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EFFECTS OF DEHYDRATION ON FLUID REGULATION IN THE

THIRTEEN-LINED GROUND SQUIRREL AND LABORATORY RAT

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EFFECTS OF DEHYDRATION ON FLUID REGULATION IN THE

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DEDICATION

To the "partner of my life" Ata, whose encouragement
and understanding have been most helpful in my work.

To my daughter Mitra and my son Paymon.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

EFFECTS OF DEHYDRATION ON FLUID REGULATION IN THE
THIRTEEN-LINED GROUND SQUIRREL AND LABORATORY RAT

INTRODUCTION

Changes in the fluid compartments of mammals experiencing dehydration can be divided approximately into three categories. In those animals fitting the first category, the plasma loses proportionally more water than either cellular or interstitial spaces. Second, interstitial and cellular spaces lose disproportionally larger quantities of water while plasma loses relatively little. Third, water is lost proportionally from all fluid compartments. These patterns were established in studies of man, domestic animals and the larger laboratory species. There is little information on volume regulation in rodents, particularly those species which normally dwell in semiarid or desert regions. The objective of this study is to compare aspects of volume regulation and related functions in the thirteen-lined ground squirrel (Citellus tridecemlineatus), whose distribution includes arid regions, and the laboratory rat (Rattus norvegicus).

1

2

Literature Review

The three patterns by which fluid compartments change or "shift" during dehydration are well exemplified in man, the camel and the donkey. Studies concerning these forms will be reviewed followed by similar studies on other forms.

<u>Man</u>, <u>Camel</u>, <u>Donkey</u>. When a man is dehydrated in a fairly short period of time, as by sweating profusely, the water lost from the plasma is disproportionately greater than that lost from cells or interstitial fluid. In extensive studies conducted in simulated desert conditions with military personnel, Adolph (1947) showed that men who lost 1 to 11% of their body weight by rapid sweating experienced a decrease in plasma volume which was out of proportion to total water losses. The plasma volume diminished 2.5 times that expected from the total water loss. In these subjects the serum concentrations of chloride and nonprotein nitrogen and the percentage of red blood cells increased in the same proportion as plasma volume decreased. Sugar concentration increased more than was expected from the loss of water from the blood. Adolph's results have been verified (Robinson, 1949; Saltin and Koslowski, 1963).

There are detrimental consequences of the pattern of fluid shift shown by man. The increases in red cell and

protein concentrations produce an increase in blood viscosity. The increased blood viscosity in combination with the reduced blood volume necessitates greater cardiac effort. The tissues must be supplied by a smaller volume of more viscous blood. The point may come when the blood cannot supply metabolic needs or function adequately to dissipate body heat (Schmidt-Nielsen, 1964).

Experiments on water deprivation in the camel (Camelus dromedarius) were reported by Schmidt-Nielsen (1964), and Macfarlane et al. (1963). The distribution in water loss in a camel deprived of water is quite different from that of man. Schmidt-Nielsen (1964) showed that a camel that lost 17% of its original weight had the greatest proportional loss in the interstitial fluid (38%). The decrease in intracellular fluid was 24% and the smallest proportional loss was from plasma (8.8%). Macfarlane et al. (1963) deprived a camel of water at a temperature of 41°C (causing the loss of 20.5% of the original weight in 9 days). Of the total weight loss, the extracellular space lost only 15.6%, plasma fluid 5.2% and the interstitial fluid 9.3%. Thus there is a relatively small loss of plasma in the dehydrated camel. Macfarlane points out that the slow rate of weight loss during exposure to high heat (41°C) may allow adjustment of fluid space.

Dill (1938) found in a dehydrated donkey that plasma chloride concentration increased 12.4% from normal. Since chloride is ubiquitous throughout the body fluids this indicated a water loss from all compartments. Dill observed no rise in plasma protein concentrations even though plasma volume decreased. He related the constancy of plasma protein to its passing from the capillary system into the interstitial spaces, whereas Schmidt-Nielsen (1964) believes that the capillary wall is impermeable to protein and thus protein concentration increases with water loss. Schmidt-Nielsen suggests that increased plasma protein draws water from the interstitial spaces into the blood; thus plasma regains its concentration at the expense of interstitial fluid.

Dog, Sheep. When dehydration is produced rapidly in the dog by heating in short term exposure (3-4 hrs.) the water loss is mainly from extracellular water (Kanter, 1953). As the period of dehydration and exposure to heat progresses the water deficit becomes increasingly greater and the intracellular volume decreases rapidly. Kumar and Lahiri (1963), studying the body fluids of dogs deprived of water for 48 hours at high temperature, found that dehydration produced loss of fluid from both plasma and interstitial spaces with a relatively greater loss from the latter. They concluded

that during hyperthermia, fluid shifts from the cellular spaces into the plasma and to a lesser extent into the interstitial spaces. Elkinton and Taffel (1942) studied the exchange of salt and water in dogs experiencing prolonged water deprivation. Water loss was out of proportion in comparison to salt loss; serum concentrations of sodium and chloride, therefore, rose progressively for many dogs. The total water loss greatly exceeded extracellular water loss and indicated a substantial intracellular water loss.

The changes in the water compartments of Merino sheep deprived of water differ according to the rate of dehydration (Macfarlane et al., 1961); at 42°C, 12% of the total weight loss was plasma water, but at 29°C, plasma loss was only 3% of the total weight loss. Interstitial fluid loss was about 35% of the total fluid loss at 42°C, or 2.9 times more than plasma loss, and was 21% at 29°C, or 7 times more than plasma loss. In both cases more than half of the water loss was from the intracellular compartment. Thus, the rate of transfer from one compartment to another is controlled by the environmental temperature (Macfarlane et al., 1961).

Rodents. General investigations have clarified some factors in the water balance of rodents. These factors include the effect of water deprivation on survival time,

weight loss, water content, urine and plasma concentrations.
Schmidt-Nielsen and Schmidt-Nielsen (1948) showed that
heteromyids (e.g., kangaroo rat, Dipodomys merriami and
others) lived and gained weight on a regimen of no drinking
water and a diet consisting of a dry grain, whereas white
rats lost weight on this regimen and died after losing 50%
of their initial body weight. In these experiments the
kangaroo rats survived with little change in water content,
whereas the white rats lived for 20 days, and had lost 52%
of their original water content at the time of death.
Schmidt-Nielsen and Schmidt-Nielsen (1948) showed that the
heteromyids had no increase in plasma concentrations of
electrolytes and urea, while living on a dry diet, whereas
white rats showed increased plasma concentrations. Electro-
lytes were excreted in very high concentrations in the
kangaroo rat, but the white rat could not excrete electro-
lytes at the same rate as they were formed. Plasma concen-
trations in the rats increased to a level that was apparently
intolerable.

Investigations on the effects of water deprivation
on fluid compartments and characteristics of rodents are
limited. Heller (1949), using adult rats, withdrew water
and food for 24 hours and found no changes in the

extracellular fluid volume, particularly the plasma volume,
and its ionic concentration. He concluded that volume was
maintained by a shift of intracellular water into the extra-
cellular spaces and by reabsorption of water by the kidney.
Oppositely, Bintz and Riedesel (1967) found that blood be-
came dehydrated when rats were deprived of water to 30% of
their original body weight.

The Problem: Dehydration and Volume Regulation in Rodents

The ability of rodents to live in arid and semiarid
areas has excited interest for many years. A conspicuous
feature in the biology of desert animals is their adaptation
to an environment with little or no supply of drinking water.
The physiological mechanisms which enable some rodents to
live with a scarcity of water are only partly understood.
Major problems are still unsolved. In those species ex-
amined a notable characteristic is the ability to form highly
concentrated urine. This characteristic has been well
documented (for reviews, see Schmidt-Nielsen, 1964; Chew,
1965). However, studies concerning dehydration have utilized
species which pant or sweat in order to thermoregulate in
high environmental temperatures (see previous sections). To
our knowledge rodents do not use water for heat regulation;

rather, many escape the heat of the day by their crepuscular and nocturnal habits (Schmidt-Nielsen, 1964). Thirteen-lined ground squirrels attracted our attention because they inhabit moderate to arid areas, subsisting on green grasses, herbs, flower heads, and insects (Davis, 1960). It is presumed that these animals consume water when it is available.

The questions presented by this situation are: To what degree can this animal remain in water balance? And how well does this rodent regulate fluid volume as a function of dehydration? These questions are of interest for the following reasons:

1. Ground squirrels, which dwell in environments where water is not always available, may have evolved means enabling them to resist water deprivation.

2. Since these ground squirrels hibernate, there may be some related physiological adaptation to water deprivation (negative water balance). In that hibernation involves periods with minimal or no exchanges between the animal and environment in terms of nutrients, water and wastes, it is similar to the removal of drinking water. Both situations represent reduced water intake over extended periods of time.

The animals selected for this study were two rodent species: the thirteen-lined ground squirrel, _Citellus tridecemlineatus_, which is a wild species living in moderate to arid areas (Davis, 1960) and possibly experiencing dehydration in the natural environment, and the laboratory rat, _Rattus norvegicus_, which has evolved in an environment where water is usually available as desired. Since the laboratory rat is commonly used for experimentation, it would be reasonable to compare results from the wild rodents with those from these laboratory animals.

Apparently nothing is known about dehydration and water balance of _Citellus tridecemlineatus_[1] and no information exists about its fluid volume regulation as a function of dehydration. In this respect there is also a scarcity of data for laboratory rats. In view of these facts, it seemed worth while to test the effect of acute dehydration on the body fluids and osmotic-ionic properties of the plasma and urine.

This study was designed to compare these two species of rodents. The first experiment in the study obtained information describing the general responses of squirrels to

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[1] Henceforth for the sake of brevity squirrel is used in place of _Citellus tridecemlineatus_, and rat is used in place of _Rattus norvegicus_.

water restriction and drinking NaCl solutions. Using the data obtained from the first experiment, a second experiment was performed. In the latter experiment squirrels and rats were deprived of water or given NaCl drinking solutions for the primary purpose of determining the influence of these treatments on the plasma and total body water. Secondarily, the influence of the treatments on urine concentration was determined.

MATERIAL AND METHODS

Experimental Plan

The study consisted of two subdivisions: 1) An experiment using squirrels, with the objective of describing general responses to water restriction and to drinking NaCl solution. 2) A comparative experiment in which squirrels and rats were exposed to water deprivation and salt-drinking in order to find the influence of these treatments upon certain characteristics of the body fluids and urine. Adult animals of both sexes were utilized. The squirrels, weighing 132-290 g, were collected from the University Golf Course (Norman, Cleveland County, Oklahoma). Rats, weighing 177-400 g, were from a colony of hybrid King-Holtzman rats (sometimes called the Stanley-Gumbreck strain), which is maintained at the Oklahoma Medical Center. All animals were housed in individual cages, and fed Purina Laboratory Chow. They were kept at room temperature. A total of 52 squirrels and 52 rats were used in the study. The work was conducted over the period from March, 1966, through March, 1968.

