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EFFECT OF MEDIUM TONICITY ON TRANSEPITHELIAL H^+–HCO_3^- FLUXES IN RAT PROXIMAL TUBULE

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SUMMARY

1. The effect of luminal and capillary perfusion with hypotonic or hypertonic solutions containing 25 mM $NaHCO_3$ or NaH_2PO_4 plus NaCl, K^+, Ca^{2+}, Mg^{2+} and acetate at an osmolality of 100 or 500 mosmol kg^{-1} on rat proximal H^+ secretion was estimated by monitoring luminal pH with Sb microelectrodes. The results were compared to perfusions with the same ionic concentration in which tonicity was adjusted to 300 mosmol kg^{-1} with raffinose.

2. The kinetics of acidification of luminally injected bicarbonate buffer permits evaluations of H^+–HCO_3^- fluxes as well as stationary pH gradients; the kinetics of alkalinization of luminally injected acid phosphate buffer indicates H^+–HCO_3^- back-fluxes from blood to lumen.

3. In alkalinization experiments, luminal perfusion with hypotonic solution during presence of blood in capillaries or hypotonic capillary perfusion leads to a decrease of stationary pH, an increase of alkalinization half-time and consequently a decrease of passive H^+–HCO_3^- backflux.

4. In alkalinization experiments, during luminal and/or capillary perfusions with hypertonic solutions, no significant differences in the stationary pH, alkalinization half-time and H^+–HCO_3^- backflux were found.

5. During acidification experiments, with both hypo- and hypertonic perfusions, no significant differences in stationary pH, acidification half-time and H^+–HCO_3^- flux were observed.

6. Luminal perfusion with hypotonic solution increases specific epithelial resistance in the presence of blood in capillaries. Luminal perfusion with hypertonic solution does not change this parameter.

7. Volume changes, measured by the split-drop method, are slow during the first 30 s and do not explain the increased alkalinization half-time during luminal perfusion with hypotonic solution, since this is the period of fastest pH change.

8. Luminal perfusion with hypotonic solution decreases apparent H^+ permeability in the presence of blood or hypotonic solution in capillaries. Hypertonic solutions in all experimental conditions had no significant effect on this parameter.

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9. The data indicate that decrease of tonicity of fluids in contact with proximal tubule epithelium affects passive H^+–HCO_3^- backflux, which proceeds in part through the shunt path, while acidification (H^+ secretion), which is transcellular, is not affected by extracellular tonicity.

INTRODUCTION

The mechanism of renal tubular acidification depends on active H^+ transport, and on passive shunt fluxes of H^+ and of HCO_3^- (Malnic, 1987). While the former, represented by Na^+–H^+ exchange and by an ATP-driven proton pump in proximal tubules are transcellular mechanisms, the latter are thought to proceed largely by the paracellular path. The magnitude of the active mechanism depends on the H^+ and Na^+ gradients across the luminal membrane, and H^+ secretion is therefore a gradient-dependent mechanism. In addition it requires the transfer of base formed within the cellular compartment across the basolateral cell membrane. Base extrusion is effected in large part by HCO_3^-–Na^+ co-transport in a 3:1 proportion (Yoshitomi, Burckhardt & Frömter, 1985; Soleimani, Grassl & Aronson, 1987). The magnitude of the passive processes contributes to the determination of transepithelial H^+ gradients and acidification rates.

Tonicity of fluids bathing epithelial tissues has been shown to affect trans- and paracellular solute and fluid movement in a number of tissues. Luminal and capillary perfusion with hypotonic solutions leads to an increase of transepithelial resistance of proximal tubules (De Mello, Lopes & Malnic, 1976), ascribed to a reduction in the width of the intercellular shunt path. In general, renal tubules respond to hypertonic media by osmotic shrinkage (Montrose-Rafizadeh & Guggino, 1990); in hypotonic media, cell swelling occurs, followed by a volume-regulatory decrease of cell volume toward control conditions (Gagnon *et al.* 1982; Welling & Linshaw, 1988).

