
15
10
5
MONTHS
M
A
M
J
J
A
S
Lab
▨ Black-tailed prairie dogs
☐ White-tailed prairie dogs
(8)
(11)
(7)
(7)
(7)
(6)
(12)
(11)
(5)
12
(2)
(7)
10
(2)
(8)

laboratory animals, but these differences were not significant. Plasma urea in white-tailed prairie dogs during July and August was greater than in dehydrated animals and was also greater than the March sample for that species. Plasma samples were not collected from white-tailed prairie dogs in June.

Kidneys.--Because a difference in ability to produce a concentrated urine was observed between the two species of prairie dogs (Fig. 6), examination of kidney structure was made to see if a morphological correlation existed. An extensive morphological study by Sperber (1944) demonstrated that mammals of xeric habitats tend to have kidneys with a longer renal papilla than species from more hydric habitats. Since that study, others have shown that the length of the renal papilla is related to habitat and the ability of the animal to concentrate urine (Vimtrup and Schmidt-Nielsen 1952, Schmidt-Nielsen and O'Dell 1961, MacMillen and Lee 1969, Schmid 1972). Also, the counter current multiplier hypothesis, which employs the loops of Henle as an exchange system to create increased osmotic concentration in the kidney from cortex to papilla, has become widely accepted with little doubt about its validity (Ullrich, Kramer, and Boylan 1961). Relative medullary thickness of prairie dog kidneys was determined according to Sperber's calculations to see if morphological differences exist. Black-tailed prairie dogs exhibited a greater relative medullary thickness (6.32 ± 0.16, n=54) than did white-tailed prairie dogs (5.85 ± 0.13, n=40).

Because of the small variation in plasma concentration within each species and between species, urine/plasma ratios (Table 9) followed identical seasonal patterns as urine osmolality for each respective species (Fig. 6) and therefore are not illustrated here. Months of maximum and minimum U/P ratios for each species are compared along with laboratory values in Table 4. There was no difference between U/P ratios in September during which the highest values were observed for the two species. However, black-tailed prairie dogs had considerably lower U/P ratios in July than white-tailed prairie dogs did in June, the lowest months for each species, respectively. Maximum field U/P ratios for white-tailed prairie dogs was above that for dehydrated animals but this difference was not significant. Urine/plasma values for dehydrated black-tailed prairie dogs was greater than the maximum field sample (September) for that species but again the difference was not significant.

