A Component of Fluid Absorption Linked to Passive Ion Flows in the Superficial Pars Recta

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ABSTRACT We studied salt and water absorption in isolated rabbit superficial proximal straight tubules perfused and bathed with solutions providing oppositely directed transepithelial anion gradients similar to those which might obtain in vivo. The perfusing solution contained 138.6 mM Cl^- and 3.8 mM HCO_3^- (pH 6.6) while the bathing solution contained 113.6 mM Cl^- and 25 mM HCO_3^- (pH 7.4); the system was bubbled with 95% O_2-5% CO_2. At 37°C, net volume absorption (J_v nl min^{-1} mm^{-1}) was 0.32 ± 0.03 (SEM); V_e, the transepithelial voltage (millivolts; lumen to bath), was +3.1 ± 0.2. At 21°C, V_e rose to +3.7 ± 0.1 and J_v fell to 0.13 ± 0.01 (significantly different from zero at $P < 0.001$); in the presence of 10^{-4} M ouabain at 37°C, V_e rose to +3.8 ± 0.1 and J_v fell to 0.16 ± 0.01 ($P < 0.001$ with respect to zero). In paired experiments, the ouabain- and temperature-insensitive moieties of J_v and V_e became zero when transepithelial anion concentration gradients were abolished. Titrametric determinations net chloride flux at 21°C or at 37°C with 10^{-4} M ouabain showed that chloride was the sole anion in an isotonic absorbate. And, combined electrical and tracer flux data indicated that the tubular epithelium was approximately 18 times more permeable to Cl^- than to HCO_3^-. We interpret these results to indicate that, in these tubules, NaCl absorption depends in part on transepithelial anion concentration gradients similar to those generated in vivo and in vitro by active Na^+ absorption associated with absorption of anions other than chloride. A quantitative analysis of passive solute and solvent flows in lateral intercellular spaces indicated that fluid absorption occurred across junctional complexes when the osmolality of the lateral intercellular spaces was equal to or slightly less than that of the perfusing and bathing solutions; the driving force for volume flow under these conditions depended on the fact that σ_{HCO_3} exceeded σ_{Cl}.

INTRODUCTION

The modes of NaCl absorption by the mammalian proximal nephron are not wholly understood. Since the relevant transport processes may differ sub-

stantially in various functional or morphologic nephron sites, characteristics of salt and water absorption for a given nephron segment may be inferred only by explicit evaluation. This paper deals with salt and water transport in the pars recta, a nephron segment not ordinarily accessible for study with micropuncture techniques.

In isolated rabbit superficial cortical proximal straight tubules perfused and bathed with symmetrical HCO_3^- Krebs-Ringer solutions (HCO_3^- KR; Table I) or comparable buffers, the spontaneous transepithelial potential difference is approximately -1.3 mV, lumen negative (1–4). Isotonic volume absorption may be rationalized quantitatively by net lumen to bath Na^+ flux (1), and both Na^+ (1) and fluid absorption (1, 3) are inhibited completely either by cooling or by ouabain. It has been inferred, on the basis of these and other observations, that fluid absorption in the case of symmetrical perfusing and bathing solutions depends on active, or conservative, transport processes involving Na^+ (1, 3, 4).

It is evident that such arguments provide no direct information on the mechanism of NaCl absorption, and studies from this (1) and other laboratories (3, 4) on volume absorption from the isolated proximal straight tubule have not clarified the question. For example, differences between $^{36}Cl^-$ fluxes from lumen to bath and from bath to lumen in tubules perfused and bathed with HCO_3^- KR solutions are not sufficiently large to permit measurement of the net Cl^- flux, if any, accompanying Na^+ absorption (1). Moreover, the Cl^- content of tubular fluid rises appreciably during volume absorption, and, under these conditions, the transepithelial potential difference may be slightly lumen positive rather than lumen negative (1). Stated in another way, lumen to bath Cl^- gradients in superficial proximal straight tubules are attended by electrical forces favoring passive Na^+, rather than passive Cl^-, absorption. In this context, Rector et al. (5) first suggested that NaCl absorption from rat proximal tubular fluid might be driven by lumen to bath Cl^- gradients resulting from preferential active absorption of $NaHCO_3$. A number of subsequent observations have provided additional support for this view (6–17).

The present studies were designed to evaluate, both experimentally and analytically, the characteristics of fluid absorption in superficial proximal straight tubules perfused and bathed with solutions providing lumen to bath Cl^- gradients similar to those which might obtain in vivo. We interpret the results for these conditions to indicate that Cl^- absorption may depend largely on Cl^- concentration gradients similar to those generated in vivo (18–20) and in vitro (1, 14) by active Na^+ transport associated with absorption of anions other than Cl^-, and that a significant fraction of fluid absorption in these nephron segments may be referable to dissipative forces, more specifically, oppositely directed concentration gradients of relatively permeant and impermeant anions. A preliminary report of some of these observations has appeared previously (21).

METHODS

The techniques utilized for studying transport processes in proximal straight tubules isolated from superficial regions of rabbit renal cortex are quite similar to those developed originally by Burg et al. (2, 3, 22). Details of the methodology as utilized in our laboratory have been presented previously (1); unless otherwise indicated, these techniques were utilized without modification in the present experiments.

Stated briefly, 2.5–4.0-mm segments of proximal straight tubules were obtained by gentle teasing from outer regions of rabbit renal cortex, i.e., from nephrons arising from superficial cortical glomeruli. (Thus, the present experiments provide no information about transport events in proximal straight tubules arising in juxtamedullary regions, which are more permeable to Na^+ than to Cl^- [23] while the converse is true for superficial proximal straight tubules [1].) The tubules were sucked into holding and collecting pipets in a thermoregulated (±0.5°C) chamber and perfused at rates of 4–20 nl min^{-1} with a microsyringe pump in series with a perfusion pipet. The latter was advanced approximately 0.2 mm into the tubule lumen.

It is particularly relevant to indicate the degree of mechanical mixing in the bath, since it has been argued (16) that bulk phase unstirred layers may be significant in the isolated tubule preparation. As described previously (1, 24), when the bath was bubbled with an appropriate gas, oscillatory movements of the tubules occurred at a rate of 60–200/min and convective mixing of minute particles in the bath was evident as near as 2×10^{-4} cm from the tubules; under these conditions, tracer permeability coefficients having magnitudes of $10–15 \times 10^{-4}$ cm s^{-1} were not affected by 10-fold increments in the viscosities of the perfusate and bath (24). Since the permeability coefficients in question for the present studies (i.e., for Na^+ and Cl^-) are smaller in magnitude than 10^{-4} cm^{-1} s^{-1} (1), it seems probable that bulk phase unstirred layers were a negligible factor in the present experiments.

The constituents of the various perfusing and bathing solutions are listed in Table I. The Cl^- content and pH of fluid samples from late proximal convolutions are, respectively, higher and lower than in plasma (18–20), and the absorptive capacities of proximal convolutions for glucose (25), and possibly amino acids (14), are appreciably greater than in proximal straight tubules. Thus, the Cl^- KR perfusate, containing 138.6 mM Cl^-, 3.8 mM HCO_3^- at pH 6.6, and 13.3 mM urea but no glucose or alanine, was intended to resemble tubular fluid which might be expected to enter superficial straight segments in vivo. We note in this connection that the luminal Cl^- concentration seen by the rabbit pars recta in vivo is not known. In our earlier experiments, we observed that, when identical proximal straight tubules were perfused and bathed with symmetrical HCO_3^- KR solutions, the Cl^- concentration in collected fluid rose from 113.6 to 132.1 mM as a consequence of active Na^+ absorption associated with perferential absorption of anions other than Cl^- (1). In the present experiments, a higher Cl^- concentration was chosen for the Cl^- KR solutions to permit reproducible and reliable measurements of net fluid absorption which were significantly different from zero in the presence of ouabain or cooling.