The aim of this work was to contribute to a better understanding of the effect of medium tonicity on transepithelial H^+–HCO_3^- fluxes in proximal tubules. The effect of luminal and capillary perfusion with hypo- or hypertonic solutions containing 25 mM $NaHCO_3$ or NaH_2PO_4 plus NaCl, K^+, Ca^{2+}, Mg^{2+} and acetate at an osmolality of 100 or 500 mosmol kg^{-1} on proximal acidification or alkalinization was estimated by monitoring luminal pH with Sb microelectrodes. The results were compared to experiments in which tonicity was maintained at 300 mosmol kg^{-1} with raffinose. The results indicate that the mechanism of H^+ secretion is based on a pump–leak system, suggesting that the leak occurs through the intercellular spaces, structures that are probably affected by tonicity alterations. The difference between the data obtained during acid and alkaline perfusions suggests that hypotonicity impairs passive flux of H^+ and/or HCO_3^- across the paracellular shunt path, while active secretory fluxes, which are transcellular, are not affected by extracellular tonicity.

METHODS

Wistar rats weighing between 240 and 360 g were used in the present experiments. They were fed a standard pellet diet and had access to food and water up to the time of the experiment. They were anaesthetized with intraperitoneal sodium pentobarbitone, 40 mg kg^{-1} body weight, and after the experiments they were killed with an additional dose of anaesthetic. The animals were prepared

according to standard *in vivo* micropuncture and microperfusion techniques (Malnic & Mello Aires, 1971). In brief, the kidney was exposed by a lumbar approach, carefully dissected from perirenal fat, and immobilized within an acrilic holder which allowed free access of renal vessels, by means of 3% agar in saline. The kidney was illuminated by a quartz rod and superfused with light mineral oil at 37 °C. During the experiments, the rats received a venous infusion of saline containing 3% mannitol at a rate of 0·05 ml min^{-1}. A jugular vein was cannulated for fluid infusion, and a carotid artery for collection of blood samples. Acidification and alkalinization kinetics were measured as described previously (Malnic & Mello Aires, 1971; Giebisch, Malnic, De Mello & Mello Aires, 1977). In short, a proximal tubule loop was punctured by a double-barrelled micropipette made out of Theta glass (B & D Optical Systems, Spencerville, MD, USA), in order to inject Sudan-black coloured castor oil through one barrel and perfusion solution through the other. A few loops downstream the tubule was punctured by an antimony pH microelectrode (Vieira & Malnic, 1968). A fluid column was injected and kept stationary by oil blocking, in contact with the pH microelectrode. pH was measured by means of a Keithley model 615 electrometer, recorded on a Beckman Dynograph model RS, the data being digitalized and processed by a Hewlett-Packard model 2313-B analog-to-digital converter within a 9603A computing system. After luminal injection of a bicarbonate-containing droplet, H^+ secretion caused a reduction of bicarbonate concentration, which equilibrated to a stationary value in an exponential fashion. The half-time of this concentration change was a measure of the rate of H^+ secretion, and allowed calculation of transepithelial bicarbonate (or H^+) fluxes. When a droplet of acid (pH 5·5) phosphate-buffered solution is injected, passive H^+ flux out of the lumen or HCO_3^- flux into the lumen will reduce acid phosphate levels with a kinetics similar to that of bicarbonate. When blood flows through capillaries, HCO_3^- backflux will be an important component of passive flux, while during capillary perfusion with phosphate buffered solutions H^+ backflux will be the predominant flux. (Lang, Quehenberger, Greger, Silbernagl & Stockinger, 1980). H^+–HCO_3^- flux or H^+–HCO_3^- backflux across tubular epithelia were obtained by the relation:

$$J = \frac{r}{2}\frac{\ln 2}{t_{\frac{1}{2}}}[B_o - B_s],$$

where J is H^+–HCO_3^- flux or H^+–HCO_3^- backflux in nmol cm^{-2} s^{-1}, r the tubule radius in μm, $t_{\frac{1}{2}}$ acidification or alkalinization half-time in s, B_o and B_s initial and steady-state buffer base concentration, i.e. bicarbonate concentrations calculated from pH and systemic P_{CO_2} (Mello Aires, Lopes & Malnic, 1990), or alkaline phosphate calculated from pH and assuming constant total phosphate levels. Sb microelectrodes were calibrated in HPO_4^- buffer Ringer solution similar in composition to perfusion solutions adjusted to pH 6·5, 7·0 and 7·4, with appropriate anion correction when measuring bicarbonate-containing solutions (Lopes, Mello Aires & Malnic, 1981).

