Lithium-Induced Nephrogenic Diabetes Insipidus: In Vivo and In Vitro Studies

IRWIN SINGER, DONALD ROTENBERG, and JULES B. PUSCHETT with the technical assistance of ELIZABETH A. FRANKO

From the Renal-Electrolyte Sections, Departments of Medicine, Veterans Administration and University of Pennsylvania Hospitals and the University of Pennsylvania School of Medicine, Philadelphia Pennsylvania 19104

ABSTRACT The physiological basis for the polyuria and polydipsia occurring in some manic-depressive patients treated with lithium salts was studied in vivo and in vitro. Three lithium-treated polyuric patients, in whom other causes of a concentrating defect were excluded, had abnormal urinary concentrating abilities after a standard water depreviation test. Two of these patients failed to respond to exogenous vasopressin (ADH) and one had a subnormal response. The abilities of these patients to excrete solute-free water (C_{H_2O}) was comparable to normal subjects during steady-state water diuresis, suggesting no gross abnormalities in sodium transport. However, each of these patients demonstrated abnormally low capacities to reabsorb solute-free water ($T^c_{H_2O}$) under hydropenic conditions after administration of hypertonic saline and vasopressin. These in vivo findings demonstrate at least a nephrogenic basis for the diabetes insipidus syndrome manifested by these three patients.

The defect in water transport was further characterized in toad urinary bladders in vitro. Short-circuit current (I) and water flow (W) were studied under basal, ADH-stimulated, and cyclic adenosine 3',5'-monophosphate (c-AMP)-stimulated conditions. Increasing mucosal [Li^+] progressively inhibited basal I, and both I and W induced by ADH. Significant inhibition of basal and ADH-induced I was observed at mucosal [Li^+] < 1.1 mEq/liter, and of ADH-induced W at mucosal [Li^+] = 11 mEq/liter. On the other hand, at these lithium concentrations, neither c-AMP-stimulated W nor I was inhibited. Increasing serosal [Li^+] produced significant inhibition of basal I only at [Li^+] at least 50-fold greater than at the mucosal (urinary) surface. These in vitro studies confirm that mucosal lithium inhibits the action of ADH, but not c-AMP. Hence, lithium appears to be a significant inhibitor of ADH-stimulated water flow, probably acts from the urinary surface, and appears to exert its effect at a site biochemically proximal to c-AMP action.

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INTRODUCTION

Lithium carbonate is a new orally active agent currently enjoying widespread use for the treatment of the manic phase of manic-depressive disorders. Listed among the less commonly occurring side effects of the administration of lithium salts are polyuria and polydipsia (1). Recently, both clinical observations (2, 3) and preliminary data obtained in experimental animals (4, 5) suggest that lithium can produce a reversible nephrogenic diabetes insipidus syndrome. However, the precise pathophysiology of the renal defect remains undefined.

Persistent excretion of large volumes of hypotonic urine, as occurs in some patients treated with lithium carbonate, could be due to (*a*) psychogenic water drinking, (*b*) central diabetes insipidus, and (*c*) nephrogenic diabetes insipidus. These possibilities were evaluated by a standard water-deprivation test, followed by vasopressin administration, with determination of the maximum urinary concentrating ability (U_{max}).[1] The ability of the kidney to reabsorb solute-free water ($T^c_{H_2O}$) was evaluated under hydropenic conditions after the administration of hypertonic saline; this method of estimating $T^c_{H_2O}$ depends on the permeability of the collecting ducts to

[1] *Abbreviations used in this paper:* ADH, vasopressin; C_{H_2O}, capacity of the kidney to excrete solute-free water; C_{In}, clearance of inulin; C_{PAH}, clearance of *p*-aminohippurate; c-AMP cyclic adenosine 3'-5'-monophosphate; GFR, glomerular filtration rate; I, short-circuit current; PAH, *p*-aminohippurate; $T^c_{H_2O}$, capacity of the kidney to reabsorb solute-free water; U_{max}, maximum urinary osmolality; U_{min}, minimum urinary osmolality; V, transepithelial potential difference; W, water flow.

water, the capacity of the ascending limb of the loop of Henle to transport sodium, and the extent to which tubular fluid equilibrates with the cortical and medullary interstitium (6). The ability of the kidney to excrete solute-free water (C_{H_2O}) was determined under steady-state water diuresis; this method of estimating C_{H_2O} depends on the volume of fluid presented to, and the sodium transport occurring in, the distal nephron (7).

Since there are many factors which can underlie any abnormalities in human clearance studies, an in vitro preparation was used to evaluate the effects of lithium on ion transport and water flow more precisely. The isolated toad urinary bladder has often been used as a physiological model for the distal portions of the mammalian nephron (8). In these studies, sodium transport was evaluated by studying short-circuit current, and water flow was studied both volumetrically and gravimetrically. Both basal and ADH-induced transport were investigated in the presence and absence of lithium.

A preliminary report of some of these results has been presented elsewhere (9).

METHODS

In vivo studies

Studies were performed on three polyuric male subjects ranging in age from 27 to 53 yr. Each of these patients were hospitalized on the psychiatric ward of the Philadelphia Veterans Administration Hospital with a diagnosis of manic-depressive psychosis. They were all treated with lithium carbonate in divided doses, ranging from 1500 to 2400 mg/day. They were taking unrestricted (approximately 4 g) sodium diets and had normal glomerular filtration rates (90–105 ml/min). Other causes of a concentrating defect, including therapy with other drugs, hypercalcemia, hypokalemia, thyroid disease, and hypertension, were excluded. Since each of the patients complained of polyuria and polydipsia for the first time after the introduction of lithium therapy, this drug was suspected despite serum lithium levels which were consistently within the accepted therapeutic range of 0.5 to 1.5 mEq/liter (1).

After informed written consent was obtained, each patient underwent a series of three studies: (*a*) determination of maximal urinary concentrating ability (U_{max}) after water deprivation and exogenously administered vasopressin; (*b*) determination of the maximal free-water clearance (C_{H_2O}) and formation of a maximally dilute urine (U_{min}); and (*c*) determination of the maximal solute-free water reabsorption ($T^c_{H_2O}$) during hydropenia and hypertonic saline diuresis. (Five normal men served as controls and were evaluated in a similar manner to the three patients.)