Influence of water restriction and NaCl solutions on body weight and urine concentration in squirrels. This experiment was conducted to determine:

1. The degree to which these animals can drink NaCl solutions and remain in water balance.

2. To what degree these animals can maintain water balance on restricted quantities of drinking water.

3. The ability of the kidney to concentrate urine.

Maintenance or lack of maintenance of body weight was taken to indicate balance between water intake and output. If the body weight decreased while the animal received a particular treatment, it was concluded that the animal either had lost water or had reduced its food or water consumption.

Twenty-eight squirrels were used in this study. The animals were divided into three groups:

1. Ten animals received various concentrations of salt solution in the following sequence: 0.1 N NaCl, April 26-May 4; 0.2 N, May 4-May 19; 0.4 N, May 19-June 3; 0.3 N, June 3-June 20; and 0.45 N, June 20-July 14, 1966.

2. Nine animals received restricted rations of water (in the form of weighed portions of

cucumber, assuming that it is 97% water); 10%

(of body weight), April 26-May 4; 15%, May 4-

June 9; 20%, June 9-June 29; 5%, June 29-July 10;

and none during July 10-July 14, 1966.

3. A control group of nine animals received no treat-
ment and served for comparison with both experi-
mental groups.

Body weights were recorded to the nearest 0.1 g.
Urine was collected while the animals were receiving 0.45 N
NaCl solution or were deprived of water. At this time the
animals were losing weight. The procedure used for the col-
lection of urine was as follows: The animal was placed in
a 2 lb. coffee can with detachable wire cloth bottom. The
can containing the animal was then placed over a Petri dish,
thus allowing urine droplets to fall through the wire cloth
into the Petri dish. The Petri dishes were water-proofed
with paraffin. The wire cloth was painted with enamel to
make it hydrophobic. Upon collection the urine was frozen
and stored in a deep freeze until analysis. Concentrations
of potassium, sodium, and chloride and osmolality of the
urine were measured (see following section).

The influence of water deprivation and NaCl drinking
on fluid spaces and osmotic-ionic properties of plasma and

<u>urine</u> <u>in</u> <u>squirrels</u> <u>and</u> <u>rats</u>. The purpose of this experiment was to determine the influence of water deprivation and NaCl drinking on:

1. Plasma volume and body water content.

2. Blood percentage of red cells, and concentrations of plasma protein, sodium, potassium, and chloride.

3. Urine osmolality, sodium, potassium, and chloride concentrations.

A total of 52 squirrels and 52 rats were used in this experiment. Twenty-eight of these squirrels were the same animals which had been used in the first division of this study a year previously.

The animals were divided into three groups:

1. Nineteen rats and eighteen squirrels were deprived of water.

2. Nineteen rats and fourteen squirrels were given drinking solution of 0.4 N, NaCl.

3. Fourteen rats and 20 squirrels received no treatment and served as controls.

Treatments were acute. That is, there was no gradual restriction of water or gradual introduction of NaCl solutions. Animals were exposed suddenly to both treatments.

The experimental animals were exposed to the treatments until body weight was 75-80% of the original. Urine samples were taken before and after treatment. Finally, the animals were prepared for measurement of plasma volume and blood *collection*.

Control animals received water and food as desired. The three groups, two experimental, one control, were processed simultaneously. Weights were measured periodically.

The results of this experiment were analyzed statistically by a single classification analysis of variance for unequal sample size. A priori tests were used to test for differences between means (Sokal and Rohlf, 1968).

Plasma Volume Determination

Dye dilution method. Plasma volume was determined by the dye dilution method. The dye used was Evans Blue (T-1824, Evans Blue injection, U.S.P., 0.5% aqueous solution, General Diagnostics Division, Warner-Chilcott, Morris, N.J.). Subsequent reference to the dye will be "T-1824".

T-1824 stock standards were made by diluting the commercially prepared aqueous solution with saline. Stock standards of 1:250 were used with the squirrels and some of the rats (Sept. 18, 1967 to Feb. 19, 1968), and dilutions of

1:500 were used with the remainder of the rats (Feb. 19 to March 24, 1968). These dilution ratios were determined by the range of the dye concentrations of plasma samples. The stock standards could be used for several weeks. The spectral absorption of 180 μl of the 1:250 stock standard was found to be the same whether diluted with 20 μl of whole plasma or of plasma diluted 1:10 with saline. Since the spectral absorption of dyed plasma, whether diluted or not, was identical, plasma concentrations were determined with plasma diluted 1:10 with saline. Standards, blanks and samples were prepared in triplicate by pipetting 20 μl of plasma (undyed in standards and blank, dyed in samples) into microtest tubes to which 180 μl of stock standard or saline was added. Optical density was determined with a spectro-colorimeter (Beckman Model 151) at 640 mμ. The absorption of 640 μl was found to be maximal for standards and samples from both species. At least 100 μl of the samples were transferred into the cuvette. The same cuvette was used on all measurements, insuring that the depth through which the light beam passed remained constant and that the cuvette used for the samples and standards was identical.

Since T-1824 gradually disappears from the blood spaces it is necessary to extrapolate the initial optical

density or that optical density representing the concentration that would theoretically exist when the T-1824 is distributed throughout the fluid compartment (Elkinton and Danowski, 1955). The optical density of plasma samples taken at 10, 30, 60 minutes after dye injection was plotted against time. Usually a straight line could be drawn through the points. In two cases the slope of the line was calculated by regression analysis. The initial optical density (A_O) was extrapolated by extending the line to the Y axis (Fig. 1). The plasma volume was calculated from the following formula:

$$\text{Plasma volume, ml} = DxVxK/A_Oxd_1/d_2$$

where D is the dilution factor of stock standard, V is ml of 0.5% T-1824 injected, K is the optical density of plasma standard, A_O is the initial optical density in plasma sample (dyed plasma), $d_1 = 200/180$ is dilution of the plasma standard and $d_2 = 200/20$ is the dilution of plasma samples. d_1/d_2 is then $= 1/9$.

Catheterization and blood collection. A carotid catheter permitted blood sampling and dye injection. The method was primarily that of Popovic and Popovic (1960). The animal was anesthetized with ether, and placed on a board with head and forelegs secured by adhesive tape. A

Fig. 1. Disappearance curve of T-1824 in blood of rats. The ordinate shows the optical density of plasma sample (dyed plasma) and the abscissa represents time (minutes) after T-1824 injection. The points on the solid line are the average of three plasma samples. The dotted line is drawn as extension of the disappearance curve of T-1824, to extrapolate the initial optical density.
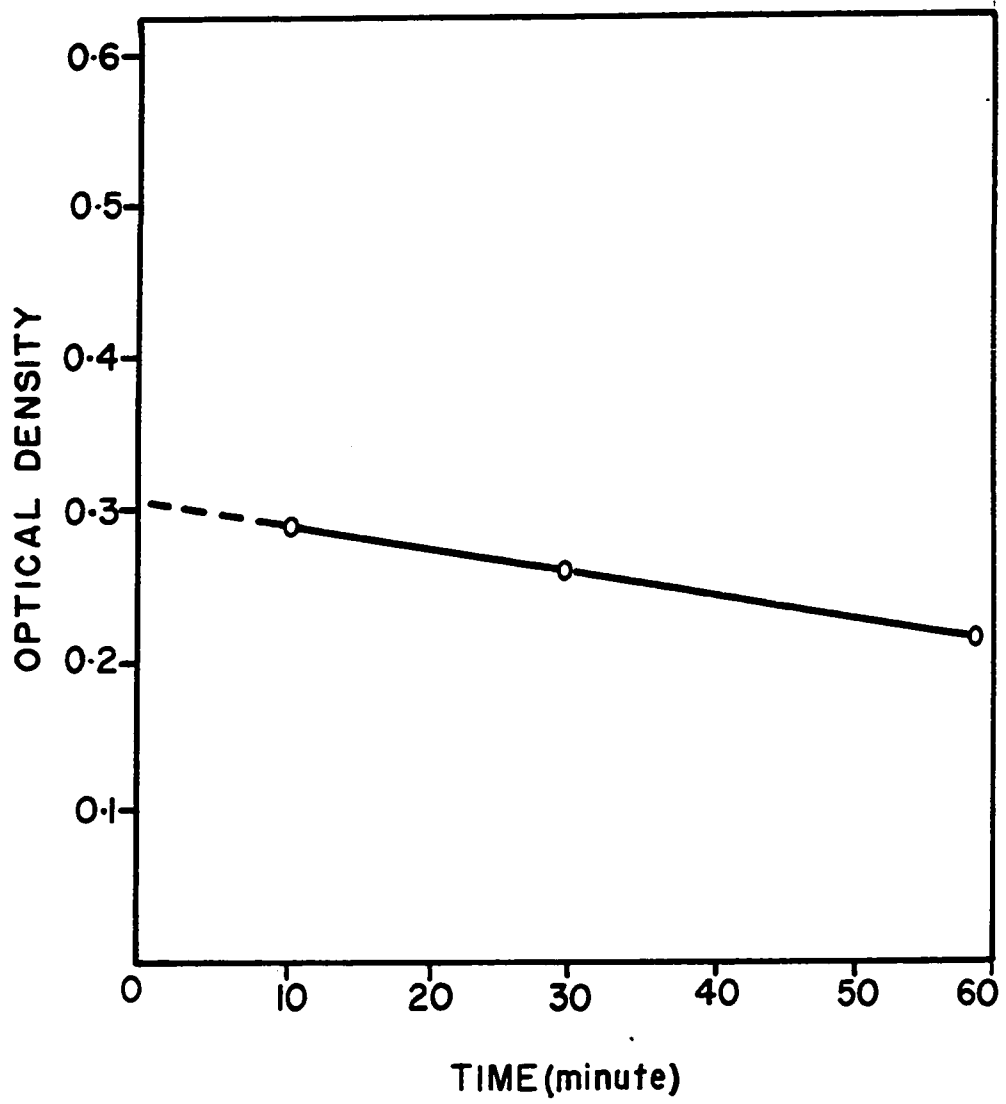
median ventral skin incision was made extending from
mandibular papilla to the lower border of the submaxillary
gland (approximately 3 cm). The left carotid artery was
located between the sternohyoideus, hyoideus and omohyoideus
muscles by using a smooth curved probe. With another probe
placed underneath, a short length (approximately 1½ cm) of
the left common carotid artery was exposed and carefully
dissected free of surrounding tissues, nerves and blood ves-
sels. To provide a smooth, clean field, a length of folded
adhesive tape was placed underneath the exposed carotid. A
nylon thread ligature was tied as close to the head as pos-
sible to stop blood flow. Another thread (Deknatel, size 1)
was placed around the artery near the chest and the artery
occluded by clamping the ends of the thread with a hemostat
and pulled sufficiently taut to occlude the artery. A small
transverse incision not greater than a third of the carotid
diameter was made towards the anterior end of the vessel
with micro-dissecting scissors (Clay-Adams). This portion
of the vessel was kept in a stretched position by a forceps
while the incision was made. At this time a 3X Binoc-Loupe
was used to magnify the carotid artery. The open end of a
heparinized (1000 U.S.P. units/ml Abbott) thin wall poly-
ethylene tube (P.E. 50) 13 cm long which had been clamped on