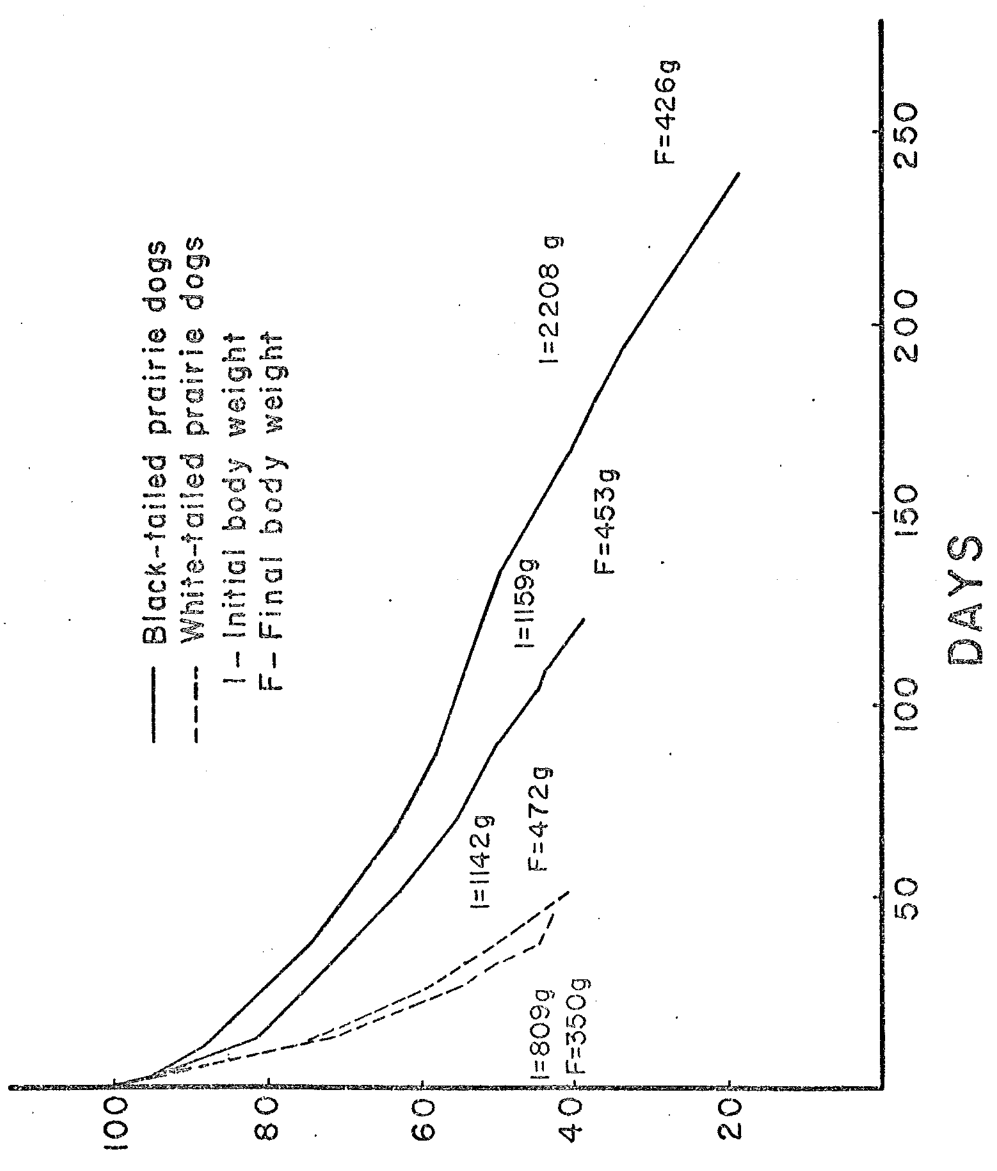
Body weight under water deprivation.--When deprived of water, white-tailed prairie dogs lost weight more rapidly and survived for a shorter period of time than did black-tailed prairie dogs (Fig. 11). Both species were able to tolerate weight losses of over 50% initial body weight. The two white-tailed prairie dogs survived to 43% and 41% initial body weight and the two black-tailed prairie dogs survived to 39% and 19% initial body weight. Few species have been reported to tolerate over a 50% weight loss. Chew (1951) reported a 36-53% weight loss in _Peremyscus leucopus_. Schmidt-Nielsen _et al_. (1948) reported a 52.6% weight loss in _Rattus norvegicus_ and Bredahl (1969) reported a 52.6% weight loss in _Spermophilus franklinii_. No animal has been reported to tolerate

TABLE 9. Field and laboratory urine/plasma ratios in black-tailed and white-tailed prairie dogs. Numbers in parentheses represent the number of animals from which the samples were taken.

	Black-tailed prairie dogs			White-tailed prairie dogs		
Condition	$\bar{X} \pm (T_{0.975})(S.E.)$	N	range	$\bar{X} \pm (T_{0.975})(S.E.)$	N	range
Field (maximum month)	8.76 ± 0.63 (September)	9	7.76-1067	8.05 ± 1.02 (September)	8	6.66-10.76
Field (minimum month)	2.92 ± 0.62 (July)	7	1.92-4.12	6.22 ± 0.59* (June)	12	4.13-7.34
Lab w/o H_2O	9.09 ± 0.62	11(2)	7.58-10.51	6.80 ± 1.26	10(2)	3.45-8.74

*U/P values not paired. Mean plasma concentration for all field animals was used to compute June U/P.

FIGURE 11. Response of body weight to water deprivation in black-tailed and white-tailed prairie dogs.



Black-tailed prairie dogs
White-tailed prairie dogs
I – Initial body weight
F – Final body weight
I=2208 g
F=426g
I=1159g
F=453g
I=1142g
F=472g
I=809g
F=350g
DAYS
50
100
150
200
250
PERCENT INITIAL BODY WEIGHT
100
80
60
40
20

a weight loss equal to that of the prairie dogs in this study. The largest black-tailed prairie dog started the water deprivation at a body weight (2208 grams) which was larger than most field body weights. However, the other black-tailed prairie dog and both white-tailed prairie dogs started the experiment at weights similar to those of field animals. Adult body weights of black-tailed prairie dogs collected in this study averaged 1144.8 grams and adult white-tailed prairie dogs averaged 962.2 grams. No body fat was apparent on animals of either species at time of death after water deprivation.

Discussion

A primary difference between habitats of the two species of prairie dogs studied here is the amount of precipitation each area receives (Table 6). Bowman, North Dakota, normally receives 10.0 cm more precipitation than does Laramie, Wyoming, where black-tailed and white-tailed prairie dogs, respectively, were collected. A prominent feature of the precipitation pattern is that Bowman normally receives 6.7 cm more rainfall during May and June which accounts for most of the additional precipitation for that area. During the remainder of the year, the difference in precipitation is not great although Bowman usually receives slightly more.