Studies of concentrating ability (U_{max}). Under constant observation, the patients were deprived of fluid for a period of 12 hr, or until they had lost 3–4% of body weight (whichever occurred first). Blood and urine samples were obtained for determination of osmolality at the outset of the study, and at 4-hr intervals until the study was terminated. At the end of the water deprivation period, 10 U of aqueous vasopressin (Pitressin; Parke, Davis & Co., Detroit, Mich.; lot #KB121) was administered subcutaneously. Urine specimens were collected for another 1–2 hr, at which time blood was again obtained, and the osmolalities of all specimens were determined.

Studies of diluting ability (U_{min}) *and free-water clearance* (C_{H_2O}). Food was withheld after the midnight before the study, but the patient was allowed water. The patient voided at 8 a.m., and base line studies of blood and urine were obtained; the patient remained in a recumbent position thereafter, except when voiding. Standard priming doses of inulin and *p*-aminohippurate (PAH) were given intravenously, and a sustaining infusion of physiological saline containing both agents was initiated at a rate of 1.0 ml/min. After a 45–60 min equilibration period, a urine specimen was obtained, and a 20 ml/kg body weight oral water load was administered over a 20–30 min period. Urine volumes were determined every 10–30 min, and an equivalent volume of tap water was replaced orally, with an additional 0.7–1.0 ml/min to replace insensible losses. This procedure continued until steady-state urine flow was achieved. Blood samples were obtained at approximately 30- to 45-min intervals throughout the study.

Studies of solute-free water reabsorption ($T^c_{H_2O}$). Measurement of $T^c_{H_2O}$ during hypertonic (3%) saline diuresis was performed as follows. Both food and water were omitted after the midnight before the study, and no oral intake was allowed except for 5–10 ml water taken with a regularly scheduled dose of lithium. The patient voided at 8 a.m. and assumed recumbency for the remainder of the study after base line studies were obtained, as above. Priming and sustaining doses of inulin and PAH were given in an infusion containing vasopressin, and the solution was administered at a rate calculated to deliver 360 mU/hr. After a 40–45 min equilibration period, the patient voided and an intravenous infusion of 3% saline was begun at a rate of 10–12 ml/min. Urines were collected every 10–30 min, and blood samples were collected every 45–60 min throughout the study.

Specimen analysis. Blood and urine were analyzed for sodium and potassium by flame photometry. Osmolalities were determined with an Advanced Osmometer (Advanced Instruments Inc., Newton Highlands, Mass.; Model 68-3L). Inulin and PAH were measured by methods described previously (7). Glomerular filtration rates and effective renal plasma flow rates were determined by the clearances of inulin (C_{in}) and PAH (C_{PAH}), respectively.

Calculations. Osmolar clearance (C_{osm}), free water clearance (C_{H_2O}), and tubular reabsorption of solute-free water ($T^c_{H_2O}$) were determined from the following formulae: $C_{osm} = U_{osm}V/P_{osm}$; $C_{H_2O} = V - C_{osm}$; $T^c_{H_2O} = C_{osm} - V$; where V = urine flow rate (ml/min), U_{osm} = urine osmolality, and P_{osm} = plasma osmolality (mOsm/kg).

In vitro studies

Urinary bladders were excised from doubly pithed female toads, *Bufo marinus*, obtained from the Dominican Republic (National Reagents, Bridgeport, Conn.). The excised hemibladders were placed in continuously aerated NaCl-Ringer's solution at room temperature (22–24°C) for varying times before use. NaCl- Ringer's solution contained: NaCl, 104.1 mmoles/liter; KCl, 3.6 mmoles/liter; $CaCl_2$, 0.7 mmoles/liter; and Na_2HPO_4, 0.7 mmole/liter; pH, 7.8–8.2; osmolality, 220 mOsm/kg water. Lithium and choline Ringer's solutions were made by isotonic, isohydric substitution for sodium, as appropriate to the experimental conditions. (Choline chloride, ChCl, was obtained from Eastman Organic Chemicals, Rochester, N. Y.).

A double chamber, similar to those described by Sharp and Leaf (10) and based on the techniques of Ussing and Zerahn (11), was used to study transepithelial potential difference and short-circuit current. In all chamber experiments, the hemibladders were mounted across the entire chamber, so that half of a single hemibladder isolated its own serosal and mucosal compartments (cross-sectional areas of 2.3 cm^2). In this manner, a single hemibladder provides an experimental and a control quarter bladder. The transepithelial potential difference (V) and the short-circuit current (I) across each quarter-bladder were measured intermittently by methods described previously (12), using Keithley 200 B Electrometers and a Weston 622 Microammeter, respectively. In all cases V and I were followed until a stable base line was obtained for at least 20 min before any experimental manipulations. V, I, and water flow were usually determined at intervals of 5 min or less. Bladders with base line $V < 5.0$ mv or $I < 5.0$ μa were discarded in all experiments.

A double chamber with closed mucosal compartments and horizontally mounted pipettes, similar to that described previously (12), was used for simultaneous measurements of electrical properties [2] and water flow (compartment cross-sectional areas of 2.0 cm^2). Paired quarter-bladder experiments with either control water flow rate greater than 2.0 μl/min, or whose control quarter bladder failed to respond to vasopressin (at least 6-fold increase in water flow rate) were not used. The mucosal compartments contained the appropriate Ringer's solution diluted in half with distilled water to provide the osmotic gradient. Solutions in each compartment (volume, 5.0 ml) were changed by draining and refilling with the next solution.

Osmotic water flow was measured also in paired hemibladders mounted on glass tubes ("bags") as described by Bentley (13). The appropriate Ringer's solution was diluted in half with distilled water, and placed inside each hemibladder to bathe the mucosal surface (volume, 5.0 ml); the serosal surface of each hemibladder was bathed in isotonic NaCl-Ringer's solution. Weight loss was measured for at least 30 min before vasopressin was added. Paired hemibladder experiments whose control hemibladder failed to respond to vasopressin (see above) were not used.

After a stable base line was obtained in both chamber (quarter-bladder) and bag (hemibladder) experiments, either vasopressin (ADH; Pitressin, 20 U/ml; Parke, Davis & Co., Detroit, Mich.), or dibutyryl-3',5'-cyclic adenosine monophosphate (c-AMP; Schwarz Bio Research Inc., Orangeburg, N. Y.) was added to the serosal medium (final concentration: 100 mU/ml for ADH; 1.0 mmole/liter for c-AMP), and the peak induced water flow rate was determined (within 40 min).

