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THE EFFECTS OF ANIONS ON FLUID REABSORPTION FROM THE PROXIMAL CONVOLUTED TUBULE OF THE RAT

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SUMMARY

1. Fluid reabsorption from surface proximal tubules of the rat was measured *in vivo* using stationary microperfusion techniques. Reabsorptive rate (J_v) was measured from droplets containing chloride as the main reabsorbable anion and when chloride was substituted by bromide, iodide, nitrate, acetate, isethionate or methylsulphate in either the tubular lumen alone or in both lumen and peritubular capillaries.

2. In tubules with an intact blood supply, droplet volume decreased in a manner best described by a single exponential and substitution of chloride by nitrate or bromide had no effect on J_v. Substitution by iodide or acetate inhibited J_v by approximately 17% but substitution by methylsulphate or isethionate caused droplets to transiently increase in volume before shrinkage which was itself inhibited by approximately 50%. The inhibitory action of isethionate was found to be concentration dependent.

3. Recollection and analysis of droplets which were initially free of chloride, containing either nitrate or isethionate, showed that chloride entered these droplets, but that the initial rate of chloride entry was greater for nitrate than isethionate droplets.

4. When tubules and capillaries were perfused with chloride solutions containing no bicarbonate, J_v was reduced to about 20% of the value when peritubular capillary blood flow was intact. Substituting chloride in the tubular and capillary perfusion revealed a sequence for supporting fluid reabsorption that was identical to that when chloride was substituted in tubule fluid alone: bromide $\equiv$ nitrate $>$ iodide $\equiv$ acetate $>$ isethionate. Addition of 2·0 mmol l^{-1} NaCN reduced the reabsorptive flux to zero.

5. The results of this study are consistent with transcellular transport of anions across the proximal tubular epithelium. The pathways for anion transport are likely to involve a series of non-selective mechanisms such as anion exchangers.

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INTRODUCTION

Reabsorption of fluid from the mammalian proximal tubule is a purely passive process driven by the transport of solute. The proximal convoluted tubule (PCT) exhibits axial heterogeneity with respect to reabsorption of the major filtered solutes. Almost all the glucose, amino acids, phosphate and bicarbonate is reabsorbed in the early PCT with equivalent amounts of sodium and water (Giebisch & Aronson, 1986). The early reabsorption of bicarbonate at the expense of chloride, raises the concentration of chloride in tubular fluid to exceed that in peritubular capillary plasma (Atherton, 1977) and the mid–late PCT predominantly reabsorbs sodium chloride and water. It is known that anions play a critical role in supporting normal sodium and fluid reabsorption (Green & Giebisch, 1975; Neumann & Rector, 1976; Green, Bishop & Giebisch, 1979) but the transport mechanisms for anions and the links between anions, sodium and water movement are not fully understood.

The purpose of the present *in vivo* micropuncture study was to examine the mechanism of anion reabsorption from the mid–late PCT in the rat. A component of chloride reabsorption from this segment occurs passively through the paracellular pathways in response to the favourable electrochemical gradient (Frömter, 1977; Alpern, Howlin, Preisig & Wong, 1985). However, approximately two-thirds is reabsorbed actively across proximal tubular cells (Lucci & Warnock, 1979; Chantrelle, Cogan & Rector, 1985) by a process which is electrically neutral in both the rabbit (Baum & Berry, 1984) and the rat (Howlin, Alpern, Berry & Rector, 1986). Several possible mechanisms have been suggested to account for electrically neutral reabsorption of salt from the proximal tubule including (1) an electroneutral coupled sodium–chloride co-transport and/or (2) the parallel operation of the sodium–hydrogen exchanger, known to be present in the proximal tubule (Aronson, 1983), with one or more anion exchangers. These exchangers would transport chloride into the cell in exchange for cellular anions such as bicarbonate, hydroxyl or formate (Schild, Giebisch & Green, 1988).

Sodium–chloride co-transport and anion exchange mechanisms in several tissues differ both with respect to their sensitivity to blocking agents and their affinity for anions (Warnock, Greger, Dunham, Benjamin, Frizzell, Field, Spring, Ives, Aronson & Seifter, 1984). Sodium–chloride co-transporters have a high chloride specificity and do not readily accept anions other than chloride (Lauf, McManus, Haas, Forbush, Duhm, Flatman, Saier & Russell, 1987) whereas anion exchangers have a relatively low anion specificity and can transport a number of different anions (e.g. bromide, iodide, phosphate, sulphate, (Gunn, 1977; Stein, 1986). One way to distinguish between the two possible mechanisms in the proximal tubule would be to assess the anion dependency of fluid reabsorption. The ability of various anions to support fluid reabsorption would be consistent with the operation of an anion exchanger rather than a sodium–chloride co-transport mechanism for salt reabsorption.

