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Dependence of Water Movement on Sodium Transport in Kidney Proximal Tubule: A Microperfusion Study Substituting Lithium for Sodium

B. Corman, N. Roinel, and C. de Rouffignac
Département de Biologie, Centre d'Etudes Nucléaires de Saclay, B.P. n° 2, 91190 Gif-sur-Yvette, France

Summary. The relationship between water and sodium movements through the mammalian proximal convoluted tubule was investigated by substituting lithium for sodium. Proximal convoluted rat Kidney tubules were perfused *in vivo* with a Ringer solution containing 107 meq/liter lithium and 42 meq/liter sodium. Several micropunctures were made along the same nephron, and [^{3}H] inulin, [^{14}C] glucose, ^{22}Na, osmolality, Na, Mg and Cl were determined on each sample. Measurements of ^{22}Na showed that sodium and lithium diffusion rates were practically identical throughout the entire epithelium. A one- for-one exchange of sodium for lithium induced a negative transepithelial net flux of Na from plasma to lumen. However, despite this negative flux, a positive net water movement was measured from lumen to plasma. This movement was proportional both to glucose reabsorption and to the rise in the chloride concentration, two mechanisms known to be dependent on the transcellular movement of sodium. It was therefore concluded that the net water flux was a function of the unidirectional transcellular net flux of Na.

Rabbit proximal convoluted tubules were perfused *in vitro* with a solution containing 75 meq/liter Li and 75 meq/liter Na on both the luminal and peritubular sides. Under these conditions, the water reabsorption rate dropped to half its control value. Water movement was therefore a function of the external sodium concentration, which in turn probably regulates the intracellular Na concentration.

Key words: Water fluxes, Na fluxes, proximal tubule microperfusion, Li substitution, rat kidney

It is now generally agreed that water movement through the proximal convoluted tubule of mammals is dependent on Na transport. Removal of all the Na from the external medium abolishes the water flux [4, 15, 17, 32]. The same result is obtained by inhibiting basolateral Na-K-ATPase activity with ouabain or by depriving the bath of potassium [4, 6]. Nevertheless, the relationship between net water fluxes and the external Na concentration is not yet clear. In previous works, the following protocols have been used to reduce external Na concentration: (i) replacement of Na on the luminal side by a nonpermeable substance such as sucrose or mannitol [25, 37]; (ii) replacement of Na on both sides of the epithelium by cations like Li, K or choline [4, 15, 17, 32], and (iii) lowering of the external Na concentration without replacement [8]. In the first two cases, the solution was iso-osmotic, whereas it was hypo-osmotic in the third.

These methods yielded different items of information relating to the coupled sodium and water fluxes. Results from protocols *ii* and *iii* showed that the water movements depend to some extent on the extracellular sodium concentration. Results from protocol *i* showed that water fluxes follow the net flux of Na when the latter was replaced from the luminal side by a nonpermeable substance. The key point of the coupling between water and sodium movement in luminal substitution is whether the element replacing Na is permeable or not. We therefore attempted in the present series to examine both the Na and water fluxes by replacing Na on the luminal side with a widely permeable cation, lithium. For this purpose, proximal convoluted rat tubules were perfused *in vivo* and several micropunctures were made along the same nephron. In a second experimental series, the Na concentration was lowered on both sides of rabbit proximal tubules during *in vitro* microperfusion with Li substitution, and the resulting water flux was measured.

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Materials and Methods

In vivo Microperfusion

Experiments were carried out on Saclay female Wistar rats, weighing 180 to 220 g. The animals were fed a standard laboratory diet (U.A.R., 91360 Villemoisson-sur-Orge, France) and had free access to water until the experiments started. They were starved for 18 hr prior to surgical procedure. Anesthesia was induced by intraperitoneal injection of sodium pentobarbital (Nembutal, Abbott, 5 mg/100 g body weight). Animals were then placed on a heated table to maintain body temperature at 37° C. Tracheotomy was performed leaving the thyroid gland untouched. One catheter (PE 20 Intramedics Adam) was inserted into the jugular vein for infusion of 0.9% sodium chloride solution at 20 µl/min and for injection of lissamine green. Another catheter (PE 50) was inserted into the right femoral artery to monitor arterial blood pressure (Telco, France).