RESULTS

In Vivo Studies

The data summarizing the responses of the three lithium-treated patients studied to maximal hydropenia and vasopressin administration are presented in Fig. 1. In our laboratory, normal subjects evaluated according to this protocol will develop a urine osmolality greater than 800 mOsm/kg H_2O after fluid deprivation alone; U_{osm} rises little or not at all after vasopressin. Patient H. S., whose defect was the most severe, still developed a urine osmolality hypotonic to plasma after 12 hr of fluid deprivation and showed no response to vasopressin. H. W., whose urine osmolality was slightly greater than that of plasma, also did not respond to vasopressin. C. McC.'s maximal concentrating ability was below normal, and while urine osmolality did rise during vasopressin administration, maximal U_{osm} was still subnormal. In Fig. 2, the ability of the patients to excrete solute-free water, C_{H_2O}, an index of sodium transport in the distal nephron, is compared with the results obtained in five normal subjects. Each point represents the mean of two or three collection periods during steady-state maximal water diuresis. At similar rates of distal sodium delivery, expressed as the urine flow rate divided by the GFR (14), patients and normal subjects formed comparable amounts of free water per 100 ml of glomerular filtrate. In each instance the patients were also able to achieve a normal minimum urine osmolality of < 90 mOsm/kg H_2O.

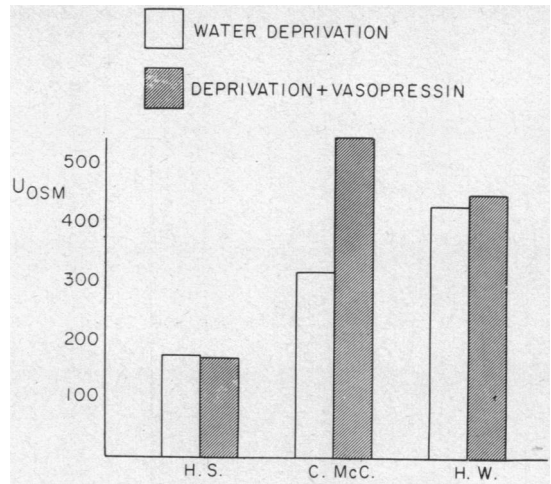

FIGURE 1 Effects of water deprivation and vasopressin on urine osmolality. The urine osmolalities are indicated on the ordinate (U_{osm}, mOsm/kg) and the three patients are identified on the abscissa. The responses of each patient to water deprivation (open bars) and water deprivation plus vasopressin (hatched bars) are shown. Only one patient (C. McC.) responded to vasopressin and this response is subnormal.

The capacity of these patients to reabsorb free water ($T^c_{H_2O}$) was measured during increasing rates of sodium and water delivery to the distal nephron. In Fig. 3, free water reabsorption is plotted against increasing osmolar clearance for three normal subjects and for the three patients. $T^c_{H_2O}$ was clearly abnormal in all three

[2] The current measurements with an osmotic gradient were only used to document the presence of lithium inhibition and to monitor bladder viability in these water-flow studies. Although the qualitative current results with a gradient were similar to those obtained without a gradient, all of the data in the Results section was obtained without a gradient.

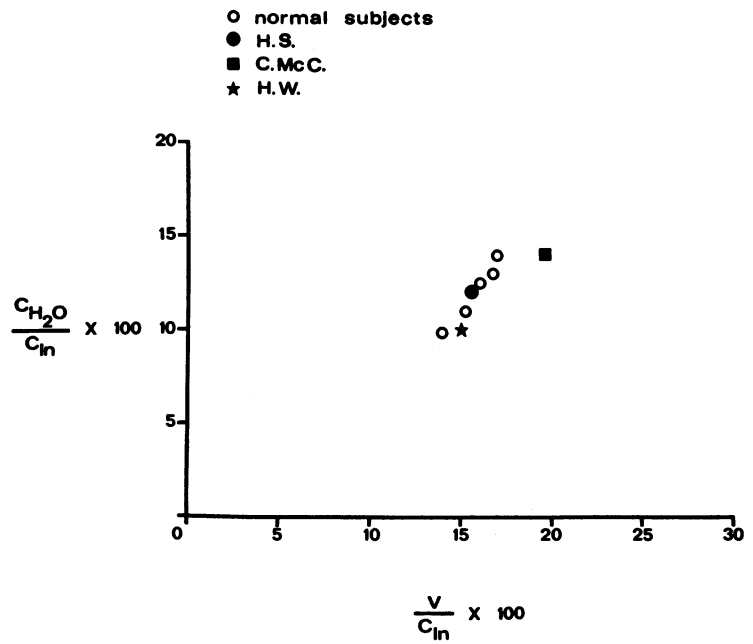

FIGURE 2 Comparison of free water clearance in lithium-treated patients and normal control subjects. The free water clearance (C_{H_2O}, ml/min) divided by glomerular filtration rate (GFR, ml/min) is on the ordinate and the urine flow rate (V, ml/min) is on the abscissa. The three patients are represented by different solid symbols and the five control subjects by open symbols.

patients, even at low levels of osmolar clearance. Moreover, patients H. S. and H. W. were actually excreting free water throughout the entire study, despite continued administration of hypertonic saline and vasopressin.

IN VITRO STUDIES

1. Effects of lithium on base line electrical characteristics

a. Mucosal lithium. Substitution of lithium for increasing fractions of the mucosal sodium resulted in significant decreases in both electrical potential difference (V) and short-circuit current (I) when the mucosal [Li^+] was greater than 1.1 mEq/liter. These electrical responses were usually observed within 10 min, and reached a maximum change within 20 min after the substitution; the responses usually persisted at these new levels for at least 60 min when compared to control quarter-bladders with choline replacing the same fraction of the mucosal sodium. (In this manner, both the experimental and the control quarter-bladders had the same mucosal [Na^+] and ionic strength.) At concentrations below 11.0 mEq/liter, these electrical responses to mucosal lithium could be reversed to within 90% of their base line values before lithium substitution. (After 20 min of exposure to Li^+ or Ch^+, vasopressin was added to the serosal medium, and the effects of lithium on the ADH response were determined [see section 2].) The typical protocol for lithium substitution experiments is given below:

Period:	1	2	3
Experimental quarter-bladder (E)	Na^+	Li^+	ADH
Control quarter-bladder (C)	Na^+	Ch^+	ADH

The base line electrical responses for each quarter-bladder were evaluated by dividing the value for V or I in period 2 by the corresponding value in period 1; the effect of lithium was compared to choline [3] by taking the ratio the experimental value to the control value (E/C) and expressing the result as a per cent of the control ($\Delta\%$). Formally, if the period 1, 2, and 3 values for the experimental quarter-bladder are a, b, and c, and the corresponding values for the control quarter-bladder are d, e, and f, then: base line $\Delta\% = 100\ [(b/a)/(e/d) - 1]$. (In a similar manner, ADH-induced $\Delta\% = 100\ [(c/b)/(f/e) - 1]$.) Values are expressed as the mean ±SEM.