In the present experiments the effect on fluid reabsorption of substituting chloride with a variety of different anions has been assessed. The results demonstrate that anions other than chloride can support fluid reabsorption to varying extents, favouring the operation of anion exchangers rather than sodium–chloride co-

transport in proximal tubule cell membranes. However, if simple rheogenic sodium transport were to operate in the rat PCT, anions may be reabsorbed passively, and the results may reflect the anion permeability of the paracellular pathways.

METHODS

Experiments were performed on male Sprague–Dawley rats weighing 200–250 g which were starved overnight but allowed free access to water. Rats were anaesthetized with an intraperitoneal injection of 100–110 mg kg body weight^{-1} Inactin (5-ethyl-5(1′-methyl-propyl)-2-thiobarbiturate; Byk Gulden, Hamburg), placed on a heated table set to maintain rectal temperature at 38 °C and prepared for micropuncture as previously described (Bishop, Green & Thomas, 1979). Two catheters (PP 10) were placed in the left jugular vein for infusion of saline (20 μl min^{-1}) and injection of 10% Lissamine Green which was used to assess proximal tubular transit time (measured from the first flush of the kidney surface to the arrival of the dye at the end of the proximal tubules). Kidneys with a transit time greater than 12 s were rejected. The ureter was sectioned to allow free drainage of urine.

Group 1: stationary microperfusion: luminal anion substitution

Fluid reabsorptive rate was measured from proximal convoluted tubules *in vivo* using the stationary microperfusion (split-drop) technique. Straight surface segments of superficial proximal tubules were punctured with double-barrelled glass micropipettes. Split-drops were made using the method of Gertz (1963) with modifications suggested by Gyory (1971). Castor oil stained with Sudan Black was injected from one barrel of the pipette to block tubular flow and Ringer solution injected from the second barrel to produce the fluid droplet. Shrinkage of droplets was filmed using a Beaulieu R16 camera at 2 frames s^{-1} and sequences recorded on Ektachrome 7252 film. Filmed sequences were subsequently analysed using a 'Magiscan' (Joyce-Loebl, Gateshead) image analysis programme (Garland, Brunt, Taylor & Green, 1979) which measured droplet length and tubular diameter (2 r) for each frame and then calculated the time taken for half the volume of the fluid in the drop to be reabsorbed ($t_{\frac{1}{2}}$). Fluid reabsorptive rate was calculated using the formula of Gertz (1963):

$$J_v \text{ (nl min}^{-1} \text{ mm length}^{-1}) = (0{\cdot}693\ \pi r^2)/t_{\frac{1}{2}}.$$

Fluid reabsorptive rate was measured from split-drops containing either chloride Ringer solution (control) or solutions in which chloride was completely substituted by bromide, iodide, nitrate, acetate, methylsulphate or isethionate. The composition of the fluids is given in Table 1. Chloride Ringer solution had a chloride concentration similar to that in tubular fluid in mid–late PCT.

Group 2: recollection of split-drops: rate of chloride influx

In these experiments the rate of chloride influx into initially chloride-free droplets was assessed. Proximal tubules were punctured with double-barrelled micropipettes as described above and very long split-drops were made with either nitrate or isethionate Ringer solution (Table 1). Ringer solution was held in the lumen of the tubule for timed intervals of 5, 15, 25 or 35 s and then recollected using a glass micropipette of tip diameter 8–10 μm filled with castor oil stained with Sudan Black. Droplets were rapidly aspirated and a small column of castor oil was drawn into the tip of the pipette to avoid contamination of the collected sample. Samples were stored either in their collection pipettes or under water-equilibrated paraffin oil. The samples were analysed for chloride by the electrotitrimetric method of Ramsey, Brown & Croghan (1955) on the same day the experiment was performed.

Group 3: stationary microperfusion with perfusion of peritubular capillaries: bilateral anion substitution

In these experiments fluid reabsorptive rate was measured from split-drops whilst the surrounding peritubular capillaries were simultaneously perfused. The tubule and capillary solutions were chloride, bromide, nitrate, acetate and isethionate Ringer solution (composition given in Table 1). Identical solutions were placed in the tubule and the capillaries to assess the

potential of the various anions to support fluid reabsorption in the complete absence of chloride. Straight surface segments of superficial proximal convoluted tubules were punctured with double-barrelled micropipettes and stained castor oil injected from one barrel to block tubular flow. Peritubular capillary stars, at a distance of 40–60 μm from the punctured tubular segments, were perfused using single-barrelled micropipettes with a tip diameter of 6–8 μm connected to a continuous infusion pump (SRI 5201, Edenbridge, Kent) with a 250 μl Hamilton syringe delivering 1–2 μl min^{-1}. Capillaries were perfused for at least 1 min prior to making split-drops. Measurement of droplet shrinkage and calculation of fluid reabsorptive rate was performed as described above. Stationary microperfusions were only accepted if the droplet fell completely within the area of capillary perfusion for the duration of droplet shrinkage and this was subsequently checked by visual examination of the filmed sequence.