the other end with a hemostat was then inserted slowly
through the incision. When the catheter had entered about
0.5 cm a ligature (surgical silk, No. 1, Deknatel) was tied
loosely around the carotid and inserted catheter, anterior
to the stretched central part of the artery. The catheter
was then gently pushed deeper for a distance of 3 cm until
the aortic arch was reached. The ligature around the
catheter was then tightened to a degree sufficient to fix
the catheter but not to stop blood flow in the catheter. A
second adjoining ligature was tied tightly to strengthen the
first one. A blunted end of a 23 gauge needle fixed to a
1/cc tuberculin syringe (graduated in 1/100cc with Luer Lok
tip was inserted into the lumen of the catheter for sampling
or injections. The hemostat was removed only when making
injections or withdrawing samples. Prior to withdrawing a
blood sample the hemostat was removed to drain the saline
from the catheter and fill it with blood. Two drops of blood
were wasted to assure that the saline was removed. Prior to
the dye injection, 1.0 ml of blood was withdrawn for prepa-
ration of the plasma blank, plasma standard, and other
analyses (protein, Na, K, and Cl).

Three fixed-volume syringes calibrated to eject 0.3,
0.16, 0.098 ml were used for dye injection. For squirrels,

the volume of the injectate was 0.3 ml (Sept. 18 to Oct. 10,
1967) and 0.15 ml (Oct. 10, 1967 to Jan. 29, 1968). For
rats the volume of the injectate was 0.1 ml (Jan. 12 to Feb.
15, 1968) and 0.15 ml (Feb. 15 to March 24, 1968). These
volumes were determined by the range of the dye concentration
on plasma samples.

All syringes and needles used for blood sampling
were rinsed with sterile heparin solution (1000 U.S.P. units/
cc Abbott) and dried. The needle was inserted into the
catheter one minute prior to sampling and the blood in the
catheter was mixed by repeated withdrawal and injection.
0.5 ml blood samples were drawn and transferred to 400 μl
polyethylene microtest tubes at 10, 30, and 60 minutes after
dye injection. These and the blank samples were centrifuged
for 5 minutes (Microfuge, Beckman Model 152). The superna-
tant plasma was then transferred to other microtest tubes.
During the squirrel experiment the supernatant was recentri-
fuged with a refrigerated automatic Sorvall centrifuge for
30 minutes at a speed of 15,000 R.P.M. at 3°C temperature,
in order to separate fats and plasma in the occasionally
lipemic plasma. Hematocrit capillaries (Clay-Adams, red tip
fire polished heparinized Yankee, 75 mm X I.D. 1.1-1.2 mm)
were filled in triplicate, and after centrifugation, the

percentage of red blood cells was determined. The micro

hematocrit centrifuge used was the International Equipment

Company Model MB. The percentage of red cells was measured

with an International Equipment Company Model C R Micro-

capillary reader.

Plasma Protein

Plasma protein content was measured by an ultramicro

adaptation (Beckman Technical Bulletin No. 6074 D) of the

biuret method of Kingsley (1939), and Gornall, Bradawill and

David (1949), using 50 μ1 of sample in place of 5 μ1 of

sample. The protein standard solution was crystalline

Bovine albumin (Armour Pharmaceutical Company, 10 mg protein

nitrogen/ml in 3 ml ampules). The maximum of absorption was

found to be 540 mμ, for standard protein, and plasma protein

of rats and squirrels, thus the absorbance of unknown and

standard were read at 540 mμ, using a spectrocolorimeter

(Beckman/Spinco Model 151) against a blank set at zero ab-

sorbance. The following equation is used to obtain grams of

total protein per 100 milliliters of plasma:

$$\text{gm total protein/100 ml plasma} = V/S \times P$$

where V is the optical density of plasma (unknown), S is the

optical density of protein standard, P is grams of protein

in 100 ml standard which is calculated by multiplying

protein nitrogen (mg per ml) by 0.625. The modified biuret

reagent (Beckman Technical Bulletin No. 6074D) was used.

This reagent was stable for several weeks.

Chloride, Sodium, Potassium, and Osmolality

Urine and plasma chloride were measured by an auto-

matic chloride titrator (Aminco-Cotlove) using $10 \mu 1$ samples

and NaCl standards. Sodium and potassium were measured by

flamephotometry (Baird-Atomic Model KY-3). Urine osmolality

was measured using a vapor pressure osmometer (Mechrolab

Model 301A). Some of the highly concentrated urine samples

were diluted 5 times in order to permit osmolality measurements.

Body Water

After each plasma volume determination the animal was

killed by over anesthesia. The carcass was weighed and

frozen.

Water content was determined by desiccation. Ini-

tially the carcass was minced and homogenized by a blendor

(Waring Products Corp. Model 700B). The knife used in minc-

ing the animal and the blendor lid and sides were washed with

warm water that was collected in the blendor. The homogenate

was poured into a weighed beaker. The blendor was rinsed

2 - 3 times with warm water, the rinse being collected in the beaker. The carcass was then dried to constant weight. Total water content was calculated by subtracting the dry weight from the original body weight. A giant extraction apparatus (Soxhlet, Size G, ACE glass) was used for fat extraction. The dried carcass was transferred from the beaker into a weighed extraction thimble. The beaker was rinsed with petroleum ether (Baker Chemical) to aid transferral. The thimble with carcass was extracted for 24 hours. After the ether evaporated the thimble and residue were weighed and the fat free body water calculated.

Fat free water content % = (W / O - F)100

Total water content % = (W / O)100

Where W is water content of the animal

O is the original weight before freezing

F is the extracted fat.

RESULTS

NaCl Drinking and Water Restriction
in Squirrels

Weight changes during NaCl drinking. At the begin-
ning of the experiment the average body weight of the ex-
perimental animals was 8% higher than the control weight,
but was not statistically different ($p > 0.1$). While receiv-
ing 0.1N and 0.2N NaCl solutions the squirrels maintained
weight (Fig. 2). When the NaCl concentration was increased
to 0.4N the weight decreased rapidly for eleven days, then
stabilized at 78.7% of the control weight. This decline was
statistically significant ($p < 0.01$). Weight increased when
0.3N was given and stabilized at 90% of the control weight.
The body weight decreased rapidly and significantly at 0.45N
and never stabilized, during 24 days of treatment.

Weight changes during water restriction. While re-
ceiving a daily ration of cucumber equivalent to 10% of the
body weight, weight declined initially but increased on the
7th day (Fig. 3). When the ration was 15% of the body weight,
weight declined, but not enough to be statistically different

Fig. 2. Weight changes in squirrels while drinking NaCl solution. The periods in which particular normalities of NaCl were given are enclosed by vertical arrows. The ordinate represents the body weight changes (per cent control). The abscissa shows days of treatment. Each point represents the average response.

Fig. 3. Weight changes in squirrels while receiving limited rations of water. The periods in which particular quantities of water were given are enclosed by vertical arrows, and the weight of the ration is shown as per cent body weight, along the abscissa. The ordinate represents the body weight changes (per cent control). The abscissa shows days of treatment. Each point represents the average response.
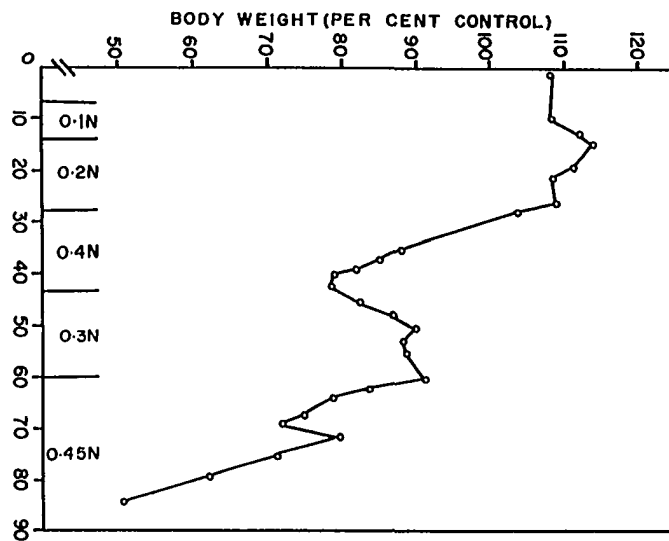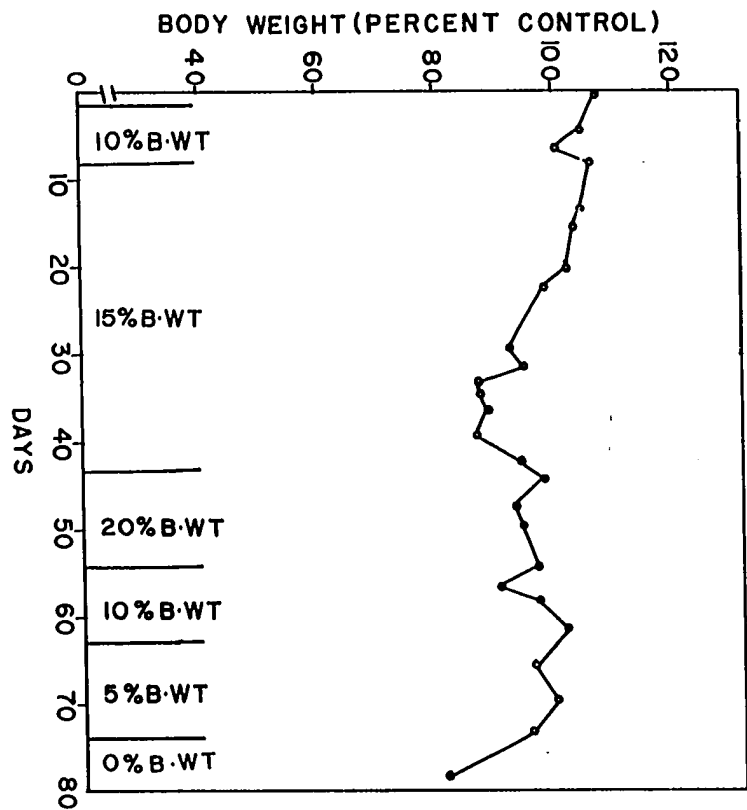
(0.5 > p > 0.1), and then stabilized between days 33 and 42.
There was a weight gain after the cucumber ration was in-
creased to 20% of body weight. During the 10% and 5% periods
the slight weight decrease which occurred initially was again
followed by a weight increase. When deprived of water com-
pletely a sustained and significant weight loss occurred, and
there was no indication of further stabilization.

