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 \(\sigma_{HCO_3}\) exceeded \(\sigma_{Cl}\).

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 HCO₃⁻ Krebs-Ringer solutions (HCO₃⁻ 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 ³⁶Cl⁻ fluxes from lumen to bath and from bath to lumen in tubules perfused and bathed with HCO₃⁻ 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 NaHCO₃. 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 × 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 HCO₃⁻ 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 I⁻/CO₃⁻ 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 preferential 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⁻²) 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 | Na Cl | Na acetate | NaHCO₃ | Na isethionate | NaCl | NaHCO₃ | Na isethionate | NaCl |
|----------------|-----------|------|-------|------------|--------|----------------|------|--------|----------------|------|
| NaCl           | 105       | 150  | 50    | 0          | 0      | 25             | 105  | 50     | 0              | 0    |
| NaHCO₃         | 25        | 50   | 3.8   | 0          | 0      | 10             | 25   | 0      | 0              | 0    |
| Na isethionate | 0         | 98   | 0     | 0          | 98     | 0              | 0    | 0      | 0              | 0    |
| Na acetate     | 10        | 10   | 0     | 0          | 0      | 10             | 0    | 0      | 0              | 0    |
| Na₂HPO₄        | 3.2       | 3.2  | 0     | 0          | 3.2    | 3.2            | 3.2  | 3.2    | 3.2            | 3.2  |
| NaH₂PO₄        | 0.8       | 0.8  | 1.6   | 0.8        | 0.8    | 1.6            | 0.8  | 0.8    | 0.8            | 0.8  |
| KCl            | 5         | 5    | 0     | 0          | 5      | 5              | 0    | 0      | 0              | 0    |
| CaCl₂          | 1.8       | 1.8  | 0     | 0          | 1.8    | 1.8            | 0    | 0      | 0              | 0    |
| K₂SO₄          | 0         | 0    | 0     | 0          | 0      | 0              | 0    | 0      | 0              | 0    |
| CaSO₄          | 0         | 0    | 0     | 0          | 0      | 0              | 0    | 0      | 0              | 0    |
| MgSO₄          | 8.3       | 8.3  | 1     | 1          | 8.3    | 8.3            | 0    | 0      | 0              | 0    |
| Glucose        | 1         | 1    | 0     | 0          | 1      | 1              | 0    | 0      | 0              | 0    |
| L-Alanine      | 5         | 5    | 0     | 0          | 5      | 5              | 0    | 0      | 0              | 0    |
| Urea           | 0         | 0    | 13.3  | 0          | 0      | 0              | 0    | 0      | 0              | 0    |
| Albumin        | 0         | 0    | 0     | 0          | 0      | 6%             | 6%   | 6%     | 6%             | 6%   |
| pH             | 7.4       | 7.4  | 6.6   | 7.4        | 7.4    | 7.4            | 7.4  | 7.4    | 7.4            | 7.4  |

The various Krebs-Ringer (KR) solutions were bubbled with 95% O₂-5% CO₂; the Na isethionate and NaCl solutions were bubbled with 100% O₂. All solutions were adjusted to 300 mosmol liter⁻¹.

The method for measuring transepithelial potential differences (Vₑ; 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**

$J_e$ and $V_e$: Effects of Ionic Gradients, Cooling, and Ouabain

In earlier studies (1), we observed that, in tubules perfused and bathed with $\text{HCO}_3^-$ $\text{KR}$ solutions at 37°C, $J_e$ and $V_e$ were, respectively, $0.46 \pm 0.03$ nl min$^{-1}$ mm$^{-1}$ and $-1.13 \pm 0.05$ mV, lumen negative; both $J_e$ and $V_e$ remained nearly constant for approximately 210 min. Changes in $J_e$ 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, $J_e$ and $V_e$ were reproducibly and consistently indistinguishable from zero.

