1971

Study of renal function in the hibernating ground squirrel (Citellus columbianus)

Richard M. Moy
The University of Montana

A STUDY OF RENAL FUNCTION IN THE HIBERNATING GROUND SQUIRREL, (CITELLUS COLUMBIANUS)

by

Richard Marshall Moy

B.A., University of Montana, 1966

Presented in partial fulfillment of the requirements
for the degree of Master of Arts

UNIVERSITY OF MONTANA

1971

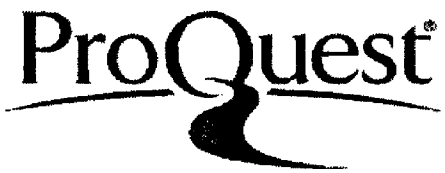
Approved by:

Chairman, Board of Examiners

Dean, Graduate School

May 10, 1971

Date



ACKNOWLEDGMENTS

I wish to thank Dr. E. W. Pfeiffer for his guidance, assistance and tolerance he gave throughout this study. I am grateful to Dr. James R. Templeton for his helpful suggestions in preparation of this thesis.

I am also indebted to Drs. Pfeiffer, Templeton and L. G. Browman for reading the manuscript. I would also like to give special thanks to Roger Gambs for his guidance on statistics and Ivan Johnson for his help in preparation of the manuscript.

This study was supported by a grant from the National Institutes of Health (to Dr. Pfeiffer).

TABLE OF CONTENTS

Page

INTRODUCTION - 1

MATERIALS AND METHODS- - - - - - - - - - - - - - - - - - - -12
Animals -12
Procedure -12
(1) Hibernating Animals - - - - - - - - - - - - -12
(2) Arousing Animals- - - - - - - - - - - - - - -13
(3) Dehydrated Animals- - - - - - - - - - - - - -14
Tissue Slice Analysis - - - - - - - - - - - - - - -15
Statistical Analysis- - - - - - - - - - - - - - - -20

RESULTS
I. Urea Concentrations - - - - - - - - - - - - - -21
(a) Renal Tissue Slices- - - - - - - - - - - -21
(b) Urine- - - - - - - - - - - - - - - - - - -23
(c) Plasma - - - - - - - - - - - - - - - - - -25
II. Sodium Concentrations - - - - - - - - - - - -25
(a) Renal Tissue Slices- - - - - - - - - - - -25
(b) Urine- - - - - - - - - - - - - - - - - - -27
(c) Plasma - - - - - - - - - - - - - - - - - -27
III. Potassium Concentrations- - - - - - - - - - -28
(a) Renal Tissue Slices- - - - - - - - - - - -28
(b) Urine- - - - - - - - - - - - - - - - - - -28
(c) Plasma - - - - - - - - - - - - - - - - - -28
IV. Ammonia Concentrations- - - - - - - - - - - -29
V. Osmolalities- - - - - - - - - - - - - - - - -29
VI. Temperature vs. Time in Arousing Squirrels- - - - - - - - - - - - - - - -29
VII. Papillary Sodium-Urea Relationships in Arousing Squirrels- - - - - - - - -30

DISCUSSION -76

SUMMARY- -83

LITERATURE CITED -84

LIST OF FIGURES

Page

Figure 1a--Lateral view of the kidney- - - - - - - - -18
1b--Cross section of the kidney - - - - - - - - -18
1c--Parasagittal view of the kidney - - - - - -18

Figure 2--Scatter diagram of renal urea concentrations from hibernating and dehydrated squirrels- - - - - - - - - - - - - - - - - - -35

Figure 3--Scatter diagram of renal urea concentrations from arousing urine formers and arousing nonurine formers - - - - - - - - - - - - - - - -41

Figure 4--Papillary TF/P and U/P urea ratios and rectal temperatures from arousing urine formers- -45

Figure 5--Papillary/urine urea ratios from arousing and dehydrated squirrels - - - - - - - - - -47

Figure 6--Urea, sodium and potassium concentrations in plasma of hibernators, arousers, and dehydrated squirrels - - - - - - - - - - - -49

Figure 7--Scatter diagram of renal sodium concentrations from hibernating and dehydrated squirrels- - - - - - - - - - - - - - - - - - -54

Figure 8--Scatter diagram of renal sodium concentrations from arousing urine formers and arousing nonurine formers- - - - - - - - - -60

Figure 9--Scatter diagram of renal potassium concentrations in hibernating and dehydrated squirrels- - - - - - - - - - - - - - - - - -64

Figure 10--Scatter diagram of renal potassium concentrations in arousing urine formers and arousing nonurine formers- - - - - - - - - -66

Figure 11--Time vs. temperature in arousing squirrels- - - - - - - - - - - - - - - - - -73

Figure 12--Relationship of sodium and urea concentrations in the right and left kidneys of arousing squirrels - - - - - - - - - - - - -75

LIST OF TABLES

TABLE Page

1. Urea Concentrations in Tissue Slices, Plasma, and Urine from Dehydrated Squirrels - - - - - - -32

2. Urea Concentrations in Tissue Slices, Plasma, and Urine from Hibernating Squirrels- - - - - - -33

3. Urea U/P TF/P, and Muscle/P Ratios from Hibernating Squirrels - - - - - - - - - - - - - - -36

4. Urea U/P, TF/P, Muscle/P, and Papillary/U Ratios from Dehydrated Squirrels - - - - - - - - - - - - -37

5. Urea Concentrations in Tissue Slices, Plasma and Urine from Arousing Squirrels (Nonurine Formers)- - - - - - - - - - - - - - - - -38

6. Urea Concentrations in Tissue Slices, Plasma, and Catheter Urine from Arousing Squirrels (Urine Formers) - - - - - - - - - - - - - - - - - -39

7. Urea U/P, TF/P and Muscle/P Ratios in Arousing Squirrels (Nonurine Formers)- - - - - - - - - - -42

8. Urea U/P, TF/P, Muscle/P, and Papillary/U Ratios from Arousing Squirrels (Urine Formers) - - - - -43

9. Urea, Sodium, and Potassium Concentrations in Kidney Tissue Slices, Plasma, and Urine from One Squirrel Entering Hibernation - - - - - - - -50

10. Sodium Concentrations in Renal Tissue Slices, Plasma, and Urine from Hibernating Squirrels -51

11. Sodium Concentrations in Renal Tissue Slices, Plasma, and Urine from Dehydrated Squirrels - - -52

12. Sodium TF/M and P/M Ratios from Hibernating Squirrels -55

13. Sodium TF/M and P/M Ratios from Dehydrated Squirrels -56

LIST OF TABLES (Continued)

TABLE Page

14. Sodium Concentrations in Tissue Slices, Plasma and Urine from Arousing Squirrels (Urine Formers) - - - - - - - - - - - - - - - - - -57

15. Sodium Concentrations in Tissue Slices, Plasma and Urine from Arousing Squirrels (Nonurine Formers)- - - - - - - - - - - - - - - - - -58

16. Sodium TF/M, P/M and Catheter Urine/M Ratios from Arousing Squirrels (Nonurine Formers) - - - - - -61

17. Sodium TF/M, Urine/M and P/M Ratios from Arousing Squirrels (Nonurine Formers) - - - - - -62

18. Potassium Concentrations in Tissue Slices, Plasma and Urine from all Four Groups - - - - - -67

19. Urine Sodium, Potassium, and Urea Concentrations and Urine Osmolality in Dehydrated Squirrels -71

INTRODUCTION

The Columbian ground squirrel (Citellus columbianus), like all mammals during stressful environmental conditions of water shortage, must be able to produce a urine hypertonic to its body fluid to maintain an internal osmotic balance. However, for six months of the year the ground squirrel differs from normal homeothermic mammals by lowering its body temperature, becoming torpid, and hibernating; but during this inactive dormancy the ground squirrel must still maintain its internal environment. Fisher and Manery (1965) recently reported, "the literature available regarding excretion and the role of the kidney is conflicting and generally inadequate for a comprehensive interpretation of kidney function during hibernation." The physiological mechanism by which the squirrel concentrates tubular fluid during active homeothermy must be reviewed before considering renal function during hibernation.

Hargitay and Kuhn (1951) postulated that urine is concentrated via a countercurrent multiplier mechanism where the loop of Henle acts as the countercurrent multiplier system which creates a progressive increase in osmotic concentration from the medulla to the papilla. Wirz et al. (1951) using a tissue slice technique showed that urea and sodium were responsible for this gradient. Wirz also

demonstrated (1953) that the osmotic concentration of blood at the tip of the papilla was as concentrated as the urine. Berliner et al. (1958) proposed that the vasa recta parallel the loop of Henle and act as a countercurrent diffusion exchange.

The mechanism by which urea is concentrated in the medulla is not completely understood. The current theory asserts that urea movement is passive, following a diffusion gradient (Berliner, 1958; Levinsky et al., 1959; and Lassiter, 1961), and that urea entering the collecting ducts has a concentration many times higher than the glomerular filtrate or plasma. Active transport of sodium out of the ascending loop of Henle pulls urea passively into the medullary interstitial space creating an urea gradient from the cortex to papilla (Bottschalk and Mylle, 1959).

Studies by Schmidt-Nielsen (1958) on kangaroo rats and white rats, camels (1967), and sheep (1959) demonstrated that the clearance of urea varies with the protein content of the diet. In camels and sheep, the change cannot be related to the changes in glomerular filtration rate, plasma urea concentration or osmotic load, suggesting that the excretion of urea is regulated at the tubular level. Schmidt-Nielsen (1959) postulated that the urea is actively transported either out of the loop of Henle or out of the collecting ducts. Bray and Preston (1961) and Truniger and Schmidt-Nielsen (1964) agreed that the mammalian collecting ducts might actively reabsorb urea.

Clapp (1966) found that rats fed low-protein diets, while in mannitol diuresis, would increase their inulin TF/P ratio (ratio of the inulin in the renal tubular fluid to the inulin in the plasma) in the collecting ducts, while the urea TF/P ratio is dropping. Goldberg et al. (1966-1967) obliterated the normal gradient of electrolytes from the kidney, causing a decrease in the reabsorption of free water from the collecting ducts. However, the cortio-medullary gradient persisted and urea was found to be more concentrated in the papilla than in the urine, suggesting active transport of urea.

No information is known as to whether active transport of urea occurs or how the countercurrent multiplier and exchange systems function in hibernating animals.

Arousing from hibernation in the spring, Citellus columbianus does not normally drink water, but relies upon the succulent vegetation for moisture (Shaw, 1925e). The ground squirrel feeds almost exclusively on green vegetation, such as butter cup blossoms, blue grass (Poa), alfalfa, Balsam root (Balsamorhiza), and grain. During August and early September the Columbian ground squirrel changes to a drier but more fat-providing diet, eating seeds, oats, wheat, and wild lettuce (Lactuna). Body weight rapidly increases. Hoffman (1964) postulated that this diet is a necessary prerequisite for successful hibernation as it accumulates body fats. However, on this drier diet the

ground squirrel without access to drinking water must still be able to conserve body water. In late August or early September, the Columbian ground squirrel enters hibernation.

The ground squirrel's remarkable ability to maintain osmotic equilibrium during the season of dormancy (204 days to 220 days for males and females, respectively) has fascinated many investigators. Kayser (1961), Beers and Richard (1956), and Pengelly and Fisher (1961), studied changes in body weight of hibernators through the hibernating season. Kayser (1965) reported that loss of body weight is dependent on the number of arousals and length of awake periods. Kayser (1962) pointed out that a single arousal and period of wakefulness consumes more heat and energy than many days in hibernation and that during the arousal periods 90 percent of the total heat production and metabolic weight loss occurs with only 10 percent occurring during torpor. Kayser (1952) and Pengelley and Fisher (1961) suggested that this weight loss is primarily a loss of water formed by the oxidation of the stored fat. Oxidation of 1.00 gram of fat yields 1.08 grams of water. The retention of this water would cause a lethal dilution of the body fluids. Therefore, these data suggest that a hibernating squirrel must micturate during an arousal. This has been confirmed in many studies (Passmore, 1967 and Fisher and Manery, 1965). Metabolism during torpor produces very little water from fat oxidation and most of this can be lost by evaporation from the

respiratory surfaces.

Nonprotein nitrogenous material (NPN) is excreted as an end product of metabolism. Benedict and Lee (1938) reported that a 1500 gram marmot in deep hibernation excretes an average of about 7 mg NPN per week. Pengelley and Fisher (1961) indicated that 9.8 mg of NPN would be produced over a 14-day period in a 270 gram ground squirrel and if not excreted would equal 0.70 mg per day and would progressively increase in the blood. Passmore (1967) found an increase of 0.09 mMoles of body urea per day in hibernating Citellus comumbianus. He also reported that the hibernating cycle (between arousals) varied from 7 to 14 days. Earlier, Klar (1938) showed that the NPN rises between arousals. Popovic (1955) observed that the blood urea of rats rose from 48 to 70 mg percent during 14 hours of hypothermia. Stefanovic (1954) reported similar findings. Kristofferson (1963) found a marked increase in serum urea level during the hibernating cycle of hedgehog, (Erinaceus europaeus). His data indicated that at the low metabolic rate found in deep torpor, metabolic end products, mainly urea, accumulates in the blood stream. Kristofferson reasoned that the increased urea level in hibernating animals depends on the marked diminished ability of the kidney to remove contaminating metabolites of nitrogen from the body.

Urea is known to be toxic to most mammals in high concentrations. Fisher (1964) reported that 75 mg of urea

injected into a ground squirrel (Citellus lateralis) in deep hypoeuthermic torpor usually results in arousal within 24 hours. Fisher and Manery (1967) concluded that an increase in NPN would be a powerful natural stimulus to initiate arousal and to cause diuresis in the hibernator. Furthermore, Kayser (1966) reported that urea is the principle end product of NPN metabolism in the marmot. Passmore (1968) reported similar findings in Citellus columbianus.

Hong (1957) reported that urine production in torpid ground squirrels is drastically reduced. However, he pooled data from four hibernating ground squirrels and from eight artifically cooled controls and unfortunately made no distinction between the two groups. Hong concluded that urine flow in hibernating mammals was 0.0012 percent of the body weight per hour. Using this value, the calculated urine flow of a 225 gram animal would be thirteen grams per 200 days. This is not possible because a 225 gram ground squirrel in hibernation must lose approximately 82 grams of water in 192 days to maintain an osmotic balance (see Fisher and Manery, 1965).

The literature also presents conflicting data concerning micturition by hibernating squirrels. Popovic (1964) reported that Citellus tridecemlineatus urinates at a body temperature of 12°-13°C. However, Zimny (1960) found that this species in a metabolic cage is fully awake before producing any urine. Johnson (1931) reported that no one

has sacrificed animals during arousal and determined whether the bladder was full, and they felt that full bladders of hibernating animals is not directly associated with arousal. Pengelley and Fisher (1961) showed that there is weight loss during the active period of hibernation (which lasted from 12 to 24 hours) but none during torpor. They also found that the volume of bladder urine of hibernating Citellus lateralis was quite variable and was not correlated with the length of torpor. They suggested that the urine in the bladder was released in the preceding active period after urination. Passmore (1967) reported similar findings.

The available literature concerning urea levels in plasma, urine, and kidneys of torpid and active ground squirrels is conflicting. Fisher (1964) found lower plasma urea concentrations in torpid Citellus lateralis than in active ones. Conversely, Kristofferson (1963) reported that there is a significantly higher urea concentration in the blood of dormant hedgehogs, (Erinaceus europeaus). Zimny and Bourgeois (1960) found that urea obtained by bladder puncture from torpid Citellus tridecemlineatus was not significantly less concentrated than voided urine urea collected from active individuals. Carpenter (1938) reported that the rate of urea excretion of one Marmota monax was reduced by fasting, but during torpor only a moderate further decrease occurred. Scott and Fisher (1967) collected urine samples from the bladders of hibernating

ground squirrels Citellus richardsoni and Citellus lateralis half-way or more through the hibernating cycle and found no reduction in any urine titer.

