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Research Article

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Urine Concentration and Dilution in Hypokalemic and Hypercalcemic Dogs

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ABSTRACT The urine-concentrating mechanism was studied in chronic hypokalemia (seven dogs given a low K^+ , high $NaCl$ diet plus injections of deoxycorticosterone acetate [DOCA]) and chronic hypercalcemia (seven dogs given vitamin D). In the potassium-depleted dogs, muscle, serum, and urine K^+ fell markedly, but glomerular filtration rate (GFR) and body weight varied little. Maximum urine osmolality fell in all dogs (mean decrease = 45%); however, solute-free water reabsorption (T^{CH_2O}) at high rates of solute excretion remained normal in three of four dogs. Free water excretion (C_{H_2O}) increased normally or supranormally as a function of increasing Na^+ delivery to Henle's loop in six dogs so tested.

Hypercalcemia of several weeks duration caused a decrease in both GFR (mean 36%) as well as in maximum urine osmolality (mean 57%). Maximum T^{CH_2O} was not invariably depressed; in fact, when the values were adjusted for the reduced number of functioning nephrons (T^{CH_2O}/C_{In}), four of seven studies were normal. C_{H_2O}/C_{In} increased normally (or supranormally) with increasing fractional Na delivery to Henle's loop in four of five dogs.

I conclude that the lowered maximum urine osmolality in these hypokalemic and hypercalcemic dogs was not related to abnormal water reabsorption from the collecting ducts. Although not specifically measured in this study, it is very likely that solute accumulation in the renal medulla was reduced. This probably was not caused by abnormal delivery of sodium to, nor reabsorption of sodium from Henle's loop. It is likely that a more subtle defect exists in the countercurrent mechanisms for establishing a steep concentration gradient in

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the renal medulla. In the few hypercalcemic dogs in whom GFR was very low, I believe that injury to, and blockage of medullary tubules could account for most of the reduction in maximum U_{Osm} . Although not specifically ruled out, there is no evidence here to suggest that high serum Ca^+ or low serum K^+ per se causes a defect in sodium and water reabsorption in the mammalian nephron.

INTRODUCTION

The renal responses to chronic hypokalemia and hypercalcemia have been studied extensively, in spontaneously occurring disease in human patients and in a variety of experimental animal models. The most consistently observed alteration in renal function that occurs under both conditions is the loss of the ability to concentrate the urine maximally (1, 2). This defect sometimes has been accompanied by an alteration in those mechanisms which control thirst and its satiation, leading to polydipsia and polyuria; however, in many instances sodium and water balance is not grossly deranged (1). Particularly with respect to experimentally induced hypokalemia in dogs the defect in water metabolism may become obvious only under artificial circumstances: either during a long period of total water and solute deprivation, such as that encountered during a measure of maximum urinary concentrating ability (U_{max}), or during an intense osmotic diuresis after a period of dehydration and vasopressin administration. If the defect is primarily a laboratory phenomenon, then given normal amounts of sodium and water, affected dogs would not be expected to develop serious or life-threatening dehydration. Glomerular filtration rate (C_{In}) is usually low in chronic hypercalcemia, but may not be changed in hypokalemic nephropathy (3).

In general terms, two mechanisms for the defect in the formation of U_{max} are possible, and evidence has

been accumulated to favor both. One mechanism involves an alteration in the permeability response of the collecting ducts to the action of vasopressin. The fact that in affected dogs and human subjects, urine hypotonic to plasma may be excreted during an osmotic diuresis, has led some investigators to conclude that this mechanism is of importance in these species (4-6). The other mechanism involves an alteration in the function of the countercurrent multiplier or exchanger in the inner zone of the renal medulla. This latter mechanism apparently plays a predominant role in rodents, since osmotic equilibration between collecting ducts and surrounding vasa rectae does occur (7, 8), and total solute concentration of the papilla is low (9-11).

The purpose of the present investigation is to study those pathophysiologic mechanisms which might be responsible for the decreased ability to concentrate the urine during water and solute deprivation in potassium-depleted and vitamin D-intoxicated dogs. It is shown that, so long as C_{In} is unchanged or only slightly depressed, the lowered U_{max} in both conditions is most likely due to a lowered solute concentration in the inner zone of the renal medulla. Evidence is presented to suggest that this depressed concentration is due to neither deficiency in sodium delivery to, nor absorption from, the ascending limb of Henle's loop. Instead it is more likely that a subtle defect in the process for countercurrent multiplication or exchange is present.

METHODS

17 Class I¹ female mongrel dogs, weighing between 15 and 20 kg were maintained on a diet of Purina Dog Chow. During a control period of several weeks one or more measurements of U_{max} , maximum free water clearance (C_{H_2O}), and maximum negative free water clearance ($T^{C_{H_2O}}$) were made in each dog. Following this, all dogs were placed on one of two experimental regimens. (a) Hypokalemia was induced in nine dogs by feeding a synthetic low potassium diet (Nutritional Biochemicals, Corp.) providing 80-90 g of protein, 2-3 g of sodium chloride, and less than 1 mEq of potassium per day. The diet contained all essential vitamins. In addition, 1 or 4 ml DOCA (5 or 20 mg) in oil (Percorten, Ciba) was given intramuscularly and in some cases 9 g of sodium chloride orally, 5 days per wk for a period of 1-3 months. The DOCA and sodium chloride were not administered for 5-7 days before any measurement of U_{max} . If urinary concentrating ability was not diminished by greater than 25%, DOCA and salt were again administered for a second (and, in some cases, a third or a fourth) period of 1-2 wk. In some dogs who tired quickly of the bland low potassium diet, beef or chicken soup (containing about 1 mEq of potassium per serving) was used as a flavoring agent. (b) Hypercalcemia was induced in eight dogs by oral administration of 150,000 U of crystalline vitamin D₂ (Drisdol, Winthrop Laboratories, New York) 5 days per week for 10-27

¹ Class I dogs at this institution are short haired, clean, well-nourished animals of good disposition which have been screened for the presence of worms, anemia, and heart or renal disease. The dogs used in this study all excreted a urine of greater than 1600 mOsm during a test of U_{max} .

days, until serum calcium began to rise or until U_{max} fell. Thereafter, vitamin D was given two to three times per wk until the studies during hypercalcemia were completed.