Seasonal urine osmolality (Fig. 6) in the two species of prairie dogs reflected their respective environments with regard to precipitation. The low urine osmolality of black-tailed prairie dogs in July was probably a result of the increased rainfall at that time. Urine osmolality for white-tailed prairie dogs, on the other hand, was slightly lower in June but not significantly below urine osmolality in March or July for that species. This corresponded to the slight increase in precipitation at Laramie during the early summer. Both species of prairie dogs had similar urine concentrations for the rest of the periods of collection. This was, in part, reflective of the similar amounts of precipitation received at both locations at those times. Virtually all water available to both species probably was preformed water in their natural foods. No free surface water was observed in areas of either species and probably seldom if ever occurs. King (1955) reported that while black-tailed prairie dogs readily accepted grain in traps, they would ignore water dishes or even fill them with dirt. However, he did report pregnant and lactating females eating snow and suggested that they may be dependent on it. Urine osmolality in March was lower than in September for both species, but the difference was significant only for black-tailed prairie dogs. Because snow was present in March at both areas, it may have accounted for the lower urine osmolality at that time.

Urine osmolality of dehydrated black-tailed prairie dogs in the laboratory was greater than that of any field sample indicating that under natural conditions, this species was not maximally stressed for water conservation. However, urine concentrations for white-tailed prairie dogs for all periods of collection except June were not different from maximum urine concentration in dehydrated animals. Thus, white-tailed prairie dogs were apparently stressed close to maximum for water balance under natural conditions for much of the time.

Urine from dehydrated black-tailed prairie dogs was greater in concentration than urine from dehydrated white-tailed prairie dogs. This was substantiated by kidney structure and U/P ratios. Black-tailed prairie dog kidneys had a greater relative medullary thickness and dehydrated black-tailed prairie dogs had a greater U/P than dehydrated white-tailed prairie dogs. Urine/Plasma from dehydrated black-tailed prairie dogs was also greater than field U/P for both species but these differences were not significant.

The concentration of potassium in the urine (Fig. 7) of both species was associated closely with urine osmolality. This was the major component to influence the variation in seasonal trends of urine osmolality for both species. The only exception to this was the March sample for black-tailed prairie dogs, which was no different from the July potassium concentration for that species. During that month, urea (Fig.8) compensated for the low potassium concentration in black-tailed prairie dogs. There evidently was a relative dietary difference or at least a metabolic difference when considering potassium and protein utilization in March for black-tailed prairie dogs, compared to the rest of the periods, as evidenced by the decreased amount of potassium and increased amount of urea in the urine.

Urine urea concentration was quite constant during all collecting periods for both species except the March sample for black-tailed prairie dogs. This means that during times of lowest urine osmolality, urea comprised a larger proportion of urine solute. Conversely, when urine osmolality was highest, urea comprised a small proportion of the urine solute. Lassiter _et al_. (1961) reported and Danielson _et al_. (1970) confirmed that the collecting ducts in the mammalian kidney were responsible for urea recirculation, which was greatest in animals on a low protein diet. Walser (1970) reported that ammonia nitrogen resulting from urea degradation may be used for protein synthesis. Whether or not the relatively low urea concentrations in August and September for both species studied here represented urea recirculation and subsequent degradation to simpler nitrogen compounds for protein synthesis remains to be demonstrated. However, ammonia excretion (Fig. 9) in both species of prairie dogs did not disagree with the above idea. That is, if ammonia nitrogen was to be used for protein synthesis it would thus become a possible "asset" rather than a "liability" and probably would be excreted in minimal amounts. Ammonia was excreted in much smaller amounts in both species during August and September. If ammonia excretion is compared as percent of urine osmolality, the difference between the March-June-July vs the August-September samples is even more pronounced. Likewise, plasma urea concentrations (Fig. 10) from field animals are compatable with this concept. If urea is to be recirculated for possible degradation and subsequent protein synthesis, then higher concentrations would be expected in the plasma also. This was best observed in black-tailed prairie dogs in August and September.

Dehydrated animals produced higher urine urea concentrations than did field animals except for the March sample from white-tailed prairie dogs, which was lower albeit not significantly different from that of the dehydrated white-tailed prairie dogs. Urine potassium from dehydrated animals was lower than most field samples for both species. Potassium was evidently higher in natural foods than in the Purina Chow used in this study. Even if urine sodium concentration from dehydrated animals is added to potassium from the same animals, the combined electrolyte concentration does not equal urine potassium concentration for most field samples.

