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THE INFLUENCE OF PROTEIN INTAKE

ON

RENAL FUNCTION IN THE PORCUPINE (ERETHIZON DORSATUM)

by

Lowell D. Neudeck

B.A. UNIVERSITY OF MONTANA, 1964

Presented in partial fulfillment of the requirements for the degree of

Master of Arts

UNIVERSITY OF MONTANA

1968

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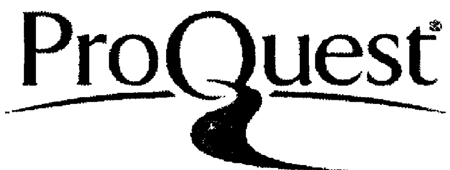

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TABLE OF CONTENTS

PAGE

INTRODUCTION 1

MATERIALS AND METHODS 8
- Animals 8
- Test Diets 8
- Preparation 11
- Slice Technique 12
- Blood and Urine Collection 12
- Tissue Analysis 15
- Tissue Dilution Factors 16
- Anatomical Studies 16
- Statistical Analysis 17

RESULTS 18
- Renal Anatomy 18
- Urea Concentrations 18
  - Renal Tissue Slices 18
  - Urine 18
  - Plasma 26
  - Serum 26
- Sodium and Potassium Concentrations 26
  - Renal Tissue Slices 26
  - Urine 35
  - Plasma 35
  - Serum 35
- Ammonia Concentrations 35
  - Urine 35
- Osmolalities and Urine-Plasma Ratios 35
  - Urine 35
- Tissue Water Content 38
- Weight Loss 38

DISCUSSION 41

SUMMARY 46

LITERATURE CITED 47

LIST OF TABLES

TABLE | PAGE

1. Composition of Diets 9
2. Urea Concentrations in Tissue, Plasma, Serum, and Urine 23
3. Sodium Concentrations in Tissue Slices, Plasma, Serum, and Urine 29
4. Potassium Concentrations in Tissue Slices, Plasma, Serum, and Urine 32
5. Plasma, Urine, and Serum Osmolalities: U/P Ratios . . . 36
6. Terminal Urine Ammonia Concentrations 37
7. Percent Weight Loss During Dehydration 39
8. Tissue Water Content 40

LIST OF FIGURES

FIGURE PAGE

1. External lateral drawing of the porcupine kidney 14
2. Frontal drawing of the porcupine kidney 14
3. Mid-saggital drawing of the porcupine kidney 14
4. Transverse section of the porcupine kidney 20
5. Schematic drawing of transverse section of the porcupine kidney 20
6. Scatter diagram of renal urea concentrations 22
7. Scatter diagram of renal sodium concentrations 28

INTRODUCTION

Mammals excrete salt and their principle nitrogenous product, urea, almost entirely through the kidney (Bray and Preston, 1961), and mammals are unique in that, regardless of habitat, urea remains the main nitrogenous waste. During times of water conservation, the kidney must reduce water loss. If mammals could not make the urine hyperosmotic to the blood during periods of water shortage, the loss of water would be deleterious (Schmidt-Nielsen, 1962). For these reasons, Schmidt-Nielsen (1962) postulated that the countercurrent system developed in urea excretors with a need for water conservation.

In 1934, Gamble, *et al*., first suggested that urea can make the urine more concentrated when it is the major solute. They found that an equivalent load of urea, by contrast with several inorganic salts, was excreted in a substantially smaller urine volume, i.e., less water was required to excrete urea. Because Gamble's animals were allowed water *ad libitum*, others (Gilman, 1937) suggested that his experiments may have indicated the influences of urea and electrolytes on thirst rather than on water requirements. Radford (1959) and, particularly, Crawford, *et al*. (1959), countered criticism of Gamble's work by showing that the rat requires less water to excrete urea than is required to excrete for nonurea solutes and that by elevating urea in the urine it may also reduce the amount of water necessary to excrete nonurea solutes.

Mammals use electrolytes and urea, the major urinary solutes, to create the osmotic gradient in the renal medulla. This gradient of urea concentration begins in the tissues of the cortex and increases through the medulla of antidiuretic animals (Ullrich and Jarausch, 1956; Levinsky, *et al*., 1959).

As early as 1959, Schmidt-Nielsen and O'Dell posited that urea added to the electrolytes in the medulla enabled the kidney to concentrate urine by elevating the osmotic pressure of the medullary tissue, thereby creating a higher diffusion gradient to enhance reabsorption of water from the collecting ducts. Micropuncture studies of Lassiter, *et al*. (1961), showed that approximately one half of the filtered urea leaves the proximal tubule of the nondiuretic rat. However, the early distal convoluted tubules contain as much or more urea than was filtered, which suggests that it enters the descending limb of the loop of Henle. Ginnebault, *et al*. (1966), found that large amounts of urea are added to the loop of Henle in a nondiuretic desert rodent, *Meriones showi*. By means of small polyethylene catheters, Hilger, *et al*. (1958), reported that fluid traveling down the collecting ducts was concentrated by reabsorption of not only water, but of solutes, chiefly sodium ions and urea, as well. Thirty-five percent of the filtered urea diffuses out of the collecting ducts (Hilger, 1958). The studies of Lassiter, *et al*. (1961), and Gottschalk, *et al*. (1963), support Hilger's assumption that urea is recirculated through Henle's loop and distal tubules. Apparently that urea added to the loop fluid and recirculated, comes from the collecting ducts (Gottschalk, 1959; Ullrich, 1963; Lassiter, 1961). Berliner (1958) postulated that the countercurrent exchanger system of the vasa recta concentrates urea in the medulla. Jaenike (1964) also emphasized the importance of the vasa recta in retaining medullary fluid ur which adds to the total solute in the tissue fluid. Apparently this trapped and recirculated urea adds to the other osmotically active solutes in the renal interstitium which creates the medullary osmotic gradient.

The importance of urea in concentrating urine appears well established (Schmidt-Nielsen, 1958). However, researchers do not agree how urea is concentrated in the mammalian renal medulla. The current theory of passive diffusion (Berliner, 1958; Levinsky, et al., 1959; Lassiter, 1963) asserts that tubular fluid entering the collecting duct during antidiuresis has a urea concentration many times higher than the glomerular filtrate and, therefore, the plasma. The osmotic gradient, created by the sodium pump of the countercurrent system, causes water to move across the tubular wall into the medulla. The loss of water further concentrates urea in the fluid until a urea gradient, in the tubular fluid, is created which allows urea to diffuse passively into the medulla. Schmidt-Nielsen and O'Dell (1958) suggest that the thick ascending limb of Henle's loop actively reabsorbs urea. Schmidt-Nielsen (1958) postulated that urea is actively transported either into the loop of Henle or out of the collecting duct and would, therefore, be concentrated by the multiplier system of Henle's loop and the exchanger system of the vasa recta. In 1959, Schmidt-Nielsen and O'Dell postulated that in the sheep kidney, active tubular regulation occurred in the thick ascending limb of the loop of Henle. Bray and Preston (1961) suggested that the mammalian collecting duct actively reabsorbed urea; and Truniger and Schmidt-Nielsen (1964) concurred.

Schmidt-Nielsen has received support for her active transport theory. Clapp (1966) reported that while the ratio of the inulin in the renal tubular fluid to plasma (TF/P) was rising in the collecting ducts of low protein fed rats undergoing mannitol diuresis, the urea TF/P ratio was dropping. The net loss of urea from the collecting ducts, against a

higher papillary concentration, suggests active transport. Lassiter, et al. (1966), reported urea concentrations in the vasa recta that were 10% higher than in the collecting duct fluid at the same level of the papilla and concluded that some area in the papilla between the cortical distal tubule and the end of the collecting duct probably reabsorbed the urea. Goldberg, et al. (1966 and 1967), obliterated the normal gradient of electrolytes from the kidney and thereby the reabsorption of free water from the collecting ducts. Under these conditions the corticomedullary gradient not only persisted but the concentration of urea was higher in the papilla than in the urine.

