# Mechanism of NaCl and Water Reabsorption in the Proximal Convoluted Tubule of Rat Kidney

## ROLE OF CHLORIDE CONCENTRATION GRADIENTS

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A B S T R A C T The role of chloride concentration gradients in proximal NaCl and water reabsorption was examined in superficial proximal tubules of the rat by using perfusion and collection techniques. Reabsorptive rates  $(J_v)$ , chloride concentrations, and transtubular potential difference were measured during perfusion with solutions (A) simulating an ultrafiltrate of plasma; (B) similar to (A) except that 20 meq/liter bicarbonate was replaced with acetate; (C) resembling late proximal fluid (glucose, amino acid, acetate-free, low bicarbonate, and high chloride); and (D) in which glucose and amino acids were replaced with raffinose and bicarbonate was partially replaced by poorly reabsorbable anions (cyclamate, sulfate, and methylsulfate).

In tubules perfused with solutions A and B,  $J_r$ were 2.17 and 2.7 nl mm<sup>-1</sup> min<sup>-1</sup> and chloride concentrations were 131.5±3.1 and 135±3.5 meg/liter, respectively, indicating that reabsorption is qualitatively similar to free-flow conditions and that acetate adequately replaces bicarbonate. With solution C,  $J_v$  was 2.10 nl mm<sup>-1</sup> min<sup>-1</sup> and potential difference was  $+1.5\pm0.2$  mV, indicating that the combined presence of glucose, alanine, acetate, and bicarbonate per se is not an absolute requirement. Fluid reabsorption was virtually abolished when tubules were perfused with D solutions;  $J_v$  was not significantly different from zero despite sodium and chloride concentrations similar to plasma; chloride concentration was  $110.8 \pm 0.2$  meg/liter and potential difference was -0.98 mV indicating that chloride was close to electrochemical equilibrium.

These results suggest the importance of the chloride gradient to proximal tubule reabsorption in regions where actively reabsorbable solutes (glucose, alanine, acetate, and bicarbonate) are lacking and provide further evidence for a passive model of NaCl and water transport.

## INTRODUCTION

The mechanism of sodium chloride and water reabsorption by the proximal convoluted tubule of the mammalian kidney has not been clearly established. Although it is generally accepted that net reabsorption depends on both active and passive transport processes, the precise identification of the active and passive components of tubular fluid reabsorption remains in question.

One widely accepted view suggests that the active transport of sodium from lumen to blood is the primary transport process and provides the driving forces for passive solvent and chloride movement (1-3). The principle evidence supporting this hypothesis has been the reported negative transtubular potential difference (PD)<sup>1</sup> and the ability of the tubules in the presence of a nonreabsorbable solute to lower the sodium concentration in the tubular fluid below that in plasma. Another fundamentally similar view suggests that proximal salt and water reabsorption is mediated by an active neutral NaCl transport process. This conclusion is based on studies where the reabsorption of chloride could not be accounted for by the prevailing electrochemical gradients for this ion (4, 5).

In contrast, Rector and co-workers, Kokko et al., and Barrat et al. (6-8) have proposed that only that fraction of sodium reabsorption which is coupled to H<sup>+</sup> secretion is active and that reabsorption of NaCl and

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<sup>&</sup>lt;sup>1</sup>Abbreviation used in this paper: PD, potential difference.

water is effected primarily by passive forces. According to this hypothesis, reabsorption of the glomerular ultrafiltrate occurs in two phases. In the first 15-20% of the proximal tubule there is almost complete reabsorption of glucose (9) and amino acids (10) by active transport processes and significant bicarbonate removal as a result of active H<sup>+</sup> secretion (11). Associated with the active reabsorption of these solutes is iso-osmotic reabsorption of water and attendant rise in chloride concentration. The concentration gradients for glucose, amino acids, bicarbonate, and chloride, generated and maintained by active processes, could give rise to two important forces for the passive reabsorption of NaCl in more distal regions of the proximal convoluted tubule where actively reabsorbable solutes are lacking. First, if the reflection coefficient for NaCl is less than those for glucose, amino acid, and NaHCO<sub>3</sub>, an effective osmotic pressure difference exists across the tubule wall. This difference would promote the flow of a NaCl solution from lumen to blood. Second, the chloride concentration gradient between tubular fluid and plasma generates a positive PD which would drive sodium out of the lumen. Data supporting the passive model of NaCl and water reabsorption has been the finding that the transtubular PD is negative only in the earliest parts of the proximal tubule (8, 12, 13) and is dependent on the presence of glucose and amino acids (8, 14), while in the latter parts of the proximal tubule the PD is positive and is due to the diffusion of chloride down its concentration gradient (8). A similar hypothesis has recently been proposed by Frömter et al. (15).