Net fluid transport (J_v, nl min^{-1} mm^{-1}) i.e., the difference between perfusion and collection rates, was measured as described previously using exhaustively dialyzed inulin-methoxy-^3H as the volume marker (1). It should be noted that the sign conven-

TABLE I

COMPOSITION OF SOLUTIONS

	Perfusate				Bath			
	HCO_3^- KR	Cl^- KR	Na isethionate	NaCl	HCO_3^- KR	Cl^- KR	Na isethionate	NaCl
	mM	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*
NaCl	105	127.8	50	150	105	127.8	50	150
$NaHCO_3$	25	3.8	0	0	25	3.8	0	0
Na isethionate	0	0	98	0	0	0	98	0
Na acetate	10	10	0	0	10	10	0	0
Na_2HPO_4	3.2	1.6	3.2	3.2	3.2	1.6	3.2	3.2
NaH_2PO_4	0.8	2.4	0.8	0.8	0.8	2.4	0.8	0.8
KCl	5	5	0	0	5	5	0	0
$CaCl_2$	1.8	1.8	0	0	1.8	1.8	0	0
K_2SO_4	0	0	0	0	0	0	0	0
$CaSO_4$	0	0	0	0	0	0	0	0
$MgSO_4$	1	1	0	0	1	1	0	0
Glucose	8.3	0	0	0	8.3	8.3	0	0
L-Alanine	5	0	0	0	5	5	0	0
Urea	0	13.3	0	0	0	0	0	0
Albumin	0	0	0	0	6%	6%	6%	6%
pH	7.4	6.6	7.4	7.4	7.4	6.6	7.4	7.4

The various Krebs-Ringer (KR) solutions were bubbled with 95% O_2-5% CO_2; the Na isethionate and NaCl solutions were bubbled with 100% O_2. All solutions were adjusted to 300 mosmol $liter^{-1}$.

tion in the present paper differs from that used previously (1); in this paper, a positive sign denotes volume efflux from lumen to bath. The internal diameter of these tubules is 22.3×10^{-4} cm (1); thus, rates of fluid absorption may be converted from nl min^{-1} mm^{-1} to cm^3 s^{-1} cm^{-2} by the factor 2.38×10^{-5} cm min mm nl^{-1} s^{-1}. J_v measurements in the present experiments were in the range 0.1–0.45 nl min^{-1} mm^{-1} for tubules 2.5–4.0 mm in length; for isolated proximal convoluted tubules, J_v values in the range of 1.0 nl min^{-1} mm^{-1} have been reported for segments 0.5–1.5 mm in length (12, 3, 26). In other words, although J_v is smaller for straight tubules than for proximal convolutions, absolute differences between perfusion and collection rates, and hence the precision of J_v measurements, are comparable in the two segments.

The method for measuring transepithelial potential differences (V_e; millivolts, lumen with respect to bath) was identical to that used previously (1). Observed potential differences were corrected for liquid junction potentials with a form of the Henderson equation (27) modified in terms of ionic activities (28); the mobility of isethionate was obtained from Caldwell (29). Since the length constant for the pars recta is

approximately 10^{-2} cm (4), V_e measurements in the present paper provide direct information only about electrical events vicinal to the inner perfusion pipet.

Chloride concentrations in perfusing and collected fluids were measured with a microtitration method (30). Computer programs were written in Basic Language and were run on a remote terminal of an IBM model 370-155 computer system (International Business Machines Corp., Armonk, N. Y.) at the Division of Biophysical Sciences, University of Alabama in Birmingham, Birmingham, Ala. Other reagents, isotope counting techniques, and chemical determinations were as described previously.

Measurements in a given tubule were used to compute a mean value for that tubule; generally, there were four measurements per tubule for a given set of experimental conditions. The mean values for individual tubules were then used to calculate a mean value ± SEM for a number of tubules. The results were expressed in this manner. When control and experimental observations were made within the same tubule, P values for mean paired differences were computed from the Student t test by comparing differences to zero.

RESULTS

J_v and V_e : Effects of Ionic Gradients, Cooling, and Ouabain

In earlier studies (1), we observed that, in tubules perfused and bathed with HCO_3^- KR solutions at 37°C, J_v and V_e were, respectively, 0.46 ± 0.03 nl min^{-1} mm^{-1} and −1.13 ± 0.05 mV, lumen negative; both J_v and V_e remained nearly constant for approximately 210 min. Changes in J_v and V_e produced either by cooling to 21°C or by adding 10^{-4} M ouabain to the bath, reported previously (1) and summarized here as a frame of reference for succeeding data, are presented graphically in Fig. 1 A and B. These illustrations show clearly that, either in the presence of 10^{-4} M ouabain or at 21°C, J_v and V_e were reproducibly and consistently indistinguishable from zero.

The transport characteristics of proximal straight tubules were different when Cl^- KR replaced HCO_3^- KR in the perfusate but not in the bath. Table II indicates that, under these conditions, volume absorption at 37°C in the absence of ouabain was accompanied by approximately a 3.0-mV transepithelial potential difference, which was lumen positive rather than lumen negative, in accord with earlier observations and/or suggestions from this and other laboratories (1, 5, 14, 17). In the presence of 10^{-4} M ouabain or at 21°C, neither J_v nor V_e was zero. Rather, J_v fell to values in the range of 0.13–0.16 nl min^{-1} mm^{-1}, which constituted 40–48% of the total volume flow observed at 37° without ouabain, and V_e rose approximately 0.7 mV. Both J_v and V_e for these two conditions differed significantly from zero ($P < 0.001$). It is evident that, for these circumstances, J_v and V_e may be resolved into components which are either sensitive or insensitive to ouabain or cooling. We assume that the sensitive and insensitive moieties represent events coupled to, respectively, active and passive transport processes.

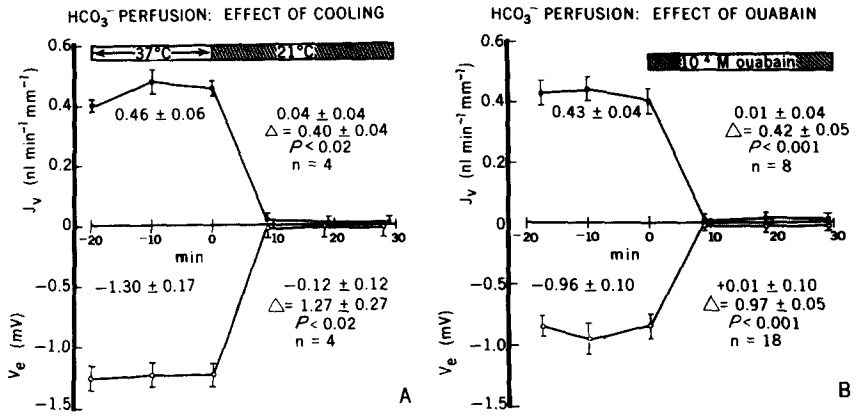

FIGURE 1. The effect of cooling to 21°C (A) or 10^{-4} M ouabain in the bath (B) on J_v and V_e when the perfusate and bath contain symmetrical HCO_3^- KR solutions (Table I). The mean values ± SEM and mean paired differences (Δ) are indicated in the figure. The P values for mean paired differences are indicated in the figure. The control values of J_v and V_e differed significantly from zero ($P < 0.001$ for all cases); the values of J_v and V_e, either with 10^{-4} M ouabain or at 21°C, were indistinguishable from zero ($P > 0.5$ for all cases). Adapted from (1).

A comparison of Fig. 1 A and B with Table II indicates that volume absorption associated with passive transport processes should be dependent on transepithelial anion gradients. In order to evaluate this possibility, two sets of experiments were carried out. In the first set (Fig. 2, Table III), J_v and V_e were measured, in a given tubule, under the following conditions: when perfusate and bath contained, respectively, Cl^- KR and HCO_3^- KR, either at 37 or at 21°C, and when perfusate and bath both contained Cl^- KR at 21°C. The order in which measurements were made was varied at random. In the second set of experiments, J_v and V_e were measured, in a given tubule, under the following conditions: when perfusate and bath contained, respectively, Cl^- KR and HCO_3^- KR at 37°C, when perfusate and bath again contained, respectively, Cl^- KR and HCO_3^- KR at 37°C, and 10^{-4} M ouabain was added to the bath, and when perfusate and bath each contained either Cl^- KR or HCO_3^- KR at 37°C with 10^{-4} M ouabain in the bath. Control observations without ouabain were always carried out first; subsequently, in the presence of ouabain, the order in which Cl^- gradients were established or abolished was varied at random.