A representative paired quarter-bladder experiment of this kind is shown in Fig. 4, and the results of similar experiments at different concentrations of mucosal lithium are summarized in Fig. 5 for base line electrical

[3] Choline substitution alone had no significant effects on short-circuit current. For example, at [Li^+] = 11 mEq/liter, $\Delta I = -2.8 \pm 15\%$; n = 17; NS (ADH and c-AMP experiments).

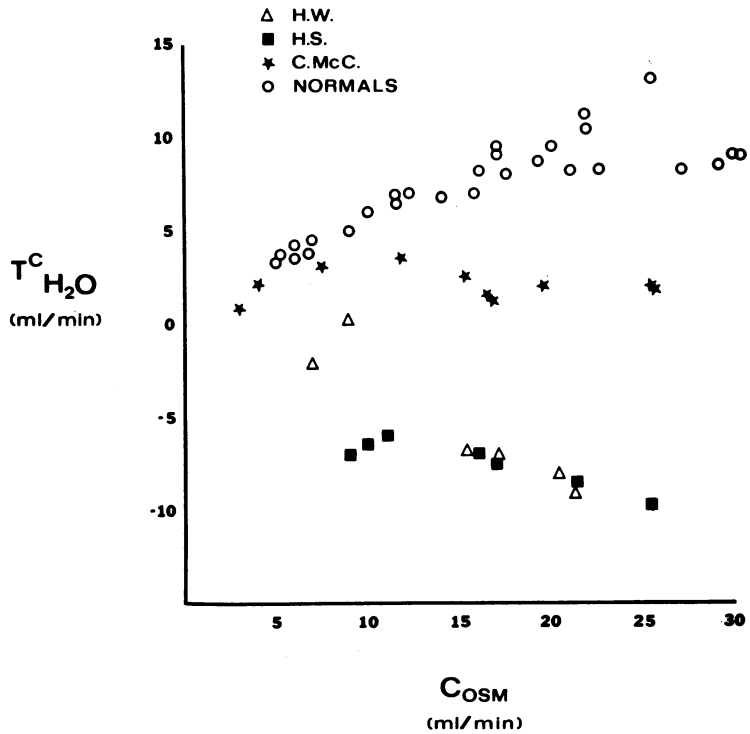

FIGURE 3 Comparison of free water reabsorption ($T^{c}_{H_2O}$) in lithium-treated patients and normal control subjects. Both positive and negative values for $T^{c}_{H_2O}$ (ml/min) are represented on the ordinate. Osmolar clearance (C_{osm}, ml/min) is indicated on the abscissa. The normal subjects are represented by open circles and the different patients by the other symbols.

effects. There was significant inhibition of both V and I at mucosal [Li^+] as low as 1.1 mEq/liter,[4] and maximal inhibition was observed above 11.0 mEq/liter. Nearly total replacement of the mucosal Na^+ with Li^+ produced no greater inhibition. Experimental quarter-bladders containing [Li^+] = 104.1 mmoles/liter, [Na^+] = 1.4 mmoles/liter, were compared to controls containing the usual NaCl-Ringers's solution with [Na^+] = 105.5 mmoles/liter; $\Delta I\% = -36\pm7\%$; n = 11, $P < 0.001$.)

Further studies suggested that the inhibitory effect of mucosal Li^+ appeared rather sharply between 0.55 and 0.7 mEq/liter. At mucosal [Li^+] = 0.7 mEq/liter, the mean ±SEM inhibitions of V and I were $-5\pm7\%$ (NS) and $-13\pm4\%$ ($P < 0.005$), respectively, for 13 experiments. Since the changes in V and I were roughly proportional at each concentration, there was little or no change in resistance.

b. Serosal lithium. Substitution of increasing concentrations of Li^+ for Na^+ in the serosal medium was without any significant effect until very high Li^+ concentrations were achieved; at a serosal [Li^+] = 55 mEq/liter, $\Delta I\% = -24\pm10\%$ (n = 8; $P < 0.05$). At these high concentrations of serosal Li^+, a significant movement of Li^+ from the serosal to the mucosal medium cannot be excluded; since similar degrees of inhibition were observed with mucosal [Li^+] < 1.1 mEq/liter, it is possible that the apparent "serosal" action is derived from back-diffusion to the mucosal surface. Simultaneous substitution of Li^+ for Na^+ symmetrically in both serosal and mucosal media was not significantly different from mucosal substitution alone; when [Li^+] = 11.0 mEq/liter, $\Delta I\% = -27\pm7\%$ (n = 7; $P < 0.01$).

2. Effects of lithium on vasopressin (ADH) responsiveness

a. Short-circuit current. Mucosal [Li^+] up to 5.5 mEq/liter had no additional inhibitory effect above that which could be accounted for by inhibition of the lithium period; (see section 1*a*.) However, at a mucosal [Li^+] of 11.0 mEq/liter substitution of Li^+ for Na^+ had a significant additional effect on the ADH-induced current response; a typical experiment is shown in Fig. 4, and the results are summarized in Fig. 6, right. Nearly total replacement of the mucosal Na^+ with Li^+ produced a sim-

[4] Seven of these 15 experiments were carried on to ADH, and are shown in Table I. (See also Fig. 5.)