The present experiments were designed to evaluate the role of the chloride concentration gradient in the mechanism of proximal tubular reabsorption. If the actively reabsorbable components of the glomerular ultrafiltrate (glucose, amino acids, and bicarbonate) were replaced by nonreabsorbable solutes, transepithelial chloride gradients could not be generated. Under these conditions the passive model would predict a proximal reabsorptive rate near zero even though tubular fluid sodium and chloride concentrations were equal to those in plasma. In contrast, if proximal reabsorption were driven primarily by active transport of sodium, either as the ion or as neutral NaCl, reabsorption would be expected to continue in the absence of a chloride gradient.

The effect of the chloride gradient on reabsorption was examined by using perfusion and collection techniques in individual proximal tubules of the rat kidney. Tubules were perfused with a solution simulating an ultrafiltrate of plasma, a solution resembling late proximal tubular fluid (glucose, amino acid, acetatefree, low bicarbonate, and high chloride) and solutions in which glucose and amino acids were replaced by

raffinose and bicarbonate was partially replaced by poorly reabsorbable anions (cyclamate, sulfate, and methylsulfate). The solutions resembling ultrafiltrate and late proximal fluid were both reabsorbed at rates comparable to those reported for free-flow micropuncture studies, 2.17 and 2.10 nl mm<sup>-1</sup> min<sup>-1</sup>, respectively, while solutions substituted with raffinose and poorly reabsorbed anions were not significantly reabsorbed despite the fact that their sodium and chloride concentrations were similar to those in plasma. These results are best explained in terms of the passive model of NaCl and water reabsorption.

## **METHODS**

Studies were performed on male Sprague-Dawley rats weighing 120-320 g. The animals were anesthetized by an intraperitoneal injection of Inactin (Promonta, Hamburg, Germany) 120 mg/kg body weight and prepared for micropuncture. Body temperature was maintained at 38°C by adjusting the heating of the micropuncture table. The left jugular vein was cannulated for infusion of 1.2 ml/h Ringer's solution and injection of Lissamine green (0.03 ml, 10% in saline). Only animals with proximal transit times less than 12 s were used. Blood pressure was measured continuously from the carotid artery by using a Statham transducer (model P23Db, Statham Instruments Div., Gould Inc., Oxnard, Calif.) and a Sanborn recorder (Sanborn Co., Cambridge, Mass.). The left kidney was exposed by flank incision and placed into a Lucite holder. The surface of the kidney was superfused with mineral oil maintained at 38°C and preequilibrated with water and a gas mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Before micropuncture an arterial blood sample was taken (150  $\mu$ l) for estimation of pH, PCO<sub>2</sub>, [HCO<sub>3</sub><sup>-</sup>] (blood gas analyzer, model 165, Corning Scientific Instruments, Medfield, Mass.) and plasma osmolality (vaporpressure osmometer, model 5100, Wescor, Inc., Logan, Utah).

Proximal convolutions of surface nephrons were punctured (pipets 8-10  $\mu$ m OD) and perfused at a constant rate of 14.3±0.1 nl/min (n = 44) with a thermally insulated Hampel microperfusion pump (16) (Wolfgant Hampel, Berlin, W. Germany). The tubule was blocked with castor oil proximal to the perfusion site. Timed total collections of perfused fluid were made at a more distal puncture site by maintaining a small droplet of injected mineral oil in a constant position.

The constancy of the pump perfusion rate was examined in two ways. The pump rate was determined each day in vitro by pumping perfusion fluid into a counting vial for a precisely timed interval. To determine whether intrapipet pressure influenced the perfusion rate pipets with tips ranging from 2 to 15  $\mu$ m were used. The pump rate was measured with these pipets before and after breaking the pipet tips (tip size 50-100  $\mu$ m). Variation in pump rate between the different sized pipets and before and after breaking the tips was less than 1.5%. The pump rate, therefore, was pressure indpendent and unlikely to be influenced by the small pressures within the tubule or changes in tip resistance that might occur during puncture. To test this point further and to ascertain whether there might be leaks around the pipet tip during in situ microperfusion inulin recoveries in the oil-blocked microperfused tubules were determined. The inulin recoveries were 99  $\pm 2.7\%$  when either perfusion solution A or D (see below) was