During the induction of nephropathy, U_{max} was studied at weekly or biweekly intervals. Once a defect in U_{max} was established, one or more measurements of maximum C_{H_2O} excretion or $T^{C_{H_2O}}$ excretion (or both) were made. Each dog was used as its own control. After completion of these studies three of the hypokalemic and four of the hypercalcemic dogs were allowed to recover. Recovery was defined as return of the serum K^+ or Ca^{++} to normal and elevation of U_{max} to or toward normal.

U_{max} studies. Food and water were removed at 7:30 a.m. on the 1st day of the study and in the afternoon the dog was walked to encourage voiding; the urine was discarded. She was given 5 U of vasopressin in oil, and placed in a metabolic cage overnight. Urine was collected after spontaneous voiding sometime within the next 18 hr, or the dog was catheterized on the morning of the 2nd day. After the first urine collection, some dogs were left in the metabolic cage without food or water throughout the 2nd day and given an additional 5 U of vasopressin. Another urine specimen was collected over the 2nd night or during the morning of the 3rd day. In some instances, dogs were loaded with urea for 3-4 days before beginning of water deprivation. 10 g of urea were mixed with the diet and eaten under observation or given as a solution directly into the oropharynx. In the hypercalcemic dogs in whom GFR was reduced, this dose was reduced to 5 g.

Diuretic studies. Measurement of $T^{C_{H_2O}}$ was begun after 24 hr of water and food deprivation, in the morning of the 2nd day of a test of U_{max} . 0.5 cc vasopressin in oil was given 16 hr, and again immediately before the study. 5% mannitol in saline (123 mEq/liter) was infused initially at 3 ml/min; the rate was slowly increased until osmolar clearance (C_{Osm}) exceeded 20 ml/min or until urine volume reached a maximum. C_{H_2O} was studied after 24 hr of food but not water deprivation. Over approximately 45 min 400-600 ml of 2.5% dextrose and water were infused and when the urine osmolality reached a minimum value (usually less than 60 mOsm/kg H₂O) the first collection was made. Urine was negative for glucose during all collections. Thereafter, half-normal saline was infused at a rate adjusted to 5-10 ml/min greater than urine flow. Collections were made at intervals of 30-45 min until urine volume reached a maximum or began to fall. A markedly positive balance of sodium was achieved in all dogs.

The dogs were lightly anesthetized with sodium pentathol and rectal temperature was kept at 38°C with heating pads during all diuretic studies. At low urine flow rates, collections were 20 min, otherwise they were 10 min in duration with mid-point blood samples. The bladder was carefully emptied before and at the end of each period by manipulation of the catheter or air washout. Inulin was infused at 2 cc/min in isotonic saline ($T^{C_{H_2O}}$ studies) or in 2.5% dextrose in water (C_{H_2O} studies), to which potassium chloride was added to provide 90 μ Eq/min of K^+ (45 μ Eq/min in hypokalemic dogs). 900 μ U/hr of aqueous vasopressin was infused throughout the $T^{C_{H_2O}}$ studies.

Urine and plasma were analyzed for osmolality (Advanced Osmometer) and sodium and potassium (Instrumentation Laboratory, Inc., flame photometer). Inulin was measured using the anthrone method. Plasma inulins were maintained in the range of 70-90 mg/100 ml and the method used was approximately 100 times more sensitive to fructose than to glucose. Plasma glucose was usually in the range of 100-200 mg/100 ml and this was neglected in the calculation of C_{In} . Muscle potassium and sodium were measured on the residue

TABLE I
Average Values Obtained in Seven Dogs before and after Potassium Depletion*

	Hydropenia				Diuresis			
	P _K	U _K	U _{Osm}	U/P _{Osm}	U _K V	Excreted K ⁺ Filtered K ⁺	Initial C _{In}	Highest C _{In}
	mEq/liter	mEq/liter	mOsm/kg H ₂ O		μEq/min	%	ml/min	ml/min
Controls	3.90† ±0.27	224 ±95	1902 ±235	6.25 ±0.83	38 ±13	17 ±6	65.1 ±13.2	79.3 ±10.9
Hypokalemia	2.10 ±0.25	1.4 ±0.6	1055 ±183	3.45 ±0.62	2.2 ±1.3	2 ±2	62.9 ±15.1	79.5 ±14.1

* P_K and U_K = plasma and urine potassium concentrations; U_{Osm} = urine osmolality; U/P = urine to plasma ratio; U_KV = potassium excretory rate during first collection period of study; C_{In} = inulin clearance.

† Values ± standard deviation in this and subsequent tables.

after vacuum heat drying and double extraction with ethyl-petroleum ether.

The relationship between C_{H₂O} excretion and urine volume (V) was plotted for each individual study on each dog. Regression analysis was performed on the pooled data by an IBM 1130 computer, using an equation of the form $y = b_1x + b_2x^2 + b_0$. Initially it was determined that in each case the quadratic term was, and the intercept (b₀) term was not significantly different from zero (F test). The data were then recalculated using the origin as the y intercept. The sd given with each equation refers to the standard deviation of the estimate of y.