In accord with Table II, the results in Fig. 2 and Table III show that, at 21°C, lumen to bath Cl^- gradients resulted in values of J_v and V_e, respectively, 0.15 nl min^{-1} mm^{-1} and +3.78 mV, which were consistently different from zero. However, when the Cl^- gradient was abolished, J_v and V_e at 21°C were both indistinguishable from zero, either as individual data for particular tubules (Fig. 2), or as mean values for all tubules tested (Table III). Similar

TABLE II

TRANSPORT CHARACTERISTICS OF THE PARS RECTA
Cl^- KR PERFUSATE AND HCO_3^- KR BATH

Ouabain	T	J_v	V_e
M	°C	*nl min⁻¹ mm⁻¹*	*mV*
0	37	0.32±0.02	+3.03±0.16
0	21	0.13±0.01	+3.72±0.10
Mean paired difference		0.19±0.03 $P < 0.001$ (n = 12)	0.66±0.11 $P < 0.001$ (n = 20)
0	37	0.33±0.04	+3.16±0.16
10^{-4}	37	0.16±0.01	+3.85±0.14
Mean paired difference		0.17±0.01 $P < 0.001$ (n = 11)	0.69±0.11 $P < 0.001$ (n = 19)

The perfusate and bath contained, respectively, Cl^- KR and HCO_3^- KR. A positive value for J_v denotes volume efflux from lumen to bath; V_e is expressed for the lumen with respect to the bath. All values of J_v and V_e shown in the table differed from zero at $P < 0.001$. The results are explained as described in Methods.

results obtained in the presence of 10^{-4} M ouabain. For lumen to bath Cl^- gradients, the ouabain-insensitive values of J_v and V_e (Fig. 3; Table IV) were comparable to those observed with larger numbers of tubules either in the presence of ouabain (Table II) or at 21 °C (Tables II, III: Fig. 2), and when Cl^- gradients were abolished, the ouabain-insensitive components of J_v and V_e were indistinguishable from zero, either in individual tubules (Fig. 3) or as mean values (Table IV). In short, the ouabain- and temperature-insensitive moieties of J_v and V_e became zero in the absence of Cl^- and HCO_3^- gradients. It should be noted that the Cl^- KR perfusate contained urea while the Cl^- KR bath contained alanine plus glucose (Table I). Since J_v was zero when the perfusate and bath contained these solutions (Figs. 2, 3; Table III, IV), fluid absorption in the presence of ouabain or at 21 °C did not depend on concentration gradients for these solutes.

J_v and V_e at 21°C Using Simplified Media

Observations from a number of laboratories indicate that proximal tubules are appreciably more permeable to Cl^- than to HCO_3^- (1, 5, 9, 10, 15–17). Thus, volume absorption dependent on transepithelial anion gradients (Tables II–IV; Figs 2, 3) might be rationalized, at least in part, in terms of the unequal passive permeability properties of these anions. According to this view, the ouabain- and temperature-insensitive moieties of J_v should be proportional in

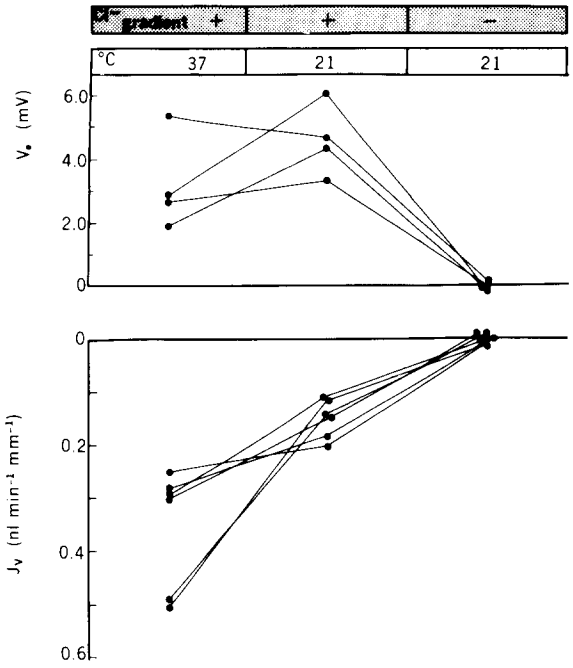

FIGURE 2. The effect of abolishing transepithelial anion concentration gradients on the temperature-insensitive components of J_v and V_e. Each point represents an individual tubule; the lines connect measurements in the same tubule for the three experimental conditions. In the panels marked Cl^- gradient, the perfusate and bath contained, respectively, Cl^- KR and HCO_3^- KR. The Cl^- gradient was abolished by changing the bath to Cl^- KR. The order in which measurements were made was varied at random in the different tubules, and all measurements were completed within 2 h after initiating perfusion.

magnitude to transepithelial Cl^- and HCO_3^- gradients and independent of pH gradients per se. The argument could not be tested directly with KR solutions, since the latter were bubbled with 95% O_2–5% CO_2, and variations in Cl^- and HCO_3^- concentrations resulted in concomitant pH changes (Table I). Consequently, we measured J_v and V_e using simplified media bubbled at 21° with 100% O_2 and containing Na^+, Cl^-, the relatively impermeant anion isethionate, and the buffer pair $HPO_4^=/H_2PO_4^-$. Under these conditions, transepithelial Cl^- and isethionate gradients were established while the pH of both luminal and bathing solutions was 7.4. The results of these experiments, summarized in Table V, include several noteworthy factors.

First, a lumen to bath Cl^- gradient and an oppositely directed isethionate gradient were necessary and sufficient to produce fluid absorption and lumen positive voltages, without a pH gradient and in the absence of other KR solution constituents, i.e., K^+, Ca^{++}, Mg^{++}, acetate, urea, alanine, and glucose. Second, lumen to bath Cl^- concentration gradients resulted in fluid

TABLE III

EFFECT OF ABOLISHING Cl^- GRADIENTS ON THE TEMPERATURE-INSENSITIVE COMPONENTS OF J_v AND V_e

Perfusate	Bath	T	J_v	ΔJ_v	V_e	ΔV_e
		°C	nl min^{-1} mm^{-1}		mV	
Cl^- KR	HCO_3^- KR	37	0.34±0.04 ($P < 0.001$)		3.35±0.22 ($P < 0.01$)	
				0.20±0.05 ($P < 0.02$)		0.43±0.26 ($P > 0.5$)
Cl^- KR	HCO_3^- KR	21	0.15±0.02 ($P < 0.001$)		3.78±0.16 ($P < 0.01$)	
				0.14±0.06 ($P < 0.01$)		3.85±0.19 ($P < 0.01$)
Cl^- KR	Cl^- KR	21	−0.01±0.02 ($P > 0.5$)		−0.08±0.05 ($P > 0.5$)	
			($n = 6$)		($n = 4$)	

This table presents a statistical analysis of the experiments shown in Fig. 2. The P values under individual J_v and V_e measurements indicate the levels at which these values differed from zero. Mean paired differences and P values for the mean paired differences are listed in the ΔJ_v and ΔV_e columns.

transport from lumen to bath, i.e., fluid absorption. The same magnitude Cl^- gradient, when directed from bath to lumen, resulted in volume flow from bath to lumen, i.e., fluid secretion. In other words, transepithelial gradients of Cl^- and isethionate produced symmetrical rather than rectified fluid transport; for the case of fluid secretion, V_e was −7.84 mV, lumen negative. Finally, fluid transport was related to the magnitude of the Cl^- gradient. When the lumen to bath Cl^- gradient at pH 7.4 was 100 mM, J_v and V_e were, respectively, 0.41 nl min^{-1} mm^{-1} and +8.2 mV (Table V); at 21°C using KR solutions, a 25 mM lumen to bath Cl^- gradient produced J_v and V_e values of, respectively, 0.15 nl min^{-1} mm^{-1} and +3.78 mV (Table III). We recognize the difficulties involved in quantitative comparisons among relatively small numbers of tubules under different sets of conditions. Yet, to a first approximation, the results in Table II and V indicate that J_v and V_e were proportional to the magnitudes of oppositely directed anion gradients.

Cl^- Balance During Passively Linked Fluid Absorption

When proximal straight tubules were perfused and bathed with symmetrical HCO_3^- KR buffers at 37°C, the Cl^- content of tubular fluid rose, during volume absorption, from 113.6 to 132.1 meq $liter^{-1}$ (1). Moreover, the total $^{36}Cl^-$ flux from bath to lumen or in the opposite direction was four to five times greater than Na^+ absorption (1), and it was not possible to measure net Cl^- flux reliably in these tubules with tracer techniques. In the present studies, we used a chemical balance method to evaluate net Cl^- flux.