TABLE IComposition of Perfusion Solutions

Solution	A	В	С	$D_1$	$D_2$	$D_3$
Cations,						
meq/liter						
Na <sup>+</sup>	145.5	145.5	154.1	145.5	145.5	145.5
K+	5	5	5	5	5	5
Mg <sup>++</sup>	1	1	1	1	1	1
Ca <sup>++</sup>	1.8	1.8	1.8	1.8	1.8	1.8
Anions,						
meq/liter						
Cl-	113.6	113.6	149.7	113.6	113.6	113.6
HCO <sub>3</sub> -	25	5	5	5	5	5
Acetate	7.5	27.5	_			
Phosphate	4	4	4	4	4	4
Cyclamate		_	_	27.5	_	_
Sulfate	1	1	1	1	13.75	1
Methyl-						
sulfate	_	_	_	—	—	27.5
Nonelectrolytes,						
mol/liter						
Glucose	5	5		—	_	
Alanine	5	5	—	_	_	_
Urea	5	5	5	5	5	5
Raffinose	_	_	—	10	23.75	10

used. Since the pump rate is not sensitive to pressure and is not associated with significant leaks at the puncture site the in vitro pump calibration is an accurate measure of the *in situ* perfusion rate. For this reason all subsequent calculations of reabsorptive rate utilized the in vitro pump rate and inulin concentrations determined on aliquots of the collected fluid.

Solutions used for the tubular perfusion are shown in Table I. Solution A ("complete" solution) simulates an ultrafiltrate of rat plasma. Solution B ("acetate" solution) is identical to solution A except that 20 meg/liter bicarbonate was replaced by acetate. This solution is expected to test the specific role of bicarbonate in fluid reabsorption. Solution C ("high chloride" solution) resembles late proximal tubular fluid in that all glucose, alanine, and acetate and 20 meg/liter bicarbonate were replaced with NaCl. In solutions  $D_1$  ("cyclamate" solution),  $D_2$  ("sulfate" solution), and  $D_3$  ("methylsulfate" solution) 20 meq/liter bicarbonate and all the acetate were replaced by cyclamate, sulfate, and methylsulfate, respectively, while glucose and alanine were replaced by raffinose. Additional raffinose was added to solution  $D_2$  to maintain isotonicity. Solutions B, C,  $D_1$ ,  $D_2$ , and  $D_3$  contained 5 meg/liter bicarbonate, a value close to the equilibrium concentration for bicarbonate in the proximal convoluted tubule (17). In addition, all solutions contained 11.4 µCi/ml [14C]inulin (New England Nuclear, Boston, Mass.) and 0.1% FD & C green dye no. 3. For all perfusion solutions, the salts were weighed to give the concentrations in Table I when diluted to volume; however, they were not diluted completely to volume and thus constituted a slightly hypertonic stock solution. To prevent water flow secondary to an osmotic gradient, the osmolality of the perfusion solution was adjusted to within ±3 mosmol/Kg H<sub>2</sub>O of the plasma osmolality of each experimental animal by addition of distilled water.

Aliquots of collected samples were measured in calibrated constant bore glass capillaries. Samples and standards were transferred into minicounting vials with 1.5 ml 0.1 N acetic acid and 6.5 ml of Aquasol (New England Nuclear) and

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counted in a liquid scintillation counter (Nuclear Chicago, Des Plaines, Ill., Mark II). In separate collections, where perfusion length was not measured, the chloride concentration of tubular fluid was determined by using the microcoulometric technique described by Ramsay et al. (18). At the end of each experiment arterial blood was collected for measurement of plasma chloride concentration (Buchler-Cotlove chloridometer, Buchler Instruments Div., Searle Analytic, Inc., Fort Lee, N. J.).

The length of the perfused segment was determined as follows. The perfused tubule was injected with latex injection compound (General Biological Supply House, Inc., Chicago, Ill.). The kidney was incubated overnight at  $4^{\circ}$ C in HCl (concentrated HCl diluted 1:1 with H<sub>2</sub>O). Afterwards a small wedge of macerated kidney containing the injected tubule was placed in a Petri dish containing tap H<sub>2</sub>O. The latex cast was dissected free, placed on a microscope slide, and photographed at 100 magnification with a Polaroid camera (Polaroid Corp., Cambridge, Mass.). The length of the cast was measured from the photograph. The system was calibrated by photographing a slide micrometer in similar fashion.

In a separate group of animals transepithelial electrical PD were measured by a technique similar to that described by Barratt et al. (8). Glass pipets with tip diameters between 3 and 5  $\mu$ m OD and tip resistances around 1 M ohm were filled with 3 M KCl colored with 0.5% Lissamine green. The electrodes were inserted into a Teflon chamber filled with 3 M KCl connected to a calomel electrode. Hydraulic pressure in the chamber could be adjusted by changing the fluid level in a side arm connection. On the reference side of the circuit a calomel electrode made contact to a Ringer's bath into which the rat's cut tail was dipped. The calomel electrodes were connected to an electrometer (Keithley Instruments Inc., Cleveland, Ohio model 602), the output of which was recorded (Honeywell Inc., Minneapolis, Minn. Electronic 194). A zero reading was obtained with the tip of the exploring electrode positioned in the thin film of interstitial fluid on the kidney surface. Before reading, the pressure in the chamber holding the electrode was adjusted so that a minute stream of green fluid was ejected to clear the tip; the pressure was then readjusted to stop flow at which time the zero reading was taken. The tubule was then punctured downstream from the perfusion pipet and intraluminal location was confirmed by observation of clear tubular fluid entering the electrode tip. Pressure in the chamber was raised sufficiently to stop entry of luminal fluid into the electrode and then the transtubular PD was read. Immediately after withdrawal the zero reading was rechecked after expelling any residual tubular fluid from the tip. Measurements of PD were discarded if they were not stable within 0.3 mV for at least 1 min.