RESULTS

Control observations. Class I dogs at this institution, as can be seen from Table I, have inulin clearance values in the upper range of normal reported for anesthetized dogs (12). The U_{max} values reported here are also in the upper range of normal, despite the fact that in most instances the urine collected was formed over a 12–18 hr period beginning only 8 hr after the initiation of water and food deprivation.² It was noted repeatedly

that the longer periods of dehydration (up to 48 hr) with additional injections of vasopressin did not lead to higher values for U_{max} in normal animals. Urea loading in eight normal dogs did not result in any significant elevation of the U_{max} (Table II). With this treatment, four dogs had higher and four had lower values for U_{max} when compared to the control values.

The results of water diuresis studies during the control period, shown in Figs. 1, 3, and 4, are similar to those reported elsewhere (13). The equation describing the control observations in Fig. 1 is $y = 0.791x - 0.014x^2$ (±1.056 sd); the respective control observations in the hypercalcemic animals (not presented

TABLE II
Effect of Urea Loading on Average Values for Urine Concentration in Eight Normal and Four Hypokalemic Dogs

	U _{Osm}	U/P _{Osm}
Controls	2019 ±297	6.56 ±1.06
Urea loaded	1932 ±208	6.39 ±0.76
Hypokalemic	1105 ±160	3.65 ±0.44
Urea loaded	1122 ±61	3.65 ±0.18

² Occasionally during the concentration study a urine of low osmolality (rarely even hypotonic to plasma) was voided. In most instances this could be related to fright in the animal. Accordingly, most urines collected were voided spontaneously in a metabolic cage overnight. If catheterization in the morning was necessary, the dogs were taken from this cage to an adjoining room, quickly anesthetized, and the bladder immediately emptied.

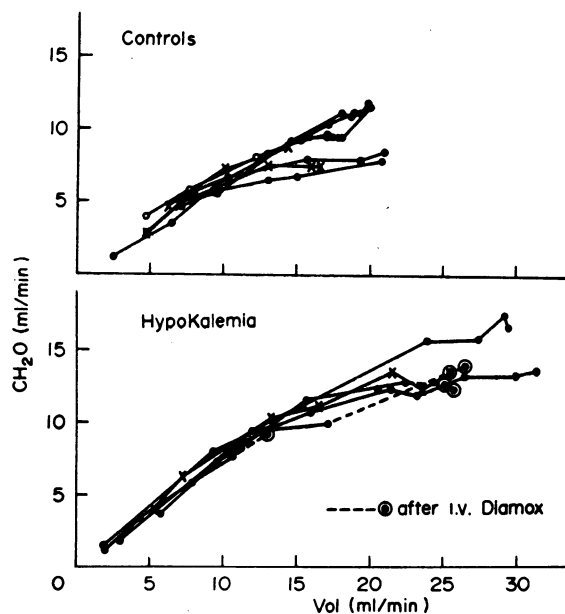


FIGURE 1 Free water excretion in hypokalemia. C_{H₂O} excretion is plotted as a function of urine volume (= delivery of Na⁺-containing fluid to Henle's loop). Diamox was used in one study to suppress reabsorption in the proximal tubule.