Urine sodium (Table 7) constituted a small part of urine osmolality and probably represented a small amount of animal material (mainly insects) in the diet (Kelso 1939, King 1955, Koford 1958, Smith 1958). Sodium was higher in most white-tailed prairie dog urine samples than in black-tailed prairie dog samples. There could have been several reasons for this, possibly including a higher incidence of palatable insects in the white-tailed prairie dog habitat. Alternatively, perhaps insects helped to supply water to the more water stressed white-tailed prairie dogs. A possible correlation of animal material in the diet of gray and red squirrels with reproduction has been suggested (Goodrum 1940, Bakken 1952, C. Smith 1968). A similar correlation for urine sodium was observed in the tree squirrels discussed previously. However, there was no correlation of urine sodium with any reproductive activity in prairie dogs. Further exploration into the role of sodium in small herbivorous rodents is needed. Urine sodium in laboratory animals was much higher than field samples due to the high NaCL content of the Purina Chow.

Plasma concentrations of field animals were similar for both species except that plasma sodium concentration in white-tailed prairie dogs was low. Trauma may cause a sodium-potassium shift in the plasma which results in a decrease in sodium and an increase in potassium (Friedman and Friedman 1963). The shooting of field animals in this study may explain why sodium was lower and potassium was higher in field animals than in hydrated laboratory animals. An additional factor that may have caused higher sodium and consequently higher plasma osmolality in hydrated laboratory animals was the relatively high salt content of the laboratory food.

Dehydrated animals demonstrated higher total plasma and plasma sodium concentration as expected.

The ability of black-tailed prairie dogs to survive longer without water (Fig. 11) further substantiated their superior adaptation to negative water balance. Animals of both species ended the experiment at similar body weights. The absence of body fat at time of death indicated that both species were able to survive on metabolic water until fat reserves were depleted. However, because white-tailed prairie dogs were not able to concentrate urine as well, they had to use body fat at a greater rate to obtain the necessary metabolic water.

It is interesting that black-tailed prairie dogs, although living in a more mesic environment, are better adapted to xeric conditions than are white-tailed prairie dogs. Black-tailed prairie dogs do not hibernate (King 1955, Koford 1958, Smith 1958, Tileston and Lechleitner 1966), whereas white-tailed prairie dogs probably do hibernate (Stockard 1930, Tileston and Lechleitner 1966, Bakko and Brown 1967). These authors also reported that although white-tailed prairie dogs were observed above ground for 8 months of the year (late February to October), individuals were active above ground for only 4-5 months. Adults appear above ground in late February or early March and return permanently to their burrows in mid-July or August. Reproduction occurs in March. Juveniles appear above ground in mid-June and remain active through October. Therefore, adult and juvenile white-tailed prairie dogs are feeding together on the available vegetation for only about one month.

This period coincides with the most productive period of plant growth as governed by precipitation for that area. Survival of juvenile white-tailed prairie dogs under the dry conditions of late summer and fall probably indicates that adults also could survive above ground. However, there may not be enough succulent vegetation to maintain the entire population (both age classes) of each colony through the dry period whereas there is enough for the juveniles. If an extremely dry period should occur, the adults would have a better chance of surviving to the spring reproductive period because they put on body fat during the "lush green" period followed by a retreat to cool, moist, underground burrows. Thus, white-tailed prairie dogs have evidently adapted to xeric conditions by means of torpor (Fisher and Manery 1967). Spending a substantial period of time underground each year has probably precluded the more social nature observed in black-tailed prairie dogs.

Although white-tailed prairie dogs live in socially structured colonies (Tileston and Lechleitner 1966), they are not as highly organized as are black-tailed prairie dogs (King 1955, Koford 1958, Smith 1958, Tileston and Lechleitner 1966). Furthermore, density within the colony is greater for black-tailed prairie dogs. For example, Tileston and Lechleitner (1966) reported 21.9 burrow entrances per acre in a white-tailed prairie dog town with a maximum density of 3.4 animals per acre compared to 41.9 burrow entrances in a black-tailed prairie dog town with a maximum density of 12.9 animals per acre. They also reported that the maximum density of white-tailed prairie dogs was observed for only about one month while the maximum density of black-tailed prairie dogs was observed until late fall with only a gradual decline. Clark (1969) reported an average density of 1.34 white-tailed prairie dogs per acre in Wyoming. King (1955) calculated an average density of 8.9 black-tailed prairie dogs per acre in South Dakota.