Pfeiffer (1968) suggested that certain structures in the medulla and pelvis may play a role in the exchange of urea, sodium chloride, and water between the medullary interstitium and the pelvic urine. He places mammalian pelvic types into two groups, 1) pelves without fornices and secondary pyramids (Type I) and 2) pelves with fornices and secondary pyramids (Type II). The Type I pelvis (Aplodontia, single calyx; the beaver and pig, multiple calyces) has no folds or extensions which form fornices; the medulla is covered with a thick, transitional epithelium; and only the medullary outer zone is present. The Type II pelvis (rat, dog, opposum, and sheep) possesses pelvic evaginations which penetrate deep into the medulla, separating the outer zone tissue from the cortical tissue except at the furthest penetration where outer zone tissue completely surrounds the fornices. In addition, Type II possesses a well-developed medulla with inner and outer zones covered with a simple cuboidal epithelium. The outer zone of the medulla is broken up by the pelvic folds, into blocks of tissue, resembling compressed buttresses,

called secondary pyramids. Thus the pelvic urine is separated from the deeper portions of outer zone tissue only by the thin squamous, cuboidal epithelium.

In many mammals, the ability to concentrate the urine is influenced by protein intake (dog: Rabinowitz & Kellogg, 1963; rat: Gamble, et al., 1934; opossum: Plakke & Pfeiffer, 1965), i.e., they are able to excrete urea more efficiently than electrolytes (Schmidt-Nielsen, et al., 1961). Other mammals have a constant osmotic ceiling of urine (Aplodontia: House, Pfeiffer and Braun, 1963; beaver and pig: Schmidt-Nielsen, et al., 1961), and these species excrete electrolytes more effectively than urea (Schmidt-Nielsen, et al., 1961). Pfeiffer (1968) reports that species in the former group possess Type II pelves and the latter group is composed of species with Type I pelves. Urine osmolality is equivalent to that of the papilla, therefore, a greater urine concentrating ability results with an increased accumulation of papillary solute (Schmidt-Nielsen, Pfeiffer and Robinson, 1966). They found much higher papilla urea concentrations in dogs (Type II pelves) and determined that these concentrations were influenced by dietary protein. Aplodontia did not increase papilla urea concentration with dietary protein increases. In both animals, the papillary sodium concentrations were not influenced by diet. They suggest that urea may have moved from pelvic urine into the medulla. Gertz, et al. (1966a), reported that solute and water in the pelvic urine can be added to the papillary capillaries and loops of Henle of the inner and outer zone of the mammalian kidney. This means that the exchange of water and solutes between the urine and renal papilla not only takes place through

the collecting duct walls but also across the epithelium covering the papilla.

Pfeiffer's pelvic classification (1968) was based on the presence or absence of secondary pyramids and specialized fornices, and the correlation of the specialized pelvis with the ability to concentrate urea. The high urea concentration in the pelvic urine favors movement of urea into the medulla and he postulated that the presence of fornices and secondary pyramids facilitated this movement. Since pelvic urine is hypertonic to the interstitial fluid of the outer medulla in anti-diuretic animals, water should move into the pelvic urine from the outer zone. This would further concentrate the outer zone tissue and thereby the inner zone tissue. Higher medullary tissue concentrations make possible higher urine osmolalities.

Species with Type I pelves, such as the beaver, Castor canadensis, and the mountain beaver, Aplondontia rufa, are usually found in or near fresh water. Animals with Type II (e.g., man, dog, sheep, rat and opossum) usually do not inhabit such moist environments.

The porcupine was selected for this study because its life history suggests an ability to survive in a wide range of environments from moist to dry. It feeds primarily on ground vegetation, dry grasses and succulent water plants in the summer, and moves gradually to tree food, bark and pine needles in the winter (Costello, 1966; Taylor, 1935). Sperber (1944) shows that animals living in moist environments have relatively lower cortico-medullary ratios than animals in dry habitats. The longer the loop of Henle, the greater is the degree of urine concentrating ability according to the present countercurrent hypothesis

(Schmidt-Nielsen, 1958). This relationship is practical since Sperber also noted that thicker medullas tended to have a greater percentage of long loops of Henle while kidneys with relatively thinner medullary thicknesses have fewer long looped nephrons and more of the shorter, cortical nephrons. However, no data has been reported on renal function in the porcupine and very little information on renal morphology is available. Gerhardt (1914) reported a papilla present in the porcupine, but Sperber (1944) described a short crest kidney. Furthermore, superficial anatomical observations by Plakke (personal communication) suggested the presence of an inner medullary zone. The porcupine, therefore, would appear to be a good concentrator of urine.

The purposes of this study are to determine:

1. The ability of the porcupine to concentrate its urine under experimental conditions, so that comparisons can be made with previously studied mammals.
2. The effect of changes in protein intake on urine concentration.
3. The ability of the renal medulla to create an osmotic gradient under conditions of high and low protein diets.
4. And to describe the principal features of its renal morphology.

MATERIALS AND METHODS

ANIMALS

The thirteen porcupines, *Erethizon dorsatum* (Cuvier), used in this study were live trapped without injury in Madison, Missoula, and Ravalli Counties, Montana. The animals were captured during the months of April through November. They averaged 8.0 kg in weight with a range of 4.3 to 12 kg. The animals were kept indoors, in metal holding cages, and fed Ceretana Commercial ration with carrots, lettuce and apples serving as moist supplements. Water was made available *ad libitum*.

TEST DIETS

Two five-animal groups were each fed diets of different protein content. One diet was high in protein and the other was low in protein. The group on the high protein diet ate either Ceretana rabbit ration, in pellet form, or a special high protein test diet (Table 1). The low protein group had either a special protein deficient test diet or lettuce and carrots (Table 1). The special test diets, in pellet form, were supplied by General Biochemicals Incorporated of Chagrin Falls, Ohio, and were made according to the specifications of Bodil Schmidt-Nielsen of Case Western Reserve University. All dry diets were supplemented with small quantities of lettuce and carrots. Animals were fed their respective diets for at least fourteen days before experimentation. Three other porcupines were sacrificed within 48 hours after capture to determine renal solute concentrations in animals on a natural diet. These three porcupines were given carrots and lettuce *ad libitum* prior to testing.

Table 1. COMPOSITION OF DIETS

A. Special High Protein Test Diet*

Ingredients	% Composition
Vitamin Free Test Casein	70.00
Corn Starch	13.00
Primex	14.00
Salt Mix. XIV	3.00

B. Special Protein Deficient Test Diet*

Ingredients	% Composition
Vitamin Free Test Casein	8.00
Corn Starch	13.00
Cane Sugar	62.00
Primex	14.00
Salt Mix. XIV	3.00

C. High Protein and Protein Deficient Diets*

Ingredients	g/100 lbs.
Alpha Tocopherol	10.215
Calcium Pantothenate	2.043
Carotene-in-Oil 39	67.00
Choline Chloride	272.40
Inositol	13.62
Menadione	0.1020
Niacin	27.24
Pyridoxine HCl	0.953
Viosterol (400,00 u/g)	3.000
Thiamine HCl	0.953

D. Lettuce and Carrots+

Ingredients	% Composition
Protein	1.20
Sodium	0.0012-0.0031

* Data obtained from General Biochemicals, Chagrin Falls, Ohio.
\+ Data obtained from Watt and Merrill (1950).

Table 1--Continued

E. Ceretana Complete Rabbit Ration

Crude Protein, minimum	16.00%
Crude Fat, minimum	2.50%
Crude Fiber, maximum	16.00%

Ingredients

Ground Barley	Dicalcium Phosphate
Wheat Bran	Defluorinated Phosphate
Wheat Shorts	Bentonite
Wheat Germ	Manganese Sulfate
Wheat Flour	Ferrous Sulfate
Soybean Meal	Ferrous Carbonate
Dehydrated Alfalfa Meal	Iron Oxide
Suncured Alfalfa Meal	Copper Oxide
Animal Fat	Cobalt Carbonate
Cane Molasses	Potassium Iodide
Citric Acid	Zinc Sulfate
Vitamin B-12	Calcium Carbonate
D-Activiated Animal Sterol	Zinc Oxide
Vitamin A	Sodium Chloride

PREPARATION

The animal was anesthetized with ether, and a 2-3 ml sample of blood was removed by heart puncture (Plasma 1). The animal was then placed in a collection cage with food and water *ad libitum* for 24 hours.