Bullard (1964) concluded that the circulation through the kidney may be minimal during hibernation. Hong (1957) showed similarly that renal blood flow was markedly reduced due to an increased resistance. The decreased cardiac output, bradycardia, (heart rate approximately 1/30-1/50 of normal) and the low diastolic blood pressure (10-40 mm of Hg) suggested that the kidney does not form any appreciable amount of urine during torpor (Lyman, 1959). However, Lyman stated that he does not know what the blood pressure changes were in the renal artery or glomerulus during hibernation and arousal (personal communication).

Zimny and Bourgeois (1960) studied the histology of the nephron with the light microscope in Citellus tridecemlineatus and observed vaso congestion in the glomeruli. Using electron microscopy, they described decreased porosity of the membrane of the renal corpuscle. Also the basement membrane of the glomerulus was thickened with swelling of the podocyte foot processes of the glomerular membrane. Zimny suggested from these data that no urine would be formed during hibernation. Amon et al. (1965) described similar findings in the kidneys of the hibernating dormouse, (Glis glis). Recently, however, Amon (1967) has reported that normal ultra-structural proportions of glomerular loops are

found in renal glomeruli of young dormice previous to the first hibernation and that the hibernation produced irreversible structural and permeability changes.

There are few data on the concentrations of electrolytes (sodium and potassium) in plasma, urine, renal tissue fluid and muscle of torpid, arousing, and dehydrated ground squirrels. Kayser (1961) reported that the potassium concentration in the plasma of hibernating ground squirrels was lower, higher, or remained constant as compared to the potassium concentration in the plasma of active ground squirrels. Christians (1961) reported that serum K of woodchucks decreased from approximately 6 meq/liter to 3 or 4 meq/liter. However, Raths (1962) showed an elevation of serum K from approximately 5.8 meq/l in the aroused hamster to approximately 8 meq/l during torpor. Eliassen (1963) also showed a rise in serum K in hibernating hedgehogs from about 4 meq/l to 7 meq/l. Other workers have indicated an increase in the potassium concentrations during hibernation (see Fisher and Manery, 1965).

Suomalainen (1960) reported an appreciable enhancement of the serum Na concentration during hibernation (171-183 meq/l). These concentrations are somewhat higher than the normal range for hibernators. In contrast Klar (1938) reported a slight increase from 97-114 to 110-122 meq/l, but his values are low for most mammals. Biorek et al. (1956b) found the same Na concentrations during hibernation as during

homeothermy. Serum Na of active awake hamsters is approximately 160 meq/l during late winter compared with 145 meq/l during other seasons (Raths, 1962). Denyes and Hassett (1960) found the same concentration of serum Na (152 meq/l) in hibernating hamsters as in normal animals fasted just prior to the experiment. These data indicate a slight elevation in serum K concentrations during hibernation, whereas serum Na concentrations remain relatively constant.

The concentration of K in diaphragm tissue of hamsters increases slightly during torpor (from 119 to 121 meq/l) and increased (from 103 to 118 meq/l) in ground squirrels, according to Willis, (1962 and 1964a). He also reported an increase in the Na concentration of diaphragm muscle of hamsters and ground squirrels during torpor as compared to the active aroused period (60 to 80 meq/l and 51 to 66 meq/l, respectively). Eliassen (1961) consistently found a marked rise in muscle K in hibernating hedgehog (from about 95 meq/l to 120 meq/l) in deep hibernation which dropped to 90 in non hibernators; whereas little or no change occurred in the Na concentrations. Muscle dehydration occurred during deep hibernation and accounted for higher K concentrations during hibernation (Fisher and Manery, 1965).

According to a recent review (Fisher and Manery, 1965), only one report of renal function in a mammal that hibernates has been published and no clear picture of nitrogen excretion or renal function during hibernation exists.

Therefore, the objectives of this study are threefold:

1. To compare the solute gradients in the kidneys of torpid, arousing and active dehydrated ground squirrels.
2. To compare urea and sodium concentrations in urine and renal tissue water of arousing and active squirrels.
3. To provide further evidence as to whether or not urine is formed by the kidney during hibernation.

MATERIALS AND METHODS

Animals

This study utilized 21 Columbian ground squirrels, (*Citellus columbianus*), either captured from Pattee Canyon in Missoula County, Montana, or obtained from the Rocky Mountain Laboratory at Hamilton, Montana. The animals weighted from 300 to 650 grams. The squirrels, kept indoors in metal cages, were fed alfalfa pellets and provided water *ad libitum*. Animals were randomly chosen for three experimental procedures. Eight squirrels were sacrificed during torpor. Two arousing squirrels were sacrificed before they formed urine and five arousing squirrels were sacrificed after they formed urine. Five active squirrels were dehydrated before they were sacrificed. One squirrel was sacrificed while entering torpor. The three experimental groups were treated as follows:

1. Hibernating squirrels

Ground squirrels were induced to hibernate by placing them in screen cages, 33 cm x 13 cm x 8 cm, containing burlap nests in a refrigerator at a constant ambient temperature of 7°C. From August to early March, squirrels would readily to into hibernation. From April to August the squirrels would not hibernate and the experimental procedure had to be discontinued.

A typical experimental procedure began by removing the hibernating squirrel from the refrigerator three to eight days after the onset of torpor. The rectal temperature was taken with Schultheus quick-registering thermometer, weight to the nearest 1.0 gram recorded with a triple beam balance, and sex determined. The squirrel was killed by decapitation, a mid-line ventral incision was made, and the thoracic and abdominal cavities were opened to expose the heart and the urinary bladder. Two ml of blood were aspirated into a heparinized syringe from the animal's beating heart. The blood was then placed in a polyethylene micro-test tube and centrifuged and plasma removed. A urine sample was similarly aspirated from the urinary bladder, placed in a micro-test tube and frozen. The kidneys were also removed from the animal, as described below. The entire procedure took less than five minutes. Determinations showed that the amount of sodium in heparin was minimal and did not affect the sodium plasma results.

2. Arousing squirrels

The torpid squirrel was removed from the refrigerator and the time was recorded for the beginning of arousal. A Schultheus thermometer recorded the rectal temperature initially and then a thermister connected to a Brown Temperature Recorder was inserted into the rectum to record the continuous changes in the body temperature during the remainder of the experiment. The ground squirrel was tied

ventral side up to a surgical board and prepared for surgery. A local anesthetic, Lidocaine, was injected both into the abdominal muscles and into the abdominal cavity approximately five minutes before a Laporotomy was performed anterior to the pubic symphsis. A 1.5 inch supra-pubic abdominal incision exposed the bladder, which was emptied of urine by aspirating it into a syringe. A catheter (Pe.10) was reduced approximately 1/3 to 1/4 the original diameter and inserted into the right ureter and tied with a suture. The animal was allowed to arouse normally at room temperature. Additional dosages of Lidocaine and, later in the experiment, Surital were injected (intrathoracic) whenever it appeared to be needed. The rectal temperature and time were noted at: the initiation of the experiment, at each administration of Surital or Lidocaine, at the first urine flow in the catheter, and at death. The first 5-10 µl of urine sample was discarded and 1 to 3 samples were collected at approximately 15-minute intervals for analysis before the animal was sacrificed by decapitation. A terminal blood sample was obtained by heart puncture and the kidneys removed as described below.

3. Dehydrated squirrels

A squirrel was placed in a round metabolism cage without food and water. A large funnel equipped with screening was placed beneath the cage to channel urine uncontaminated by feces into a beaker filled with mineral

oil and a few thymol crystals. The squirrel concentrated urine maximally within 8 to 10 hours after dehydration began (Passmore 1968). (A few squirrels seemed to lose their concentrating ability after 9 hours of dehydration.) A terminal urine sample was obtained, 35 minutes before sacrificed by gently blowing on the squirrel which induced evacuation of the bladder. The squirrel and cage were gently placed into a carbon dioxide chamber until the squirrel's death. The carbon dioxide chamber maintained the squirrel in a relatively normal physiological state until death, and the squirrel was not handled during the procedure or excited. The thoracic and abdominal cavities were opened, blood and urine samples were taken via heart and bladder punctures respectively, and the kidneys removed. These procedures took less than four minutes after death.

Tissue Slice Analysis

Each kidney was removed from the intact squirrel for all three procedures, simultaneously with death and immediately sliced. The renal fascia (perirenal fat) was removed from around the outer extremeties of the kidneys. The kidneys were sliced with a sharp razor blade. The sectioning of the kidney is described in figure 1 a & b. The mid-sagittal sections were immediately frozen and stored in dry ice and acetone. A piece of abdominal muscle was also frozen at this time.

The mid-sagittal sections were carefully removed from

the dry ice and acetone and sliced into six sections as follows (Fig. 1c): two cortex zones (C1 and C2), two outer medulla zones (OZ1 and OZ2), and two inner medulla zones (IZ1 and IZ2), with IZ2 representing the tip of the papilla.

Micro-test tubes were weighed on a Mettler rapid pan balance to the nearest 0.0001 gram. Approximately 200 µl of ammonia-free water (triple distilled) were placed in each tube except in the tube which was to receive the papilla, where 100 µl of ammonia-free water were used because of the small size of the papillary tissue. The micro-test tubes were again weighed to determine the amount of water in each tube. The frozen kidney slices were then placed into micro-test tubes. The renal tissue weights ranged from 1.2 mg to 100 mg. The test tubes were placed into boiling water for 5 to 10 minutes to destroy the tissue enzymes. Weighing the test tubes before and after boiling indicated insignificant water loss by evaporation. The test tubes were placed in the refrigerator at 5°C for 24 hours to permit diffusion of the electrolytes and urea. They were stored at a -20°C until analyzed.

Urea concentrations in the tissue fluid were expressed in mMoles/Liter and sodium and potassium concentrations were expressed in mEq/Liter. To correct for the added water, a dilution factor was determined using the following formula:

$$\text{Dilution Factor} = \frac{\text{Wt. of tissue water + diluent}}{\text{Wt. of tissue water}}$$

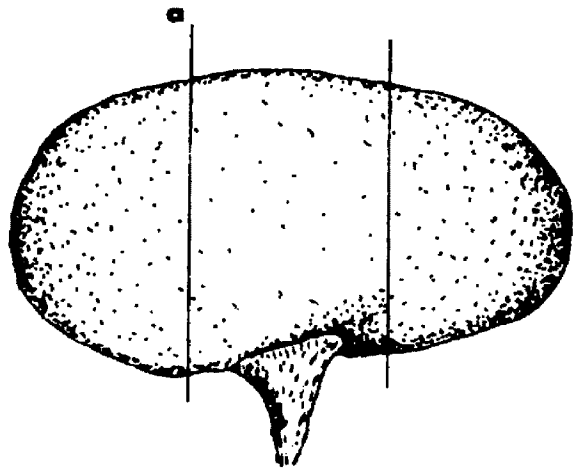


Fig. 1a

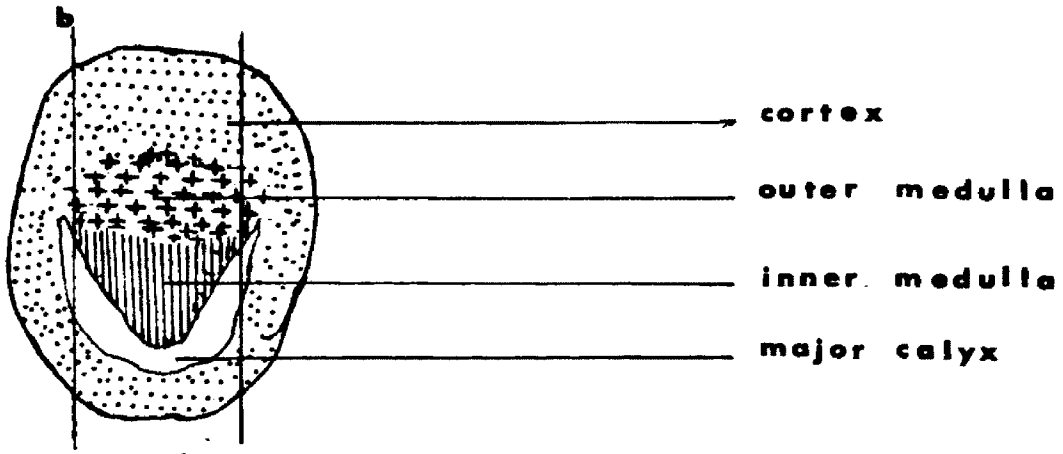


Fig. 1b

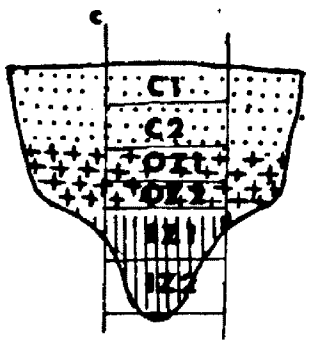


Fig. 1c

The dilution factor ranged from 2.9 in the cortex (C1 and C2) and abdominal muscle to 184 in the tip of the papilla (IZ2). The tissue water was estimated to be 80.0 percent of the total tissue weight (Schmidt-Nielsen and O'Dell, 1959).

Osmolal concentrations of urine were determined cryoscopically, using a Fiske osometer (model B.). Sodium and potassium were analyzed in a Lithium internal standard flame Photometer (Beckman model 105). Urea and ammonia were determined by an ultra-micro adaptation of the method of Fawcett and Scott (Beckman, 1962) using a Beckman spectro-colorometer, Model 151. The ammonia nitrogen present in the urine was substracted from the total nitrogen (NPN) to obtain the concentration of urea nitrogen. All urea urine concentrations were calculated using the following formula:

$$\text{NPN concentration - ammonia nigrogen concentration} \times \frac{60}{28} = \frac{\text{urea mg\%}}{6.0} = \text{mMoles/l urea}$$

The urea determinations for the tissue slices and the plasma were calculated using the same procedure except that the ammonia determination was disregarded because of its small concentrations therein.

Two known urea concentrations as standards were analyzed with every group of determinations to check the precision of the technique and three to four known standard controls were run after every sodium and potassium analysis.

Human serum and urine samples, obtained from the Hyland Laboratories, were also analyzed for urea, sodium, and potassium and these concentrations were compared with those determined by the Hyland Laboratories.

Statistical Analysis

The solute concentrations from the six renal zones for the right and left kidneys of squirrels within a group are plotted on the X axis against equal distances between renal zones on the Y axis. Two straight regression lines are calculated from the scatter diagram for the right and left kidneys. The slope of the line was determined from the regression coefficients using the equation $Y = \bar{y} + b(x-\bar{x})$. The paired sample t-test was used to determine variations between the mean solute concentrations from kidneys and urine samples within a group, and the t-test was used for comparing mean solute concentrations from kidneys and urine samples between groups. A two-factor analysis of variance was used for comparing the papillary TF/P urea ratio for the right and left kidneys of every arousing squirrel against papillary TF/Plasma urea ratio from the other arousing squirrels. A one-factor analysis of variance was used to compare plasma urea, sodium and potassium concentrations between hibernating, arousing and dehydrated squirrels.

RESULTS

I. Urea concentrations

A. Renal Tissue Slices

The kidneys of the torpid ground squirrels had little or no urea gradients extending from the cortex to the papilla (Table 2). The urea concentrations for the right kidney did not differ significantly ($P > 0.05$) from the left kidney. The calculated linear regression line for the right kidney was similar to that of the left with slopes of 0.052 and -0.406, respectively (Fig. 2). In contrast, the kidneys of the dehydrated squirrels had pronounced urea gradients (Fig. 2). The slopes for the right and left kidneys of the active dehydrated squirrels were 64.36 and 70.87, respectively, and the urea gradient of the right kidney did not differ significantly ($P > 0.05$) from that of the left kidney. Urea concentrations in the papillae of hibernators were significantly lower ($P < 0.025$) than those in papillae of dehydrated squirrels. The mean tutular fluid-plasma ratios at the tip of the papilla (IZ2TF/P) are 1.55 and 2.23 for the right and left kidneys, respectively, of hibernators (Table 3) and 37.25 and 38.00 for the right and left kidneys of dehydrated active squirrels (Table 4).