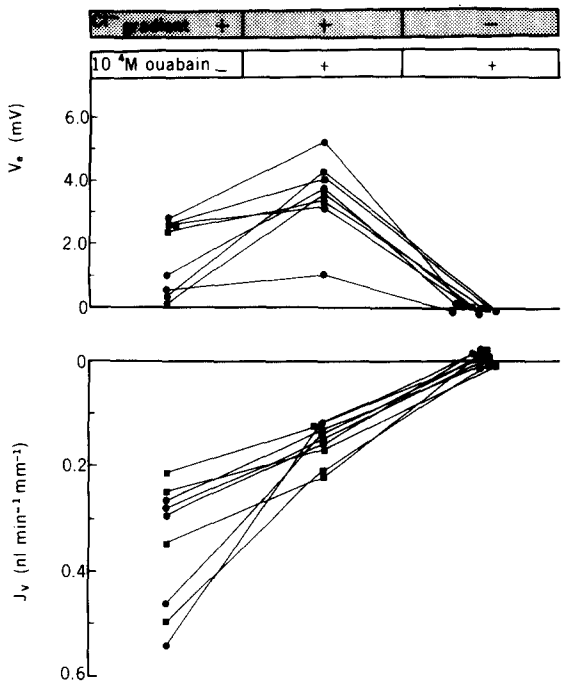

FIGURE 3. The effect of abolishing transepithelial anion concentration gradients on the ouabain-insensitive components of J_v and V_e. The lines connect measurements on the same tubule for the three experimental conditions. In the panels marked Cl^- gradients the perfusate and bath contained, respectively, Cl^- KR and HCO_3^- KR. The squares and circles indicate experiments in which anion gradients were abolished by changing, respectively, either the perfusate to HCO_3^- KR or the bath to Cl^- KR.

TABLE IV

EFFECT OF ABOLISHING Cl^- GRADIENTS ON THE OUABAIN-INSENSITIVE COMPONENTS OF J_v AND V_e

Perfusate	Bath	Ouabain	J_v	ΔJ_v	V_e	ΔV_e
		M	*nl min^{-1} mm^{-1}*		*mV*	
Cl^- KR	HCO_3^- KR	0	0.35±0.04 ($P < 0.001$)		2.81±0.13 ($P < 0.02$)	
				0.18±0.05 ($P < 0.001$)		0.59±0.13 ($P < 0.01$)
Cl^- KR	HCO_3^- KR	10^{-4}	0.17±0.01 ($P < 0.001$)		3.40±0.14 ($P < 0.001$)	
				0.19±0.02 ($P < 0.001$)		3.41±0.15 ($P < 0.001$)
Cl^- KR or HCO_3^- KR	Cl^- KR HCO_3^- KR	10^{-4}	−0.01±0.02 ($P > 0.5$)	($n = 9$)	−0.01±0.15 ($P > 0.5$)	($n = 8$)

This table presents a statistical analysis of the experiments shown in Fig. 3. The results are expressed as in Table II. The data for abolishing Cl^- gradients by using either a Cl^- KR bath or an HCO_3^- KR perfusate have been grouped together, since the results for these two conditions were, as shown in Fig. 3, indistinguishable.

TABLE V

VOLUME ABSORPTION DEPENDENT ON PASSIVE ANION GRADIENTS WITH SIMPLIFIED PERFUSING AND BATHING MEDIA

Perfusate	Bath	J_v	V_e
		nl min⁻¹ mm⁻¹	*mV*
NaCl	Na isethionate	0.41±0.04	+8.2±1.30
NaCl	NaCl	−0.02±0.02	+0.1±0.01
Mean paired difference		0.43±0.05 ($P<0.002$)	8.3±1.00 ($P<0.001$)
		($n=5$)	
Na isethionate	NaCl	−0.40±0.06	−7.84±1.20
NaCl	NaCl	−0.04±0.02	−0.14±0.14
Mean paired difference		0.35±0.04 ($P<0.001$)	7.70±1.20 ($P<0.001$)
		($n=5$)	

The NaCl and Na isethionate perfusing and bathing solutions are described in Table I.

In the experiments summarized in Table VI, the perfusate and bath contained, respectively, Cl^- KR and HCO_3^- KR, both at 21°C; fluid absorption (i.e., the difference between perfusion and collection rates) was in the range 0.09–0.32 nl min^{-1} mm^{-1}. The mean Cl^- content of the collected fluid was slightly less than that of the perfusate; the mean paired difference between perfusate and collected fluid Cl^- concentrations was 2.73 ± 0.74 eq $nl^{-1} \times 10^{-12}$ ($P < 0.05$). The net passive lumen to bath Cl^- fluxes, computed from the chemical balance data, are listed in the column labeled observed J_{Cl} (Table VI). The column labeled predicted J_{Cl} lists values for net Cl^- flux calculated by assuming that Cl^- was the sole anion in an isotonic fluid absorbate. The mean paired difference between observed and predicted values of J_{Cl} was 0.35 ± 0.18 eq min^{-1} $mm^{-1} \times 10^{-11}$ (Table VI), which was not significantly different from zero ($P > 0.1$). Thus, for an isotonic process, Cl^- was virtually the sole anion in absorbed fluid.

Anion Permeability Coefficients

Ion gradient-dependent transepithelial voltages in these (1) and other (15, 16, 31–33) electrically leaky renal tubules may be described empirically by the expression:

$$V_e = \sum_{i=1}^{n} t_i E_i, \qquad (1)$$

where t_i is the transference number of the ith ion, and E_i, the equilibrium

TABLE VI

Cl^- BALANCE DURING VOLUME ABSORPTION DEPENDENT ON TRANSEPITHELIAL ANION GRADIENTS

Tubule length	Perfusate		Collected Fluid		J_{Cl}		ΔJ_{Cl}
	[Cl^-]	Rate	[Cl^-]	Rate	Observed	Predicted	(observed-predicted)
mm	*eq nl*$^{-1}$ × 10^{12}	*nl min*$^{-1}$	*eq nl*$^{-1}$ × 10^{12}	*nl min*$^{-1}$	*eq min*$^{-1}$ *mm*$^{-1}$ × 10^{11}		
2.8	140.5	11.42	136.8	10.52	5.90	5.16	+0.74
3.2	139.5	11.55	138.5	11.11	2.27	2.20	+0.07
2.9	138.0	5.06	133.5	4.73	2.30	1.78	+0.52
3.3	142.0	5.63	141.4	5.14	2.20	2.39	−0.19
2.7	142.1	6.07	138.5	5.83	2.04	1.43	+1.61

Mean paired difference: +0.35±0.18
$P>0.10$

The perfusate and bath contained, respectively, Cl^- KR and HCO_3^- KR, bubbled with 95% O_2-5% CO_2 at 21 ± 0.5°C. Cl^- concentrations in perfusing and collected fluids were measured with a microtitration method (29). Samples of collected fluid were taken at 7–10-min intervals. In a given tubule, alternating samples of collected fluid were used for J_v and [Cl^-] determinations, with the first and last samples being used for J_v measurements; four to five J_v measurements and three to four [Cl^-] determinations were made in each tubule.

potential of the ith ion, is given by:

$$E_i = \frac{RT}{Z_i F} \ln \frac{C_i^b}{C_i^l}, \tag{2}$$

where Z_i is the valence of the ith ion, R, T, and F have their usual meaning, and C_i^l and C_b^i are activities of the ith ion in, respectively, luminal and bathing solutions. The sum of Na^+, Cl^-, and HCO_3^- concentrations in KR solutions was, at a minimum, 10-fold greater than the sum of other ionic constituents. Thus, for these solutions:

$$t_{Na} + t_{Cl} + t_{HCO_3} \simeq 1. \tag{3}$$

In proximal straight (1) and convoluted (32, 33) tubules, the relation:

$$\frac{P_i}{P_j} \simeq \frac{t_i \bar{C}_j}{t_j \bar{C}_i}, \tag{4}$$

obtains experimentally, where P_i and P_j are ionic permeability coefficients, and $\bar{C}_i$ and $\bar{C}_j$ are the arithmetic means of the concentrations of the ith and jth solute in luminal and bathing solutions. For superficial straight tubules (1), P_{Na} and P_{Cl} computed from tracer fluxes were, respectively, 0.23×10^{-4} and 0.73×10^{-4} cm^{-1} s^{-1}; and, t_{Na}/t_{Cl}, estimated from electrical measurements, was 0.3 (as noted previously for these tubules [1], P_{Na}, P_{Cl}, and the P_{Na}/P_{Cl} ratio

are the same at 21 or at 37°C). Thus, these data, together with electrical potential differences produced at 21°C by opposing transepithelial anion gradients (Table II) may be used to compute P_{HCO_3}. The results, shown in Table VII, indicate that the tubular epithelium was virtually impermeable to HCO_3^-, with respect to either Na^+ or Cl^-. These data do not necessarily provide information about the P_{HCO_3}/P_{Cl} ratio at 37°C, since V_e at that temperature depended on active as well as passive ion transport processes. However, E_{HCO_3} was appreciably greater in magnitude than E_{Cl} (Table VII); thus, any value of V_e greater than zero is consistent with a P_{HCO_3}/P_{Cl} ratio appreciably less than unity. Since V_e was uniformly lumen positive and not very different at 37 or 21°C (Table II), it seems probable that these tubules were much more permeable to Cl^- than to HCO_3^- both at 21 and 37°C.