TABLE 1. Composition (mmol l^{-1}) of perfusion solutions

Perfusion Ringer solution...	Chloride	Bromide	Iodide	Nitrate	Acetate	Methyl-sulphate	Isethionate		
NaCl	155	—	—	—	—	—	—	140	153·5
NaBr	—	155	—	—	—	—	—	—	—
NaI	—	—	155	—	—	—	—	—	—
$NaNO_3$	—	—	—	155	—	—	—	—	—
$NaCH_3COOH$	—	—	—	—	155	—	—	—	—
$NaCH_3SO_4$	—	—	—	—	—	155	—	—	—
$NaCH_2OHCH_2SO_3$	—	—	—	—	—	—	155	15	1·5
K_2HPO_4	2	2	2	2	2	2	2	2	2
KH_2PO_4	2	2	2	2	2	2	2	2	2
$CaSO_4$	1·5	1·5	1·5	1·5	1·5	1·5	1·5	1·5	1·5

All solutions were adjusted to pH 6·8 with NaOH or H_3PO_4 and gassed with 100% O_2 prior to use.

Statistics

Statistical comparisons between control (chloride Ringer solution) and experimental (chloride-substituted Ringer solution) data were performed by Dunnet's modified t test for multiple comparisons. Single comparisons, where appropriate, were performed by Student's t test for unpaired groups. Results are presented as means ± S.E.M. and values of $P < 0.05$ are reported as statistically significant.

RESULTS

Group 1: stationary microperfusion: luminal anion substitution

In these experiments fluid reabsorptive rate was measured from chloride Ringer solution split-drops (controls) and from droplets in which chloride was completely substituted by bromide, iodide, nitrate, acetate, methylsulphate or isethionate (Table 1) in blood-perfused tubules. Fluid reabsorption from chloride Ringer solution was 2·22 ± 0·10 nl mm^{-1} min^{-1} which compares favourably with reabsorptive rates reported using continuous microperfusion with solutions of similar chloride concentration (Neumann & Rector, 1976; Green *et al.* 1979). None of the anions substituting for chloride affected the mean tubular radius (r) measured during split-drop shrinkage (Table 2) and any reduction in reabsorptive rate corresponded to an increased reabsorptive half-time ($t_{\frac{1}{2}}$). Representative examples of individual droplets shrinking are shown in Fig. 1*A* and *B*. In the case of bromide, nitrate, iodide and acetate substitution, droplet shrinkage could be described by a single-exponential decline. Results of all the droplets are summarized in Table 2. Substitution of chloride by bromide and nitrate had no significant effect on fluid reabsorption but

TABLE 2. Effect of luminal anion substitution on reabsorptive half-time, tubular radius and reabsorptive rate

Tubular perfusate		$t_{\frac{1}{2}}$ (s)	r (μm)	J_v(nl mm^{-1} min^{-1})
NaCl	(59)	23·18±1·16	18·96±0·35	2·22±0·10
NaBr	(20)	25·86±2·19	19·19±0·49	2·17±0·22
NaI	(28)	34·58±3·28*	19·81±0·41	1·84±0·16*
$NaNO_3$	(23)	22·34±1·83	18·83±0·71	2·21±0·11
$NaCH_3COOH$	(24)	30·24±1·71*	19·72±0·39	1·83±0·13*
$NaCH_3SO_4$	(17)	47·98±3·88**	18·66±0·49	1·06±0·10**

* $P < 0·05$ when compared with NaCl perfusate.
** $P < 0·01$ when compared with NaCl perfusate.
Numbers in parentheses are the number of tubules.
Methylsulphate is calculated for the reabsorptive phase only (see text).