Capillary perfusion was performed applying pressure to the solution contained in micropipettes of about 12 μm tip diameter made out of thin-walled glass tubing of 2 mm o.d. Pressure was applied connecting a compressed air gas tank to the pipette holder, and regulated so as to allow perfusion of an area of 50–100 μm diameter, recognized by the blanching of the cortical surface. For perfusion, a star vessel was punctured, and care was taken to position the studied tubule segment about half way between the tip of the perfusion pipette and the border of the perfused area.

Changes in volume of a luminal fluid column were measured by a modification of the Gertz split-droplet method (Malnic, Silva Neto, Stamopoulos & Mello Aires, 1979). The meniscus correction was made as described by Gyory (1971). Transepithelial electrical resistance was measured using double-barrelled Ling–Gerard microelectrodes filled with 3 M KCl, which determined effective resistance by measuring voltage changes in one barrel due to current pulses injected by the other barrel, and then calculating specific wall resistance as previously described (De Mello, Lopes & Malnic, 1976). Apparent permeability to H^+ (p_H) was calculated by the relationship:

$$p_H = \frac{J_H}{\Delta[H^+]} \times \frac{\exp(EF/RT) - 1}{EF/RT},$$

where J_H is H^+ backflux obtained from alkalinization experiments at luminal pH of 6·2 and capillary perfusion with phosphate-buffered solution, $\Delta[H^+]$ the transepithelial H^+ concentration difference (pH 7·4 *vs.* 6·2) and E the transepithelial potential difference. F, R and T have their conventional meanings.

TABLE 1. Composition of luminal perfusion solutions

	Acidification experiments			Alkalinization experiments		
Solutions (mM)	S1	S2	S3	S4	S5	S6
NaCl	25	25	25	15	15	15
$NaHCO_3$	25	25	25	—	—	—
KCl	—	—	—	5	5	5
NaH_2PO_4	—	—	—	25	25	25
$CaCl_2$	—	—	—	1	1	1
$MgSO_4$	—	—	—	1·2	1·2	1·2
Sodium acetate	—	—	—	5	5	5
Raffinose	200	400	—	200	400	—
pH	8	8	8	5·5	5·5	5·5
Osmolality (mosmol kg^{-1})	300	500	100	300	500	100

TABLE 2. Composition of capillary perfusion solutions

	Acidification experiments*			Alkalinization experiments		
Solutions (mM)	S7	S8	S9	S10	S11	S12
NaCl	105	105	20	15	15	15
$NaHCO_3$	20	20	20	—	—	—
KCl	5	5	5	5	5	5
Na_2HPO_4/NaH_2PO_4	10	10	10	25	25	25
$CaCl_2$	1	1	1	1	1	1
$MgSO_4$	1·2	1·2	1·2	1·2	1·2	1·2
Sodium acetate	5	5	5	5	5	5
Raffinose	—	200	—	200	400	—
pH	7·4	7·4	7·4	7·4	7·4	7·4
Osmolality (mosmol kg^{-1})	300	500	100	300	500	100

* These solutions were pre-equilibrated with 40 mmHg CO_2 in air.

The solutions used for luminal and capillary perfusion at different osmolality in acidification and alkalinization experiments are given in Tables 1 and 2. The groups of experiments studied were selected to combine several possibilities of effect of medium tonicity on transepithelial H^+ and HCO_3^- fluxes. Some of these solutions were also used to study the effect of medium tonicity on changes of volume of a luminal fluid column and specific epithelial resistance.

pH and P_{CO_2} of blood were measured at intervals of 30–60 min during the experiments by a Radiometer PHM72-MK2 pH meter–blood gas meter.

Data are presented as means ± S.E.M. Differences between groups were evaluated by Student's paired *t* test or, when more than two groups were compared, by analysis of variance and the Scheffé contrast test (Snedecor & Cochran, 1967).

RESULTS

During the experiments, acid–base data for blood were: pH, 7·42 ± 0·04; P_{CO_2}, 39·7 ± 2·1 mmHg and HCO_3^-, 25·4 ± 1·7 mmol l^{-1} (± S.E.M., $n = 60$).

In the first series of experiments, the effect of changing lumen and/or capillary tonicity on luminal pH during perfusions with bicarbonate or phosphate-buffered solutions was studied. Both during bicarbonate perfusions, where the injected fluid was acidified by H^+ secretion, and acid phosphate perfusions, where the fluid was alkalinized due to passive H^+ efflux from or HCO_3^- influx into the lumen, stationary pH, half-time of buffer concentration change and net H^+–HCO_3^- fluxes were obtained.