The left kidney was exposed through a flank incision and placed in a Lucite cup without removing the renal capsule. The kidney surface was bathed with mineral oil at 37° C and illuminated with an optic fiber connected to a xenon arc lamp. Mean proximal transit time was measured before each microperfusion by injecting 50 µl of a 2.5% lissamine green solution through the jugular catheter. The proximal transit time was taken as the interval between the appearance of the dye within the vascular bed and the clearing of all the proximal tubules. Microperfusion was only performed when the proximal transit time was less than 16 sec, a value comparable to that reported by others using a similar end point [30, 33].

Tubular Microperfusion

Twelve proximal convoluted tubules were microperfused in 10 rats with a pump (Hampel, Frankfurt) connected to a micropipette with a tip diameter of 10 µm, according to the method originally described by Sonnenberg and Deetjen [31].

Early proximal convolutions were detected by tubular injection of a small bolus of saline solution colored with 2.5% lissamine green. When the nephron had more than four loops on the surface and a sufficiently fine geometry for perfusion and collection, the tubule was drawn. The nephrons selected had from 4 to 9 convolutions accessible to micropuncture (mean = 7) and the segments studied were 1.5 to 3.8 mm long (mean = 2.6 mm). The tubular perfusion pump was set to deliver 15 nl/min. It was allowed to run for at least 10 min before the perfusion pipette was introduced into the tubular lumen. The tubular perfusate contained no dye at all, and the perfusion was performed in the orthograde direction. Blockade of the tubule was ensured by injecting castor oil stained with Sudan-black, which filled the loops up to the perfusion pipette. After 3 to 6 min equilibration, the fluid was collected from the most distal surface loop of the proximal tubule and, when possible, each loop was punctured (Fig. 1). The last micropuncture sample was generally collected from a point as close as possible to the tip of the perfusion pipette. Fluid withdrawal lasted from 1 to 3 min and the volumes collected varied from 5 to 15 nl. The entire operation required from 10 to 40 min. One to two tubules were perfused per kidney, and three to six loops were punctured per tubule. At the end of each experiment, tubules were filled with Microfil (Canton Biomedical Products Inc.), and the silicone rubber casts were dissected out in order to measure the distance between the perfusion site and each puncture site.

In preliminary experiments, the rate of delivery of the pump was checked *in vitro* by measuring the amount of [^{3}H] inulin delivered into a counting vial over a 10-min period. With a nominal value of 15 nl/min, we obtained 15.0 ± 0.3 nl·min^{-1} ($n = 36$). The value of *in vivo* determinations performed by collections of total fluid delivered was 14.8 ± 0.4 nl/min ($n = 16$). In view of this good agreement between *in vitro* and *in vivo* measurements, we decided to use the nominal value for calculations, i.e., 15 nl/min.

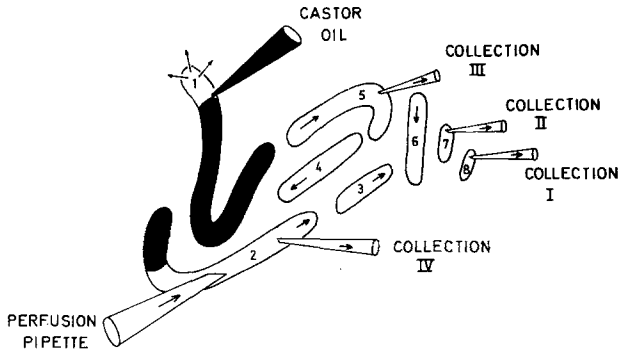

Fig. 1. Protocol used for *in vivo* microperfusion of rat proximal tubule. At the kidney surface, a proximal tubule with numerous convolutions was selected. The glomerular filtrate was allowed to escape through holes made in loop *1*. An oil block between loops *1* and *2* prevented contamination of the perfused solution by the natural filtrate. Several collections were performed in a retrograde direction, from the most distal convolution up to loop *2*

