Distal nephron function of the rat during lithium chloride infusion

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Distal nephron function of the rat during lithium chloride infusion. Chronic lithium (Li) administration in the rat leads to reduced renal concentration ($T^c_{H_2O}$) without alterations in renal dilution (C_{H_2O}). To examine the acute effects of lithium on $T^c_{H_2O}$ and C_{H_2O}, rats were infused with a solution composed of 1% sodium chloride and 1% lithium chloride or a 0.225% lithium chloride solution at rates from 0.06 to 0.5 ml/min. In six rats, infusion of the sodium-lithium solution resulted in marked inhibition of $T^c_{H_2O}$ at any level of osmolar clearance (C_{Osm}) when compared to animals receiving 2.2% sodium chloride alone. Administration of amiloride, 40 or 80μg/kg BW/min throughout the experiment, to ten rats infused with the sodium-lithium solution, resulted in marked improvement of $T^c_{H_2O}$ to C_{Osm} relationship. Infusion of 0.255% lithium chloride to nine rats undergoing water diuresis led to marked reductions in C_{H_2O} as a function of distal delivery (V) when compared to rats receiving 0.225% sodium chloride alone. Infusion of amiloride did not improve the C_{H_2O} to V relationship in ten rats undergoing hypotonic lithium chloride infusion. These studies suggest that lithium interferes with sodium chloride reabsorption in the distal nephron, particularly the loop of Henle, and also reduces the permeability of the collecting duct to water. Amiloride, probably by interfering with lithium transport into collecting duct cells, corrects partially the $T^c_{H_2O}$ defect by preventing water permeability changes. The lack of effect of amiloride in C_{H_2O} studies may relate to the fact that collecting duct permeability is already at its lowest. The studies suggest that lithium ions retard chloride reabsorption in the ascending limb as inferred from the C_{H_2O} and $T^c_{H_2O}$ studies. Both chloride and sodium ions appear to be required for normal loop function.

Fonctionnement du néphron distal chez le rat au cours de la perfusion de LiCl. L'administration chronique de lithium au rat conduit à une diminution du pouvoir de concentration ($T^c_{H_2O}$) sans modification de la capacité de dilution (C_{H_2O}). Pour étudier les effets aigus du lithium sur $T^c_{H_2O}$ et C_{H_2O} des rats ont été perfusés avec une solution composée de 1% de NaCl et 1% de LiCl ou une solution à 0,225% de LiCl à des débits de 0,06 à 0,5 ml/min. Chez six rats la perfusion de la solution Na-Li a eu pour conséquence une diminution importante de $T^c_{H_2O}$ à tous les niveaux de clairances osmolaires (C_{Osm}) par comparaison avec des animaux qui recevaient seulement du NaCl à 2,2%. L'administration d'amiloride, 40 ou 80μg/kg/min tout au long de l'expérience, à dix rats perfusés avec la solution Na-Li a eu pour résultat une amélioration nette de la relation $T^c_{H_2O}$-C_{Osm}. La perfusion de LiCl à 0,225% à neuf rats soumis à une diurése aqueuse a dèterminté une rèduction importante de C_{H_2O} en fonction du débit distal (V) par comparaison avec des rats recevant du NaCl à 0,225% seulement. L'administration d'amiloride n'a pas amélioré la relation entre C_{H_2O} et V chez dix rats soumis à une perfusion hypoosmotique de LiCl. Ces études suggèrent que le Li interfère avec la réabsorption de NaCl dans le néphron distal, en particulier l'anse de Henle, et réduit aussi la perméabilité du canal collecteur à l'eau. L'amiloride corrige partiellement le déficit de $T^c_{H_2O}$ en empêchant les modifications de perméabilité à l'eau, probablement par l'action de l'amiloride sur le transport de Li dans les cellules des canaux collecteurs. L'absence d'effet de l'amiloride dans les expériences concernant C_{H_2O} peut être due au fait que la perméabilité des canaux collecteurs est déjà minimale. Ces études suggèrent que Li réduit l'absorption de Cl dans la branche ascendante, comme cela peut être déduit des expériences concernant C_{H_2O} et $T^c_{H_2O}$. Le fonctionnement normal de l'anse semble exiger à la fois Na et Cl.

