Volume Reabsorption, Transepithelial Potential Differences, and Ionic Permeability Properties in Mammalian Superficial Proximal Straight Tubules

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ABSTRACT This paper describes experiments designed to evaluate Na⁺ and Cl⁻ transport in isolated proximal straight tubules from rabbit kidneys. When the perfusing solution was Krebs-Ringer buffer with 25 mM HCO₃⁻ (KRB) and the bath contained KRB plus 6% albumin, net volume reabsorption (Jᵥ, nl min⁻¹ mm⁻¹) was -0.46 ± 0.03 (SEM); Vₛ, the spontaneous transepithelial potential difference, was -1.13 ± 0.05 mV, lumen negative. Both Jᵥ and Vₛ were reduced to zero at 21°C or with 10⁻⁴ M ouabain, but Jᵥ was not HCO₃⁻ dependent. Net Na⁺ reabsorption, measured as the difference between ²²Na⁺ fluxes, lumen to bath and bath to lumen, accounted quantitatively for volume reabsorption, assuming the latter to be an isotonic process, and was in agreement with the difference between lumen to bath ²²Na⁺ fluxes during volume reabsorption and at zero volume flow. The observed flux ratio for Na⁺ was 1.46, and that predicted for a passive process was 0.99; thus, Na⁺ reabsorption was rationalized in terms of an active transport process. The Cl⁻ concentration of tubular fluid rose from 113.6 to 132.3 mM during volume reabsorption. Since Vₛ rose to +0.82 mV when tubules were perfused with 138.6 mM Cl⁻ solutions, Vₛ may become positive when tubular fluid Cl⁻ concentrations rise during volume reabsorption. The permeability coefficients Pₐ and P_Cl computed from tracer fluxes were, respectively, 0.23 × 10⁻⁴ and 0.73 × 10⁻⁴ cm s⁻¹. A Pₐ/P_Cl ratio of 0.3 described NaCl dilution potentials at zero volume flow. The magnitudes of the potentials were the same for a given NaCl gradient in either direction and Pₐ/P_Cl was constant in the range 32–139 mM NaCl. We infer that the route of passive ion permeation was through symmetrical extracellular interfaces, presumably tight junctions, characterized by neutral polar sites in which electroneutrality is maintained by mobile counterions.
INTRODUCTION

One of the primary functions of the proximal tubule is isotonic reabsorption of a major fraction of glomerular filtrate. It seems established that, in the proximal convoluted tubule, the process depends on active sodium transport (1-8), accompanied in mammalian (9, 10) species by acidification of tubular fluid. Approximately 80-90% of filtered bicarbonate is reabsorbed in the mammalian proximal convoluted tubule (9, 10), and sodium and fluid reabsorption are at least in part bicarbonate dependent (11, 12).

A number of studies have documented the electrical leakiness of the proximal convoluted tubule. The transepithelial electrical resistance is approximately 5 ohm-cm² (8, 13-15) and the length constant is 0.005-0.01 cm (3, 8, 13, 15). Since the electrical resistances of luminal and peritubular plasma membranes of proximal tubular cells are relatively high, in the vicinity of 10⁴ ohm-cm² (16), it has been inferred that the low transepithelial resistance of the proximal convoluted tubule is due to ionic shunting through tight junctions (14, 17, 18), in accord with models proposed for the main route of passive ion permeation in frog skin (19), gall bladder (20, 21), and small intestine (22). Both tracer fluxes and electrical measurements indicate that the shunt pathway in the proximal convoluted tubule is slightly more permeable to Na⁺ than to Cl⁻ (11, 14, 15, 18, 23).

In the amphibian proximal convoluted tubule, the transepithelial potential difference (Vₑ, mV) is approximately −15 mV, lumen negative (7, 18, 24). In the mammalian proximal convoluted tubule, Vₑ is appreciably less, in the range −1.0 to −6.0 mV, lumen negative (14, 15, 25, 26); the observations of Kokko and Rector (27, 28) and Lutz et al. (29) indicate that the sign and magnitude of Vₑ depend on the presence of glucose, amino acids, and bicarbonate in tubular fluid.

Less information exists concerning the straight portion, or pars recta, of the proximal tubule, which is not readily accessible to micropuncture. Studies with isolated perfused segments of this portion of the nephron have shown that: the rate of isotonic volume reabsorption and the magnitude of Vₑ in the proximal straight tubule are less than in the proximal convoluted tubule (6, 8, 30); the electrical resistances of the proximal straight tubule and the proximal convoluted tubule are comparable (8); and the reabsorptive capacity of the proximal straight tubule for glucose is appreciably smaller than that of the proximal convoluted tubule (31).

In order to evaluate some of the characteristics of transport processes in proximal straight tubules, we measured volume reabsorption, spontaneous transepithelial potential differences, single salt dilution potentials at zero volume flow, and ²²Na⁺ and ³⁶Cl⁻ fluxes in isolated proximal straight tubules.
from rabbit kidney. The results of these studies indicate that fluid reabsorption in tubules perfused and bathed with KRB buffers containing 25 mM HCO₃⁻ is referable to active Na⁺ transport; and that passive ion permeation involves neutral polar extracellular sites, more permeable to Cl⁻ than to Na⁺. Preliminary reports of some of these results have appeared elsewhere (32, 33).

**METHODS**

The techniques for studying transport processes in segments of proximal straight tubules were quite similar both to those described originally by Burg et al. (6, 26, 34, 35) and to those utilized in this laboratory for the study of segments of cortical collecting tubules (36-38). Female New Zealand white rabbits, 1.5-2.0 kg in weight, were decapitated without pretreatment. The left kidney was excised rapidly, stripped of its capsule and sliced into 1-3-mm thick cross sections. The sections were transferred to rabbit serum (Microbiological Associates, Inc. Bethesda, Md.), which was bubbled continuously with 95% O₂-5% CO₂ at room temperature while the tubules were dissected freehand.

Segments of superficial cortical proximal straight tubule were cut from the region distal to the highly convoluted portion of the proximal tubule, and before the tubule thinned into the descending limb of Henle's loop. All proximal straight tubule segments had a uniform outside diameter and originated primarily from the cortex or, occasionally, the outermost region of the medulla. The tubule segments were 2-4 mm in length, having outside diameters in the range of 35-40 × 10⁻⁴ cm. The internal diameter of the tubules was 22.3 ± 0.19 × 10⁻⁴ cm (SEM, 80 tubules). Three of these tubules had internal diameters less than 20 × 10⁻⁴ cm (respectively, 15, 18, and 19 × 10⁻⁴ cm), and none of the tubules had an internal diameter greater than 25 × 10⁻⁴ cm. The segments appeared similar to proximal convoluted tubules except that they were somewhat less dense and had only occasional bends like those seen throughout the length of proximal convoluted tubules. The isolated tubules were transferred in a droplet of rabbit serum to a thermoregulated perfusion chamber (36), volume approximately 1.2 ml, containing a bathing medium bubbled with an appropriate gas (see below).