TABLE VII

PERMEABILITY COEFFICIENT FOR HCO_3^-

Perfusate	Bath	V_e	E_{Cl}	E_{HCO_3}	t_{Na}	t_{Cl}	t_{HCO_3}	P_{HCO_3}
		mV						*cm sec*$^{-1}$ × *10*^{4}
Cl^- KR	HCO_3^- KR	+3.72	+5.04	−47.7	0.23	0.77	0.005	0.04

The values of V_e are the mean data from Table II for the indicated perfusate and bath at 21°C. Ionic transference numbers were calculated from Eqs. 1-3, taking $t_{Na}/t_{Cl} = 0.3$ (1). P_{HCO_3} was calculated from Eq. 4, taking $P_{Na} = 0.23 \times 10^{-4}$ cm s^{-1} and $P_{Cl} = 0.73 \times 10^{-4}$ cm s^{-1} (1).

$P_{isethionate}$ was measured directly using [^{14}C]isethionate fluxes. The perfusate and bath contained, at 21°C, HCO_3^- KR solutions (Table I) in which 10 mM Na isethionate replaced an isosmolar amount of NaCl. In five tubules, $P_{isethionate}$, computed from bath to lumen fluxes of [^{14}C]isethionate, was $0.023 \pm 0.006 \times 10^{-4}$ cm s^{-1}, approximately 30 times less than 0.73×10^{-4} cm s^{-1}, the comparable value for P_{Cl} (1).

DISCUSSION

The experiments in this paper were intended to evaluate the possibility (5) that salt and water absorption in proximal nephrons could be driven by passive as well as by active transport processes. Several observations indicate that the hypothesis may be applicable, under appropriate conditions, to superficial proximal straight tubules.

When the perfusate and bath contained symmetrical HCO_3^- KR solutions, J_v and V_e were zero, either at 21°C or in the presence of ouabain (Fig. 1; [1]); similar observations have been reported by others both for isolated straight and convoluted proximal tubules (3). In contrast, when the perfusate and bath contained, respectively, Cl^- KR and HCO_3^- KR, ouabain or cooling inhibited fluid transport only partially, and transepithelial electrical potential

differences, initially lumen positive, rose slightly (Table II). The ouabain- and temperature-insensitive volume flows and voltages became zero when transepithelial anion gradients were abolished (Figs. 2, 3; Tables III; IV), and comparable transport phenomena occurred using simplified perfusing and bathing media at a constant pH (Table V). The tubular fluid Cl^- content fell slightly during volume absorption at 21 °C, and, for an isotonic process, Cl^- was, within experimental error, virtually the sole anion in absorbed fluid (Table VI). It is reasonable to suppose that Na^+ accompanied Cl^-, since Na^+ was the principal cation in KR solutions and the only cation in NaCl/Na isethionate solutions (Tables I, V).

We conclude that, with luminal Cl^- KR and bathing HCO_3^- KR solutions, transepithelial Cl^- concentration gradients and lumen-positive voltages provided driving forces for, respectively, Cl^- and Na^+ absorption. We consider next a model system for analyzing these transport processes.

Analytical Model

It is widely believed that isotonic fluid transport in electrically leaky epithelia involves a standing osmotic gradient in lateral intercellular spaces (34); the hypothesis, a derivative of the dual-membrane model proposed by Curran and MacIntosh (35) and analyzed quantitatively by Patlak et al. (36), assumes that active salt transport into unstirred intercellular spaces raises the total osmolality of the latter, producing a driving force for solvent flow from cells to tissues. In the present context, a comparable argument may be formulated.

Passive ion transport in the superficial pars recta (1), as in a number of other epithelia (15, 16, 32, 33, 37–42), involves a predominantly extracellular route. So it may be that the ouabain- and temperature-insensitive component (Tables II, VI) of net lumen to bath Cl^- flux, and accompanying cation flux, traversed junctional complexes and lateral intercellular spaces. According to this view, the ouabain- and temperature-insensitive component of fluid absorption (Table II), subsequently termed J_v^P, may be rationalized in terms of two classes of explanations. First, passively transported solute might raise the total osmolality in intercellular spaces and fluid might enter the latter through extracellular and cellular pathways. Alternatively, since junctional complexes were more permeable to Cl^- than HCO_3^- (Table VII), unequal concentrations of Cl^- and HCO_3^- in lumen and intercellular spaces might have provided a driving force for J_v^P at equal osmolalities.

Such a "passive-flow" model, like the standing gradient mechanism (34), pictures the intercellular space as an unstirred layer. However, the present proposal differs from the mechanism of Diamond and Bossert (34) in four respects: first, junctional complexes are not closed ends; second, solute enters lateral spaces by passive rather than active transport processes; third, solvent

may enter lateral spaces through extracellular as well as cellular routes, rather than from the cellular compartment exclusively; and fourth, depending on differences between reflection coefficients for Cl^- and HCO_3^- osmotic fluid transport from lumen to lateral intercellular spaces might occur with the same osmolalities in luminal, cellular, intercellular, and bath compartments.

A schematic diagram of the model to be considered is illustrated in Fig. 4. An unstirred channel of unspecified geometry separates luminal and bathing solutions, and is bounded on either side by a cellular compartment; 1 cm^2 of luminal membrane surface area contains n homogeneous channels. The aqueous solutions contain the three principal ions of KR solutions: $Na^+ = 1$, $Cl^- = 2$, and $HCO_3^- = 3$; the ith ion concentrations in lumen, cell, channel, and bath are designated as, respectively, C_i^l, C_i^c, C_i^x, C_i^b.

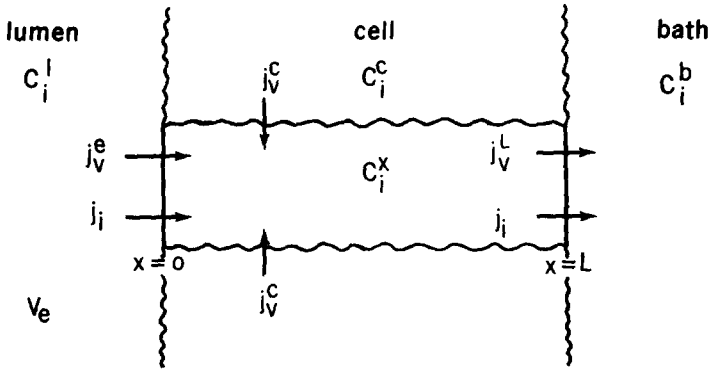

FIGURE 4. Schematic diagram for analyzing the "passive-flow" model. The flows J_i j_v^e, j_v^c and J_v^L are taken to be positive with increasing x.

Each channel is separated from the lumen and bath by membranes at, respectively, $x = 0$ and $x = L$. The observations of Welling et al. (43) indicate that peritubular basement membranes of proximal straight tubules have remarkably high coefficients of hydraulic conductivity and are moderately permeable to albumin. Consequently, we assume that σ_i^L, the reflection coefficient of the ith solute at the right-hand membrane, was zero, and that $C_i^L = C_i^b$. Since passive ion permeation in these tubules involves an extracellular route (1), it is probable that junctional complexes are appreciably more permeable to ions than lateral or basilar membranes. Thus, as in the standing gradient model (34), the membranes separating cellular and channel compartments were taken to have reflection coefficients (σ_i^c) of unity for Na^+, Cl^-, and HCO_3^-.

