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A DISSERTATION

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1969

FUNCTIONAL ADAPTATIONS IN THE WATER ECONOMY

OF *PEROMYSCUS MANICULATUS NEBRASCENSIS*

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INTRODUCTION

Peromyscus maniculatus is the most widespread North American rodent (Hall and Kelson, 1959), (Fig. 1). Within its range are habitats which are considered mesic to xeric. P. maniculatus, a cricetid, has been collected in such arid conditions as Carlsbad, New Mexico, the panhandle of Oklahoma, and the Painted Desert in Arizona. It has also been taken in mesic areas such as the coastal regions of Canada, Washington, Oregon, and California (Hall and Kelson, 1959). P. m. nebrascensis ranges throughout central United States occurring in the western two-thirds of Nebraska and Oklahoma, western one-half of Kansas and South Dakota, southwestern corner of North Dakota, and northern panhandle of Texas (Fig. 1). The habitat of this subspecies is mesic and is periodically subject to drought. Since this animal may experience water shortage or dehydration in its natural environment there is a possibility of it being adapted to xeric conditions.

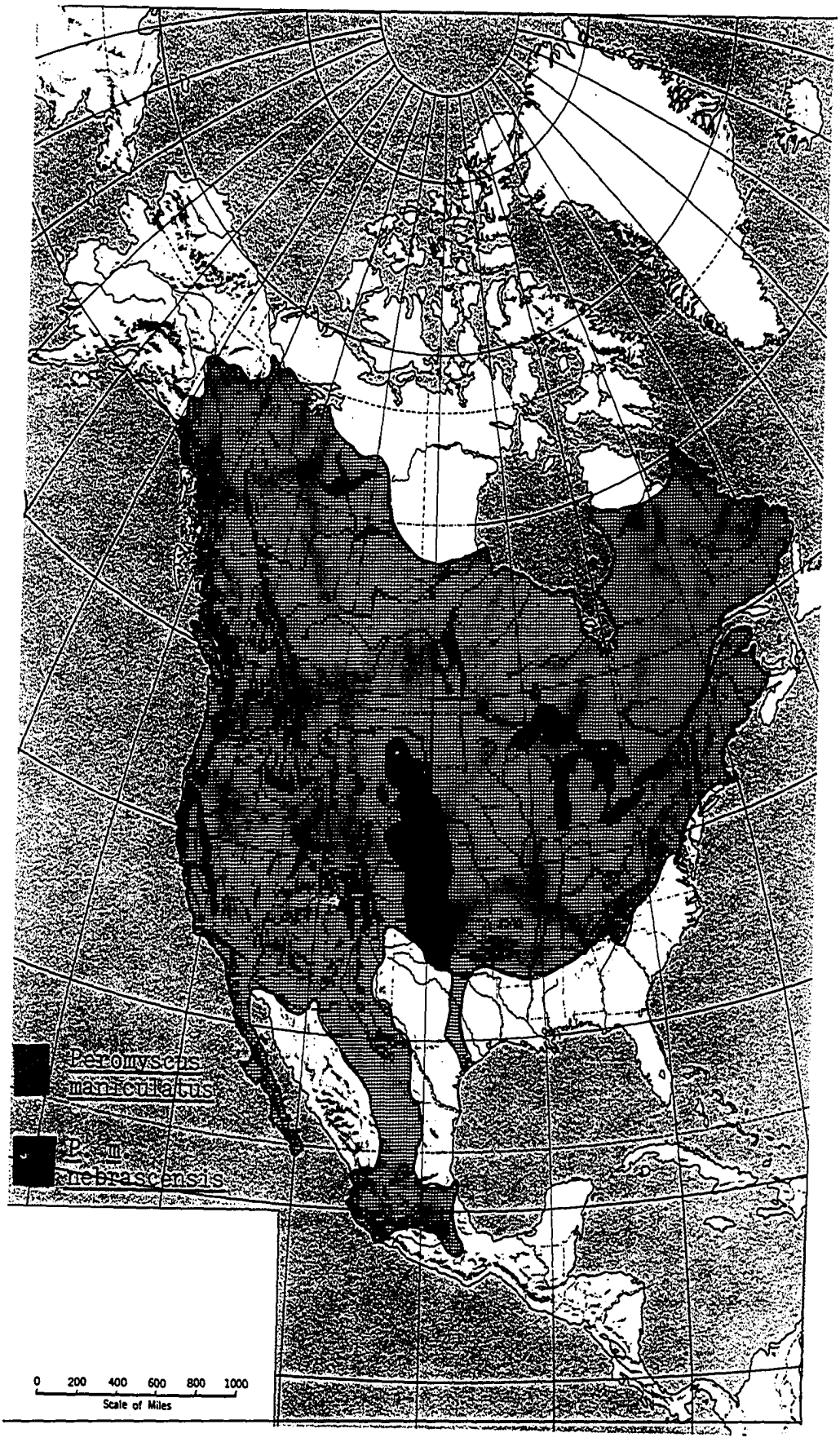

Fig. 1.--Distribution of Peromyscus maniculatus and P. m. nebrascensis (Hall and Kelson, 1959).

An animal exposed to water shortage must reduce water loss to remain in water balance. In an animal in water balance the avenues of water loss (fecal, urinary, evaporative) are balanced by water intake (free water, metabolic water, bound water). The degree to which one or all of the avenues of water loss can be reduced may be critical to the survival of the animal during times of water shortage.

Literature Review

There are no data on the ability of P. m. nebrascensis to acclimate (the functional compensation over a period of days to weeks in response to a single environmental factor only, as in controlled experiments) (Folk, 1966) or acclimatize (the functional compensation over a period of days to weeks to a complex of environmental factors, as in seasonal or climatic changes) (Folk, 1966) to water deprivation. How effectively this animal can adapt by acclimation or acclimatization to water deprivation should be reflected by changes in urine concentrations and/or volume, fecal water, and possibly evaporative water loss (EWL).

Chew (1965) summarized data on the daily ad lib water intake for six subspecies of P. maniculatus. These studies were done in the laboratory under various conditions of temperature, humidity, and diet. P. m. bairdi had an ad lib water intake of 3.0 ml/day at an average ambient

temperature (T_A) of 20-25 C and on a diet of "air-dry food" (Lindeborg, 1952). Dice (1922) showed a water intake of 1.7 ml/day for the same subspecies while on a diet of whole wheat where the average T_A was 21 C. At an average T_A of 20-25 C and on a diet of "air-dry food" ad lib water consumption for P. m. blandus, P. m. nebrascensis, and P. m. gracilis was 1.9, 2.6, and 2.7 ml/day respectively (Lindeborg, 1952). At an average temperature of 24.7 C and a mean R.H. of 56.2% and on a diet of Purina Chow P. m. sonoriensis had an ad lib water intake of 2.7 ml/day (French, 1956). The ad lib water intake for P. m. nubiterrae was 1.8 ml/day (Odum, 1944). Neither temperature, diet, or R.H. were given. The humidity and type of diet are seldom specified but are essential if comparisons are made.

The tolerance to water restriction in P. m. nebrascensis is not known. Lindeborg (1952) studied water restriction in the genus Peromyscus and showed a direct correlation between habitat and ability to withstand water deprivation. Xeric species were able to survive longer on the lowest water rations and maintained a greater percentage of their original weight than mesic species. A significant difference between the number of visits to the drinking device and in total amounts of water drunk was shown for P. maniculatus (mesic form) and P. eremicus (xeric form), but there was no difference in these same two factors between subspecies within either species (Ross, 1930). This

relationship between aridity and the amount of moisture required does not always show a direct relationship. In restricted food and water studies, Hudson and Rummel (1966) showed that Liomys salvani, a tropical species (mesic environment), was able to maintain body weight without free water and produced a more concentrated urine than L. irroratus, a subtropical species (xeric environment).

Water restriction has also been studied by providing animals various concentrations of NaCl drinking solutions (salt loading). The tolerance of P. m. nebrascensis to increasing concentrations of NaCl solutions is not known, but the ability to survive salt loading has been determined for several other species. Often the degree of tolerance to salt loading is correlated to the environment of the population under study. Fisler (1962) found that a P. m. rubidus inhabitating an area near a salt marsh survived 160 days drinking sea water, while two P. m. gambelii (first generation laboratory stock from a population on California Mountain in the central Sierra Nevadas) died at 12 and 67 days. Schmidt-Nielsen and Schmidt-Nielsen (1950a) showed that Dipodomys merriami (a desert rodent) could maintain weight on salt solutions as concentrated as sea water. Bartholomew and Hudson (1959) obtained similar results with the antelope ground squirrel, Citellus leucurus, which also dwells in deserts. Adaptation to salt-water drinking in desert species is probably related to water conservation

through the ability of the kidney to produce a concentrated urine. P. floridanus was unable to survive on sodium chloride solutions above 0.35M and unlike the desert species mentioned above required either free water or succulent food supplements (Fertig and Layne, 1963).

Urine concentrations and volume changes of water deprived animals have been studied in the laboratory (Chew, 1951, 1965; Hudson, 1962; Hudson and Rummel, 1966; Bentley, 1955; Haines and Schmidt-Nielsen, 1967), showing that urine volume decreases with water deprivation and/or the urine concentration increases. Haines and Schmidt-Nielsen (1967) induced Mus musculus to produce average maximal urine concentrations of 5.0 osmoles by feeding a high protein diet and restricting water intake. Urine concentrations above 5.0 osmoles are not uncommon in some desert rodents. The ability of many of these rodents to subsist on a dry diet is at least partially due to their capability of producing highly concentrated urine. No species of Peromyscus have been found to survive on air-dry food. Such desert dwelling species as P. eremicus and P. m. sonoriensis require drinking water or succulent food (Ross, 1930; French, 1956). No measurements of urine osmolality, sodium, chloride, and potassium concentrations for P. maniculatus were found in the literature.

Urine concentrations of animals in the field are seldom determined. The difficulties involved in studying

the effects of day to day weather changes and their effect on the microhabitat of small mammals has primarily limited acclimatization studies to man and farm animals (Refer to review by Hart, 1957). In contrast, acclimation studies are abundant and cover a wide variety of physiological functions in a number of species.