It is well recognized that the chronic administration of lithium to man and to lower species may result in a defect in urine concentration [1–5]. This defect is characterized, in both man and rat, by normal free water generation during hypotonic saline diuresis in the presence of an inability to sustain a normal free water reabsorption-to-osmolar clearance relationship during hypertonic saline infusion [1, 2]. Studies performed in primates during acute lithium infusion, enough to raise serum lithium between 1.0 and 5.0 mEq/liter, have yielded similar results [6]. The normal free water clearance in the presence of a reduced capacity for free water reabsorption strongly suggests that lithium exerts its primary renal effect on the collecting duct. Of interest is the finding that amiloride, an agent known to prevent the epithelial actions of lithium *in vitro*, is able to partially prevent the changes induced by this ion on free water reabsorption [6].

The purpose of the present study was to examine further the effects of acute lithium infusion on the renal concentration and diluting function in the rat, a species in which this has not been well defined. In addition, an attempt has been made to raise dramatically the filtered load of lithium with the hope of determining what effect, if any, reduction in the concentration of sodium without reductions in the con-

centration of chloride reaching the ascending limb may have on its function. This was felt to be an important point in view of the demonstration that chloride appears to be actively transported by the ascending limb [7, 8]. To achieve high serum concentrations of lithium and to examine its effects on the diluting and concentrating process, rats were infused with lithium chloride solutions of two different osmolalities. The effect of amiloride on the response to the lithium infusions has also been examined.

Methods

Female, Wistar rats, weighing 200 to 300 g, were used in all experiments. Animals were housed in individual cages and were allowed food and water *ad libitum*. The day before the experiment, water was withdrawn from animals to be used in free water reabsorption ($T^c_{H_2O}$) experiments, while food was withdrawn from animals to be used in free water clearance (C_{H_2O}) experiments. For C_{H_2O} experiments, rats were given a volume of distilled water equal to 3% of their body weight by orogastric tube. The total amount of water was administered in three equal volumes 30 min apart the morning of the experiment.

Five groups of rats were studied. In the rats of groups II and IV (see in the following), under light ether anesthesia, catheters were placed in both femoral veins for infusions and in a femoral artery for blood sampling. Urine samples were collected from a bladder catheter. The rats were then placed in a restraining cage and were allowed to awaken to insure a good water diuresis. An infusion of ^{3}H-inulin was begun, and an hour was allowed to elapse for equilibration. Thereafter, a continuous infusion of ^{3}H-inulin was administered at a rate of 0.01 ml/min throughout the experiment. All other solutions were infused at rates varying from 0.06 to 0.5 ml/min. Groups I, III, and V were handled similarly except that they were anesthethized with sodium pentobarbital throughout the experiment.

Group I consisted of six rats kept without water for 24 hr and infused with a solution containing 1% sodium chloride and 1% lithium chloride (osmolality = 780 mOsm). Group II consisted of nine rats undergoing water diuresis infused with a solution containing 0.255% lithium chloride (osmolality = 110 mOsm). Group III consisted of ten rats receiving the sodium-lithium solution, as in group I, but in addition a constant infusion of amiloride, 40μg/kg BW/min (four rats) or 80μg/kg/min (six rats) throughout the length of the experiment. Group IV consisted of ten rats undergoing water diuresis receiving hypotonic lithium chloride solution, as in group II, and in addition a constant infusion of amiloride, 40μg/kg/min (five rats) and 80μg/kg/min (five rats), as in group III. Group V consisted of four rats receiving 2.2% sodium chloride (osmolality = 780 mOsm) but otherwise handled in identical fashion to group I. Half of the rats in all groups, except II and IV, received i.m. one unit of vasopressin (Pitressin tannate) in oil.