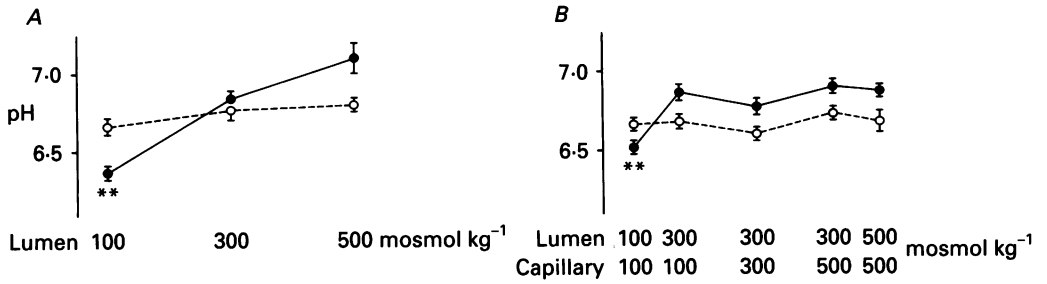

Fig. 1. Stationary luminal pH (pHs) during acidification of luminally injected bicarbonate buffer columns (○) or alkalinization of luminally injected phosphate buffer columns (●). *A*, luminal perfusion with hypo- or hypertonic solutions. *B*, luminal plus capillary perfusion with hypo- or hypertonic solutions. $^{**}P < 0{\cdot}01$.

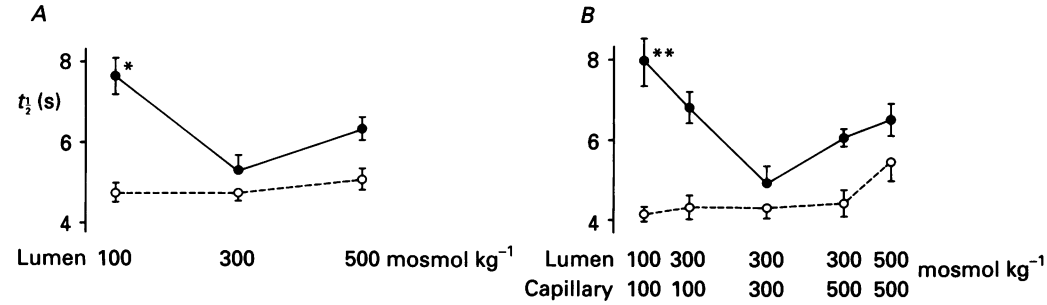

Fig. 2. Acidification (○) and alkalinization (●) half-time ($t_{\frac{1}{2}}$) during luminal (*A*) or luminal plus capillary (*B*) perfusion with solutions of different tonicity. $^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$.

Stationary pH values obtained during luminal or luminal plus capillary perfusion with hypo- and hypertonic solutions during acidification and alkalinization experiments are given in Fig. 1. The results show that during alkalinization experiments the luminal perfusion with hypotonic solutions leads to a decrease of stationary pH in the absence of capillary perfusion, i.e. with blood in peritubular capillaries ($6{\cdot}36 \pm 0{\cdot}046$ ($n = 45$) *vs.* $6{\cdot}82 \pm 0{\cdot}043$ ($n = 43$) at 300 mosmol kg^{-1}, $P < 0{\cdot}01$) or hypotonic capillary perfusion ($6{\cdot}52 \pm 0{\cdot}042$ ($n = 59$) *vs.* $6{\cdot}76 \pm 0{\cdot}033$ ($n = 49$), $P < 0{\cdot}01$). In alkalinization experiments during luminal and/or capillary perfusions with hypertonic solutions and in the acidification experiment, during both medium tonicity alterations, no significant differences in stationary pH were observed.

The maintenance of lower stationary pH during hypotonic perfusions might be due either to increased H^+ secretion or to a decreased passive leak of H^+ and/or HCO_3^-. The data on acidification and alkalinization half-time can be used to distinguish between these possibilities.