The composition of the solution perfused was the following (in mmol per liter): LiCl, 107; $NaHCO_3$, 25; KCl, 5; Na_2HPO_4, 2; NaH_2PO_4, 0.5; $CaCl_2$, 1.5; $MgSO_4$, 0.5; Na_2SO_4, 1.5; sodium acetate, 10; glucose, 7; alanine, 5; and urea, 5. Before use, the solution was bubbled with a 95% O_2 and 5% CO_2 gas mixture. Ten µCi of [^{3}H] methoxy inulin (150 µCi/mg, NENC) was added to 100 µl of solution which was kept under mineral oil saturated with both water and the above gas mixture. The batches of [^{3}H] methoxy inulin were systematically tested by checking that the renal clearance of the product was identical to that of unlabelled inulin.

Ten µCi of ^{22}NaCl (ICV carrier free) was also added in tracer doses to the perfusion solution. In nine out of twelve perfused tubules, [^{14}C] glucose (Service des Molécules Marquées, Saclay, S.R.A. = 300 mCi/mmol) was also added to the solution. The osmotic pressure of the perfused solution was checked daily during experimentation. Mean value was 298.8 ± 1.9 mosmol/kg H_2O ($n = 8$).

Analytical Procedures

Forty-seven samples were collected from 12 tubules. On both the perfusate and micropuncture samples, the following determinations were made: osmotic pressure by microcryometry [28], sodium and magnesium concentrations with an MS-46 Cameca electron-microprobe [26–27] and radioactivity by liquid scintillation counting (Intertechnique, France). Chloride was measured by coulometry [29].

Calculations. According to a previous report [25], the present microperfusion method may cause leakage of ^{22}Na in the first 0.1 mm of the tubule. To check whether this occurred in our experiments, fluid samples were collected very close to the perfusion pipette. The composition of this fluid, expressed as fluid over perfusate (F/P) concentrations, was: F/P inulin 0.98 ± 0.01, $F/P^{22}\text{Na} = 0.84 \pm 0.02$, $F/P^{14}\text{C}$ glucose $= 0.90 \pm 0.02$, and $F/P\text{Na} = 1.44 \pm 0.05$. These data confirm Morel and Murayama's hypothesis that there is a ^{22}Na leak at the tip of the perfusion pipette. In addition, a small leak of ^{14}C glucose also occurred at this point, but no net movement of water was detected. As regards the Na concentration, an exchange with Li was observed at the perfusion

site; some Na from plasma went into the lumen, and the Na concentration increased from 42 to 62 meq/liter (F/P Na:1.44). The calculated F/P Li at this point was 0.84, a value identical to that for ^{22}Na. Fluxes were therefore calculated between the different puncture sites and not between the puncture sites and the pump, to avoid over-estimating flux values.

The following symbols and units are used:

V: water flow rate (nl/min)
ΦH_2O: net flux of water (nl/min)
ΦNa: net flux of sodium (peq/min)

In the following equations, Na and In refer respectively to the concentrations of sodium and inulin. Subscript 0 refers to the perfusion solution and subscripts 1 and 2 designate two successive puncture sites. Fluxes between two successive puncture sites were calculated on the basis of the following equation:

Net water flux:

$$\Phi H_2O = V_0 \left(\frac{In_0}{In_1} - \frac{In_0}{In_2}\right) \quad (1)$$

Net Na flux:

$$\Phi Na = V_1\ Na_1 - V_2\ Na_2 = V_0 \left(\frac{In_0}{In_1} Na_1 - \frac{In_0}{In_1} Na_2\right). \quad (2)$$

In vitro Microperfusion

Proximal convoluted tubules were dissected from the outer and midcortical regions of the rabbit kidney and perfused according to Burg et al. [3]. Female New Zealand white rabbits weighing 1.5–2.0 kg were used. After they had been killed, their left kidney was cut into thin slices, and segments of proximal convoluted tubule were dissected at room temperature in a bath containing an artificial albumin-free solution. The tubules were transferred to a chamber and perfused with a pipette system similar to the one previously described [6]. They were slowly heated to 37.5° C, and 20 min were allowed for equilibration before determinations were made.