Maintenance of highly organized societies by black-tailed prairie dogs probably requires nearly continuous interaction of the entire colony. Therefore, this species apparently has adapted physiologically to survive the rigors of a moderately arid environment without having to retreat to the confines of a burrow for extended periods.

GENERAL DISCUSSION AND SUMMARY

Because all four species studied are members of the family Sciuridae, it would be worthwhile to compare physiological adaptations to water balance in tree squirrels to that in prairie dogs. All data discussed here have been presented previously in this paper.

Table 10 compares some of the major measurements determined in this study for the four species. The difference in water availability for the two groups in their respective habitats is illustrated by the fact that means of all measurements from prairie dogs were greater than those for squirrels except when comparing relative medullary thickness in white-tailed prairie dogs to that in red squirrels, minimum field urine osmolality of black-tailed prairie dogs to that of gray squirrels, and minimum U/P ratios of black-tailed prairie dogs to that of gray squirrels.

Phylogenetic relationships show that tree squirrels (tribe Sciurini) and ground squirrels (tribe Marmotini) separated during Oligocene times which was quite early in the history of the sciurids (Black 1963). At that time the Great Plains probably consisted of mesic forests and grasslands with an average annual precipitation estimated to be about 50 cm per year (MacGinitie 1953). During this time, semiarboreal chipmunk-like mammals (_Tamias_) were thought to inhabit the area (Black 1963). Subsequent gradual upheaval of the Rocky Mountains reduced annual precipitation to present levels (Table 6) and the rather extensive woodland areas gave way to shortgrass prairies. This probably resulted in strong selective

TABLE 10. Comparison of kidney relative medullary thickness, urine osmolality, U/P ratios, and body weight loss and survival time under dehydration stress for gray and red squirrels and black-tailed and white-tailed prairie dogs. $[\bar{X} \pm (T_{0.975})(S.E.)]$

Measurement	Gray squirrels	Red squirrels	Black-tailed prairie dogs	White-tailed prairie dogs
Kidney rmt	5.15 ± 0.8	5.79 ± 0.12[a] 5.95 ± 0.12[b] 5.32 ± 0.23[c]	6.32 ± 0.16	5.85 ± 0.13
Field - maximum mOsm	1402 ± 182.4 (Mar-Apr)	1367 ± 211.0 (Nov-Dec)	2480 ± 210.9 (Sept)	2381 ± 305.2 (Sept)
Lab - maximum mOsm	1783 ± 98.7	1787 ± 213.5	2837 ± 84.3	2365 ± 114.2
Field - minimum mOsm	643 ± 232.6 (July)	309 ± 147.1 (July)	825 ± 225.1 (July)	1829 ± 188.5 (June)
Lab - minimum mOsm	912 ± 84.1	564 ± 41.7	1795 ± 321.4	1363 ± 245.6

TABLE 10 (cont.). Comparison of kidney relative medullary thickness, urine osmolality, U/P ratios, and body weight loss and survival time under dehydration stress for gray and red squirrels and black-tailed and white-tailed prairie dogs. $[\bar{X} \pm (T_{0.975})(S.E.)]$

Measurement	Gray squirrels	Red squirrels	Black-tailed prairie dogs	White-tailed prairie dogs
Field - maximum U/P	4.52 ± 0.61 (Mar-Apr)	4. 74 ± 0.64 (Nov-Dec)	8.76 ± 0.63 (Sept)	8.05 ± 1.02 (Sept)
Lab - maximum U/P	4.44 ± 1.01	4.16 ± 0.80	9.09 ± 0.62	6.80 ± 1.26
Field - minimum U/P	2.10 ± 1.03 (July)	1.14 ± 0.60 (July)	2.92 ± 0.62 (July)	6.22 ± 0.59[d] (June)
% initial body weight at time of death	70.4 ± 4.9	74.4 ± 3.1	43, 41[e]	39, 19[e]
Days survived w/o water	6.8 ± 2.0	2.0 ± 0.0	122, 238[e]	44, 50[e]

[a] Total animals collected.

[b] Animals from coniferous and mixed hardwoods habitats.

[c] Animals from river bottom habitats.

[d] U/P ratios not paired. Mean plasma concentration for all field animals used to compute U/P.