Sodium, potassium, and lithium concentrations in plasma and urine were determined by flame photometry (Beckman), while the chloride concentration was determined in a chloridometer (Cotlove). Plasma and urine osmolality was measured by freezing point depression. Radioactivity in aliquots of plasma and urine was determined by scintillation counting after appropriate corrections for quenching. Statistical analysis was performed by standard methods (Student's *t* test).

Results

The administration of vasopressin to some rats did not affect the results; therefore, rats differing only in this respect are not discussed separately.

Table 1. Glomerular filtration rate (GFR), plasma and urine electrolytes in Groups I to IV[a]

	GFR *ml/min*	P_{Na} *mEq/liter*	P_K *mEq/liter*	P_{Cl} *mEq/liter*	$U_{Na}V$ *μEq/min*	U_KV *μEq/min*	P_{Li} *mEq/liter*	$U_{Li}V$ *μEq/min*
Free water reabsorption ($T^c_{H_2O}$) experiments								
Group I (without amiloride)	2.13 ± 0.10	141.2 ± 0.83	5.09 ± 0.24	148 ± 0.92	36.61 ± 3.40	3.71 ± 0.32	32.2 ± 0.91	30.24 ± 3.25
Group III (with amiloride)	2.30 ± 0.10	140.6 ± 0.81	5.05 ± 0.17	147 ± 0.83	31.18 ± 2.35	1.01 ± 0.12	33.1 ± 0.90	32.16 ± 2.17
	NS	NS	NS	NS	NS	$P < 0.01$	NS	NS
Free water clearance (C_{H_2O}) experiments								
Group II (without amiloride)	2.56 ± 0.13	120.9 ± 0.83	5.44 ± 0.22	106 ± 0.96	24.53 ± 1.87	6.27 ± 0.45	9.4 ± 0.23	8.17 ± 0.98
Group IV (with amiloride)	2.78 ± 0.11	126.2 ± 1.03	5.61 ± 0.16	108 ± 0.87	24.30 ± 1.32	1.14 ± 0.12	9.7 ± 0.34	8.04 ± 0.87
	NS	$P < 0.01$	NS	NS	NS	$P < 0.01$	NS	NS

[a] Mean ± SEM. All values during individual experiments were meaned. These means were utilized for the values in the table. Abbreviations used are: P_{Na} = plasma sodium, P_K = plasma potassium, P_{Cl} = plasma chloride, $U_{Na}V$ = urinary sodium excretion, U_KV = urinary potassium excretion, P_{Li} = plasma lithium, $U_{Li}V$ = urinary lithium excretion, NS = not significant.

Table 2. Maximal plasma lithium concentration achieved (mEq/liter)[a]

Free water reabsorption experiments:	
Group I (without amiloride)	54.4 ± 1.1
Group III (with amiloride)	53.8 ± 0.9
Free water clearance experiments:	
Group I (without amiloride)	16.3 ± 0.5
Group IV (with amiloride)	15.5 ± 0.4

[a] Mean ± SEM.

Glomerular filtration rate (GFR), plasma and urine electrolytes. As shown in Table 1, in rats receiving sodium chloride plus lithium chloride (hypertonic) solutions, amiloride did not influence GFR, which remained stable throughout the experiment, and did not change plasma sodium, potassium, lithium, or chloride. The average values for sodium and lithium excretion did not differ in rats receiving amiloride from those which did not. By contrast, potassium excretion was distinctly lower during amiloride infusion. The results during infusion of hypotonic lithium chloride solution were identical to those observed during hypertonic infusions except that plasma sodium was higher during amiloride administration. The reason for this finding is not clear. Table 2 depicts the values of serum lithium concentration at the end of the experiments. Plasma lithium concentration was at least three times higher in the C_{H_2O} than in the $T^c_{H_2O}$ experiments. In the rats receiving 2.2% saline, GFR averaged 2.28 ± 0.4 ml/min throughout the experiment; plasma sodium averaged 189 ± 0.9 mEq/liter, and chloride averaged 149 ± 0.95 mEq/liter. The values for these parameters for the rats undergoing 0.225% saline infusion [1] were: GFR, 2.68 ± 0.32 ml/min; plasma sodium, 134 ± 0.8 mEq/liter; and plasma chloride, 102 ± 0.9 mEq/liter. It appears clear that filtered load of sodium was lower in rats infused with either 0.225% or 1% lithium than in those receiving sodium chloride solutions. Filtered chloride was comparable in all groups.