The transport characteristics of proximal straight tubules were different when $\text{Cl}^-$ $\text{KR}$ replaced $\text{HCO}_3^-$ $\text{KR}$ 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 $J_e$ nor $V_e$ was zero. Rather, $J_e$ 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°C without ouabain, and $V_e$ rose approximately 0.7 mV. Both $J_e$ and $V_e$ for these two conditions differed significantly from zero ($P < 0.001$). It is evident that, for these circumstances, $J_e$ 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.
HCO₃⁻ PERFUSION: EFFECT OF COOLING

Figure 1. The effect of cooling to 21°C (A) or 10⁻⁴ M ouabain in the bath (B) on Jₑ and Vₑ when the perfusate and bath contain symmetrical HCO₃⁻ 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 Jₑ and Vₑ differed significantly from zero (P < 0.001 for all cases); the values of Jₑ and Vₑ, 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), Jₑ and Vₑ were measured, in a given tubule, under the following conditions: when perfusate and bath contained, respectively, Cl⁻ KR and HCO₃⁻ 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, Jₑ and Vₑ were measured, in a given tubule, under the following conditions: when perfusate and bath contained, respectively, Cl⁻ KR and HCO₃⁻ KR at 37°C, when perfusate and bath again contained, respectively, Cl⁻ KR and HCO₃⁻ 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 HCO₃⁻ 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 Jₑ and Vₑ, 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, Jₑ and Vₑ 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 HCO₃⁻ KR BATH

| Ouabain | T  |  Jₒ | Vₒ |
|---------|----|-----|-----|
| M       | °C |  µl 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     | 0.66±0.11 |
|         |    | P < 0.001     | P < 0.001 |
| (n = 12)|    |               |        |
| 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     | 0.69±0.11 |
|         |    | P < 0.001     | P < 0.001 |
| (n = 11)|    |               |        |

The perfusate and bath contained, respectively, Cl⁻ KR and HCO₃⁻ KR. A positive value for Jₒ denotes volume efflux from lumen to bath; Vₒ is expressed for the lumen with respect to the bath. All values of Jₒ and Vₒ 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 Jₒ and Vₒ (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 Jₒ and Vₒ 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 Jₒ and Vₒ became zero in the absence of Cl⁻ and HCO₃⁻ gradients. It should be noted that the Cl⁻ KR perfusate contained urea while the Cl⁻ KR bath contained alanine plus glucose (Table I). Since Jₒ 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.

Jₒ and Vₒ at 21°C Using Simplified Media

Observations from a number of laboratories indicate that proximal tubules are appreciably more permeable to Cl⁻ than to HCO₃⁻ (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 Jₒ should be proportional in
Figure 2. The effect of abolishing transepithelial anion concentration gradients on the temperature-insensitive components of \( J_e \) 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 \( \text{Cl}^- \) gradient, the perfusate and bath contained, respectively, \( \text{Cl}^- \) KR and \( \text{HCO}_3^- \) KR. The \( \text{Cl}^- \) gradient was abolished by changing the bath to \( \text{Cl}^- \) 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 \( \text{Cl}^- \) and \( \text{HCO}_3^- \) 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% \( \text{O}_2 \)-5% \( \text{CO}_2 \) and variations in \( \text{Cl}^- \) and \( \text{HCO}_3^- \) concentrations resulted in concomitant pH changes (Table I). Consequently, we measured \( J_e \) and \( V_e \) using simplified media bubbled at 21\(^\circ\) with 100% \( \text{O}_2 \) and containing \( \text{Na}^+ \), \( \text{Cl}^- \), the relatively impermeant anion isethionate, and the buffer pair \( \text{HPO}_4^-/\text{H}_2\text{PO}_4^- \). Under these conditions, transepithelial \( \text{Cl}^- \) 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 \( \text{Cl}^- \) 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., \( \text{K}^+ \), \( \text{Ca}^{++} \), \( \text{Mg}^{++} \), acetate, urea, alanine, and glucose. Second, lumen to bath \( \text{Cl}^- \) concentration gradients resulted in fluid
TABLE III