The effects of water restriction on EWL have been overlooked, but it is known that some rodents can reduce pulmonary water losses. Jackson and Schmidt-Nielsen (1964) explained the mechanism that allows an animal to establish a reduced nasal temperature and thereby reduce pulmonary water loss as a "countercurrent heat exchanger with intermittent flow in a single tube instead of the usual continuous flow in two tubes." Factors that have been shown to affect respiratory water loss of small mammals are ambient temperature and the absolute humidity of the inspired air (Getz, 1968; Jackson and Schmidt-Nielsen, 1964). Total EWL (pulmonary plus cutaneous) has been shown to increase with an increase in ambient temperature (Chew, 1951, 1965; Hudson, 1962; Hudson and Rummel, 1966); to increase with a decrease in R.H. (Chew and Dammann, 1960); to decrease with a decrease in free water intake (Chew, 1951; Hudson and Rummel, 1966; Shield, 1968); and to increase with an increase in body weight (Chew, 1965). The total amount of moisture evaporated from the pulmonary surface area and the skin accounts for an appreciable water loss. Chew (1955) found the total EWL

of P. m. sonoriensis on ad lib food and water intake to be equal to one-fourth to one-half the total water loss under normal laboratory conditions. The above study (Chew, 1955) is the only EWL study on a subspecies of P. maniculatus, and it did not consider the possibility of reduced water loss upon acclimation to water deprivation.

Metabolic rate and body temperature may be related to the ability to withstand water restriction. Entry into hypothermia was shown for P. eremicus (MacMillen, 1965), Baiomys taylori (Hudson, 1965), and Perognathus longimembris (Bartholomew and Cade, 1957) at room temperature with food and water deprivation. Dormancy is usually discussed in terms of the ability of an animal to become hypothermic for extended or short periods in lieu of maintaining high metabolic rates during periods of food shortage. Regardless of the reduced energy expenditure these features may be adaptive in dry desert conditions in that they reduce this expenditure of moisture. MacMillen (1965) stated that aestivation in P. eremicus is effective in reducing water loss in this species and may account for its occurrence in dry habitats. McNab and Morrison (1963) described a reduction in metabolism for desert and chaparral subspecies of Peromyscus which may contribute to their ability to survive arid environments. The environmental conditions necessary to bring about a reduction in metabolism for these arid subspecies of Peromyscus are not known.

The Problem

Since P. maniculatus is widely distributed one might expect it to tolerate water restriction as well as some desert animals that can remain in water balance by reducing the avenues of water loss when deprived of water. The objective of this study was to determine the ability of one subspecies of P. maniculatus to withstand and adapt to water deprivation. Literature deficiencies for the genus Peromyscus were noted in the following areas: Metabolism studies involving food and/or water restriction; acclimation to reduced water intake and concomitant urine concentrations; ad lib water intake under specified conditions; acclimation to increasing concentrations of NaCl solutions and how this affects renal efficiency; the effects of water deprivation on EWL; changes in urine concentrations of acclimatized animals. The following questions are in response to these deficiencies and indicate the scope of the study.

1. Do acute water and/or food deprivation affect the metabolism, total body water content, and urine concentrating ability of P. m. nebrascensis?
2. What is the ad lib water intake of this subspecies under specified conditions of temperature, humidity, and diet?
3. To what degree can P. m. nebrascensis acclimate to reduced water intake? Which avenues

of water loss are reduced?

4. How effectively can this subspecies adapt to salt loading? Is renal efficiency increased with acclimation to increasing concentrations of NaCl solutions?
5. Are pulmonary and cutaneous EWL reduced upon acclimation to water deprivation? How much water is obligated to evaporation in nonacclimated and acclimated animals?
6. Do urine concentrations of P. m. nebrascensis change during the seasons of the year?

METHODS AND MATERIALS

Experimental Plan

A series of five experiments was conducted:

1. Metabolism studies were conducted on twenty mice subjected to acute and total water and/or food restriction to determine if P. m. nebrascensis becomes hypothermic in response to water and/or food deprivation.
2. Ad lib water consumption was determined for mice under controlled laboratory conditions. Then, six of these mice were gradually acclimated to water deprivation to determine the minimum daily water requirements.
3. A series of increasing concentrations of salt solutions was given to six mice to determine the ability to tolerate salt loading. Urine concentrations were determined during acclimation to water deprivation and salt loading to evaluate renal efficiency.
4. Pulmonary and cutaneous evaporative water losses were determined for a control group of six mice

and a group of four mice acclimated to water restriction.

5. Urine samples were collected from field animals over a years' interval to determine if P. m. nebrascensis produces a concentrated urine during periods of possible water shortage in the natural environment.

Experimental Animals

All animals used in these experiments were captured in Sherman live traps from August, 1967, through July, 1968. The animals were trapped on an abandoned air strip of Max Westheimer Field, North Campus, University of Oklahoma, Norman. Only animals used in determining seasonal urine concentrations were used for more than one type of experimental measurement.

Urine Collection and Analysis

To collect urine samples, the mice were placed in hardware cloth restraining cages. The cages were positioned over disposable petri dishes filled with paraffin oil. Any sample voided within the first hour was discarded to eliminate possible diuretic samples. Samples from controls were usually obtained within the first four hours of collection. In water deprived animals longer collection periods were required. Urine samples were transferred from beneath the paraffin oil with micropipettes, and were placed in

polyethylene micro test tubes (Beckman Laboratory Instrumentation No. 314326) and frozen.

Chloride concentrations were determined with an Aminco-Cotlove automatic chloride titrator. Osmotic activity was measured with a vapor pressure osmometer (Mechrolab model 301A). Ten µl urine samples were diluted with 50 µl of distilled water for all osmolality determinations. Sodium and potassium determinations were made by flame photometry (Baird Atomic model KY 3).

Metabolism

This experiment was conducted from September 25, 1967, through December 9, 1967. All animals were taken from the field during the months of August and September. Animals were allowed to adjust for two weeks to the experimental diet, temperature, humidity, and the confinement of the cage. Separate metabolism cages were provided for each animal. The animals were housed in an environmental chamber at 20 ± 1 C, 50 ± 2% R.H. A commercial test diet (sucrose 68%, vitamin free casein 18%, vegetable oil 8%, brewers yeast 2%, salt mixture 4%) (Nutritional Biochemicals Corporation, Cleveland, Ohio) was used as a food source. A one per cent purified agar gel provided the water supply. Agar was continuously available to those animals on ad lib water consumption.

The mice were randomly divided into four groups (AC, AD, BC, BD) consisting of five mice per group. Table I gives the treatment for each group. Oxygen consumption was

measured with a closed circuit apparatus (Haines, 1968). The apparatus consisted of an animal chamber, air circulating pump, CO_2 absorbent (Metalime, McKesson) and recording spirometer. The response of the apparatus was linear when air was withdrawn at rates (0.191 ml/min) as slow as one-tenth the oxygen consumption of a mouse.

TABLE I

Experimental treatment of animals used in metabolism experiment

	A WATER	B NO WATER
FOOD C	AC	BC
NO FOOD D	AD	BD

The period that an animal was in the system was approximately three hours. After one hour of equilibration, the oxygen consumption of the animal was measured for about two hours. The time at which each animals' oxygen consumption was measured each day was the same until the experiment was terminated. Animals were weighed on a Mettler top-loading balance (Model P 1200) immediately after each measuring period. The temperature throughout any measurement never varied more than ± 0.5 C. The barometric pressure, temperature of the system, time interval of measurement, body weight, and the volume of oxygen consumed were recorded. From these values oxygen consumption (cc O_2/g hr at S.T.P.) was calculated.

At least one urine sample was taken from each group each day. The osmolality of each sample was determined.

Total body water content was measured for each mouse. The animals that did not die during testing were killed by over anesthesia and frozen with the animals that had died during testing. Later the carcass was thawed, minced, and homogenized (Waring Blendor model 700B). The homogenate was dried to a constant weight and the dry weight was subtracted from the original body weight to determine total body water. Fat free body water was obtained by extracting fat with petroleum ether in a soxhlet apparatus for 24 hours. The fat free residue was weighed and the fat free body water calculated.

Acclimation to a Reduced Water Intake

Twelve field animals were randomly divided into two groups of six experimentals and six controls and placed in individual gallon jar cages. The jars were fitted with hardware cloth lids to allow for ventilation. The animals were housed in a windowless room where temperature, R.H., and photoperiod were controlled (30 ± 2 C, 55 ± 10% R.H., 12 hr ± 30 min). A one per cent purified agar gel was used as a water source. Agar gel was used so that accurate amounts of water could be given to each animal (± 0.1 g). Purina Lab Chow provided the food (protein 23.4%, fat 3.78%, nitrogen free extract 50.58%, fiber 4.86%, ash 7.70%). In order to determine ad lib water intake the animals were transferred

from the gallon jar cages into individual 2 lb coffee cans. Each can was fitted with a device for measuring water consumption. The device consisted of a water bottle, drinking tube, and a spillage collection tube partially filled with paraffin oil. Blank drinking apparatuses were weighed daily to ascertain the weight loss due to evaporation. The difference in the original weight of the apparatus and water minus the amount due to evaporation and spillage is the daily water intake of the animal. After normal water intake was determined the animals were returned to the gallon jar cages. The controls were then given an amount of agar equal to the ad lib water intake per day. Experimentals were given reduced amounts of agar in a stepwise manner equal to one-half, one-fourth, and one-eighth ad lib water intake. Reduction of the agar ration was begun only when the experimentals' weight had stabilized on the previous agar regime.

All animals were weighed on a Sartorius top-loading balance (Model 2204) to the nearest 0.1 g at least every third day. When weight stabilized, on each agar regime, urine samples were collected. A stable weight was not attained by the one-eighth ad lib experimentals and so the agar ration was increased to 0.6 ± 0.1 g. This was an increase of 0.15 g and within limits of error still approximated one-eighth ad lib. Urine samples from controls were taken at the same time as experimentals. Osmolality,

chloride, sodium, and potassium concentrations were determined for each urine sample.

Acclimation to Increased Concentrations of NaCl Solutions

Twelve animals were randomly divided into groups of six controls and six experimentals. Controls received distilled water throughout the experiment. Experimentals received increasing concentrations of NaCl solutions (0.1, 0.2, 0.3, 0.35, and 0.4M). Other environmental conditions were the same as those used in the acclimation experiment. Animals were weighed at least every third day. Urine collections and analyses were made on each salt water regime. Drinking records were also taken.