Fractional free water clearance and reabsorption during lithium chloride infusion. In Figure 1, fractional free water reabsorption is plotted against fractional osmolar clearance for group I rats. The two arbitrarily drawn curves enclose most of the points obtained in the four rats in Group V. As may be seen, infusion of the sodium-chloride solution at increasing rates resulted in considerable rises in C_{Osm}, but most values for $T^c_{H_2O}$ fell outside the normal range. Similar results were obtained when the hypotonic lithium chloride solution was infused into rats undergoing water diuresis. Fractional free water clearance is plotted in Figure 2 against fractional urine flow, an index of distal delivery. The hatched curve represents

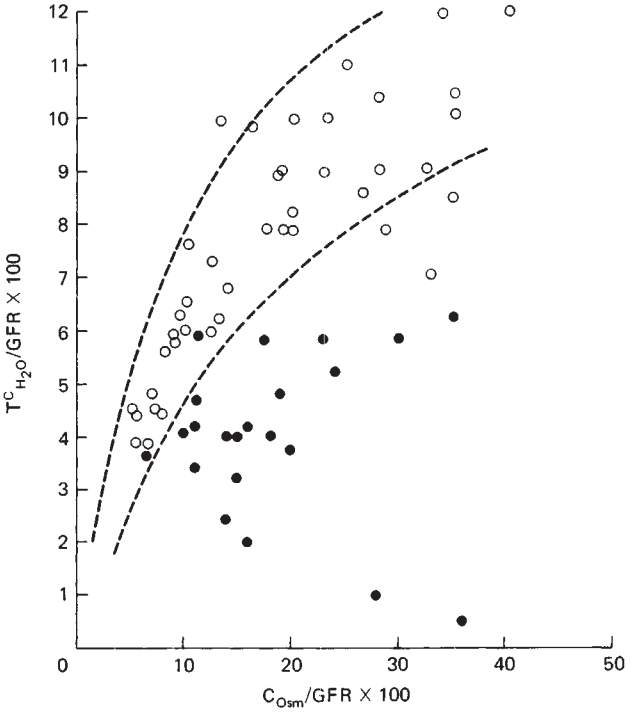

Fig. 1. *Relationship between fractional free water reabsorption ($T^c_{H_2O}$) and fractional osmolar clearance (C_{Osm}) in anesthetized rats.* The *open circles* are the values obtained by infusion of 2% sodium chloride (N = 4). The *broken curves* are arbitrarily drawn to enclose most of the values. The *solid dots* are the values observed in six rats inflused with the lithium chloride-sodium chloride solution.

part of the normal range of the relationship between these two parameters, as determined in normal Wistar rats infused with a sodium chloride solution of

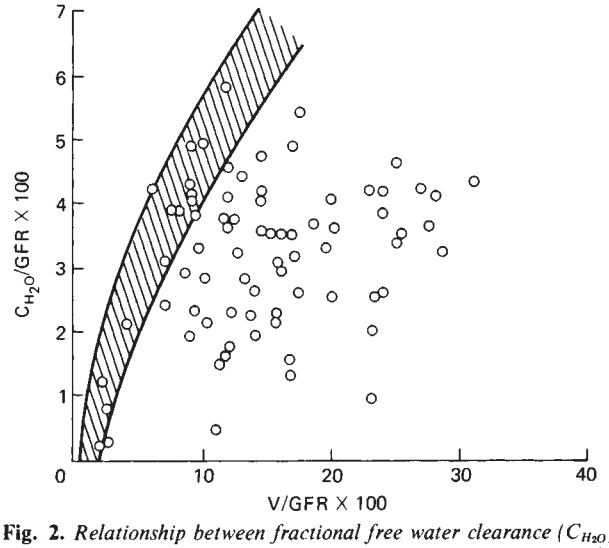

Fig. 2. *Relationship between fractional free water clearance (C_{H_2O}) and fractional urine flow (V).* The *curve* represents values previously obtained in normal awake Wistar rats [see Ref. 1] infused with hypotonic saline; the *open circles* represent values obtained under similar conditions during hypotonic lithium chloride infusion.