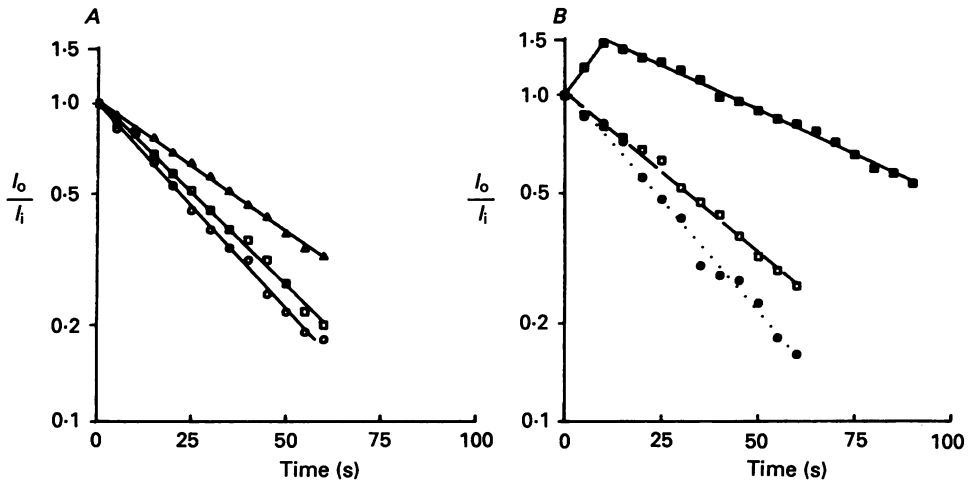

Fig. 1. The change in length (corrected as suggested by Gyory (1971) for the meniscus error) of representative shrinking droplets containing: *A*, ○, chloride; □, bromide; ▲, iodide; and *B*, ●, nitrate; □, acetate; ■, methylsulphate as the reabsorbable anion plotted semilogarithmically against time. l_o, length at any time; l_i, initial length. Regressions shown in other figures are also corrected for meniscus error.

both iodide and acetate substitution reduced reabsorptive rate by approximately 17%.

When chloride was substituted by the larger anion methylsulphate the volume of the injected droplet initially increased, reflecting net movement of fluid into the tubule lumen. After the initial swelling phase of 10–12 s, net reabsorption of fluid from the droplet occurred. The shrinking phase could again be described by a single exponential; a representative example of the change in volume of a droplet containing methylsulphate is shown in Fig. 1*B*. The rate of fluid reabsorption (calculated by taking the maximum length achieved during the swelling phase as the first frame in the sequence) was reduced by 53% when compared to chloride Ringer solution.

The concentration dependence of the effect of isethionate was tested to assess whether this large organic anion may have a direct inhibitory effect on transport

TABLE 3. Effects of various luminal concentrations of isethionate on reabsorptive half-time, tubular radius and reabsorptive rate

Tubular perfusate	$t_{\frac{1}{2}}$ (s)	r (μm)	J_v(nl mm^{-1} min^{-1})
0 isethionate 155 mmol l^{-1} NaCl (59)	23·18±1·16	18·96±0·35	2·22±0·10
1·5 mmol l^{-1} isethionate 153·5 mmol l^{-1} NaCl (23)	24·60±2·00	19·20±0·50	2·00±0·20
15 mmol l^{-1} isethionate 140 mmol l^{-1} NaCl (28)	28·60±1·70*	18·10±0·70	1·50±0·09**
155 mmol l^{-1} isethionate 0 NaCl (18)	52·59±6·49**	18·90±0·54	1·06±0·10**

* $P < 0·05$ when compared with chloride perfusate.
** $P < 0·01$ when compared with chloride perfusate.
Numbers in parentheses are the number of tubules.

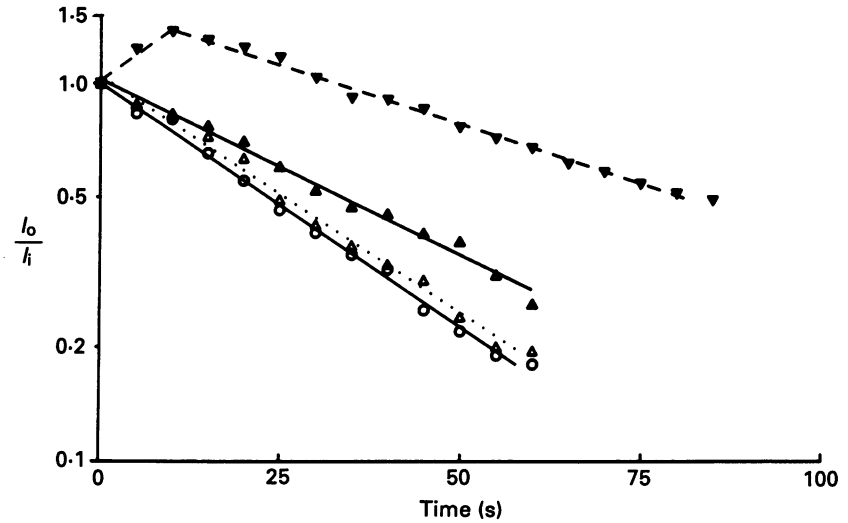

Fig. 2. The effect of different concentrations of isethionate on droplet shrinkage. Shown are representative examples of droplets containing chloride (○) and where chloride was replaced by 1·5 (△), 15 (▲) or 155 (▼) mmol l^{-1} isethionate. l_o, length at any time; l_i, initial length.