The control solution was composed of (in mmol per liter): NaCl, 110; $NaHCO_3$, 25; KCl, 5; $MgSO_4$, 1.2; $CaCl_2$, 1.8; Na acetate, 4.0; Na citrate, 1.0; alanine, 6.0; glucose, 5.5; Na_2HPO_{41}, 3.0; and NaH_2PO_4, 1.0. The bathing medium consisted of the same artificial solution to which albumin was added to a final concentration of 6%. The albumin thus added was a concentrated solution (35% bovine albumin, Sigma Chemical Co.) that had been dialyzed at 10° C for 18 hr against the artificial solution. The pH was adjusted to 7.4 after bubbling with a 95% O_2 and 5% CO_2 gas mixture. Osmolality was adjusted to 300 mosmol/kg H_2O. The experimental solutions were prepared in the same way as the control solution except that 75 meq/liter of sodium chloride was replaced by 75 meq/liter of lithium chloride. The albumin added to the experimental bathing medium was always dialyzed against the experimental solution. ^{125}I-iodothalamate (Abbot) was added to the solution perfused (activity=100 μCi/ml) and used as a volume marker for measuring the rate of fluid absorption (J_v) [6]. Results were expressed in nl·mm^{-1}·min^{-1}. All the experiments followed the same protocol, which included control, experimental, and post-control periods. Twenty minutes were allowed for equilibration before and after each experimental period. The fluid reabsorption rate was measured for each of four or more consecutive collections and is expressed as the mean of these determinations. No statistical changes were observed between the first and last collections in any group of experiments. Data are given as the mean ±SE of the results for individual tubules. Statistics were obtained by paired t-test.

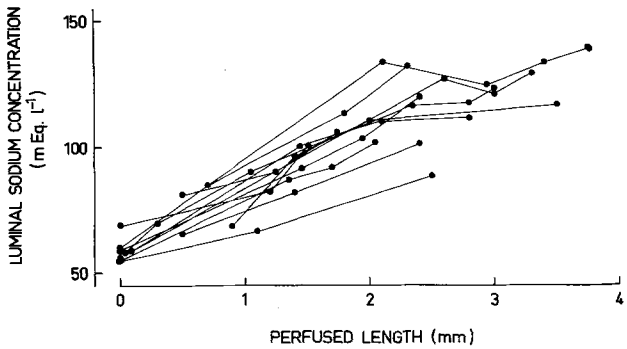

Fig. 2. Luminal Na concentration expressed in milliequivalents per liter in each sample collected, *vs.* the length of the perfused segment (millimeters). Each point represents one collection; samples from the same tubule are joined by a solid line. The Na concentration rose as the Li concentration fell; the osmotic pressure of the fluid remained constant

Results

In vivo Microperfusion Experiments

Intraluminal sodium concentration. The luminal sodium concentration was found to increase all along the tubular segments perfused with Li solution. As depicted in Fig. 2, the mean concentration of sodium at the perfusion site was 62±3 meq/liter and rose to 140 meq/liter for the longest segments. The osmotic pressure of the samples was found to be constant all along the tubule and equal to that of the perfused solution, i.e., 300.7±1.3, $n=41$, *vs.* 298.8±1.9 mosmol·liter^{-1}, $n=8$. This indicates that the Na concentration in the lumen increased as the Li concentration decreased, so that the sum of Na+Li was constant.

Intraluminal ^{22}Na and Li concentrations. Assuming that the sum of [Na]+[Li] remained constant and equal to the perfusion value (149 meq/liter), the intraluminal Li concentration was calculated as 149-[Na] in each sample punctured. When expressed as the fluid over perfusate concentration ratio (F/P), a good correlation was found between F/P ^{22}Na and F/P Li: $y=0.93\ x+0.07$, $r=0.957$, $n=47$ (*see* Fig. 3).