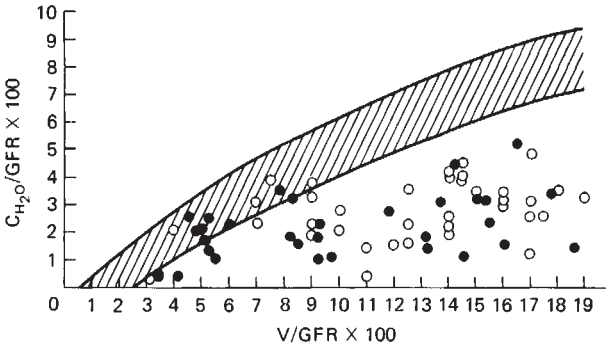

Fig. 3. *Relationship between fractional free water clearance (C_{H_2O}) and fractional urine flow (V) in rats given amiloride in addition to hypotonic lithium chloride infusion.* The *open circle* represents administration of 40 μg/kg/min of amiloride; the *closed circle,* 80 μg/kg/min of amiloride. The *hatched curve* is essentially the same as in Figure 2.

similar tonicity to that of the lithium chloride solution utilized in this group of rats. It is evident that despite marked increases in distal delivery (V), in most cases, C_{H_2O} failed to rise proportionately, so that most values fell outside of the normal curve.

Fractional free water clearance (C_{H_2O}) *and reabsorption* ($T^c_{H_2O}$) *during lithium chloride plus amiloride infusion.* The administration of amiloride to animals infused with hypotonic lithium solutions did not appreciably alter the inhibition of C_{H_2O} at any level of delivery (V/GFR × 100). This was the case whether 40μg/kg/min or 80 μg/kg/min were administered. Analysis of the results shown in Figure 3 demonstrated that the relationship between C_{H_2O} and V during low dose amiloride (C_{H_2O} = 0.07 + 7.44) was not different in slope from that during high dose amiloride (C_{H_2O} = 0.05V + 2.13). Furthermore, regression line analysis of the relationship between C_{H_2O} and V during amiloride infusion, regardless of dose, disclosed that the slope was not different (C_{H_2O} = 0.15V + 0.48) from that without amiloride (C_{H_2O} = 0.12V + 1.51) in the range of V, where data from Figure 3 can be compared to that of Figure 2 (V = 2 to 20% of GFR). It should be pointed out that when C_{H_2O} + C_{Cl} (clearance of chloride) was utilized, instead of V as delivery term, although slopes and intercepts of individual data groups changed, the results were essentially identical.[a]

The results of amiloride infusion during the lithium chloride-sodium chloride infusion resembled those during hypotonic infusion in that no difference existed between low ($y = 0.06X + 2.38$) and high dose effects ($y = 0.05X + 2.63$). On the other hand as shown in Figure 4, amiloride infusion (combined data of high and low dose) significantly improved the $T^c_{H_2O}$ to C_{Osm} relationship. Although it did not completely correct the concentrating defect at high C_{Osm}, most of the points at C_{Osm} below 10% fell within the normal curve. The slope of the relationship between $T^c_{H_2O}$ and C_{Osm} are similar with ($y = 0.05X + 3.26$) and without amiloride ($y = 0.06X + 1.76$), but the intercept was significantly different ($P < 0.01$).

[a] The equations describing the relationship of C_{H_2O} to C_{H_2O} + C_{Cl} without amiloride was $y = 0.14X + 1.65$, while that during amiloride infusion was $y = 0.17X + 0.50$. The equation in rats infused with hypotonic saline is $y = 0.56X + 1.5$.