[e] Individual data.

pressure for adaptation to increasingly terrestrial life. Mammals capable of making the shift from forest and forest-edge habitats to the grasslands were favored. Meanwhile, the more arboreal forms followed the retreat of the mesic forests. Consequently, Black placed chipmunks (*Tamias*) in an intermediate position between tree squirrels and ground squirrels and presumed that the chipmunk-like ancestor gave rise to both tree squirrels and ground squirrels during this habitat divergence in Oligocene times. With this significant divergence one would expect major differences to appear between the tree squirrels and ground squirrels with respect to water balance physiology as well as the obvious morphological changes. These have been brought out in this study.

Within the tree squirrels, *Tamiasciurus* shows signs of continued radiation from *Sciurus*. *Tamiasciurus* became generically distinct from *Sciurus* during late Pliocene (Black 1963). Bryant (1945) reported that although skull differences were hardly sufficient to warrant generic difference, reproductive differences were considered of generic significance. Moore (1959) placed red squirrels in a separate tribe (Tamiasciurini) from gray squirrels based on reproductive differences, mainly, a bacculum was lacking in red squirrels. Black (1963), however, placed both gray and red squirrels in the tribe Sciurini. Layne (1954b) reported that a rudimentary bacculum does exist in red squirrels, the reduction of which represents an advanced degree of specialization. Because red squirrels occupy so many habitats within their range, it is expected that this species has wide adaptability. Adaptation of kidney structure to environment, as shown in this study, supports this.

Although, it is not known to what extent this adaptability represents genetic versus behavioral differences between populations.

Black (1963) reported that ground squirrel radiation is recent and still in progress. Prairie dogs (_Cynomys_) probably became generically distinct from other ground squirrels (_Spermophilus_) in late Pliocene or early Pleistocene. He cites _Cynomys_ as becoming highly specialized in a short time. Cade (1964) reported that torpor is primitive in mammalian history and is being lost or submerged as a vestigal trait. The superior water concentrating ability of black-tailed prairie dogs over white-tailed prairie dogs is probably instrumental for this species to be able to survive in their environment without relying on torpor to escape the environmental rigors. This in turn has allowed a social organizational development that surpasses that of white-tailed prairie dogs. Based on chromosome structure, Nadler _et al_. (1971) reported a higher degree of specialization in black-tailed prairie dogs than in white-tailed prairie dogs. They correlated this with the greater social specialization observed in black-tailed prairie dogs. Thus, genetic radiation in black-tailed prairie dogs is probably proceding at a greater rate than in the more conservative white-tailed prairie dogs.

LITERATURE CITED

Bakken, Z. 1952. Interrelationships of *Sciurus carolinensis* (Gmelin) and *Sciurus niger* (Linneaus) in mixed populations. Ph. D. thesis. Univ. of Wisconsin. 188p.

Bakko, E. B., and L. N. Brown. 1967. Breeding biology of the white-tailed prairie dog, *Cynomys leucurus*, in Wyoming. J. Mamm., 48: 100-112.

Baxter, J. S., and J. M. Yoffey. 1948. The post-natal development of renal tubules in the rat. J. Anat., 82: 189-197.

Black, C. C. 1963. A review of the North American tertiary Sciuridae. Bull. Mus. Comp. Zool. Harv., 130: 109-248.

Blount, R. F., and I. H. Blount. 1968. Adaptive changes in size of renal papilla with altered function. Texas Reports on Biol. and Med., 26: 473-484.

Bredahl, E. A. 1969. A study of water balance in hibernating and non-hibernating *Citellus franklinii*. Ph. D. thesis, Univ. of North Dakota. 99p.

Brown, L. G., and L. E. Yeager. 1945. Fox squirrels and gray squirrels in Illinois. Bull. Ill. Nat. Hist. Surv., 23: 449-532.

Bryant, M. D. 1945. Phylogeny of Nearctic Sciuridae. Amer. Mid. Nat., 33: 257-390.

Cade, T. J. 1964. The evolution of torpidity in rodents. Ann. Acad. Sci. Fenn., Series A, IV, 71: 78-112.

Carpenter, R. E. 1966. A comparison of thermoregulation and water metabolism in the kangaroo rats *Dipodomys agilis* and *Dipodomys merriami*. Univ. Calif. Publ. Zool., 78: 1-36.

Chew, R. M. 1951. The water exchange of some small mammals. Ecol. Monogr., 21: 215-225.

________. 1965. Water metabolism of mammals. Pp. 43-178. In: Physiological Mammalogy II, W. V. Mayer and R. G. Van Gelder, eds. Acad. Press, New York. 326p.

Clark, T. W. 1969. A study of the population dynamics of a colony of white-tailed prairie dogs (*Cynomys leucurus*) in Wyoming. Rocky Mt. Div., A.A.A.S. May 8, 1969, Colorado Springs, Colorado.