Volume flow may enter a channel both at $x = 0$ and through cells; all of the transported fluid leaves the channel at $x = L$. Designating the volume flow leaving the kth channel at L as j_v^L and volume flows entering the kth channel

through extracellular and cellular pathways as, respectively, j_v^e and j_v^c, we have:

$$j_v^L = j_v^e + \int_0^L j_v^c \, dx. \tag{5}$$

Defining β as the fraction of j_v^L entering at $x = 0$,

$$j_v^e = \beta j_v^L. \tag{6}$$

In the absence of active transport, ion transport involves an extracellular route (1) and, in the steady state, j_i, the flux of the ith ion in the kth channel through a plane at x normal to the direction of solute transport, is:

$$j_i = -\frac{D_i}{\kappa}\left[\frac{dC_i^x}{dx} + \frac{Z_i F}{RT} C_i^x \frac{dV}{dx}\right] + j_v^x C_i^x, \tag{7}$$

where κ is a tortuosity factor (44), D_i is the free diffusion coefficient for the ith ion, and j_v^x is the volume flow through the kth channel at x; from Eqs. 5 and 6, j_v^x might include both an extracellular component and the total fluid transported from cells to the kth channel between zero and x. Defining A^k as the cross-sectional area of the kth channel, we have:

$$f_m = \sum_{k=1}^{n} \frac{A^k}{1 \text{ cm}^2}, \tag{8}$$

where f_m is the fractional area of channels in 1 cm² of luminal membrane area (45). Accordingly, J_i (eq s^{-1} cm^{-2}), the flux of the ith ion for a 1-cm² luminal surface area, is:

$$J_i = -\frac{D_i f_m}{\kappa}\left[\frac{dC_i^x}{dx} + \frac{Z_i F}{RT} C_i^x \frac{dV}{dx}\right] + J_v^x C_i^x, \tag{9}$$

where J_v^x is the net volume flux crossing x for a 1-cm² luminal surface area. The electroneutrality conditions are:

$$J_1 = J_2 + J_3, \tag{10}$$

and

$$C_1^x = C_2^x + C_3^x. \tag{11}$$

The terms f_m and κ may be used to define α, a geometric factor relating the diffusion resistance of the epithelial cell layer, exclusive of luminal surfaces, to that of an equivalent thickness of free solution, as:

$$\alpha = \frac{\kappa}{f_m}. \tag{12}$$

We know of no morphometric or kinetic data which define explicitly either α, κ, or f_m for proximal mammalian renal tubules. Nor is it possible, in our view, to assign particular geometric models to intercellular spaces on the basis of current electron photomicrographs (46) of isolated proximal tubules from rabbits. However, it may be feasible to set approximate limits for α in this epithelium.

The electrical resistance of isolated straight tubules perfused and bathed in solutions comparable to HCO_3^- KR buffers is approximately 5 Ω-cm^2 (4). For a specific resistance of 60 Ω-cm (47), a 7.5 $\times$ 10^{-4}-cm thick layer (i.e., the thickness of tubular epithelium [1]) of HCO_3^- KR buffer would have a resistance of 0.045 Ω-cm^2. Stated in another way, for passive ion permeation through an extracellular route, the resistance of lateral spaces could be, at a maximum, 111 times greater than that of an equivalent thickness of HCO_3^- KR buffer if junctional complexes made no contributions to the observed resistance of 5 Ω-cm^2. Since the HCO_3^-/Cl^- selectivity ratio in these tubules (Table VII) was far less than might be expected for free solution, we consider that junctional complexes contributed at least in part to the total resistance to ion permeation. Accordingly, we choose:

$$1 \leq \alpha \leq 100$$

as reasonable limits.

We now consider a solution of the flow-diffusion problem which predicts, from bulk phase parameters and observed solute and solvent flows, the concentration and volume profiles within a channel. Eqs. 9–12 may be solved simultaneously to yield:

$$\frac{dC_1^x}{dx} = \frac{1}{2}\left\{\left(\frac{\alpha}{D_1} + \frac{\alpha}{D_3}\right)(J_v^x C_1^x - J_1) + \left(\frac{\alpha}{D_2} - \frac{\alpha}{D_3}\right)(J_v^x C_2^x - J_2)\right\}, \quad (13)$$

$$\frac{dC_2^x}{dx} = \frac{1}{2}\left\{\left(\frac{\alpha}{D_1} - \frac{\alpha}{D_3}\right)(J_v^x C_1^x - J_1)\frac{C_2^x}{C_1^x} + \left[\frac{2_\alpha}{D_2} - \left(\frac{\alpha}{D_2} - \frac{\alpha}{D_3}\right)\frac{C_2^x}{C_1^x}\right](J_v^x C_2^x - J_2)\right\}, \quad (14)$$

and

$$\frac{dV}{dx} = \frac{RT}{F}\left[\frac{J_v^x \alpha}{D_1} - \frac{1}{C_1^x}\frac{dC_1^x}{dx} - \frac{J_1^x \alpha}{D_1 C_1^x}\right]. \quad (15)$$

Defining S^k as the surface area of the kth channel, the gradient of volume flow is:

$$\frac{dJ_v^x}{\kappa\, dx} = P_{f_c} \bar{V}_w f_m \sum_{k=1}^{n} \frac{S^k}{A^k}\left[\sum_{i=1}^{3}(C_i^x - C_i^c)\right], \quad (16)$$

where $\bar{V}_w$ (cm^3 mol^{-1}) is the partial molar volume of water, and P_{f_c} (cm s^{-1}) is the osmotic water permeability of the membrane separating cellular and space compartments. If we let:

$$P'_{f_c} = \kappa^2 P_{f_c} \sum_{k=1}^{n} \frac{S^k}{A^k}, \tag{17}$$

Eq. 16 becomes:

$$\frac{dJ_v^x}{dx} = \frac{P'_{f_c}}{\alpha} \bar{V}_w \left[\sum_{i=1}^{3} (C_i^x - C_i^c) \right], \tag{18}$$

where P'_{f_c} has the dimension s^{-1}. Eqs. 13, 14, 15, and 18 may be integrated numerically (48) to yield: C_i^o, the solute concentration at $x = 0$, V^o, the electrical potential difference between the bath and $x = 0$, and P'_{f_c}. The required inputs are C_i^b, D_i, J_i^p, J_i, L, β, and α, and, assuming that σ_i^c was unity for Na^+, Cl^-, and HCO_3^-, the total osmolality within cells, which we take to be identical to that in luminal and bathing solutions.

In the case of ouabain-insensitive volume flow measurements with Cl^- KR and HCO_3^- KR in, respectively, perfusate and bath (Tabe II): J_v^p was 0.16 nl min^{-1} mm^{-1} and V_e was 3.85 mV; J_{Cl}, computed as in Table VI for this value of J_v^p, was 5.6×10^{-10} eq s^{-1} cm^{-2}; and we assume that $J_{Na} \simeq J_{Cl}$. From Table VII and earlier experiments (1): $P_{Na} = 0.23 \times 10^{-4}$ cm s^{-1}; $P_{Cl} = 0.73 \times 10^{-4}$ cm s^{-1}; and $P_{HCO_3} = 0.04 \times 10^{-4}$ cm s^{-1}. These data will now be considered in terms of the numerically integrated Eqs. 13, 14, 15, and 18, and the variable parameters α and β.