The control body weights increased an average of
14.9% during the 84 day research period.

Urine osmolality. In both experimental groups the
urine osmolality was significantly greater than control
values. The highest urine osmolality obtained was 3.8
osmolal, and was found in the urine of the water-restricted
group (Table 1).

TABLE 1

Urine osmotic concentration of squirrels, provided water as
desired (control), chronically deprived of water, or given
0.45N NaCl solution to drink. The mean $\pm$ S.E.(N) is given.

	Osmolality Osm/1	Range Osm/1
Control	0.53 $\pm$0.05(12)	0.225-0.80
Water Deprived	3.15* $\pm$0.20(4)	2.7 -3.80
NaCl Drinking	0.95* $\pm$0.06(8)	0.8 -1.40

*Probability, control vs. experimental, $P < 0.005$.

Volume and Osmotic Regulation
in Squirrels and Rats

Rates of weight loss. Acute water deprivation or
0.4N NaCl solutions for drinking were the treatments used to
disturb water balance. Treatment was continued until the
animals had lost 20-25% of the original body weight. Rats
lost this much weight in an average of 5 days acute water
deprivation and 10 days NaCl drinking (Fig. 4). Squirrels
had greater tolerance than rats; an average of 23 days water
deprivation and 29 days NaCl drinking elapsed before the
squirrels lost 20-25% of the original body weight. Individ-
ual variation in the ability to tolerate water deprivation
and NaCl solutions was particularly evident in the squirrels,
no more than 16.5% of the sample losing 20-25% of the orig-
inal body weight on a single day (NaCl drinking, day 23, Fig.
4).

Plasma volume and electrolytes. Acute water depriva-
tion caused plasma volume to decrease in both species. On
the contrary, plasma volume did not decrease in animals given
NaCl solutions to drink. The average plasma volume of water
deprived rats was 21% less than the average plasma volume of
the controls (Fig. 5, Table 2). In the water deprived group
the per cent of red blood cells increased to the same degree

Fig. 4. Percentages of the rats and squirrels los-
ing 20-25% of the original body weight plotted against days
of treatment. The upper figure is for animals experiencing
water deprivation, the lower figure for animals receiving
NaCl solution.
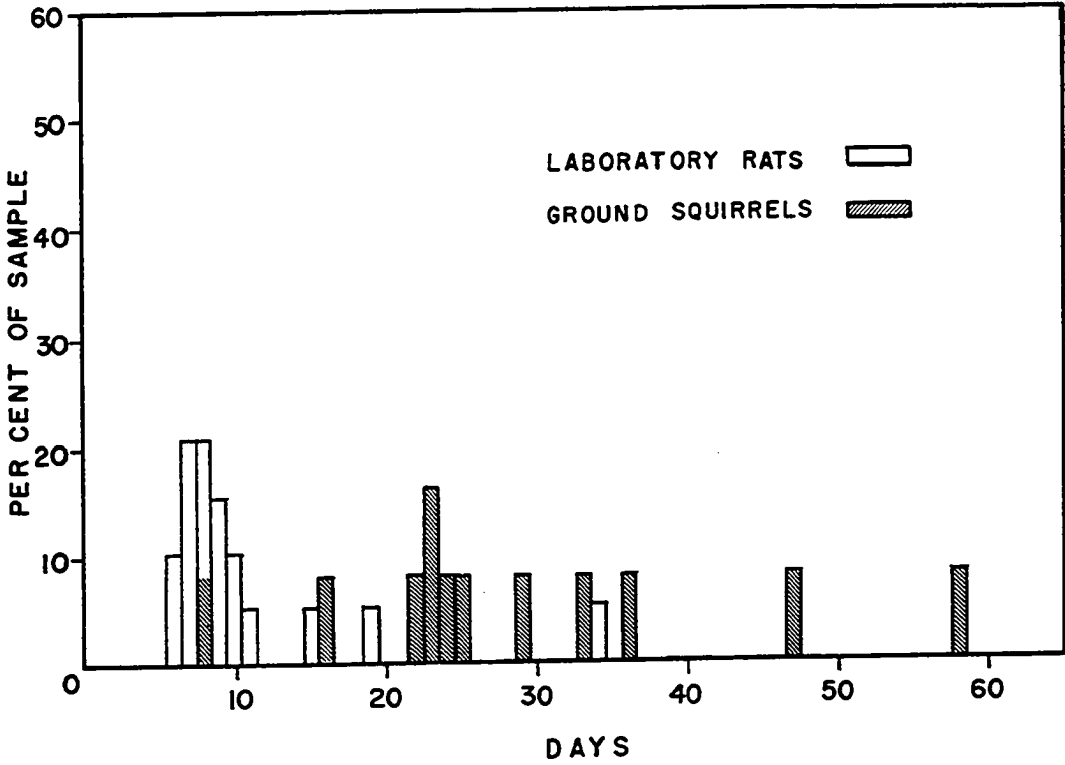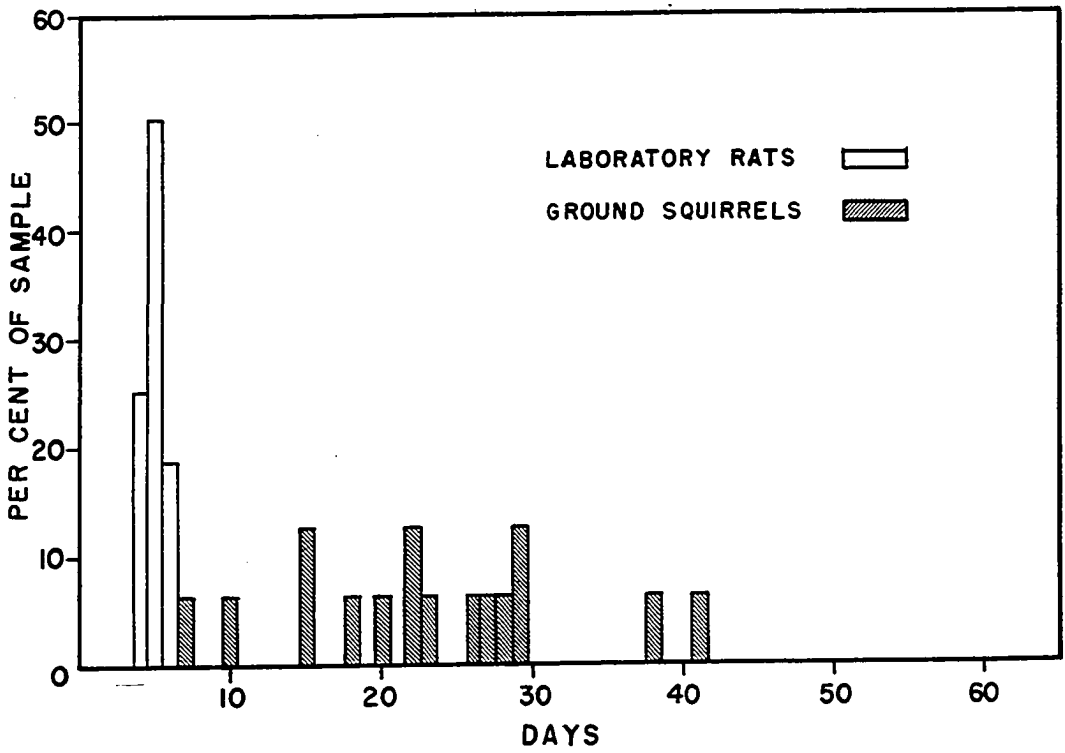
TABLE 2

Plasma volume, per cent red cells, and plasma protein of rats and squirrels, provided water as desired (control), acutely deprived of water or given NaCl solution to drink. The mean $\pm$ S.E.(N) is given.

	Plasma Volume % Body Weight	Hematocrit % Cells	Protein mg/100ml
RAT			
Control	3.13 $\pm$0.15(12)	45.77 $\pm$0.34(12)	6.07 $\pm$0.11(12)
Water Deprived	2.46** $\pm$0.10(15)	55.22* $\pm$0.60(16)	6.19 $\pm$0.24(17)
NaCl Drinking	3.26 $\pm$0.15(15)	44.67 $\pm$0.65(13)	5.81 $\pm$0.08(16)
SQUIRREL			
Control	3.17 $\pm$0.24(10)	47.9 $\pm$0.79(18)	8.66 $\pm$0.78(8)
Water Deprived	2.63* $\pm$0.13(12)	52.1* $\pm$1.47(13)	7.56 $\pm$0.50(9)
NaCl Drinking	2.93 $\pm$0.17(10)	49.3 $\pm$1.56(12)	8.31 $\pm$0.50(8)

*Probability, control vs. experimental, $P < 0.05$.

**Probability, control vs. experimental, $P < 0.005$.

(20%) as plasma volume decreased (Table 2). Similarly, squirrels deprived of water had a lower plasma volume than control animals; average plasma volume was 17% less in the water deprived squirrels than in the controls (Fig. 6, Table 2) and the per cent red blood cells was 8% greater.

Water deprivation resulted in a more pronounced decrease in plasma volume and increase in per cent blood cells in rats than in squirrels. In rats proportionate changes were found in plasma volume and per cent red blood cells, whereas in squirrels the per cent red blood cells increased less than would be expected from the loss of volume from the plasma. —

Plasma volume in rats and squirrels drinking NaCl solution was not significantly different from controls (p $>$ 0.1) (Table 2 and Figs. 5 and 6), and per cent red blood cells was unaffected by NaCl drinking (Table 2). Plasma protein remained unaffected in both species whether water deprived or drinking NaCl solution (Table 2).

Plasma ions rose in concentration as a result of both treatments (Table 3). Chloride was 10% greater in the water deprived rats than in controls and sodium increased similarly (Table 3, Fig. 5). Chloride and sodium in water deprived squirrels tended to increase 9.2% and 5.5%

TABLE 3

Plasma concentration in rats and squirrels, provided water as desired (control), acutely deprived of water, or given NaCl solution to drink. The mean $\pm$ S.E.(N) is given.