Net Na fluxes. The transepithelial net Na flux was calculated between two puncture sites in each tubule. It was found to be negative, i.e., it proceeded from plasma to lumen (Fig. 4). At the end of the longest perfused segments, the Na concentration was high and little Li remained in the lumen; the negative net Na flux changed to a positive net Na flux in these segments (Fig. 4).

Net water fluxes. Despite the negative net Na flux through the epithelium, net water fluxes were found to be positive, i.e., they proceeded from lumen to

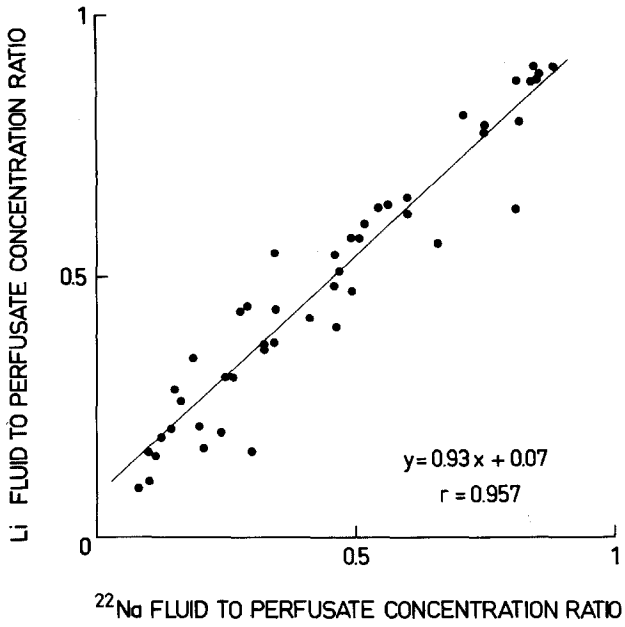

Fig. 3. Li concentration in luminal fluid expressed as the fluid over perfusate concentration (F/P) *vs.* the ^{22}Na concentration expressed in the same way (F/P)

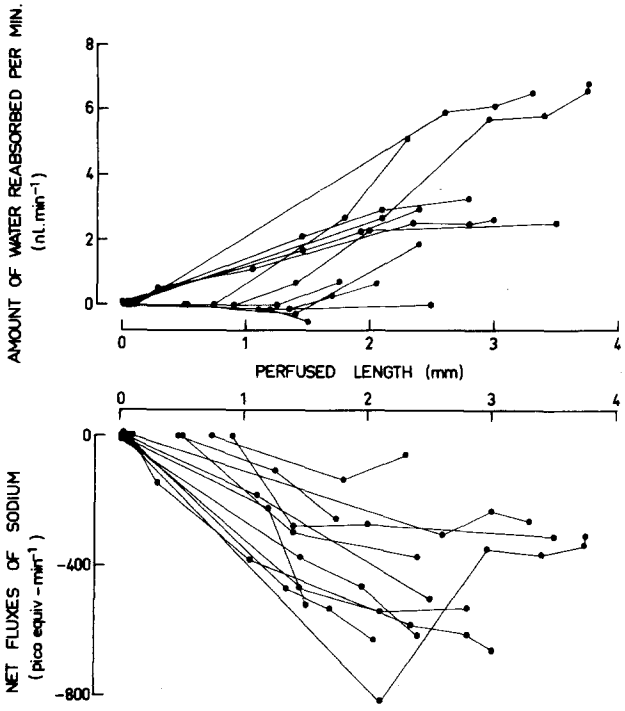

Fig. 4. *Upper panel:* Amount of water reabsorbed in nl/mn *vs.* the length of rat proximal tubule perfused. Each point represents one sample; samples from the same tubule are joined by a solid line. Positive values for net water fluxes refer to lumen-to-plasma movements/mm. *Lower panel:* Net Na fluxes expressed in peq/mm as a function of the length perfused. Different samples from the same tubule are joined by a solid line. Negative values for net Na fluxes refer to plasma-to-lumen movements. Data from the upper and lower panels were obtained from the same tubules. Positive net water fluxes were observed despite negative Na fluxes