Evaporative Water Loss

Evaporative water loss from the respiratory surfaces and head skin areas was separated from the evaporation of the rest of the body. Hereafter these two divisions of EWL are referred to as pulmonary or respiratory and cutaneous or insensible perspiration respectively. EWL and oxygen consumption were measured simultaneously according to the method described by Shield (1968). A brief description of the method follows. The animal was placed in a restraining chamber divided into head and body compartments. The two compartments were separated by air-inlet plates and a stockade that held the animal in place and sealed off the two compartments. With the animal in the restraining chamber

the entire device was submerged into a temperature controlled water bath (29 ± 0.5 C). Dry air was supplied to each compartment and the amount of moisture lost was determined gravimetrically by the difference in weight of separate head and body drying tubes filled with indicator silica gel. Oxygen consumption was determined by a recording spirometer (Warren and Collins Incorporated with Gilson model MSP recorder). A variable speed tubing pump (Masterflex Variable Speed Pump, Cole-Palmer) maintained air flow at a constant rate. Environmental conditions prior to EWL measurements were the same as those used in the acclimation experiments. Four experimentals acclimated to water restriction and six controls were used for EWL measurements.

Urine Concentrations of Field Animals

Urine samples from field animals were taken during August and September, January, April, and July of 1967-'68. These time periods were chosen to represent the seasons of the year. Traps were placed in the field just before dark and collected the following morning thereby allowing an animal to be in the trap as short a time as possible. The animals were transported to the laboratory, removed from the traps and placed in hardware cloth cages. Urine samples were taken according to the method previously described. The animals appeared calm and seldom urinated when transferred. Handling was reduced to a minimum but urine samples

that obviously resulted from fright diuresis were discarded. Urine analyses were done as in previous experiments.

Mean, variance, standard deviation, and standard error for all experiments were calculated by an IBM 360 (Model 40) computer.

RESULTS

Metabolism

The changes in oxygen consumption, body weight, and urine osmolality resulting from the study on acute food and/or water deprivation are summarized in Figs. 2, 3, 4. Group AC (controls) maintained their body weight and showed only slight fluctuations in mean oxygen consumption (Figs. 2, 3). Mean urine osmolality fluctuated from 1.76 osmoles to 3.97 osmoles but did not show the magnitude of change that was seen in the experimental groups (Fig. 4). Individual urine osmolality ranged from 1.62 osmoles to 5.47 osmoles. None of the controls died.

The group receiving no food and ad lib agar (AD) showed a rapid decline in mean body weight and oxygen consumption (Figs. 2, 3). Oxygen consumption increased just prior to death in three of the five animals. All of the animals in this group may have exhibited the same rise before death but the experimental plan did not always allow this to be observed. Some animals died either during testing or in the 24 hr period between individual tests. Mean urine osmolality never exceeded 1.60 osmoles and was

minimally 0.60 osmoles when the experiment was terminated with the death of the last animal on the fourth day. Individual urine osmolality ranged from 0.60 osmoles to 3.18 osmoles. One animal died on the second day, one on the third day, and three on the fourth day.

Mice receiving no agar and ad lib food (BC) did not decrease mean oxygen consumption until the last three days of testing and never reached a level less than 2.6 ml O_2/g hr (Fig. 3). However, body weight continued to decline (Fig. 2). The rise in oxygen consumption before death observed in six of the ten animals in groups AD and BD was not observed in any of the animals in this group. Mean urine osmolality ranged from 2.87 osmoles to 6.82 osmoles. Individual urine osmolality ranged from 2.87 osmoles to 7.00 osmoles. Mice in this group survived the longest of the three experimental groups. One animal survived 12 days. The first animal died on the seventh day, a second on the ninth day, and two on the eleventh day.

Group BD (no water and no food) showed a rapid decline in mean oxygen consumption until the fifth day of testing (Fig. 3). Only one animal was alive after the fifth day and this animal showed an increase in oxygen consumption and urine osmolality until death (Figs. 3, 4). Two other mice in this group showed similar increases in oxygen consumption and urine osmolality just before death. The rise in urine osmolality was not observed in any other

Fig. 2.--Weight in mice during a metabolism study while acutely deprived of food and/or water. AC refers to controls, food and agar ad lib; AD to group receiving no food and ad lib agar; BC to group receiving no agar and ad lib food; BD to group receiving neither agar nor food. The ordinate is body weight. The abscissa shows days of treatment. Each point is the average.

Fig. 3.--Oxygen consumption in mice during a metabolism study while acutely deprived of food and/or water. The ordinate is oxygen consumption (ml O_2/g hr). The abscissa shows days of treatment. Each point is the average. For the treatment of each group see the legend of Fig. 2.

Fig. 4.--Urine osmolality changes in mice during a metabolism study while acutely deprived of food and/or water. The ordinate is urine osmolality (osm/l). The abscissa shows days of treatment. Each point is the average. For the treatment of each group see the legend of Fig. 2.

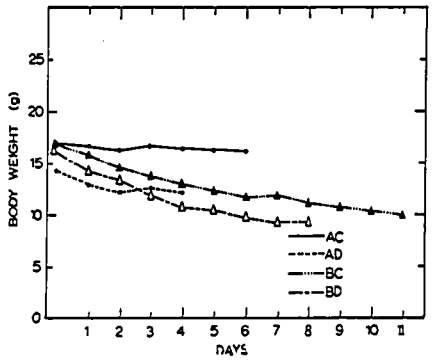

Fig. 2

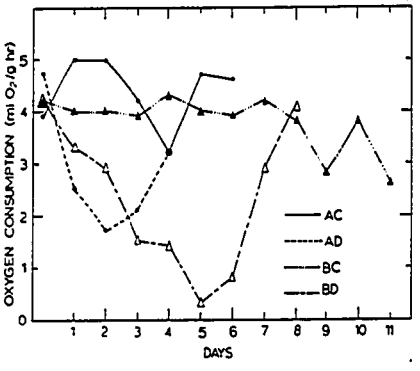

Fig. 3

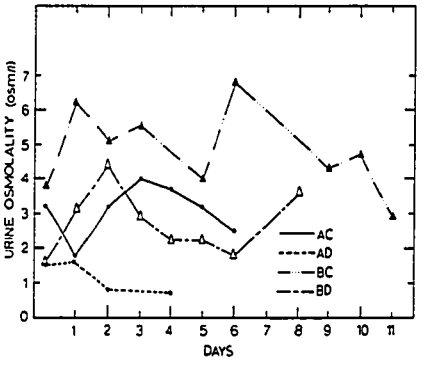

Fig. 4

experimental group. Mean urine osmolality ranged from 1.62 osmoles to 4.40 osmoles (single observation on day eight). Individual urine osmolality ranged from 1.55 osmoles to 4.40 osmoles. Body weight declined throughout the experiment which terminated on the eighth day with the death of the last animal. One animal died on the fourth day, and three died on the fifth day.

All three experimental groups averaged less fat-free water than controls (Table II). There was no significant difference between groups in either total body water or fat-free water ($.20 > P > .10$). The percent total water was lower in controls than in experimentals since body fat was greatly reduced in experimentals. It is noteworthy that among the experimental groups, group BC survived the longest and had the lowest percentage of total body water and fat-free body water.

Acclimation To Reduced Water Intake

Ad lib water consumption was determined to be 3.6 ± 0.3 ml/day. Upon reduction to one-half ad lib an immediate decline in body weight occurred (Fig. 5). Twenty-two days later body weight began to stabilize and the mean was 98.4% of the original weight when urine was sampled and a further reduction in agar to one-fourth ad lib was initiated (Fig. 5). Weight stabilization on one-fourth ad lib required approximately 12 days (88.6% original) and urine samples were taken nine days later. Weight stabilization

did not occur at one-eighth ad lib and one animal died on the tenth day of that regime. On the tenth day, the agar ration was increased to 0.6 g (± 0.1 g) and all experimental animals stabilized at 71.5% of the original weight within six days. This slight increase in agar was within the experimental error of the agar weights and is referred to as one-eighth in all of the figures.

TABLE II

Total and fat-free body water of mice, expressed as percentage of total body weight and percent of lean body weight. The mean ± S.E. are given.

Group	$B.W._I$ (g)	Total H_2O (%)	Fat-Free H_2O (%)
AC*	17.0	62.4 ± 1.5	70.7 ± 0.54
AD	14.2	67.8 ± 2.0	68.9 ± 1.4
BC	16.9	64.5 ± 0.58	66.3 ± 0.45
BD	16.2	67.7 ± 0.63	68.2 ± 0.63

$B.W._I$ = Initial body weight

AC = Controls

AD = No food, ad lib agar

BC = No agar, ad lib food

BD = No food, no agar

*Each group consisted of 5 animals.

The results of urine analyses are summarized in Figs. 6, 7, 8, 9. There was no significant increase in mean osmolality with decreased water intake (Fig. 6), ($P > .50$).

Fig. 5.--Weight changes in mice on ad lib water and reduced water intake. The ordinate is body weight (g). The abscissa shows days of treatment. Weight changes are shown for mice on ad lib, one-half ad lib, one-fourth ad lib, and one-eighth ad lib agar. Agar was used as a water source.

Fig. 6.--Urine osmolality in mice on ad lib water and mice acclimated to water restriction. The ordinate is urine osmolality (osm/1). The abscissa is divided according to agar regime. Vertical line = range, horizontal line = mean, boxes = 95% confidence limits, unshaded boxes = controls, shaded boxes = experimentals.