The kidneys of the arousing nonurine formers had no urea gradients (Table 5) and were quite comparable to

the torpid hibernators ($P > 0.05$). The slopes for the right and left kidneys of arousing nonurine formers were 0.072 and 1.528, respectively, and the urea gradient of the right kidney did not differ significantly ($P > 0.05$) from that of the left (Fig. 3). However, during the arousal process, as the squirrel began to form urine the gradient of urea was rapidly restored. The arousing urine formers had distinct urea gradients (Table 6). The slopes for the right and left kidneys were 46.73 and 37.73, respectively, and the urea gradients for the right kidney did not differ significantly ($P > 0.05$) from the left. However, the urea gradients of the arousing urine formers differed significantly from those of the nonurine formers ($P < 0.001$) and the urea gradients of each arousing urine former varied significantly from the other arousing urine formers ($P < 0.05$) (Fig. 12). The mean papillary tissue fluid to plasma ratios of urea (IZ2TF/P) for the right and left kidneys of the nonurine formers were 1.32 and 2.19, respectively, whereas, the mean IZ2TF/P ratios of urea for the right and left kidneys of the arousing urine formers were 26.40 and 23.06 (Table 7 and Table 8), indicating that the arousing urine formers are capable of producing a hypertonic urine.

Both the urea gradients and the slope value for the right kidney of arousing urine formers were slightly higher than the urea gradients of the dehydrated active squirrels (Table 4 and Table 8). However, both the urea gradients

and slope value for the left kidney of the arousing urine formers resembled the urea gradients of both the right and left kidneys of the dehydrated squirrels. The slope for the right kidney, 48.85, was relatively larger than the slope for the left kidney, 36.90, but the urea gradients did not differ significantly ($P > 0.05$) (Fig. 2 and Fig. 3).

One squirrel sacrificed as it was entering torpor had a very pronounced urea gradient (Table 9).

B. Urine

The mean urea concentration in the bladder urine of the hibernators (Table 2) averaged slightly lower than that found in the terminal bladder urine of the dehydrated squirrels (Table 1), but the difference was not significant ($P > 0.05$). In contrast, the catheter urine urea to plasma urea ratios (U/P) of arousing urine formers were significantly lower ($P < 0.005$) (Table 8) than the terminal bladder urea to plasma urea ratios of antidiuretic squirrels (Table 4). The urea concentrations in the terminal catheter urine samples for all five arousing formers were consistently lower than their respective papillary urea concentrations (Table 6, Table 8, and Fig. 4), whereas in dehydrated squirrels the opposite held true.

The arousing urine formers were sacrificed at three different intervals (15, 28 and 40 minutes), after the first observation of urine flow in the ureteral catheter (Fig. 11). Arousing urine former No. 15 was sacrificed fifteen minutes

after the beginning of urine flow in the catheter and only one 10 µl sample of urine was collected. Both the papillary tissue fluid to plasma ratios (IZ2TF/P) of urea and the catheter urine and plasma ratios (U/P) of urea were above 24, whereas, arousing urine former No. 17 and No. 25, both sacrificed 40 minutes after urine was observed flowing in the catheter, had much smaller IZ2TF/P ratios of urea (13 and 16, and 12 and 8.90) indicating that urine former No. 15 was capable of concentrating urea more readily than either urine former No. 17 and No. 25 (Table 8). Three consecutive 10 µl catheter urine samples were collected at 15-minute intervals in urine former No. 17 and the urea concentrations rose appreciably in the second sample from 67.5 mM/l to 122.0 mM/l, but dropped somewhat in the third sample to 117 mM/l. Possibly this squirrel had completed the osmotic gradient before the terminal catheter urine sample was collected. Urine formers Nos. 25 and 16, both sacrificed 40 minutes after the beginning of catheter urine flow, showed increasing concentration of urea in two consecutive catheter urine samples. However, the terminal U/P ratio of urea for squirrel No. 16 was more than three times that of squirrel No. 25 (Table 8). Furthermore, the papillary tissue fluid to catheter urine ratios (IZ2TF/U) of urea for arousing urine formers was always appreciably greater than unity, whereas, this ratio for the dehydrated active squirrels was always less than unity (Fig. 5).

The two arousing nonurine formers did not produce any observable urine from the right ureteral catheter and the terminal bladder urine concentrations of urea were found to be in the range of terminal bladder urine urea concentrations of hibernators.

C. Plasma

The mean plasma urea levels of torpid, dehydrated, and arousing urine forming squirrels did not differ significantly ($P > 0.05$) (Fig. 6). In fact, the mean urea concentrations of all three groups ranged from 8.0 mM/l to 9.0 mM/l and twice the standard errors for the three respective groups were -2.08, -1.57, and -1.39. The mean plasma concentration of urea (11.4 mM/l) for the arousing nonurine formers was appreciably higher (Table 5). However, only two squirrels were in this group and perhaps more urea plasma data are needed from arousing nonurine formers.

II. Sodium concentrations

A. Renal Tissue Slices

The kidneys of all torpid ground squirrels had little or no sodium gradients extending from the cortex to the tip of the papilla (Table 10). The slopes for the right and left kidneys were 1.89 and 0.57, respectively, and the two sodium gradients did not differ significantly ($P > 0.05$) (Fig. 7). However, the dehydrated squirrels had very pronounced sodium gradients extending from the cortex to the papilla (Table 11). The mean sodium concentrations of zones (C1 and C2) did not

differ appreciably. The slope for the right kidney, 41.65, was slightly greater than the slope for the left kidney, 38.31, but the sodium gradient for the right kidney did not differ significantly ($P > 0.05$) from that of the left (Fig. 7). The sodium gradients of kidneys of the dehydrated squirrels were significantly higher ($P < 0.05$) than the sodium gradients of the hibernators. No sodium TF/M gradients existed in hibernators, whereas, the sodium TF/M gradients of dehydrated squirrels were very pronounced (Table 12 and Table 13). Squirrel No. 22 was dehydrated too long a time and the osmolality began to decrease in the urine samples collected during the dehydration period and the sodium IZ2TF/M ratio for its right and left kidneys were only 3.00 and 3.36, respectively. The mean sodium IZ2TF/M ratios for both kidneys of dehydrated squirrels were still considerably higher with slopes of 8.65 and 8.26. The renal zone concentrations of squirrel No. 22 were included in the calculations of the slopes.

The sodium gradients of kidneys of the arousing urine formers were very pronounced but varied considerably between squirrels, possibly suggesting that the sodium gradient, as well as the urea gradient, might be correlated with the osmotic state of the squirrel. The TF/M ratio of sodium in four of the five squirrels increased proportionally with the P/M ratio of sodium (Table 16). (The development of the sodium and urea gradients are compared on page 30.)

The arousing nonurine formers did not have a renal sodium gradient (Table 15). The slopes for the two arousing non-urine formers were 0.212 and 3.857 for the right and left kidneys, respectively (Fig. 9), and the slopes did differ significantly ($P < 0.025$) from slopes of kidneys of arousing urine formers.

Animal No. 11 was sacrificed at 21°C, as it was entering hibernation and the kidneys had distinct sodium gradients (Table 9).

B. Urine

The mean sodium concentration from the terminal bladder urine of hibernating squirrels averaged 37 mEq/l, and the mean sodium concentration from the bladder of dehydrated squirrels was slightly higher, 44 mEq/l (Table 11). However, the concentrations varied considerably between squirrels (Table 14). The sodium concentration in the catheter urine samples progressively increased when consecutive urine samples were collected. However, in many cases, the urine samples were too samll to permit determination of sodium concentration. The mean terminal U/P ratio of sodium for three of the five arousing urine formers was 0.76.

C. Plasma

The mean sodium concentration in the plasma of hibernators did not differ significantly ($P > 0.025$) from that in the plasma of both the arousing urine formers and

the dehydrated squirrels. However, the mean plasma sodium concentration of arousing urine formers was significantly lower ($P < 0.05$) than that of dehydrated squirrels (Fig. 6).

III. Potassium concentrations

A. Renal Tissue Slices

No potassium concentration gradient extending from the cortex to the papilla existed in kidneys of squirrels of any group (Table 18). The potassium levels in the corresponding zones of each group did not differ significantly ($P > 0.05$). The regression lines for both the right and left kidneys of all groups did not differ significantly ($P > 0.05$) (Fig. 9 and Fig. 10). The slopes for the regression lines of all groups ranged from -2.711 to 4.899.

B. Urine

The concentration of potassium in the urine of hibernators was not determined. The urine potassium concentrations of the dehydrated squirrels varied considerably (Table 18) with the mean value slightly higher than 232 mEq/l. The potassium concentrations in the terminal catheter urine of arousing urine formers was low, with a mean of 92 mEq/l (Table 18). The bladder urine potassium concentration of two arousing nonurine formers averaged 266 mEq/l (Table 18).

C. Plasma

The mean potassium concentration in the plasma of hibernators did not differ ($P > 0.05$) significantly from

that in the plasma of arousing urine formers and dehydrated squirrels, whereas the arousing urine formers' mean potassium level in the plasma was significantly lower ($P < 0.001$) than that of the plasma of dehydrated squirrels (Fig. 6) (Table 18). The mean plasma potassium concentrations for the torpid, arousing and dehydrated squirrels were 4.9, 4.0, and 5.6 mEq/l, respectively, and twice the standard error for the three groups were ±1.16, ±0.09, and ±0.06, respectively.

IV. Ammonia Concentrations

Unappreciable amounts of ammonia were found in the plasma and renal tissue slices. Approximately 5% of the nitrogenous substances in the urine of all squirrels was ammonia.

V. Osmolality

Osmolality was only determined on terminal urine samples obtained from the bladders of dehydrated squirrels. The mean osmolality was 1530 mOsm/l (Table 19). Squirrel No. 17 was dehydrated fifteen hours and had an osmolality of 690 mOsm/l, whereas squirrel No. 19 was dehydrated only nine hours and had an osmolality of 2800 mOsm/l.

VI. Temperature vs. Time in Arousing Squirrels

The mean rectal temperature (T_B°C) of hibernating squirrels at the beginning of the arousal process was 8°C with a range of 6.0 to 12.0°C (Table 2). Rectal temperatures of two arousing nonurine formers, sacrificed 70 and 152

minutes after the initiation of the arousal process, were 21°C and 33°C, respectively. These squirrels were not passing urine through the right ureteral catheter (Fig. 11). Four arousing urine formers started passing urine through the ureteral catheter 75 to 135 minutes after the onset of arousal at T_B ranging from 23°C to 31°C. Squirrel No. 26 was removed from the refrigerator and placed immediately on an ice pack for one-half hour. This squirrel started to pass urine 175 minutes after the onset of arousal, at a rectal temperature of 29°C. The five arousing urine formers were allowed to raise their T_B from 2.4° to 7.0°C after the beginning of ureteral urine flow before they were sacrificed.

VII. Papillary Sodium Urea Relationships in Arousing Urine Formers

The ratio of papillary tubular fluid sodium to abdominal muscle sodium increased proportionally to the ratio of papillary tubular fluid urea to plasma urea (IZ2TF/P) in four arousing squirrels (Fig. 12). When the urea TF/P ratio was high in a squirrel, so was the corresponding sodium TF/M ratio, except in squirrel No. 26 in which the sodium TF/M ratio did not increase proportionally with the urea U/P ratio. The TF/M ratio of sodium was the same in both the right and left kidney for all arousing squirrels. The IZ2TF/P ratio of urea in the right kidney did not differ significantly ($P > 0.05$) ($F = 0.77$) from the IZ2TF/P ratio of urea in the left kidney of all squirrels, but the

IZ2TF/P ratio of urea for both right and left kidneys did differ significantly between squirrels ($P < 0.05$) ($F = 7.81$). The sodium and urea concentrations were not consistently higher in one kidney than in the other. For example, squirrels Nos. 16, 25, and 26 had a higher urea concentration in the right kidney than in the left kidney (Table 6), and squirrels Nos. 15, 25, and 26 had higher sodium concentrations in the right kidney than in the left kidney (Table 14). Therefore, the urea and sodium gradients seemed to develop simultaneously as the squirrel aroused. Catheterizing the right ureter did not alter the sodium or urea gradients in the right kidney as compared to the urea and sodium gradients of the left kidney.

Table 1

UREA CONCENTRATIONS IN TISSUE SLICES, PLASMA AND URINE

DEHYDRATED SQUIRRELS

Right Kidney	mMoles/Liter					
Squirrel No.	18	19	21	22	23	mean
C1	12.9	18.2	16.7	17.5	32.4	19.5
C2	15.7	35.9	25.0	19.9	36.2	26.5
OZ1	26.9	93.7	66.1	55.3	163.9	81.2
OZ2	67.7	199.3	150.7	130.5	**	137.0
IZ1	122.3	92.6	188.4	172.1	418.6	238.8
IZ2	188.8	580.6	203.5	137.1	551.8	332.4
Left Kidney						
C1	14.2	17.3	16.2	19.5	14.7	16.4
C2	16.6	38.1	19.7	22.5	25.0	24.4
OZ1	48.7	136.8	66.1	36.6	169.2	91.5
OZ2	84.3	346.6	118.3	88.5	**	159.4
IZ1	131.2	773.2	53.3	144.6	352.4	292.9
IZ2	244.1	840.8	29.4	123.7	447.1	337.0
Muscle	12.8	17.8	14.0	12.3	14.9	14.4
Plasma	8.0	9.7	11.4	7.8	8.2	9.0
Bladder Urine	430.0	1120.5	555.6	865.7	831.4	760.6
Temp. at Sacrifice °C	37.2	36.8	36.5	35.9	37.1	36.7

(no samples = **)

Table 2

UREA CONCENTRATIONS IN TISSUE SLICES, PLASMA AND URINE

HIBERNATING SQUIRRELS

mMoles/Liter

Right Kidney

Squirrel No.	3	5	7	8	9	10	12	mean
C1	11.9	18.4	11.5	4.2	13.3	7.3	11.2	11.1
C2	7.3	14.8	8.5	5.5	14.1	6.0	6.9	9.0
OZ1	10.9	13.9	9.5	**	13.4	6.1	5.0	9.8
OZ2	8.4	17.3	7.9	**	18.5	5.8	2.9	10.2
IZ1	9.5	13.2	10.3	**	19.4	6.2	1.6	10.0
IZ2	11.4	17.0	10.3	**	24.3	7.0	3.7	12.3
Left Kidney								
C1	**	20.6	10.9	8.1	12.9	8.7	6.9	11.3
C2	**	15.1	9.9	5.9	15.4	6.7	6.0	9.8
OZ1	**	17.4	9.9	9.0	13.9	5.2	6.9	10.4
OZ2	**	31.1	14.2	5.3	16.6	6.6	5.7	13.2
IZ1	**	13.1	9.0	14.8	27.1	5.7	5.1	12.5
IZ2	**	24.9	**	10.0	30.5	**	**	21.8
Muscle	12.5	22.6	12.6	**	13.5	9.6	7.1	13.0
Plasma	11.6	11.8	7.3	8.0	9.1	3.5	4.6	8.0
Bladder Urine	719.1	690.1	517.3	**	1028.0	328.9	190.8	579.0
Temp. at Sacrifice °C	8.0	12.0	7.0	6.0	6.5	7.5	6.0	7.6

(no samples = **)

Figure 2. Scatter diagram of urea concentrations in kidney tissue slices of hibernators and anti-diuretic homeotherms. Straight line regression drawn by the equation $Y = \bar{y} + b(x - \bar{x})$, where b = 0.052 and 1.406 for the hibernators right and left kidney respectively, and b = 64.360 and 70.868 for the anti-diuretic homeotherms right and left kidney respectivly.