Food and water were withheld for a period of 18-26 hours to ensure maximum urinary concentration at the time of sacrifice. Previous experiments had indicated that the urine of dehydrated porcupines would reach peak osmolality values during this period. Animals that urinated voluntarily within 18-26 hours after start of dehydration were sacrificed 25 minutes after urination. No method could be found to induce the porcupine to urinate, therefore, when an animal did not voluntarily micturate during the 18-26 hour time period, the experiment was stopped and the animal returned to its cage and given food and water *ad libitum*. That animal was not used again for at least two weeks. Bottles, with a urinofecal separator, were placed beneath the cage to collect any urine voided during the dehydration period. Mineral oil and a few crystals of thymol were placed in the bottles to prevent evaporation and bacterial decomposition, respectively.

Approximately 25-30 minutes after the first voluntary micturition, the animal was placed in a container continuously perfused with carbon dioxide gas. The carbon dioxide anesthesia maintained the animals in a relatively normal physiological state until death, and the renal shutdown caused by excitement and operative procedures was eliminated as much as possible. The thoracic and abdominal cavities were rapidly opened, the renal vessels ligated, and both kidneys quickly removed.

SLICE TECHNIQUE

Each kidney was stripped of its capsule and sectioned with a sharp razor blade. Transverse cuts, perpendicular to the crest, were made at the junction of the pelvis and cortex to remove all cortex except that which was directly above the papilla and around the middle portion (Figure 1). Next, longitudinal cuts were made in the paramedian plane on either side of the midline (Figure 2). These cuts removed the lateral cortex and left a mid-saggital section approximately 4 mm thick with cortex, underlying medulla, and intact papilla tip (Figure 3). The triangular mid-saggital section was immediately frozen in acetone and dry ice to a temperature of approximately -76° C to avoid postmortem changes. A piece of abdominal muscle was also cut and frozen at this time. Measurable changes in tissue osmolality between the time of death and freezing of the tissue were held to a minimum by completing the procedure within four-five minutes.

BLOOD AND URINE COLLECTION

After the renal sections were frozen, a final blood sample was taken from the still beating heart with a heparinized syringe (Plasma 1). In some of the later experiments, nonheparinized blood was aspirated into both heparinized and nonheparinized syringes. The sodium in serum obtained from the nonheparinized blood (Serum 2) was measured as a check for sodium contamination, if any, by the heparin in the plasma (Plasma 2). All heparinized blood samples were centrifuged and the clear plasma supernatant fluid removed for later analysis. Since carbon dioxide anesthesia did not cause terminal, spontaneous micturition, terminal urine samples were taken directly from the bladder. Plasma, serum and urine samples were stored at -20° C until analysis could be done.

Figure 1. External, lateral view of kidney. The straight lines indicate the first two cuts made to expose the papilla. The end pieces are discarded.

Figure 2. Frontal view at approximately (a) of Figure 1. The next cuts are indicated by straight lines. The middle section (c) was frozen for tissue solute analysis. The tissue within (b) and (d) was used for water content analysis.

Figure 3. The mid-saggital section (c), of Figure 2, that was used for tissue analysis. The seven sections that were analyzed are indicated by horizontal lines.

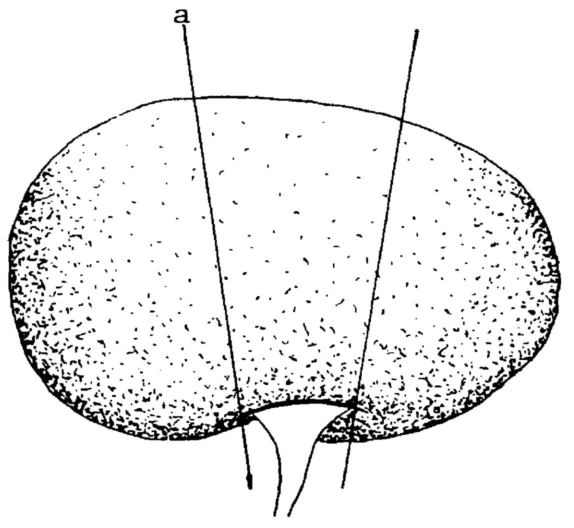


Figure 1.

b c d
cortex
outer zone
inner zone

Figure 2.

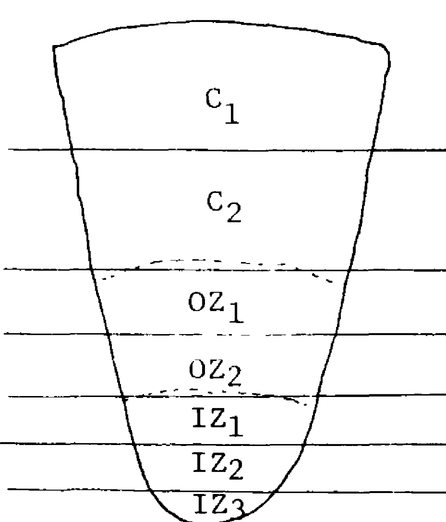


Figure 3.

TISSUE ANALYSIS

The mid-saggital slices were cut, while in the frozen state, into seven sections, perpendicular to the axis of the papilla, as follows: two cortex zones (C1 and C2), two outer medulla zones (OZ1 and OZ2), and three inner medulla zones (IZ1, IA2, and IZ3). The tip of the papilla is IZ3 (Figure 3). These zonal sections, still frozen, were halved, placed in tared microtest tubes with 190-200 mg of ammonia-free, demineralized water, sealed, and reweighed with a Mettler rapid pan balance to determine tissue weight. The tissue weight ranged from 4-134 mg. The test tubes were placed in boiling water for 5-10 minutes to kill the tissue and release the electrolytes and urea for diffusion. This procedure provided four sections per zone from each animal for analysis. The test tubes were frozen at -20° C until analyzed.

Osmolal concentrations of plasma, serum, and urine were determined cryoscopically, using a Fiske osmometer (Model G). Sodium and potassium were analyzed in a lithium internal standard flame photometer, (Beckman, Model 105). Urea and ammonia were measured by an ultramicro adaptation of Fawcett and Scott (Beckman, 1962) which allows urease to release nitroprusside and hypochlorite to form a blue color. This color, which is a linear function of urea concentration, was measured by the Beckman spectro-colorimeter, Model 151. All analytical procedures required extreme care to obtain the highest possible accuracy.

For quality control, concentrations of sodium, potassium and urea in Hyland Laboratory preanalyzed samples of human urine and serum were occasionally determined. Electrolyte and urea concentrations were measured in each tissue section, and analytical precision was checked by

comparison of the four sections of each zone. The final figure given for each zone of an animal is the average of the four sections of that zone.

TISSUE DILUTION FACTORS

All concentrations in the tissue are expressed as mM/l or meq/l of tissue water. To correct for tissue water, the dilution factor was determined according to the following formula:

$$\text{Dilution Factor} = \frac{\text{Wt. of Tissue Water + Diluent}}{\text{Wt. of Tissue Water}}$$

Tissue water weight was determined from wet and dry weights of the same kidneys and muscle used for solute analysis. After the center portions of the kidneys were frozen, small cortical and medullary samples from remaining kidney were removed (Figure 2). These samples, 3-4 mm wide, were put into tared flasks, weighed to 0.1 mg on a rapid pan balance, and dried to constant weight (within 0.3 mg).