	Chloride (m Eq/1)		Sodium (m Eq/1)		Potassium (m Eq/1)	
RAT						
Control	104.9	$\pm$0.59(13)	153.9	$\pm$2.42(12)	3.28	$\pm$0.36(12)
Water Deprived	116.5***	$\pm$1.49(19)	168.5**	$\pm$2.48(17)	4.04	$\pm$0.74(17)
NaCl drinking	126.6***	$\pm$3.06(16)	175.9***	$\pm$4.27(18)	2.93	$\pm$0.21(18)
SQUIRREL						
Control	103.1	$\pm$1.69(15)	158.6	$\pm$4.92(15)	9.4	$\pm$1.87(15)
Water Deprived	115.2*	$\pm$3.15(16)	166.7	$\pm$4.39(19)	8.7	$\pm$1.27(19)
NaCl drinking	122.7**	$\pm$8.39(13)	174.6*	$\pm$7.98(11)	8.7	$\pm$1.95(11)

*Probability, control vs. experimental, $0.1 > P > 0.05$.

**Probability, control vs. experimental, $0.01 > P > 0.005$.

***Probability, control vs. experimental, $P < 0.005$.

Fig. 5. Plasma volume and concentration in rats pro-
vided water as desired (control), acutely deprived of water
or given NaCl solution to drink. The right ordinate repre-
sents the electrolyte concentration (mEq/l). The left ordi-
nate represents plasma volume (ml/100g weight). The horizon-
tal line in each bar represents the average response. The
dotted line is drawn through the average plasma volume at
each treatment. Vertical lines are standard errors.

Fig. 6. Plasma volume and concentration in squir-
rels provided water as desired (control), acutely deprived
of water or given NaCl solution to drink. The right ordi-
nate represents the electrolyte concentration (mEq/l). The
left ordinate represents plasma volume (ml/100g weight).
The horizontal lines in each bar represent the average re-
sponse. The dotted line is drawn through the average plasma
volume at each treatment. Vertical lines are standard
errors.

PLASMA CONCENTRATIONS
AND VOLUME

CONTROL   WATER DEPRIVED   NaCl DRINKING

PLASMA CONCENTRATIONS
AND VOLUME

CONTROL   WATER DEPRIVED   NaCl DRINKING
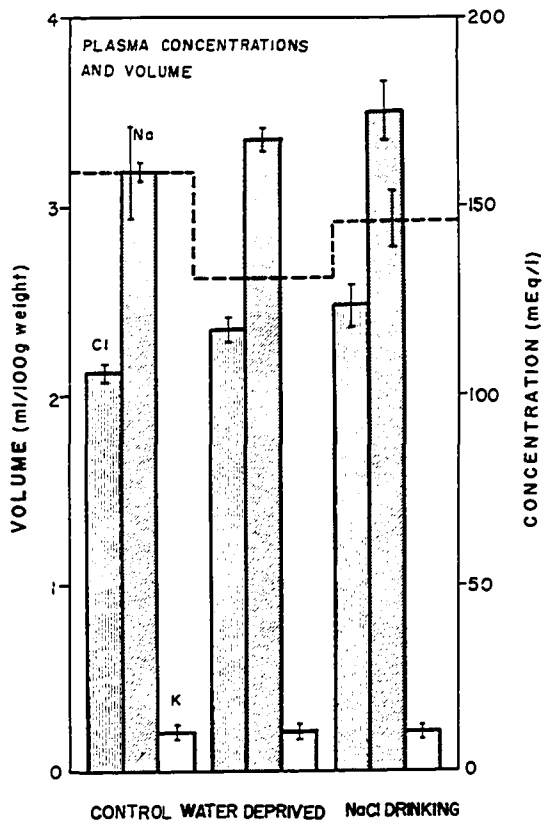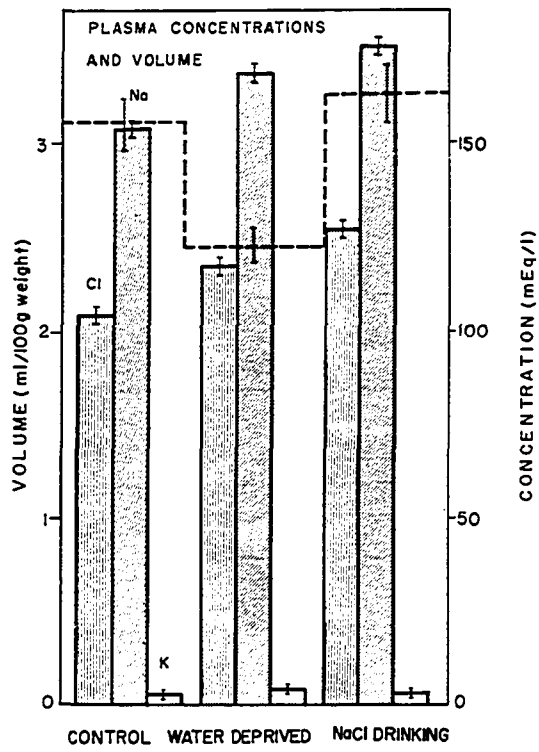
respectively (Table 3, Fig. 6) but without statistical significance. In the rats which drank NaCl solution chloride and sodium rose in concentration by 20% and 14% respectively (Table 3, Fig. 5) as compared to controls, and similarly, plasma chloride was 11.7% higher in squirrels drinking NaCl solution than in controls, and sodium concentration increased by 10.4% ($0.1 > p > 0.05$). Therefore, water deprivation and NaCl drinking caused a statistically significant increase in plasma sodium and chloride in the rats whereas the plasma concentrations of sodium and chloride in the water deprived squirrels and of sodium in squirrels drinking NaCl solution stayed rather constant. Treatment had no effect on potassium concentration; however, squirrels showed an average of 9.40 mEq/l as compared to 3.28 mEq/l in rats (Table 3).

Urine concentrations. The data are summarized in Table 4. Urine chloride decreased by 60% in water deprived rats as compared to controls. Sodium followed a similar course, decreasing by 56%. Potassium concentration increased by 61% (Table 4, Fig. 7). Oppositely, urine chloride was 304% greater in the water deprived squirrels than in controls and urinary sodium increased by 116% (Table 4, Fig. 8). Urinary potassium increased by 166% when squirrels were deprived of water. Osmolality of urine in both species deprived of water

TABLE 4

Urine concentration of rats and squirrels, provided water as desired (control), acutely deprived of water or given NaCl solution. The mean $\pm$ S.E.(N) is given.

	Chloride mEq/1		Sodium mEq/1		Potassium mEq/1		Osmolality Osm/1	
RAT								
Control	105.6	$\pm$13.3(34)	105.5	$\pm$21.4(34)	119.2	$\pm$19(34)	0.98	$\pm$0.09(33)
Water Deprived	38.9**	$\pm$ 4.7(13)	45.9	$\pm$ 4.8(15)	193.2**	$\pm$16.8(15)	2.77***	$\pm$0.12(15)
NaCl Drinking	420.3***	$\pm$33.6(13)	378.0***	$\pm$19.0(11)	49.2**	$\pm$18.6(11)	1.23*	$\pm$0.18(11)
SQUIRREL								
Control	35.5	$\pm$ 6.6(13)	89.1	$\pm$13.4(17)	75.8	$\pm$16.9(17)	0.77	$\pm$0.10(16)
Water Deprived	143.5**	$\pm$29.6(10)	192.5	$\pm$59.6(8)	202.3***	$\pm$37.0(8)	2.39***	$\pm$0.12(10)
NaCl Drinking	439.5***	$\pm$74.2(5)	501.9***	$\pm$52.5(12)	37.1	$\pm$ 6.6(12)	1.07	$\pm$0.18(6)

*Probability, control vs. experimental, $0.1 > P > 0.05$.

**Probability, control vs. experimental, $P < 0.05$.

***Probability, control vs. experimental, $P < 0.005$.

Fig. 7. Urine concentration of rats provided water as desired (control), deprived of water or given NaCl solution to drink. The left ordinate represents electrolyte concentration (mEq/l). The right ordinate represent osmolality (Osm/l). Each horizontal line represents the average response. The vertical lines on the center of each bar are the standard errors.

Fig. 8. Urine concentration of squirrels provided water as desired (control), deprived of water or given NaCl solution to drink. The left ordinate represents electrolyte concentration (mEq/l). The right ordinate represents osmolality (Osm/l). Each horizontal line represents the average response. The vertical lines on the center of each bar are the standard errors.

URINE CONCENTRATIONS AND OSMOLALITY

CONTROL WATER DEPRIVED NaCl DRINKING

URINE CONCENTRATIONS AND OSMOLALITY

CONTROL WATER DEPRIVED NaCl DRINKING
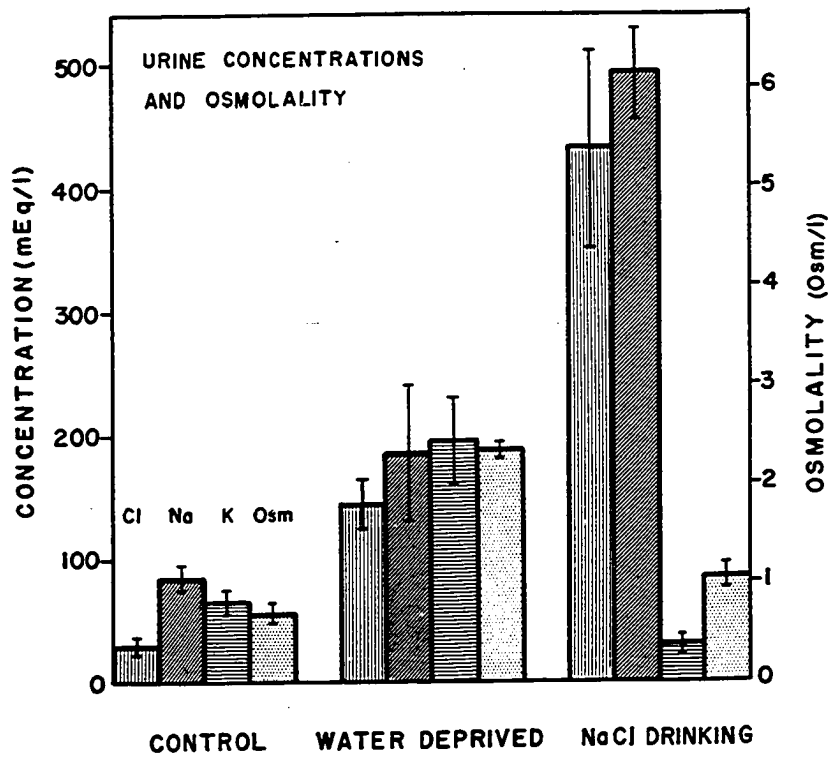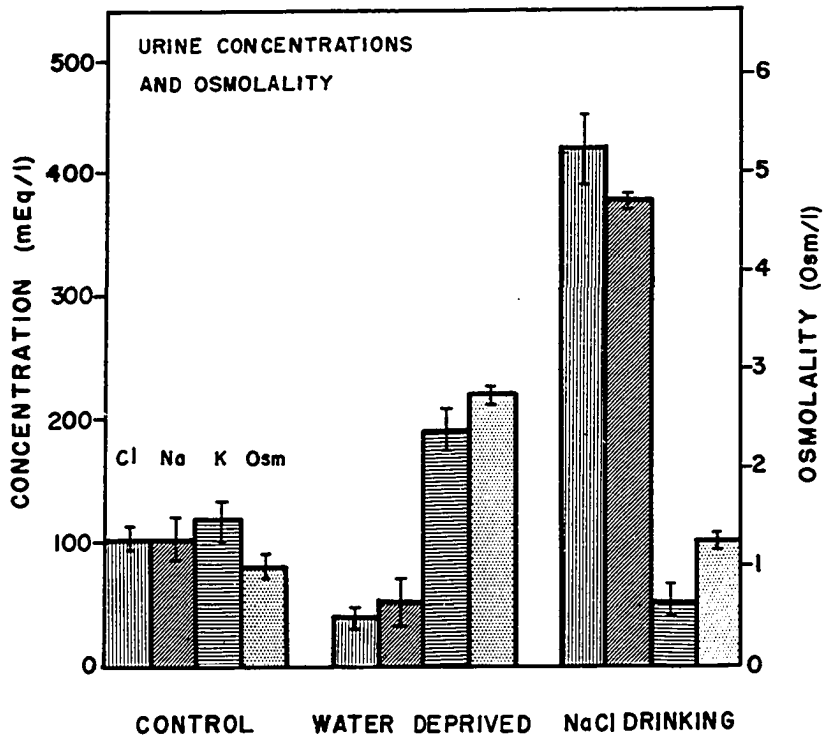
was significantly greater than control values. The highest