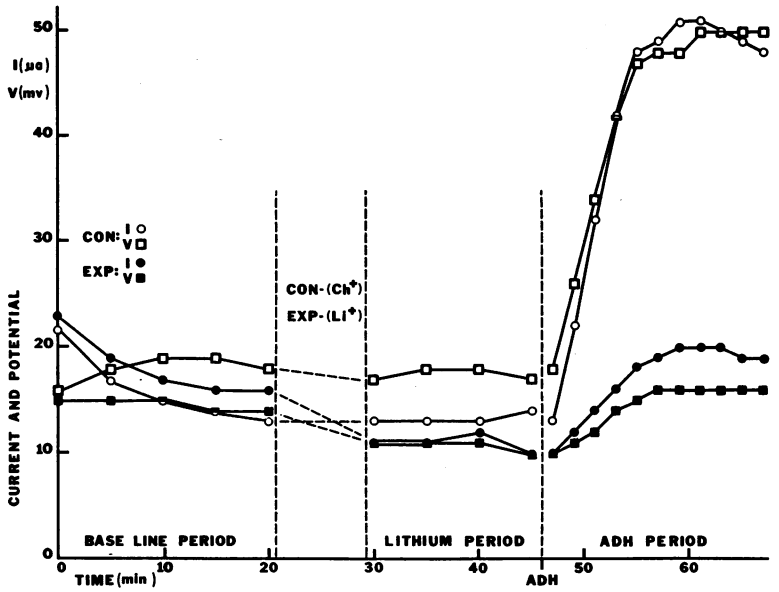

FIGURE 4 Electrical effects of mucosal lithium. The short-circuit current (I, μa, circles) and potential difference (V, mv, squares) are indicated on the common ordinate, and the time (min) along the common abscissa. After a period of base line stabilization (20 min), the solutions were drained and replaced (first pair of vertical dashed lines; 20–29 min). Both V and I for the control quarter-bladder (open symbols) were virtually unaffected by replacing a small fraction of the mucosal Na^+ with Ch^+ ($[Ch^+] = 11$ mmoles/liter). On the other hand, both V and I for the experimental quarter-bladder (solid symbols) were decreased by replacing the same fraction of the mucosal Na^+ with Li^+ ($[Li^+] = 11$ mmoles/liter). Subsequent addition of vasopressin (ADH at 46 min) resulted in large increases in both V and I for the control quarter-bladder, but much smaller increases for the experimental quarter-bladder, even when compared to the values observed during the lithium period.

ilar degree of additional inhibition (solutions as in section 1*a*, $\Delta I\% = -34 \pm 9\%$; $n = 6$; $P < 0.02$).

Substitution of Li^+ for Na^+ in the serosal medium had no additional inhibitory effect on the ADH-induced current response (i.e., above that which should be accounted for by base line inhibition). For example, at serosal $[Li^+] = 55.0$ mEq/liter, $\Delta I\% = +22 \pm 9\%$ ($n = 8$; NS).

b. Water flow. When studied in paired quarter-bladders (volumetric chamber method), substitution of Li^+ for Na^+ in the mucosal medium was without effect on base line water flow in response to a 2:1 osmotic gradient. (In each case, the experimental quarter- or hemibladder had Li^+ substituted for Na^+ before dilution, and the control had Ch^+ substituted for the same fraction of the mucosal Na^+.)

However, ADH-induced water flow was significantly inhibited when the initial mucosal $[Li^+]$ was 11.0 mEq/liter before dilution. A typical experiment of this type is shown in Fig. 7, left, and the results are summarized in Fig. 6, left. Increasing the initial mucosal $[Li^+]$ to 55.0 mEq/liter before dilution was without any further inhibitory effect ($\Delta W\% = -16 \pm 8\%$; $n = 12$; $P > 0.05$).

These results were confirmed in paired hemibladder experiments (gravimetric bag method). With an initial mucosal $[Li^+] = 11.0$ mEq/liter before dilution, the mean ±SEM inhibition of ADH-induced water flow was $-46 \pm 10\%$ ($n = 9$; $P < 0.005$). Although this degree of inhibition is much greater than that observed in chamber experiments, the difference may be accounted for by the much larger surface area utilized to observe flow in bag experiments. However, even with the more sensitive gravimetric method, reduction of the initial mucosal $[Li^+]$ to 5.5 mEq/liter before dilution failed to produce significant inhibition of the ADH-induced water flow response ($\Delta W\% = -33 \pm 19\%$; $n = 10$; $P > 0.1$).

3. Effects of lithium on cyclic adenosine 3′, 5′-monophosphate (c-AMP) responsiveness

a. Short-circuit current. Mucosal Li^+ at concentrations of 1.1 mEq/liter and of 11.0 mEq/liter, which produced significant inhibition of base line current at both concentrations, and additional inhibition of ADH-responsiveness at the higher concentration, failed to inhibit the response to c-AMP. These results are summarized in comparison to ADH in Fig. 8, right.

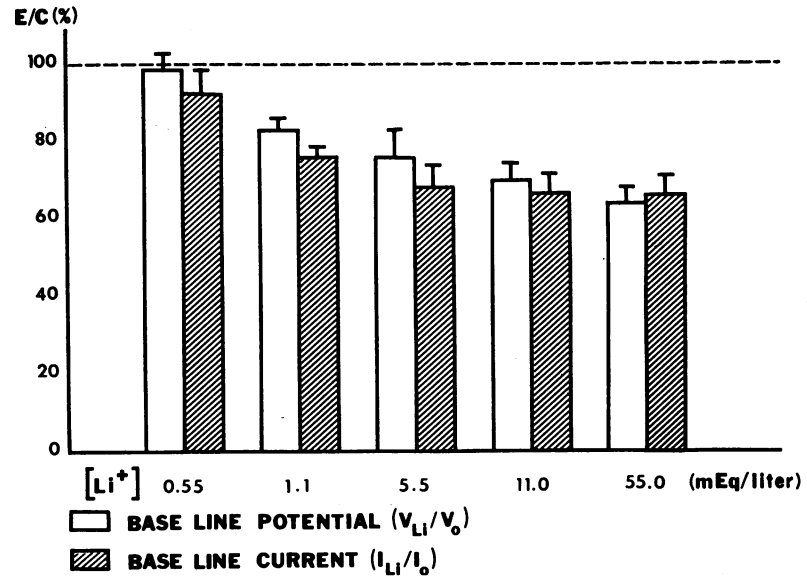

FIGURE 5 Base line inhibition by lithium. The inhibition (mean ±SEM) of both potential difference (open bars) and short-circuit current (hatched bars) produced by increasing concentrations of mucosal lithium is indicated as the ratio (E/C) of the experimental (Li^+) to the control (Ch^+) quarter-bladders, expressed as a per cent of the control; the dashed line at 100% represents no difference between the paired experimental and control quarter-bladders. The significance of each of these paired differences (Student *t* test) is given below:

[Li^+]	0.55	1.1	5.5	11.0	55.0
n =	15	15	18	29	16
(V) $P <$	NS	0.001	0.005	0.001	0.001
(I) $P <$	NS	0.001	0.001	0.001	0.001

(Each value for E or C is obtained from the ratio of the lithium period [V_{Li} or I_{Li}] to the corresponding base line period [V_o or I_o] see Fig. 4 and section 1*a*). Seven of the 15 experiments at [Li^+] = 1.1 mEq/liter were carried on to ADH, and are shown in Table I. Although there is significant inhibition ($P < 0.001$) of base line current, there is no inhibition of ADH-induced current at this concentration of lithium.

b. Water flow. Mucosal Li^+ at initial concentrations of 11.0 mEq/liter likewise failed to inhibit the c-AMP-induced water flow response. These c-AMP results from paired quarter-bladders are contrasted with those obtained for ADH-induced responses in Fig. 8, left. Even the more sensitive gravimetric method, with the same mucosal [Li^+] and gradient, failed to show inhibition of the c-AMP-induced response ($\Delta W\% = +17 \pm 17\%$; n = 11; NS); these c-AMP observations are in sharp contrast to the lithium inhibition of ADH-induced water flow, described above.

DISCUSSION

Lithium-induced concentrating defects, although only recently described, are probably not uncommon (5). To investigate the mechanisms responsible for the lithium-induced renal concentrating defect, we utilized current models of the urinary concentration and dilution mechanisms (6). As the glomerular filtrate courses through the ascending limb of the loop of Henle, sodium is transported into the interstitium in excess of water. In the absence of ADH, the hypotonic fluid thus entering the early distal tubule may be further diluted by the reabsorption of sodium chloride at relatively water-impermeable cortical diluting sites, and a dilute urine is excreted. In the presence of ADH, the passive movement of solute-free water from the isotonic tubular fluid in the collecting duct to the hypertonic medulla results in excretion of a concentrated urine. Thus, renal concentrating defects in the presence of circulating ADH may be produced by (*a*) depressions in GFR which limit the delivery of sodium-containing fluid to the loop of Henle and the collecting duct, (*b*) defective sodium transport in the ascending limb of the loop of Henle, (*c*) abnormal medullary blood flow which disturbs the osmotic gradient, and (*d*) depressed responsiveness of the distal tubular epithelium and collecting duct to ADH.

Each of our three patients clearly demonstrated a concentrating defect when challenged with prolonged hydropenia plus vasopressin administration. Of interest is the

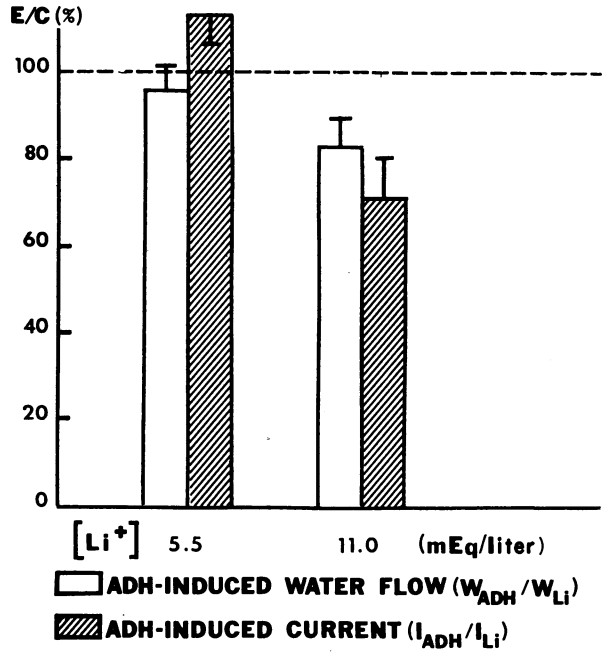

FIGURE 6 ADH responses in the presence of lithium. The inhibition (mean ±SEM) of both ADH-induced water flow (open bars) and ADH-induced current (hatched bars) by different initial concentrations of mucosal lithium is expressed as in Fig. 5. Each value of E or C is obtained from the ratio of the ADH-induced peak (W_{ADH} or I_{ADH}) to the corresponding lithium period (W_{Li} or I_{Li}). The significance of each of these paired differences (Student's t test) is given below ([Li^+] before 1:2 dilution in water flow studies only).

	Water flow		Current	
[Li^+]	5.5	11.0	5.5	11.0
n =	11	15	11	9
$P <$	NS	0.05	NS	0.02

range of concentrating abnormalities observed; the maximum U_{osm} obtained by hydropenia alone ranged from 150 to 450 mOsm/kg. This implies a spectrum of responses of the distal nephron to ADH. Two patients failed to respond to ADH at all; one patient (H. W.) demonstrated a significant, though blunted, response to ADH.

To differentiate among the possible causes of the renal concentrating defect, the patients were studied under conditions of maximal sustained water diuresis to test their ability to excrete free water and thus estimate distal sodium transport at the diluting sites. Since the ability of our patients to excrete free water compared favorably to a group of normal subjects used as controls, we conclude that sodium transport in the ascending limb of the loop of Henle and the other diluting segments was grossly normal. However, studies during hypertonic saline and vasopressin infusions revealed a decreased $T^c_{H_2O}$ at low and moderate rates of sodium delivery. These data suggest that the lithium-induced concentrating defect in man is most likely due to impaired water flow across the collecting duct epithelium rather than abnormal sodium pumping by the ascending limb of the loop of Henle. Our studies do not exclude, as additional mechanisms, either the inability to maintain an osmotic gradient in the medulla or abnormal sodium transport by the distal nephron at higher rates of sodium delivery than were achieved in these studies.