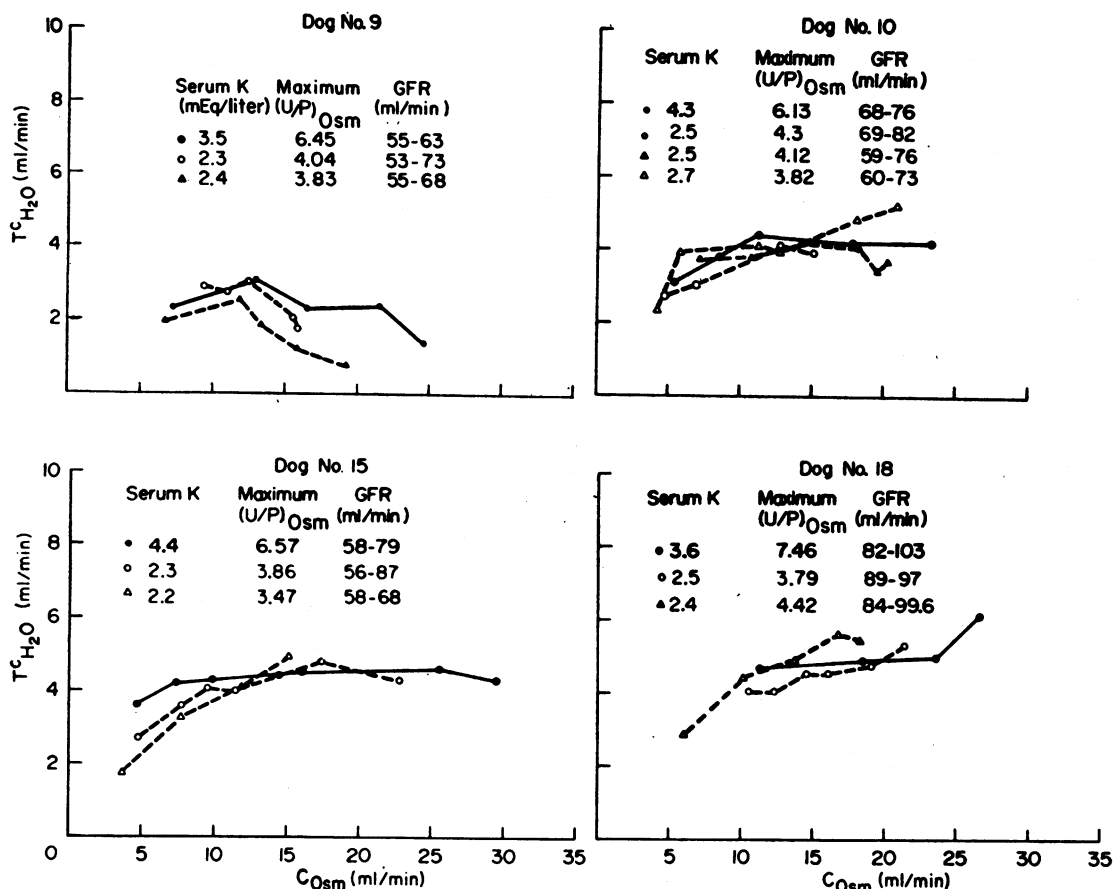


FIGURE 2 Free water reabsorption in hypokalemia, plotted as a function of C_{Osm} (= delivery of isotonic fluid to the collecting ducts). Solid lines are control data, dotted lines are data obtained in hypokalemic animals. Tabular data indicate the serum K^+ concentration, maximum $(U/P)_{Osm}$, and range of GFR of each dog at the time $T^{C_{H_2O}}$ was measured.

graphically) fit a similar equation, $y = 0.903x - 0.016x^2$ (± 0.798 SD). The equation for the control observations in Fig. 4 is $y = 0.946x - 0.016x^2$ (± 1.360 SD). In most instances, large increases in C_{In} and V/C_{In} and C_{Osm}/C_{In} were achieved. Thus despite the lack of prior extracellular fluid volume loading, infusion of hypotonic saline apparently caused large decreases in reabsorption in the proximal tubule, and large increases in delivery of sodium and water to the diluting sites. In a few dogs, such large increases in V and C_{In} were not achieved; this was reproducible in multiple studies on the same dogs. No explanation for this was apparent.

The hydropenia-osmotic diuresis studies (Figs. 2 and 5) are noteworthy to the extent that in the nine studies in these normal dogs (and several more in other animals not reported here) $T^{C_{H_2O}}$ either rose or remained stable up to quite high rates of solute excretion. This is in contrast to studies that show falling $T^{C_{H_2O}}$ (to the point of C_{H_2O} formation) at high C_{Osm} reported by others (14, 15). In two dogs, (Nos. 19 and 20), oral

water and sodium loading in the evening before the study without administration of vasopressin in oil, resulted in low U_{Osm} but no impairment in $T^{C_{H_2O}}$ formation once vasopressin infusion was begun (see Fig. 5, dog 20). In two other dogs it was shown that $T^{C_{H_2O}}$ formation as related to C_{Osm} was nearly identical whether the loading solute was hypertonic mannitol or hypertonic saline. This has been reported previously by others (16).

Hypokalemia. Low potassium, high sodium synthetic diet, and intramuscular DOCA administration to nine dogs resulted in rapid development of hypokalemia and in a few cases, episodic muscle weakness. Two animals died suddenly without any obvious explanation other than severe hypokalemia. However, the seven survivors appeared healthy and were normally active and playful. Of these, four had no change in weight, while two sustained a modest weight loss (-9% and -13%). The diet was usually taken well, particularly after the addition of soup as a flavoring agent. Measure-

TABLE III
Recovery from Hypokalemic or Hypercalcemic Nephropathy

Dog No.	Control			Experimental			Recovery		
	U/P _{osm}	U _{osm}	P _K	U/P _{osm}	U _{osm}	P _K	U/P _{osm}	U _{osm}	P _K
6	5.86	1875	3.6	3.98	1290	1.9	6.00	1800	3.6
9	6.45	1940	3.5	3.83	1140	2.4	6.03	1810	4.2
10	5.43	1630	4.3	3.82	1120	2.7	5.69	1740	3.4
Mean	5.91			3.88			5.91		
	U/P _{osm}	U _{osm}	P _{Ca} *	U/P _{osm}	U _{osm}	P _{Ca}	U/P _{osm}	U _{osm}	P _{Ca}
2	6.38	1915	8.1	2.97	890	14.6	6.37	1910	10.5
8	5.56	1780	9.2	2.32	695	12.9	5.69	1700	8.4
12	6.82	2135	8.6	3.08	920	12.6	5.40	1610	10.4
14	5.44	1730	10.1	2.16	668	12.1	5.48	1600	8.9
Mean	6.05			2.63			5.74		

* P_{Ca} = Plasma Ca⁺⁺ concentration in mg/100 ml.

ment of muscle electrolytes in four of the hypokalemic dogs revealed that intracellular potassium decreased from a mean of 37.5 to 28 mEq/100 g fat-free dry solids (minus 25%). In the same animals, muscle sodium increased on the average 27%.

The first five of the nine animals inadvertently were given only 1 cc (5 mg) of DOCA 5 days per wk and evidence of nephropathy (a low U_{max}) took much longer to develop in these animals than in the four dogs given a daily dose of 4 cc of DOCA. In four of these first five dogs, normal or near normal values for U_{max} were observed 11 days, 3 months, 3½ months, and 2 months after institution of the low potassium diet and low-dose DOCA administration, despite the fact that urinary potassium was nearly absent and plasma potassium low. In one of these five dogs a significant decrease in U_{max} was not seen until nearly 2 months after DOCA was discontinued, and two of the dogs died before a persistent reduction in U_{max} was observed. If this diet were deficient in protein, one would anticipate the development of a lowered U_{max} in a much shorter period of time (15). An inability to concentrate the urine normally was observed in all seven surviving animals and the minimum values for U_{max} are given in Table I. It should be emphasized that DOCA was discontinued in every instance at least 1 wk before these measurements of U_{max}.