EFFECT OF ABOLISHING Cl⁻ GRADIENTS ON THE TEMPERATURE-INSENSITIVE COMPONENTS OF Jₑ AND Vₑ

| Perfusate | Bath | T  | Jₑ  | ΔJₑ | Vₑ  | ΔVₑ |
|-----------|------|----|-----|-----|-----|-----|
| Cl⁻ KR    | HCO₃⁻ KR | 37 | 0.34±0.04 | 3.35±0.22 | 0.20±0.05 | 0.43±0.25 |
|           |       |    | (P < 0.001) | (P < 0.01) | (P < 0.02) | (P > 0.5) |
| Cl⁻ KR    | HCO₃⁻ KR | 21 | 0.15±0.02 | 3.78±0.16 | 0.14±0.06 | 3.85±0.19 |
|           |       |    | (P < 0.001) | (P < 0.01) | (P < 0.01) | (P < 0.01) |
| Cl⁻ KR    | Cl⁻ KR | 21 | 0.01±0.02 | 0.08±0.05 | (P > 0.5) | (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 Jₑ and Vₑ 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 ΔJₑ and ΔVₑ 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, Vₑ 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, Jₑ and Vₑ 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 Jₑ and Vₑ 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 Jₑ and Vₑ 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 HCO₃⁻ 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 ^3⁵Cl⁻ 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

| Perfusate  | Bath       | Ouabain | $J_v$ (nl min$^{-1}$ mm$^{-3}$) | $\Delta J_v$ | $V_e$ (mV) | $\Delta V_e$ |
|------------|------------|---------|--------------------------------|--------------|------------|-------------|
| Cl$^-$ KR  | HCO$_3^-$ KR | 0       | 0.35±0.04 (P < 0.001)          | 2.81±0.13 (P < 0.02) | 0.59±0.13 (P < 0.01) |
| Cl$^-$ KR  | HCO$_3^-$ KR | 10$^{-4}$ | 0.18±0.05 (P < 0.001)          | 3.40±0.14 (P < 0.001) | 3.41±0.15 (P < 0.001) |
| Cl$^-$ KR  | Cl$^-$ KR   | 10$^{-4}$ | -0.01±0.02 (P > 0.5)          | -0.01±0.15 (P > 0.5) |
| or HCO$_3^-$ KR | HCO$_3^-$ KR | (n = 9) |                                      |              |

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       | $J_e$  | $V_e$ |
|-------------|------------|--------|-------|
| 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 | 8.3±1.00 |
|              |            | (P<0.002) | (P<0.001) |
| 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 | 7.70±1.20 |
|              |            | (P<0.001) | (P<0.001) |

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 HCO₃⁻ 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 × 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 × 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:

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

where $t_i$ is the transference number of the $i$th ion, and $E_i$, the equilibrium
TABLE VI

Cl⁻ BALANCE DURING VOLUME ABSORPTION DEPENDENT ON TRANSEPITHELIAL ANION GRADIENTS

| Tubule length (mm) | Perfusate [Cl⁻] (mM) | Collected Fluid [Cl⁻] (mM) | J(Cl⁻) (nl min⁻¹ X 10⁻⁵) | Observed Rate (nl min⁻¹) | Predicted Rate (nl min⁻¹) | ΔJ(Cl⁻) (observed-predicted) (nl min⁻¹) |
|-------------------|----------------------|---------------------------|--------------------------|--------------------------|--------------------------|------------------------------------------|
| 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 HCO₃⁻ KR, bubbled with 95% O₂-5% CO₂ 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 Jₖ and [Cl⁻] determinations, with the first and last samples being used for Jₖ measurements; four to five Jₖ measurements and three to four [Cl⁻] determinations were made in each tubule.

The potential of the ith ion, is given by:

\[ E_i = \frac{RT}{Z_i F} \ln \frac{C_i^l}{C_i^b}, \]  

(2)

where \( Z_i \) is the valence of the ith ion, \( R \), \( T \), and \( F \) have their usual meaning, and \( C_i^l \) and \( C_i^b \) are activities of the ith ion in, respectively, luminal and bathing solutions. The sum of Na⁺, Cl⁻, and HCO₃⁻ concentrations in KR solutions was, at a minimum, 10-fold greater than the sum of other ionic constituents. Thus, for these solutions:

\[ t_{Na} + t_{Cl} + t_{HCO_3} \approx 1. \]  

(3)

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

\[ \frac{P_i}{P_j} \approx \frac{t_i C_j}{t_j C_i}, \]  