*Calculations*. The reabsorptive rate,  $J_V$  (nl mm<sup>-1</sup> min<sup>-1</sup>), is given by the equation:

$$J_{v} = \frac{V_{o}}{X} \left( 1 - \frac{P}{C} \right), \tag{1}$$

where  $V_o$  is the perfusion rate (nl/min), X is the length of perfused segment (mm), P is the inulin concentration in perfusate (cpm/ml), and C is the inulin concentration in collected fluid (cpm/ml). All results are expressed as mean ± SEM. Significance was tested by null hypothesis by using the *t*-distribution (19).

The diffusive flux of an ion  $(J_i)$  was calculated by the following equation:

 $J_i(\text{peq mm}^{-1} \text{ min}^{-1}) = P_i(C_L - C_p) + P_i(\bar{C}_i Z_i F R^{-1} T^{-1} E_T), (2)$ 

where  $P_i$  is the permeability of ion *i* (nl mm<sup>-1</sup> min<sup>-1</sup>),  $C_L$  is



FIGURE 1 Relation between reabsorptive rate and length of perfused segment in tubules perfused with "Complete" Ultrafiltrate-like solution (solution A) ( $\odot$ ), "Acetate" solution (solution B) ( $\triangle$ ), and "High Chloride" solution (solution C) ( $\bigcirc$ ). The calculated regression equation for these data is y = -0.39 + 2.49x, with an r value of 0.87.

the luminal concentration of ion *i* (peq nl<sup>-1</sup>),  $C_p$  is the plasma water concentration of ion *i* (peq nl<sup>-1</sup>),  $\bar{C}_i$  is the mean concentration of ion  $i\left(\frac{C_L + C_p}{2}\right)$ ,  $E_T$  is the transtubular PD (mV), and *F*, *R*, *T*, and *Z* have their usual meaning.

## RESULTS

The rats had a mean plasma osmolality of  $299.3\pm0.8$  (n = 44), arterial blood pH of  $7.39\pm0.01$ , PCO<sub>2</sub> of 42.3  $\pm0.7$  mm Hg, and bicarbonate of 24.6 meq/liter. The measured plasma chloride concentration was 103  $\pm0.6$  meq/liter which, when corrected for a plasma water content of 94% and a Donnan factor of 1.05,

gives an ultrafiltrate chloride concentration of 115.5 meq/liter.

The absolute reabsorptive rates in nanoliter per minute obtained with solutions A, B, and C are plotted against perfused tubule length in Fig. 1. There was a highly significant linear correlation with an intercept close to zero and a slope of 2.49 nl mm<sup>-1</sup> min<sup>-1</sup>.

In tubules perfused with a complete solution (solution A) the reabsorptive rate was 2.17 nl mm<sup>-1</sup> min<sup>-1</sup> (Table II). In our laboratory weight-matched Sprague-Dawley rats have absolute proximal reabsorptive rates of 15–20 nl/min. In this species the length of the proximal convoluted tubule is 5.5-6.5 mm, accordingly the free-flow reabsorptive rate is 2.5-3.0 nl mm<sup>-1</sup> min<sup>-1</sup>. Thus, the reabsorptive rates obtained with perfusion solution A are only slightly lower than free-flow values. In 16 additional collections the measured chloride concentration was 131.5  $\pm 3.1$  meq/liter. The rise in chloride concentration indicates preferential reabsorptive mechanism qualitatively similar to free-flow conditions.

Removal of bicarbonate and replacement with acetate had only a slight effect on the rate of proximal reabsorption. In tubules perfused with the "acetate" solution (solution *B*) the reabsorptive rate was 2.74 nl mm<sup>-1</sup> min<sup>-1</sup> (Table II). Although this rate is higher than that obtained with the complete solution, it is apparent from Fig. 1 that the reabsorptive rates with *B* solution overlap completely with those obtained with *A* solution. In eight separate samples the concentration of chloride was  $135.8 \pm 3.5$  meq/liter, indicating preferential reabsorptive rate and the rise in chloride concentration suggest that acetate adequately replaces bicarbonate in proximal fluid reabsorption.