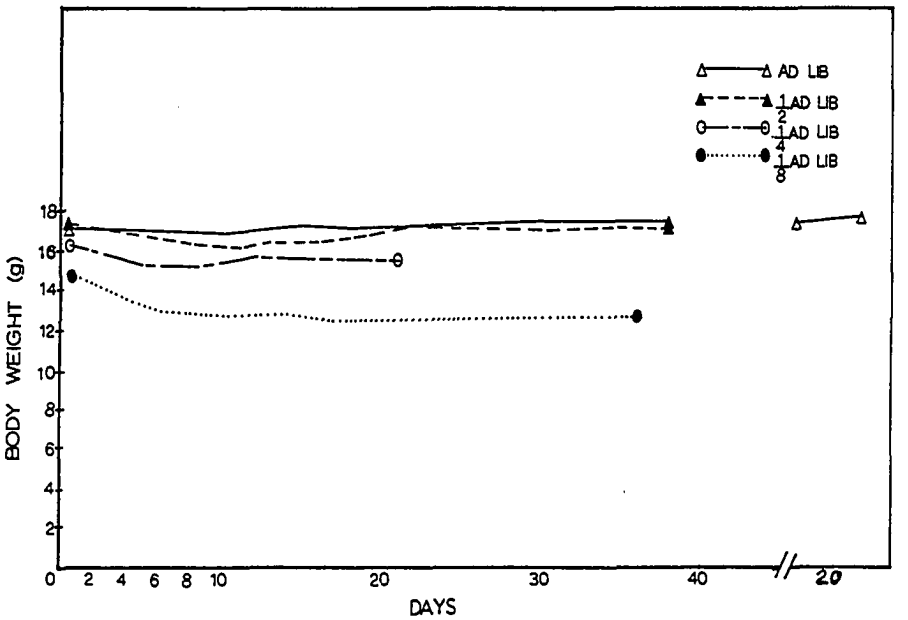
AD LIB
1/2 AD LIB
1/4 AD LIB
1/8 AD LIB
BODY WEIGHT (g)
DAYS
0 2 4 6 8 10 20 30 40 20
2 4 6 8 10 12 14 16 18

Fig. 5

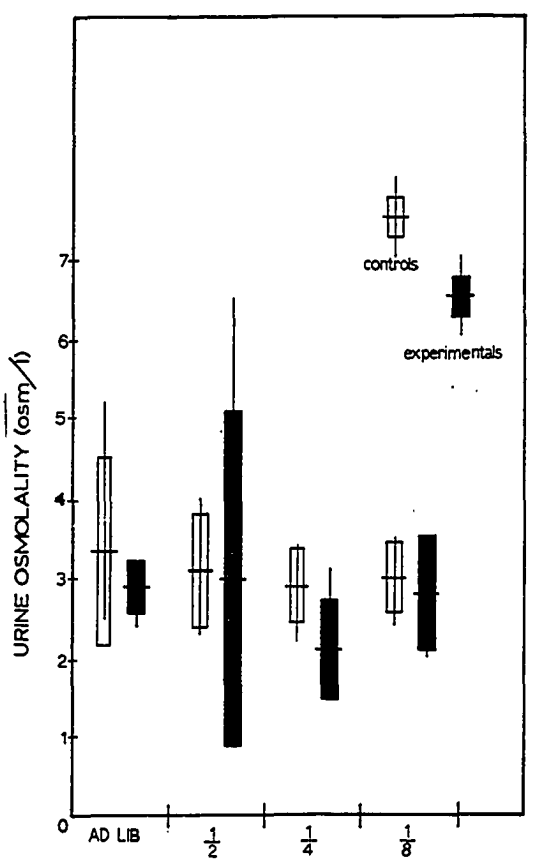
controls
experimentals
URINE OSMOLALITY (osm/l)
AD LIB
1/2
1/4
1/8
0 1 2 3 4 5 6 7

Fig. 6

Fig. 7.--Urine chloride in mice on ad lib water and in mice acclimated to water restriction. The ordinate is urine chloride (mEq/1). The abscissa and the graphical analysis are the same as for Fig. 6. Any box enclosed by a broken line indicates that the confidence limits extend below zero.

Fig. 8.--Urine sodium in mice on ad lib water and in mice acclimated to water restriction. The ordinate is urine sodium (mEq/1). Refer to Fig. 6 for description of abscissa and graphical analysis.

Fig. 9.--Urine potassium in mice on ad lib water and in mice acclimated to water restriction. The ordinate expresses urine potassium (mEq/1). Refer to Fig. 6 for description of abscissa and graphical analysis.

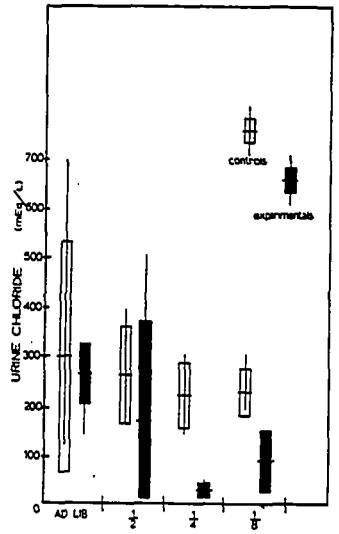

Fig. 7

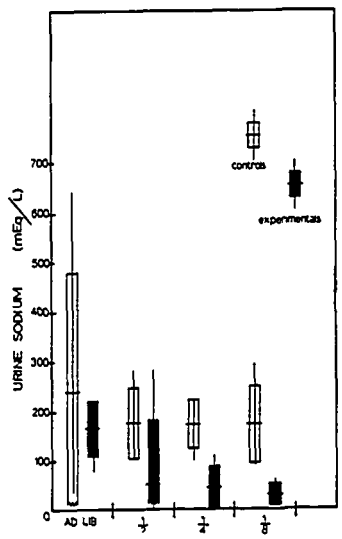

Fig. 8

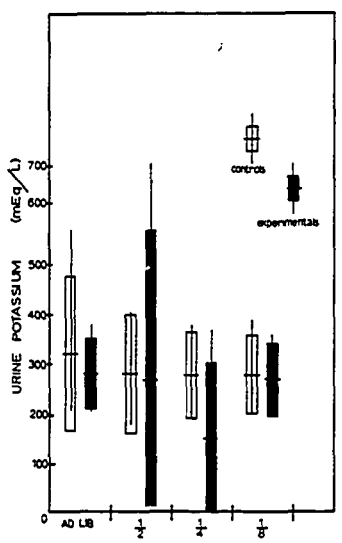

Fig. 9

Duplicate samples were taken at the lower agar intakes and osmolality was determined on freshly collected unfrozen samples to determine if storage and freezing affected the osmolality. Identical values were obtained for samples that were checked before and after freezing. Duplicate samples also gave similar results. The highest osmolality recorded for any individual animal (6.52 osmoles) was in the one-half ad lib experimental group. Controls ranged from 5.24 osmoles to 2.20 osmoles, while experimentals ranged from 1.63 osmoles to 6.52 osmoles. Urine chloride and sodium showed a definite decrease with decreased water intake (Figs. 7, 8). The lowest mean chloride was recorded in the one-fourth ad lib experimental group (29 mEq/1). This compares with 218 mEq/1 for the control group. Individual control values ranged from 690 to 120 mEq/1. Experimentals ranged from 500 to 15 mEq/1. The lowest mean sodium concentration was recorded in the one-eighth ad lib experimental group (32 mEq/1). The lowest mean control value was 171 mEq/1. Controls ranged from 640 to 40 mEq/1. Experimentals ranged from 280 to 10 mEq/1.

There was no significant difference in mean potassium concentration between controls and experimentals (Fig. 9), ($P > .50$). Control values ranged from 570 to 180 mEq/1. Experimentals ranged from 700 to 20 mEq/1.

Although total urine volumes were not quantified, it was evident that urine volume decreased with a decrease in

Fig. 10.--Weight changes in mice on distilled water (0.0M) and increasing concentrations of NaCl solutions (0.1, 0.2, 0.3, 0.35, and 0.4M). The ordinate is body weight (g). The abscissa is divided according to each NaCl concentration. Above the abscissa is the number of days for each salt solution. Each point represents the average of the animals on each drinking solution.

Fig. 11.--Urine osmolality in mice on distilled water (0.0M) and increasing concentrations of NaCl solutions (0.1, 0.2, 0.3, 0.35, and 0.4M). The ordinate is urine osmolality (osm/1). The abscissa is described in Fig. 10. The explanation of graphical analysis is the same as for Fig. 6.

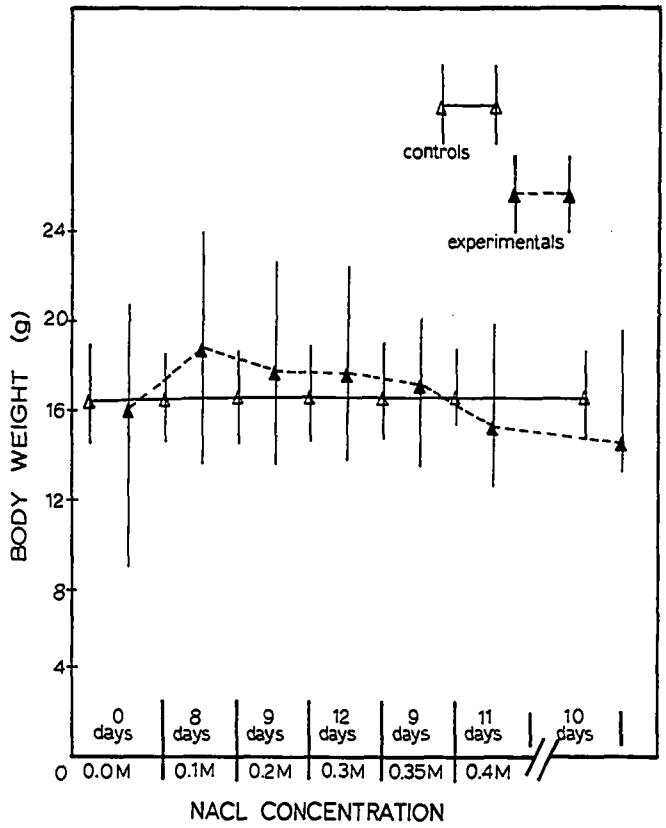
controls
experimentals
BODY WEIGHT (g)
24
20
16
12
8
4
0
0 days
8 days
9 days
12 days
9 days
11 days
10 days
0.0M
0.1M
0.2M
0.3M
0.35M
0.4M
NACL CONCENTRATION

Fig. 10

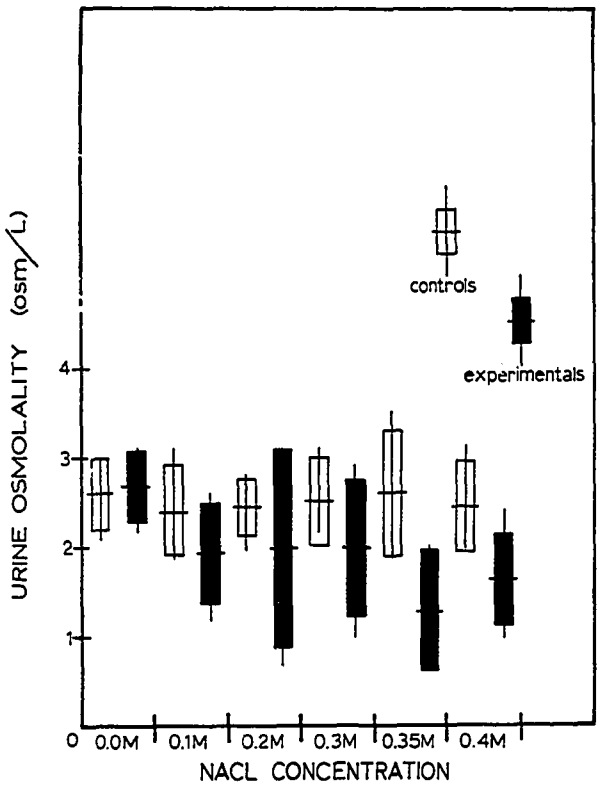
controls
experimentals
URINE OSMOLALITY (osm/L)
4
3
2
1
0
0.0M
0.1M
0.2M
0.3M
0.35M
0.4M
NACL CONCENTRATION

Fig. 11

agar rations. This decrease in urine volume from controls was evidenced by the time needed for collection of adequate amounts of urine for analysis.