Once present, the abnormally low U_{max} usually persisted on the low potassium diet alone without further DOCA administration. Thus, in five dogs studied on multiple occasions following discontinuation of DOCA, the low U_{max} persisted for periods of up to 2 months (at this point the other studies were usually complete and either potassium repletion was instituted or the animal was sacrificed.) Although during complete water

deprivation for up to 24 hr these dogs exhibited moderate hypostheuria, they manifested neither significant polydipsia nor polyuria.³ In two dogs, U_{max} tended to return toward control values repeatedly, always associated with a rise in plasma potassium. This was interpreted as being due to nearly complete retention of the small amount of dietary potassium available, as well as shifting of potassium from the intra- to extracellular compartment. It should be noted that urinary excretion of potassium fell to near zero in the hypokalemic dogs.

In all dogs the protein and sodium intake appeared to be adequate; and indeed C_{osm} averaged 0.56 ml/min and urea excretion 120 mmoles/24 hr, values not different from those seen in normal dogs (0.53 ml/min and 117 mmoles/24 hr) during the elaboration of a maximally concentrated urine. Nevertheless, the addition of urea or sodium chloride to the regimen was tested to determine if this maneuver would raise U_{max}. As can be seen from Table II, urea had no effect on U_{max} in four dogs. In two dogs addition of 2–5 g of sodium chloride to the low potassium diet for 5–10 days likewise had no effect.

In the few dogs tested, a mild metabolic acidosis was present presumably because the chloride intake (diet + sodium chloride supplements) was high (17).

Finally, three dogs were allowed to recover: one dog on a regular diet with potassium chloride supplements and two dogs on potassium supplements alone, with continuation of the low potassium diet. Note from Table III, that recovery was nearly complete in all three animals.

³ At an average C_{osm} of 0.5 ml/min, a decrease in U_{max} to the degree shown in the table only would increase the average urine flow from approximately 115 ml to 209 ml/24 hr.‡

TABLE IV
Average Values Obtained in Seven Dogs before and after Vitamin D Intoxication

	Hydropenia			Diuresis	
	P _{Ca}	U _{Osm}	U/P _{Osm}	Initial C _{In}	High C _{In}
Controls	9.64 ±0.83	1864 ±164	6.01 ±0.55	69.8 ±12.7	88.7 ±14.7
Hypercalcemia	13.0 ±0.9	794 ±106	2.59 ±0.39	44.8 ±11.7	61.5 ±13.9

U_{max} was measured several days before and several days after each diuresis study in the hypokalemic dogs to make certain that the U_{max} was near its low point and relatively stable. From Fig. 1 it can be seen that C_{H₂O} formation (i.e., sodium reabsorption in the loop of Henle) at low and at high rates of urine flow (i.e. low and high rates of delivery of sodium-containing fluid to the diluting site) was normal in all six hypokalemic dogs when compared to control values. Indeed, in four dogs, once the animals were hypokalemic,⁴ it was possible to achieve greater increases in delivery and in fractional delivery to the loop of Henle during hypotonic saline loading; consequently, greater amounts of C_{H₂O} were excreted. The equation describing the observations in Fig. 1 is $y = 0.886x - 0.013x^2$ (± 0.979 SD). In one dog, whose filtration rate was lower than that found in

⁴ Administration of 45 μ Eq of potassium per minute over the course of a diuretic study delivered only a few mEq of potassium in total. Serum K did not rise during these studies.

the control state, maximum C_{H₂O} excretion and urine flow were slightly lower; however, in this dog maximum C_{H₂O}/C_{In} was normal.

In agreement with the water diuresis studies, sodium reabsorption in the loop of Henle, as estimated from the maximum rate of free water reabsorption during an osmotic diuresis, was normal in three of four dogs, and only moderately reduced in one dog (Fig. 2). Multiple studies during chronic hypokalemia of as long as 6 months duration, revealed no change in maximum T^{C_{H₂O}} excretion to suggest a defect either in sodium reabsorption in Henle's loop or in water reabsorption in the collecting ducts. In the one dog (No. 9, Fig. 2) in which T^{C_{H₂O}} formation was low, hypotonic urine was not excreted even at an osmolar clearance equal to 28% of the filtration rate. Moreover, in this one dog, when free water formation was partially blocked with chlorothiazide, T^{C_{H₂O}} formation nearly doubled.

Histologic study of multiple sections of the kidney from two hypokalemic dogs failed to reveal the characteristic lesions which have been described in hypokalemic rodents and man (1). No lesions on light microscopy in hypokalemic dogs have been found by others (1, 3, 17).

Hypercalcemia. Administration of large doses of vitamin D to eight dogs resulted in prompt elevation of plasma calcium levels (to 11.5–14.6 mg/100 ml) and deterioration of renal function, including a moderate to profound fall in C_{In} and loss of the ability to concentrate the urine normally (Table IV). Three dogs became anorectic and lost weight after variable periods of time, and of these, one dog was not studied at all after vita-