Discussion

Infusion of lithium chloride exerted a remarkable inhibitory effect on both free water reabosrption and free water clearance. The magnitude of this change was comparable regardless of the delivery term utilized to describe the nature of the C_{H_2O} or $T^c_{H_2O}$ curve. For C_{H_2O} studies, both V and (C_{H_2O} + C_{Cl}) were utilized and yielded similar results. The same was true of C_{Osm} in the case of $T^c_{H_2O}$ studies. Conceptually, in view of the comparable levels of filtered chloride in those studies and those employing sodium chloride solutions, the term containing C_{Cl} might be the most appropriate. Nevertheless, it appears that utilization of the C_{Cl} is only necessary when distal delivery of an unreabsorbable anion is increased [9, 10].

The filtered load of lithium was purposely increased to examine the effect of high delivery of this

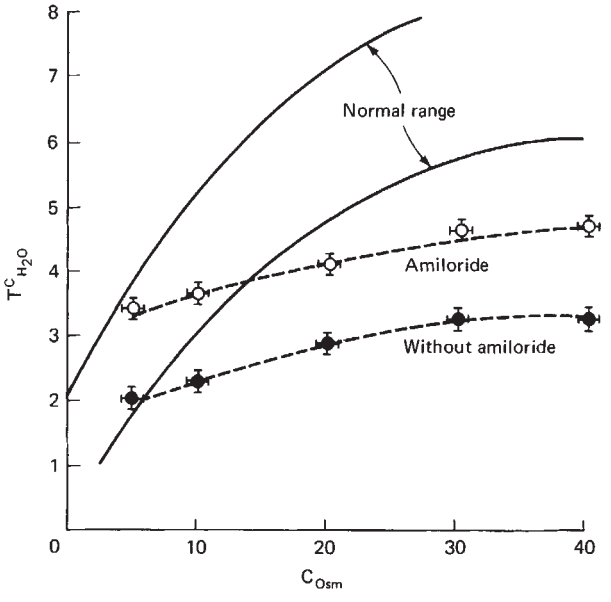

Fig. 4. *Results of infusion of amiloride on relationship between fractional free water reabsorption ($T^c_{H_2O}$) and fractional osmolar clearance* (C_{Osm}). Each *point* represents the mean of all $T^c_{H_2O}$ values at different ranges of C_{Osm} (0 to 5; 6 to 10; 11 to 20; 21 to 30; 31 to 40) (see text).

cation to the loop of Henle. Since plasma chloride concentration was comparable to that observed when hypotonic or hypertonic sodium chloride is infused, the substrate for the proposed "pump" in the ascending limb was normal. This suggests either that lithium inhibited loop reabsorption of chloride (and sodium) or that it acted as a nonreabsorbable solute. Experiments of the renal handling of lithium have suggested that this ion is principally reabsorbed in the proximal tubule [11, 12]. In a recent study, Steele, Durgeon, and Larmore [13] have examined the effect of diuretics on lithium reabsorption in an attempt to localize the major sites of its reabsorption in the rat. The results of these studies confirm that the proximal tubule is a major site of lithium reabsorption. Based on the additive effect of acetazolamide and furosemide on lithium excretion (separately, they led to equivalent increases in the fractional excretion of lithium), they concluded that significant reabsorption may take place in the loop of Henle. It is possible, therefore, that influx of lithium ions into ascending limb cells interfered with the net transcellular transport of solute. This could be the case since efflux of lithium ions from other epithelial cells is slow [14, 15] and retards sodium ionic transport [16]. Furthermore, lithium does not substitute well for sodium as an activator of the sodium-potassium-adenosine triphosphatase (Na^+-K^+-ATPase) in rat kidney [17] or in frog skin epithelium [18][b]. It is conceivable then that the inhibition of C_{H_2O} as delivery rose was the result of interference with transcellular solute transport in the ascending limb and more distal segments of the nephron. This suggestion is supported by the results of the $T^c_{H_2O}$ experiments. Although interference with free water reabsorption by lithium is known to be, in part, mediated by inhibition of the effect of vasopressin (ADH) on the collecting duct [18–20], this cannot entirely explain the changes observed. Administration of amiloride, which has its principal effect on the collecting duct, only *partially* corrected the abnormality in free water reabsorption. In epithelial analogues of the collecting duct, amiloride reduces lithium influx into cells to very low values [16]. Moreover, studies in the toad bladder [18] have shown that amiloride in a final concentration of 10^{-5} M abolishes completely the inhibitory effect of lithium (at concentrations of 11 mEq/liter) on ADH-induced, cyclic adenosine-5-monophosphate-mediated, short circuit current and osmotic water flow. This suggests that the improvement in the $T^c_{H_2O}$ curve during amiloride was the result of its blocking of lithium entry into collecting duct cells. The persistence of a defect in $T^c_{H_2O}$ must have been the result of inhibition of solute transport in the ascending limb of the loop of Henle, where amiloride does not appear to have an effect. This has been shown in the rat [21] as well as in the isolated thick ascending limb of rabbit [22]. A lack of effect of amiloride in the loop of Henle can be deduced from the results during free water clearance in the present studies, since amiloride had no effect whatever on the inhibition seen in that group of rats.