Acclimation To Increasing Concentrations of NaCl Solutions

Controls maintained their weight throughout the experiment (Fig. 10). Experimentals rapidly increased in weight when placed on 0.1M salt solution, but steadly declined on successively more concentrated salt regimes. Weight stabilization usually occurred within one week after initiation of each successively more concentrated salt solution. Two animals out of six failed to stabilize their weight on 0.4M and died within sixteen days after beginning this salt water regime.

The results of urine analyses after acclimation to increasing concentrations of sodium chloride are summarized in Figs. 11, 12, 13, 14. Urine osmolality decreased with increasing concentrations of sodium chloride solutions (Fig. 11). A significant decrease in osmolality in the experimentals was observed at 0.35M and 0.4M ($.01 < P < .05$). Control values ranged from 1.92 to 3.54 osm/1. The greatest osmolality recorded for an experimental animal was 3.10 osm/1 at the 0.2M sodium chloride concentration. The lowest osmolality (0.68 osm/1) for the experimental animals was recorded at 0.2 and 0.35M.

Fig. 12.--Urine chloride (mEq/1) in mice on distilled water and increasing concentrations of NaCl solutions described in Fig. 10. The abscissa is described in Fig. 10, the explanation of graphical analysis is the same as for Fig. 6.

Fig. 13.--Urine sodium (mEq/1) in mice on distilled water and increasing concentrations of NaCl drinking solutions described in Fig. 10. The abscissa is described in Fig. 10, the explanation of graphical analysis is the same as for Fig. 6.

Fig. 14.--Urine potassium (mEq/1) in mice on distilled water and increasing concentrations of NaCl drinking solutions described in Fig. 10. The abscissa is described in Fig. 10, the explanation of graphical analysis is the same as for Fig. 6.

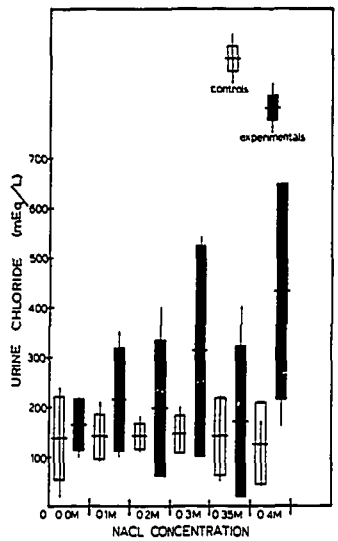

Fig. 12

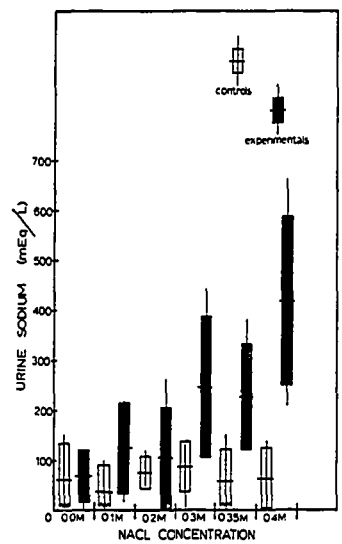

Fig. 13

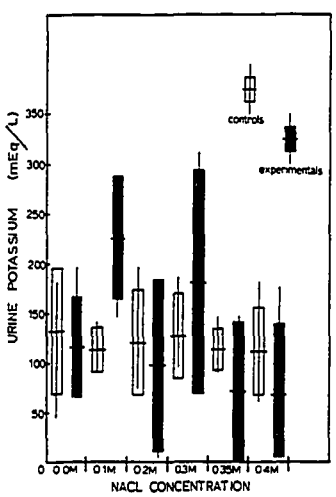

Fig. 14

Urine chloride and sodium concentrations increased with increasing molar concentrations of drinking solutions (Figs. 12, 13). A significant increase in sodium concentration in the experimentals was observed at 0.3M, 0.35M, and 0.4M ($.01 < P < .05$). This was also shown for chloride concentrations on the 0.3M and 0.4M drinking solutions (.01 P .05). Control sodium values ranged from 5 to 150 mEq/1. Experimental values ranged from 20 mEq/1 at 0.1M to 660 mEq/1 at 0.4M. The greatest mean sodium concentration was 418 mEq/1 and occurred on the 0.4M regime. Control chloride concentrations ranged from 20 to 240 mEq/1. Experimental chloride concentrations ranged from 40 to 640 mEq/1. The greatest mean chloride concentration was 430 mEq/1 and occurred on the 0.4M regime.

Urine potassium was more variable than sodium or chloride and showed a slight decrease with increasing concentrations of sodium chloride drinking solutions (Fig. 14). Experimental values ranged from 5 mEq/1 on the 0.2, 0.35, and 0.4M regimes to 310 mEq/1 on the 0.2M regime. The greatest mean potassium concentration for experimental animals was 224 mEq/1 on the 0.1M regime. The lowest mean experimental value (67 mEq/1) occurred on the 0.4M drinking solution. Control potassium concentrations ranged from 40 to 195 mEq/1.

Evaporative Water Loss

Table III gives the milligrams of water evaporated per cc of oxygen for six control animals and four animals acclimated to water restriction. The acclimated animals showed the greatest reduction in evaporative water loss through the skin. However, both pulmonary and cutaneous avenues were reduced. The mean total mg H_2O/cc O_2 lost by acclimated animals was almost 50% of controls (0.570 vs. 1.039).

TABLE III

Evaporative water loss per unit oxygen consumption for controls and mice acclimated to water restriction. Mean ± S.E. and (N) are given for pulmonary, cutaneous, and total evaporative water loss. Agar was used as a water source.

mg H_2O/cc O_2			
Group	Pulmonary	Cutaneous	Total
Controls (6)	0.624±0.040	0.415±0.058	1.039±0.077
Acclimated (4)	0.426±0.033	0.144±0.011	0.570±0.038

The mean oxygen consumption per hour did not show a significant difference between the two groups (Table IV). If oxygen consumption were reduced in the acclimated group, this would account for even a greater reduction in total water lost. In a twenty-four hour period, assuming equal oxygen consumption in both groups, the acclimated animals would conserve slightly more than 0.2g of water. The magnitude of water conservation becomes evident when one considers that free water intake was 0.6g/day.

TABLE IV

Oxygen consumption for the two groups of mice described in Table III. Mean ± S.E. are given.

$cc O_2$/ hr	
Controls (6)	Acclimated (4)
19.87 ± 2.34	22.85 ± 2.03

Urine Concentrations of Field Animals

Table V summarizes the results of urine analyses for field samples taken during August and September, January, April, and July, 1967-'68. The greatest mean urine osmolality was recorded for samples taken in August and September. Urine osmolality decreased in January. April samples showed a significant decrease in osmolality from samples taken in August and September (Fig. 15), ($.01 < P < .05$). Sodium and chloride concentrations showed the same trend as urine osmolality (Figs. 16, 17). The greatest sodium and chloride concentrations were obtained in August and September and the lowest concentrations were obtained in April. Sodium concentrations in April were not significantly lower than in August-September and July ($.10 < P < .20$).

Potassium values were variable, however they did show the same high values for August and September and low values for April (Fig. 18). There was no significant difference found for potassium during August-September and January and July ($.20 > P > .10$). A significant decrease in

urine potassium concentration was noted for April samples as compared to August and September values ($.01 < P < .05$).

TABLE V

Urine osmolality, sodium, chloride, and potassium concentrations for field animals during the seasons of the year. Mean ± S.E. and (N) are given.

Month	Osmolality	Sodium	Chloride	Potassium
Aug. and Sept. (10)	4.02 ± 0.2	178 ± 34	173 ± 22	441 ± 48
Jan. (10)	3.55 ± 0.3	129 ± 22	143 ± 14	303 ± 27
Apr. (10)	3.09 ± 0.1	86 ± 8	123 ± 19	206 ± 9
July (10)	3.55 ± 0.1	157 ± 18	157 ± 15	253 ± 35