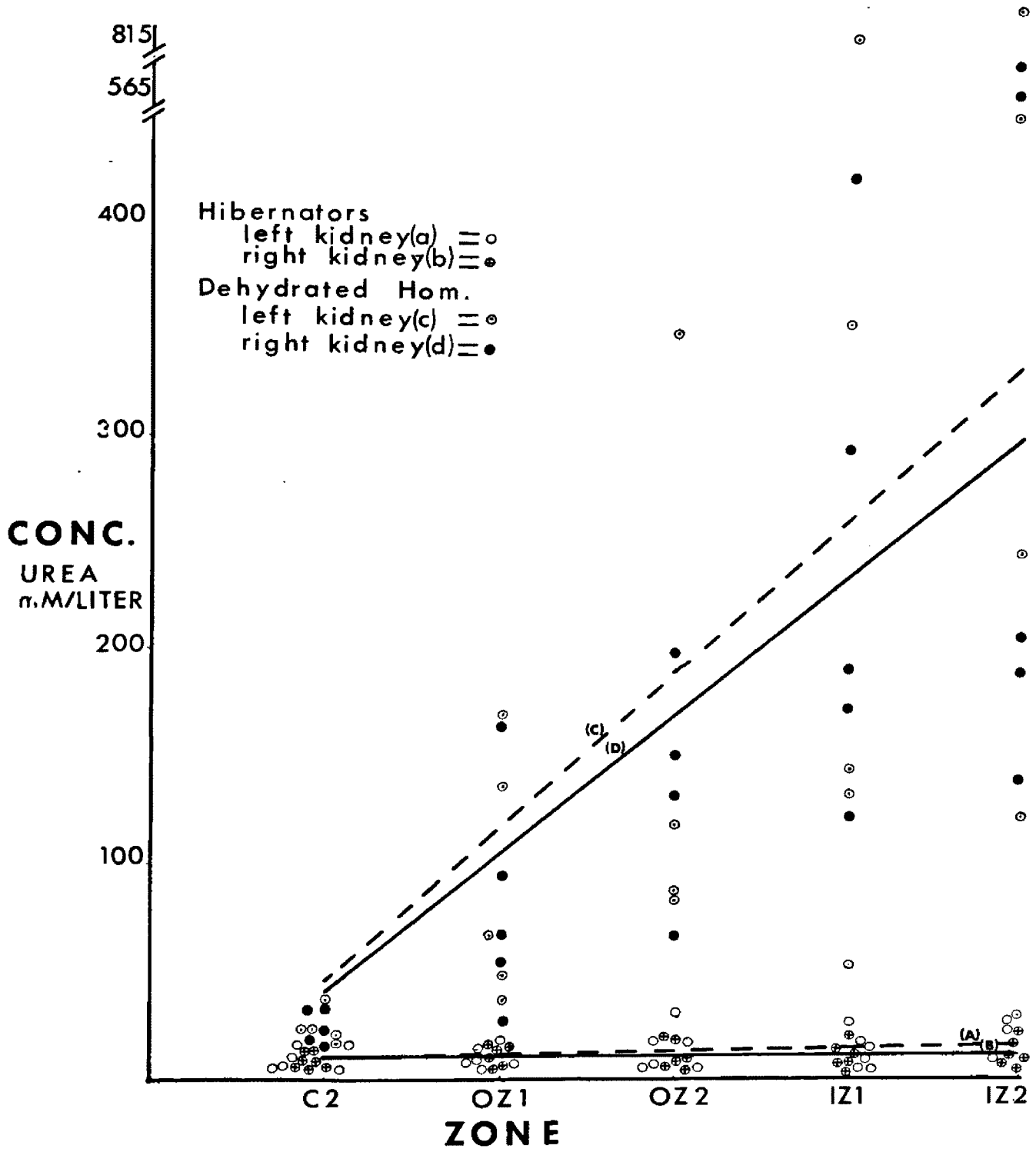


Table 3

UREA U/P, TF/P AND MUSCLE/P RATIOS

HIBERNATING SQUIRRELS

Squirrel No.	3	5	7	8	9	10	12	mean
Right Kidney TF/P								
C1	1.02	1.56	1.58	.52	1.46	2.09	2.43	1.52
C2	.53	1.26	1.16	.68	1.54	2.01	1.49	1.24
OZ1	.94	1.18	1.30	**	1.47	1.74	1.08	1.28
OZ2	.73	1.48	1.08	**	2.04	1.66	.64	1.27
IZ1	.82	1.12	1.42	**	2.13	1.78	.36	1.27
IZ2	.98	1.44	1.41	**	2.67	2.00	.81	1.55
Left Kidney TF/P								
C1	**	1.74	1.49	1.00	1.42	2.50	1.49	1.61
C2	**	1.28	1.35	.73	1.70	1.90	1.31	1.30
OZ1	**	1.48	1.36	1.12	1.53	1.49	1.51	1.41
OZ2	**	2.63	1.94	.66	1.83	1.90	1.23	1.70
IZ1	**	1.12	1.23	1.84	2.98	1.62	1.11	1.65
IZ2	**	2.12	**	1.24	3.34	**	**	2.23
U/P (bladder)	62.1	58.6	70.9	**	112.9	94.0	41.4	73.3
Muscle/P	1.08	2.25	1.80	**	1.47	3.08	1.41	2.21

(no samples = **)

Table 4

UREA TF/P, U/P AND MUSCLE/P RATIOS AND PAPILLARY/URINE RATIOS

DEHYDRATED SQUIRRELS

Squirrel No.	18	19	21	22	23	mean
Right Kidney TF/P						
C1	1.61	1.88	1.46	2.24	3.97	2.23
C2	1.96	3.70	2.19	2.55	4.41	2.96
OZ1	3.36	9.66	5.80	7.09	19.99	9.18
OZ2	8.46	20.55	13.22	16.73	**	14.74
IZ1	15.29	30.16	16.53	22.06	51.05	27.02
IZ2	23.60	59.86	17.85	17.57	67.29	37.23
Left Kidney TF/P						
C1	1.77	1.78	1.42	2.50	1.79	1.85
C2	2.07	3.92	1.73	2.88	3.05	2.73
OZ1	6.09	14.10	5.80	4.69	20.63	10.26
OZ2	10.54	35.73	10.38	11.35	**	17.00
IZ1	16.40	80.78	4.67	18.54	42.97	32.66
IZ2	30.51	86.68	2.58	15.86	54.52	38.00
U/P (Bladder)	53.75	115.51	48.74	110.99	101.39	86.08
Muscle/P	1.60	1.83	1.23	1.57	1.82	1.61
RT. IZ2TF/U	.44	.52	.37	.16	.66	.43
LT. IZ2TF/U	.57	.75	.05	.14	.54	.41

(no samples = **)

Table 5

UREA CONCENTRATIONS IN TISSUE SLICES, PLASMA AND URINE

AROUSING NONURINE FORMERS

Squirrel No.	13	14	mean
Right Kidney			
C1	12.1	21.6	16.8
C2	12.4	17.6	15.0
OZ1	11.9	22.1	17.0
OZ2	11.5	22.3	16.9
IZ1	12.2	22.9	18.6
IZ2	8.3	23.4	15.8
Left Kidney			
C1	10.7	20.3	15.5
C2	12.8	19.9	16.3
OZ1	14.5	27.6	21.5
OZ2	9.9	23.2	16.5
IZ1	19.1	27.1	23.1
IZ2	19.5	26.6	23.5
Muscle	15.0	17.1	16.0
Plasma	7.3	15.5	11.4
Bladder Urine	907.4	1095.4	1001.4
Temp. at Sacrifice °C	21.0	33.0	27.0

Table 6

UREA CONCENTRATIONS IN TISSUE SLICES, PLASMA AND CATHETER URINE

AROUSING URINE FORMERS

mMoles/Liter

Squirrel No.	15	16	17	25	26	mean
Right Kidney						
C1	13.1	21.3	26.3	23.7	21.3	21.1
C2	11.9	24.5	30.6	23.9	32.1	24.6
OZ1	21.4	69.2	68.1	59.7	54.9	54.7
OZ2	57.7	141.1	106.9	89.0	129.9	104.9
IZ1	213.7	331.3	112.0	100.3	224.8	196.6
IZ2	215.2	373.2	153.9	114.3	322.7	235.8
Left Kidney						
C1	22.5	25.9	29.5	21.3	24.2	24.7
C2	40.4	24.5	28.1	19.8	31.4	28.8
OZ1	39.1	64.1	50.4	25.4	51.9	46.2
OZ2	62.1	146.1	88.0	63.8	171.1	106.2
IZ1	75.9	207.7	168.4	109.8	180.1	148.4
IZ2	237.9	277.7	189.0	84.9	240.0	205.9
Muscle	12.4	13.3	12.2	**	10.9	13.8
Plasma	7.3	8.0	11.5	9.5	10.6	9.4
Bladder Urine	300.0	**	**	269.0	572.0	382.0
Cath. Urine 1	179.9***	168.2	67.5	29.5	212.0***	
Cath. Urine 2		207.0***	122.0	79.0***		
Cath. Urine 3			117.0***			159.0***
Temp. at Sacrifice °C	28.0	28.0	33.4	32.0	29.0	30.1

(no samples = **)
(terminal catheter urine samples = ***)

Figure 3. Scatter diagram of urea concentrations in kidney tissue slices of arousing nonurine formers and arousing urine formers. Straight line regression drawn by the equation $Y = \bar{y} + b(\bar{x} - x)$, where $b = 46.725$ and 37.743 for the arousing urine formers right and left kidneys respectively and $b = 0.072$ and 1.528 for the arousing nonurine formers right and left kidneys.

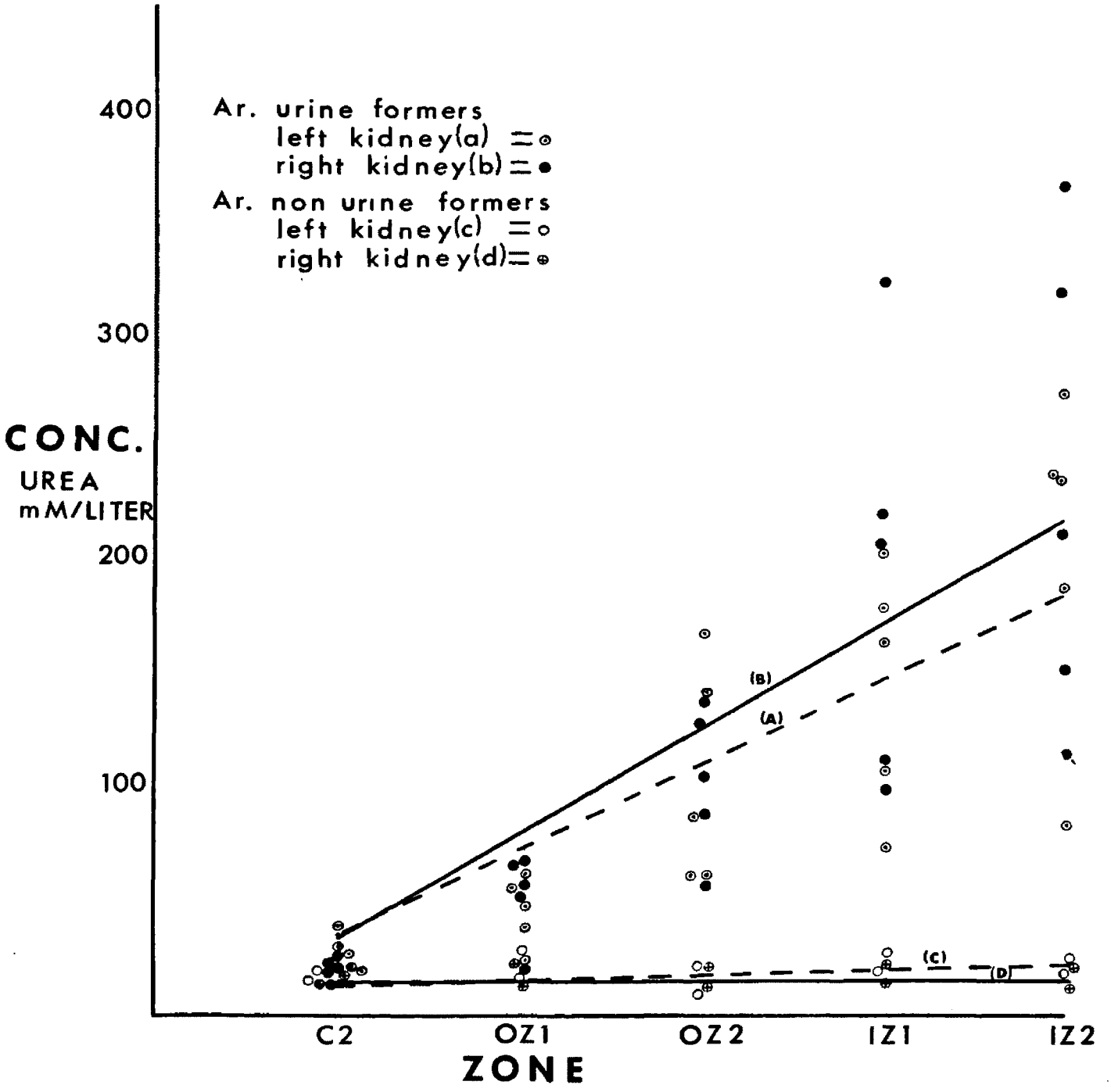


Table 7

UREA TF/P, U/P, AND MUSCLE/P RATIOS

AROUSING NONURINE FORMERS

Squirrel No.	13	14	mean
Right Kidney TF/P			
C1	1.66	1.39	1.52
C2	1.70	1.13	1.41
OZ1	1.63	1.42	1.52
OZ2	1.57	1.44	1.50
IZ1	1.67	1.48	1.57
IZ2	1.14	1.51	1.32
Left Kidney TF/P			
C1	1.47	1.31	1.39
C2	1.75	1.28	1.51
OZ1	1.99	1.78	1.88
OZ2	1.36	1.50	1.43
IZ1	2.62	1.75	2.18
IZ2	2.67	1.72	2.19
U/P (Bladder urine)	124.3	70.7	97.5
Muscle/P	2.05	1.10	1.57

Table 8

UREA TF/P, U/P AND MUSCLE/P RATIOS AND PAPILLARY/URINE RATIOS

AROUSING URINE FORMERS

Squirrel No.	15	16	17	25	26	mean
Right Kidney TF/P						
C1	1.79	2.66	2.29	2.24	2.01	2.25
C2	1.63	3.06	2.66	2.52	3.03	2.58
OZ1	2.93	8.65	5.92	6.28	5.18	5.79
OZ2	7.90	17.64	9.30	9.37	12.25	11.29
IZ1	29.27	41.41	9.81	10.56	21.20	22.45
IZ2	29.48	46.65	13.38	12.03	30.44	26.40
Left Kidney TF/P						
C1	3.08	3.24	2.56	2.24	2.28	2.68
C2	5.53	3.06	2.44	2.08	2.96	3.21
OZ1	5.36	8.01	4.38	2.67	4.90	5.06
OZ2	8.51	18.26	7.65	6.72	16.14	11.46
IZ1	10.40	25.96	14.64	11.56	16.99	15.91
IZ2	32.59	34.71	16.44	8.94	22.64	23.06
U/P (*)	24.64	25.87	10.17	8.31	20.00	17.80
Muscle/P	1.70	1.66	1.06	2.12	1.02	1.51
RT. IZ2TF/P(**)	1.20	1.80	1.31	1.45	1.52	1.46
LT. IZ2TF/P(**)	1.32	1.34	1.61	1.07	1.19	1.31

(* = Terminal Catheter urine and plasma ratio of urea)
(** = Papillary tissue fluid and terminal catheter urine ratio of urea)