A schematic diagram of the perfusion setup, described in detail elsewhere (36), is illustrated in Fig. 1. Each end of a tubule was sucked into a holding pipet, and a concentric perfusion pipet (inside diameter = 16-18 × 10⁻⁴ cm) was advanced into the lumen. The other end of the perfusion pipet was fitted with a three-way stopcock to permit changes in the composition of the perfusing solution. The holding pipet on the collecting end of the tubule contained Sylgard 184 liquid silicone rubber (Dow Corning Corp., Midland, Mich.) to provide an electrical seal at the tubule-glass junction; oil was added above the collected fluid to prevent evaporation. Perfusion was ordinarily driven by a constant delivery microsyringe pump (Sage Instruments Div. Orion Research, Cambridge, Mass., model 255-3) at a rate of 10-35 nl min⁻¹; in the experiments listed in Table X, perfusion was driven by hydrostatic pressure, in the range of 1-25 cm H₂O.
Perfusing and Bathing Solutions

The usual perfusing solution was a modified isotonic Krebs-Ringer bicarbonate buffer (KRB) described by Kokko (28) containing: 105 mM NaCl, 25 mM NaHCO₃, 10 mM Na acetate, 5 mM KCl, 4 mM NaH₂PO₄, 1.8 mM CaCl₂, 1.0 mM MgSO₄, 8.3 mM glucose, and 5.0 mM L-alanine, adjusted to 300 mosmol·liter⁻¹, pH 7.4, and bubbled continuously with 95% O₂-5% CO₂. Two types of bathing solutions were used in most experiments: either rabbit serum or an isotonic KRB buffer containing 6% (wt/vol) bovine serum albumin (fraction V, Sigma Chemical Co., St. Louis, Mo.). Each solution was adjusted to 300 mosmol·liter⁻¹, pH 7.4, and bubbled continuously with 95% O₂-5% CO₂.

The bathing solution was usually exchanged at 10-min intervals to minimize evaporation, and all the bathing solutions used during a given experimental period were pooled for measurements of ³H leakage (see below). In some cases (Tables V-VIII), the bathing solutions contained either ³²Na⁺ or ³⁶Cl⁻ and were not exchanged. Instead, 7.5-15.0 µl of water were added to the bath every 10 min to maintain the measured Na⁺ concentration of the bathing solutions at a constant level.

Changes in the composition of the perfusing and/or bathing solutions are indicated in the text. In experiments where HCO₃⁻ was omitted from both the perfusing and bathing solutions (Fig. 2, Table IX), the system was gassed entirely with 100% O₂ rather than 95% O₂-5% CO₂.

Measurement of Net Volume Reabsorption

After mounting the tubule and initiating perfusion, the temperature of the perfusion chamber was raised to 37 ± 0.5°C over a 5-10-min period using a temperature regulator described previously (36). The perfusion solution contained a labeled volume marker in order to measure both perfusion rate and leakage of perfusion solution to the bath (inulin-methoxy-³H; lots 281-227, 281-257, 511-200, 511-124, 622-156; New England Nuclear, Boston, Mass.). In order to eliminate radioactivity unbound to
inulin, 5 mC of tracer were dissolved in 5 ml water and the solution was dialyzed, using Visking dialysis tubing, for 36–48 h against 1 liter of water at 0–4°C with frequent changes of the dialysis bath. Approximately 50% of the radioactivity was lost to the dialysis bath. The dialyzed fluid was stored at 4°C, and 0.2–1.0 ml were evaporated to dryness as needed for addition to perfusing solution at a specific activity of 25–200 μC ml⁻¹. Using this technique, the rate of leakage, estimated from the appearance of ³H in bathing solutions, was less than 1% of the perfusion rate in the experiments reported in this paper.

Collections of perfused tubular fluid were made at 7–8-min intervals with a constant bore capillary tube calibrated with inulin-methoxy-³H to contain approximately 3.2 nl/mm length. Net volume reabsorption ($J_v$) was computed from the difference between perfusion rates and collection rates (36). The net volume flows were expressed either as nl min⁻¹ mm⁻¹ (tubule length) or as cm³ s⁻¹ cm⁻²; a negative sign for $J_v$ denotes volume efflux from lumen to bath. It should be noted that surface area measurements provide an erroneously low estimate of luminal membrane area, owing to the brush border arrangement of the luminal surfaces of proximal tubules.

**Electrical Measurements**

A schematic representation of the setup for measuring transepithelial potential differences ($V_e$, mV) is illustrated in Fig. 1. Electrical connections to the perfusing and bathing solutions were made through 4% agar-salt bridges. Two different types of salt bridges were used. When either the perfusing and/or bathing solutions contained HCO₃⁻ and the system was gassed with 95% O₂-5% CO₂, the salt bridges contained isotonic KRB and were equilibrated with the same solution, which was gassed with 95% O₂-5% CO₂. In those experiments where HCO₃⁻ was omitted from both the perfusing and bathing solutions and the system was gassed with 100% O₂, the salt bridges contained isotonic Krebs-Ringer buffer in which HCO₃⁻ had been replaced by Cl. The bridges were connected to calomel-KCl reference electrodes (Beckman Instruments, Inc., Fullerton, Calif.) with Tygon tubing containing 3 M KCl. The calomel-KCl electrodes from the perfusing and bathing solutions were attached to, respectively, the positive and negative inputs of a high impedance differential electrometer (model 3233/15; Burr-Brown Research Corp., Tucson, Ariz.), so that $V_e$ was expressed for the lumen with respect to the bath. The output of the electrometer was displayed on a single channel strip chart recorder (model A-5; Varian Associates, Instrument Div., Palo Alto, Calif.). The voltage drift in the system was in the range of ±0.1 mV/8 h.

In principle, no correction for liquid junction potentials should be required when perfusing and bathing solutions and salt bridges are identical in composition; the values of $V_e$ reported for such experiments in this paper represent uncorrected transepithelial potential differences. However, it has been pointed out that, in instances where asymmetry potentials between saturated KCl bridges vary with time, such bridges may be inadequate for measuring transepithelial potential differences of small magnitude (39). We note in this regard that, in the present studies, the asymmetry potentials checked at the beginning and end of each experiment were routinely stable to ±0.1 mV/6 h when the perfusing pipet and bathing solution contained, respec-
tively, isotonic KRB and isotonic KRB plus 6% albumin, and no tubule was in place; similar results obtained in the case of bicarbonate-free solutions. Moreover, the results in Table II and Figs. 2 and 3 indicate clearly that, when volume reabsorption was abolished either with 10^{-4} M ouabain (Table II) or at 21 °C (Table II; Figs. 2 and 3), the transepithelial potential difference was reproducibly indistinguishable from zero in the absence of salt concentration gradients. Thus, we are confident that the liquid junction potentials between KCl bridges and agar-salt bridges were, for all practical purposes, both time invariant and a negligible source of experimental artifact.

In those experiments where the perfusing and/or bathing solutions contained salt concentrations unequal to those in the corresponding agar-salt bridges, $V_{j}$ was corrected for liquid junction potentials according to the Henderson equation (40) modified in terms of activities (39):

$$V_{1-2} = \frac{RT}{F} \sum_{i} \left( \frac{u_i}{z_i} \right) \left( a_i^1 - a_i^2 \right) \ln \frac{\sum_{i} a_i^1 u_i}{\sum_{i} a_i^2 u_i},$$

where $V_{1-2}$ is the potential difference for solution 1 with respect to solution 2, $R$ is the gas constant, $T$ is the absolute temperature, $F$ is Faraday's number, and, $z_i$, $u_i$, and $a_i$ are, respectively, the valence, mobility, and activity of the $i$th ionic species. Ionic mobilities were obtained from Moore (41) and activity coefficients (except for HCO$_3^-$) from Latimer (42). The activity coefficient of NaHCO$_3$ is not known. However, since the activity coefficients of uni-univalent Na salts differ slightly (42), the activity coefficient of NaHCO$_3$ was taken to be, as an approximation, equal to that for NaCl.