Cochran, W. W., and G. M. Cox. 1964. Experimental Designs. John Wiley and Sons, Inc., New York. 611p.

Danielson, R. A., B. Schmidt-Nielsen and C. Hohberger. 1970. Micropuncture study of the regulation of urea excretion by the collecting ducts in rats on high and low protein diets. Pp. 375-384. In: Urea and the Kidney, B. Schmidt-Nielsen and D. W. S. Kerr, eds. Excerpta Medica Foundation, Amsterdam. 495p.

Davis, D. S., and F. B. Golley. 1963. Principles in Mammalogy. Reinhold Publ. Corp., New York. 335p.

Davson, H., and M. C. Eggleton. 1968. Starling's Principles of Human Physiology. Lea and Febiger, Philadelphia, 14th ed.

Dolbeer, R. A. 1973. Reproduction in the red squirrel (*Tamiasciurus hudsonicus*) in Colorado. J. Mamm., 54: 536-540.

Finley, R. B., Jr. 1969. Cone caches and middens of *Tamiasciurus* in the Rocky Mountain region. Misc. Publ., Univ. Kansas Mus. Nat. Hist., 51: 233-273.

Fisher, K. C., and J. F. Manery. 1967. Water and electrolyte metabolism in heterotherms. Pp. 235-279. In: Mammalian Hibernation III, K. C. Fisher, *et al.*, eds. American Elsevier Publ. Co., New York.

Friedman, S. M., and C. L. Friedman. 1963. Effects of ions on vascular smooth muscle. In: Handbook of Physiology, section 2; Circulation, vol. II, W. F. Hamilton and P. Dow, eds. Amer. Physiol. Soc., Washington, D.C.

Goodrum, P. D. 1940. A population study of the gray squirrel in eastern Texas. Texas Agr. Expt. Sta. Bull., no. 591. 34p.

Gunderson, H. L., and J. R. Beer. 1953. The Mammals of Minnesota. Univ. of Minnesota Press, Minneapolis. 190p.

Hall, E. R., and K. R. Kelson. 1959. The Mammals of North America. Ronald Press, New York. 2 vol.

Hamilton, W. J., Jr. 1939. Observations on the life history of the red squirrel in New York. Amer. Mid. Nat., 22: 732-745.

Hatt, R. T. 1943. The pine squirrel in Colorado. J. Mamm., 24: 311-345.

Irving, L., H. Krog, and M. Monson. 1955. The metabolism of some Alaskan animals in summer and winter. Physiol. Zool., 28: 173-185.

Jameson, E. W., Jr., and R. A. Mead. 1964. Seasonal changes in body fat, water, and basic weight in *Citellus lateralis*, *Eutamias speciosus*, and *E. amoenus*. J. Mamm., *45*: 359-365.

Kelso, L. H. 1939. Food habits of prairie dogs. U. S. D. A. Circ., no. 529. 15p.

Kemp, G. A., and L. B. Keith. 1970. Dynamics and regulation of red squirrel (*Tamiasciurus hudsonicus*) populations. Ecology, 51: 763-779.

King, J. A. 1955. Social behavior, social organization and population dynamics in a black-tailed prairie dog town in the Black Hills of South Dakota. Contrib. Lab. Vert. Biol., no. 67, Univ. of Michigan. 123p.

Klugh, A. B. 1927. Ecology of the red squirrel. J. Mamm., 8: 1-32.

Koford, C. B. 1958. Prairie dogs, white faces and blue grama. Wildl. Monogr., no 3. 78p.

Lassiter, W. E., C. W. Gottschalk and M. Mylle. 1961. Micropuncture study of net transtubular movement of water and urea in nondiuretic mammalian kidney. Am. J. Physiol., 200: 1139-1147.

Layne, J. N. 1954a. The biology of the red squirrel, *Tamiasciurus hudsonicus* loquax (Bangs), in central New York. Ecol. Monogr., *24*: 227-267.

________. 1954b. The os clitoridis of some North American Sciuridae. J. Mamm., 35: 357-366.

MacGinitie, H. D. 1953. Fossil plants of the Florissant beds, Colorado. Carnegie Inst. Wash. Publ., no. 599. 198p.

MacMillen, R. E. 1972. Water economy of nocturnal desert rodents. Symp. Zool. Soc. Lond., 31: 147-174.

MacMillen, R. E., and A. K. Lee. 1969. Water metabolism of Australian hopping mice. Comp. Biochem. Physiol., 28: 493-514.