In tubules perfused with "high chloride" solution simulating late proximal fluid (solution C) the reab-

 TABLE II

 The Mean Reabsorptive Rates in Perfused Rat Superficial

 Proximal Convoluted Tubules

Solution	Number of tubules	Perfused length	Reabsorptive rate	Tubular fluid chloride
		mm	nl min <sup>-1</sup> mm <sup>-1</sup>	meqlliter
Α	18	1.72±0.9(SD)	2.17±0.20(SEM)	$131.5 \pm 3.1(n = 16)(SEM)$
В	12	$2.04 \pm 1.0$	$2.74 \pm 0.18$	$135.8 \pm 3.5 (n = 8)$
С	23	$1.73 \pm 0.50$	$2.10 \pm 0.12$	
$D_1$	22	$1.96 \pm 0.96$	*0.16±0.09	$110.8 \pm 1.2(n = 19)$
$D_2$	16	$2.05 \pm 0.78$	$0.29 \pm 0.07$	
$D_3$	12	$1.73 \pm 0.79$	$*0.14 \pm 0.11$	

\* Not statistically different from zero.

sorptive rate was 2.10 nl mm<sup>-1</sup> min<sup>-1</sup> (Table II). This value is not different from the rate obtained with the complete solution (Fig. 1). In tubules perfused with solution C, transtubular PD were measured to define the electrical driving force for ion movement. The observed transtubular PD was  $+1.5\pm0.2$  mV (lumen positive), which agrees well with the value of  $+1.6\pm0.2$  mV obtained in randomly punctured late proximal tubules.

Microperfusion studies with the D solutions specifically examined the importance of the chloride concentration gradient to proximal salt and water reabsorption. Replacement of glucose and alanine with raffinose and acetate and bicarbonate with poorly reabsorbable anions had a profound effect on proximal reabsorption. The reabsorptive rates were 0.16 nl  $mm^{-1}$  min<sup>-1</sup> with "cyclamate" solution (solution  $D_1$ , Table II), 0.29 nl mm<sup>-1</sup> min<sup>-1</sup> with "sulfate" solution (solution  $D_2$ , Table II), and 0.14 nl mm<sup>-1</sup> min<sup>-1</sup> with "methylsulfate" solution (solution  $D_3$ , Table II). Only the reabsorptive rate with "sulfate" solution was significantly different from zero. In tubules perfused with "cyclamate" solution (solution  $D_1$ ), the measured concentration of chloride in collected fluid was 110.8  $\pm 1.2$  meg/liter (n = 19) and the transtubular PD was  $-0.98 \pm 0.2$  mV (n = 15). From the Nernst equation and a plasma ultrafiltrate chloride concentration of 115 meg/liter, the calculated equilibrium concentration of chloride was 110.9 meg/liter. Thus, under these conditions chloride was close to electrochemical equilibrium across the tubular epithelium. Since the perfusion fluid chloride concentration of 113.6 meg/liter was slightly above the equilibrium concentration, a few experiments were performed with a cyclamate solution in which the chloride concentration was reduced to 110.6 meg/liter and the cyclamate concentration proportionately increased to 30.5 meg/liter. The reabsorptive rate obtained with this solution was zero  $(-0.02\pm0.06 \ (n = 8) \ nl \ mm^{-1} \ min^{-1})$ .

It is possible, therefore, that the small apparent reabsorptive rates observed with solutions  $D_1$ ,  $D_2$ , and  $D_3$  were due to a small electrochemical gradient for chloride. In addition, if the substituent anions were not completely impermeant, passive anion movement would result in apparent reabsorption.

## DISCUSSION

Fluid was rapidly reabsorbed from superficial proximal convoluted tubules of the rat kidney when perfused with "complete", "acetate", or "highchloride" solutions. In contrast, when tubules were perfused with solutions which had the reabsorbable organic solutes and bicarbonate replaced by nonpermeant counterparts, no significant volume reabsorption

occurred despite the fact that these solutions contained sodium and chloride concentrations equal to those in plasma. These data suggest that proximal reabsorption of volume depends on either the presence of actively reabsorbable solutes (glucose, amino acids, acetate, and bicarbonate) or a high chloride concentration in tubular fluid. Furthermore, they argue against the active transport of sodium as the principal driving force for reabsorption of fluid free of the actively reabsorbable constituents.

Experiments with the "complete" or "acetate" perfusion solutions do not provide specific information regarding the mechanism of reabsorption of buffered salt solutions containing organic solutes; however, when considered with other data, certain points are suggested. When proximal tubules were perfused with a fluid simulating an ultrafiltrate of plasma (solution A), the reabsorptive rate was  $2.17 \text{ nl} \text{ mm}^{-1} \text{ min}^{-1}$ (Fig. 1), a value similar to that obtained in freeflow micropuncture studies. Reabsorption was found not to be specifically dependent on the presence of bicarbonate since replacement of all but 5 meg/liter with acetate resulted in a similar reabsorptive rate, 2.7 nl mm<sup>-1</sup> min<sup>-1</sup>. The measured chloride concentration of the collected fluid was significantly higher than the injected concentration after perfusion with both the "complete" and "acetate" solutions (see Results). Preferential reabsorption of bicarbonate and acetate, as well as glucose and alanine, would best account for the observed rise in tubular fluid chloride concentration in these experiments. On the basis of other studies (20-22) the preferential reabsorption of both bicarbonate and acetate appears to involve H<sup>+</sup> secretion and subsequent nonionic diffusion of the acid form of the buffer ion.