ANATOMICAL STUDIES

Liquid latex (Fisher Scientific Company, Catalogue No. L-14, 62%) was injected into the renal pelvis, *in vivo*. This commercial latex was previously diluted 2-3 times with demineralized water and stored in an airtight jar. At the time of injection, latex was aspirated from just under the surface of the diluted mixture to avoid aspirating large lumps which had settled deeper into the mixture. The latex was aspirated into a 1-ml syringe through a 25-cm piece of polyethylene tubing (PE 20) fitted to a 26-gauge needle. The upper portion of the ureter was cut approximately 5-cm distal from the kidney, and the PE tubing was introduced until the tip of the tubing had reached the papilla tip. To prevent pelvic distortion, the tubing was not tied into the ureter so that excess latex could freely escape. One to 2 ml of latex was

injected into the pelvic cavity under sufficient pressure to move the syringe. The tubing was slowly removed, the ureter tied, and the kidney removed from the animal. The renal tissue was macerated in concentrated HCl for 18-24 hours, and the resulting pelvic and ureteral cast rinsed with water.

One kidney from a freshly killed porcupine was sectioned at eight microns and stained using Groat's modification of Harris' Hematoxylin stain.

STATISTICAL ANALYSIS

Zonal tissue solute concentrations of each group were graphed against the estimated distance of the slice from the mid-cortex. The resulting scatter diagrams permitted a line of best fit to be drawn through the points. The slope of the line was plotted by determining the regression coefficients and was calculated by the equation $Y = a + bX$. The paired sample t-test was used to determine the effect of dehydration plasma levels, within a group, whereas the t-test was used for comparison of plasma and urine differences between groups.

RESULTS

RENAL ANATOMY

The porcupine kidney has a large medullary region with a clearly defined inner and outer zone. Histological sections and latex casts revealed that secondary pyramids and fornices are lacking (Figure 4) and therefore, the porcupine kidney has a simple, uncomplicated pelvis without folds or extensions similar to the Type I pelvis (Figure 5). However, it resembles Type II in that it possesses a well developed inner zone of the medulla.

UREA CONCENTRATIONS

Renal Tissue Slices.--The kidneys of all porcupines had pronounced urea gradients extending from the outer cortex through the renal papilla (Table 2). When the urea concentration is plotted against the renal zones and a line of best fit made to the data, using the equation, $Y = a + bX$, the regression coefficients for the high protein (H.P.) group differ significantly ($P < .001$) from those of the low protein (L.P.) group (Figure 6). For H.P., the slope of the straight line relating Y to X, b, is 20.34, and the Y intercept, a, is -105.86. For L.P., the slope is 6.22 and the Y intercept is -23.99.

Urine.--The concentration of urea in the terminal urine of the H.P. animals was significantly higher ($P < .05$) than that of the L.P. group (Table 2). The urine urea concentrations of the natural (N.P.) group (Table 2) and the other groups did not differ significantly ($P > .05$). The ratio of urea in the tissue fluid of IZ3 to the urine urea concentration (IZ3 TF/U) differed slightly but not significantly in the three groups (Table 2). In fact, the means of the ratios of the

ups were exactly the same.

Figure 4. Cross section through the center of the porcupine kidney showing the inner (IZ) and outer zone of the medulla (OZ). There are no specialized fornices and secondary pyramids. Arrow indicates pelvic musculature extending deep into kidney.

Figure 5. Schematic drawing of the porcupine kidney sectioned transversely with the major zones indicated. Horizontal lines indicate the extent of the seven slices analysed.

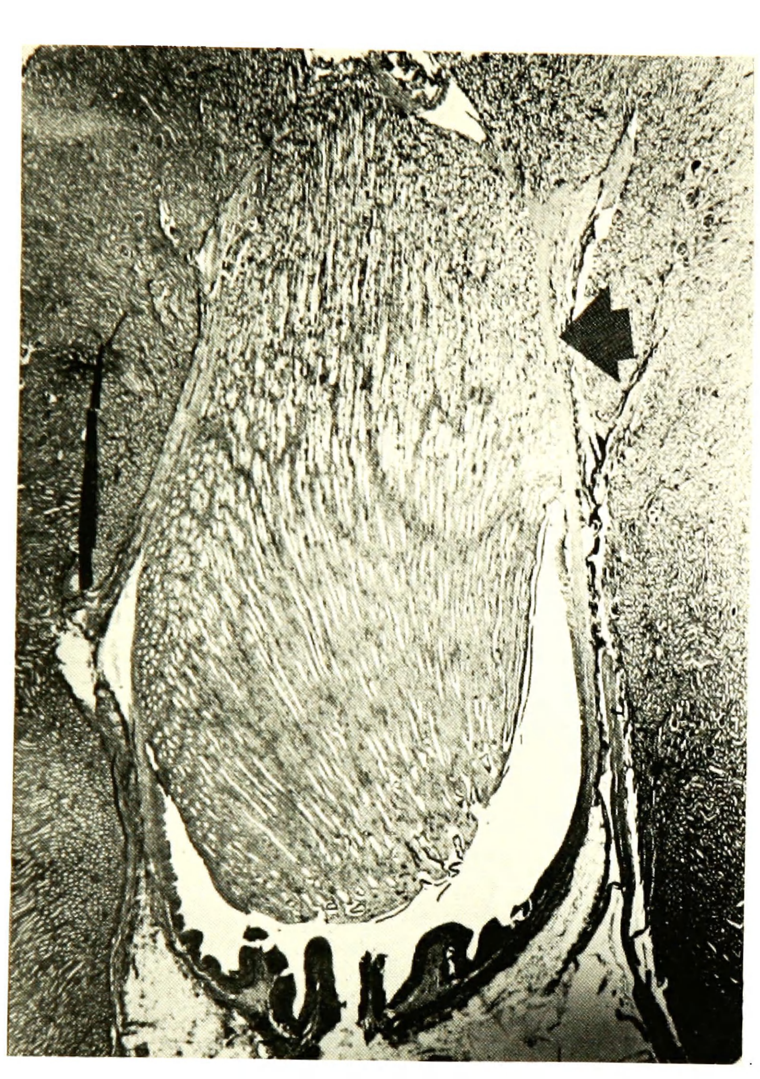
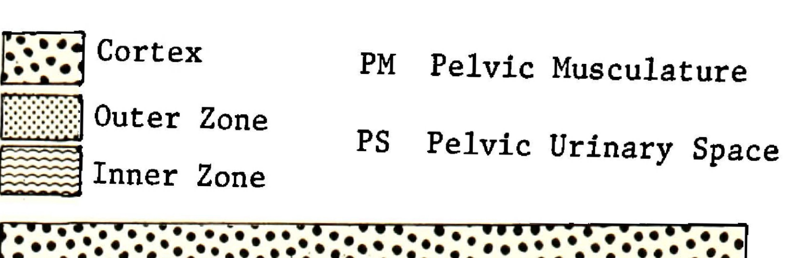