(4)

obtains experimentally, where \( P_i \) and \( P_j \) are ionic permeability coefficients, and \( C_i \) and \( C_j \) 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, \( 0.23 \times 10^{-4} \) and \( 0.73 \times 10^{-4} \, \text{cm}^{-1} \, \text{s}^{-1} \); 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 $P_{\text{HCO}_3^-}$. The results, shown in Table VII, indicate that the tubular epithelium was virtually impermeable to $\text{HCO}_3^-$, with respect to either $\text{Na}^+$ or $\text{Cl}^-$. These data do not necessarily provide information about the $P_{\text{HCO}_3^-}/P_{\text{Cl}^-}$ ratio at 37°C, since $V_e$ at that temperature depended on active as well as passive ion transport processes. However, $E_{\text{HCO}_3^-}$ was appreciably greater in magnitude than $E_{\text{Cl}^-}$ (Table VII); thus, any value of $V_e$ greater than zero is consistent with a $P_{\text{HCO}_3^-}/P_{\text{Cl}^-}$ 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 $\text{Cl}^-$ than to $\text{HCO}_3^-$ both at 21 and 37°C.

| Perfusion | Bath | $V_e$ | $E_{\text{Cl}^-}$ | $E_{\text{HCO}_3^-}$ | $t_{\text{Na}}$ | $t_{\text{Cl}^-}$ | $P_{\text{HCO}_3^-}$ |
|-----------|------|-------|-------------------|-------------------|--------------|----------------|-----------------
| CI$^-$ KR | HCO$^-$ 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 $t_{\text{Na}}/t_{\text{Cl}^-} = 0.3$ (1). $P_{\text{HCO}_3^-}$ was calculated from Eq. 4, taking $P_{\text{Na}} = 0.23 \times 10^{-4}$ cm s$^{-1}$ and $P_{\text{Cl}^-} = 0.73 \times 10^{-4}$ cm s$^{-1}$ (1).

$P_{\text{isethionate}}$ was measured directly using $[1^4\text{C}]$isethionate fluxes. The perfusate and bath contained, at 21°C, HCO$^-$ KR solutions (Table I) in which 10 mM Na isethionate replaced an isosmolar amount of NaCl. In five tubules, $P_{\text{isethionate}}$ computed from bath to lumen fluxes of $[1^4\text{C}]$isethionate, was 0.023 ± 0.006 $\times 10^{-4}$ cm s$^{-1}$, approximately 30 times less than 0.73 $\times 10^{-4}$ cm s$^{-1}$, the comparable value for $P_{\text{Cl}^-}$ (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$^-$ KR solutions, $J_e$ 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, CI$^-$ KR and HCO$^-$ 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 HCO3- KR solutions,
transepithelial Cl- concentration gradients and lumen-positive voltages pro-
vided 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 McIntosh (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 ab-
sorption (Table II), subsequently termed Jp, 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 com-
plexes were more permeable to Cl- than HCO3- (Table VII), unequal con-
centrations of Cl- and HCO3- in lumen and intercellular spaces might have
provided a driving force for Jp 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 HCO₃⁻ 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 cm² 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 HCO₃⁻ = 3; the ith ion concentrations in lumen, cell, channel, and bath are designated as, respectively, $C^l_i$, $C^c_i$, $C^c_i$, and $C^b_i$.

![Schematic diagram for analyzing the "passive-flow" model. The flows $J^e_i$, $J^f_i$, $J^g_i$, and $J^h_i$ are taken to be positive with increasing x.](image)

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 $\sigma^r_i$, the reflection coefficient of the ith solute at the right-hand membrane, was zero, and that $C^b_i = C^c_i$. 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 ($\sigma^r_i$) of unity for Na⁺, Cl⁻, and HCO₃⁻.