**Tracer Fluxes**

$^{36}$Cl$^-$ was obtained as an aqueous solution of the Na$^+$ salt (New England Nuclear, Boston, Mass. lot no. 11911; specific activity = 40 mCi/g). Because of the low specific activity of $^{36}$Cl$^-$, the Na$^{36}$Cl solution was evaporated to dryness and dissolved in a buffer identical either to isotonic KRB (for lumen to bath $^{36}$Cl$^-$ fluxes) or isotonic KRB plus 6% albumin (for bath to lumen $^{36}$Cl$^-$ fluxes) but containing no NaCl. Na$^+$, K$^+$, Cl$^-$ concentrations, osmolalities, and pH's were carefully determined on the tracer solutions and adjusted as necessary. The final $^{36}$Cl$^-$ concentration of such solutions was approximately 25 μCi ml$^{-1}$ in either perfusing or bathing solutions. $^{22}$Na was obtained as the carrier free Cl$^-$ salt from New England Nuclear Corp. (lots 3731, 6731, 1072); 50–100 μCi were added to each milliliter of perfusing or bathing solution with no further adjustment.

Unidirectional $^{22}$Na$^+$ or $^{36}$Cl$^-$ fluxes from lumen to bath were carried out as described previously for nonelectrolyte fluxes (36, 38). The mean specific activities of $^{22}$Na$^+$ (Tables V and VI) and $^{36}$Cl$^-$ (Table VII) in the perfusing solutions were, respectively, 1.8 × 10$^{11}$ and 2.8 × 10$^{14}$ cpm·eq$^{-1}$.

In lumen to bath tracer fluxes, the tracer concentration in the bath was uniformly less than 1% of the tracer concentration in the perfusing solution. Thus, following the analysis described previously (36), an apparent efflux constant $K_i$ (cm s$^{-1}$), including
terms for passive and active ion transport and exchange diffusion, was computed from
the expression:
\[
K_i = \frac{V_{in} - V_{out}}{A} \left[ \ln \left( \frac{C_{in}^{*} / C_{out}^{*}}{C_{in}^{*} / C_{out}^{*}} \right) + \frac{1 + \sigma_i}{2} \right],
\]
where \( V_{in} \) and \( V_{out} \) (cm\(^3\) s\(^{-1}\)) are, respectively, collection and perfusion rates; \( A \) (cm\(^2\)) is lumen surface area; \( C_{in}^{*} \) and \( C_{out}^{*} \) (cpm cm\(^{-3}\)) are the concentrations of the \( i \)th tracer ion in, respectively, perfusing fluid and collected fluid; and \( \sigma_i \) is the reflection coefficient. As pointed out previously (36), the \((1 + \sigma_i/2)\) term is small with respect to the logarithmic term in brackets in Eq. 2; thus, \( K_i \) values computed for the range \( 0 \leq \sigma_i < 1.0 \) differ only slightly. Since the lumen to bath tracer fluxes for \(^22\)Na\(^+\) (Tables V and VI) and \(^36\)Cl\(^-\) (Table VII) were measured under conditions of net volume reabsorption, the data were calculated assuming \( \sigma_i = 0 \).

The lumen to bath ion fluxes (\( J_{l-b}^{i} \), eq s\(^{-1}\) cm\(^{-2}\), Tables V-VII) were computed according to the expression:
\[
J_{l-b}^{i} = K_i C_l,
\]
where \( C_l \), the mean ionic concentration in the tubule lumen, may be approximated by the arithmetic mean of the ionic concentrations in the perfusing and collected fluids (6). The concentration of Cl\(^-\) in collected fluid was obtained from Table IV. Since net Na\(^+\) reabsorption accounted quantitatively for net volume flows assuming isotonic reabsorption (Table V), \( C_{Na} \) was taken to be the Na\(^+\) concentration in perfusate.

Bath to lumen tracer fluxes were carried out as described previously (38). The mean specific activities of \(^22\)Na\(^+\) and \(^36\)Cl\(^-\) in the bathing solutions were, respectively, \( 2.5 \times 10^{11} \) and \( 2.0 \times 10^{11} \) cpm·eq\(^{-1}\). Two maneuvers were used to avoid significant tracer accumulation in the luminal fluid: first, relatively high perfusion rates (25–30 nl min\(^{-1}\) for \(^22\)Na\(^+\) fluxes and 35–40 nl min\(^{-1}\) for \(^36\)Cl\(^-\) fluxes), and second, in the case of \(^36\)Cl\(^-\) fluxes, shorter tubule segments, approximately 1.0 mm in length. The tracer concentration in collected fluid under these conditions was, at a maximum, 10% of the tracer concentration in the bath, which remained constant. If it is assumed that the arithmetic mean provides a reasonable index to the average luminal tracer concentration, the tracer backflux from lumen to bath due to active and passive transport was \( \leq 5\% \) of the bath to lumen flux. Accordingly, bath to lumen ion fluxes (\( J_{b-l}^{i} \), eq s\(^{-1}\) cm\(^{-2}\)) were calculated neglecting the backflux component according to the expression:
\[
J_{b-l}^{i} = \frac{P_{out}^{*} C_{out}^{*}}{A X_l^{*}},
\]
where \( X_l^{*} \) is the tracer specific activity in the bath (cpm·eq\(^{-1}\)).

The bath to lumen tracer fluxes were carried out during volume reabsorption when spontaneous transepithelial potential differences were present. Neglecting an entrainment term for coupling of solute and solvent flows, and assuming a negligibly small
tracer backflux from lumen to bath, one may compute ionic permeability coefficients $(P_i, \text{cm s}^{-1})$ from the bath to lumen tracer fluxes and the expression (43):

$$P_i = \frac{-J_i^{b 	o l}RT}{V_i Z_i F} \left[ \frac{1 - \exp\frac{Z_i F V_s}{RT}}{C_i} \right],$$

(5)

where $C_i$ (eq cm$^{-3}$) is the concentration of the $i$th solute in the bath. By taking $V_s$ to be zero and applying L'Hospital's rule, Eq. 5 becomes the familiar Fick equation:

$$P_i = \frac{-J_i^{b 	o l}}{C_i}.$$  

(6)

The observed value for $V_s$ during net volume reabsorption from isotonic KRB perfusing solution was $-1.13$ mV (Table I). However, as will be indicated in the text, it is likely that, under such conditions, the transepithelial potential difference varied along the length of the tubule. Accordingly, the ionic permeability coefficients reported in this paper were computed according to Eq. 6. Values of $P_i$ calculated in this manner differed by $\leq 5\%$ from values of $P_i$ computed from the same tracer flux data and Eq. 5, assigning arbitrary values of $\pm 3$ mV to $V_s$, i.e., considerably in excess of the magnitudes of $V_s$ observed in these experiments.

**Microchemical Determinations**

Chloride concentrations in collected fluid were measured using a microtitration method (44).

**Statistical Analyses**

Measurements in a given tubule were used to compute a mean value for that tubule; generally, there were four to six measurements per tubule. The mean values for individual tubules were then used to calculate a mean value $\pm$ standard error of the mean (SEM) for a number of tubules. The results were expressed in this manner. The parentheses in the tables give, first, the number of individual observations, and second, the number of tubules. 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. The number of control and experimental observations carried out in a given tubule was either the same or differed by one observation. The $P$ values for differences between means of unpaired observations were computed from the standard Student $t$ test.