Figure 2 gives acidification and alkalinization half-time during luminal or luminal plus capillary perfusion with solutions of different tonicity. Net H^+ secretion and H^+–HCO_3^- backflux across tubular epithelia during the same conditions are given in Fig. 3. In the alkalinization experiments, the data show that

luminal perfusion with hypotonic solution increases alkalinization half-time with blood in capillaries (7·64 ± 0·474 s ($n = 45$) *vs.* 5·31 ± 0·307 s ($n = 43$), $P < 0·05$) or during hypotonic capillary perfusion (7·87 ± 0·606 s ($n = 59$) *vs.* 4·96 ± 0·199 s ($n = 49$), $P < 0·01$), and consequently decreases the H^+–HCO_3^- backflux with blood

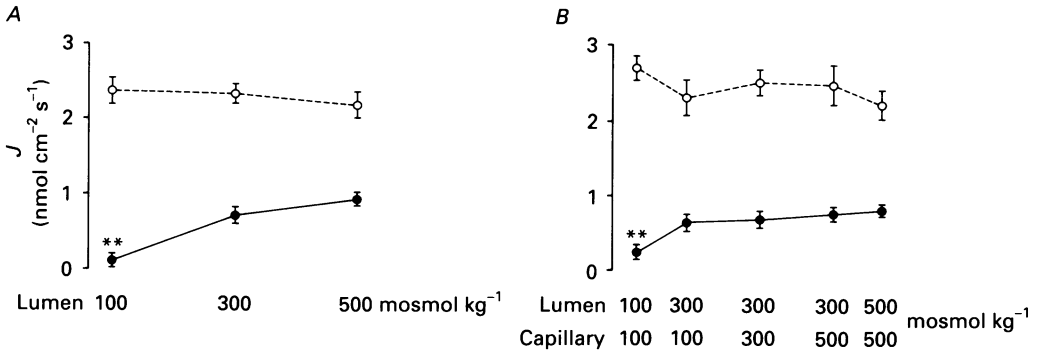

Fig. 3. H^+–HCO_3^- fluxes (○) and H^+–HCO_3^- backflux (●) across tubular epithelium during luminal (*A*) or luminal plus capillary (*B*) perfusion with solutions of different tonicity. $^{**}P < 0·01$.

in capillaries (0·109 ± 0·053 nmol cm^{-2} s^{-1} ($n = 45$) *vs.* 0·724 ± 0·100 nmol cm^{-2} s^{-1} ($n = 43$), $P < 0·01$) or with hypotonic capillary perfusion (0·251 ± 0·056 nmol cm^{-2} s^{-1} ($n = 59$) *vs.* 0·681 ± 0·067 nmol cm^{-2} s^{-1} ($n = 49$) $P < 0·01$). In perfusions with hypertonic solutions no significant differences in the alkalinization half-time or H^+ backflux were found. During the acidification experiments, in both medium tonicity alterations, no significant differences in the acidification half-time and net H^+ flux were observed. These experiments indicate that only passive backflux is affected by hypotonicity, since only alkalinization (and no acidification) half-time as well as net fluxes were modified by this condition.

The observed reduction in H^+–HCO_3^- backfluxes may be caused by cell swelling during hypotonic perfusion, and the consequent narrowing of the paracellular pathway. In order to evaluate this possibility by an independent method, transepithelial resistance was measured by electrophysiological techniques.

Figure 4 gives specific epithelial resistance of proximal tubular epithelium at different luminal tonicity but constant ionic composition. The results show that during hypotonic luminal perfusion during blood capillary perfusion, proximal epithelial specific resistance is significantly increased compared to isotonic perfusions (8·63 ± 1·34 Ω cm^2 ($n = 41$) *vs.* 4·52 ± 0·82 Ω cm^2 ($n = 41$), $P < 0·01$). Luminal perfusion with hypertonic solution does not change this parameter significantly. Since the electrical resistance across proximal tubule epithelium is mostly due to the paracellular path, these results are compatible with the decrease of H^+–HCO_3^- backfluxes during perfusions with hypotonic solutions being due to narrowing of the paracellular pathway.

To investigate if volume variations of the luminally injected fluid columns could affect the concentrations of buffer components and thereby the measured pH, we

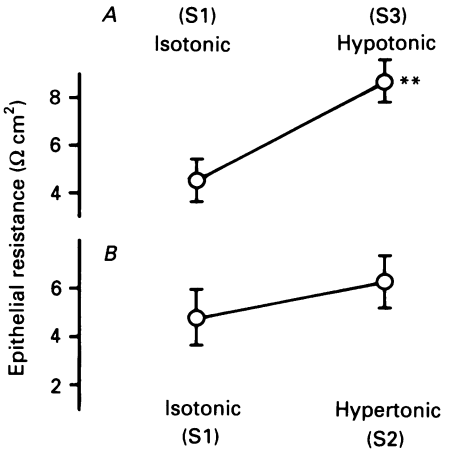

Fig. 4. Specific epithelial resistance of proximal tubular epithelium at different luminal tonicity but constant ionic composition. *A*, $n = 41$; *B*, $n = 49$. $^{**}P < 0{\cdot}01$ against isotonic perfusion; solution number, (S*n*).