urine osmolalities obtained were 3.66 osmolal from a water

deprived rat and 3.25 osmolal from a water deprived squirrel.

The averages of urine chloride and sodium in NaCl

drinking rats were 300% and 250% greater than control values.

Potassium excretion decreased by 58%. Similarly squirrels

drinking NaCl solution excreted more sodium and chloride and

tended to decrease their potassium excretion; excretion of

sodium was 463% greater and chloride was 1138% more than in

controls.

Potassium excretion in squirrels tended to decrease

but not enough to be statistically significant (p > 0.1).

Osmolality of urine in rats and squirrels drinking NaCl solu-

tion tended to increase (Table 4, Figs. 7 and 8). The high-

est urine osmolalities obtained were 2.85 in the rat and

1.80 osmolal in the squirrel.   —

Body water. The average fat free body water in water

deprived rats was 4.9% lower than in the controls (Table 5,

Fig. 9). In water deprived squirrels, total body water de-

creased 4.0% and fat free body water decreased 3.9% (Table 5,

Fig. 10). Total body water increased in both rats and

squirrels when drinking solutions of NaCl (Table 5, Figs. 9

and 10). However, fat free body water decreased 3.3% in rats

43

TABLE 5

Total and fat free body water of rats and squirrels, provided water as desired (control), acutely deprived of water or given NaCl solution to drink. Expressed as percentage of total body weight and per cent of lean body weight. The mean ± S.E.(N) is given.

	Total Body Water %		Fat Free Body Water %	
RAT				
Control	66.39	±0.76(7)	73.49	±0.79(7)
Water Deprived	66.73	±2.23(5)	69.82**	±1.57(5)
NaCl Drinking	67.82***	±0.97(7)	71.04*	±0.73(7)
SQUIRRELS				
Control	39.56	±1.33(14)	73.08	±0.31(14)
Water Deprived	37.94**	±1.71(7)	70.19***	±0.23(7)
NaCl Drinking	43.33***	±1.76(7)	70.45***	±0.58(7)

*Probability, control vs. experimental, 0.1 > P > 0.05.

**Probability, control vs. experimental, P < 0.05.

***Probability, control vs. experimental, P < 0.005.

Fig. 9. Total and fat free body water, per cent of original body weight of rats provided water as desired (control), acutely deprived of water or given NaCl solution to drink. The ordinate represents water concentration (per cent original weight). Each horizontal line of the bar represents the average response, vertical lines on the center of each bar are the standard errors.

Fig. 10. Total and fat free body water, per cent of original body weight of squirrels provided water as desired (control), acutely deprived of water or given NaCl solution to drink. The ordinate represents water concentration (per cent original weight). Each horizontal line of the bar represents the average response, vertical lines on the center of each bar are the standard errors.

WATER CONTENT (% original weight)

0   40   50   60   70

CONTROL

WATER DEPRIVED   TOTAL

NaCl DRINKING

FAT FREE

WATER CONTENT(% original weight)

0   60   70   80

CONTROL

WATER DEPRIVED   TOTAL

NaCl DRINKING

FAT FREE
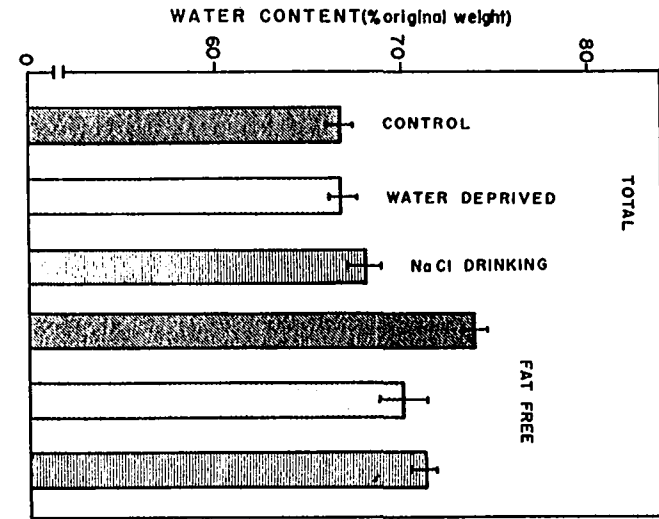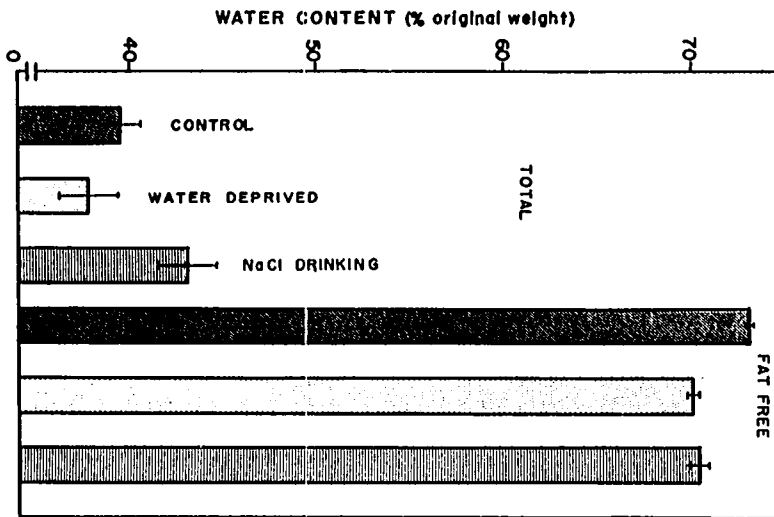
and 3.6% in squirrels as a result of drinking NaCl solutions
(Table 5, Figs. 9 and 10).

DISCUSSION

Water Restriction and NaCl Drinking
in Squirrels

This part of the study was conducted to determine
the degree to which these animals can drink salt solutions
and restricted quantities of drinking water and remain in
water balance. From the stepwise shape of the weight curves
(Figs. 2 and 3) it was concluded that the squirrels, al-
though their weight was reduced, were able to tolerate and
remain in water balance while drinking NaCl solutions as
concentrated as 0.4N or while receiving as little as 5% of
the body weight per day in water (i.e., cucumbers). This
indicated that the species had the ability to adjust water
balance. The data on urine osmolality show that the squir-
rels can produce fairly concentrated urine (3.8 osmolal).

Volume and Osmotic Regulation in
Squirrels and Rats

The major purpose of this experiment was to determine
how these rodent species regulate fluid volume as a function
of dehydration. Both species were exposed to two distinct

types of dehydration: dehydration by water deprivation and
dehydration by drinking 0.4N NaCl solution. The results of
these experiments show that these animals responded differ-
ently to the two types of dehydration. In fact, about the
only similarity between the two conditions is that implied
by the term "dehydration," that is, the animals lost water.
Dehydration resulting from water deprivation was character-
ized by a reduction in plasma volume, subsequently a dis-
turbance in circulation. Dehydration resulting from drink-
ing NaCl solution resulted in no change in plasma volume,
but an exchange of water from the intracellular and/or
interstitial spaces into the plasma volume.

Plasma volume and electrolytes. The results clearly
show that a deficit of water caused by water deprivation in
rats and squirrels was accompanied by a decrease in plasma
volume and an increased concentration of per cent red blood
cells, sodium and chloride. Generally these responses are
similar to those of the water deprived donkey (Dill, 1938),
dogs (Elkinton and Taffel, 1942; Kanter, 1954), man (Adolph,
1947), camel (Schmidt-Nielsen and Schmidt-Nielsen, 1952),
Merino sheep (Macfarlane et al., 1961), and steers (Bianca
et al., 1965). The rats were more sensitive to water depri-
vation than squirrels as evidenced by the greater changes in

plasma; water deprivation resulted in a more pronounced decrease in plasma volume and increase of per cent red blood cells in rats than in squirrels.

The various plasma constituents did not respond uniformly to water deprivation, possibly indicating independent regulation. Varying increases in the concentration of blood constituents in response to water deprivation have been found by Macfarlane et al. (1961) in Merino sheep, and Bianca et al. (1965) in steers. The per cent red blood cells, chloride, and sodium concentrations of plasma increased in water deprived rats as the consequence of the smaller plasma volume. The rise in the per cent red blood cells amounted to 20% and most probably represents relative increases brought about by loss of water from the plasma. The rise in the per cent red blood cells and the tendency of sodium and chloride to increase in the plasma of water deprived squirrels indicates water loss from the plasma. Differently from water deprived rats, the per cent red blood cells in water deprived squirrels increased less than would be expected from the loss of volume from the plasma. Since chloride is ubiquitous in the body fluids (Schmidt-Nielsen, 1964), the increase in the concentration of this ion in water deprived rats, and the tendency of this ion to increase in water deprived squirrels

suggests a water loss from all compartments, and an increase in ionic concentrations throughout the body fluids. Bianca et al. (1965) speculated that the increase of sodium and chloride and the decrease in volume of the plasma result in its hypertonicity. This hypertonicity may help to hold water and maintain circulating blood volume (Chew, 1965).