**Results**

**Net Volume Reabsorption and Transepithelial Potential Differences**

Table I lists the values for net volume reabsorption ($J_n, \text{nl min}^{-1} \text{mm}^{-1}$) and spontaneous transepithelial potential differences ($V_s, \text{mV}$) at $37^\circ \text{C}$ for isotonic perfusing and bathing media. When the bath contained KRB plus $6\%$
TABLE I

TRANSPORT PROPERTIES OF PROXIMAL STRAIGHT TUBULES

| Perfusion solution | Bath            | $J_e$ (nl min$^{-1}$ mm$^{-1}$) | $V_e$ (mV) |
|--------------------|-----------------|-------------------------------|------------|
| KRB                | KRB + 6% albumin| $-0.46\pm0.03$ (480; 80)     | $-1.13\pm0.05$ (396; 68) |
| KRB                | rabbit serum    | $-0.48\pm0.03$ (130; 24)    | $-1.35\pm0.10$ (180; 32) |

$P > 0.5$ $P > 0.10$

Net volume efflux and spontaneous transepithelial potential differences are expressed as the mean value ±SEM for a number of tubules, as described in Methods; the first term in parentheses is the number of either flux periods (for $J_e$) or observations (for $V_e$); the second term in parentheses is the number of tubules. A negative sign for $J_e$ indicates volume efflux from lumen to bath. $V_e$ denotes the voltage in the lumen with respect to the bath. All experiments were carried out at 37 ± 0.5°C.

Albumin, $J_e$ and $V_e$ were, respectively, $-0.46 \pm 0.03$ nl min$^{-1}$ mm$^{-1}$ and $-1.13 \pm 0.05$ mV; and the values of $J_e$ and $V_e$ were not significantly different when the bath contained rabbit serum instead of KRB plus 6% albumin. Although the data are not shown in Table I, $J_e$ and $V_e$ were also unaltered when an ultrafiltrate of rabbit serum, rather than KRB, was used as the perfusing solution (six tubules). Table II illustrates that both $J_e$ and $V_e$ were reduced to values indistinguishable from zero either by cooling to 21°C or by 10$^{-4}$ M ouabain to the bath. These data (Tables I and II) are in close agreement with the results obtained previously for isolated proximal straight tubules by other workers (6, 8, 26, 30). The magnitudes of both $J_e$ and $V_e$ in proximal straight tubules were appreciably less than in proximal convoluted tubules, where values of, respectively, $-0.8$ to $-1.2$ nl min$^{-1}$ mm$^{-1}$ and $-3.5$ to $-5.6$ mV have been reported (6, 8, 26–30).

Kokko and Rector (27, 28) and Lutz et al. (29) observed that $V_e$ in proximal convoluted tubules became less negative when glucose or alanine were omitted from perfusing solutions. Table III shows that, in proximal straight tubules, the mean paired differences for either $J_e$ (five tubules) or $V_e$ (six tubules) were not significantly different from zero when alanine plus glucose were either present or absent from the perfusing solutions. A reduction in the magnitude of either $J_e$ or $V_e$ equal to or greater than 30% would have been detected within 95% confidence limits. Thus, the present experiments do not exclude the possibility that omission of glucose and alanine from the perfusing solutions resulted in changes in $J_e$ or $V_e$ of smaller magnitude.

Two other characteristics of $J_e$ and $V_e$ measurements should be noted. The proximal straight tubule segments used in the present experiments were 2–4 mm in length, while $J_e$ measurements reported in proximal convoluted tubules were carried out on 0.5–1.5-mm long segments (6). Thus, although
Table II

| T°C | Ouabain | Jₖ | Vₑ |
|-----|---------|----|----|
| 37  | 0       | -0.44±0.06 (21; 4) | -1.40±0.17 (28; 4) |
| 21  | 0       | -0.04±0.04 (23; 4)  | -0.12±0.12 (25; 4)  |

Mean paired difference: 0.40±0.04 (21; 4) 1.27±0.27 (28; 4)

P<0.02  P<0.02

Table III

| Perfusing solution | Jₖ | Vₑ |
|--------------------|----|----|
| Complete KRB       | -0.46±0.03 (30; 5) | -1.24±0.33 (20; 6) |
| KRB - alanine, -glucose | -0.40±0.03 (29; 5) | -1.13±0.28 (20; 6) |

Mean paired difference: 0.06±0.04 0.11±0.28

P>0.2  P>0.5

Jₖ and Vₑ were first measured under control conditions (37°C, ouabain absent) identical to those described in Table I for an isotonic KRB plus 6% albumin bath. Subsequently, in the same tubule, either the temperature was reduced to 21 ± 0.5°C or 10⁻⁴ M ouabain was added to the bath. The results are expressed as described in Methods and in Table I.

The magnitude of Jₖ was smaller in proximal straight tubules (Table I) than in proximal convoluted tubules (6, 26, 30), the absolute differences between perfusion and collection rates, and hence the reliability of Jₖ determinations, were comparable in the two different segments. Second, Lutz et al. (8) recently reported that the length constant for both proximal straight and proximal convoluted tubules was approximately 0.01 cm, in close agreement with earlier observations during in vivo micropuncture (3, 13, 15). Accordingly, Vₑ measurements indicate transepithelial potential differences in the vicinity of the inner perfusion pipet (Fig. 1), but not necessarily along the length of the nephron segment.
**Cl⁻ Content of Collected Fluid**

Table IV illustrates that the Cl⁻ concentration in fluid collected during net volume reabsorption was 132.1 ± 0.98 meq liter⁻¹, 18.5 meq liter⁻¹ higher than in the perfusing solutions. The present experiments do not indicate directly whether this rise in Cl⁻ concentration was associated with preferential reabsorption of acetate or HCO₃⁻ with Na⁺. If it is assumed that the increment in the chloride concentration of collecting fluid was due entirely to preferential bicarbonate reabsorption mediated by carbonic anhydrase (45), the HCO₃⁻ concentration of collected fluid was 6.5 meq liter⁻¹, corresponding at 37°C to a pCO₂ of approximately 40 mm Hg. Thus, for complete hydration of tubular CO₂ to H₂CO₃ (45), the pH of collected fluid computed from the Henderson-Hasselbalch equation was 6.83, slightly less than the pH of fluid samples from micropuncture of late proximal convoluted tubules in mammalian species (10, 47).

**Table IV**

| Jᵥ | Vᵥ | [Cl⁻] |
|----|----|-------|
| nl min⁻¹ mm⁻¹ | mV | meq liter⁻¹ |
| -0.55 | -1.41 | 132.1 ± 0.98 (24; 5) |

This table lists the mean values of Jᵥ and Vᵥ, measured under the conditions described in Table I for an isotonic KRB plus 6% albumin bath, for tubules in which the Cl⁻ concentration of collected fluid was measured. The results are expressed as mean values ± SEM for the number of tubules. The first term in parentheses is the number of determinations, and the second term the number of tubules.

**22Na⁺ and ¹⁴Cl⁻ Fluxes**

In the proximal convoluted tubule, net Na⁺ reabsorption accounts quantitatively for isotonic volume reabsorption (1-6). Table V presents data from a series of experiments in which unidirectional ²²Na⁺ fluxes, either from lumen to bath or from bath to lumen, were carried out concomitantly with measurements of net volume reabsorption. The observed net Na⁺ reabsorption, computed from the difference (P < 0.02) between unidirectional ²²Na⁺ fluxes, was 1.52 ± 0.12 × 10⁻⁹ eq s⁻¹ cm⁻², in excellent agreement with 1.56 × 10⁻⁹ eq s⁻¹ cm⁻², the value for net Na⁺ lumen to bath flux predicted for isotonic reabsorption of -0.49 ± 0.06 nl min⁻¹ mm⁻¹, the mean value for Jᵥ in these experiments.