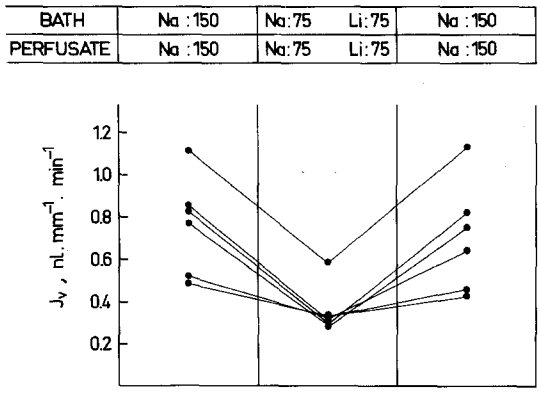

Fig. 5. Effect of 75 meq/liter Li and 75 meq/liter Na in perfusate and bath on fluid absorption (J_v) in rabbit proximal convoluted tubules

plasma (Fig. 5). Large variations in the water reabsorption rate were observed from one tubule to another. Except for two tubules with no reabsorption, net water fluxes varied from 0.44 to 2.20 nl/min/mm (mean $=1.14 \pm 0.20$ nl/min/mm).

Magnesium, chloride and ^{14}C glucose concentrations. The Mg concentration along the perfused segments increased with water reabsorption. The correlation between F/P Mg and F/P inulin was $y=0.84\,x+0.03$, $r=0.799$, $n=43$, and indicated that Mg and inulin behaved similarly, a sign that the ^3H inulin used reflected water movements well. A positive correlation was also found between F/P Cl and F/P inulin: $y=0.25\,x+0.84$, $r=0.801$, $n=43$, indicating that the rise in the chloride concentration was also linked to the water reabsorption rate, probably through preferential reabsorption of bicarbonate. The mean increase in chloride concentration was 3.0 meq/liter per millimeter of proximal tubule. The glucose concentration in the perfused solution was 7 mM/liter; at this concentration, there is almost no backflux of glucose through the proximal tubule [11] and the disappearance of ^{14}C glucose can be considered as an indication of the unidirectionnal flux of glucose from lumen to plasma. In these experiments, F/P ^{14}C glucose decreased as F/P inulin increased all along the perfused segments. The negative correlation observed between these two parameters was $y=-1.09\,x+1.92$, $r=0.922$, $n=37$, showing that water movement and glucose reabsorption were closely connected.

In vitro Microperfusion Experiments

The fluid reabsorption rate was 0.76 ± 0.09 nl/min/mm during the control period and fell to 0.36 ± 0.05 nl/min/mm ($p < 0.005$) during the experimental period of Li substitution for Na. The percentage of drop in J_v observed during the experimental period was

49% of the control value. Throughout periods of Li perfusion lasting up to 120 min, no tendency for J_v to decrease was observed. The effects of Li were reversible regardless of the length of the experimental periods: post-control values for J_v (0.71 ± 0.10 nl/min/mm) were not statistically different from control values.

Discussion

In contrast to the results of experiments on snake [10] or *Necturus* proximal tubule [36], Burg, working on rabbit tubule [4], and Green and Giebisch [15] and Gyory [17], on rat tubules, demonstrated that water reabsorption dropped to zero when all the Na on both sides of the tubule was replaced by Li, choline, tetramethyl-ammonium, or mannitol. Furthermore, inhibition of basolateral Na-K-ATPase activity [4, 6] indicated that water reabsorption depended not only on the presence of Na, but also on its transport.

Of the methods used earlier to clarify the relationship between various external sodium concentrations and water movements (replacement of Na by a nonpermeable molecule or by a cation or simply the lowering of the Na concentration – *see* introduction) Na replacement by lithium was chosen in the present work on the following grounds.