mechanisms. It has been shown previously that substitution by 25 mmol l^{-1} sodium cyclamate inhibited fluid reabsorption by 70% (Green *et al.* 1979); an effect much greater than would be expected from the relative concentration of cyclamate. Individual examples of shrinkage of droplets containing isethionate are shown in Fig. 2 and the results are summarized in Table 3. Complete replacement of chloride by isethionate produced changes in droplet volume that were qualitatively and quantitatively similar to replacement by methylsulphate. Over the first 10–12 s droplet volume increased due to net fluid secretion at a rate of 1·39±0·14 nl mm^{-1} min^{-1}. After the swelling phase, droplet shrinkage occurred which could be described by a single exponential. The rate of fluid reabsorption was reduced by 53% when chloride was completely replaced with isethionate. Substitution of 15 mmol l^{-1} chloride with isethionate reduced fluid reabsorption by 32% but substitution of

1·5 mmol l^{-1} had no effect. Droplet swelling was not observed when 15 or 1·5 mmol l^{-1} chloride was replaced with isethionate.

The results show that the preferential sequence for anions in supporting sodium and fluid reabsorption when substituted for chloride at the luminal membrane is chloride ≡ bromide ≡ nitrate > iodide ≡ acetate > methylsulphate ≡ isethionate.

Group 2: recollection of split-drops: rate of chloride influx

The initial increase in droplet volume when the less permeant anions methylsulphate and isethionate substituted for chloride could be a consequence of chloride

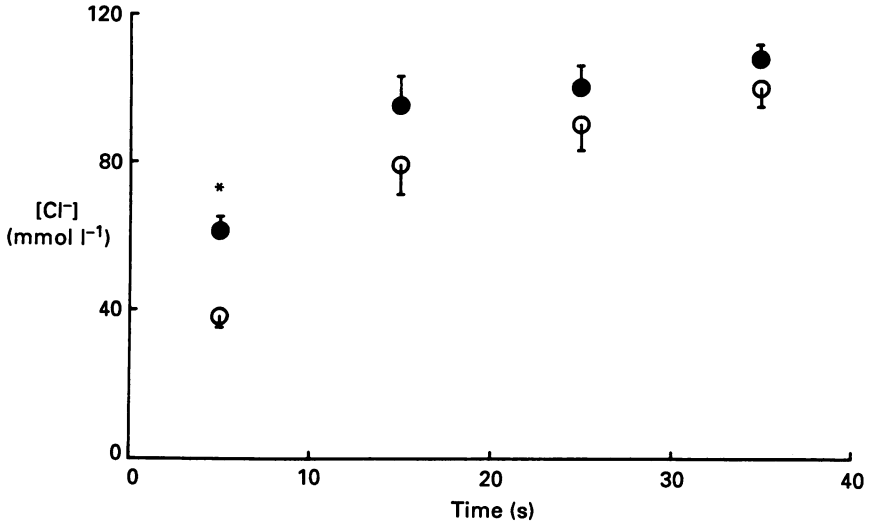

Fig. 3. The change in chloride concentration of initially chloride-free droplets where chloride was replaced by either isethionate (○) or nitrate (●), plotted against the time of recollection. Results are plotted as means ± S.E.M. *$P < 0.05$ by unpaired t test.

diffusion from peritubular capillary plasma and proximal tubular cells into the chloride-free droplet down its concentration gradient (Malnic & De Mello Aires, 1970). This movement of chloride would be accompanied by sodium and equivalent amounts of fluid. The rate of reabsorption of the anion substituted for chloride would determine whether there was net movement of fluid in a reabsorptive or secretory direction. The rate of chloride influx into initially chloride-free drops was assessed by recollecting droplets after timed periods in the tubule and analysing them for chloride. Droplets where chloride was replaced with nitrate, an anion which supports fluid reabsorption at a rate equivalent to chloride and does not induce droplet swelling, were compared with droplets where chloride was completely substituted by isethionate. The results are shown in Fig. 3.