Table VI compares values for unidirectional ²²Na⁺ fluxes in the same tubule before and after adding 10⁻⁴ ouabain to the bath. Several factors are relevant.
The experimental conditions were the same as those listed in Table I for an isotonic KRB plus 6% albumin bath. The observed net flux of sodium (net $J_{1 Na}^{j-b}$) was computed as the difference between the values for unidirectional $^{22}Na$ fluxes in the two directions. The predicted net flux of sodium was computed from the mean value for volume efflux in these experiments, listed in the table, assuming isotonic volume reabsorption. The results are expressed as described in Table I and in Methods.

First, the mean paired difference between lumen to bath $^{22}Na^+$ fluxes before and after exposure to ouabain was $1.20 \pm 0.30 \times 10^{-9}$ eq s$^{-1}$ cm$^{-2}$ ($P < 0.05$), in reasonable agreement with $1.52 \pm 0.12 \times 10^{-9}$ eq s$^{-1}$ cm$^{-2}$, the net lumen to bath $Na^+$ flux computed from the difference between $J_{Na}^{1}$ and $J_{Na}^{j-b}$ (Table V). Second, the ratio $J_{Na}^{1}/J_{Na}^{j-b}$ was 1.46 (Table V), in accord with 1.29, the ratio of lumen to bath $^{22}Na^+$ fluxes before and after inhibition of net volume reabsorption (Table VI). Third, the value of $P_{Na}$ computed from bath to lumen $^{22}Na^+$ fluxes during isotonic volume reabsorption (Table V) was $0.23 \pm 0.03 \times 10^{-4}$ cm s$^{-1}$ (Table VIII). A comparable value of $P_{Na}$, $0.29 \times 10^{-4}$ cm s$^{-1}$, may be computed from the lumen to bath $^{22}Na^+$ fluxes in the presence of $10^{-4}$ M ouabain (Table VI). Accordingly, it seems reasonable to conclude that $P_{Na}$, and accordingly passive $Na^+$ transport, was not detectably different in the presence and absence of net volume reabsorption.

Volume reabsorption in the proximal straight tubule (Table I) is approximately 40–50% of that in the proximal convoluted tubule (6), and, as will be
indicated below (Table VIII), the passive Na$^+$ permeability of the proximal convoluted tubule is greater than that of the proximal straight tubule. It follows that the unidirectional flux of Na$^+$ from lumen to bath must be greater in proximal convolutions than in the pars recta. In the present studies, $J_{Na}^{i-b}$ was $4.86 \pm 0.26 \times 10^{-9}$ eq s$^{-1}$ (Table V); the comparable value reported for proximal convoluted tubules is $20.0-25.1 \times 10^{-9}$ eq s$^{-1}$ cm$^{-2}$ (6).

The unidirectional $^{36}$Cl fluxes during net volume reabsorption are listed in Table VII. A comparison of Tables V and VII indicates clearly that the $^{36}$Cl fluxes in a given direction were substantially larger than the corresponding $^{22}$Na fluxes. The difference between unidirectional $^{36}$Cl fluxes, $1.19 \pm 1.45 \times 10^{-9}$ eq s$^{-1}$ cm$^{-2}$, was not statistically significant ($P > 0.5$). Moreover, a comparison of Tables V and VII illustrates that the magnitude of net Na$^+$ reabsorption was approximately $15-20\%$ of the Cl$^-$ flux in either direction, i.e., within the range of the standard deviation for Cl$^-$ flux measurements. Thus, these tracer experiments provide no direct information about the magnitude of net Cl$^-$ reabsorption.

Ionic permeability coefficients (Table VIII) computed from the bath to lumen tracer fluxes show clearly that proximal straight tubules were three times more permeable to Cl$^-$ than to Na$^+$. In the proximal convoluted tubule, the ratio $P_{Na}/P_{Cl}$ estimated either isotopically or from electrical measurements, is slightly in excess of unity (11, 14, 15, 18, 23). The value of $P_{Na}$ com-

| TABLE VII |
| UNIDIRECTIONAL $^{36}$Cl FLUXES DURING ISOTONIC VOLUME REABSORPTION |
| $J_{Cl}^{i-b}$ | $J_{Cl}^{b-i}$ | $J_{Cl}^{i-b} - J_{Cl}^{b-i}$ |
| eq $s^{-1} \times 10^{9}$ | eq $s^{-1} \times 10^{9}$ | eq $s^{-1} \times 10^{9}$ |
| 9.47$\pm$1.41 (25; 5) | 8.28$\pm$0.33 (14; 4) | 1.19$\pm$1.45 |

The experimental conditions were the same as those listed in Table I for an isotonic KRB plus 6% albumin bath. The values of $J_{Cl}^{i-b}$ and $J_{Cl}^{b-i}$ are expressed as described in Methods and in Table I. The difference between $J_{Cl}^{i-b}$ and $J_{Cl}^{b-i}$ was not statistically significant.

| TABLE VIII |
| IONIC PERMEABILITY COEFFICIENTS IN PROXIMAL STRAIGHT TUBULES |
| $P_{Na}$ | $P_{Cl}$ |
| cm $s^{-1} \times 10^{4}$ | cm $s^{-1} \times 10^{4}$ |
| 0.23$\pm$0.03 (14; 4) | 0.73$\pm$0.03 (14; 4) |

$P_{Na}$ and $P_{Cl}$ were computed from, respectively, the unidirectional bath to lumen fluxes of $^{22}$Na (Table V) and $^{36}$Cl (Table VII) according to Eq. 6.
puted by Kokko et al. (6) from bath to lumen $^{22}\text{Na}^+$ fluxes in proximal con-
voluted tubules, $0.7-1.2 \times 10^{-4} \text{ cm s}^{-1}$, is approximately fourfold greater than
$0.23 \pm 0.03 \times 10^{-4} \text{ cm s}^{-1}$, the value of $P_{\text{Na}}$ in the present experiments (Table VIII). Clearly, the ratio $P_{\text{Na}}/P_{\text{Cl}}$ is lower in proximal straight tubules (Table VIII) than in proximal convoluted tubules (11, 14, 15, 18, 23) primarily because $\text{Na}^+$ is less permeable in proximal straight than in proximal convoluted tubules.