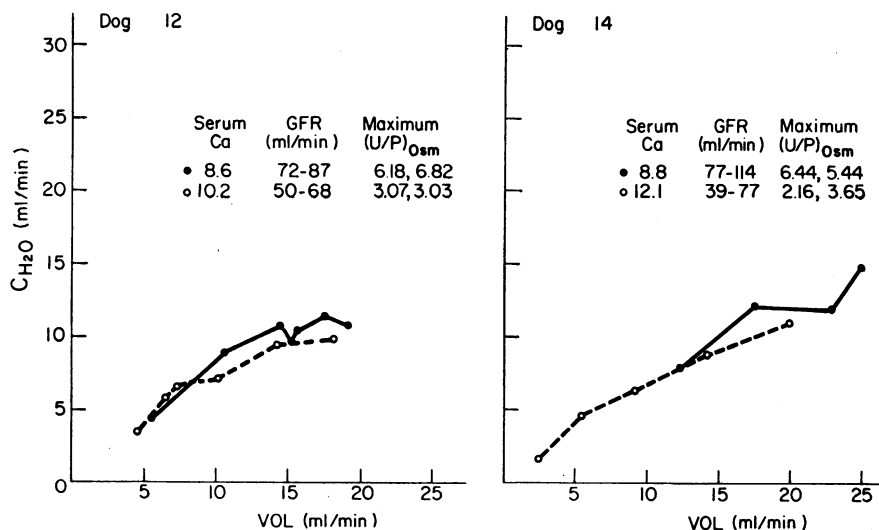


FIGURE 3 Free water excretion in hypercalcemia. C_{H₂O} plotted as a function of urine volume in two dogs, during a control period (solid line) and a hypercalcemic period (dashed line). The serum Ca⁺⁺ in dog 12 was higher than 10.2 mg/100 ml a few days before this study.

min D administration was begun. Renal function tended to remain abnormal long after vitamin D was discontinued and the serum calcium returned to normal. T^{CH_2O} in one dog (No. 14) was studied twice when C_{In} was only modestly depressed, and once when C_{In} was severely depressed.

U_{max} was low in all dogs irrespective of the state of nutrition (Table IV). The magnitude of the depression in U_{max} did not correlate with the values for serum calcium, but did correlate with the degree to which C_{In} was depressed. Many studies of U_{max} in hypercalcemia were done after urea loading and as in the hypokalemic dogs, the depression in U_{max} could not be related to either an abnormal urea or osmolar excretion rate.

Four dogs were allowed to recover and of these U_{max} returned completely to control levels in three, approximately 6–10 wk after vitamin D was discontinued (Table III).

Diuresis studies. Hypotonic saline loading resulted in nearly normal increases in C_{H_2O} excretion as a function of increases in urine flow in three of five hypercalcemic dogs. Two of these are illustrated in Fig. 3. The maximum delivery of solute and water (V) to the diluting site was low in four of five dogs, presumably as a result of the moderate to marked reduction in C_{In} . However, fractional delivery of solute (V/C_{In}) was normal or supranormal in all five dogs, and fractional free water excretion (C_{H_2O}/C_{In}) was normal in four, only slightly reduced in one hypercalcemic dog (Fig. 4). The equation for the pooled experimental data in Fig. 4 is $y = 1.010x - 0.020x^2 (\pm 1.621 \text{ SD})$, which is not significantly different from the equation for the control data. Seven studies of T^{CH_2O} formation in five dogs were

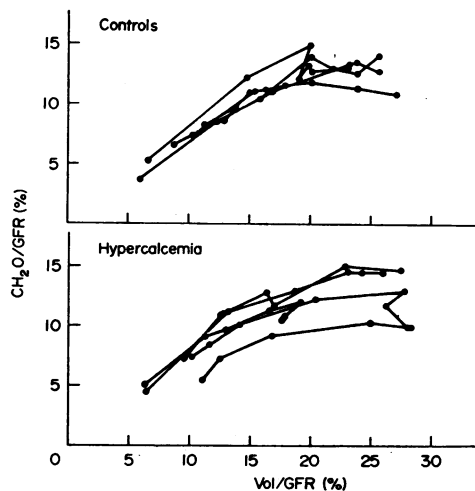


FIGURE 4 Fractional free water excretion in hypercalcemia. C_{H_2O} as a function of urine volume, both expressed as a percentage of the C_{In} , in five dogs before and after the induction of vitamin D intoxication.

performed at a time when serum calcium was elevated and U_{max} was depressed. Unfortunately in all but one study C_{In} was moderately or profoundly depressed, thus complicating the interpretation of these studies. Nevertheless, in three studies in two dogs, maximum T^{CH_2O} formation was normal or near normal (Fig. 5); in those three studies and in one additional study (dog 19), T^{CH_2O}/C_{In} was also normal. In only one study was free water (hypotonic urine) excreted at high urine flow rates (dog 14, Fig. 5); this dog had a markedly low C_{In} at the time. A moderate reduction in T^{CH_2O} formation

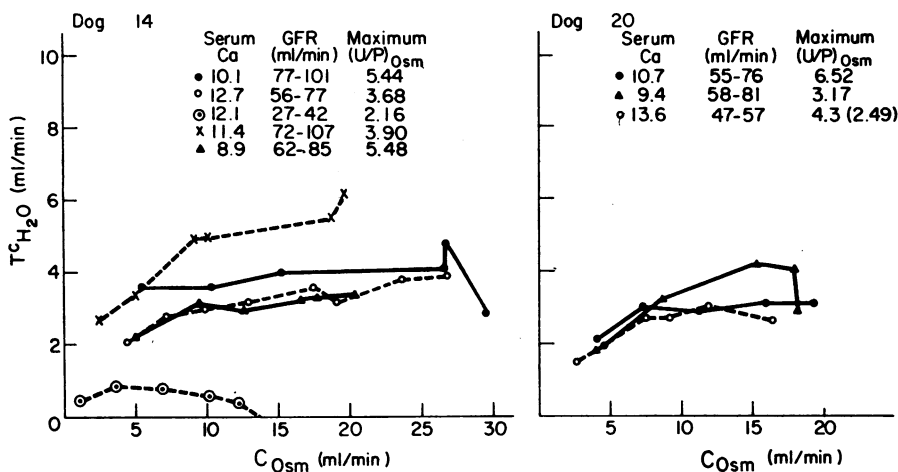


FIGURE 5 Free water reabsorption in hypercalcemia, plotted as a function of C_{Osm} in two dogs. Dashed lines signify studies performed while dogs were vitamin D intoxicated, while solid lines signify control observations. ▲ in dog 14 signifies a study after recovery; note GFR was not back to normal. ▲ in dog 20 refers to a study after oral water loading, before administration of vitamin D.

was not altered by intravenous administration of chlorothiazide in two of the dogs. Histologic study of sections from the kidneys of two dogs, one sacrificed during, and one sacrificed 3 wk after recovery from hypercalcemia revealed nephrocalcinosis, particularly in the renal medulla.