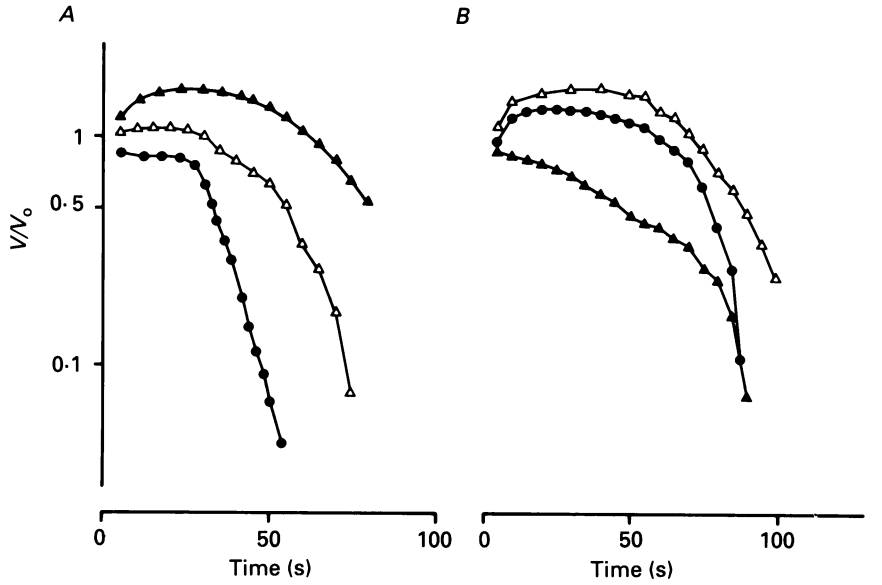

Fig. 5. Changes in volume of a luminal fluid column at different luminal (*A*) or capillary (*B*) tonicities. V = volume at time t; V_0 = initial volume; *A*, lumen osmolality of 500 ▲ (S3), 300 △ (S1) and 100 ● (S2) mosmol kg^{-1}. Capillary contains blood. *B*, lumen osmolality 300 (S1), capillary osmolality 500 ▲ (S3) 300 △ (S1) and 100 ● (S2) mosmol kg^{-1}. Composition of solutions (S*n*) given in Table 1.

measured changes of volume of luminal fluid columns at different luminal or capillary tonicities by a modification of the Gertz split-droplet method.

Figure 5 shows that when the solutions are injected into the proximal tubule lumen, their volume is altered only moderately during the first 30 s, which is the period in which most pH changes occur. Therefore, these volume variations probably do not interfere in a major way with luminal buffer concentrations.

DISCUSSION

It has been shown before that bathing epithelia with hypotonic solutions increases transepithelial resistance and reduces water permeability, while the opposite occurs in hypertonic solutions (DiBona & Civan, 1973; De Bermudez & Windhager, 1975; De Mello *et al.* 1976; Reuss & Finn, 1977). It has been suggested that these modifications might be due to alterations of the paracellular shunt path occurring in the described solutions. In hypotonic solutions the paracellular path would be reduced due to cell swelling, leading to an increase in epithelial resistance, the reverse situation occurring in hypertonic solutions due to cell shrinkage. It is well known that following swelling in hypotonic media, cells return to their normal volume by several ion transport mechanisms which characterize the process of cell volume regulation (Gagnon *et al.* 1982; Welling & Linshaw, 1988). The efflux of solute during this process is usually via K^+ loss by activation of conductance pathways. The accompanying anion loss, is less well defined but could be either Cl^- or HCO_3^- in different segments of the nephron. Thus, the modifications of the paracellular path are transient, with the parameters returning to normal after 2–10 min even if the tissue is maintained in hypotonic media (Linshaw & Grantham, 1980; Gagnon *et al.* 1982; Völkl, Paulmichl & Lang, 1988). Under our experimental conditions, in which perfusion experiments are performed acutely, over less than 1 min, the phase involving cell swelling is studied predominantly before cell volume regulation, which explains the findings in the alkalinization experiments, of a delay in passive H^+ efflux from the tubule lumen or bicarbonate influx into the lumen during lumen or lumen plus capillary hypotonicity (see Figs 2 and 3). In the present work, however, no effect of hypotonicity was observed in perfusions where H^+ secretion or HCO_3^- reabsorption were studied. These findings suggest that H^+ secretion or HCO_3^- reabsorption depends on transcellular transport mechanisms, the ions being generated within renal tubule cells and transferred to the lumen by the Na^+–H^+ exchanger (Kinsella & Aronson, 1980) or by an H^+–ATPase (Kinne-Saffran, Beauwens & Kinne, 1982) and to the interstitium by a 3:1 HCO_3^-–Na^+ co-transport (Yoshitomi, Burckhardt & Frömter, 1985; Soleimani *et al.* 1987).