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 $k$th channel at $L$ as $j^L_k$, and volume flows entering the $k$th channel...
through extracellular and cellular pathways as, respectively, \( j^e_x \) and \( j^i_x \), we have:

\[
j^e_x = j^e_0 + \int_0^x j^e_x \, dx. \tag{5}\]

Defining \( \beta \) as the fraction of \( j^e_0 \) entering at \( x = 0 \),

\[
j^e_0 = \beta j^e_x. \tag{6}\]

In the absence of active transport, ion transport involves an extracellular route (1) and, in the steady state, \( j^i_x \), the flux of the \( i \)th ion in the \( k \)th channel through a plane at \( x \) normal to the direction of solute transport, is:

\[
J_i = -D_i \left[ \frac{dC_i}{dx} + \frac{Z_i F}{RT} \frac{dV}{dx} \right] + j^i_x C_i, \tag{7}\]

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

\[
f_m = \sum_{k=1}^{n} \frac{A_k^e}{1 \, \text{cm}^2}, \tag{8}\]

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 \( i \)th ion for a 1-cm² luminal surface area, is:

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

where \( J^i_x \) 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

\[
C_1^e = C_2^e + C_3^e. \tag{11}\]

The terms \( f_m \) and \( \kappa \) may be used to define \( \alpha \), 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 \( \text{HCO}_3^- \) KR buffers is approximately 5 Ω-cm² (4). For a specific resistance of 60 Ω-cm (47), a 7.5 × 10⁻⁴-cm thick layer (i.e., the thickness of tubular epithelium [1]) of \( \text{HCO}_3^- \) KR buffer would have a resistance of 0.045 Ω-cm². 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 \( \text{HCO}_3^- \) KR buffer if junctional complexes made no contributions to the observed resistance of 5 Ω-cm². Since the \( \text{HCO}_3^-/\text{Cl}^- \) 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:

\[ \frac{dC_1^a}{dx} = \frac{1}{2} \left\{ \left( \alpha \frac{C_1^a}{D_1} + \frac{C_1^a}{D_2} \right) (J_1^a C_1^a - J_1) + \left( \frac{\alpha C_1^a}{D_2} - \frac{\alpha C_1^a}{D_3} \right) (J_2^a C_2^a - J_2) \right\}, \]  

(13)

\[ \frac{dC_2^a}{dx} = \frac{1}{2} \left\{ \left( \frac{C_2^a}{D_1} - \alpha \frac{C_2^a}{D_2} \right) (J_1^a C_1^a - J_1) \right\} \]

\[ + \left[ \frac{2 \alpha C_2^a}{D_3} - \frac{\alpha C_2^a}{D_4} \right] (J_4^a C_4^a - J_3) \right\}, \]  

(14)

and

\[ \frac{dV}{dx} = \frac{RT}{F} \left[ \frac{J_1^a \alpha}{D_1} - \frac{1}{C_1^a} \frac{dC_1^a}{dx} - \frac{J_2^a \alpha}{D_2 C_2^a} \right]. \]  

(15)

Defining \( S^a \) as the surface area of the \( k \)th channel, the gradient of volume flow is:

\[ \frac{dJ_1^a}{\kappa dx} = P_{t, w} \bar{v}_w f_m \sum_{k=1}^{n} S^a \left[ \sum_{i=1}^{n} \frac{S^a_i}{A_k} (C_i^a - C_i^a) \right], \]  

(16)
where $P_w$ (cm$^3$ mol$^{-1}$) is the partial molar volume of water, and $P_{f_r}$ (cm s$^{-1}$) is the osmotic water permeability of the membrane separating cellular and space compartments. If we let:

$$P_{f_r}' = kP_{f_r}\sum_{i=1}^{N} \frac{S_i}{A_i},$$

Eq. 16 becomes:

$$\frac{dJ_{f_r}'}{dx} = \frac{P_{f_r}'}{\alpha} \tilde{V}_{w} \left[ \sum_{i=1}^{N} (C_i' - C_i) \right],$$

where $P_{f_r}'$ has the dimension s$^{-1}$. Eqs. 13, 14, 15, and 18 may be integrated numerically (48) to yield: $C_0'$, the solute concentration at $x = 0$, $V_{w}$, the electrical potential difference between the bath and $x = 0$, and $P_{f_r}'$. The required inputs are $C_i$, $D_i$, $J_i$, $L$, $\alpha$, and, assuming that $\sigma_i'$ 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$^-$ K$^+$ and HCO$_3^-$ K$^+$ in, respectively, perfusate and bath (Table II): $J_{f_r}'$ was 0.16 nl min$^{-1}$ mm$^{-1}$ and $V_{w}$ was 3.85 mV; $J_{Cl}$, computed as in Table VI for this value of $J_{f_r}'$, was $5.6 \times 10^{-10}$ eq s$^{-1}$ cm$^{-2}$; and we assume that $J_{Na} \approx J_{Cl}$. 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 $\alpha$ and $\beta$.