DISCUSSION

It is apparent that both experimental groups of dogs had altered renal function, the principal manifestation of which was a decreased ability to maximally concentrate the urine. One can conclude with reasonable certainty that in the hypokalemic dogs the decrease in U_{\max} was due to potassium depletion rather than to some other deficiency, since potassium repletion alone returned U_{\max} to normal. In the hypercalcemic dogs it was difficult if not impossible to separate the several effects of hypercalcemia, vitamin D itself, and lowered GFR upon the concentrating mechanisms.

It should be noted that a decrease in U_{\max} , even to levels lower than those seen in the present study, is not associated necessarily with noticeable polydipsia or polyuria (such as that seen in diabetes insipidus), unless there is an associated disturbance in the thirst mechanism. The present studies are concerned only with the lowered U_{\max} as a manifestation of the experimental electrolyte nephropathy, and no studies of urine volume or urine osmolality were done during ad lib. fluid intake.

The glomerular filtration rates of the seven hypercalcemic dogs were clearly depressed during the entire period of hypercalcemia, and even after the serum calcium returned to normal levels. The mechanism of the reduction of U_{\max} under these circumstances cannot clearly be related to hypercalcemia per se, and may be similar to those mechanisms responsible for the concentration defect in subtotaly nephrectomized dogs (18). Although the high concentrations of calcium, in itself, may have reduced GFR in all nephrons equally, for example by means of some vascular mechanism, it seems more likely that the greater part of the reduction in GFR was the consequence of tubular injury. It has been shown that in hypercalcemic animals the medullary portions of many nephrons are necrotic and the lumens obstructed (19). The deposition of calcium in the medullary interstitium (19) may play a role in the development of these lesions. It is likely that disproportionate injury and reduction in flow would occur in those tubules subjected to the highest calcium concentration, i.e., those that reach deepest into the inner zone of the renal medulla. This, of course, would reduce the highest solute concentration attainable at the papillary tip, and, secondarily, reduce the U_{\max} . Although hypercalcemia per se may alter the function of the remaining tubules, there is no evidence in this study to support this notion. The normal C_{H_2O}/C_{In} and T^{CH_2O}/C_{In}

suggest at least that the maximal reabsorption of sodium and water was normal in these nephrons. However, under conditions of hydropenia, when water and solute excretion rates were low, a solute diuresis in the reduced number of nephrons may have contributed to the reduction in U_{\max} .

In the hypokalemic animals, whose glomerular filtration rates were relatively well preserved, it is appropriate to consider under two general categories the mechanisms which might be responsible for the inability to concentrate the urine normally.

I. Incomplete osmotic equilibration between the fluid in the medullary collecting ducts and the surrounding interstitium. One cause of this, excessive fluid delivery to the collecting ducts with normal (or even) supranormal but inadequate water reabsorption, is unlikely because of the fact that solute excretion was normal. A diminution in the osmotic driving force for water reabsorption, such as might occur if the walls of the collecting ducts became very leaky to solute, would result in diminished water reabsorption, dissipation of the medullary solute gradient, and perhaps incomplete osmotic equilibration. Again this is unlikely because of normal rate of solute excretion. Moreover, as measured by the maximum ability to reabsorb free water (T^{CH_2O}) the permeability of the collecting ducts to water appeared to be normal or near normal in all hypokalemic dogs (and several hypercalcemic dogs).⁵ Therefore, we conclude that, at low flow rates, the inability to concentrate the urine normally, cannot be ascribed to inadequate or incomplete water reabsorption in the collecting ducts.

The tendency for the urine osmolality to fall towards isotonicity, or even become hypotonic to plasma at high rates of solute excretion ($C_{Osm} = 15-20$ ml/min) has been interpreted by others as indicating a defect in water permeability in the collecting ducts. This phenomenon was seen in only two dogs in this study (Nos. 9 and 14) although it was a frequent finding in other studies (4-6). The excretion of hypotonic urine during osmotic diuresis does not lend itself to any simple interpretation. It need not suggest an abnormally low permeability of the collecting ducts, that is casually related to the decrease in U_{\max} . Urine hypotonic to plasma is excreted during osmotic diuresis in some normal dogs (14, 15, 20) and in dogs on low sodium

⁵ Even in the most severely affected hypercalcemic dogs, the amount of free water reabsorbed at low rates of solute excretion (early in the course of an osmotic diuresis study), was clearly in excess of that which would be necessary to form a normally high U_{\max} . This is because at the solute excretion rate invariably seen during a test of U_{\max} ($C_{Osm} = 0.5-0.6$ ml/min), in order to raise the tubule fluid osmolality from isotonicity (with plasma) to roughly 7 times isotonicity, only something of the order of 0.4-0.5 ml water must be reabsorbed per minute.