Water deprivation had no effect on the plasma potassium concentration; however, squirrels had greater plasma potassium concentrations (9.4mEq/l) than rats (3.3mEq/l). This could be due, in part, to the fact that at the time of measurement they may have been in a state preparatory for hibernation. Trusler et al, as cited by Kayser (1953) found an increase in plasma potassium in comparison with post-hibernation values in the hedgehog. Similarly, water deprivation without an increase in plasma potassium was found in man (Winkler et al., 1944a; Black et al., 1944), rats (Heller, 1949), dogs (Kanter, 1954), sheep (Macfarlane, 1961) and steers (Bianca et al., 1965). In steers, the effect of reduced dietary potassium seems to have outweighed the concentrating effect of water loss from the plasma (Bianca et al., 1965). Alternatively, Kanter (1954) explained the constancy of plasma potassium in dogs by the increased urinary loss which was found in these animals when they were deprived

of water. Either of these alternative explanations could account for the constancy of potassium observed in the present study.

Despite the decline of plasma volume in water deprived rats and squirrels, protein concentration did not increase. Such a response has been observed in man (Marriott, 1923; Black et al., 1944) donkey (Dill, 1938; Schmidt-Neilsen and Schmidt-Nielsen, 1952), rats and dogs (Wolf, 1958). Marriott (1923) reported that high concentrations of protein are usually found only when dehydration is sudden. When dehydration lasts for several days, a decrease in serum protein concentration occurs even though body weight and blood volume measurements indicate further loss of water. Some explanations may be advanced for this phenomenon (constancy of protein):

1. The possibility that the capillary wall is unusually permeable to protein is discussed by Dill (1938), who suggested that in a dehydrated donkey the protein passes from the capillary system into the interstitial space.

2. An argument against the assumed permeability of capillary membrane to protein is offered by Schmidt-Nielsen (1964) who suggested that

dehydration increases plasma protein which draws
water from interstitial space into the plasma,
thus maintaining protein concentration.

3. During water deprivation, reduced food intake
   may outweigh the concentrating effect of water
   loss from the plasma. Adolph (1947a) showed
   that food intake decreased during the course of
   water deprivation in the rat and he concluded
   that food intake in the absence of water was so
   small that it could add nothing to the net con-
   tent of the body as long as water was not
   available.

Of these possibilities, it appears that (3), the de-
creased dietary intake and subsequently the increased endog-
enous protein catabolism, is most responsible for the con-
stancy of plasma protein. Black et al. (1944) showed that
dehydration in man increased the amount of urea produced by
the body. Thus, as is seen in this study, determinations of
plasma protein may fail to indicate accurately the degree of
dehydration. The measurement of plasma volume, taken to-
gether with determinations of ionic concentrations and per
cent red cells, supplies more adequate data for the estima-
tion of the degree of dehydration.

In contrast to water deprivation, drinking NaCl
solution failed to produce significant changes of the plasma
volume in rats and squirrels. These rodents responded to
drinking NaCl solutions in much the same manner as dogs
(Winkler et al., 1944b). The procedure resulted in consider-
able addition of NaCl to the body with diminution of the
total water content. The loss of fat free body water most
clearly makes demands on the extracellular fluid. However,
not only were the normal per cent of red blood cells, protein,
and potassium concentrations maintained, but the plasma vol-
ume was also unaffected. In view of the fat free water loss,
the avoidance of haemoconcentration could only have been
achieved by a shift of intracellular water towards the plasma.
Similarly, Winkler et al. (1944b) demonstrated a depletion
of total body water, an expansion of extracellular fluid
volume and a decrease of intracellular fluid in dogs receiv-
ing hypertonic (5%) injection of saline.

Urine concentrations. Surprisingly, the urine of
rats dehydrated by water deprivation was not hypertonic with
respect to sodium and chloride in spite of increased plasma
chloride and sodium concentrations. Excretion of chloride
and sodium decreased while that of potassium was increased.
In sheep (Macfarlane et al., 1961) the opposite occurred.

When sheep were deprived of water, urinary potassium and urinary sodium decreased. However, the retention of sodium and chloride and enhanced excretion of potassium was found in dogs (Elkinton and Taffel, 1942; Kanter, 1954), rats (Heller, 1949), and man (Black et al., 1944). In water deprived rats, sodium and chloride must have been almost completely reabsorbed in the kidneys despite the increasingly high concentration of these substances in the plasma, a phenomenon similar to that noted by Kerpel-Fronius (1935).

Water deprived squirrels differed from water deprived rats by increasing sodium and chloride excretion and potassium excretion. Sufficient electrolytes were excreted to prevent a rise in concentration of the plasma. Here renal function was the major determinant of plasma composition.

Increased urinary potassium could be related to a mobilization of intracellular potassium (Kanter, 1954) which was described by Elkinton et al. (1948). Kanter (1954) suggested that the loss of intracellular potassium may be a compensatory adjustment of cellular osmoequilibrium which is produced in dehydration. Similarly, a decrease of cellular potassium without an increase in plasma potassium was demonstrated by Elkinton et al. (1948) in adult dogs deprived of

water. The preferential excretion of potassium in dogs was noted and discussed by Elkinton and Taffel (1942). These authors pointed out that by releasing intracellular potassium, water is withdrawn from the intracellular compartment, so that the extracellular phase is to the same extent preserved. Black et al. (1944) indicated that, in addition to that cellular water loss which corresponded to potassium excretion, some of the cellular water loss also corresponds to protein breakdown, but cellular water loss is more rapid than potassium and protein loss and this causes cellular dehydration.

When rats and squirrels were given 0.4N NaCl solution, highly concentrated urine was formed. In rats drinking NaCl solution, the average urine chloride and sodium were 300 and 250% greater than the control values. In squirrels drinking NaCl solution, the excretion of sodium was 463% greater than in the controls, and that of chloride was 1138% more than the control values. How do squirrels drinking NaCl solution differ in their response from rats? The data show that the ability of squirrel kidneys to excrete electrolytes is greater than that of rats. A high concentration of salts in the urine of the squirrels drinking NaCl solution not only permits the squirrel to expend moderate amounts of water for excretion, but also permits it to keep the plasma

concentration of sodium constant. The ability of the kidney
of the rat and squirrel to form osmotically concentrated
urine proved to be greater than that of man and camel. The
maximum urine osmolality observed for these species was 3.66
Osm. for rats, 3.8 Osm. for the squirrel, 2.74 for the camel
and 1.43 for man (Schmidt-Nielsen, 1964). But these
osmolalities were lower than osmolality reported for kangaroo
rats (5.5 Osm.) and the jerboa, _Jaculus jaculus_ (6.5 Osm.)
(Schmidt-Nielsen, 1964).

In rats and squirrels drinking 0.4N NaCl solution
the osmolality of the urine did not increase particularly.
The average urine osmolality of rats and squirrels which re-
ceived NaCl solutions was 1.23 and 1.08 respectively. These
low osmotic concentrations could be due to osmotic diuresis.
This is in agreement with Adolph's work (1943) on rats given
NaCl solutions. He showed that the factors which guide the
amount of fluid ingestion are related to the concentration
of the drinking salt solution. Adolph showed that when rats
were given solutions of 0.37N NaCl, it was drunk in amounts
greater than distilled water, sufficient to furnish the usual
available water and to form urine sufficient to excrete the
ingested salt.

Body water. Table 5 shows that there are no

differences among the fat free water contents of the normal
(control) laboratory rats (73.49%) and squirrels (73.08%) and
those values that Chew (1965) presented for white rats
(73.3%, 73.7%).

Dehydration caused by water deprivation or drinking
NaCl solution resulted in a significant reduction in water
content of the fat free weight of rats and squirrels. In
rats and squirrels deprived of water, the average fat free
water decreased 4.9% and 3.9% respectively. Similarly, when
rats and squirrels were drinking NaCl solution they lost
3.3% and 3.6% of their fat free water, respectively. The
average fat free water of rats and squirrels deprived of
water was 69.82% and 70.19%. In animals drinking NaCl solu-
tion these values were 71.04% for rats and 70.45% for squir-
rels. These data show that although the animals were in a
negative water balance, and a considerable amount of water
was lost, the proportion of the water in the body did not
change remarkably. Schmidt-Nielsen and Schmidt-Nielsen
(1948) showed that kangaroo rats given dry diets for various
lengths of time (14-25 days) have about the same percentage
of body water as normal animals.

Tolerance to dehydration. The ability of the squir-
rels to tolerate water deprivation proved to be much greater

than that of the rats. Tolerance obviously corresponds to the rate of water loss. The rate of water loss in squirrels was shown to be much slower than that in rats. Rats lost an average of 4.9% of their fat free water in an average of 5 days acute water deprivation, whereas squirrels lost only 3.9% of their fat free water in an average of 23 days. Similarly, when these animals were receiving NaCl solution to drink, the rats lost 3.3% of the fat free water in an average of 10 days, whereas the squirrels lost only 3.6% of their fat free water in an average of 29 days.