From these data, reabsorption of the "complete" solutions appears to be qualitatively similar to reabsorption of glomerular ultrafiltrate. In the earliest portion of the proximal convoluted tubule there is also rapid removal of glucose, amino acids, and bicarbonate and a commensurate rise in chloride concentration (9-11). In addition, there is a negative PD (8, 12, 13) which is dependent on the presence of glucose (14), amino acids (14), and sodium (23) and is ouabain inhibitable (14). These observations are consistent with the view that the initial phase of reabsorption is primarily dependent on active sodium transport. According to this hypothesis, the movement of glucose and amino acids from lumen into cell and the movement of H<sup>+</sup> from cells into lumen are all mediated by Na<sup>+</sup>-glucose, Na<sup>+</sup>-amino acid, and Na<sup>+</sup>-H<sup>+</sup> co-transport processes located in the luminal brush border membranes. The energy for these transport processes would be provided by the sodium concentration gradient between lumen and cell which is maintained by the continued active outward transport of sodium across the peritubular membrane.

Experiments in which tubules were perfused with the "high chloride" solution indicate that the combined presence of glucose, alanine, acetate and bicarbonate per se is not an absolute requirement for proximal reabsorption since their removal and replacement by NaCl resulted in a reabsorptive rate of 2.10 nl mm<sup>-1</sup> min<sup>-1</sup>. However, the actively reabsorbable solutes (glucose, amino acids, acetate, and bicarbonate) do appear to play an important permissive role in the late proximal phase of reabsorption. As will be discussed below, it is their removal that generates the chloride concentration gradient which is essential for further reabsorption of NaCl and volume.

The importance of the chloride concentration gradient in late proximal reabsorption was specifically examined in experiments with anion substitutions. In these experiments glucose and alanine were replaced by iso-osmolar amounts of raffinose, while the acetate and all but 5 meq/liter bicarbonate were replaced by equivalent amounts of poorly reabsorbable anions (cyclamate, sulfate, and methylsulfate). In this manner the concentrations of sodium and chloride in the perfusion solutions were maintained equal to their plasma concentrations. Proximal reabsorption was virtually abolished by these substitutions (Table II).

Several spurious causes for the cessation of reabsorption in these experiments must be considered. First, it seems unlikely to be due to a specific toxic effect of one of the substituent anions; similar results were obtained for all three anions. Second, it does not appear to be due to the specific absence of glucose, amino acids, acetate or bicarbonate; their removal and replacement with NaCl had little, if any, effect on the rate of reabsorption. Third, it does not appear to be due to large electrochemical gradients; the concentration of sodium and chloride in the perfusates (145.5 meq/liter and 110.8 meq/liter, respectively) were similar to plasma concentrations and the measured transtubular PD was -0.98 mV.

The failure to observe significant reabsorption in tubules perfused with the anion substituted solutions  $(D_1, D_2, \text{ and } D_3)$  despite the absence of significant concentration gradients for sodium and chloride argues against the view that late proximal reabsorption of NaCl is mediated entirely by either active sodium transport followed by electrically coupled chloride reabsorption or active neutral NaCl transport. These results, however, do suggest that in the absence of the actively reabsorbable solutes (glucose, amino acids, acetate, and bicarbonate) proximal reabsorption is dependent on the existence of a chloride concentration gradient between lumen and peritubular fluid, and thus provide further support for the passive model of NaCl and water reabsorption proposed by Rector and co-workers, Kokko et al., and Barratt et al. (6-8).

Recently, Green and Giebisch (24) and Cardinal et al. (5) have challenged the view that chloride concentration gradients play an important role in proximal reabsorption. Interpretations of these data (24, 5), however, rely on the properties of acetate as a "non stimulating" substitute for bicarbonate. Our data (Table II) indicate that acetate can qualitatively replace bicarbonate. In contrast, Green and Giebisch found that, during in situ perfusion of rat proximal tubules and peritubular capillaries, complete removal of bicarbonate and replacement with acetate reduced reabsorption from 3.3 to 1.3 nl mm<sup>-1</sup> min<sup>-1</sup>. If, in fact, the only difference between the present study and Green and Giebisch is peritubular capillary perfusion, then the failure of acetate to sustain normal reabsorptive rates might be due to the total removal of bicarbonate from both luminal and peritubular perfusion solutions. Even in their studies, however, acetate does not appear to be a completely nonreabsorbable anion. The discrepancy between sodium (198 peq<sup>1</sup> min<sup>-1</sup> mm<sup>-1</sup>) and chloride (110 peq min<sup>-1</sup> mm<sup>-1</sup>) reabsorption [Table 3 in reference 24] suggests significant acetate reabsorption. This raises the possibility that the continued reabsorption of 0.55 nl mm<sup>-1</sup> min<sup>-1</sup> observed by Green and Giebisch in the presence of an adverse chloride gradient [(C1) peritubular > (C1)lumen] and acetate, but in the absence of glucose and bicarbonate, was due to an overriding effect of sodium acetate reabsorption.