**Single Salt Diffusion Potentials**

In order to assess the relative ionic permeability properties of proximal straight tubules with electrical methods, we measured NaCl dilution potentials at zero volume flow. In these experiments, $\text{HCO}_3^-$ was replaced by $\text{Cl}^-$; the total ionic concentrations in the perfusing or bathing solutions were varied by isosmotic replacement of NaCl with mannitol. Two maneuvers (Table II) were used to maintain $J_\text{a}$ and $V_\text{a}$ at virtually zero: either the system was

![Figure 2](image-url)

**Figure 2.** NaCl dilution potentials in proximal straight tubules. $\text{Cl}^-$ replaced $\text{HCO}_3^-$ in perfusing and bathing solutions. $V_\text{a}$ was first measured, in a given tubule, when the NaCl concentrations in perfusing and bathing solutions were both 140 mM; the results, indicated in the figure as mean values SEM, were indistinguishable from zero. Subsequently, a portion of the NaCl in either the bathing solutions (open symbols) or perfusing solutions (closed symbols) was replaced isosmotically with mannitol; the NaCl concentration in these solutions was in the range 32-126 mM. The circles denote experiments at 21 ± 0.5°C; the squares denote experiments at 37°C with $10^{-4}$ ouabain in the bath. The system was gassed with 100% O$_2$; the pH was 7.4; and all other constituents of the perfusing and bathing solutions were kept constant. Each point at a given NaCl concentration ratio represents the value for an individual tubule; six tubules were used for the 21°C experiments and seven tubules for the 37°C plus $10^{-4}$ ouabain experiments. The absolute values of $V_\text{a}$, after correction for liquid junction potentials according to Eq. 1, were plotted according to Eq. 7, choosing ratios for permeability coefficients as described in the text. The magnitudes of the liquid junction potentials were approximately 25-45% of the indicated values of $V_\text{a}$. The indicated values of the slope and intercept were computed from a regression analysis of the data (correlation coefficient = 0.99).
cooled to 21°C (Fig. 2, circles) or, in 37°C experiments, $10^{-4}$ ouabain was added to the bathing media (Fig. 2, squares). The data were plotted arbitrarily according to the Goldman-Hodgkin-Katz equation (43, 47):

$$V_e = 2.3 \frac{RT}{F} \log \frac{\frac{P_{Na}}{a_{Na}} + \frac{P_{K}}{a_{K}} + \frac{P_{Cl}}{a_{Cl}}}{\frac{P_{Na}}{a_i} + \frac{P_{K}}{a_i} + \frac{P_{Cl}}{a_i}},$$

(7)

where $Na^+$, $K^+$, and $Cl^-$ were assumed to be the major current-carrying species and $a_i$ and $a_b$ refer to ionic activities in, respectively, the bath and lumen. It is relevant to note again that the value of $P_{Na}$ computed from $J'_b$ at zero volume flow (Table VI) was $0.29 \times 10^{-4}$ cm s$^{-1}$, while the value of $P_{Na}$ computed from $J'_b$ during isotonic volume reabsorption (Table V) was $0.23 \times 10^{-4}$ cm s$^{-1}$ (Table VIII). Stated alternatively, passive $Na^+$ transport was not appreciably different at zero volume flow and during volume reabsorption.

In Fig. 2, the NaCl dilution potential measurements were plotted assuming $P_{Na}/P_{Cl} = 0.30$ (Table VIII) and $P_{K}/P_{Cl} = 0.30$, i.e., that $P_{K} \approx P_{Na}$. It is evident that the magnitude of $V_e$ was the same for a given salt gradient whether the composition of the perfusing or the bathing solution was changed. The slope (56.0 mV) and intercept (0.2 mV) computed from a regression analysis of the data (correlation coefficient = 0.99) were in close agreement with the theoretical slopes of 58.2 and 61.4 mV expected from Eq. 7 at, respectively, 21°C and 37°C. The slope and intercept listed in Fig. 2 were negligibly different if the ratio $P_{K}/P_{Cl}$ was chosen to be either zero or unity, since $K^+$ concentrations in perfusing and bathing solutions were small with respect to the $Na^+$ and $Cl^-$ concentrations.

**Effect of HCO$_3^-$ on $J_e$ and $V_e$**

In proximal convoluted tubules perfused in vivo, both $J_e$ and net Na reabsorption are reduced 50-75% when HCO$_3^-$ is omitted from tubular and peritubular fluid (11, 12). Volume reabsorption in the in vitro proximal convoluted tubule is not affected by omitting HCO$_3^-$ from perfusing and bathing solutions (29); however, $V_e$ becomes positive when HCO$_3^-$ replaces Cl$^-$ in perfusing solutions (28, 29).

The effect of HCO$_3^-$ on $J_e$ and $V_e$ in proximal straight tubules is shown in Table IX. The mean paired differences between $J_e$ determinations, when HCO$_3^-$ was omitted either from perfusing solutions or from both perfusing and bathing solutions, were not significantly different from zero. Similarly, a comparison of unpaired observations on a relatively large number of tubules under control conditions (Table I) and in the absence of HCO$_3^-$ from both
perfusing and bathing solutions (Table IX) indicates that $J_s$ was not altered when both HCO$_3^-$ and CO$_2$ were omitted from perfusing and bathing media. Specifically, a change in $J_s$ equal to or greater than 20% would have been detected within 95% confidence limits.

However, both the sign and magnitude of $V_s$ were HCO$_3^-$ dependent. When HCO$_3^-$ was omitted from only the perfusing solutions, i.e., when the ionic composition of the perfusing solutions resembled that of fluid obtained from late superficial proximal convolutions (9, 10), $V_s$ reversed polarity and was $+0.82 \pm 0.10$. When HCO$_3^-$ was excluded from both perfusing and bathing solutions, $V_s$ was not significantly different than under control conditions for either paired or unpaired observations.

The Cl$^-$ concentration in the bicarbonate-free perfusing solution in Table IX was slightly higher than that in fluid collected from tubules perfused with bicarbonate-containing solutions (Table IV). Thus, the case of bicarbonate omission solely from perfusing solutions (Table IX) should provide an index to the transepithelial potential difference at the collecting end of a tubule.
segment perfused and bathed with HCO₃⁻-containing solutions. The argument could not be tested directly, since attempts to introduce voltage-measuring pipets into the lumen at both ends of tubules during volume reabsorption were not successful. Alternatively, we considered the possibility that $V_*$ changes at low perfusion rates (27) might also reflect the spontaneous transepithelial potential difference associated with higher intraluminal Cl⁻ concentrations. The results of such a representative experiment are presented in Table X. $V_*$ remained constant at perfusion rates in the range 0.5-30 nl min⁻¹, and was unaffected by perfusion pressures in the range 1-15 cm H₂O, as noted by others (27). When perfusion was stopped completely, $V_*$ rose to +0.9 mV, in agreement with the value of $V_*$ when HCO₃⁻ was omitted from perfusing but

| cm H₂O | Perfusion rate | $V_*$  |
|-------|---------------|-------|
| 15    | ~30           | -0.9  |
| 1     | ~0.5-1.0      | -0.9  |
| 0     | 0             | +0.9  |
| 15    | ~30           | -1.0  |

The table lists a representative experiment showing the effects on $V_*$ of varying, in the indicated sequence, perfusion pressure and perfusion rate. The perfusing solution was isotonic KRB and the bath contained isotonic KRB plus 6% albumin; the experimental conditions were the same as those listed in Table I.

not bathing solutions (Table IX), and returned to -1.0 mV when perfusion was reinitiated. In two other tubules not shown in Table X, $V_*$ rose to +0.6 and +0.85 mV at zero perfusion rate.

**DISCUSSION**

The experiments in this paper were designed to evaluate salt and water transport in proximal straight tubules isolated from rabbit renal cortex. When the perfusing and bathing solutions contained 25 mM HCO₃⁻; the spontaneous transepithelial potential difference was -1.13 mV (Table I); fluid reabsorption could be accounted for quantitatively by net Na⁺ flux (Table V), assuming isotonic reabsorption; and both $J_*$ and $V_*$ were reduced to zero either by $10^{-4}$ M ouabain or by cooling (Table II). These results are qualitatively similar to those obtained from both in vivo and in vitro studies on proximal convoluted tubules (1-10, 30). Quantitatively, the magnitudes of both $J_*$ and $V_*$ in isolated straight tubules (Table I) were less than half of those obtained in isolated convoluted tubules (6, 8, 27-30).