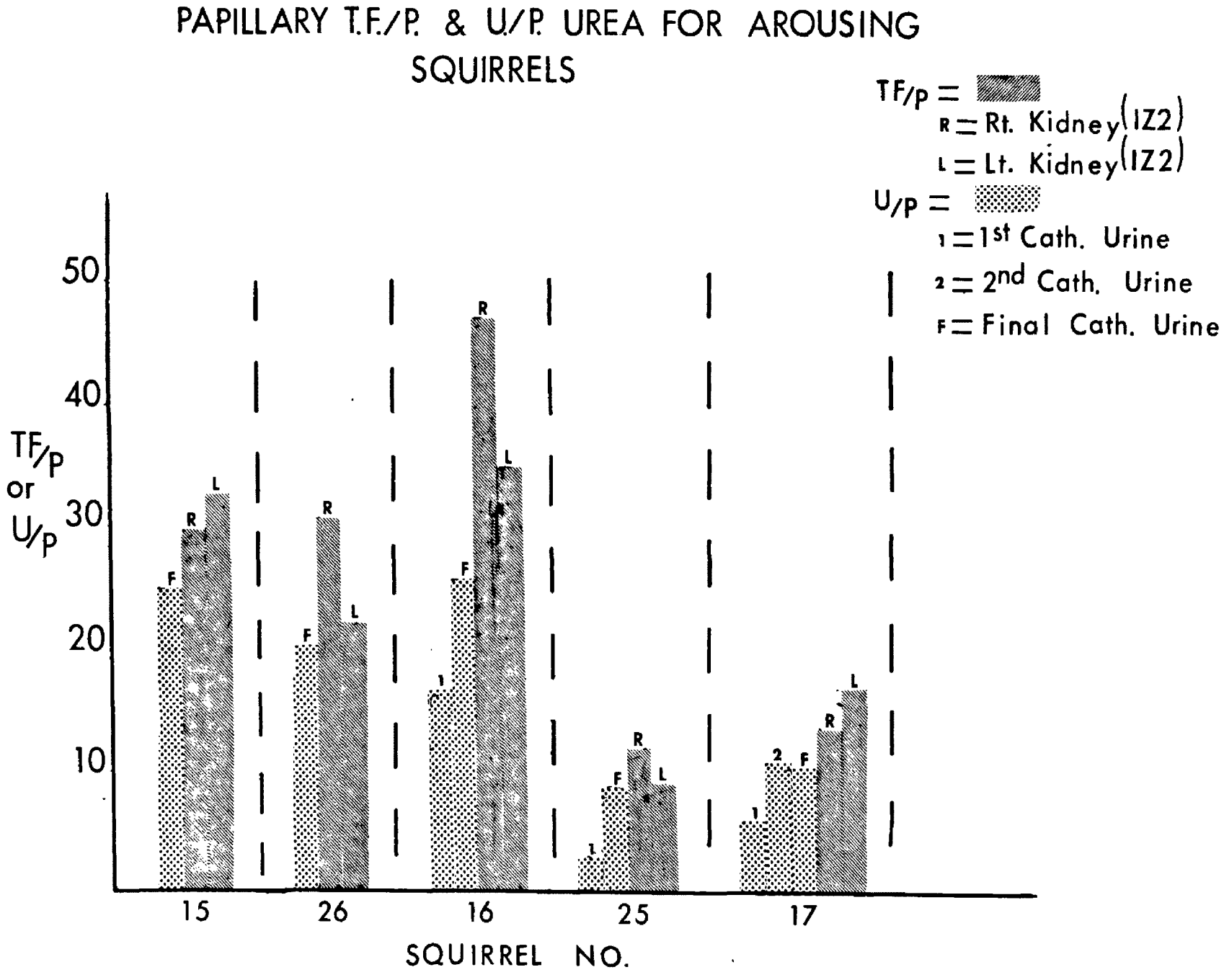


Figure 5. Body temperatures at sacrifice, and urea in the kidneys and urine of arousing and dehydrated squirrels.

UREA IN KIDNEYS & URINE OF AROUSING & DEHYDRATED SQUIRRELS

Squirrel No.	T_B °C	Cath. Urea mM/l	Papillary Urine	Bladder Urea mM/l
AR. 15	28.0	180	1.20	—
AR. 16	28.2	207	1.80	—
AR. 17	33.4	117	1.31	—
AR. 25	32.0	79	1.45	—
AR. 26	29.0	212	1.52	—
Mean	30.1	159	1.48	—
HOM. 18	37.2	—	0.44	430
HOM. 19	36.8	—	0.52	1120
HOM. 21	36.5	—	0.37	556
HOM. 22	35.9	—	0.16	866
HOM. 23	37.1	—	0.66	831
Mean	36.7	—	0.43	761

Figure 6. The levels of urea, sodium and potassium were compared in the plasma of hibernators, arousing urine formers and anti diuretic homeotherms. The darkened rectangular blocks represent two standard errors divided by the mean. The two parallel lines, outside the standard error, indicate the standard deviation.

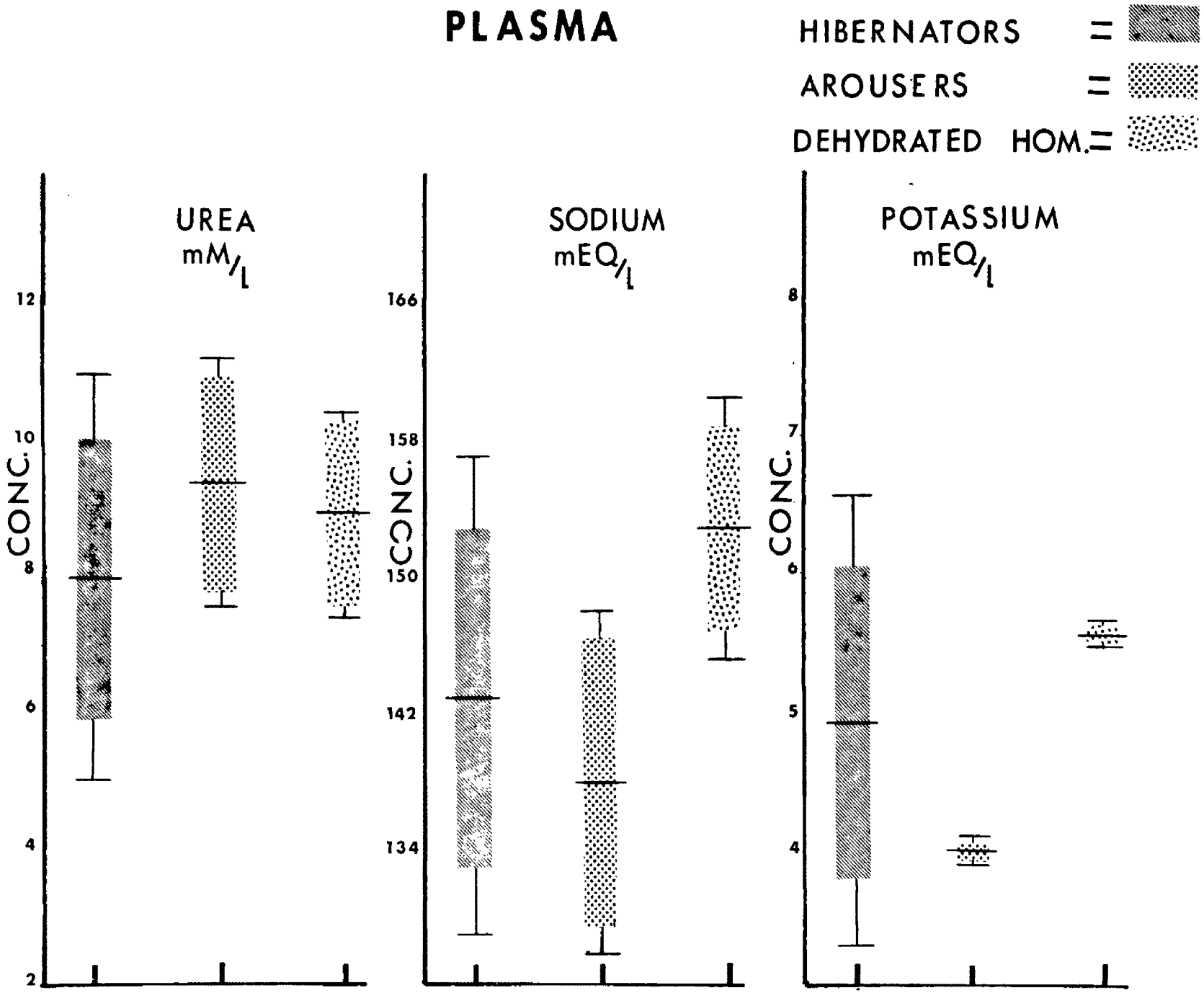


Table 9

UREA, SODIUM AND POTASSIUM CONCENTRATIONS IN KIDNEY TISSUE SLICES, PLASMA AND URINE

SQUIRREL ENTERING HIBERNATION

Squirrel No. 11

Rectal temp. 21°C

Right Kidney

Zones	Urea mM/l	Sodium mEq/l.	Potassium mEq/l.
C1	13.9	41	68
C2	19.0	42	72
OZ1	47.0	83	81
OZ2	75.0	94	67
IZ1	101.4	112	59
IZ2	128.0	105	56
Left Kidney			
C1	13.2	37	56
C2	15.2	39	51
OZ1	47.4	70	69
OZ2	69.0	80	48
IZ1	120.8	114	54
IZ2	130.4	85	31
Muscle	20.2	43	58
Plasma	7.7	143	3.2
Bladder Urine	938.7	26	81

Table 10

SODIUM CONCENTRATIONS IN RENAL TISSUE SLICES, PLASMA AND URINE

HIBERNATING SQUIRRELS

mEq/l

Right Kidney									
Squirrel No.	3	4	5	7	8	9	10	12	mean
C1	98	59	85	106	54	46	48	42	67
C2	79	63	61	96	69	60	51	55	64
OZ1	79	87	72	106	58	41	78	34	69
OZ2	60	70	81	101	61	42	70	48	67
IZ2	80	**	89	91	53	53	71	52	70
IZ2	57	86	112	110	76	60	50	69	77
Left Kidney									
C1	97	80	63	47	53	45	54	50	61
C2	70	93	55	45	41	53	58	64	60
OZ1	77	83	49	43	64	46	40	61	58
OZ2	72	41	71	49	44	58	58	66	57
IZ1	51	69	48	75	48	67	67	48	59
IZ2	46	61	71	68	42	**	95	86	67
Muscle	57	15	33	65	65	31	50	52	46
Plasma	155	152	145	151	150	120	121	149	143
Urine	41	31	30	65	42	**	**	11	37

(no samples = **)

Table 11

SOIDUM CONCENTRATIONS IN RENAL TISSUE SLICES, PLASMA AND URINE

DEHYDRATED SQUIRRELS

mEq./l

Squirrel No.	18	19	21	22	23	mean
Right Kidney						
C1	46	56	72	62	81	61
C2	55	65	74	38	86	64
OZ1	66	94	92	58	147	91
OZ2	78	139	121	73	**	103
IZ1	118	284	147	94	264	181
IZ2	181	426	328	75	390	280
Left Kidney						
C1	50	51	91	47	78	63
C2	36	81	68	47	91	65
OZ1	85	116	83	44	156	97
OZ2	68	151	112	60	**	98
IZ1	94	410	66	73	238	176
IZ2	193	546	128	84	352	261
Muscle	27	29	33	25	44	31
Plasma	148	152	145	156	162	153
Bladder Urine	4	166	42	10	**	44

(no samples = **)

Figure 7. Scatter diagram of sodium concentrations in kidney tissue slices of hibernators and anti-diuretic homeotherms. Straight line regression drawn by the equation $Y = \bar{y} + b(\bar{x} - x)$, where b = 1.889 and 0.670 for the right and left kidneys of the hibernators and b = 41.651 and 38.309 for the right and left kidneys of the anti diuretic homeotherms.

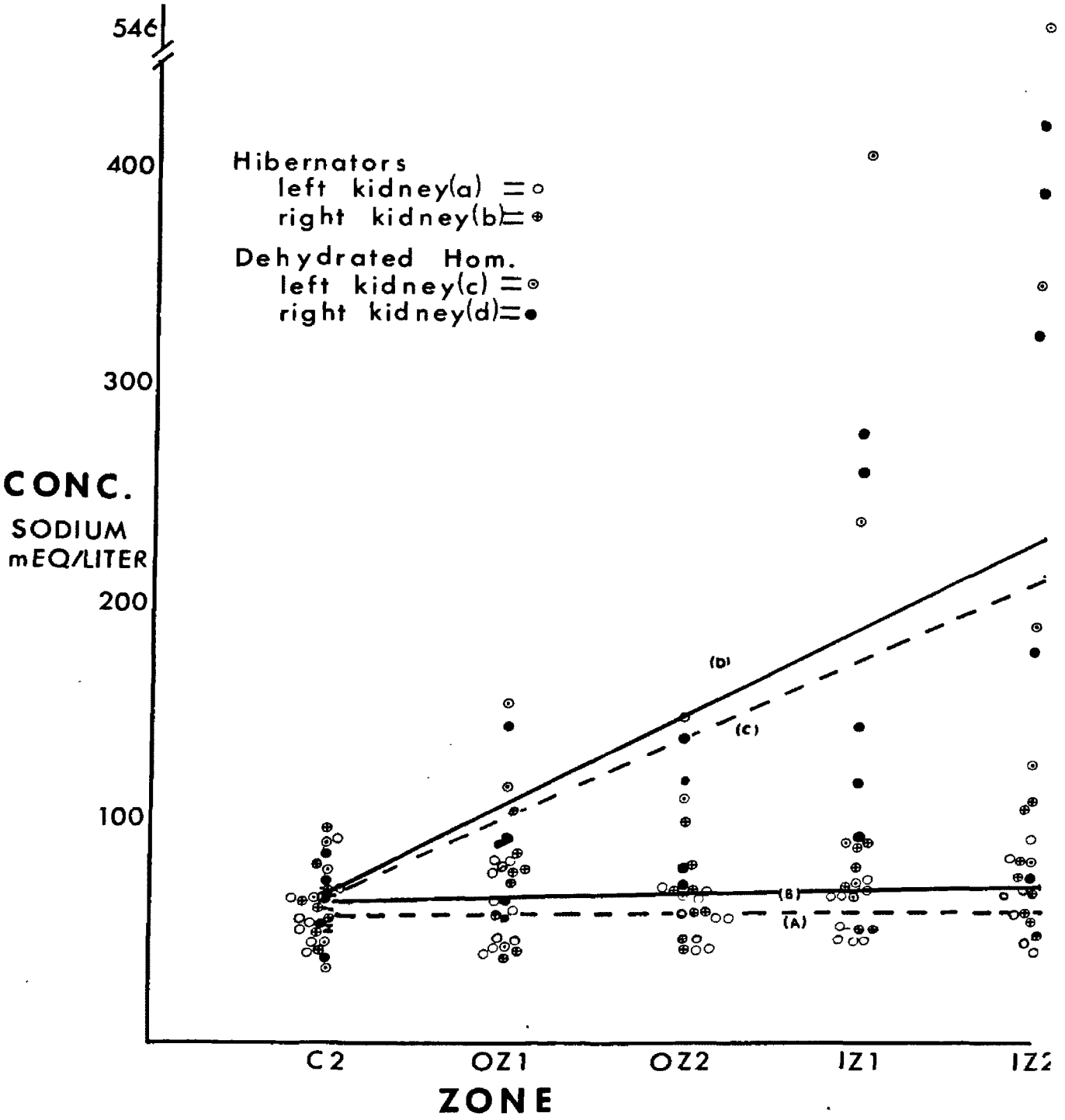


Table 12

SODIUM TF/P AND PLASMA/MUSCLE RATIOS

HIBERNATING SQUIRRELS

Right Kidney

Squirrel No.	3	4	5	7	8	9	10	12	mean
C1	1.72	3.93	2.57	1.63	.83	1.48	.96	.81	1.74
C2	1.39	4.20	1.85	1.48	1.06	1.29	1.02	1.06	1.67
OZ1	1.39	5.80	2.18	1.63	.89	1.32	1.56	.65	1.92
OZ2	1.05	4.67	2.45	1.55	.94	1.35	1.40	.92	1.79
IZ1	1.40	**	2.70	1.40	.81	1.71	1.42	1.00	1.69
IZ2	1.00	3.80	3.39	1.69	1.17	1.93	1.00	1.33	1.91
Left Kidney									
C1	1.70	5.43	1.90	.72	.82	1.45	1.08	.96	1.74
C2	1.23	6.20	1.67	.69	.63	1.71	1.16	1.23	1.81
OZ1	1.35	5.53	1.48	.66	.98	1.48	.80	1.17	1.68
OZ2	1.26	2.73	2.15	.75	.68	1.57	1.16	1.27	1.48
IZ1	.89	4.60	1.45	1.15	.74	2.16	1.34	.92	1.66
IZ2	.81	4.06	2.15	1.05	.65	**	1.90	1.65	1.72
P/M	2.72	10.13	4.39	3.23	2.31	3.87	2.42	2.86	3.88