The concentration of chloride in the recollected droplets increased in a saturable fashion over 5–35 s in the tubule. By 35 s both nitrate and isethionate droplets had equilibrated with peritubular capillary plasma chloride which has been determined previously (Atherton, 1977) to be 115 mmol l^{-1}. However, the concentration of chloride in nitrate drops after 5 s was significantly higher than in isethionate drops.

Estimation of the rate of chloride entry at 5 s (for idealized droplets of initial volume 100 pl and taking into account the relative volume changes) gives a flux of 11·8 nmol cm^{-1} s^{-1} for nitrate and 7·2 nmol cm^{-1} s^{-1} for isethionate drops. The results imply that the permeance of the anion replacing chloride at the luminal membrane dictates the rate of chloride movement in a secretory direction. This suggestion is supported by earlier work of Malnic & De Mello Aires (1970) who showed that chloride entered droplets faster when bicarbonate replaced chloride in the split-drop than when the less permeant anion sulphate replaced chloride.

TABLE 4. Effects of bilateral anion substitution on reabsorptive half-time, tubular radius and reabsorptive rate

Tubular and capillary perfusate		$t_{\frac{1}{2}}$ (s)	r (μm)	J_v(nl mm^{-1} min^{-1})
NaCl	(31)	94·6± 7·9	16·5±0·8	0·40±0·03
NaBr	(15)	121·9±11·0	18·2±0·7	0·38±0·03
$NaNO_3$	(14)	124·0±18·0	17·1±0·8	0·37±0·05
NaI	(14)	220·0±30·0**	17·8±0·5	0·24±0·03**
$NaCH_3COOH$	(14)	184·3±30·0*	15·3±0·6	0·25±0·05**
$NaCH_2OHCH_2SO_4$	(13)	228·0±36·0**	18·1±0·9	0·20±0·03**

* $P < 0·05$ when compared with control (NaCl).
** $P < 0·01$ when compared with control (NaCl).
Numbers in parentheses are the number of tubules.

Group 3: stationary microperfusion with perfusion of peritubular capillaries: bilateral anion substitution

The forces for fluid reabsorption from split-drops during luminal substitution of chloride could be generated by passive movement of anions down their concentration gradient, most probably via paracellular pathways. Alternatively anions may be transported across proximal tubule cells. To test the possibility that the anions used in this study may be reabsorbed across cells, split-drops were made and the peritubular capillaries simultaneously perfused. Solutions with the same composition were placed in the lumen and capillary, thus avoiding gradients which may drive passive movement, to assess the potential of the various anions to support fluid reabsorption in the complete absence of chloride.

When tubules and capillaries were perfused with chloride Ringer solution, fluid was reabsorbed at 0·40±0·03 nl mm^{-1} min^{-1}. A representative example of a droplet shrinking containing chloride Ringer solution is shown in Fig. 4*A*. The rate of fluid reabsorption was significantly slower during tubule and capillary perfusion with chloride Ringer solution than when the blood supply was left intact (2·22±0·10; $P < 0·001$).

When tubules and capillaries contained bromide, iodide, nitrate, acetate and isethionate Ringer solutions, split-drop shrinkage could be described by a single-exponential decline. Representative examples of individual droplets shrinking are shown in Fig. 4*A* and *B* and the data is summarized in Table 4. The proximal tubule was able to transport all the anions which had been substituted for chloride. There was no significant difference in fluid reabsorption with bromide or nitrate compared to chloride. Iodide substitution reduced fluid reabsorption by 40%, acetate by 37%

and isethionate by 50%. The initial phase of droplet swelling when isethionate replaced chloride in the tubule with an intact blood supply (Fig. 2) was never observed during tubule and capillary perfusion with isethionate. This indicates that capillary perfusion was complete in these experiments and effectively removed chloride from the peritubular space and tubular cells.

The addition of 2 mmol l^{-1} cyanide to the tubule and capillary solutions when chloride was the available anion completely inhibited fluid reabsorption (example in Fig. 4*A*). Similarly, when cyanide was added to the perfusates with all the other