The available data on volume regulation in hypertonic media are, on the other hand, conflicting. Some cells in the kidney are able to regulate volume in response to a hypertonic challenge, responding immediately to hyperosmotic media by a regulatory volume increase (RVI). Other cells require stimulation prior to exposure to hyperosmolarity to demonstrate RVI. An increase of intracellular osmolytes during RVI usually occurs with an increase of NaCl influx via the activation of parallel Na^+–H^+ and Cl^-–HCO_3^- exchangers, or Na^+–K^+–$2Cl^-$ co-transporters (Montrose-Rafizadeh & Guggino, 1990). On the other hand, in many experimental situations no volume regulatory effect has been detected in hypertonic solutions, including kidney cortex slices (Györy, Kweifio-Okai & Ng, 1981; Gilles, Duchene & Lambert, 1983), as well as isolated perfused and non-perfused proximal tubules (Gagnon *et al.* 1982; Kirk, DiBona & Schafer, 1987), that is, the cells, after shrinking in hypertonic media do not return to their original volume. The absence of an effect of hypertonic media to alter H^+–HCO_3^- transport across proximal tubule epithelium suggests that under these conditions cell shrinking might not induce significant alterations of the paracellular shunt path.

According to an electrical analog model that has been described (Amorena, Fernandes & Malnic, 1984), the observed increase in half-time of alkalinization in hypotonic media (see Fig. 2) is compatible with an increase in the resistances, both in series and parallel of the model, representing a decrease in passive conductance to

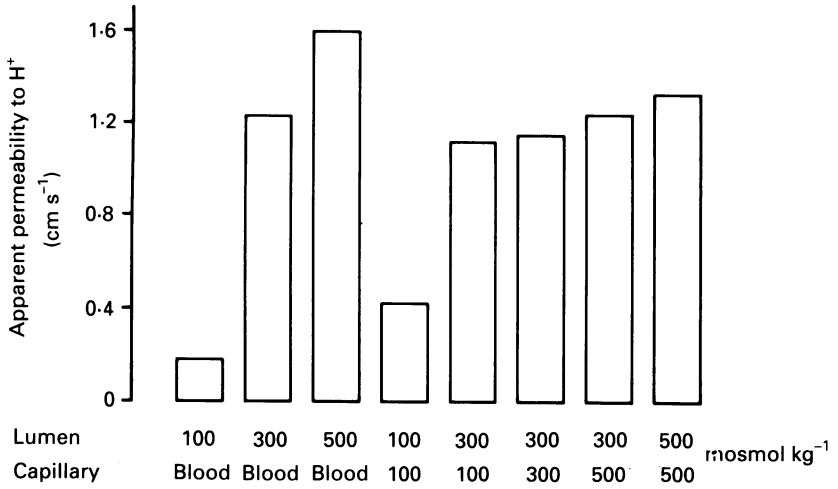

Fig. 6. Apparent permeability to H^+ of proximal tubular epithelium at different luminal and capillary tonicities, obtained from mean values of alkalinization experiments at luminal pH of 6·2.

H^+ or other buffer components like HCO_3^-, when blood is present in capillaries (Lang, Quehenberger, Greger, Silbernagl & Stockinger, 1980). In the condition of luminal and peritubular capillary perfusion, performed with phosphate-buffered solution, however, besides phosphate anions, to which the tubular epithelium has a very low permeability (Cassola & Malnic, 1977), only H^+ ions have a significant mobility in the system. Therefore, H^+ permeabilities may be calculated in such conditions from mean values of J_H, H^+ ion concentration gradients and transepithelial potential difference during alkalinization experiments. They are shown in Fig. 6, and indicate that a major reduction in this permeability is apparent only in experiments with luminal hypotonic solutions. It must be noted, however, that these permeabilities are termed apparent H^+ permeabilities since the participation of cellular buffer components in the observed pH changes cannot be discarded (Ives, 1985).