(no samples = **)

Table 13

SODIUM TF/P AND P/M RATIOS

DEHYDRATED SQUIRRELS

Squirrel No.	18	19	21	22	23	mean
Right Kidney						
C1	1.70	1.93	2.18	2.48	1.86	2.03
C2	2.04	2.24	2.24	1.52	1.97	2.00
OZ1	2.44	3.24	2.79	2.32	3.37	2.83
OZ2	2.89	4.79	3.67	2.92	**	3.57
IZ1	4.37	9.79	4.45	3.76	6.06	5.69
IZ2	6.70	14.69	9.94	3.00	8.94	8.65
Left Kidney						
C1	1.85	1.76	2.76	1.88	1.79	2.00
C2	1.35	2.79	2.06	1.88	2.09	2.03
OZ1	3.15	4.00	2.51	1.76	3.58	3.00
OZ2	2.52	5.21	3.39	2.40	**	3.38
IZ1	3.48	14.14	2.00	2.92	5.46	5.60
IZ2	7.15	18.83	3.88	3.36	8.07	8.26
Plasma/M	5.48	5.24	4.39	6.24	3.71	5.01

Table 14

SODIUM CONCENTRATIONS IN TISSUE SLICES, PLASMA AND URINE

AROUSING URINE FORMERS

mEq./l

Squirrel No.	15	16	17	25	26	mean
Right Kidney						
C1	65	21	46	99	62	59
C2	51	33	55	**	58	49
OZ1	137	50	60	112	50	82
OZ2	121	75	93	146	154	118
IZ1	214	226	123	162	**	181
IZ2	472	343	215	304	228	312
Left Kidney						
C1	80	28	51	47	28	47
C2	68	14	49	50	60	48
OZ1	81	54	59	**	112	76
OZ2	104	105	91	65	85	90
IZ1	128	194	139	99	**	140
IZ2	278	409	241	193	166	257
Muscle	49	23	36	32	58	40
Plasma	140	126	140	131	152	138
Bladder Urine	41	21	**	**	**	31
Cath. Urine 1	24***	25	**	27	39***	29***
Cath. Urine 2		**	25	**		
Cath. Urine 3			40***			

(no samples = **)
(terminal catheter urine samples = ***)

Table 15

SODIUM CONCENTRATIONS IN TISSUE SLICES, PLASMA AND URINE

AROUSING NONURINE FORMERS

mEq/l

Squirrel No.	13	14	mean
Right Kidney			
C1	73	42	57
C2	55	30	42
OZ1	66	39	52
OZ2	71	20	45
IZ1	94	38	66
IZ2	58	27	42
Left Kidney			
C1	58	44	51
C2	61	45	53
OZ1	69	44	56
OZ2	83	46	64
IZ1	90	29	59
IZ2	110	35	72
Muscle	60	60	60
Plasma	146	146	146
Bladder Urine	26	**	26

(no samples = **)

Figure 8. Scatter diagram of sodium concentrations in kidney tissue slices of arousing non urine formers and arousing urine formers. Straight line regression drawn by the equation $Y = \bar{y} + b(\bar{x} - x)$, where b = 0.212 and 3.857 for the arousing non-urine formers right and left kidneys and 48.849 and 36.896 for the right and left kidney of the arousing urine formers.

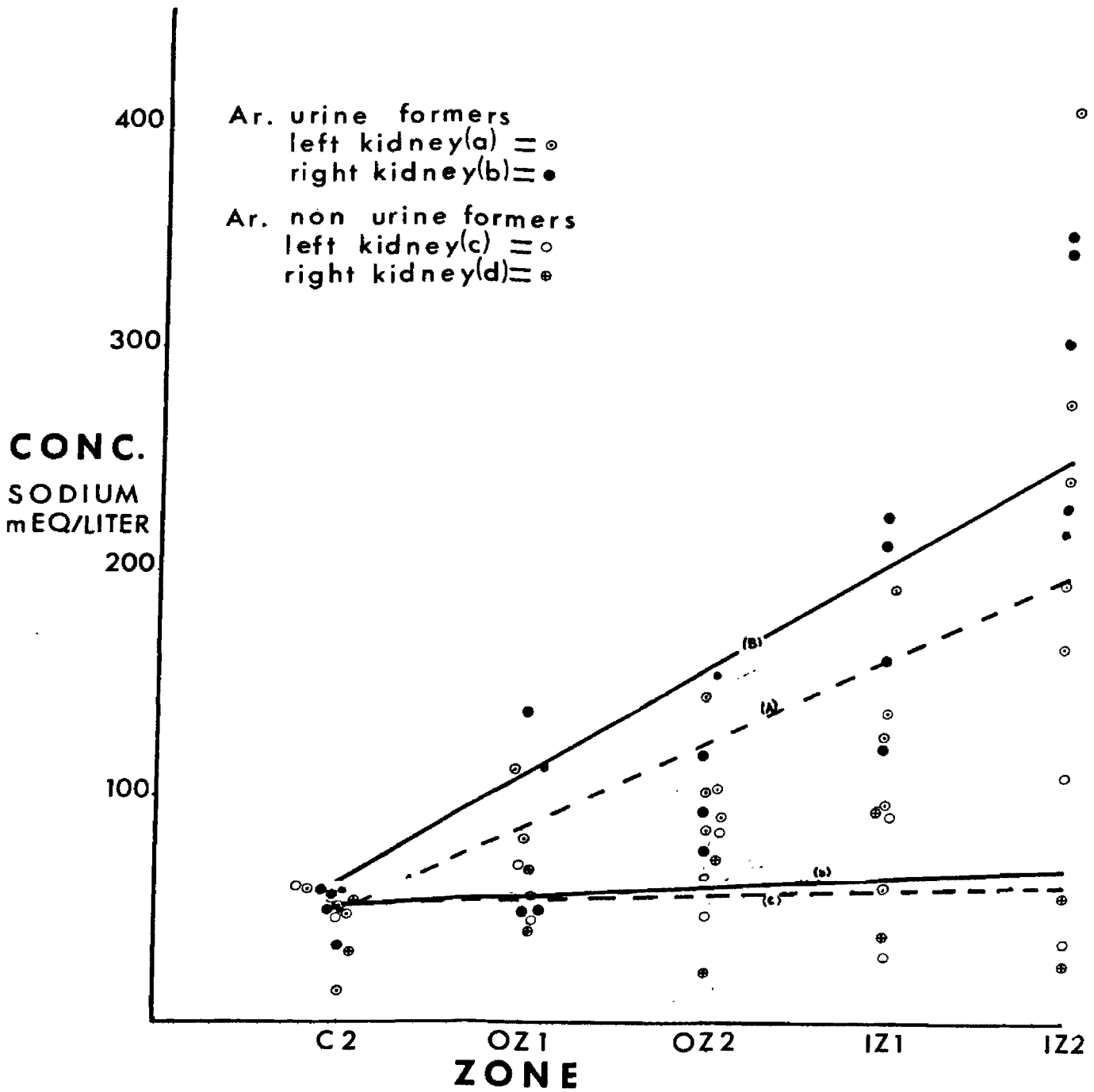


Table 16

SODIUM TF/M AND PLASMA/MUSCLE RATIOS

AROUSING URINE FORMERS

Squirrel No.	15	16	17	25	26	mean
Right Kidney						
C1	1.33	.91	1.28	3.09	1.07	1.53
C2	1.04	1.43	1.53	**	1.00	1.25
OZ1	2.80	2.17	1.67	3.50	.86	2.20
OZ2	2.47	3.26	2.58	4.56	2.65	3.10
IZ1	4.37	9.83	3.42	5.06	**	5.67
IZ2	9.46	14.91	5.97	9.50	3.93	8.75
Left Kidney						
C1	1.63	1.22	1.42	1.47	.48	1.24
C2	1.39	.61	1.36	1.59	1.03	1.20
OZ1	1.65	2.35	1.64	**	1.93	1.89
OZ2	2.12	4.56	2.53	2.03	1.46	2.54
IZ1	2.61	8.43	4.86	3.09	**	4.75
IZ2	5.67	17.78	6.69	6.03	2.86	7.81
Plasma/Muscle	2.86	5.47	3.89	4.09	2.62	3.79

(no samples = **)

Table 17

SODIUM TF/M AND PLASMA/MUSCLE RATIOS

AROUSING NONURINE FORMERS

Squirrel No.	13	14	mean
Right Kidney			
C1	1.22	.70	.86
C2	.92	.50	.71
OZ1	1.10	.65	.87
OZ2	1.18	.33	.75
IZ1	1.56	.63	1.09
IZ2	.97	.45	.71
Left Kidney			
C1	.97	.73	.85
C2	1.02	.75	.86
OZ1	1.15	.73	.94
OZ2	1.38	.77	1.07
OZ1	1.50	.48	.99
IZ2	1.83	.58	1.20
Plasma/Muscle	2.43	2.43	2.43

Figure 9. Scatter diagram of potassium concentrations in kidney tissue slices of hibernators and antidiuretic homeotherms. Straight line regression drawn by the equation $Y = \bar{y} + b(\bar{x} - x)$, where b = 1.026 and 1.130 for the right and left kidneys of the hibernators and b = 44.899 and 2.850 for the right and left kidneys of the antidiuretic homeotherms.

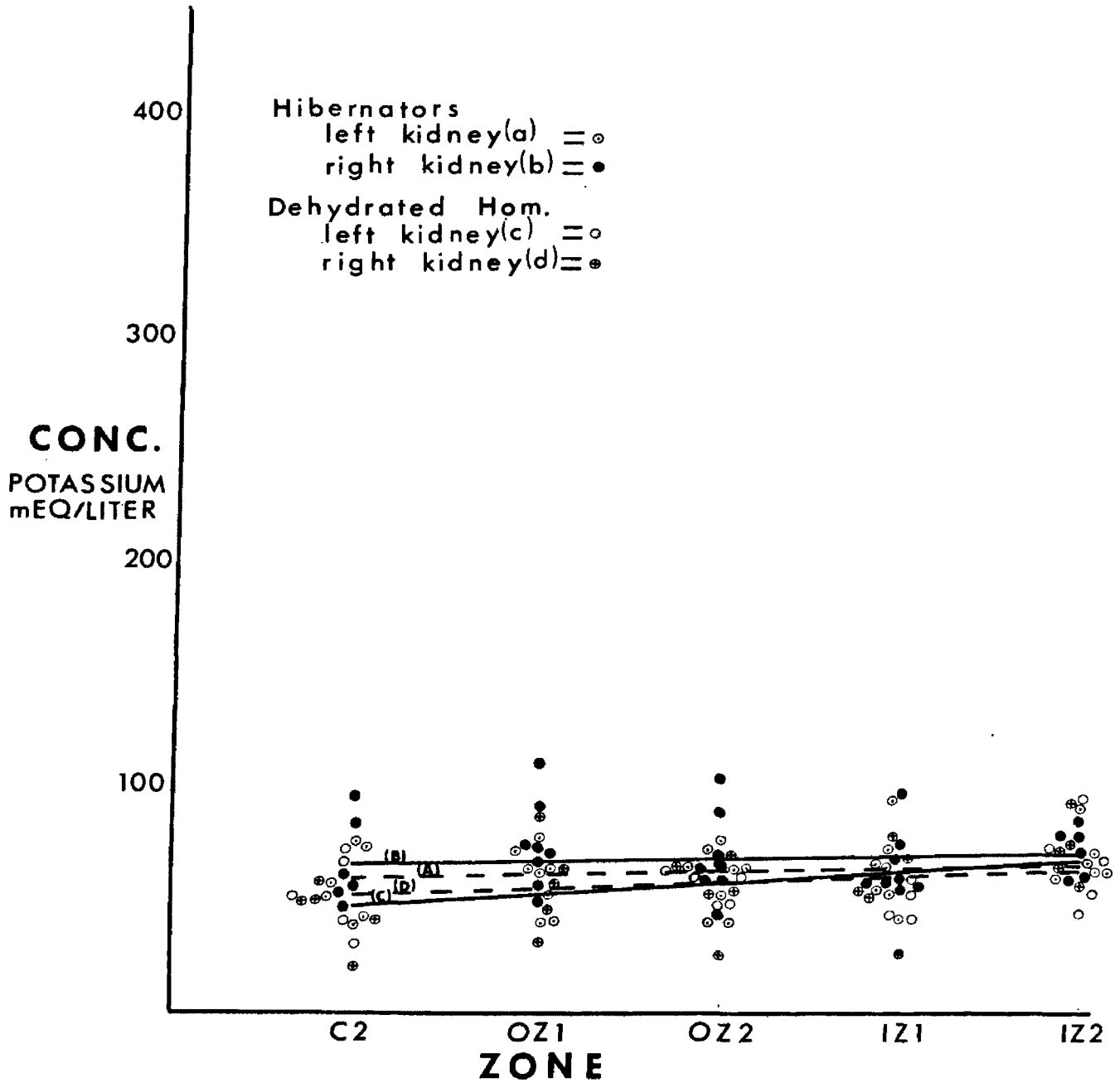


Figure 10. Scatter diagram of Potassium concentrations in kidney tissue slices of arousing nonurine formers and arousing urine formers. Straight line regression drawn by the equation $Y = \bar{y} + b(\bar{x} - x)$, where b = 2.711 and 1.742 for the right and left kidneys of the arousing nonurine formers and b = 1.945 and 1.099 for the right and left kidneys of the arousing urine formers.

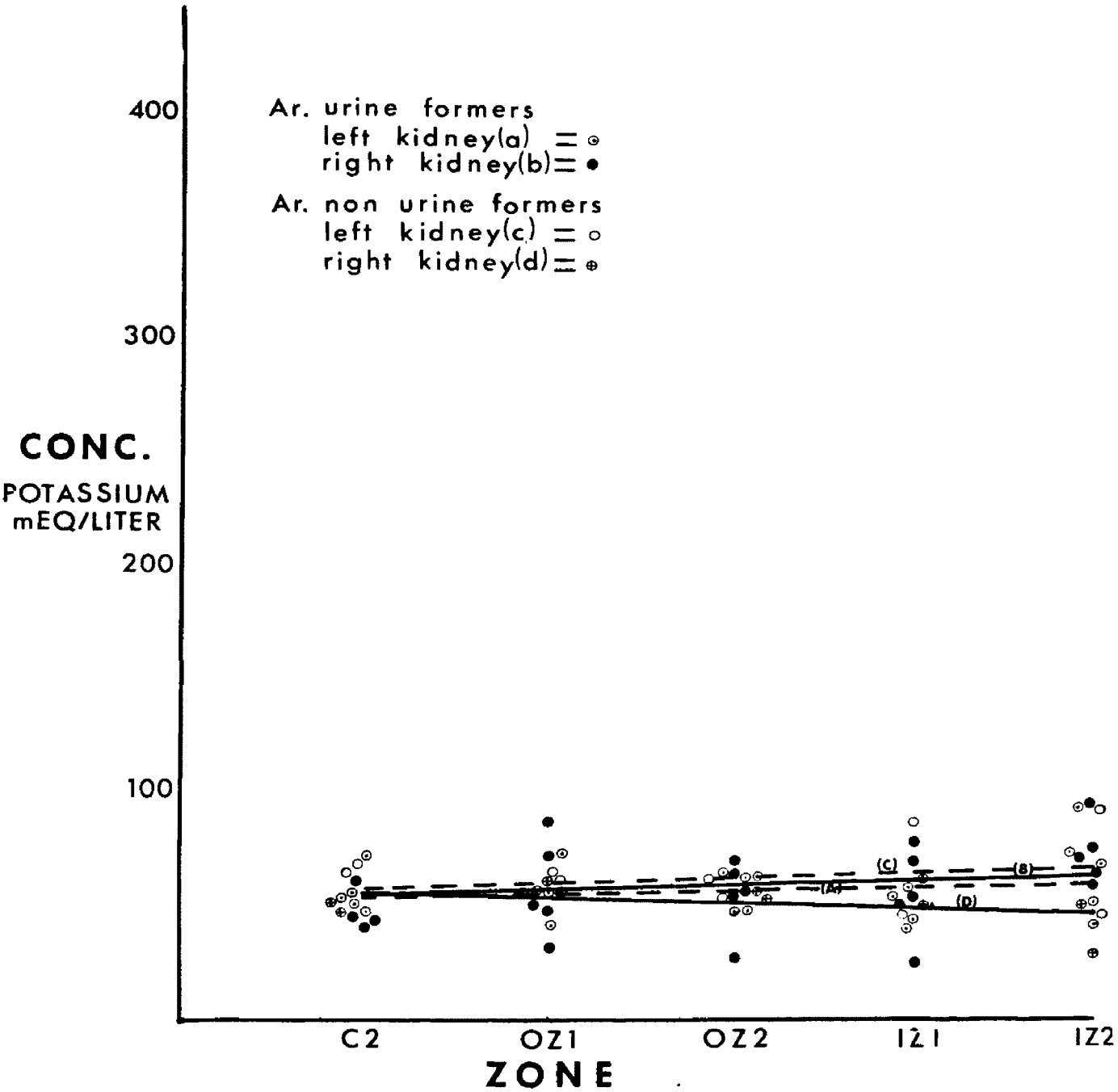


Table 18

POTASSIUM CONCENTRATIONS IN TISSUE SLICES AND PLASMA

HIBERNATING SQUIRRELS

mEq/l

Right Kidney									
Squirrel No.	3	4	5	7	8	9	10	12	mean
C1	*	72	*	62	*	60	58	62	63
C2	*	96	*	61	84	57	47	55	67
OZ1	67	111	90	73	74	50	71	57	74
OZ2	58	104	89	65	68	43	64	59	69
IZ1	62	98	75	59	67	55	61	56	67
IZ2	61	*	86	72	*	60	79	79	73
Left Kidney									
C1	89	72	69	41	*	57	*	65	65
C2	78	58	53	38	*	43	*	74	57
OZ1	64	63	52	39	78	71	38	64	59
OZ2	64	44	71	40	68	65	58	68	60
IZ1	55	94	54	42	74	63	*	66	64
IZ2	91	*	63	67	60	62	*	72	69
Muscle	45	29	30	44	75	*	*	104	54
Plasma	4.5	6.7	5.0	3.5	4.4	8.1	3.4	4.0	4.9

Table 18 (cont.)