Since the diabetes insipidus in these three lithium-treated patients was confirmed to be nephrogenic, the effects of lithium on salt and water transport by toad urinary bladders were studied to further characterize the mechanism by which lithium impairs the response of the mammalian kidney to ADH. In principle, lithium could affect salt and/or water transport by altering basal transport characteristics, ADH responsiveness, or both; furthermore, the action of lithium may be at the mucosal surface, the serosal surface, or both. The present studies demonstrate that (*a*) very low concentrations of lithium inhibit basal sodium transport, (*b*) higher concentrations of lithium inhibit ADH-induced sodium transport and water flow in response to an osmotic gradient, and (*c*) lithium probably acts at the mucosal surface.

Under basal conditions lithium is both accumulated by toad urinary bladders (15) and transported by both frog (16) and toad urinary bladders, although less well than sodium (15). It has also been suggested that lithium may compete with sodium for entry at the mucosal membrane in toad bladders (17). It might be argued that replacement of a fraction of the mucosal sodium with the less well transported lithium would then be expected to produce inhibition of the short-circuit current. Therefore, we compared the effects of lithium substitution (experimental preparations) to those of choline substitution (control preparations) for the same fraction of the sodium present. Since choline is not transported, and since up to 50% replacement of the mucosal sodium with choline has no effect on sodium transport (18), any transport of lithium would tend to minimize the observed inhibitory effect on short-circuit current. Therefore the observed inhibitory effects of lithium cannot be attributed to a reduction in the concentration of sodium present, and are likely to be underestimated to the extent that lithium is itself transported.

Lithium was found to inhibit basal short-circuit current at concentrations of 0.7 mEq/liter in the mucosal medium. Increasing inhibition was observed at increasing lithium concentrations up to 5.5 mEq/liter with maximal inhibition of 35-40% observed at this concentration; no further inhibition of basal transport was found up to 55 mEq/liter, even when compared to sodium-containing,

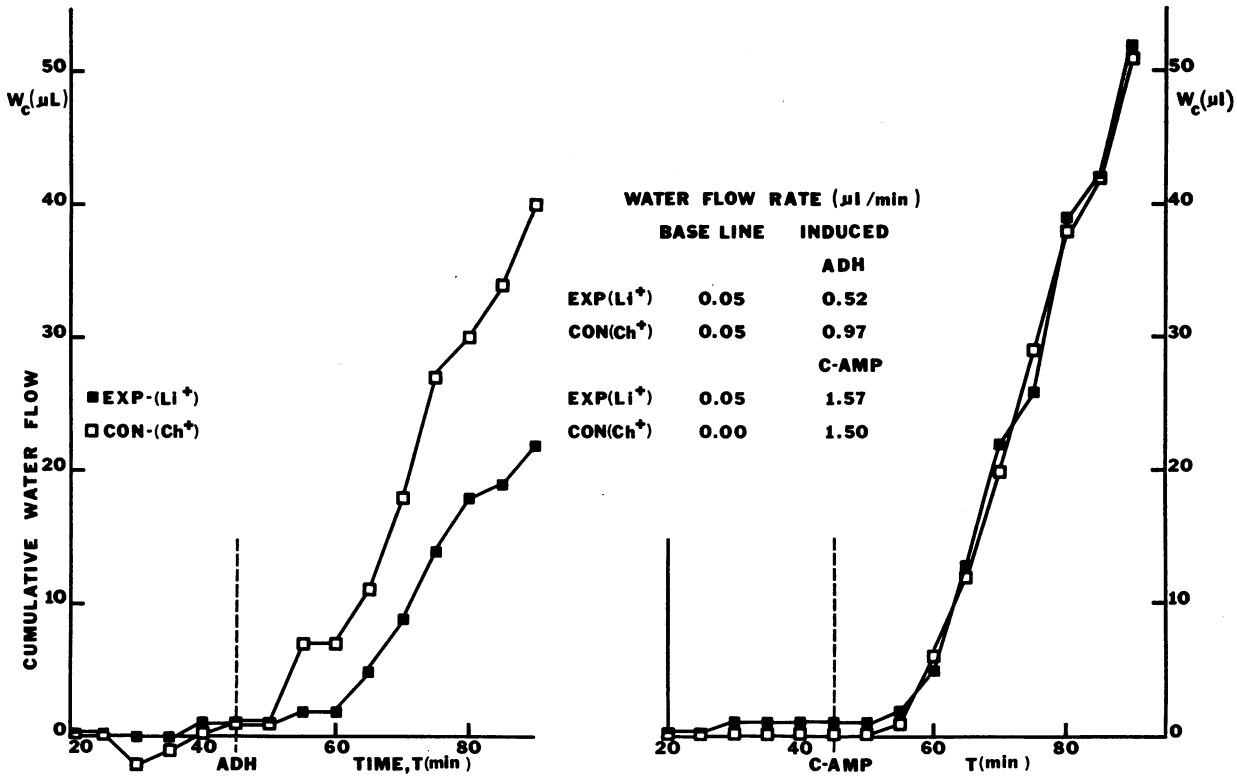

FIGURE 7 Lithium inhibition of ADH and c-AMP-induced water flow. The cumulative water flows (W, μl) for ADH-induced flow (left) and c-AMP- induced flow (right) are indicated on the ordinates. The time (min) is given on each abscissa, with the dashed vertical line at the time of addition of ADH or c-AMP. The first 20 min of stabilization (base line period in NaCl-Ringer's solution; mucosal medium 1/2 isotonic) has been omitted from the graph of each of these paired quarter-bladder experiments. At 20 min the mucosal medium was replaced with 1/2 isotonic solutions originally containing either $[Li^+] = 11.0$ mEq/liter (experimental quarter-bladders, solid symbols) or $[Ch^+] = 11.0$ mEq/liter (control quarter-bladders, open symbols). Although no effects were observed on basal water flow by this substitution, lithium clearly inhibited the response of the experimental quarter-bladder to ADH (left); in contrast, the response to c-AMP (right) was unaffected.

rather than choline-containing controls. The present study cannot distinguish between the two most likely explanations for the latter data: (*a*) lithium transport increases as its mucosal concentration rises, and (*b*) part of the sodium transport pathway is insensitive to lithium at high concentrations. However, at higher concentrations (> 11.0 mEq/liter) lithium did inhibit the short-circuit current responses to ADH significantly more than could be accounted for by proportional depression of basal transport. Since ADH can induce lithium transport across frog urinary bladders (19), the degree of inhibition of sodium transport calculated in the present experiments (15-20%) is likely to be an underestimate.