(21) or low protein diet (15). In none of these conditions is an abnormal water permeability of the wall of the collecting duct suspected. Indeed, "normal" dogs that excrete hypotonic urine at high rates of urine flow may excrete a urine of very high (normal) osmolality during a test of U_{\max} (14). Note that blocking free water formation with chlorothiazide in one hypokalemic dog elevated the reduced $T^{\text{CH}_2\text{O}}$, whereas in two hypercalcemic dogs it had no such effect. This suggests that, in the latter, in contrast to the former, solute accumulation in the medulla was severely impaired during the period of osmotic diuresis.

II. Abnormally low solute concentration in the medulla during hydropenia. This has been demonstrated by direct kidney slice analysis in hypercalcemic and hypokalemic rats and dogs (9–11). Although such measurements were not confirmed in the present studies, one can infer from the above discussion that this condition must have obtained in these animals.

One can speculate upon any number of mechanisms which would result in a lowering of solute concentration in the papilla. Inadequate delivery of solute to the medullary tubules (sodium to Henle's loop or urea to the collecting ducts) is one obvious possibility. This is considered unlikely since sodium and urea excretion rates were normal in hydropenia and rose normally after hypotonic saline loading in both groups of dogs. Nor did urea administration effect the low U_{\max} . It is, of course, impossible to make any definite statement about sodium delivery to those loops of Henle which traverse the deepest regions of the papilla. Because of their very location, presumably they are involved in the development of the highest interstitial solute concentration at the tip of the papilla. Their proper function might very well mean the difference between a urine osmolality of 1000–1200 and a urine osmolality of over 1800 mOsm/kg H_2O . It has already been mentioned that redistribution of glomerular filtrate might have occurred in the hypercalcemic animals because of injury to the deepest nephrons. In the hypokalemic dogs in whom filtration rate did not fall, it is possible, but unlikely, that some sort of redistribution of filtrate to the superficial cortical nephrons could have occurred. The phenomenon of disproportionate or disparate changes in filtration rate between superficial and deep nephrons has been documented recently (22, 23). This redistribution is presumably functional and its mechanism, which is not understood, may or may not have any relevance to the process under study. Two groups have found that aortic constriction leads to ischemia greatest in the superficial cortex (24, 25). This leads me to question the conclusions from any studies which compare normal animals whose GFR has been decreased by renal artery constriction, with experimental animals

whose GFR is spontaneously low due to the experimental lesion or disease process (26, 27).

Neither diminished potassium excretion nor diminished potassium accumulation in the medulla can account for the decrease in U_{\max} . Potassium ordinarily accounts for only a small fraction of the solute in the medulla; thus, its absence would not contribute to a significant fall in solute concentration. Disappearance of potassium from the urine regularly occurred soon after the institution of a low potassium diet, before the development of a significant fall in U_{\max} .

A decrease in the efficiency of the process for countercurrent multiplication is yet another possible mechanism. For example, this condition would obtain if there were some defect in the active transport of sodium in Henle's loop. Normal dilution of distal tubule fluid during hydropenia in hypercalcemic (8) and hypokalemic (7, 28) rats has been shown by others. In the present studies, absolute and fractional sodium reabsorption in the formation of $\text{C}_{\text{H}_2\text{O}}$ was normal up to and including very high rates of sodium delivery to the loop of Henle. The studies of $T^{\text{CH}_2\text{O}}$ formation were confirmatory. Of course, one cannot conclude from this that sodium reabsorption necessarily was normal in the deepest thin segments of Henle's loops. These thin segments may not participate in the formation of $T^{\text{CH}_2\text{O}}$ or $\text{C}_{\text{H}_2\text{O}}$ (29), but certainly must participate in the processes which determine the maximum urine osmolality (29, 30). In the deeper regions of the medulla the metabolic processes may be primarily anaerobic, and might be more susceptible to electrolyte imbalance. Unfortunately, the present studies do not bear on this question.

It is reasonable to conclude however, that since the present clearance studies did not lend any support to the notion that sodium transport is defective, the diminished U_{\max} is as likely to be a consequence of an alteration in some other aspect of the concentrating process. A slight change in water or solute permeability of either the descending or the ascending segments of Henle's loop or vasa rectae could lead to diminished solute accumulation in the papilla, and is entirely consistent with the present data. Unfortunately, such a defect would be difficult, if not impossible, to confirm directly in the dog. Another possibility has been suggested by evidence presented (in abstract form) by Gardner (31), namely a hypertrophy of papillary interstitium in the hypokalemic rat, including interstitial cells, which might alter the contiguity of vessels, and diminish the efficiency of the countercurrent multiplier and exchanger. Finally, it is possible that altered renal blood flow might play some role in the diminished deposition of solute in the renal medulla. Unfortunately, the measurement of medullary blood flow, par-

ticularly in the innermost regions, cannot easily be made (30).

In summary, we believe that these studies more clearly have defined the concentrating defect in chronic hypokalemic and hypercalcemic nephropathy. It was shown that some disturbance of the accumulation of solute in the renal medulla was likely. In contrast to results of previous studies by other authors, it was shown that this defect was present at a time when sodium reabsorption in Henle's loop apparently was normal. Without evidence to suggest a defect in sodium transport, I submit that an alteration in any one of several other processes in the medulla is as, or more likely to be present to explain the lowered U_{max} . The concentrating defect in hypercalcemia may have a similar mechanism, however, the interpretation of the data is complicated by the fact that glomerular filtration rate usually was decreased, probably due to damage to deep medullary tubules. Thus, the decreased U_{max} in this condition is likely to be of similar mechanism to that in subtotal nephrectomized animals. Although a direct tubular effect of hypercalcemia is not ruled out, neither is it suggested by the normal fractional C_{H_2O} excretion and the supranormal sodium reabsorption encountered in some of the animals.

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