The Cl⁻ concentration in tubular fluid rose 18.5 meq liter⁻¹ during volume
reabsorption (Table IV). The results in Table IX and presumably Table X indicate that, coincident with a rise in tubular fluid Cl⁻ concentration (for bathing solutions containing 25 mM HCO₃⁻), \( V_r \) became positive in sign. Thus, in the in vivo superficial mammalian nephron, it may be that \( V_r \) is positive in sign in the pars recta, if: the Cl⁻ concentration in tubular fluid entering the in vivo pars recta is appreciably higher than in glomerular ultrafiltrate (9, 10), and the in vivo pars recta is also Cl⁻ permselective.

There were also qualitative differences between straight and convoluted tubules. The results in Tables V–VIII indicate clearly that the straight tubules were three times more permeable to Cl⁻ than to Na⁺. On the other hand, convoluted tubules (11, 14, 15, 18, 23), as well as other electrically leaky epithelia such as rabbit ileum (22) or gall bladder (20), are more permeable to Na⁺ than to Cl⁻. Thus, there may be differences, as yet indeterminate, in the permselective characteristics of the rate-limiting sites for ion permeation between proximal straight tubules and these epithelia.

Another disparity between straight and proximal tubules obtains in the HCO₃⁻ requirement for volume reabsorption. A comparison of Tables I and IX shows that \( J_v \) was unaffected by removal of HCO₃⁻ from luminal and bathing fluids, while in vivo micropuncture studies have shown that the magnitude of net volume reabsorption in proximal convolutions is reduced 50–75% when HCO₃⁻ is omitted from luminal and peritubular capillary fluids (11, 12). The lack of an appreciable HCO₃⁻ effect on \( J_v \) in straight tubules may depend in part on the lower rates of active Na⁺ transport and volume reabsorption in straight than in convoluted tubules. Thus, Cl⁻ plus acetate, rather than HCO₃⁻ plus Cl⁻ transport, may be adequate to keep pace with active Na⁺ transport in straight but not convoluted tubules. We consider this explanation uncertain since Lutz et al. (29) found no effect of HCO₃⁻ removal from perfusing and bathing solutions on rates of volume reabsorption in the isolated proximal convoluted tubule; and Diamond (48) observed that, in the rabbit gall bladder, which transports Cl⁻ in preference to HCO₃⁻, isotonic fluid reabsorption was stimulated by adding bicarbonate to mucosal and serosal solutions. It is evident that additional studies will be required to clarify this question.

Net Na⁺ flux occurred against an apparent electrochemical gradient (Table I) and could be inhibited completely either by 10⁻⁴ M ouabain or by cooling (Tables II and VI). It seems reasonable to infer that Na⁺ reabsorption under the conditions for these experiments depended on an active process. Accordingly, it is relevant to compare the ratio of observed unidirectional Na⁺ fluxes with that predicted from Ússing’s flux ratio equation:

\[
\frac{J_{Na}^{v+}}{J_{Na}^{v-}} = \frac{\delta_{Na}^{v+}}{\delta_{Na}^{v-}} \exp \frac{zFV_r}{RT},
\]
where $a_{Na}$ and $V_*$ are, respectively, the mean Na$^+$ activity in the lumen and the mean spontaneous transepithelial potential difference. The applicability of Eq. 8 to the analysis of unidirectional ionic fluxes (49-51), requires, at a minimum, that the concentration and voltage parameters in perfusing and bathing solutions define adequately the dissipative forces for ion transport. In the case of volume reabsorption in electrically leaky epithelia such as proximal straight tubules, these requirements merit particular consideration.

The standing gradient model proposed by Diamond and Bossert (52) for fluid transport in gall bladder and other epithelia involves active solute transport and concomitant osmotic volume flow into lateral intercellular spaces. A further complexity arises from the suggestion of Keynes (53) that the small magnitudes of transepithelial potential differences during isotonic volume reabsorption in the gall bladder may be the consequence of attenuation of electrogenic voltages in intercellular spaces if the length constant for the latter is small. Hill and Hill (54, 55) have provided evidence for this effect in the Limonium salt gland.

It is likely, as discussed in greater detail below, that passive ion flows in proximal straight tubules involve primarily an extracellular pathway. Accordingly, we consider the possibility that the active transport-dependent forces cited above affect passive ion transport. If the mechanism proposed by Keynes were operative in the proximal straight tubule, electrical potential differences across tight junctions would exceed measured values of $V_*$, i.e., the electrical driving force for passive ion translocation across tight junctions might be greater in magnitude than expected from experimental measurements of $V_*$. Furthermore, if fluid transport in proximal straight tubules involves a standing gradient mechanism, passive ion transport in intercellular spaces could depend on coupling of passive ion flows to outward fluid transport. Thus, a combination of these factors could modify passive ion flows through tight junctions and intercellular spaces in ways not accounted for by the flux ratio equation.

An unambiguous answer to the problem is not presently available. However, in the case of proximal tubules, the following factors are relevant. The net Na$^+$ flux from lumen to bath was a relatively small component, 31.2%, of the unidirectional Na$^+$ flux from lumen to bath (Tables V-VI); thus, one might deduce intuitively that the effect of active transport processes on passive ion permeation was relatively small. A comparable conclusion may be drawn from the unidirectional Na$^+$ fluxes. The reduction in unidirectional Na$^+$ flux from lumen to bath with exposure to $10^{-4}$ M ouabain was approximately the same as the net Na$^+$ flux (Tables V and VI). This result would not be expected unless passive Na$^{++}$ transport was not detectably different in the presence and absence of fluid reabsorption. Similarly, the value of $P_{Na}$ computed from lumen to bath $^{22}$Na$^+$ fluxes during inhibition of volume reabsorption (Table
VI), $0.29 \times 10^{-4} \text{ cm s}^{-1}$, was not appreciably different from $0.23 \times 10^{-4} \text{ cm s}^{-1}$ (Table VIII), the value for $P_{Na}$ computed from bath to lumen $^{22}\text{Na}$ fluxes during volume reabsorption (Table V). Finally, a comparison of Table VIII and Fig. 2 indicates clearly that the $P_{Na}/P_{Cl}$ ratio derived from tracer fluxes during volume reabsorption was adequate to describe zero current potential differences referable to ion gradients at zero volume flow. Taken together, these observations are consistent with the view that passive $Na^+$ transport could be rationalized in terms of a simple diffusion process which was not appreciably affected either by active transport-dependent events or by obligatory exchange diffusion. Accordingly, the flux ratio equation should provide a reasonable approximation for evaluating unidirectional $Na^+$ fluxes.

In order to obtain $V_*$ for calculating the $Na^+$ flux ratio, we assume that: $V_*$ in Table I ($-1.13 \text{ mV}$) represented transepithelial potential differences at the perfusing ends of tubules; $V_*$ during $HCO_3^-$ exclusion from the perfusing solution (Table IX, $+0.82 \text{ mV}$) or at zero perfusion rate (Table X, $+0.9 \text{ mV}$) reflected $V_*$ at the collecting ends of tubules; and $V_*$ can be approximated by the arithmetic mean of $V_*$ at perfusing and collecting ends. Under these conditions, $V_*$ was $-0.16 \text{ mV}$ and the $Na^+$ flux ratio predicted from Eq. 8 was 0.99. The observed $Na^+$ flux ratio was 1.46 (Table V), i.e., 45% greater than expected for a passive process, in agreement with the results in Tables II and VI.