Whether the differences between the rats and the squirrels is due to better adaptation to dry conditions or to physiological adjustments to hibernation cannot be evaluated at this time. In my judgment, both factors are involved. Squirrels, which dwell in environments where water is not always available, may have evolved means to enable them to resist water deprivation (Macfarlane et al., 1961). Also, since squirrels hibernate, there may be a related physiological adaptation to water deprivation (negative water balance). Hibernation involves extended periods of time with minimal or no exchange between the animal and environment in terms of nutrients and waste. Thus, regarding the functional capacity of tissues and maintenance of homeostasis,
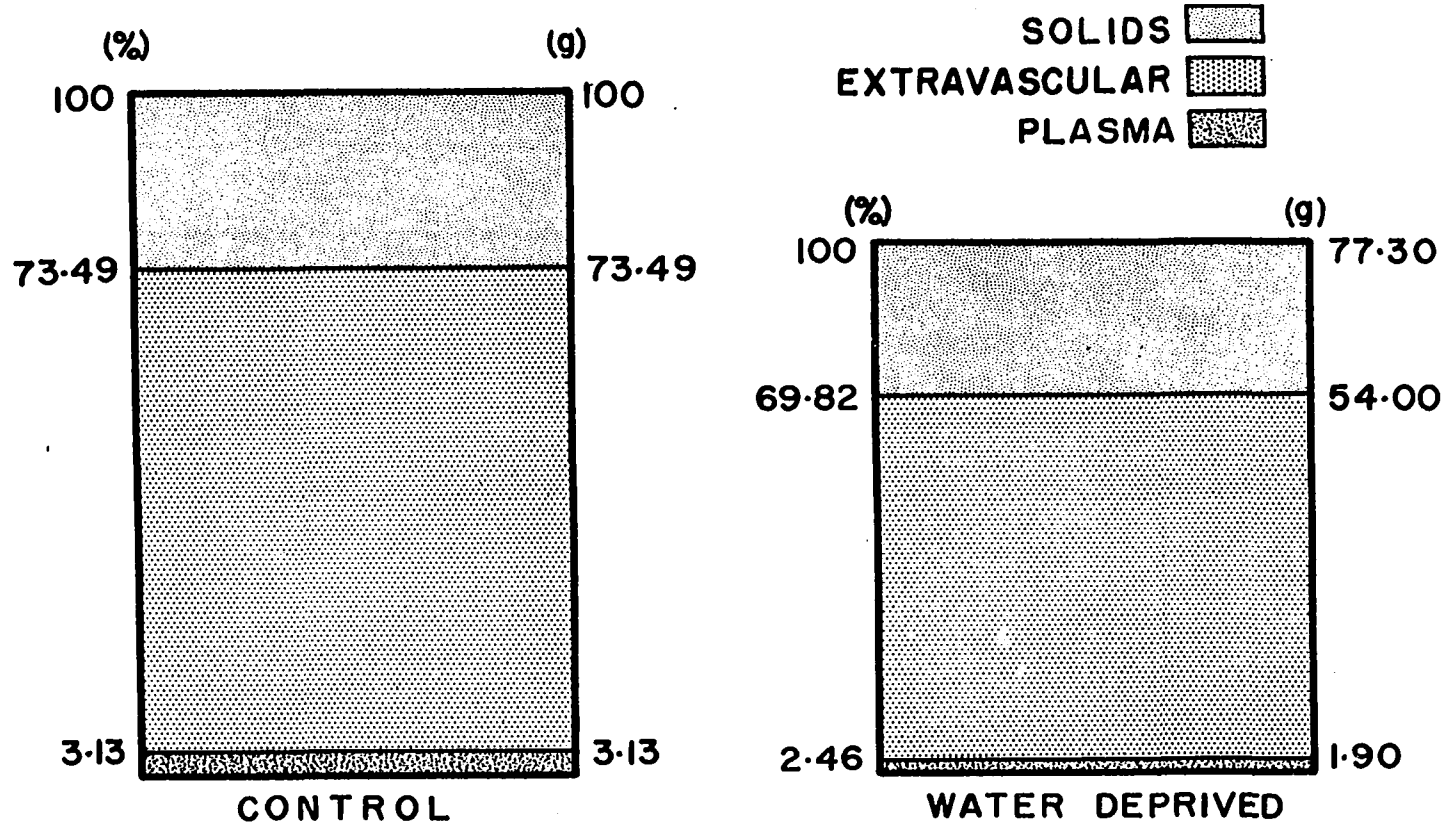
the situation of removing drinking water is similar to hibernation (Bintz and Riedesel, 1967).

Water exchange. In water deprived rats and squirrels the average weight loss at the end of experimentation was 22.7%, but the decrease in average fat free body water was only 4.9% and 3.9%, respectively. However, the following quantitative analysis of total water shows that considerable amounts of water were lost; therefore, the total water loss is of more concern than the relative changes in fat free water content.

The computation utilizes the per cent changes in body weight, fat free water content and plasma volume (Table 6, Fig. 11). The data from the water deprived rats serve for explanation of the computation. The animals lost an average of 22.7% of their original weight; thus, a hypothetical rat weighing 100 g at the beginning of the experiment would weigh 77.3 g after water deprivation. The fat free water content would be 73.49 g at the beginning and 54.00 g at the end for a water loss of 19.49 g. The plasma volume would be 3.13 g at the beginning and 1.90 g at the end for a loss of 1.23 g. The percentage decrease in the volume -of the total fluid compartment was 26.5% and in the plasma volume 39.3%. The plasma, therefore, carried more than its

Fig. 11. A diagram of the body compartments of rats provided water as desired (control), or acutely deprived of water (water deprived). The left side of the rectangle shows the values as the percentage and the right side shows the values as absolute amounts.

LABORATORY RATS

SOLIDS

EXTRAVASCULAR

PLASMA

(%)   (g)
100   100
73·49   73·49
3·13   3·13
CONTROL

(%)   (g)
100   77·30
69·82   54·00
2·46   1·90
WATER DEPRIVED

$$\frac{\text{PLASMA FLUID LOSS}}{\text{TOTAL FLUID LOSS}} = 1.5$$

proportional share in the total water loss, and the calculation shows that the rats which were deprived of water had a reduction in circulatory plasma volume which amounted to 1.5 times that expected if plasma water had been lost in proportion to the loss of the whole body. Similar analysis in squirrels deprived of water showed that the plasma carried 1.4 times more than its proportional share in the total water loss (Fig. 11). The results of the quantitative analysis of plasma and total water loss in rats and squirrels deprived of water showed that these animals responded to the withdrawal of water with a change in the fluid compartments intermediate between man and donkey. In the dehydrated man, plasma water contributed 2.5 times more than its proportional share to total water loss (Adolph, 1947), whereas in a dehydrated donkey water is lost proportionally from all fluid compartments (Dill, 1938). As mentioned above, for the rats and squirrels deprived of water, the plasma volume lost proportionally 1.5 and 1.4 times more water than the other fluid spaces.

TABLE 6

The distribution of water loss in various mammals deprived
of water.

Species	% of original body water loss	% of original plasma volume loss	Ratio
Camel (Schmidt-Nielsen, 1964)	26.0	8.8	0.3
Donkey (Dill, 1938)	12.4	12.0	1.0
Squirrel	25.7	36.0	1.4
Rat	26.5	39.3	1.5
Man (Adolph, 1947)	--	--	2.5

SUMMARY

1. The study consisted of two subdivisions: (a) An experiment in which the degree to which the thirteen-lined ground squirrel (Citellus tridecemlineatus) can drink NaCl solutions or utilize restricted quantities of drinking water was tested; (b) A comparative experiment showing how laboratory rats (Rattus norvegicus) and ground squirrels regulate fluid volume as a function of dehydration. Henceforth squirrel will refer to thirteen-lined ground squirrel and rat will refer to laboratory rats.

2. Plasma volume was determined by a dye dilution method. The dye used was T-1824, utilizing multiple sampling and extrapolation to zero time. A carotid catheter permitted blood sampling and dye injection. The percentage of red cells was determined. Plasma protein was measured by an ultramicro adaptation of the biuret method. Total and fat free water content was determined by desiccation and extraction.

3. It was found that squirrels, although their weight was reduced, were able to remain in water balance while

drinking NaCl solutions as concentrated as 0.4N or while receiving as little water (i.e., cucumber) as 5% of the body weight per day. The stepwise shape of the weight curves as a result of drinking NaCl solutions or receiving restricted quantities of drinking water (Figs. 2 and 3) indicated that the species had the ability to adjust the water balance. Data on urine osmolality show that squirrels can produce fairly concentrated urine; a maximum urine osmolality of 3.8 was produced by a water deprived squirrel.

4. In the second part of this study both species, rat and squirrel, were exposed to two distinct types of dehydration: dehydration by water deprivation and dehydration by drinking 0.4N NaCl solution. Food was available as desired. Treatment was continued until there was a loss of 20-25% of the original body weight. The animals responded differently to the two types of dehydration.

5. Dehydration resulting from water deprivation was characterized by a reduction in plasma volume; in contrast, dehydration resulting from drinking 0.4N NaCl solution failed to produce significant changes of plasma volume.

6. Per cent red blood cells, chloride, and sodium concentrations of plasma increased in water deprived rats as

the consequence of the smaller plasma volume. An increase in the per cent red blood cells, the tendency of sodium and chloride to increase in the plasma of water deprived squirrels also resulted from the plasma water loss. Water deprivation resulted in a more pronounced decrease in plasma volume and increased per cent of red blood cells in rats than in squirrels.

7. Water deprivation had no effect on plasma protein and potassium concentrations. However, squirrels had a greater plasma potassium concentration (9.4mEq/1) than rats (3.3mEq/1). Possible explanations accounting for the constancy of potassium and protein are suggested.

8. The rise in the per cent red blood cells in rats amounted to 20% control values and most probably represented relative changes brought about by loss of water from the plasma volume. Differently from water deprived rats, the per cent red blood cells in water deprived squirrels increased less than would be expected from the loss of volume of plasma.

9. Since chloride is ubiquitous in the body fluids, the increase in the concentration of this ion (10% above control) in water deprived rats and the tendency of this ion to increase (9% above control) in water deprived

squirrels suggests a water loss from all compartments, and an increase in ionic concentrations throughout the body fluids. The significance of this in the maintenance of circulatory volume is discussed.

10. Dehydration resulting from drinking 0.4N NaCl solution in rats and squirrels did not decrease the plasma volume and consequently the per cent red blood cells, protein and potassium concentration were unaffected. The procedure resulted in a considerable addition of NaCl to the body with diminution of the total water content. In view of the fat free water loss, the avoidance of haemo-concentration may have been achieved by a shift of intra-cellular water towards the plasma.

11. The urine of rats dehydrated by water deprivation was not hypertonic with respect to sodium and chloride in spite of increased plasma concentrations of chloride and sodium. The average ratio of chloride and sodium concentration in urine and plasma (U/P) of water deprived rats was 0.3 and 0.2 respectively. Water deprived squirrels differed from water deprived rats by increasing sodium and chloride excretion and potassium excretion. The average ratio of chloride, sodium, and potassium concentration in urine and plasma (U/P) was 1.2, 1.1 and 23.2

respectively.

Sufficient electrolytes were excreted in water deprived
squirrels to prevent a rise in concentration of these
electrolytes in plasma. Urine chloride, sodium and
potassium were 304%, 116% and 166% above controls, re-
spectively. The osmotic concentrations of urine from
water deprived rats (3.66 osmolal) and squirrels (3.8
osmolal) exceeded those found in the camel.

12. The ability of the kidney of squirrels drinking NaCl
solution to excrete electrolytes was greater than that
of the rats. The average urine chloride and sodium in
NaCl drinking rats were 300% and 250% greater than con-
trol values, whereas these values were 1138% and 463% in
squirrels. The high concentration of sodium in the
urine of squirrels drinking NaCl solution permits the
animal to expend moderate amounts of water for excretion,
but also permits it to keep the plasma concentration of
sodium constant. In rats and squirrels drinking NaCl
solution the osmotic concentration of the urine did not
increase particularly.

13. The two types of dehydration resulted in a significant
reduction in water content of the fat free weight of rats
and squirrels. Although the animals were in negative

water balance, and considerable amounts of water were
lost, the proportion of water in the body did not change
remarkably; rats lost 4.9% in acute water deprivation
and 3.3% after NaCl drinking; water deprived squirrels
lost 3.9% and 3.6° after NaCl drinking. Since the rate
of water loss was much less in squirrels than in rats,
the ability of squirrels to tolerate water deprivation
was greater than that of the rats. Ecological and physi-
ological significance is discussed.

14. The quantitative analysis of the distribution of water
loss in rats and squirrels showed that the plasma volume
lost 1.5 and 1.4 times more fluid than the cellular and
interstitial spaces. These values show that rats and
squirrels responded to withdrawal of water with a change
in the fluid compartments intermediate between those of
man and donkey.

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