McKeever, S. 1964. Food habits of the pine squirrel in northeastern California. J. Wildl. Mgmt., 28: 402-404.

Moore, J. C. 1959. Relationships among the living squirrels of the Sciuridae. Amer. Mus. Nat. Hist., 118: 155-206.

Mullen, R. K. 1970. Respiratory metabolism and body water turnover rates of *Perognathus formosus* in its natural environment. Comp. Biochem. Physiol., *32*: 259-265.

Nadler, C. F., R. S. Hoffman, and J. J. Pizzimenti. 1971. Chromosomes and serum proteins of prairie dogs and a model for Cynomys evolution. J. Mamm., 52: 545-555.

Nixon, C. M., D. M. Worley and M. W. McClain. 1968. Food habits of squirrels in southeastern Ohio. J. Wildl. Mgmt., 32: 294-305.

Obrink, K. J. 1955. A modified Conway unit for microdiffusion analysis. Biochem. J., 59: 134-136.

Ovington, J. D. 1962. Quantitative ecology and the woodland ecosystem concept. Pp. 103-192. In: Advances in Ecological Research, vol. I, J. B. Cragg, ed. Academic, New York. 203p.

Pearson, O. P. 1960. Habits of Microtus californicus revealed by automatic photographic recorders. Ecol. Monogr., 30: 232-249.

Schmid, W. D. 1972. Nocturnalism and variance in ambient vapor pressure of water. Physiol. Zool., 45: 302-309.

Schmidt-Nielsen, B. 1964. The excretory system. Pp. 215-243. In: Handbook of Physiology, sect. 4, Adaptation to the Environment, D. B. Dill, ed. Amer. Physiol. Soc., Washington, D. C.

Schmidt-Nielsen, B., and D. W. S. Kerr, eds. 1970. Urea and the Kidney. Excerpta Medica Foundation, Amsterdam. 495p.

Schmidt-Nielsen, B., and R. O'Dell. 1961. Structure and concentrating mechanism in the mammalian kidney. Am. J. Physiol., 200: 1119-1124.

Schmidt-Nielsen, B., and K. Schmidt-Nielsen, 1950. Evaporative water loss in desert rodents in their natural environment. Ecology, 31: 75-85.

Schmidt-Nielsen, B., K. Schmidt-Nielsen, A. Brokaw, and H. Schneiderman. 1948. Water conservation in desert rodents. J. Cell. Comp. Physiol., 32: 331-360.

Schmidt-Nielsen, K. 1964. Desert Animals. Oxford Univ. Press, London. 277p.

Selander, R. K. 1970. Behavior and genetic variation in natural populations. Amer. Zool., 10: 53-66.

Smith, C. C. 1968. The adaptive nature of social organization in the genus of tree squirrels Tamiasciurus. Ecol. Monogr., 38: 31-63.

Smith, M. C. 1968. Red squirrel response to cone failure in interior Alaska. J. Wildl. Mgmt., 32: 305-317.

Smith, R. E. 1958. Natural history of the prairie dog in Kansas. Univ. Kansas Misc. Publ., no. 16. 36p.

Sperber, I. 1944. Studies on the mammalian kidney. Zool. Bidrag. Fran Uppsala, 22: 249-431.

Stockard, A. H. 1930. Observations on the seasonal activities of the white-tailed prairie dog (Cynomys leucurus). Pap. Mich. Acad. Sci., Arts and Letters, 11: 471-479.

Tileston, J. V., and R. R. Lechleitner. 1966. Some comparisons of the black-tailed and white-tailed prairie dogs in north-central Colorado. Amer. Mid. Nat., 83: 167-185.

Ullrich, K., J. Kramer and J. W. Boylan. 1961. Present knowledge of the countercurrent multiplier system in the mammalian kidney. Prog. Card. Diseases, 3: 395-431.

Vimtrup, B., and K. Schmidt-Nielsen. 1952. The histology of the kidney of kangaroo rats. Anat. Rec., 114: 515-527.

Vorhies, C. T. 1945. Water requirements of desert animals in the Southwest. Tech. Bull. Agric. Exper. Sta., Univ. Arizona, 107: 487-525.

Walser, M. 1970. Use of isotopic urea to study the distribution and degradation of urea in man. Pp. 421-429. In: Urea and the Kidney, B. Schmidt-Nielsen and D. W. S. Kerr, eds. Excerpta Medica Foundation, Amsterdam. 495p.

Wilber, C. G., and P. F. Robinson. 1958. Blood composition in deer. J. Mamm , 39: 309-310.