Figure 4



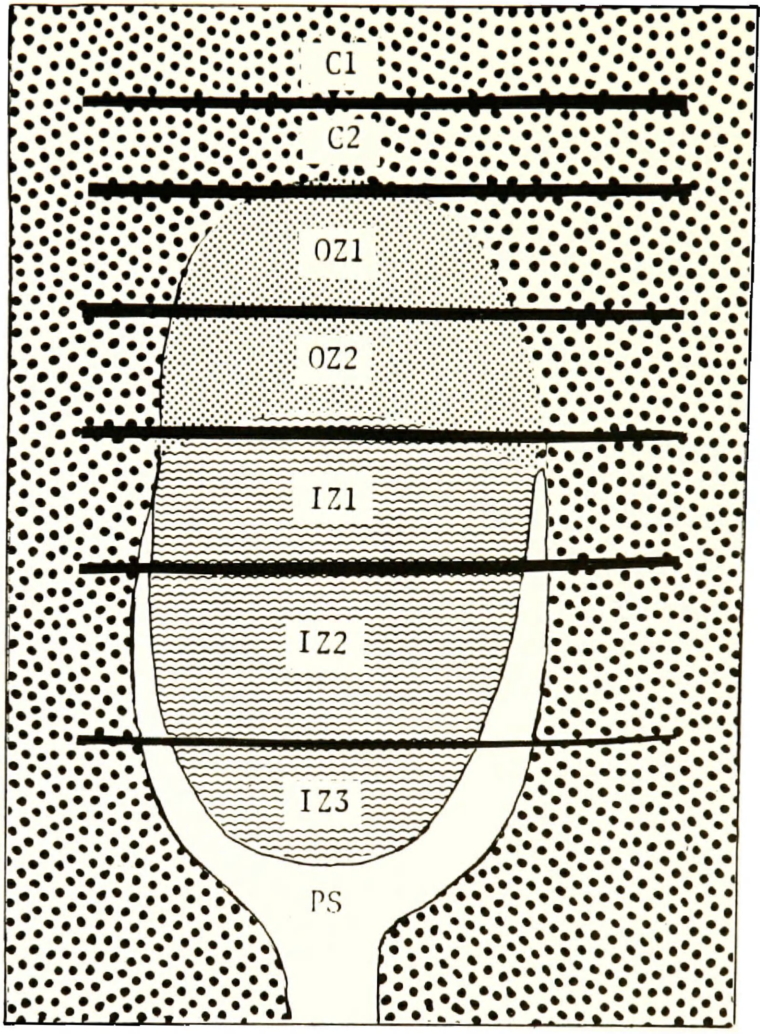


Figure 5

Figure 6. Scatter diagram of urea concentrations in kidney tissue slices of H.P. and L.P. groups. Straight line regression drawn by the equation $Y = a + bX$, where $b = 20.34$ and $a = -105.86$ for H.P. animals, and $b = 6.22$ and $a = -23.99$ for L.P. animals.

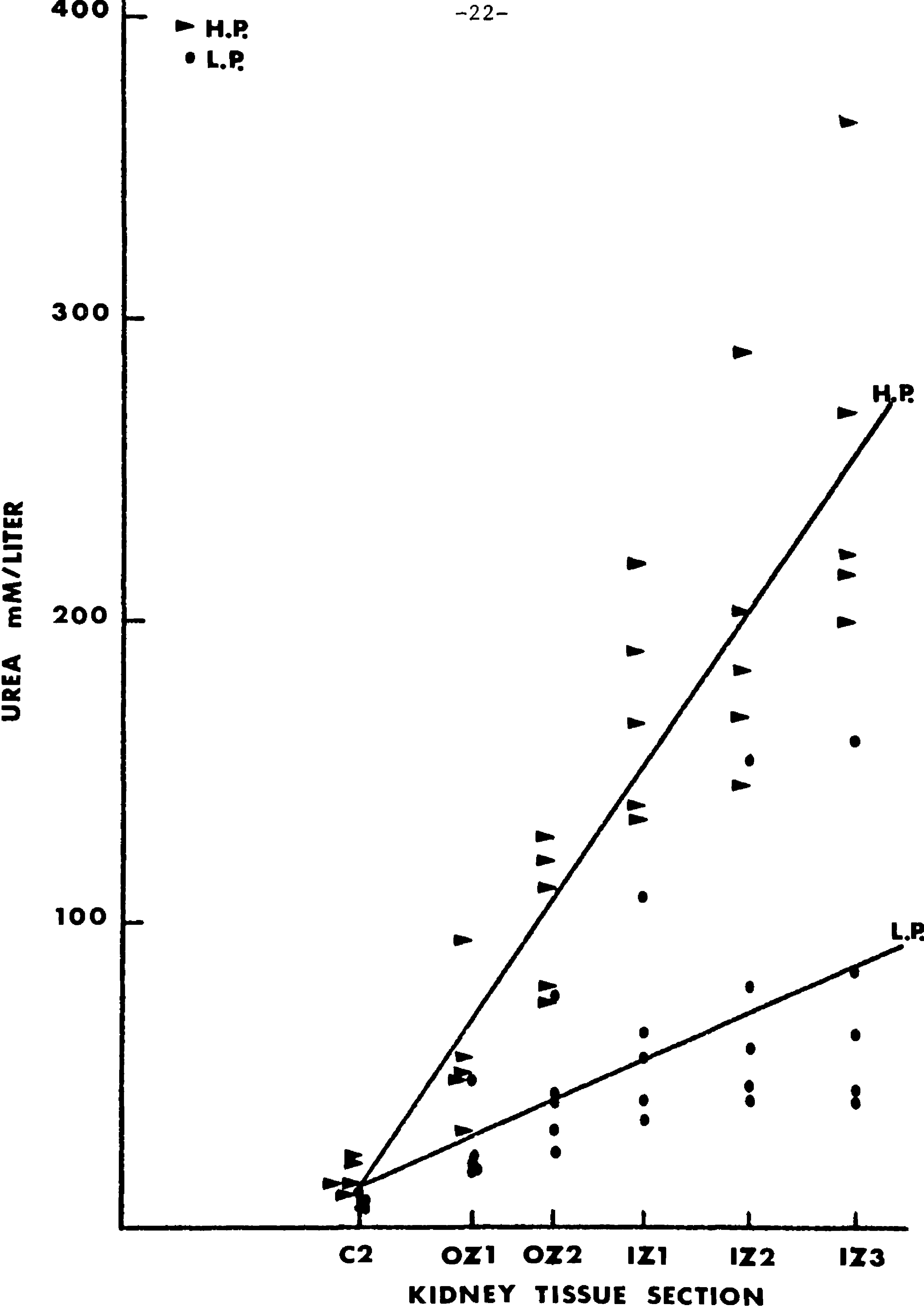


Figure 6

Table 2 UREA CONCENTRATIONS IN TISSUE, PLASMA, SERUM AND URINE

UREA mM/1

Animals on a High Protein Diet

Animal	3	6	8	12*	14*	Mean
C1	15	15	10	14	8	12
C2	21	14	12	23	14	17
OZ1	94	32	51	56	48	56
OZ2	129	74	79	112	121	103
IZ1	167	139	135	192	221	171
IZ2	146	169	185	209	291	200
IZ3	271	202	217	224	368	256
Muscle	11.6	11.3	5.4	2.9	4.6	7.2
Urine	308	210	252	184	390	269
Plasma 1	7.1	10.0	8.0	8.5	10.1	8.7
Plasma 2	5.5	7.3	5.6	8.4	5.4	6.4
Serum 2	6.3	**	**	8.9	5.3	6.8
IZ3 TF/P	49.3	27.7	38.8	26.7	68.1	42.1
IZ3 TF/U	0.88	0.96	0.86	1.22	0.94	0.97
Urea U/P	56.00	28.77	45.00	21.90	72.22	44.78

* Denotes special diet
** No sample taken

Table 2 (cont.)

UREA CONCENTRATION IN TISSUE, PLASMA, SERUM AND URINE

UREA mM/1

Animals on a Low Protein Diet

Animal	1	9*	10	13*	15*	Mean	
C1	8	6	7	8	5	7	
C2	11	9	8	9	8	9	
OZ1	24	48	21	20	19	26	
OZ2	42	76	43	32	25	44	
IZ1	55	109	64	41	35	61	
IZ2	59	155	79	47	42	76	
IZ3	64	162	85	42	45	80	
Muscle	6.2	6.2	5.1	5.8	6.3	5.9	
Urine	37	303	66	83	56	109	
Plasma 1	2.6	3.4	2.7	1.6	2.3	2.5	
Plasma 2	3.8	2.8	2.2	2.7	2.0	2.7	
Serum 2	3.4	2.7	2.5	3.1	2.0	2.7	
IZ3 TF/P	16.8	57.8	38.6	15.6	22.5	30.3	
IZ3 TF/U	1.73	0.53	1.29	0.51	0.80	0.97	
Urea U/P	9.74	108.21	30.00	30.74	28.00	41.44	(24.62)***

* Denotes special diet

*** Mean calculated without data from animal 9

Table 2 (cont.)