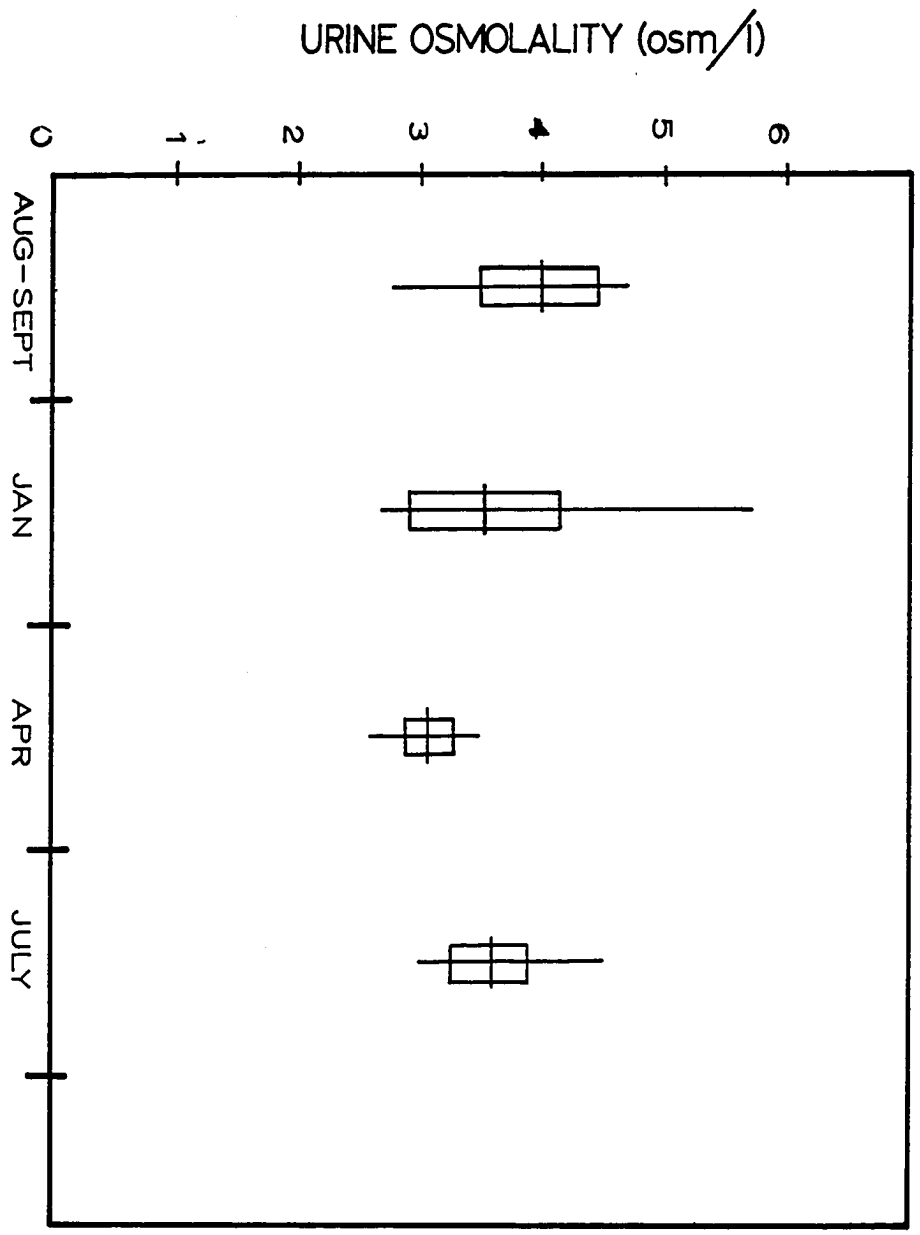

Fig. 15.--Urine osmolality in mice taken directly from the field during the seasons of the year. The ordinate shows changes in osmolality (osm/1). The abscissa is divided into the months when samples were taken. Horizontal line = mean, vertical line = range, box = 95% confidence limits.

Fig. 16.--Urine sodium (mEq/1) in mice taken directly from the field during the seasons of the year. The abscissa and graphical analysis are explained in Fig. 15.

Fig. 17.--Urine chloride (mEq/1) in mice taken directly from the field during the seasons of the year. The abscissa and graphical analysis are explained in Fig. 15.

Fig. 18.--Urine potassium (mEq/1) in mice taken directly from the field during the seasons of the year. The abscissa and graphical analysis are explained in Fig. 15.

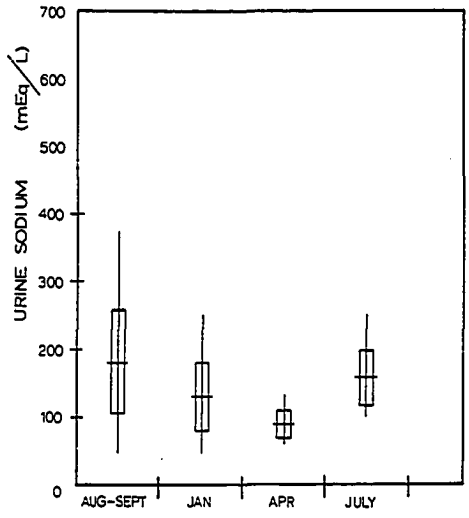

Fig. 16

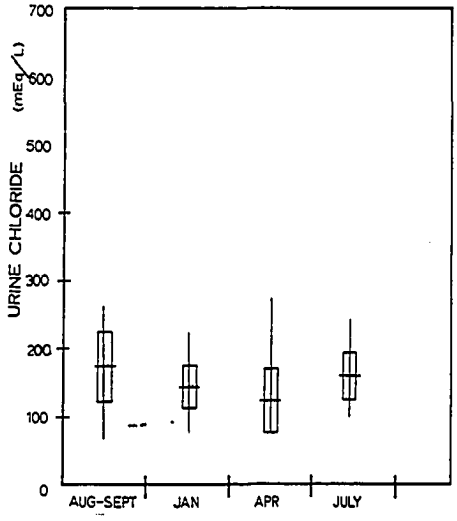

Fig. 17

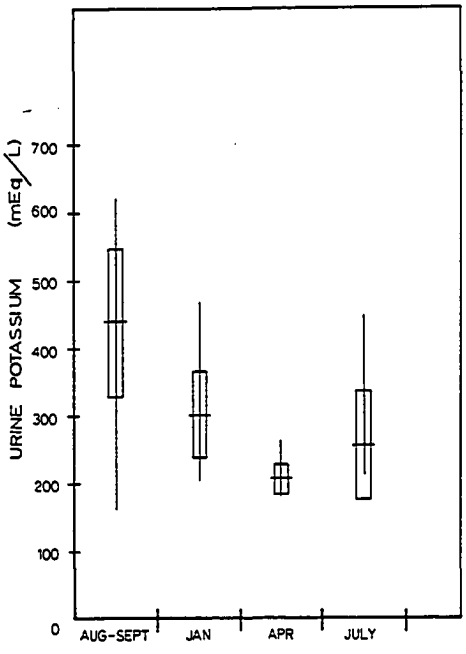

Fig. 18

DISCUSSION

Metabolism

Metabolism and body temperature in Peromyscus have been shown to vary in animals from arid and mesic environments (McNab and Morrison, 1963), the xeric species having the lower basal metabolic rates (BMR). This would tend to reduce overheating. P. m. nebrascensis acclimated to 20 C and 50% R.H. and on ad lib food and agar had a mean oxygen consumption of 4.4 ml O_2/ g hr. This value compares with predicted oxygen consumption for mesic species at this temperature (McNab and Morrison, 1963). How then would P. m. nebrascensis react to an acute deprivation of food and/or water? If the animal becomes hypothermic and reduces body temperature the amount of moisture lost would decrease and this would provide an effective means of survival during periods of water and food shortage. P. eremicus (xeric form) readily entered torpor (reduced oxygen consumption and body temperature) when deprived of food and water at ambient temperatures between 5 and 30 C, and showed a decrease in pulmocutaneous water loss (MacMillen, 1965). P. m. nebrascensis when deprived of agar but given food ad lib did

not show a reduction in oxygen consumption until the last three days of testing. An immediate reduction in oxygen consumption was observed when either food or food and agar were deprived. This would tend to indicate that the lack of food may provide the necessary stimulus to hypothermia in P. m. nebrascensis. A reduction in body temperature and oxygen consumption would be of some adaptive value for energy conservation and reduction in water loss during periods of food and water shortage.

All animals that were deprived of food and/or agar showed a continuous decrease in weight. The group deprived of both food and agar (BD) and the group with no agar and ad lib food (BC) showed an average weight loss of 43% before death. The mean survival time for these two groups was 7.7 days. A 14% average weight loss before death was observed in those animals on ad lib water and no food (AD). This group had a mean survival time of 3.4 days. This difference between groups might be due to the lower average initial weight of group AD as compared to groups BC and BD (14.2 vs. 16.5 g). Groups BD and BC compare with semidesert rodents in ability to withstand acute food and water deprivation in terms of average survival time and concomitant weight loss. MacMillen (1964) showed an average survival time of 9.1 days and a 41.3% weight loss in Neotoma fuscipes, a semidesert woodrat. Fertig and Layne (1963) showed an average weight loss of 36.6% and a mean survival time of

6.1 days for P. floridanus deprived of water. The habitat of this species is among the most xeric in Florida, consisting of dry, sandy, scrubby woodlands (Fertig and Layne, 1963).

The results of total body water and fat-free body water did not show a significant difference between controls and experimental groups. P. leucopus that died from water restriction had normal body water contents of 66% (Chew, 1951). The combined average body water content for P. m. nebrascensis in groups BC and BD which died from either food and agar or agar restriction was 66%. Chew and Hinegardner (1957) found a fat-free water content and total water content for white mice on fatal water restriction to be 68.8 and 67.8% respectively. These values compare with group BD (no food, no agar) 68.2 and 67.7%. The total fat-free water in the experimental groups averaged 3.1% lower than controls and represents some degree of dehydration.

Urine osmolality in the experimental groups showed a great degree of variation depending on the treatment. Urine osmolality in group BC showed an increase above controls and continued to have consistently high concentrations until death. An increase in urine osmolality would decrease the amount of moisture needed, and may have contributed to the long survival time in this group. This group also had the lowest fat-free water percentage of the experimental groups. The only water available to this group was free or

bound water in the food and metabolic water. The small percentage of water in the food (2.4%) and the metabolic water was inadequate to maintain life for an extended period. Chew (1951) indicated that the free water available in food may be the major means of water intake for some animals in their natural environment. The increased survival time of P. m. nebrascensis on ad lib food and no agar would tend to show that if enough water is available in their food and food intake is not restricted, free water may not be necessary for the survival of this species. Some seed eating rodents can survive on metabolic water and free water of seeds (Chew, 1961). The response of mice in group BC to acute agar restriction and ad lib food was not hypothermia but increased concentration of urine and thus more efficient utilization of metabolic water. This group would most likely compare to animals in the field since a shortage of water may be more often found than a shortage of food.

Acclimation to Reduced Water Intake and NaCl Loading

Fertig and Layne (1963) determined the ad lib water consumption to be 3.9 cc/day for P. floridanus that had been in captivity over three months. The daily water intake of P. m. nebrascensis (3.6 ml/day) can be compared with the above species since the conditions under which both were measured were similar. P. floridanus inhabits relatively xeric environments and may be subjected to periods of drought

or high protein food. P. m. nebrascensis is found in mesic environments but may experience periods of drought. In terms of ability to acclimate to water restriction, P. m. nebrascensis showed less weight loss than P. floridanus on similar water restrictions, and was able to survive at a reduced weight on higher NaCl drinking solutions. French (1956) showed that P. m. sonoriensis (a desert subspecies) was unable to maintain normal weight on 30% ad lib water intake (approx. 0.8 g/day) at 20.5-27.4 C and 51-60% R.H. Chew (1951) found that P. leucopus (a deciduous forest species) required 39% ad lib water intake (approx. 4.0 cc/day) for maintaining normal weight while at 28 C., P. m. nebrascensis required at least 50% ad lib (1.8 ml/day) to maintain body weight at 29-30 C and 50-60% R.H. while on Purina Lab Chow which was similar to the diet used by French and Chew in the studies described above. The per cent of ad lib water intake required to maintain body weight in P. m nebrascensis is higher than the per cent required by the species cited above if the absolute amounts of water are not considered. If absolute amounts of water are stated, P. m. nebrascensis requires less water for weight maintainence than P. leucopus and approximately the same amount of water for weight maintainence as P. m. sonoriensis.