Fig. 5 illustrates the values of $C_{Na}^0$ and $C_{Cl}^0$ computed for $1 \leq \alpha \leq 100$ when the parameter $\beta$ was taken to be unity. Several factors are noteworthy. First, it is evident from Fig. 5 that, while $C_{Cl}^0$ exceeded $C_{Na}^0$ slightly with increasing $\alpha$, $C_{Na}^0$ was either the same or slightly less than $C_{Na}^0$. 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 $\beta$, transcellular volume flow was zero by definition. Second, although not shown in Fig. 5, the values of $C_i'$ varied in a near-linear fashion for $0 \leq x \leq L$ and $\alpha$ 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, $V_{w}$ 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 $V_{w}$, 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 $1 \leq \alpha \leq 100$, $V_{w}$ was virtually equal to $V_{w}$ or $V_{w}$. Fourth, it might be surmised from the results in Fig. 5 that Na$^+$ permeation through
FIGURE 5. The relationship between $C_{Na}^i$, $C_{Cl}^i$, and $\alpha$. The results were computed from the numerically integrated forms of Eqs. 13, 14, 15, and 18, taking the parameter $\beta$ to be unity. For ouabain-insensitive fluid absorption (Table II) $J_F = 0.16 \, \text{nl min}^{-1} \, \text{mm}^{-1}$ and $V_s = 3.85 \, \text{mV}$; $J_{Cl}$, computed according to Table VI, was $5.6 \times 10^{-10}$ eq s$^{-1}$ cm$^{-2}$. $D_{Na}$, $D_{Cl}$, and $D_{HCO_3}$ were taken to be, respectively, $1.34 \times 10^{-5}$, $2.04 \times 10^{-5}$, and $1.2 \times 10^{-5}$ 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 $\sigma_i$ 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 $P_i$ when the parameter $\beta$ 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:

$$
\frac{1}{P'_i} = \frac{1}{P_i'} + \frac{\alpha L}{D_i},
$$

where $L$ is the observed thickness of the epithelium, $P_i'$ is the permeability coefficient of the $i$th ion across junctional complexes, $D_i$ is the free diffusion coefficient of the $i$th ion, and $\alpha$ is the geometric factor defined in Eq. 12.
Table VIII lists values of $P_{Na}^i$ and $P_{Cl}^i$ computed according to Eq. 19 for varying $\alpha$. The values of $P_{Na}^i$ and $P_{Cl}^i$ for $\alpha = 1$ are virtually identical to the observed permeability coefficients reported previously for these ions ([1]; Table VII). In the case of Cl$^-$, $P_{Cl}^i$ 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$^+$, $P_{Na}^i$ 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 $\alpha$ ON $P_{Na}^i$ AND $P_{Cl}^i$**

| $\alpha$ | $P_{Na}^i \times 10^4$ | $P_{Cl}^i \times 10^4$ |
|---------|-------------------------|------------------------|
| 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                   |

$P_{Na}^i$ and $P_{Cl}^i$ for the indicated values of $\alpha$ were computed from Eq. 8. The values of $P_{Na}^i$ and $P_{Cl}^i$ 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 $7.5 \times 10^{-4}$ cm (1). $D_{Na}$ and $D_{Cl}$ were taken to be, respectively, $1.34 \times 10^{-5}$ and $2.04 \times 10^{-5}$ 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):

$$J_i = -P_i \left( \Delta C_i + \frac{Z_i F}{R T} \bar{C}_i V^i \right) + \beta J_i^s (1 - \sigma_i) \bar{C}_i^o,$$