UREA CONCENTRATION IN TISSUE, PLASMA, SERUM AND URINE

UREA mM/1

Animals on a Natural Diet

Animal	16	17	18	Mean
C1	9	5	7	7
C2	8	6	10	8
OZ1	22	8	27	19
OZ2	43	18	58	40
IZ1	93	32	85	70
IZ2	120	33	139	97
IZ3	143	29	182	118
Muscle	5.3	4.2	3.5	4.3
Urine	194	25	324	181
Plasma 1	5.1	5.0	**	5.0
Plasma 2	2.4	1.9	5.0	3.1
Serum 2	2.7	1.9	5.2	3.3
IZ3 TF/P	59.6	15.3	36.4	37.1
IZ3 TF/U	0.74	1.16	0.56	0.82
Urea U/P	80.83	13.16	64.80	52.93

** No sample taken

Plasma.--The urea plasma levels in three of the five L.P. animals declined during dehydration but because the levels increased so greatly in the other two animals, the resulting mean is relatively higher. The plasma urea concentrations of all H.P. and N.P. animals decreased from a mean of 8.7 to 6.4 mM/l (H.P.) and 5.0 to 3.1 mM/l (N.P.) during dehydration (Table 2). In one N.P. animal an initial plasma sample was not taken. The plasma urea level means of the H.P. and the L.P. groups differed significantly ($P < .001$). The ratio of urea in the tissue fluid of IZ3 to that in the plasma (IZ3 TF/P) did not differ significantly between any of the groups (Table 2).

Serum.--The serum levels of 6.8 H.P., 2.7 L.P., and 3.3 N.P. did not differ significantly from the plasma levels of their respective groups.

SODIUM AND POTASSIUM CONCENTRATIONS

Renal Tissue Slices.--The porcupine kidney concentrates sodium to a greater degree than urea, and a sodium gradient similar to that of urea extended from the cortex through the papilla (Table 3). The regression line of the L.P. group was significantly lower ($P < .05$) than the H.P. group (Figure 7). The slope of the line is 17.34 and the Y intercept is -29.37, for the H.P. group. For the L.P. group, the slope of the line is 9.08 and the Y intercept is 22.30.

Potassium concentrations (Table 4) for the porcupine kidney increased slightly from C1 to IZ3 with an irregular pattern and only the L.P. group had a consistent pattern through the kidney tissue, however the rise was not significant ($P > .05$). The potassium concentration of the H.P. and N.P. groups were the same (59 mM/l).

Figure 7. Scatter diagram of sodium concentrations in tissue slices of H.P. and L.P. kidneys. Straight line regression drawn by equation $Y = a + bX$, where $b = 17.34$ and $a = -29.37$ for H.P. animals and $b = 9.08$ and $a = 22.30$ for L.P. animals.

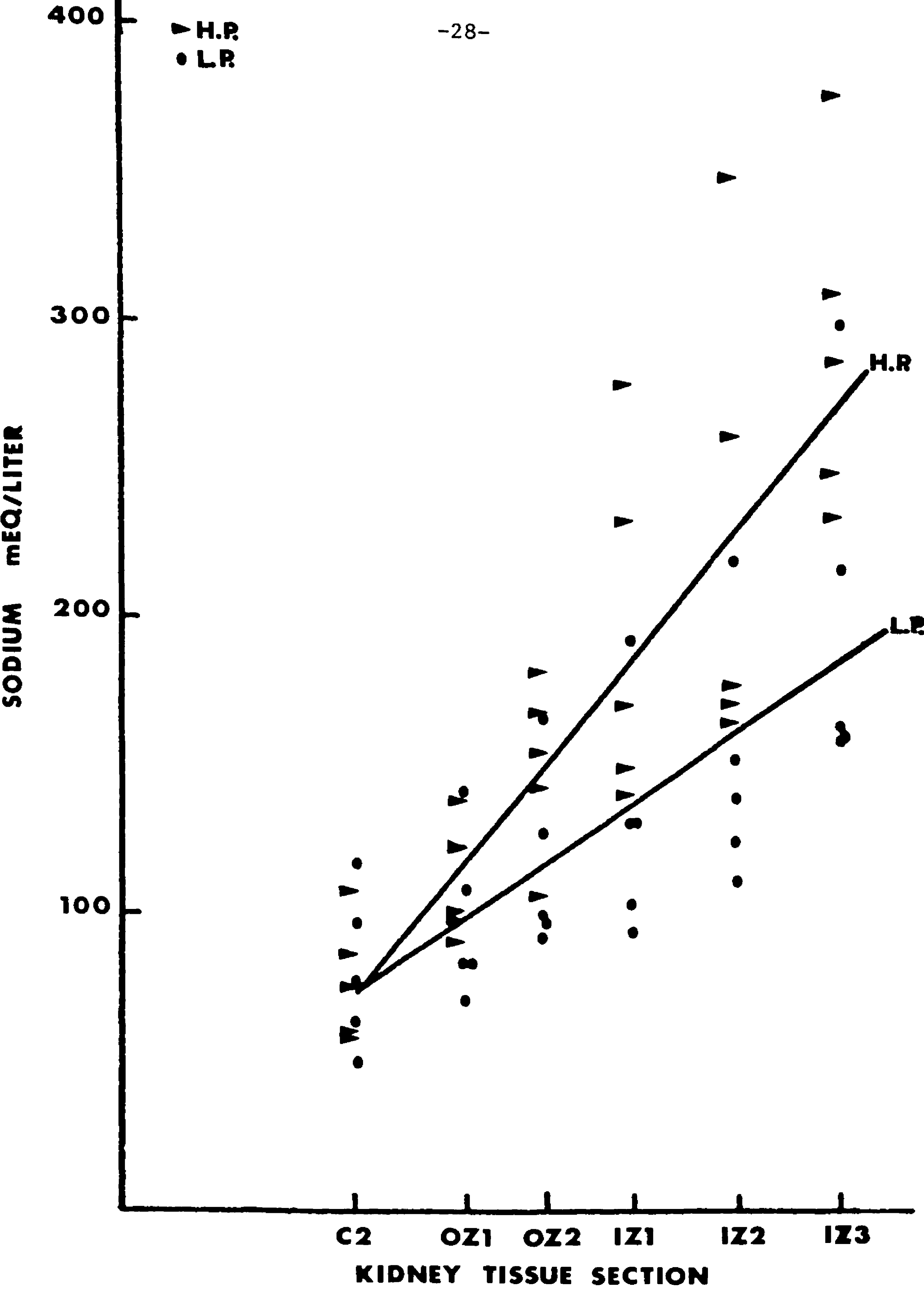


Figure 7

Table 3 SODIUM CONCENTRATIONS IN TISSUE SLICES, PLASMA, SERUM AND URINE

SODIUM mEq/1

Animals on a High Protein Diet

Animal	3	6	8	12*	14*	Mean
C1	68	80	42	103	76	74
C2	62	76	59	109	87	79
OZ1	102	98	91	124	139	111
OZ2	169	107	143	155	183	151
IZ1	150	140	171	233	279	195
IZ2	164	176	170	260	348	224
IZ3	309	233	248	286	377	291
Muscle	41	25	45	72	59	48
Urine	182	102	157	96	156	139
Plasma 1	132	126	134	128	124	129
Plasma 2	137	137	179	139	139	146
Serum 2	134	**	**	139	134	136

* Denotes special diet

** No sample taken

Table 3 (cont.)