In the study of Cardinal et al. (5) superficial proximal convoluted tubules of the rabbit, when perfused with a solution free of glucose, amino acids, and bicarbonate but containing 12 meg/liter acetate and a high chloride concentration and bathed in rabbit serum, had a transtubular PD of +1.1 mV and a reabsorptive rate of 0.81 nl mm<sup>-1</sup> min<sup>-1</sup>. When the chloride concentration gradient was obliterated by replacing the bicarbonate in the bath with chloride, the PD fell to zero but the reabsorptive rate was unchanged (0.83 nl mm<sup>-1</sup> min<sup>-1</sup>). The authors concluded that proximal NaCl reabsorption is not dependent on electrochemical gradients for chloride. However, the authors did not measure the chloride concentration in the collected fluid in these experiments; therefore, it is not certain how much, if any, chloride was reabsorbed. If acetate is reabsorbed, as our data suggest, then the preferential reabsorption of 12 meg/liter sodium acetate by a 1.4 mm segment of tubule perfused at a rate of 12 nl min<sup>-1</sup> (5) would result in a volume reabsorption of 0.7 nl mm<sup>-1</sup> min<sup>-1</sup>. In the presence of acetate, the findings of normal reabsorptive rates despite the absence of a chloride gradient do not constitute unequivocal evidence against the passive model.

The failure for significant reabsorption to occur in the absence of glucose, amino acids, acetate, bicarbonate, and chloride gradients does not exclude some component of active sodium transport. The present studies in which cyclamate was the substituent anion permit a quantitative analysis of the electrochemical gradients for sodium and chloride. The PD in these experiments was -0.98 mV, the chloride concentration in the collected fluid was 110 meg/liter, and the calculated concentration of interstitial chloride was 115 meq/liter. From these data chloride was calculated to be in diffusion equilibrium across the tubular epithelium. Thus, there does not appear to be any active secretory or reabsorptive chloride transport. In contrast to chloride, sodium was probably not in electrochemical equilibrium. The observed PD of -0.98 mV provides a significant driving force for the passive diffusion of sodium into the lumen. If one assumes no significant concentration gradient for sodium, the passive sodium influx calculated from the sodium permeability of 9.3 nl mm<sup>-1</sup> min<sup>-1</sup> (24) and the PD of -0.98 mV is 50 peg mm<sup>-1</sup> min<sup>-1</sup>. If net sodium transport under these conditions were, in fact, zero, the passive inward movement of sodium into the tubule must have been balanced by the active outward movement of 50 peq mm<sup>-1</sup> min<sup>-1</sup> of sodium. If, on the other hand, net reabsorption under these conditions were 0.2 nl mm<sup>-1</sup> min<sup>-1</sup> (excluding any possible effect of solvent drag), there would be an additional 30 peq mm<sup>-1</sup> min<sup>-1</sup> of active sodium transport for a total of 80 peg  $mm^{-1}$  min<sup>-1</sup>. The exact mechanism responsible for this small component of apparent active sodium transport is not delineated by the present studies; however, we have previously suggested (8) that it is mediated by a sodiumhydrogen exchange process which is driven by the inward passive diffusion of bicarbonate.

It is of interest to compare the active sodium transport rate calculated from tubular perfusion with the cyclamate solution to the rate estimated in other conditions. Frömter et al. (15) found that when proximal tubules of the rat were perfused with "steadystate" solutions (i.e., no net reabsorption of sodium and water) containing high concentrations of raffinose, the PD was +1.9 mV, tubular fluid chloride was close to equilibrium with that in peritubular fluid, while the tubular fluid sodium concentration was maintained 20 meg/liter less than that in the peritubular perfusion solution. In the steady-state the active outward sodium transport rate is equal to the calculated passive inward diffusion rate of 90 peg mm<sup>-1</sup> min<sup>-1</sup>. In the present studies tubules perfused with "high chloride" solution reabsorbed fluid at a rate of 2.10

nl mm<sup>-1</sup> min<sup>-1</sup>. The perfusion solution sodium concentration was 154.5 peq/nl, thus the observed rate of net sodium reabsorption was 323 peq mm<sup>-1</sup> min<sup>-1,2</sup> Although the rate of active sodium transport calculated from our cyclamate studies (50–80 peq mm<sup>-1</sup> min<sup>-1</sup>) was similar to that calculated from the studies of Frömter et al. (15) (90 peq mm<sup>-1</sup> min<sup>-1</sup>), it is clear that these estimates of active transport are much smaller than the observed rate of net sodium reabsorption in the tubules perfused with the "high chloride" solution.