On the other hand, acidification experiments did not show any significant modification of pH gradients, acidification half-time or net H^+ flux during changes in tonicity of luminal, capillary or luminal plus capillary perfusates (see Figs 1–3). In this condition, H^+ transport is mainly transcellular, and these results suggest that the series resistance of the H^+ secretory mechanism is not affected by changes in the tonicity of the medium bathing tubular cells.

Transport of H^+ and HCO_3^- appears to be involved in cell volume regulation. Hypotonic media might be expected to stimulate H^+ ion secretion via activation of Na^+–H^+ exchange (Eveloff & Warnock, 1987; Lopes & Guggino, 1987). However, it has been shown recently that transepithelial transport mechanisms may be

independent from homeostatic mechanisms regulating cell volume or cell pH. Montrose & Murer (1990) have demonstrated that the Na^+–H^+ exchangers of apical and basolateral membrane of cultured opossum kidney cells have markedly different properties, so that the basolateral exchanger, involved in homeostatic regulatory processes, may be affected differently from the apical exchanger, largely responsible for luminal acidification. This view is compatible with the present results, demonstrating absence of effect of hypo- and hypertonic solutions on transepithelial H^+ ion secretion. Thus, it appears that the most important alterations produced by modifications of medium osmolality on H^+ transport are related to the paracellular permeability to the buffer components involved.

Independent evidence for a role of the paracellular shunt path in H^+–HCO_3^- movement across epithelial was obtained by applying electrophysiological methods (Frömter, 1979; Ikonomov, Simon & Frömter, 1985). The model developed by Spring (1973) for the electrophysiological properties of proximal tubule likewise shows that transepithelial resistance is very sensitive to the geometry of lateral intercellular spaces, hypotonicity increasing and hypertonicity decreasing specific wall resistance of this segment. The increase in specific wall resistance observed when tubules were perfused with hypotonic solution (see Fig. 4) is compatible with our findings discussed above, of significantly decreased passive H^+ and/or HCO_3^- fluxes across the epithelium during perfusion with solutions of low tonicity. Similar effects of changes in tonicity upon epithelial resistance have been observed by others, in proximal (De Mello *et al.* 1976) and distal (De Bermudez & Windhager, 1975) renal tubule and in toad bladder (DiBona & Civan, 1973). In electrophysiologically tight structures like distal tubule and toad bladder, alterations of resistance upon modifications in tonicity depend on the compartment into which these solutions were added. These observations were interpreted by DiBona & Civan (1973) as evidence that the important factor determining the effect of changes in tonicity was the direction of the osmotic gradient across the epithelium. In our experiments an asymmetric effect of osmolality was not observed, but hypotonic solutions on both sides of the epithelium caused reduction in passive H^+–HCO_3^- fluxes, while hypertonic solutions had no significant effect in all experimental conditions.

One aspect of our experiments that deserves mention concerns volume variations of the injected fluid columns within the tubule lumen, which could affect by themselves the concentration of luminal buffer components and thereby the measured pH. This question was verified experimentally by measuring volume variations of isotonic, hypotonic and hypertonic fluid columns in the tubule lumen. Figure 5 shows that volume variations are slow during the first 30 s, which is the period of fastest pH change. It is, therefore, unlikely that these variations of volume would affect pH curves in an important way.

In sum, the results obtained in our experiments in proximal tubules involving modifications of luminal and peritubular osmolality indicate that active transport mechanisms resulting in tubular acidification are not affected by sizeable alteration of luminal and/or peritubular osmolality, while passive backflux of H^+ from lumen to interstitium or of HCO_3^- from blood to lumen are affected to a significant extent, leading to a decrease in the rate of pH equilibration and of apparent H^+ permeability. These modifications are accompanied by increases in epithelial resistance. Although

the nature of the morphological and functional alterations responsible for these changes has not been defined, they are compatible with some of the findings in the literature that attribute the effects of changes in osmolality on epithelial resistance and transepithelial fluid flow to modifications of cell volume and geometry of the paracellular pathway.

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