(20)

where $\sigma_i$ and $V^i$ are, respectively, the ionic reflection coefficient and the voltage across junctional complexes, $\Delta C_i$ is $(C_i^o - C_i)$, and $\bar{C}_i^o$ is, to a sufficient approximation:

$$\bar{C}_i^o = \frac{C_i^o + C_i^o}{2}.$$

The results from Eqs. 13, 14, 15, 18, and 19 provide estimates of $C_i^o$, $V^i$, and $P_i^o$ for varying $\alpha$ (Fig. 5; Table VIII). Accordingly, Eq. 20 may now be used...
to compute $\sigma_1^j$ and the contributions of diffusion and solute entrainment by solvent flow to passive ion fluxes across junctional complexes. Table IX illustrates the values of $\sigma_{Na}^j$, $\sigma_{Cl}^j$, and $\sigma_{HCO_3}^j$ computed in this manner. For $1 \leq \alpha \leq 75$, $\sigma_{Na}^j$ exceeded $\sigma_{Cl}^j$, and $\sigma_{HCO_3}^j$ was 0.97. Thus, for values of $\alpha$ 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 $\alpha$. 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**

| $\alpha$ | $\sigma_{Na}^j$ | $\sigma_{Cl}^j$ | $\sigma_{HCO_3}^j$ |
|---|---|---|---|
| 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 coefficients across junctional complexes ($\sigma_1$) were computed according to Eq. 20 from the data in Table VIII and Fig. 5.

**Table X**

| $\alpha$ | $Na^+$ | $Cl^-$ |
|---|---|---|
| | Diffusion | Entrainment | Diffusion | Entrainment |
| 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 $\alpha$. 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 $\alpha$, one may calculate ranges but not particular values for passive ion transport coefficients in junctional complexes.

The volume flow across junctional complexes is:

$$\beta J_{w}^{p} = -P_{f}^{j} \bar{V}_{w} \sum_{i=1}^{3} \sigma_{i}^{j} \Delta C_{i}^{j},$$

(21)

where $P_{f}^{j} (\text{cm s}^{-1})$ is the osmotic water permeability of junctional complexes. Table XI shows the relationship between $P_{f}^{j}$ and $\alpha$, computed from the data in Fig. 5, Table IX, and Eq. 21. For $\alpha$ 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 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.

**Table XI**

| RELATIONSHIP BETWEEN $P_{f}^{j}$ AND $\alpha$ |
|---------------------------------------------|
| $\alpha$ | $P_{f}^{j}$ |
|-------|---------|
| 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.

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 $\text{HCO}_{3}^{-}$ 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 $P_{\text{HCO}_{3}^{-}}$ (Table VII), it seems reasonable to infer that $\text{HCO}_{3}^{-}$ permeation through junctional complexes was very small.

Similarly, $\sigma_{i}^{j}$ 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 \( \sigma_i \) was slightly less than one and differed for \( \text{Na}^+ \), \( \text{Cl}^- \), and \( \text{HCO}_3^- \), 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 \( \sigma_i \) 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 \( \text{Na}^+ \) and \( \text{Cl}^- \) 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 \( \text{Na}^+ \), or in large part, for \( \text{Cl}^- \), 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 \( \text{HCO}_3^- \) and \( \text{Cl}^- \), having unequal reflection coefficients (Table IX; Eq. 21).

Relevance to Measurements at 37°C

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 \( \text{Cl}^- \) KR perfusate and \( \text{HCO}_3^- \) 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 \( \text{Na}^+ \) 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 \( \text{Na}^+ \) 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, \(C_{\text{Na}}\) may be different from that computed for the present experiments (Fig. 5). Third, at 37°C in the absence of ouabain, total \(J_e\) exceeds \(J_{p}^e\) (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 \(J_e\), rather than \(J_{p}^e\). 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 \(4 \times 10^{-2}\) cm s\(^{-1}\) atm\(^{-1}\) (43). Consequently, for values of \(J_e\) 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\(_2\)O 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\(_3\) bionic 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 \(P_{\text{HCO}_3}\) relative to \(P_{\text{Cl}}\) (Table VII) and a concomitant reduction in \(\sigma_{\text{HCO}_3}\) (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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