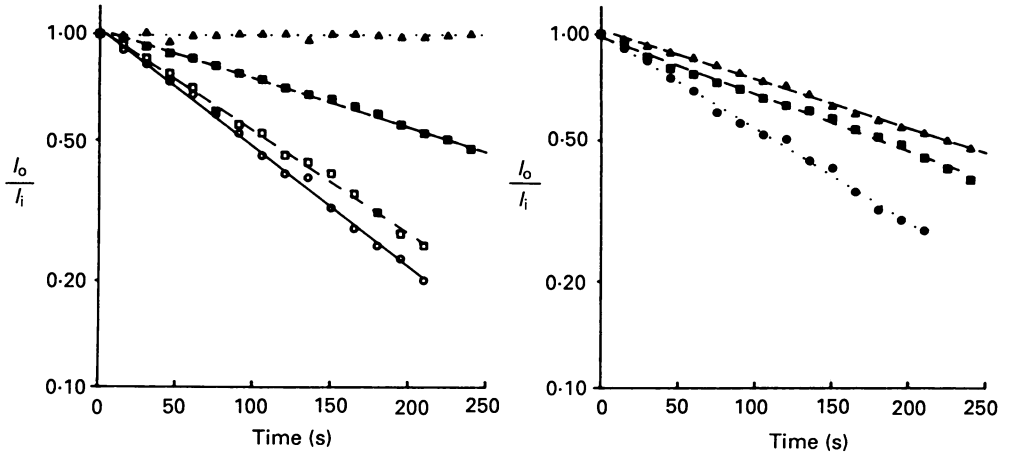

Fig. 4. The change in length of representative examples of shrinking droplets where the peritubular capillaries were perfused with identical solutions containing: *A*, ○, chloride; ▲, chloride + 2 mmol l^{-1} NaCN; □, bromide; ■, iodide; and *B*, ●, nitrate; ■, acetate; ▲, isethionate plotted semilogarithmically against time. l_0, length at any time; l_i, initial length.

anions, there was complete inhibition of fluid reabsorption (data not shown). This indicates that transport of all these anions is ultimately dependent on cell metabolism. After capillary perfusion and 1 min recovery (blood allowed to re-enter the capillaries) reabsorptive rates were similar to those found in series 1. The sequence of the relative abilities of anions to support fluid reabsorption is almost identical to that obtained when luminal substitutions alone were performed, i.e. chloride ≡ bromide ≡ nitrate > iodide ≡ acetate > isethionate.

DISCUSSION

Anion movement across proximal tubular epithelium might be across (transcellular) or between cells (paracellular). If movement is predominantly paracellular then abolition of driving gradients would inhibit anion movement. Transcellular anion movement must occur across two separate cell membranes each of which might have a number of transport systems either exchanging one anion for another or co-transporting anions and cations (Giebisch & Aronson, 1986). Conductive movement of chloride has been observed in numerous studies using brush-border vesicles

(Warnock & Yee, 1981; Sabolic & Burckhardt, 1983; Low, Friedrich & Burckhardt, 1984). However, electrophysiological studies of intact proximal tubules both *in vivo* and *in vitro* have been unable to demonstrate apical chloride conductive pathways (Frömter, Muller & Wick, 1971; Bello-Reuss, 1982).

In general, anion exchangers are rather non-specific (Gunn, 1977; Stein, 1986) while the anion specificity of true co-transporters is very narrow, bromide being the only partially substitutible anion (Chipperfield, 1980; Saier & Boyden, 1984; Lauf *et al.* 1987).

Luminal anion substitution

Interpretation of data from experiments where anion substitutions were made only in the luminal perfusate is difficult. Although solutions initially contained no chloride ions, chloride rapidly entered the lumen from cells and peritubular capillary plasma due to the favourable concentration gradient and the high chloride permeability of the tubule (Green & Giebisch, 1986). Experiments in which tubular perfusates were recollected (series 2) showed that for both nitrate and isethionate there was rapid entry of chloride, though there may have been differences in the rate at which it entered. Thus, the droplet solution is always a mixture containing chloride. In addition, of course, there is an outwardly directed gradient for the substituent anion which allows it to move down its electrochemical gradient out of the tubule.

The net movement of anions will constrain net movement of sodium and so, net fluid movement. If chloride entry is faster than the egress of the substituent anion then droplets will swell as occurred with isethionate and methylsulphate substitutions. With nitrate substitution (or bromide) the net exit of nitrate together with any chloride which had entered must exceed chloride entry by a factor of two so that normal net fluid reabsorption occurs.

It is of some interest that fluid reabsorption was affected by the presence of as little as 15 mmol l^{-1} isethionate. Previously it has been reported that similar concentrations of cyclamate inhibits transport (Green *et al.* 1979). Whether this effect is due to very low permeance of large organic anions consequently impeding sodium transport, whether these anions inhibit chloride transport *per se* or whether it is due to non-specific actions is not known. In this respect anions are known to inhibit various enzymes such as carbonic anhydrase (Maren, Rayburn & Liddel, 1976) and enzymes of the glycolytic pathway (Funder & Wieth, 1967), but not the Na^+–K^+-ATPase, at least in skeletal muscle (Edwards, Harris & Nishie, 1957).