Earlier results for Na replacement by Li showed that active Li transport along the mammalian proximal tubule did not occur through the Na carrier (4), neither did Li activate the kidney Na-K-ATPase enzyme [12, 16]. Consequently, in contrast to tight epithelium [5, 21, 34, 38], it seems that Li is not actively transported through the cells of the proximal tubule. On the other hand, some evidence has shown that lithium can diffuse widely through the mammalian proximal convoluted tubule [12, 20, 35]. Thomsen, for instance, measured the fluid over plasma Li concentration (F/P Li) along the rat proximal tubule during Li infusion into the entire animal and found that F/P Li was equal to F/P Na at the end of the proximal tubule [20].

The movements of Li could occur through the paracellular or the transcellular pathways. Most people agree with the view that across such a leaky epithelium the diffusion of sodium takes place essentially through the intercellular junction and that the magnitude of the diffusion through the baso-lateral membrane is small. Since Li is not actively transported by the Na-K-ATPase system, we therefore make the assumption in this study that the movements of Li across the epithelium are essentially due to its diffusion through the leaky junctions of the proximal tubule and not through the cells.

Further evidence for Li diffusion through the leaky epithelial junctions is given in Fig. 3. During the present experimental series, ^{22}Na and Li were perfused into the tubular lumen only and left this compartment to be diluted in an "infinite space", i.e., the peritubular capillary blood flow. We observed that Li escaped from the lumen at almost the same rate as ^{22}Na.

Since the transcellular active transport of Na carried the same proportion of Na and ^{22}Na, the fall in the luminal ^{22}Na concentration is necessarily due to the ^{22}Na escape from the lumen and its replacement by unlabeled Na moving from plasma to lumen. Clearly, this exchange largely takes place through the leaky junction, as the sodium permeability of the basolateral membrane of the cell is relatively low in comparison to that of the paracellular pathway. Similarly, whenever Li left the lumen through the highly permeable junction, it was replaced by Na moving from plasma to lumen (Fig. 2). However, our most important observation was that ^{22}Na and Li are exchanged throughout the leaky junctions at the same velocity, indicating that they have a very similar diffusion coefficient in this structure.

It may therefore be justifiably concluded that, in mammalian proximal tubule, Li is not actively transported and that it diffuses in the same way as Na. In addition, Li does not irreversibly damage the proximal tubule since *in vitro* microperfusion shows that the water reabsorption rate is identical during both the postcontrol and control periods, irrespective of the duration of Li perfusion (up to 120 min).

At this stage, it is of interest to compare our results with those of the microperfusion experiments conducted by Morel and Murayama [25] and Giebisch et al. [13] on rats and by Windhager et al. on *Necturus* [37]. These authors lowered the intraluminal sodium concentration from 150 to 85 meq/liter (rats) or from 100 to 50 meq/liter (*Necturus*) by replacing Na with sucrose or mannitol to keep the solution isoosmotic. Both groups observed that net water and Na fluxes were closely correlated. They found water secretion in the lumen when the net flux of Na was also negative from plasma to lumen (luminal Na concentration reduced to more than 30 meq/liter). This negative water net flux reversed and changed to net reabsorption when the net Na flux from lumen to plasma became positive (luminal Na concentration reduced to less than 30 meq/liter). In our experiments, the luminal Na concentration is reduced to the same extent as in the previous studies and a negative net plasma-to-lumen sodium flux was also generated, except along the last mm of 4 out of the 12 perfused tubules; but unlike the previous investigators, we never found negative net fluxes of water from lumen to plasma. Instead, positive water net fluxes were observed in most instances (Fig. 4). The difference between these

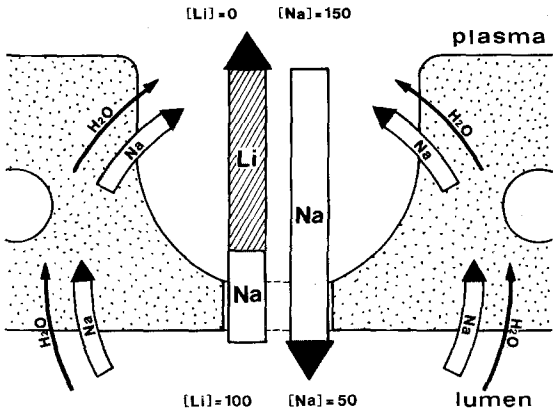

Fig. 6. Interpretation of Na and water movements during *in vivo* luminal perfusion of a Ringer Li solution. The one-for-one exchange of Li for Na probably takes place through the intercellular junctions, inducing a negative net Na flux from lumen to plasma; the drop in luminal Na concentration no doubt diminishes the entry of Na into the cell and therefore the transcellular unidirectional Na flux. If this is the case, the water movement would follow the positive transcellular Na flux and would not be affected by the negative paracellular Na flux