Fig. 5 illustrates the values of C_{Na}^0 and C_{Cl}^0 computed for $1 \leq \alpha \leq 100$ when the parameter β was taken to be unity. Several factors are noteworthy. First, it is evident from Fig. 5 that, while C_{Cl}^0 exceeded C_{Cl}^b slightly with increasing α, C_{Na}^0 was either the same or slightly less than C_{Na}^b. In other words, for these conditions, Na^+ accumulation in intercellular spaces did not occur and the osmolality of the latter was either the same or less than that in luminal, cellular, or bath compartments. It should be noted that, for a unity value of β, transcellular volume flow was zero by definition. Second, although not shown in Fig. 5, the values of C_i^x varied in a near-linear fashion for $0 \leq x \leq L$ and α in the range 1–100; since C_{Na}^0 and C_{Cl}^0 varied by approximately 1 mM from the Na^+ and Cl^- concentrations in the bath, it is evident that the concentration profiles for ions were very nearly constant along the length of the channel. Third, V^o computed together with the indicated values of C_{Na}^0 and C_{Cl}^0 in Fig. 5 was zero to 0.05 mV less than V_e, which, for these conditions, was 3.85 mV (Table II). Thus, for $1 \leq \alpha \leq 100$, 98% of the observed transepithelial voltages were referable to electrical events across junctional complexes; and, for $0 \leq x \leq L$ and $1 \leq \alpha \leq 100$, V^x was virtually equal to V_e or V^o. Fourth, it might be surmised from the results in Fig. 5 that Na^+ permeation through

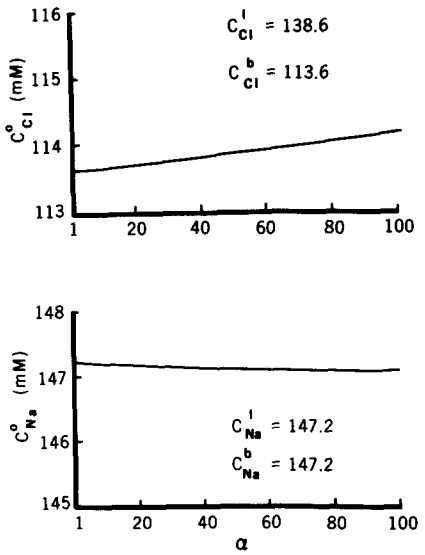

FIGURE 5. The relationship between C^o_{Na}, C^o_{Cl}, and α. The results were computed from the numerically integrated forms of Eqs. 13, 14, 15, and 18, taking the parameter β to be unity. For ouabain-insensitive fluid absorption (Table II) $J^p_v = 0.16$ nl min^{-1} mm^{-1} and $V_e = 3.85$ mV; J_{Cl}, computed according to Table VI, was 5.6×10^{-10} eq s^{-1} cm^{-2}. D_{Na}, D_{Cl}, and D_{HCO_3} were taken to be, respectively, 1.34×10^{-5}, 2.04×10^{-5}, and 1.2×10^{-5} cm^2 s^{-1} (48). From previous data (1) and Table VII, $P_{Na} = 0.23 \times 10^{-4}$ cm s^{-1}; $P_{Cl} = 0.73 \times 10^{-4}$ cm s^{-1}, and $P_{HCO_3} = 0.04 \times 10^{-4}$ cm s^{-1}.

junctional complexes was sufficiently slow to preclude the formation of hypertonic intercellular spaces, and, given the assumption that σ^c_i was unity for Na^+, Cl^-, and HCO_3^-, that fluid transport linked to passive ion flows entered intercellular spaces solely by an extracellular route. In accord with this view, the numerically integrated forms of Eqs. 13, 14, 15, and 18 yielded negative, i.e., nonreal, values of P'_{f_c} when the parameter β was taken to be less than unity and the other parameters specified in Fig. 5 were held constant.

The possibility that junctional complexes, rather than intercellular spaces, were virtually the only significant resistance to Na^+ diffusion may also be evaluated by a second, independent set of calculations. For passive ion permeation involving an extracellular route, the observed ionic permeability coefficient P_i ([1]; Table VII) for proximal straight tubules may be expressed as:

$$\frac{1}{P_i} = \frac{1}{P^j_i} + \frac{\alpha L}{D_i}, \tag{19}$$

where L is the observed thickness of the epithelium, P^j_i is the permeability coefficient of the ith ion across junctional complexes, D_i is the free diffusion coefficient of the ith ion, and α is the geometric factor defined in Eq. 12.

Table VIII lists values of P^j_{Na} and P^j_{Cl} computed according to Eq. 19 for varying α. The values of P^j_{Na} and P^j_{Cl} for $\alpha = 1$ are virtually identical to the observed permeability coefficients reported previously for these ions ([1]; Table VII). In the case of Cl^-, P^j_{Cl} rose for $\alpha \geq 20$; in other words, for $\alpha \geq 20$, a fraction of the observed resistance for Cl^- diffusion was referable to intercellular spaces. But for the less permeable species Na^+, P^j_{Na} was very nearly constant for $1 \leq \alpha \leq 100$, i.e., the transepithelial resistance to Na^+ diffusion was due almost entirely to junctional complexes rather than intercellular spaces. These data, in accord with the results in Fig. 5, are consistent with the view that, for the case of passive ion permeation, Na^+ accumulation and thus hypertonicity in intercellular spaces was an improbable event.

TABLE VIII

EFFECT OF VARYING α ON P^j_{Na} AND P^j_{Cl}

α	P^j_{Na}	P^j_{Cl}
	$cm\ s^{-1} \times 10^4$	
1	0.23	0.73
25	0.23	0.78
50	0.24	0.84
75	0.25	0.91
100	0.26	1.00

P^j_{Na} and P^j_{Cl} for the indicated values of α were computed from Eq. 8. The values of P^j_{Na} and P^j_{Cl} at $\alpha = 1$ are the permeability coefficients computed previously for these ions from unidirectional tracer fluxes ([1]; Table VII). The thickness of the epithelium was taken to be 7.5×10^{-4} cm (1). D_{Na} and D_{Cl} were taken to be, respectively, 1.34×10^{-5} and 2.04×10^{-5} $cm^2\ s^{-1}$ (47).

Solute and Solvent Flows Across Junctional Complexes

For dissipative ion transport involving an extracellular route, J_i may be expressed as (16, 49, 50):

$$J_i = -P^j_i\left(\Delta C^j_i + \frac{Z_i F}{RT}\bar{C}^j_i V^j\right) + \beta J^p_v(1 - \sigma^j_i)\bar{C}^j_i, \qquad (20)$$

where σ^j_i and V^j are, respectively, the ionic reflection coefficient and the voltage across junctional complexes, ΔC^j is $(C^0_i - C^l_i)$, and $\bar{C}^j_i$ is, to a sufficient approximation:

$$\bar{C}^j_i = \frac{C^l_i + C^0_i}{2}.$$

The results from Eqs. 13, 14, 15, 18, and 19 provide estimates of C^0_i, V^j, and P^j_i for varying α (Fig. 5; Table VIII). Accordingly, Eq. 20 may now be used

to compute σ_i^j and the contributions of diffusion and solute entrainment by solvent flow to passive ion fluxes across junctional complexes. Table IX illustrates the values of σ_{Na}, σ_{Cl}, and σ_{HCO_3} computed in this manner. For $1 \leq \alpha \leq 75$, σ_{Na}^j exceeded σ_{Cl}^j, and $\sigma_{HCO_3}^j$ was 0.97. Thus, for values of α consistent with the electrical resistance of these tubules (4), $\sigma_{HCO_3}^j > \sigma_{Cl}^j$ obtains uniformly, in agreement with electrical and/or isotopic measurements ([1]; Tables VII, VIII) of the permeation rate of these ions.

The first and second terms in Eq. 20 describe, respectively, ionic diffusion and ion entrainment by solvent flow. Thus, the results in Tables VII–IX and Fig. 5, together with Eq. 20, may be used to assess the modes of solute transport across junctional complexes for varying values of the parameter α. The calculations, shown in Table X, indicate that for $1 \leq \alpha \leq 75$, less than 10 and 20% of, respectively, Na^+ and Cl^- flux, depended on coupling of solute and solvent flows. Thus, ion transport was predominately diffusional, driven by voltage and concentration gradients.

It is relevant to note in this context that Ullrich et al. (15, 16) have used a form of Eq. 20 to compute, for rat proximal convoluted tubules, ionic reflection coefficients and the amount of NaCl transported by entrainment with volume flow. The calculations of these workers implicitly assign a value of

TABLE IX

RELATIONSHIP BETWEEN σ_i^j AND α

α	σ_{Na}^j	σ_{Cl}^j	$\sigma_{HCO_3}^j$
1	0.9	0.78	0.97
25	0.95	0.83	0.97
50	0.96	0.88	0.97
75	1.0	0.95	0.97

The values for individual ionic reflection ceofficients across junctional complexes (σ_i^j) were computed according to Eq. 20 from the data in Table VIII and Fig. 5.