It could be argued that in the $T^c_{H_2O}$ studies the concentration of lithium reaching the collecting duct (about 55 mEq/liter) was far in excess of the blocking capability of amiloride. In toad bladder, increasing mucosal lithium concentration from 11 to 55 mEq/liter does not lead to a further inhibitory effect. That this was also the case in our studies can be discerned from Figure 4. Absolute values for $T^c_{H_2O}$ were abnormal at all levels of delivery, but the inhibition was not greater at high values of C_{Osm}, although this time urine lithium concentration had risen to values about 20 times over 11 mEq/liter.

An effect of lithium on the loop of Henle has not been shown previously in either chronic [1, 2] or acute studies [6]. In those studies, lithium concentrations did not exceed 5 mEq/liter, while in the present study, concentrations two- to ten-fold greater than this were achieved.

The studies of Webb et al [6] in the monkey are directly pertinent to the present discussion. At a time when plasma lithium concentration was about 5 mEq/liter, amiloride abolished almost totally the defect in $T^c_{H_2O}$ observed throughout a wide range of C_{Osm}. In view of the concentrations of plasma lithium achieved in the free water studies (about 16 mEq/liter), values between 5 and 16 mEq/liter appear to be sufficient to produce inhibition of sodium chloride reabsorption in the loop of Henle.

Chloride is apparently the ion actively reabsorbed in the ascending limb of Henle's loop [7, 8]. In both the lithium chloride-sodium chloride and the hypotonic lithium studies, chloride was reaching the loop in concentrations similar to those one would expect during 2% or 0.225% sodium chloride, yet $T^c_{H_2O}$ and C_{H_2O} formation were inhibited. This suggests that lithium cannot substitute for sodium as the accompanying cation if distal reabsorption is to proceed unaltered.

[b] Studies of Na^+-K^+-ATPase in kidney cortex of ground squirrels have suggested that lithium may stimulate the enzyme [23]. In the presence of 20 mM potassium, 50 mM lithium produced about 31% of the total stimulation achieved with 100 mM sodium and 20 mM potassium. Furthermore, in concentrations up to 50 mM, lithium causes a miniscule activation of Na^+-K^+-ATPase in the absence of sodium and potassium. It is unlikely, therefore, that these results have any relevance to the *in vivo* conditions of the present experiments.

In summary, acute infusion of lithium into normal Wistar rats results in marked inhibition of urine dilution and concentrating capacity. Amiloride, a diuretic which prevents entry of lithium into cells, partially reverts the changes in $T^c_{H_2O}$ but does not alter the effect of lithium on C_{H_2O}. These findings indicate that a major effect of lithium is to prevent normal collecting duct response to vasporessin (ADH). In addition, the results also suggest that lithium can interfere with normal solute reabsorption by the loop of Henle and more distal nephron sites, leading to reduced C_{H_2O} and $T^c_{H_2O}$. Sodium appears to be necessary for normal solute reabsorption by the loop, whether it is transported actively *per se* or as the necessary cation secondary to active chloride reabsorption.

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