The present studies suggest that in tubules perfused with fluid simulating late proximal tubular fluid only 20-30% of total net sodium reabsorption can be attributed to active transport, and that the remainder is mediated by passive processes which are dependent on the chloride concentration gradient between tubular fluid and plasma. The chloride concentration gradient could give rise to two separate passive sodium transport processes: diffusion and convection. First, the diffusion of chloride down its concentration gradient would generate a positive PD which would cause net diffusion of sodium out of the lumen. Second, if the reflection coefficient for sodium and chloride were less than for glucose, amino acids, acetate, and bicarbonate. there would be an effective osmotic force across the tubule which would result in reabsorption of volume  $(J_{v})$  containing sodium and chloride in concentrations dependent on their reflection coefficients and luminal concentrations.

In the proximal tubule the reabsorption of water in amounts iso-osmotic to the reabsorbed NaCl has previously been attributed to local osmotic gradients in the lateral intercellular spaces generated by active sodium transport. Since active sodium transport constitutes only a small fraction of the total sodium transport in regions of the proximal tubule where actively reabsorbed solutes are lacking, this mechanism is probably not applicable. An alternative explanation for late proximal reabsorption is that the iso-osmotic reabsorption of water is a consequence of the glucose, amino acid, acetate, bicarbonate, and chloride transepithelial concentration gradients and differing membrane reflection coefficients for these solutes. If one assumes a reflection coefficient of 1.0 for glucose, amino acids, acetate, and bicarbonate and a reflection coefficient of 0.6 for sodium and chloride, the calculated effective osmotic pressure across the tubular wall is approximately 25 mosmol/Kg H<sub>2</sub>O. To achieve a reabsorptive rate of 2.3 nl mm<sup>-1</sup> min<sup>-1</sup>, the hydraulic conductivity  $(L_{\nu})$  would have to be  $8 \cdot 10^{-4}$  nl cm<sup>-1</sup>

<sup>&</sup>lt;sup>2</sup> Since the high chloride solution contains no actively reabsorbable solutes and since proximal reabsorption is iso-osmotic, it is assumed that sodium and chloride concentrations in the perfusion solution do not change during reabsorption.

s<sup>-1</sup> mm Hg<sup>-1</sup>, which is approximately half of the observed value,  $17.2. \cdot 10^{-4}$  nl cm<sup>-1</sup> sec<sup>-1</sup> mm Hg<sup>-1</sup> (25–27). An explanation for this difference may be that the reflection coefficients for glucose, amino acids, acetate, and bicarbonate are less than 1.0, and therefore that the true effective osmotic pressure across the tubule is less than the estimated value of 25 mosmol/Kg H<sub>2</sub>O.

An essential factor in the operation of this passive model is differential solute permeability of the tubule epithelium: the reflection coefficient for NaCl must be lower than that for NaHCO<sub>3</sub>, glucose, acetate, and amino acids. If the permeabilities and reflection coefficients are the same, the observed concentration gradients for the solutes will not constitute effective driving forces. Previous studies (3, 25) indicate that superficial convoluted tubules have the required differential permeability. Juxtamedullary convoluted proximal tubules, on the other hand, have relative sodium and chloride permeabilities which are different from those of superficial convoluted tubules (28). Whether the juxtamedullary nephrons have the differential chloride and bicarbonate permeabilities required for operation of the passive model, however, has not been examined.

In summary, the results of the present studies are consistent with the following model of proximal tubular reabsorption. In the earliest segments of the proximal tubule glucose, amino acids, organic acids, and bicarbonate are almost completely reabsorbed by active transport processes. This accounts for reabsorption of approximately 25% of the glomerular filtrate. In addition, these active reabsorptive processes establish the transepithelial concentration gradients for the organic solutes, bicarbonate, and chloride which play a key role in salt and water reabsorption in the latter parts of the proximal tubule where approximately 35% of the glomerular filtrate is reabsorbed. Of this latter component of reabsorbed fluid approximately one fourth is due to active sodium reabsorption and three fourths is due to the passive processes of diffusion and convection, dependent on the organic and anionic concentration gradients. In the face of continuous passive influx of the solutes, the maintenance of these gradients requires the continuous operation of metabolically driven active transport processes. In assessing total proximal reabsorption half appears to be due to active transport, while the remaining half appears to be passive.

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