TABLE X

DIFFUSIONAL AND ENTRAINED ION FLUXES ACROSS JUNCTIONAL COMPLEXES

	Na^+		Cl^-	
α	Diffusion	Entrainment	Diffusion	Entrainment
	$eq\ s^{-1}\ cm^{-2} \times 10^{10}$			
1	5.04	0.56	4.54	1.04
50	5.38	0.22	5.05	0.55
75	5.57	0.03	5.36	0.24

The diffusional and entrained components of ion flux were computed according to Eq. 20 from the data in Figs. 5 and 6 and Table VIII.

unity to the parameter α. We argue, on the basis of the results shown in Fig. 5 and Tables VIII–X that, in the absence of specific information regarding the magnitude of α, one may calculate ranges but not particular values for passive ion transport coefficients in junctional complexes.

The volume flow across junctional complexes is:

$$\beta J_v^p = -P_f^j \bar{V}_w \sum_{i=1}^{3} \sigma_i^j \Delta C_i^j, \tag{21}$$

where P_f^j (cm s^{-1}) is the osmotic water permeability of junctional complexes. Table XI shows the relationship between P_f^j and α, computed from the data in Fig. 5, Table IX, and Eq. 21. For α in the range 1–60, P_f^j was in the range 466–1,793 $\times$ 10^{-4} cm s^{-1}. The reported values for the transepithelial osmotic

TABLE XI

RELATIONSHIP BETWEEN P_f^j AND α

α	P_f^j
	$cm\ s^{-1} \times 10^4$
1	466
20	594
40	869
60	1,793

The results were completed according to Eq. 21 from the data in Fig. 5 and Table IX.

water permeability coefficient for proximal straight tubules are in the range 650–2,000 $\times$ 10^{-4}cm s^{-1} (43). Thus, P_f^j values derived from the electrical resistances (4) of these tubules are also consistent with the observed water permeability properties of these tubules.

Uncertainties of the Model

It is evident that a number of uncertainties arise in connection with the analytical model. Based on the balance data in Table VI, the computations in Fig. 5 and Tables IX–XI were carried out by assuming that there was negligible bath to lumen HCO_3^- flux. Clearly, experimental measurements of perfusion rates, collection rates and tubular fluid Cl^- concentrations, as in Table VI, are not sufficiently precise to exclude the possibility that a relatively small amount, e.g. 5%, of Cl^-/HCO_3^- exchange occurred. However, in view of the remarkably low values for P_{HCO_3} (Table VII), it seems reasonable to infer that HCO_3^- permeation through junctional complexes was very small.

Similarly, σ_i^c was taken to be unity (Eq. 16). The assumption derives from the argument, presented in detail previously (1), that passive ion permeation in these tubules involves an extracellular route. Such observations do not

necessarily exclude the possibility that σ_i^c was slightly less than one and differed for Na^+, Cl^-, and HCO_3^-, so it is possible that a portion of fluid absorption linked to passive ion flows traversed a transcellular route when the osmolality of intercellular spaces was the same or less than that of the cellular compartments (Fig. 5). It is also implicit in the assumption that σ_i^c was unity that there was negligible dissipation of electrical driving forces across the membranes separating cellular compartments and intercellular spaces. In this connection, Keynes (51) has suggested the possibility that, for a sufficiently small length constant for intercellular spaces, there may be considerable voltage attenuation in the latter. We note in this regard that, in these proximal straight tubules, externally measured transepithelial voltages are adequate to account for passive Na^+ and Cl^- flux, even in the presence of active transport processes (1).

Taken together, it seems reasonable to infer from the analytical results in Fig. 5 and Tables VIII–XI that, for fluid absorption coupled to transepithelial anion gradients (Table II), the following considerations may be applicable. If dissipative ion flows involve an extracellular route, the strikingly low electrical resistance of these tubules (4) indicates that observed resistances ([1]; Table VII) to ion diffusion were referable either entirely, for Na^+, or in large part, for Cl^-, to junctional complexes (Table VIII). Stated in another way, the diffusion resistance of lateral spaces was sufficiently small, with respect to net passive ion transport, that hypertonicity of intercellular spaces was unlikely (Fig. 5; Table VIII). Thus, volume flow linked to passive ion flows depended on oppositely directed concentration gradients for anions, specifically HCO_3^- and Cl^-, having unequal reflection coefficients (Table IX; Eq. 21).

Relevance to Measurements at 37°

It would be desirable to extend the analytical model in Fig. 4 to the case of volume absorption dependent on both active and passive transport processes, i.e., a Cl^- KR perfusate and HCO_3^- KR bath at 37°C in the absence of ouabain (Table II). Such an analysis requires, at a minimum, quantitative estimates of the anion fluxes accompanying active Na^+ transport (1) during ouabain- and temperature-sensitive fluid absorption (Table II). While our preliminary results (Schafer and Andreoli, unpublished observations) indicate that acetate is the predominant anion accompanying active Na^+ transport under these conditions, the data to date are not sufficient to warrant quantitative analysis.

A number of other caveats should be noted in considering the application of the present analytical model to the case of volume absorption dependent on combined active and passive transport processes. First, Eq. 7, which describes the entry of the *i*th ion into a channel, will require the addition of terms for

active ion transport. Second, in the case of combined active and passive transport processes, Na^+ influx into lateral intercellular spaces will exceed that observed when the tubules are cooled or exposed to ouabain. Thus, at 37°C in the absence of ouabain, C^o_{Na} may be different from that computed for the present experiments (Fig. 5). Third, at 37°C in the absence of ouabain, total J_v exceeds J^p_j (Table II). Accordingly, during combined active and passive ion transport, assessment of the entrained components of passive ion flows according to Eq. 20 will require the use of total J_v, rather than J^p_v. Fourth, as indicated previously (cf. Methods), the Cl^- concentration of tubular fluid entering the in vivo superficial proximal straight nephron of the rabbit is not known. Our earlier studies (1) showed that, when these tubules were perfused and bathed with HCO_3^- KR solutions, active Na^+ transport accompanied by preferential absorption of anions other than Cl^- raised the tubular fluid Cl^- concentration to 132.1 mM, which is slightly lower than the Cl^- content of the Cl^- KR solutions used in the present studies. Lastly, the present analytical model was formulated without regard to hydrostatic pressure terms (Eqs. 16, 18, 21). The coefficient of hydraulic conductivity of peritubular basement membranes in isolated proximal straight tubules is in excess of 4×10^{-2} cm s^{-1} atm^{-1} (43). Consequently, for values of J_v in the range 0.1–0.2 nl min^{-1} mm^{-1} (Table II), one requires negligible hydrostatic pressure gradients, i.e., 0.1 cm H_2O, between intercellular spaces and bath. Since renal interstitial and peritubular capillary hydrostatic pressures are appreciably greater, it is evident that application of the present model to salt and water transport in the intact kidney requires explicit consideration of hydrostatic pressure terms.

The present results are qualitatively in accord with experimental data indicating a HCO_3^- requirement for maximal rates of fluid absorption in proximal tubules (5–16), since the generation of transepithelial Cl^- gradients in tubules perfused with the equivalent of HCO_3^- KR solutions may depend, at a minimum, on active transport of $NaHCO_3$ (1, 5, 7–14), and possibly of amino acids, glucose (14), and acetate (1). Moreover, lumen-positive transepithelial voltages recently observed during in vivo micropuncture of proximal convolutions (17) may be rationalized, according to Table VII, in terms of Cl^-/HCO_3^- biionic gradients known to occur (18, 19) in proximal convolutions. According to this view, the results in Tables II–IV and Figs. 2 and 3 are consistent with the possibility that a significant fraction of fluid absorption in superficial proximal straight tubules may depend on passive driving forces generated by active transport processes in proximal convolutions.

Finally, the careful observations of Boulpaep (52) indicate clearly that, in proximal tubules of *Necturus* kidney, the passive permeation rates of Na^+ and Cl^- increase during volume expansion, coincident with decrements in net rates of Na^+ absorption. It is evident in this regard that an increase in P_{HCO_3} relative to P_{Cl} (Table VII) and a concomitant reduction in σ_{HCO_3} (Table IX;

Eq. 21) could, for the case of near-isotonic intercellular spaces (Fig. 5), reduce appreciably volume absorption linked to transepithelial anion gradients. The role of such a phenomenon in the regulation of fluid absorption by the in vivo proximal mammalian nephron requires further evaluation.

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