POTASSIUM CONCENTRATIONS IN TISSUE SLICES, PLASMA AND URINE

DEHYDRATED SQUIRRELS

mEq/l

Squirrel No.	18	19	21	22	23	mean
Right Kidney						
C1	33	49	47	37	64	46
C2	42	53	53	20	60	46
OZ1	47	65	58	31	87	58
OZ2	53	71	46	41	*	53
IZ1	51	66	52	41	90	60
IZ2	65	89	84	36	88	72
Left Kidney						
C1	48	52	55	30	75	52
C2	39	67	54	29	73	52
OZ1	47	68	52	27	84	56
OZ2	50	57	54	33	*	48
IZ1	86	*	30	33	88	59
IZ2	79	*	67	37	97	70
Muscle	55	70	72	39	*	59
Plasma	5.4	4.9	4.8	6.6	6.5	5.6
Bladder Urine	66	400	>190	285	*	>232

(no samples = *)

Table 18 (cont.)

POTASSIUM CONCENTRATIONS IN TISSUE SLICES, PLASMA AND URINE

AROUSING SQUIRRELS (URINE FORMERS)

mEq/l

Squirrel No.	15	16	17	25	26	mean
Right Kidney						
C1	60	37	50	87	71	61
C2	39	43	62	*	48	48
OZ1	73	49	52	87	31	58
OZ2	56	27	53	64	71	54
IZ1	69	78	54	52	25	56
IZ2	75	93	71	63	57	71
Left Kidney						
C1	75	56	62	47	39	56
C2	58	49	55	72	51	57
OZ1	56	57	56	41	74	57
OZ2	48	61	63	61	50	57
IZ1	43	60	54	41	*	49
IZ2	74	95	68	42	53	66
Muscle	60	67	56	54	56	59
Plasma	4.4	3.5	3.8	4.6	3.7	4.0
Cath. Ur. 1	80**	71	*	*	105**	
Cath. Ur. 2		*	*	*		92**
Cath. Ur. 3			*			

(no samples = *)
(terminal catheter urine sample = **)

Table 18 (cont.)

POTASSIUM CONCENTRATIONS IN TISSUE SLICES, PLASMA AND URINE

AROUSING SQUIRRELS (NON URINE FORMERS)

Right Kidney			
Squirrel No.	13	14	mean
C1	68	57	62
C2	54	47	50
OZ1	57	61	58
OZ2	56	53	54
IZ1	61	50	55
IZ2	30	52	41
Left Kidney			
C1	56	55	55
C2	70	64	67
OZ1	62	64	63
OZ2	60	53	56
IZ1	87	47	67
IZ2	92	46	69
Muscle	76	65	70
Plasma	5.6	3.0	4.3
Urine	277	255	266

Table 19

URINE SODIUM, POTASSIUM, UREA AND OSMOLALITY

IN DEHYDRATED SQUIRRELS

Squirrel No.	T_B °C.	Length of Dehydration in Hours	Urine Urea mM/l	Urine Na mEq/l	Urine K mEq/l	Urine Osmolality in mOsm
+18	37.2	15	430	3.7	66	690
19	36.8	9	1120.5	166	>400	2800
21	36.5	9	555.6	41.8	>190	1410
+22	35.9	9	865.7	10	285	1000
23	37.1	9	831	*	*	1750
MEAN	36.7	10	760	44.5	>232	1530

\+ The gradient had started to break down
* No samples.

Figure 11. Seven hibernating squirrels had their right ureter catheterized and were allowed to arouse normally. The time and rectal temperatures were recorded for the initiation of the arousal process, beginning of urine flow in the uretheral catheter, and at sacrifice. Two squirrels were killed before and five squirrels were killed after urine was observed flowing in the ureteral catheter.

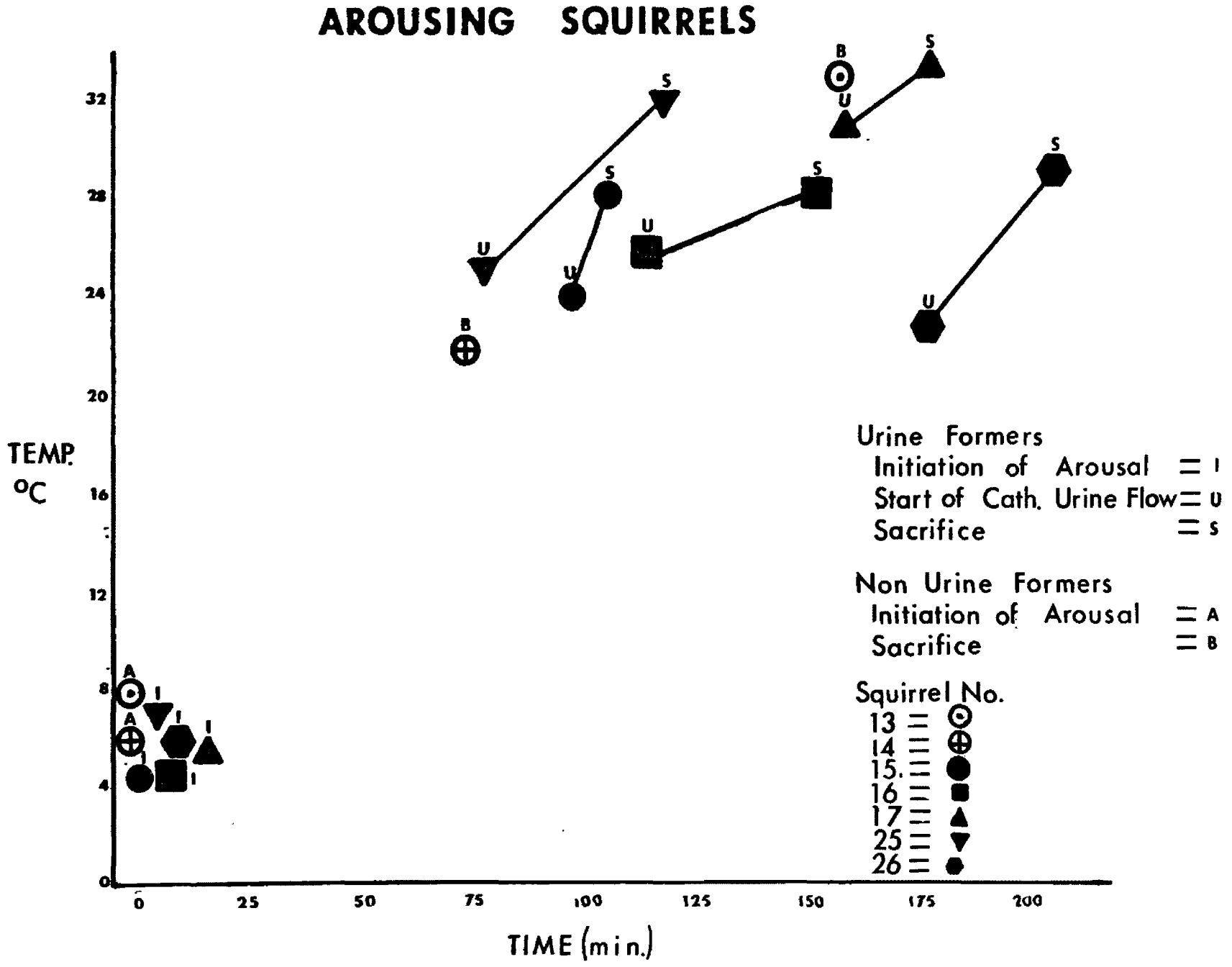
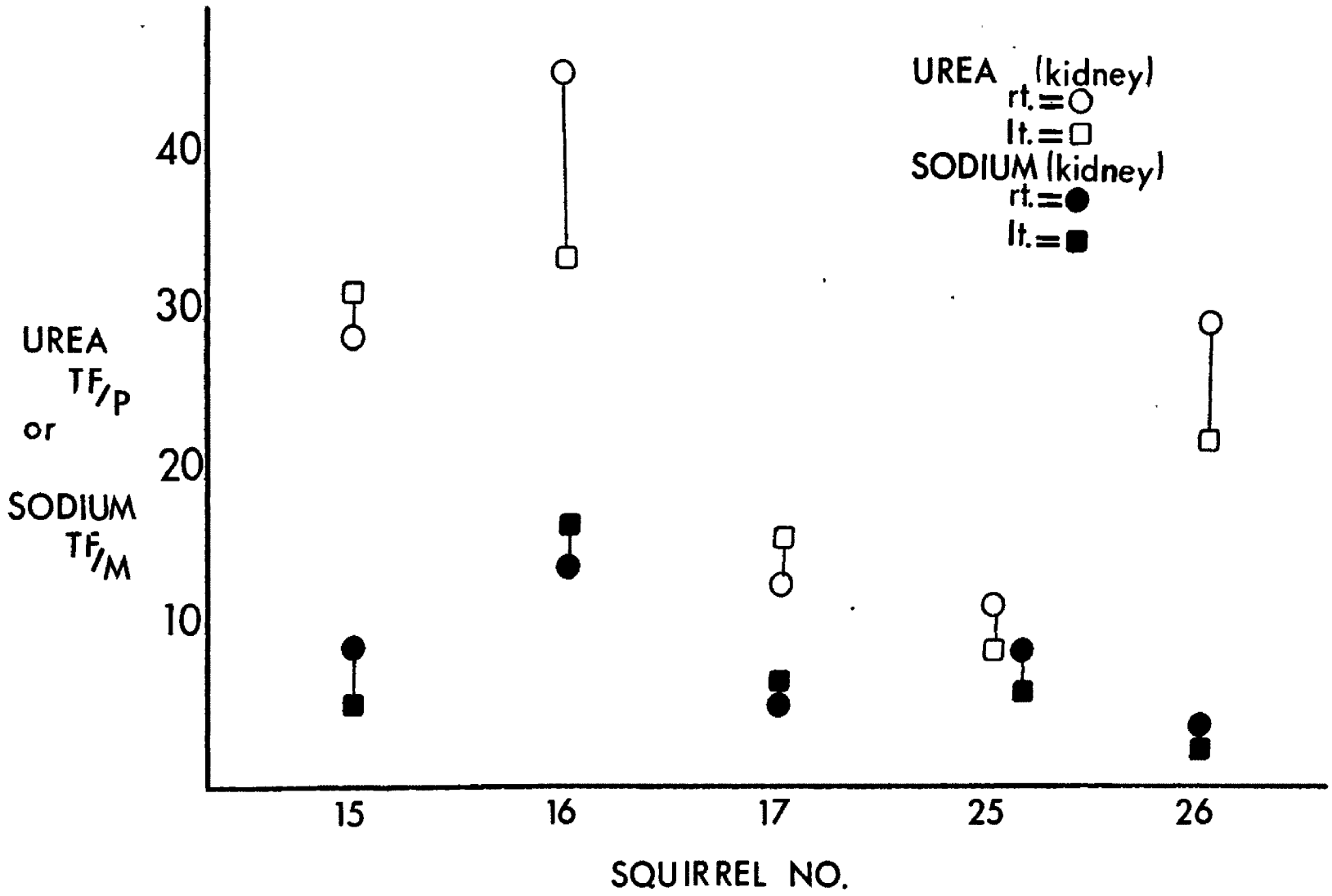


Figure 12. The ratio of papillary tubular fluid urea to plasma urea (IZ2TF/P) was compared to the ratio of papillary tubular fluid sodium to abdominal muscle sodium (IZ2TF/M). The TF/M sodium and TF/P urea ratio were plotted for both kidneys against the animal number. A two-factor analysis of variance indicated that the right and left kidney papillary urea levels of an animal were not significantly different ($P > 0.05$), whereas the papillary urea levels between squirrels differ significantly ($P < 0.05$).

PAPILLARY SODIUM - UREA RELATIONSHIP IN AROUSING SQUIRRELS



DISCUSSION

Many investigators have postulated that the kidneys of a torpid ground squirrel are non-functional or only partially functional (Fisher and Manery, 1965). Pengelley and Fisher (1961) suggested that while the urine production is minimal it is not necessarily unappreciable during hibernation. Lyman (1960) indicated that low diastolic blood pressure (10 to 40 mm Hg) in the aorta of hibernating squirrels would impede glomerular filtration. Zimny and Rigamer (1966) reported that glomerular filtration would also be inhibited by structural changes occurring in the glomeruli. Hong (1957) reported that urine production is minimal in hibernating ground squirrels. Schmidt-Nielsen (1958) postulated that a lack of glomerular filtration would eliminate an urea gradient in the kidney. However, no one has determined the actual role of the kidney during hibernation (Fisher and Manery, 1965). This study showed that ground squirrels sacrificed during the hibernation period possessed no concentration gradient of urea and sodium from the cortex to the tip of the papilla and that a gradient was developed only in those arousing squirrels producing urine.

Since no solute gradient of urea, potassium, and sodium existed in the kidneys of hibernating ground squirrels,

any urine formed during hibernation would be isotonic to the plasma. However, in this study it has been shown that concentrations of urea in the bladder urine of hibernating squirrels are similar to those found by Zimny and Bourgeois (1960) in Citellus tridecemlineatus, by Scott and Fisher (1967) in Citellus richardsoni and Citellus lateralis, and by Passmore (1967) in Citellus columbianus. There was little or no reduction in the urea content of hibernators as compared to the urea content in urine collected from dehydrated active ground squirrels. Furthermore, the concentration of urea in the bladder urine was not correlated with the length of the hibernating period (Passmore 1967) and Fisher and Manery (1965). Therefore, Pengelley and Fisher (1961) suggested that urine production occurs during the short active awake periods and again as the squirrel enters torpor. Passmore's data suggested that the longer the period of torpor, the greater the plasma urea concentration which indicates that no urine is formed to accept the urea. Such an increase in plasma urea might initiate arousal. Squirrel No. 11, sacrificed while reentering torpor at 21°C had well-developed urea and sodium gradients extending from the cortex to papilla, suggesting that the kidneys were still capable of forming a hypertonic urine.