SODIUM CONCENTRATIONS IN TISSUE SLICES, PLASMA, SERUM AND URINE

SODIUM mEq/1

Animals on a Low Protein Diet

Animals	1	9*	10	13*	15*	Mean
C1	55	114	79	66	73	77
C2	51	119	77	65	98	82
OZ1	84	142	84	71	109	98
OZ2	98	167	100	94	128	117
IZ1	94	193	132	104	132	131
IZ2	111	218	138	123	151	148
IZ3	163	299	159	160	216	199
Muscle	49	84	44	58	96	66
Urine	121	82	35	105	53	79
Plasma 1	121	126	132	132	131	128
Plasma 2	128	128	138	137	134	133
Serum 2	133	122	134	141	134	133

* denotes special diet

Table 3 (cont.) SODIUM CONCENTRATIONS IN TISSUE SLICES, PLASMA, SERUM AND URINE

SODIUM mEq/1

Animals on a Natural Diet

Animal	16	17	18	Mean
C1	64	86	67	72
C2	72	80	65	72
OZ1	82	90	94	89
OZ2	107	105	119	110
IZ1	181	99	166	149
IZ2	208	114	180	167
IZ3	254	120	247	207
Muscle	22	43	23	29
Urine	164	160	40	121
Plasma 1	136	128	**	132
Plasma 2	142	140	134	139
Serum 2	134	133	132	133

** No sample taken

Table 4 POTASSIUM CONCENTRATIONS IN TISSUE SLICES, PLASMA, SERUM AND URINE

POTASSIUM mEq/l

Animals on a High Protein Diet

Animal	3	6	8	12*	14*	Mean
C1	50	55	52	62	71	58
C2	63	57	51	58	67	59
OZ1	36	54	40	64	66	52
OZ2	76	48	30	59	56	54
IZ1	75	57	43	69	62	61
IZ2	32	57	31	71	69	52
IZ3	64	56	34	70	70	59
Muscle	90	68	57	114	120	90
Urine	107	100	117	196	200	144
Plasma 1	4.3	3.8	4.0	4.1	4.0	4.0
Plasma 2	4.7	7.5	6.5	6.5	5.1	6.1
Serum 2	9.0	**	**	7.7	7.3	8.0

* Denotes special diet

** No sample taken

Table 4 (cont.) POTASSIUM CONCENTRATIONS IN TISSUE SLICES, PLASMA, SERUM AND URINE

POTASSIUM mEq/1

Animals on a Low Protein Diet

Animal	1	9*	10	13*	15*	Mean
C1	45	67	50	51	61	55
C2	50	70	50	47	77	59
OZ1	55	66	54	51	72	60
OZ2	63	62	59	53	66	61
IZ1	55	66	61	54	66	60
IZ2	55	61	64	64	67	62
IZ3	61	64	90	64	62	68
Muscle	70	120	87	86	128	98
Urine	36	86	68	78	68	67
Plasma 1	4.4	4.5	3.5	3.0	3.8	3.8
Plasma 2	6.4	5.6	5.4	4.0	5.3	5.3
Serum 2	6.1	8.3	7.2	6.2	7.2	7.0

* Denotes special diet

Table 4 (cont.) POTASSIUM CONCENTRATIONS IN TISSUE SLICES, PLASMA, SERUM AND URINE

POTASSIUM mEq/1

Animals on a Natural Diet

Animal	16	17	18	Mean
C1	58	40	52	50
C2	57	47	61	55
OZ1	51	45	59	52
OZ2	55	41	55	50
IZ1	61	42	64	56
IZ2	58	51	67	59
IZ3	58	51	68	59
Muscle	73	81	86	80
Urine	54	68	173	98
Plasma 1	3.8	3.5	**	3.6
Plasma 2	4.4	4.2	6.1	4.9
Serum 2	8.0	7.1	8.5	7.9

** No sample taken

Urine.--In their urine, the H.P. group excreted the highest sodium levels, 96 to 182 meq/1 (Table 3). The H.P. group was significantly ($P < .05$) higher than the L.P. group (Table 3). The mean of the N.P. group did not differ significantly from the other groups and lay between them. The H.P. potassium levels were significantly higher ($P < .02$) than the L.P. group but neither group differed significantly from the N.P. group (Table 4).

Plasma.--Following dehydration all of the animals had elevated plasma sodium levels, however plasma values did not differ significantly within or between groups (Table 3). Plasma potassium concentrations increased but not significantly.

Serum.--In all of the individuals in which serum checks were taken, the sodium in Plasma 2 did not differ significantly from Serum 2 (Table 3). The serum to plasma potassium difference within the H.P. group was not significant but within the L.P. and N.P. groups, the serum potassium was significantly higher than the plasma ($P < .02$ and $P < .05$ respectively).

AMMONIA CONCENTRATIONS

Urine.--The mean ammonia concentrations (32.11 mM/1 H.P., 9.39 mM/1 L.P., and 5.75 mM/1 N.P.) in the terminal urines (Table 6) of all of the groups did not differ significantly ($P > .05$).

OSMOLALITIES AND URINE-PLASMA OSMOLAR RATIOS

Urine.--The mean osmolality of the H.P. group, 1195 mOsm/1, was almost double that of the L.P. group, 601 mOsm/1. This difference was significant ($P < .01$). However, the N.P. group did not differ significantly from the L.P. or the H.P. groups (Table 5).

Table 5 PLASMA, URINE AND SERUM OSMOLALITIES; U/P RATIOS

mOsm/l

Animals on a High Protein Diet

Animal	3	6	8	12*	14*	Mean
Urine	1445	760	1290	1145	1335	1195
Plasma 1	298	306	305	299	282	298
Plasma 2	302	320	307	320	299	310
Serum 2	328	**	**	329	312	323
U/P	4.78	2.38	4.20	3.58	4.46	3.88

Animals on a Low Protein Diet

Animal	1	9*	10	13*	15*	Mean
Urine	435	1035	596	537	400	601
Plasma 1	304	305	298	319	298	305
Plasma 2	307	305	300	326	305	309
Serum 2	301	301	301	320	301	305
U/P	1.42	3.39	1.99	1.65	1.31	1.95

Animals on a Natural Diet

Animal	16	17	18	Mean
Urine	960	458	1163	860
Plasma 1	280	288	**	284
Plasma 2	313	292	307	304
Serum 2	311	305	301	306
U/P	3.07	1.57	3.79	2.81

* Denotes special diet
** No sample taken

Table 6

TERMINAL URINE AMMONIA CONCENTRATIONS

AMMONIA mM/1

Animals on a High Protein Diet

Animal	3	6	8	12	14	Mean
Urine	26.67	48.84	79.92	1.08	4.05	32.11

Animals on a Low Protein Diet

Animal	1	9	10	13	15	Mean
Urine	5.18	3.45	29.97	5.74	2.63	9.39

Animals on a Natural Diet

Animal	16	17	18	Mean
Urine	5.49	6.16	5.60	5.75

The urine to plasma osmolar ratios (U/P) followed the pattern of the osmolalities, i.e., the H.P. group differed significantly from the L.P. group ($P < .01$) but the N.P. group did not differ significantly from the H.P. or the L.P. groups (Table 5).

TISSUE WATER CONTENT

The renal medulla of the porcupine has a significantly higher ($P < .05$) percentage of water than does the renal cortex (Table 8).

WEIGHT LOSS

For all groups, the loss of body weight did not exceed ten percent (Table 7).

Table 7

PERCENT WEIGHT LOSS DURING DEHYDRATION

Animals on a High Protein Diet

Animal	3	6	8	12*	14*	Mean
Weight**	11.36	10.45	12.78	8.27	9.09	8.39kg
Percent	6.54%	7.26%	6.40%	8.31%	8.04%	7.31%

Animals on a Low Protein Diet

Animal	1	9*	10	13*	15*	Mean
Weight**	5.79	7.75	8.75	3.97	6.34	6.52kg
Percent	8.93%	7.84%	6.09%	7.89%	11.75%	8.50%

Animals on a Natural Diet

Animal	16	17	18	Mean
Weight**	6.13	4.43	8.63	6.40kg
Percent	8.47%	9.30%	7.32%	8.36%

* Denotes special diet

** Terminal body weight in kilograms

Table 8

TISSUE WATER CONTENT

Percent of Total Weight

Renal Cortex

High Protein			Low Protein			Natural		
Animal	Right	Left	Animal	Right	Left	Animal	Right	Left
3	77.30	77.75	1	77.43	77.09	16	80.94	81.37
6	77.21	77.51	10	78.26	78.36	17	77.80	77.62
8	78.28	78.94	9*	78.48	77.68	18	81.20	77.70
12*	79.99	79.18	13*	78.02	77.89			
14*	79.14	79.48	15*	76.72	76.78			
Mean	78.3%							

Renal Medulla

High Protein			Low Protein			Natural		
Animal	Right	Left	Animal	Right	Left	Animal	Right	Left
3	79.92	80.90	1	81.75	80.98	16	83.47	83.34
6	79.96	80.50	10	81.33	81.41	17	80.58	80.83
8	81.80	82.56	9*	82.32	81.83	18	78.80	80.27
12*	83.14	81.99	13*	81.79	82.03			
14*	83.37	81.86	15*	81.42	82.13			
Mean 81.5%								

Abdominal Muscle

High Protein		Low Protein		Natural	
Animal		Animal		Animal	
3	75.10	1	76.93	16	76.97
6	75.47	10	75.50	17	76.51
8	**	9*	73.68	18	76.43
12*	74.85	13*	76.12		
14*	75.05	15*	77.11		
Mean 76.6%					

* Denotes animals on special diet

** No sample taken

DISCUSSION

The urine concentrating ability of the hydropenic porcupine is enhanced by an increase in dietary protein and is impaired when the diet is deficient in protein. The urine osmotic ceiling in animals on high protein diets was two to five times that of the plasma, whereas the ceiling of low protein animals was twice the plasma osmolality. Furthermore, the porcupine can increase papilla urea concentrations up to 70 times the plasma urea concentration. This latter ability was also impaired by dietary deficiencies of protein. It appears that the porcupine, like Type II mammals, on a high protein diet utilizes the additional urea to increase the efficiency of the urine concentrating process.