The ability of P. m. nebrascensis to survive on restricted water intake for long periods of time was not due to the ability of this subspecies to produce a concentrated

urine. There was no significant difference in urine osmolality between controls and experimentals. An increase in urine osmolality with water deprivation has been reported for several species of rodents (Chew, 1951, 1955; Hudson, 1962; Hudson and Rummel, 1966; Schmidt-Nielsen, 1964). A decrease in urine volume was noted for P. m. nebrascensis while on the restricted water intake, and was obviously an effective method in reducing the amount of water lost. A reduction in urine volume and a fecal water loss that was 19% of normal was observed in P. leucopus while on a restricted water regime (Chew, 1951).

No values of urine osmolality or concentration of sodium, chloride, and potassium could be found in the literature for Peromyscus. P. m. nebrascensis when compared to desert species is less able to tolerate water deprivation and produced an average maximal urine concentration of 3.05 osmoles as compared to osmolalities above 5.0 in Dipodomys merriami, Gerbillus gerbillus, Jaculus jaculus, and Psammomys obesus (Refer to Table XXVI in Schmidt-Nielsen, 1964).

Mice on one-fourth and one-eighth ad lib agar showed an average reduction of sodium and chloride concentrations of from five to seven times less than that of controls. A similar reduction of sodium and chloride in water deprived laboratory rats has also been shown (Mogharabi, 1968). No data on Peromyscus are available for comparison but

increased electrolyte and chloride concentrations in water deprived salt-marsh harvest mice, Reithrodontomys raviventris, has been shown (Haines, 1964). Similar increases in electrolyte and chloride concentrations have been shown for other animals given NaCl drinking solutions.

The kidney efficiency of P. m. nebrascensis was tested by giving increasing concentrations of NaCl solutions to one group of mice. Microtus ochrogaster was able to utilize a higher molarity of salt water than M. pennsylvanicus and could possibly be better adapted to the drier habitats in which it is found (Getz, 1963). With an increase in molarity of NaCl to 0.3M P. m. nebrascensis was able to maintain weight above the controls. Mice initially lost weight on 0.35 and 0.4M NaCl but eventually stabilized at 89 and 86% original weight respectively. Two mice died on the 0.4M regime and it is doubtful if P. m. nebrascensis could tolerate NaCl concentrations much above 0.4M. Six mice were placed directly on 0.4M NaCl and two of these died within six and nine days respectively. At the end of 12 days the four remaining mice were placed on 0.5M NaCl and all but one of these animals died within 12 days while on this regime. The remaining mouse lived a total of 22 days on 0.5M NaCl, and had lost 46.7% of its original weight. The ability of this subspecies to tolerate high concentrations of sodium chloride solutions does not compare with desert adapted rodents. Some desert rodents can survive while

drinking NaCl solutions more concentrated than sea water (Hudson, 1962; Winkelmann and Getz, 1962; Schmidt-Nielsen and Schmidt-Nielsen, 1950a). Reithrodontomys raviventris, an inhabitant of salt marshes, has been shown to maintain weight on sea water (Fisler, 1963). P. m. nebrascensis is probably never exposed to salt water in its natural environment and has not evolved the necessary mechanisms to adapt to salt water drinking. But when salt loading was used as an indicator of renal efficiency this species was able to void high concentrations of sodium and chloride in urine by increasing urine volume and concentrations of sodium and chloride. Although urine osmotic activity decreased in mice on 0.35 and 0.4M NaCl drinking solutions, the amount of nitrogenous wastes excreted probably remained the same. An increase in urine volume would allow the same amount of nitrogen compounds to be excreted as would be eliminated in a smaller volume but at a lower osmotic activity. P. m. nebrascensis in terms of renal efficiency as measured by ability to drink increasing concentrations of NaCl solutions, is not as efficient as some desert rodents and rodents that are exposed to sea water or eat vegetation that has a high salt content.

Evaporative Water Loss (EWL)

The total evaporative water loss of an animal is equal to pulmonary plus cutaneous evaporation, and is usually expressed in mg H_2O/cc O_2. These units express --

evaporation rate in terms of metabolism. This mode of expression reduces individual variation to the underlying process of oxygen consumption which is related to other body processes. A total evaporative rate of 1.06 mg H_2O/cc O_2 has been shown for Microtus californicus while on ad lib water intake (Church, 1966). Chew (1955) observed a total EWL of 0.96 mg H_2O/cc O_2 in P. m. sonoriensis on ad lib water. A total EWL of approximately 1.0 mg H_2O/cc O_2 for wild and lab Mus musculus on ad lib agar has been shown (Shield, 1968). The total EWL for P. m. nebrascensis on ad lib agar was 1.04 mg H_2O/cc O_2. Schmidt-Nielsen and Schmidt-Nielsen (1950b) observed total EWL in wild M. musculus, P. crinitus, and the Syrian Golden Hamster (Cricetus aureus), along with the desert rodents D. merriami, D. spectabilis, and Perognathus spp. to average about 0.5 mg H_2O/ml O_2. The reduced EWL for desert species was postulated and confirmed after calculations showed that such a mechanism was a necessity for the animals if water loss was not to exceed water intake (Schmidt-Nielsen and Schmidt-Nielsen, 1950b). It is not known whether the non-desert rodents were acclimated or acclimatized to water deprivation. This may have accounted for their lower EWL than for the same or similar species that have been determined by other workers. Carpenter in 1963 (from Hudson and Rummel, 1966) found EWL values of 0.8 and 1.2 mg H_2O/cc O_2 at 28 C for D. merriami and D. agilis respectively. The possibility of acclimation or

acclimatization is suggested because total EWL values for wild and lab M. musculus acclimated to one-eighth ad lib agar (Shield, 1968), and P. m. nebrascensis (present study) similarly acclimated have evaporation rates almost equal to those found for the species studied by the Schmidt-Nielsens.

The ability to acclimate to water restriction by decreasing EWL was not shown for M. californicus (Church, 1966). However, the need for reducing EWL in animals exposed to water deprivation is suggested in the work done by Chew, (1955) who showed an average cutaneous EWL in P. m. sonoriensis on ad lib water intake to represent 46% of the total surface loss and the total surface water loss to be from one-fourth to one-half of its total water loss. If this evaporation rate is not reduced with a reduction in water intake, the percentage of water obligated to evaporation would increase and could exceed total water intake. This would place the animal in negative water balance and would eventually cause death. The value of reduced EWL to an animal in maintaining water balance on restricted water intake was evaluated for P. m. nebrascensis (Table VI). Using the total EWL observed in this study and comparing it to total water intake would allow one to determine the percentage of total water gained that would be obligated to evaporation. Free water and the bound water in the food was determined. Since food consumption studies were not done, metabolic water was determined by estimating the amount of

food eaten per day. Food consumption of 2.54 and 1.56 g/day was found for P. m. sonoriensis at an average temperature of 24.7 C and a mean R.H. of 56.2% while on ad lib and 30% ad lib water consumption respectively (French, 1956). Fertig and Layne (1963) found an average food consumption of 3.1g/day and .05 g/g/day for P. floridanus at approximately 23 C and a mean R.H. of 56.2% while on ad lib and 11% ad lib water intake respectively. The diet used in the experiments described above was Purina Lab Chow. Although the same diet and similar temperature and R.H. were used in this study as that used by French, it is not definitely known whether food intake decreases with concomitant water reduction. Moderate water restriction in white mice caused a highly significant decrease in food intake, however extreme water restriction caused no further reduction in food intake (Chew and Kinegardner, 1957). Haines (1968) found no significant reduction in food consumption with water restriction in P. m. nebrascensis and wild house mice. Because of the discrepancy in these data, calculations to determine the amount of metabolic water were made assuming that experimentals either ate the same amount of food as controls or reduced their food intake.

The amount of oxygen required to metabolize each quantity of food was computed from caloric equivalents and should equal oxygen consumption. The average EWL per cc of oxygen times oxygen consumption per day yields the total

TABLE VI

Relationship of total water intake to evaporation in acclimated and nonacclimated animals.

Estimation of the total water intake and evaporative water loss of mice consuming 2.5 g and 1.5 g of Purina Lab Chow per day. EWL was calculated from the average mg H_2O/cc O_2 times the volume of oxygen necessary to combust the appropriate amounts of the diet.

	Nonacclimated (controls)	Acclimated	Acclimated
	2.5 g food/day	2.5 g food/day	1.5 g food/day
Water Intake/Day (g)			
Free	3.60	0.60	0.60
Oxidative	1.10	1.10	0.70
Bound	0.10	0.10	0.06
Total	4.80	1.80	1.36
EWL/Day			
mg H_2O/cc O_2	1.039	0.570	0.570
1 O_2/day	1.90	1.90	1.10
H_2O/day (g)	2.00	1.08	0.63
Water Intake Obligated to Evaporation (%)			
Nonacclimated	42%	111.1%	147.0%
Acclimated		60%	46.0%

quantity of water evaporated per day. Since a dry air system was used the quantity of moisture evaporated would represent the maximum EWL.

The amount of moisture obligated to evaporation in a normally hydrated animal was 42%. The EWL exceeded water intake for those animals acclimated to water restriction when calculations were made assuming that acclimation did not occur. Because animals acclimated to water restriction and on an estimated food intake of 2.5 g/day had reduced their EWL; only 60% of the total water intake was obligated to evaporation. This allows for the remaining 40% to be used in urinary and fecal water loss. Only 46% of the total water intake would be obligated to evaporation in acclimated animals assuming they ate 1.5 g food/day. Almost equal percentages of total water intake were available for urinary and fecal water loss in acclimated and nonacclimated animals and may help to explain why no increase in urine osmolality was found for animals in the experiment on acclimation to reduced water intake. Since the total EWL/day was calculated from the volume of oxygen necessary to combust the estimated food intake, computed oxygen consumption may not have represented the actual amount of oxygen used. The ability of an animal to utilize a given amount of food may vary among individuals and also with the degree of dehydration of the animal. If a dehydrated animal were more efficient in metabolizing food than a hydrated animal, the

dehydrated animal would be able to survive on less food and oxygen and less water would be obligated to evaporation. Whether food intake decreases with water restriction or not, P. m. nebrascensis reduces EWL on restricted water intake and therefore reduces the amount of moisture obligated to evaporation. This water savings in EWL provides more water for urinary and fecal elimination.