two types of protocols is that for a similar mean intraluminal concentration, Na was replaced by a nonpermeant molecule in the Morel and Murayama as in the Giebisch et al. studies, whereas in our case it was replaced by a widely permeant cation. We interpreted the results as signifying that the one-for-one exchange of Li and Na through the paracellular space, which was responsible for the negative net Na flux, did not affect the water movements. Consequently, we conclude that the net water and Na fluxes can be dissociated when Na is replaced by Li.

Since the transepithelial net Na flux was obviously not responsible for the water movement in these experiments, the most likely explanation for such movement is that it followed the transcellular unidirectional flux of Na (Fig. 6). In that case, the amount of Na entering the proximal tubule cells would be a function of its luminal concentration; Na would therefore be extruded on the basolateral side of the cell through the Na-K-ATPase system, in amounts proportional to its entry on the luminal side [32] and would be followed by the extrusion of an isoosmotic proportion of water.

In connection with this hypothesis, we observed that the net water flux was also closely correlated to glucose transport which is known to be transcellular and dependent on Na movement on the luminal side of the proximal cells [22]. The same interpretation might account for the increase in the chloride concentration observed concomitantly with water reabsorption. This increase is known to be due to preferential bicarbonate reabsorption, a mechanism in some way mediated by the Na-H exchange on the luminal side of the cell [23]. Here again, the water reabsorption rate seems to rely more on cell transport capacity than on transepithelial sodium movements.

To what extent lowering the extracellular Na concentration reduces the net water flux is difficult to assess from *in vivo* microperfusions. The segments perfused were randomly chosen and a large scatter in water reabsorption rates was observed from one tubule to another even under control conditions [1, 9, 18, 19, 24]. Whatever the reason for this scatter, the technical difficulty of making paired observations during *in vivo* microperfusion prompted us to perform *in vitro* microperfusion of rabbit proximal tubules. When the Na concentration was reduced to half its control value (75 meq/liter) and equal amounts of Li were substituted for Na on both sides of the tubule, the water reabsorption rate also dropped to half its control value. The effect of Li was stable for a two-hour period and reversible, thus excluding the possibility that there had simply been toxic impairment of the tubular function.

The present results are somewhat in contradiction with those obtained on *Necturus* by Spring and Giebisch [32] and on the rat by Gyory [17]; these authors found that lowering the external Na concentration by up to half its control value did not statistically change the net water and sodium fluxes. On the other hand, the results of the present lithium experiments are in good agreement with previous observations made on rabbit proximal tubule [8]. In rabbit proximal tubule perfused *in vitro*, the external Na concentration was lowered to 80 meq/liter without ionic substitution and a 36% drop in Na reabsorption was observed. We therefore conclude from this and the present studies that, in the mammals, the water and sodium movement through the proximal tubule is dependent on the external Na concentration. Two hypotheses may be proposed to explain the decrease in water and sodium transport at low external sodium concentrations:

1) The permeability of the luminal membrane, and therefore Na entry into the cell, is a function of the external Na concentration [32, 2], or

2) The intracellular pool of Na decreases proportionally with the external Na concentration; this reduction in the cellular Na concentration would affect the rate of baso-lateral Na-K-ATPase activity. The latter hypothesis is compatible with the decrease in the intracellular Na concentration measured by Grantham [14] in a similarly low Na medium and by Cooke [7], and Spring and Giebisch [32] with Li substitution.

In the case of both hypotheses, the decrease in transcellular Na transport would cause a parallel reduction in the rate of isoosmotic water reabsorption through the proximal tubule.

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