Whereas lithium is an effective inhibitor of basal sodium transport at very low concentrations in the mucosal medium, and only at very high concentrations in the serosal medium, it is likely that the inhibitory action takes place at the mucosal surface. Similarly, high serosal lithium concentrations are without effect on the ADH response, whereas lower mucosal concentrations do affect the ADH response. In fact, the serosal effect of high concentrations of lithium may be the result of back-diffusion into the mucosal medium. If applicable to man, these findings suggest that the urinary rather than the blood levels of lithium may be clinically important in the production of nephrogenic diabetes insipidus. However, the clinical study provided no evidence for impaired sodium transport, and there is no evidence for ADH-induced sodium transport in man.

On the other hand, it is clear that ADH-induced water flow does occur in man, as it does in toad urinary bladder. The present studies demonstrate significant lithium inhibition of ADH-induced water flow both in chamber (15-20%) and in bag (45-50%) preparations. Since it is likely that ADH acts through the production

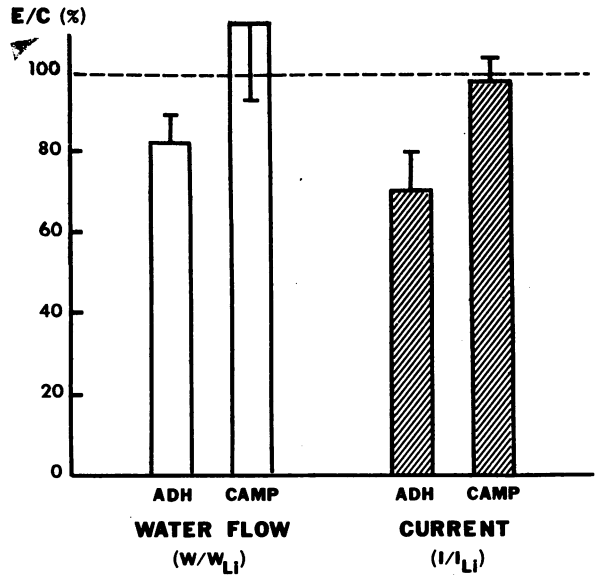

FIGURE 8 Comparative lithium inhibition of ADH and c-AMP. The inhibition (mean ±SEM) of water flow (open bars) and current (hatched bars) induced by ADH and c-AMP is expressed as in Figs. 5 and 6, with mucosal $[Li^+] = 11.0$ mEq/liter in the original solution. The significance of each of these paired differences (Student's t test) is given below:

	Water flow		Current	
	ADH	c-AMP	ADH	c-AMP
n =	15	13	9	8
$P <$	0.05	NS	0.02	NS

(Note that these ADH experiments are also shown in Fig. 6 for $[Li^+] = 11$ mEq/liter).

of c-AMP (20, 21), and since c-AMP mimics the actions of ADH to accelerate both sodium and water transport across toad urinary bladder, the effects of lithium on c-AMP-induced transport were studied. c-AMP-induced transport of either sodium or water was not significantly affected by lithium concentrations which inhibited ADH-induced transport. These data suggest that the effects of lithium on ADH-induced transport are exerted at a site biochemically proximal to the action of c-AMP. Furthermore, lithium is known to inhibit the production of c-AMP by various adenyl cyclase systems in vitro, including brain, thyroid, and kidney (22–24).

Recently, Forrest, Cohen, Torretti, and Epstein (5) reported that lithium produced nephrogenic diabetes insipidus in rats, and that c-AMP failed to increase U_{osm} above P_{osm} under these conditions. From these data, the authors suggested that lithium interfered with the action of ADH at a site beyond the production of c-AMP. Although these findings with different doses in a different preparation cannot be entirely reconciled with our results, a significant rise in U_{osm}, even if not above P_{osm}, may mean a subnormal but significant increase in water transport was induced by c-AMP. Our findings are also supported by a recent study of rabbit kidney adenyl cyclase, where a direct assay of c-AMP production showed that lithium inhibited ADH interaction with adenyl cyclase (24). The methods employed in that study effectively exclude the alternative possibility that lithium increases c-AMP destruction (25). The possibility that lithium also inhibits c-AMP action (5) is not excluded.

In conclusion, our in vivo study has documented impaired ADH-induced water flow across the collecting ducts underlying nephrogenic diabetes insipidus in three patients treated with lithium carbonate for manic-depres-

TABLE I

*Effect of Lithium on Base Line and ADH-Induced Electrical Characteristics**

Expt. No.	Experimental quarter-bladder						Control quarter-bladder						$\frac{Li^+/Base}{Ch^+/Base}$		$\frac{ADH/Li^+}{ADH/Ch^+}$	
	Base line		Lithium		ADH		Base line		Choline		ADH					
	V	I	V	I	V	I	V	I	V	I	V	I	V	I	V	I
1	22	34	20	25	39	104	27	34	27	31	62	124	0.909	0.806	0.849	1.040
2	24	26	13	11	26	29	17	21	10	14	24	41	0.922	0.635	0.833	0.900
3	24	27	15	14	32	36	13	42	12	33	29	93	0.667	0.659	0.882	0.912
4	22	19	16	12	30	28	18	17	24	21	48	69	0.545	0.510	0.938	0.710
5	15	14	7	5	16	13	8	8	4	4	10	10	0.934	0.714	0.914	1.040
6	64	104	50	53	59	132	28	30	24	24	40	53	0.911	0.636	0.708	1.127
7	14	11	10	6	15	10	34	43	34	39	54	104	0.714	0.601	0.945	0.624
Mean	26.4	33.6	18.7	18.0	31.0	50.3	20.7	27.9	19.3	23.7	38.1	70.6	0.800	0.652	0.867	0.908
±SEM	6.0	11.2	5.1	5.9	5.3	16.7	3.2	4.9	3.8	4.2	6.4	13.8	0.055	0.032	0.029	0.064
Δ%													−20.0	−34.8	−13.3	−9.2
±SEM													±5.5	±3.2	±2.9	±6.4

* Mucosal $[Li^+]$ or $[Ch^+] = 1.1$ mEq/liter; V = potential difference (mv); I = short-circuit current (μa).

sive disorders. In vitro studies with toad urinary bladders suggest that lithium exerts its action on the renal concentrating mechanism from the urinary surface, and that the mechanism of action is at least in part inhibition of ADH-stimulated production of c-AMP.

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