Acclimatization

It would appear from the metabolism experiment that P. m. nebrascensis in the laboratory meets food and/or food and water shortages by entering into a torpid state for short periods of time. If food were available and water were absent, P. m. nebrascensis increased its renal efficiency and could survive for extended periods. Data from urine concentrations of field animals may indicate a similar response to food and/or food and water shortages in the animals natural environment. The highest mean urine osmolality was recorded for animals taken in August and September when water shortage would be most likely to occur. July urine samples showed a higher concentration than January or April samples. The amount of moisture in January and April would normally be greater than it would be in July. Urine potassium concentrations were higher in August-September and July than they were in April and January. This may indicate an increased consumption of plant material during the absence of free water. If the plant material had

sufficient moisture, P. m. nebrascensis could survive. No animals were taken during the latter part of July or the first part of August but adult animals were readily available in late August and early September. This scarcity of animals in late July and August may be due to the high temperatures and decrease in free and bound water. The absence of free water or decreased water in food coupled with the high temperatures may be the necessary stimuli to induce aestivation or periods of torpor in P. m. nebrascensis. This does not agree with laboratory findings on metabolism, but laboratory and field responses of animals may differ.

SUMMARY

1. This study was divided into five areas in order to determine any functional adaptation to water shortage in the deer mouse Peromyscus maniculatus nebrascensis.
    a. The effects of acute and total food and/or water deprivation on oxygen consumption, urine concentration, and total and fat-free body water.
    b. The ability of this subspecies to acclimate to water restriction.
    c. The renal efficiency of this subspecies as determined by tolerance to salt loading.
    d. The effects of water restriction on evaporative water loss.
    e. Changes in urine concentrations in field animals acclimatized to seasonal changes.
2. For the metabolism experiment twenty mice were randomly divided into four groups of five each. The treatment for each group is given. Mice given food and water ad lib averaged 4.4 ml O_2/g hr and maintained body weight. Mice deprived of food and/or food and water decreased oxygen consumption or became hypothermic and showed a

consistent decrease in body weight. Mice deprived of water and given food ad lib decreased body weight but maintained a high oxygen consumption and survived longer than any other experimental group. Urine osmolality varied with treatment and was greater in mice deprived of water and receiving ad lib food than in any group. The lowest osmolality was recorded for mice on ad lib water and no food. Total and fat-free body water were not significantly different in any of the groups in the metabolism experiment.

3. The daily water intake for P. m. nebrascensis was 3.6 ml/day at 29-30 C and 50-60% R.H. and on a diet of Purina Lab Chow. Reduction to one-half, one-fourth, and one-eighth ad lib water intake was accomplished by giving a one per cent purified agar gel that could be weighed with an accuracy of ± 0.1g. Mice lost weight on restricted water regimes but stabilized their weight at 98.4, 88.6 and 71.5% original on each decreasing regime respectively. Mice acclimated to water restriction did not show an increase in urine osmolality. An average osmolality of 3.05 osmoles was found. Individual osmolality reached a maximum of 6.52 osmoles for one animal on one-half ad lib agar. A definite decrease in urine sodium and chloride concentrations was observed at the one-fourth and one-eighth ad lib regime. Urine

potassium concentrations did not show any significant difference between controls and experimentals.

4. In the experiment on acclimation to salt loading twelve mice were randomly divided into six controls and six experimentals. The controls received distilled water and the experimentals were acclimated to increasing molarities of NaCl solutions (0.1, 0.2, 0.3, 0.35, and 0.4M). Controls maintained weight throughout the experiment. Experimentals gained weight on 0.1M NaCl but lost weight on each more concentrated salt regime. Weight stabilization occurred on 0.35 and 0.4M salt solutions at 89.4 and 86.2% original respectively. Urine osmolality decreased with increasing concentrations of NaCl solutions. A significant decrease in osmolality was observed in the experimentals at 0.35 and 0.4M drinking solutions. Urine sodium and chloride concentrations increased with increasing molar concentrations of drinking solutions. Urine potassium concentrations showed a slight decrease with an increase in concentration of drinking solutions. Desert rodents are more efficient in their ability to drink high concentrations of NaCl solutions than P. m. nebrascensis. The highest molarity of NaCl tested was 0.5M and all mice died on this regime. Four mice out of six were able to survive at a reduced weight on 0.4M NaCl.

5. In the evaporative water loss experiment mice acclimated to water restriction reduced their total EWL to almost 50% of controls. A total of 42% of the total water gained was obligated to evaporation in normally hydrated animals. If EWL were not reduced in acclimated animals the water obligated to evaporation would exceed water gained and the animal would be in negative water balance. Since EWL was reduced in animals acclimated to reduced water intake, only 46-60% of the total water gained was obligated to evaporation and this left a minimum of 40% for urinary and fecal water loss.
6. Urine samples were taken from field animals during the seasons of the year. Urine osmolality, sodium, chloride, and potassium concentrations were determined. August and September urine samples had the highest mean osmolality followed by July, January and April in that order. April urine samples showed a significant decrease in osmolality from samples taken in August and September. Sodium and chloride concentrations showed the same trend as urine osmolality. Although urine potassium concentrations were variable, they did show high values for August and September and low values for April.

LITERATURE CITED

Bartholomew, G. A. and T. J. Cade. 1957. Temperature regulation, hibernation, and aestivation in the little pocket mouse, Perognathus longimembris. J. Mamm. 38: 60-72.

Bartholomew, George A. and Jack W. Hudson. 1959. Effects of sodium chloride on weight and drinking in the Antelope Ground Squirrel. J. Mamm. 40(3): 354-360.

Bentley, P. J. 1955. Some aspects of the water metabolism of an Australian marsupial Setonyx brachyurus. J. Physiol. 127: 1-10.

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

_____. 1955. Skin and respiratory water losses of Peromyscus maniculatus sonoriensis. Ecology. 36: 463-467.

_____. 1961. Water metabolism of desert-inhabiting vertebrates. Biol. Reviews. 36(1): 1-31.

_____. 1965. Water metabolism of mammals in: Physiological Mammalogy. Edited by William Mayer and Richard Van Gelder. 2: 43-178. Academic Press, New York.

Chew, R. M. and A. E. Dammann. 1960. Evaporative water loss of small vertebrates, as measured with an infrared analyzer. Science. 133: 384-385.

Chew, R. M. and R. T. Hinegardner. 1957. Effects of chronic insufficiency of drinking water in white mice. J. Mamm. 38: 361-374.

Church, R. L. 1966. Water exchanges of the California vole, Microtus californicus. Physiol. Zool. 39(4): 326-340.

Dice, L. R. 1922. Some factors affecting the distribution of the prairie vole, forest deer mouse, and prairie deer mouse. Ecology. 3: 29-47.

Fertig, Daniel S. and James N. Layne. 1963. Water relationships in the Florida mouse. J. Mamm. 44(3): 332-334.

Fisler, G. F. 1962. Ingestion of sea water by Peromyscus maniculatus. J. Mamm. 43(3): 416-417.

_____. 1963. Effects of salt water on food and water consumption and weight of harvest mice. Ecology. 44(3): 604-608.

Folk, Edgar G., Jr. 1966. Introduction to Environmental Physiology. Lee and Febiger, Philadelphia. pp. 24-25.

French, R. L. 1956. Eating, drinking, and activity patterns in Peromyscus maniculatus sonoriensis. J. Mamm. 37: 74-79.

Getz, Lowell L. 1963. A comparison of water balance of the prairie and meadow voles. Ecology. 44(1): 202-207.

_____. 1968. Relationship between ambient temperature and respiratory water loss of small mammals. Comp. Biochem. Physiol. 24: 335-342.

Haines, H. 1964. Salt tolerance and water requirements in the salt-marsh harvest mouse. Physiol. Zool. 37(3): 266-272.

_____. 1968. Personal communication.

Haines, H. B. and K. Schmidt-Nielsen. 1967. Water deprivation in wild house mice. Physiol. Zool. 40(4): 424-431.

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

Hart, J. S. 1957. Climatic and temperature induced changes in the energetics of homeotherms. Revue Canadienne De Biologie. 16: 133-174.

Hudson, J. W. 1962. The role of water in the biology of the antelope ground squirrel. U. Calif. Publ. Zool. 64(1): 56.

Hudson, J. W. 1965. Temperature regulation and torpidity in the pygmy mouse, Baiomys taylori. Physiol. Zool. 38(3): 243-254.

Hudson, J. W. and J. A. Rummel. 1966. Water metabolism and temperature regulation of the primitive heteromyids, Liomys salvani and Liomys irroratus. Ecology. 47: 345-354.

Jackson, Donald C. and Knut Schmidt-Nielsen. 1964. Counter-current heat exchange in the respiratory passages. Proc. Nat. Aca. Sci. 51(6): 1192-1197.

Lindeborg, R. G. 1952. Water requirements of certain rodents from xeric and mesic habitats. Contr. Lab. Vert. Biol. Univ. Mich. 58: 1-32.

MacMillen, Richard E. 1964. Population ecology, water relations, and social behavior of southern California semidesert rodent fauna. U. Calif. Publ. Zool. 71:— 66 pp.

_____. 1965. Aestivation in the cactus mouse, Peromyscus eremicus. Comp. Biochem. and Physiol. 16: 227-248.

McNab, B. K. and P. R. Morrison. 1963. Body temperature and metabolism in subspecies of Peromyscus from arid and mesic environments. Ecol. Monogr. 33: 63-82.

Mogharabi, Forough. 1968. Ph.D. Dissertation. Effects of dehydration on fluid regulation in the thirteen-lined ground squirrel and laboratory rat. Univ. of Okla. Norman, Okla.

Odum, E. P. 1944. Water consumption of certain mice in relation to habitat selection. J. Mamm. 25: 404-405.

Ross, L. G. 1930. A comparative study of daily water intake among certain taxonomic and geographic groups within the genus Peromyscus. Biol. Bull. 59: 326-328.

Schmidt-Nielsen, K. 1964. Desert Animals. Oxford University Press.

Schmidt-Nielsen, B. and K. Schmidt-Nielsen. 1950a. Do Kangaroo rats thrive when drinking sea water? Amer. J. Physiol. 160: 291-294.

Schmidt-Nielsen, B. and K. Schmidt-Nielsen. 1950b. Pulmonary water loss in desert rodents. Amer. J. Physiol. 162(1): 31-36.

Shield, C. 1968. Unpublished M.S. Thesis. Effects of water deprivation on evaporative water loss in Mus musculus. Univ. of Okla. Norman, Okla.

Winkelmann, John R. and Lowell L. Getz. 1962. Water balance in the Mongolian Gerbil. J. Mamm. 43(2): 150-154.