This study showed conclusively that urine starts to form during the arousal process. However, the time interval from the initiation of the arousal process to the actual

observation of urine flow in the catheter varied among arousing squirrels. Also the rectal temperature at first urine flow varied among arousing squirrels. These data are consistent with those for blood pressure changes during arousal. Lyman (1960) reported that the systolic blood pressure can exceed 180 mm Hg before the temperature of the heart reaches 37°C in Citellus tridecemlineatus, indicating that the blood pressure could be high enough for glomerular filtration to occur before the completion of the arousal process.

Two arousing non-urine formers, Nos. 13 and 14, did not produce urine in the ureteral catheter and did not have urea or sodium gradients in their kidneys. Presumably, the blood pressure was still too low for glomerular filtration and/or some other factor was needed to increase the permeability of the glomeruli for filtration. The two nonurine formers were sacrificed 70 and 152 minutes after the onset of the arousal process. Squirrel No. 14 (rectal temperature of 33°C) did not form any urine after 152 minutes of arousing.

Five arousing squirrels (rectal temperatures ranging from 23° to 31°C) with their right ureter catheterized, produced urine 90 to 135 minutes after the onset of the arousal process and had distinct urea and sodium gradients. Squirrel No. 15, sacrificed only 15 minutes after urine was observed flowing through the right ureteral catheter, had an extremely well-developed renal gradient of solutes,

whereas squirrels No. 17 and 25, sacrificed 40 minutes after urine flow began, had considerably smaller sodium and urea gradients. It is quite evident that the five arousing urine formers restored their urea and sodium gradients but at different rates, presumably only after the beginning of glomerular filtration. Furthermore, squirrels Nos. 16 and 25, both sacrificed 40 minutes after urine was first noticed flowing in the catheter, had different papillary urea TF/P ratios (Table 8). Perhaps the squirrel's ability to rebuild the solute gradient depends upon such factors as the length of time the squirrel remains in deep torpor and/or the extend of dehydration of the squirrel. The urea and sodium gradients developed simultaneously as the hibernating squirrels began to arouse, but apparently not until glomerular filtration occurs.

Catheterization of the right ureter had little effect on the sodium and urea gradients in the right kidney as compared to the left kidney. The onset of urine flow in squirrel No. 26 was delayed when the squirrel was placed on an ice bath for 1/2 hour immediately after being removed from the refrigerator. Recently during a different study in this laboratory many hibernating squirrels have been placed on an ice bath and allowed to arouse and, in each case, onset of urine formation took longer than in squirrels that were not cooled but allowed to arouse normally. Therefore, it seems reasonable to assume that formation

of urine is not directly correlated with time, but with blood pressure.

Two or three consecutive catheter urine samples were collected from three arousing ground squirrels. The catheter urine urea and sodium levels showed a progressive rise in concentration indicating increased urine concentration and a progressive rise in the gradient of renal solutes in all squirrels. The concentrations of sodium and urea in the catheter urine samples were always lower than those found in the bladders of dehydrated and hibernating squirrels. This is almost certainly due to low osmolality of the urine, although direct measurements of osmolality were not made.

As a ground squirrel arouses from torpor, restoration of the solute gradient must be rapid to conserve body water. Lever (1965) postulated that mammals faced with dehydration, similar to the condition of the Columbian ground squirrel during hibernation will reabsorb urea in the kidney to help control water loss. Schmidt-Nielsen (1961) and Bray and Preston (1961) postulated that urea is pulled out of the collecting ducts into the descending loop of Henle and recirculated increasing the hypertonicity of the papillary tissue fluid in antidiuretic mammals. However, the actual transport mechanism of urea remains obscure.

The urea ratio of papillary fluid to terminal catheter urine (IZ2TF/U) in the arousing urine formers is always greater than unity, whereas the urea ratio of papillary

fluid to bladder urine (IZ2TF/U) in the dehydrated squirrels is always less than unity, suggesting that urea is being treated differently in the two groups. The kidneys of the arousing squirrels might be actively transporting urea from the collecting ducts into the descending loop of Henle. This could account for the higher urea concentration in the tip of papilla than in the urine. However, the terminal catheter urine sample was collected over a 15-minute period and therefore the IZ2TF/U may not represent the true concentration of urea in urine at the time the kidney was removed. Data from squirrel No. 17 further suggested active transport of urea. The urea concentration in the third catheter urine sample was lower than that of the second sample indicating that the squirrel produced urine with a lower urea concentration than that found in the papillary tissue fluid. Although more data are needed, the evidence favors the view that during arousal the renal tubules reabsorb urea against a concentration gradient while in dehydrated homeothermic squirrels, urea apparently moves into the medullary tissue fluid by diffusion only.

The urea concentration in the plasma of hibernating, arousing and dehydrated squirrels did not differ significantly in Citellus columbianus. However, in hibernating ground squirrels the plasma urea levels varied considerably, increasing as the length of the hibernating period increased. These findings are in agreement with those of Passmore (1968)

in Citellus columbianus, and Kristofferson (1963), who reported similar findings in the hedgehog (Erinaceus europaeus).

The sodium concentrations in the plasma of hibernating squirrels were not significantly different from those of dehydrated and the arousing squirrels. These findings are supported by Biorek et al., (1956). Although, other studies of hibernating squirrels showed that the plasma sodium concentration may increase, decrease, or remain the same relative to normal homeothermic squirrels (see Manery and Fisher, 1956). This study indicated that a significant difference only exists between plasma sodium concentrations of arousing and dehydrated squirrels. Apparently the internal osmotic environment may vary among squirrels thereby affecting the sodium levels in the plasma.

The potassium concentrations in the plasma also did not differ significantly in arousing, dehydrated and hibernating squirrels, but the dehydrated squirrels' plasma potassium concentration was significantly higher than that in arousing squirrels.

SUMMARY

1. No urine is produced in the ground squirrel (Citellus columbianus), during deep hibernation.

2. Urine formation begins during the arousal process.

3. No potassium gradient exists in the kidney at any time.

4. No sodium or urea gradient exists in the kidney while the animal remains in deep hibernation, and the gradients probably do not develop until after the beginning of glomerular filtration.

5. My data suggest that urea is actively transported out of the collecting ducts into the papillary tissue fluid as the squirrel begins to rebuild its medullary solute gradient of urea and sodium, but more studies are needed to determine this conclusively.

6. The sodium and urea gradients seem to develop simultaneously in the arousing squirrel.

7. Plasma potassium and sodium concentrations did not differ significantly between the arousing, hibernating and dehydrated ground squirrels.

LITERATURE CITED

Amon, H. 1967. Untersuchungen an der Niere des Siebenschlafers (Glis glis L.) im Winterschalf und im sommerlichen Wachzustand--IV. Electronenmikroskopische Befunde an Glomerula von Jungtieren. Z. Zellforsch. 76, 349-354. Cited by: Zimny, M. L. 1968. Glomercular Ultrastructure in Kidneys from some Northern Mammals. Comp. Biochem. Physiol., 1968, Vol. 27, pp. 859-863.

_____, Kuhnel, W. and Pelry, U. G. 1965. Untersuchungen an der Niere des Siebenschlafers (Glis glis L.) im Winterschalf und im sommerlichen Wachzustand--III. Die glomerulare Permeabilitatsslugerung und ihre submikroskopische Grundlage. Z. Zellforsch. 65, 777-789. Cited by: Zimny, M. L. 1968. Glomercular Ultrastructure in Kidneys from some Northern Mammals. Comp. Biochem. Physiol., 1968, Vol. 27, pp. 859-863.

Beers, J. and Richards, A. G. 1956. Hibernation in the big brown bat. J. Mammalogy. 37, 31-41.

Benedict, F. G. and Lee, R. C. 1938. Hibernation and marmot physiology. Carnegie Institute of Wash. Publ. 497, Waverly Press, Baltimore.

Berliner, R. W., N. G. Levinsky, D. G. Davidson and M. Eden. 1958. Dilution and concentration of the antidiuretic hormone. Am. J. Med. 24, 730-744.

Biorck, G., Johansson, B. W. and Verge, S. 1956b. Some laboratory data on hedgehogs, hibernating and non hibernating. Acta. Physiol. Scand. 37, 281-294.

Bray, G. A. and Agnes S. Preston. 1961. Effect of urea on urine concentrations in rats. J. Clin. Invest. 40, 1952-1960.

Bullard, R. W. 1964. Changes in regional blood flow and blood volume during arousal from hibernation. Ann. Acad. Sci. Fenn. Series A, IV, 71, 65-76.

Carpenter, T. M. 1938. The partition of urinary nitrogen of fasting and hibernating woodchucks (Arctomys monax). J. Biol. Chem. 122, 343-347.

Christian, J. J. 1961. Potassium deficiency in marmots during hibernation. Science. 134, 390.

Clapp, J. R. 1966. Renal tubular reabsorption of urea in normal and protein depleted rats. Am. J. Physiol. 210, 1304-1308.

Eliassen, E. 1963. The serum and potassium contents of serum and urine in the hibernating hedgehog. Arbok Univ. Bergen, Mat.-Nat., 6.

Eliassen, E. and Leivestad, H. 1961. Sodium and potassium content in the muscles of hibernating animals. Nature, Lond. 192, 459-460.

Fisher, K. C. 1964. On the mechanism of periodic arousal in the hibernating ground squirrel. Ann. Acad. Sci. Fenn., Ser. A. IV, 71, 141-156.

Fisher, K. C. and J. Manery. 1965. Water and electrolyte metabolism in Heterotherms. International Symposium in Natural Mammalian Hibernation. 3d. 235-239.

Goldberg, M. 1967. Uphill transport of urea in the dog kidney: effects of certain inhibitors. J. Clin. Invest. 46, 388-399.

Gottschalk, C. W. and Margaret Mylle. 1959. Micropuncture study of the mammalian urinary concentration mechanisms: evidence for the counter current hypothesis. Am. J. Physiol. 196, 927-936.

Hoffman, R. A. 1964. Speculations on the regulation of hibernation. Ann. Acad. Sci. Fenn. Ser. A, IV, 71, 199-216.

Hong, S. K. 1957. Renal function during hypothermia and hibernation. Am. J. Physiol. 188, 137-150.

Jamison, R. L., C. M. Bennet and R. W. Berliner. 1967. Counter current multiplication by the thin loops of Henle. Am. J. Physiol. 212, 357-366.

Jaenike, J. R. 1964. The relative rates of urea and water permeation in the distal nephron in the intact kidney. J. Clin. Invest. 43, 45-55.

Johnson, G. E. 1931a. Hibernation in mammals. Q. Rev. Biol. 6, 439-461.

Kayser, C. 1952a. La depense d'energie des mammiferes hibernants pendant toute la duree da l'hibernation. Archs. Sci. Physiol. 6, 193-212.

_____, C. 1961. The physiology of natural hibernation. Pergamon Press. New York.

_____, 1965. In physiological mammalogy II. Academic Press. New York.

Klar, E. 1938. Zur kenntnis des chemismus des Wintershlafs. Z. ges. exp. med. 104, 105-115. Cited by: J. S. Willis. 1965. Cold adaptation of activities of tissues of hibernating mammals. International Symposium on Natural Mammalian Hibernation. 3rd, 356-381.

Kristofferson, R. 1963. Urea levels in blood and tissues of hibernating and nonhibernating hedgehogs. Nature Lond. 197, 402-403.

Lassiter, W. E., C. W. Gottschalk and M. Mylle. 1961. Micropuncture study of net trans tubular movement of water and urea in nondiuretic mammalian kidney. Am. J. Physiol. 200, 1139-1146.

Lever, A. F. 1965. The basa recta and counter current multiplication. Acta. Med. Scand. Vol. 178, Supp. 434.

Levinsky, N. G., D. G. Davidson and R. W. Berliner. 1959. Effects of reduced glomercular filtration on urine concentrations in the presence of antidiuretic hormone. J. Clin. Invest. 38, 730-740.

Lyman, C. P. and Dawe, A. R. (eds.) 1960. Mammalian hibernation. Proceedings of the first international symposium on natural mammalian hibernation. Bull. Mus. comp. Zool. Horv. 124.

Passmore, J. 1968. Urea excretion in the hibernating Columbian ground squirrel. Master's Thesis, University of Montana, Missoula, Montana.

Pengelley, E. T. and K. C. Fischer. 1961. Rythmical arousal from hibernation in the golden-mantled ground squirrel. (Citellus lateralis tescorum). Can. J. Zool., 39, 105-120.

Popovic, V. P. 1964b. In discussion following paper by Fisher, 1964. On the mechanism of periodic arousals in hibernating ground squirrels. Ann. Acad. Fenn. Ser. A, IV, 71, 141-156.

_____, 1955. De l'hypothermie lethargique prolongue. Archs. Sci. Physiol. Paris. 9, 219-229.

Raths, P. 1962. Ueber das Serum-Natrum-Koleum and Kalzium des winter schlafenden und hypothermischen Hamsters. Z. Biol. 113, 113-204.

Schmidt-Nielsen, B. 1955. Urea excretion in white rats and kangaroo rats as influenced by excitement and diet. Am. J. Physiol., 181, 131-139.

Schmidt-Nielsen, B., K. Schmidt-Nielsen, T. R. Houpt, and S. A. Jarum. 1957. Urea excretion in the camel. Am. J. Physiol. 188, 477-484.

Schmidt-Nielsen, B. and R. O'Dell. 1958. Effect of diet on distribution of urea and electrolytes in kidneys of sheep. Am. J. Physiol. 197, 856-860.

Schmidt-Nielsen, B. 1958. Urea excretion in mammals. Physiological Rev. 38, 139-168.

Schmidt-Nielson, B. and Roberta O'Dell. 1959a. Distribution and active uptake of urea in renal tissues. (Abstr.) Fedn. Proc., 18, 138.

_____. 1961. Interdependence of urea and electrolytes in production of a concentrated urine. Am. J. of Physiol. Vol. 200:No. 6.

Scott and Fisher. 1967. (Unpublished). Cited by: Fisher, K. C. and Manery, J. 1965. Water and electrolyte metabolism in heterotherms. International Symposium on natural mammalian hibernation. 3d, 235-279.

Shaw, W. T. 1925e. The food of the ground squirrel. Amer. Nat. 59, 250-264.

Smith, H. W. 139-40. Physiology of renal circulation. The Harvey Lectures. 35, 166.

Stefanovic, M. P. 1954. Sur la composition chimique du sang du rats dans l'hypothermie lethargique. Bull. Soc. Chim. Biol. 36, 1151-1161. Cited by: Fisher, K. C. and J. Manery. 1965. International Symposium on natural mammalian hibernation. 3ed, 235-279.

Suomalaeinen, P. 1960. Stress and neurosecretion in the hibernating hedgehog. Bull. Mus. Comp. Zool. Harv. 124, 271-283.

Truniger, B. and Bodel Schmidt-Nielsen. 1964. Intrarenal distribution of urea and related compounds: effects of nitrogen intake. Am. J. Physiol. 207, 271-978.

Willis, J. S. 1964a. Potassium and sodium content of tissues of hamsters and ground squirrels during hibernation. Science. 146, 546-547.

_____. 1964b. Uptake of potassium at low temperatures in kidney cortex of hibernating mammals. Nature, London. 204. 691-693.

Wirz, H. 1953. Der osmotische durch des blute in der nieron papille. Helv. Physiol. Pharmac. Acta. 11, 20-29.

Wirz, H. B. Hargity, and W. Kuhn. 1951. Lokalisation des Kunzentrierungs prozesses in der Niere durch direkte Kryoskopie. Helv. Physiol Pharmac. Acta. 9, 196-207.

Zimny, M. L. and C. Bourgeois. 1960. Histochemical localization of some enzymes in the kidney of hibernators. J. Cell Comp. Physiol. 56, 93-101.

Zimny, M. L. and Rigamer, E. 1966. Clomercular ultrastructure in the kidney of a hibernating animal. Anat. Rec. 154, 87-94.