The pelvic structure of the porcupine is unlike the two mammalian pelvic types described by Pfeiffer (1966 and 1968). It has a well developed inner and outer zone but lacks the secondary pyramids and specialized fornices. I suggest that a third group should be formed and added to Pfeiffer's classification of the pelvic types (1968). Type I (Pfeiffer, 1968) would include species with a simple and uncomplicated pelvic type with only outer zone tissue, e.g., Aplodontia (Pfeiffer, 1968). The Type III (Pfeiffer's Type II) would include animals with secondary pyramids, specialized fornices, and a well developed inner zone, e.g., dog and rat (Pfeiffer, 1968). Type II, the new group, would have an uncomplicated pelvis but also would have well developed inner and outer zones, e.g., Erethizon, and Ondatra (Zahn, 1968). Type I species and some of the Type II species cannot enhance urine osmotic ceiling with increased protein intake (Aplodontia and Ondatra) while other Type II species (Erethizon) can. This breakdown is more detailed

and could indicate the pattern of adaptation of the mammalian kidney during movement from moist to dry habitats.

In the porcupine kidney, the epithelium separating the inner zone of the medulla from the pelvic urine is a simple, squamous or cuboidal type like that in Type II and this suggests that exchanges of water and solute between pelvic urine and medullary interstitium are possible (Figure 4). Physiologically, the porcupine resembles Type II kidneys in that it can reach relatively high urine to plasma (U/P) osmolal ratios and renal tissue fluid to plasma (TF/P) urea concentrations. Type I species such as the beaver and *Aplodontia* cannot. The maximum U/P osmolal ratio in Type I is approximately two during hydropenia, and renal papilla urea concentrations range from 80 mMols/l in the beaver (Schmidt-Nielsen and O'Dell, 1961) to 14 mMols/l in *Aplodontia* (Schmidt-Nielsen, Pfeiffer and Robinson, 1966).

Ullrich, *et al*. (1961), suggested that urea diffuses from the collecting ducts to the loops of Henle and there is recycled. However, sources other than the tubular fluid may contribute to the urea gradient in the medulla. Gertz, *et al*. (1966b), reported that urea and water move readily from pelvic fluid into the medulla which could modify the quantity of urea in the papillary interstitium. The urea added to the medulla would be trapped and concentrated, thereby increasing the total osmotic concentration of the medulla and making possible higher urine concentrations. Pfeiffer (1968) suggested that solutes, including urea, might also move into the medullary tissue from the pelvic urine through the epithelium of specialized pelvic fornices (Type II). Since the pelvic urine is more concentrated than the outer zone tissue, water would move

passively across the secondary pyramids from the medulla into the pelvic urine. This would further concentrate the medullary interstitium and make possible even higher urine osmolalities.

Zahn (1968) suggested that the inner zone of the muskrat is needed to concentrate urea and the secondary pyramids and specialized fornices are necessary to enhance the urine osmotic ceiling with increased protein intake. The porcupine does not have secondary pyramids and specialized fornices, yet its urine osmotic ceiling is enhanced with increased protein intake. A factor that could explain the porcupine's ability to elevate urine osmolality with protein intake is perhaps the unusually long and extensive pelvic musculature extending deep into the kidney (Figures 4 and 5). Boyarsky (1964) cited pelvic muscle studies which suggest that peristaltic filling and emptying of the pelvis occur in a regular fashion, and Gertz, et al. (1966), reported hydrostatic pressure changes of 1-5 mm Hg. Pfeiffer (personal communication) postulates that this extensive pelvic mesculature may create pressure gradients which force water and solute, especially urea, into the medulla across the thin cuboidal epithelium covering the medulla's pelvic exposure. Since urine urea concentration is higher than that of the lower medulla (IZ3 TF/U urea ratios less than one), urea could thereby be effectively filtered into the medulla by pelvic hydrostatic pressure. During periods of low protein intake, therefore, the medullary electrolyte concentrations contribute more to the total papilla solute concentration which is necessary for reabsorption of water from the collecting ducts.

Grain eating desert rodents which have no access to drinking water must excrete their metabolic wastes, urea and salt, in a minimum of water,

while another desert rodent (Psammomys obesus) has a succulent natural diet which is high in salt concentration and therefore must excrete large volumes of a highly concentrated urine (Schmidt-Nielsen, 1965). While the other desert rodents can concentrate urea better than electrolytes, Psammomys does just the opposite (Schmidt-Nielsen, 1962). The Psammomys' kidney has 100% long looped nephrons reaching to the tip of a long and broad papilla (Sperber, 1944). The length of the multiplier system allows the animal to achieve high urine concentrations, and since the papilla is long and broad, large volumes of fluid are concentrated in the collecting ducts (Schmidt-Nielsen, 1965). The porcupine has a broad papilla and shows moderate increases in papilla electrolyte concentrations on a high protein diet. This suggests that, like Psammomys, the porcupine may have a natural diet high in electrolytes. Additional anatomical studies on the porcupine are necessary to determine if the porcupine has the percentage of long looped nephrons required for large volume urine concentration. Because the porcupine can utilize urea to obtain high urine osmolalities and has a broad papilla which makes available large volumes of collecting duct fluid for concentration, the animal could feed on a natural diet high in electrolytes and protein and excrete large volumes of a moderately concentrated urine.

Cizek and Nocenti (1967) reported that under a variety of experimental conditions, all mammals tested had a medullary water content significantly higher than the cortex. The porcupine exhibited this pattern. Since, as Cizek and Nocenti point out, electrolyte concentrations are generally expressed in terms of tissue water, total electrolytes in the medulla may be greater than believed. When they expressed the electrolyte

concentrations in terms of dry weight, all electrolyte contents increased. This indicates that the concentrations and therefore, the gradients of urea and sodium may be steeper than generally reported. Also a potassium gradient in the porcupine may exist, if analyses of potassium were on the basis of dry weight instead of wet weight.

The moderate ability of its kidney to concentrate urine allows the porcupine to survive in relatively xeric habitats. Present work indicates, however, that the porcupine cannot live in extremely dry situations for any length of time. The nocturnal habits of the porcupine aid the animal in avoiding high temperatures in environments where water is not abundant. Studies of the protein and electrolyte content of its natural food, throughout the year, would determine if the porcupine has a natural source of protein food, high in electrolyte content, with which to concentrate urine and thereby conserve water.

SUMMARY

1. The porcupine kidney lacks specialized fornices and secondary pyramids but possesses a well developed inner medullary zone.
2. The porcupine fed a high protein diet creates a large urea gradient in the medulla. Low protein intake reduces this gradient significantly.
3. The porcupine can establish a large sodium gradient in the renal medulla and this gradient is enhanced with increased protein in-take.
4. No potassium gradient exists in the porcupine kidney.
5. The porcupine is a good concentrator. Studies suggest the kidney may be designed for handling a large dietary intake of electro-lytes. More data is needed on the composition of the natural diet.

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