Bilateral anion substitutions

Total substitution of chloride in both tubular and capillary perfusates allows a simple model for study of anion transport since there are no concentration gradients for the substituent anion and chloride ions cannot enter the tubule lumen. Two major findings are immediately obvious; first that there is a reduction in the absolute rate of volume absorption and second that the ability of anions to support fluid movement is the same as in unilateral substitution experiments.

The reduction in total net volume absorption can be ascribed to a number of mechanisms. Removal of bicarbonate from the peritubular capillaries has long been

known to reduce proximal tubular reabsorption (Ullrich, Radtke & Rumrich, 1971; Green & Giebisch, 1975; Ullrich, Capasso, Rumrich & Papavassiliou, 1977). Whether this is due to a direct action of bicarbonate on transcellular sodium transport; generation of an osmotic gradient due to the difference in reflection coefficients of chloride and bicarbonate (Green & Giebisch, 1984, 1986) or due to alteration of intracellular pH and hence ionic permeabilities is not known. In addition, there was no protein in the peritubular perfusate; this is known to reduce the rate of reabsorption (Green, Windhager & Giebisch, 1974).

In spite of the low reabsorptive rate, all the anions studied were able to support fluid transport to some extent. When arranged in order of ability to support fluid transport the sequence was very similar to anion permeability sequences of a variety of nerve and muscle membranes (Wright & Diamond, 1977; Aickin & Brading, 1985) and the ability to support fluid transport by the gall bladder epithelium (Whitlock & Wheeler, 1967).

The mechanism for anion transport is still obscure, however. The wide anion specificity that we have demonstrated argues against the involvement of a classical Na–Cl or Na–K–2Cl co-transporter since such systems demonstrate narrow anion specificity (Chipperfield, 1980; Saier & Boyden, 1984; Lauf *et al.* 1987). Conductive movement of chloride is a common observation in studies which have utilized brush-border membrane vesicles (Warnock & Yee, 1981; Burnham, Munzesheimer, Rabon & Sachs, 1982; Sabolic & Burckhardt, 1983) and a variety of anion channels in nerve and muscle have been shown to conduct even large organic anions (Edwards, 1982). However, it has not so far been possible to demonstrate chloride conductances in intact cells using electrophysiological techniques (Frömter *et al.* 1971; Bello-Reuss, 1982). The anion channel blocker anthracene-9-carboxylic acid has little effect on fluid transport when applied at either the luminal (Green & White, 1985) or peritubular (Baum & Berry, 1984) membrane, suggesting that transcellular conductive movement of chloride does not play a major role in normal fluid absorption.

A system of anion exchangers of low specificity would explain the results we have observed. The classical anion exchange protein of red cell membranes (Band 3) transports a wide variety of anions including some large organic anions (Stein, 1986). However, transport rates vary widely even amongst the halides (Wieth, 1979). Other exchange processes have been described in both brush-border and basolateral membrane vesicles derived from kidney cortex (Warnock & Yee, 1981; Low *et al.* 1984) and more recently, chloride–formate and chloride–oxalate exchangers have been characterized in brush-border vesicles (Karniski & Aronson, 1985, 1987). These exchangers accept a wide variety of anions in lieu of chloride including most of the anions tested in this study and are inhibited by anion transport inhibitors, such as SITS and DIDS which are known to inhibit fluid transport by this epithelium (White, Greenwood & Green, 1988). Whether such exchangers are operative under the conditions of our experiments is unknown. However, dependence of anion exchange on some intracellularly produced and recycled metabolite as proposed by Karniski & Aronson (1985) would allow net transport of anion.

A further possibility remains. In the proximal straight tubule of rabbit, sodium is actively reabsorbed in a rheogenic fashion (Schafer, Troutman, Watkins & Andreoli,

1978). Rheogenic sodium transport generates a transepithelial electrical gradient which acts to drive chloride out of the tubule (presumably via the paracellular pathway) and thus supports fluid transport. If this were the case in our experiments net transport would depend on the passive permeability of the epithelium for each anion. We have not measured transepithelial potential difference in the present study, but under similar conditions Frömter (1977) has shown that the potential difference is not different from zero. Furthermore, Frömter *et al.* (1971) have reported the anion permeability sequence of the proximal tubule to be $P_{Cl} > P_{Br} > P_I$. From these permeabilities we would have expected droplets containing anions of a lower permeance than chloride such as bromide and iodide, to initially swell; in unilateral substitutions swelling was never observed.

In summary, our observations argue against the paracellular pathway as the main pathway for anion transport but support the idea that transport proceeds transcellularly across the proximal tubule via a series of non-selective pathways such as anion exchangers.

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