Spontaneous transepithelial potentials in the gall bladder, approximately 1.4 mV lumen positive, are opposite in sign to those expected for passive $Cl^-$ reabsorption, and it has been suggested that an active coupled pump mediates neutral $NaCl$ transport (56, 57). In the present experiments, the rates of volume reabsorption were the same in the presence and absence of $HCO_3^-$ (Tables I and IX). Thus, a neutral salt transport model for proximal straight tubules would require that $NaCl$ or $Na$ acetate pumping accelerated sufficiently when $HCO_3^-$ was omitted from perfusing and bathing solutions to maintain an invariant rate of fluid transport. Alternatively, we interpret these observations to indicate that net volume reabsorption depended primarily on the rate of active $Na^+$ transport.

The values of $V_*$ in the salt dilution experiments were opposite in sign but equal in magnitude when the composition of either the perfusing or bathing solutions was changed (Fig. 2). Stated in another way, $V_*$ was expressed across a symmetrical interface having the same $P_{Na}/P_{Cl}$ ratio on either side. Since the ionic permeability properties of luminal and peritubular membranes differ (18, 58), we conclude, in accord with comparable suggestions for other electrically leaky epithelia (14-22), that the values of $V_*$ in Fig. 2 were referable to an extracellular interface, presumably tight junctions.

The $P_{Na}/P_{Cl}$ ratio estimated from tracer fluxes (Table V-VIII), when the $Na^+$ and $Cl^-$ concentrations were, respectively, 144 and 113.6 mM, was adequate to describe $NaCl$ dilution potentials (Fig. 2), when the $NaCl$ concentra-
tions in either the perfusing or bathing media were as low as 32 mM. Stated in another way, $P_{Na}/P_{Cl}$ was independent of ionic concentrations in the external solutions. Ionic partition into a membrane containing fixed charges should be regulated in part by a Donnan distribution and hence be dependent on ionic concentrations in the external solutions (51, 59, 60). Thus, it is difficult to reconcile the observations in Tables V–VIII and Fig. 2 with a permeation pathway containing a relatively high density of fixed charges.

Alternatively, the $P_{Na}/P_{Cl}$ ratio of 0.30 for proximal straight tubules, approximately half of the Na/Cl mobility ratio in free solutions, may depend on ion permeation through weakly charged or neutral polar sites, in accord with earlier descriptions of ion permeation pathways in gall bladder (20, 61) and rabbit ileum (22). We note in this regard that electroneutrality should be required for ion permeation through tight junctions, since the thickness of the latter (200–400 Å [62]) in proximal tubules far exceeds the Debye length one calculates for dielectric constants of 3 (oil) or 81 (water) and the salt concentrations in these experiments. For tight junctions lacking charged sites, we expect that electroneutrality will depend on mobile counterions.

It is relevant to consider the validity of expressing the NaCl dilution potentials in Fig. 2 in terms of the arbitrarily chosen Goldman-Hodgkin-Katz expression. In an ion permeation pathway containing no charged sites and sufficiently thick to require microscopic electroneutrality, the potential distribution is logarithmic and the electric field is not constant (47, 61). Thus, a description of the results in Fig. 2 according to Eq. 7 is a seeming paradox. This apparent discrepancy may be resolved by considering that, for the relatively small magnitudes of salt dilution potentials listed in Fig. 2 (which are comparable to those noted in proximal convoluted tubule [14, 15], small intestine [22], and gall bladder [20]), $V_s$ may be described empirically by a number of different expressions.

In Fig. 3, $V_s$ values from Fig. 2 were plotted according to a form of the Planck-Henderson equation:

$$V_s = \frac{2.3RT}{F} \log \frac{a_{NaCl}^i}{a_{NaCl}^0}$$

assuming that: the route of ion permeation contained no charged sites; microscopic electroneutrality obtained, so that, at any point, the Na$^+$ and Cl$^-$ concentrations were the same; and ionic partition coefficients were concentration independent. There was close agreement between the line drawn from a regression analysis of the data and the theoretical line predicted from Eq. 9 for $P_{Na}/P_{Cl} = 0.30$. Thus, the salt dilution potentials in the present experiments could be described equally well either by Eq. 7 or by Eq. 9. Fig. 3 also shows that, particularly at the higher NaCl activity ratios, there was considerable
disparity between experimental values of $V_*$ and those predicted for a $P_{Na}/P_{Cl}$ ratio of either 0.2 or 0.5. These observations provide additional support for the hypothesis that $P_{Na}/P_{Cl}$ was independent of external NaCl concentration and hence that the ion permeation pathways contained a negligibly small density of charged sites.

It is evident that the present experiments leave unresolved a number of issues. As indicated previously, the results in Table III are not adequate for evaluating whether or not omitting alanine and glucose from perfusion solutions resulted in relatively small changes, i.e., approximately 25%, in the magnitudes of $J_*$ and $V_*$. Similarly, it was not possible with the tracer techniques utilized in the present experiments to define adequately the net Cl$^-$ flux, if any, which accompanied net Na$^+$ flux (Table IV). It might be argued that, since the tubular fluid concentration rose during volume reabsorption (Table IV), net Cl$^-$ reabsorption was less than net Na$^+$ reabsorption. According to this view, the actual Cl$^-$ flux ratio might be slightly in excess of unity, i.e., consistent with the prediction of Eq. 8 for a passive process, since unidirectional Cl$^-$ fluxes in either direction were five to six times greater than net Na$^+$ flux (Tables V, VII). However, evaluation of such an hypothesis requires measurements of net Cl$^-$ reabsorption by other techniques.

Finally, the origin of the spontaneous transepithelial potential differences observed in the present experiments is uncertain. At least two classes of events, singly or in unison, may have accounted for these spontaneous transepithelial potential differences. First, if fluid in intercellular spaces was hypertonic due to active ion influx from luminal fluids, i.e., if a standing gradient mechanism
(52, 57) was operative in proximal straight tubules, \( V_* \) might have depended in part on the Cl\(^-\) concentration ratio between luminal and lateral intercellular space fluids. Thus, the positive sign of \( V_* \) when HCO\(_3^-\) was omitted from luminal but not bathing solutions (Table IX) might be rationalized by assuming that the luminal Cl\(^-\) concentration remained approximately constant and greater than that in lateral intercellular spaces, which were exposed to a bathing solution containing 113.6 mM Cl\(^-\) and 25 mM HCO\(_3^-\). Alternatively, the results in Tables I, II, V, and VI, taken together with the flux ratio calculations, are consistent with the possibility that volume reabsorption depended primarily on active Na\(^+\) transport. Consequently, the negative values of \( V_* \) observed when the ionic compositions of perfusing and bathing solutions were the same (Tables I, IX) might have depended in part on an electrogenic Na\(^+\) pump shunted by high conductance intercellular pathways.

Clearly, an explicit analysis of the contributions of diffusion potentials, electrogenic Na\(^+\) pumping, and possibly other factors, to spontaneous trans-epithelial potential differences in the pars recta will require detailed information on a number of issues including, at a minimum: the composition of fluid in lateral intercellular spaces under various conditions of volume reabsorption, estimates of electromotive forces arising across luminal and/or lateral-basal plasma membranes as a consequence of active Na\(^+\) transport and/or ion diffusion, quantitation of the rates of net HCO\(_3^-\) and net Cl\(^-\) reabsorption with respect to net Na\(^+\) transport, and measurements of the electrical resistances of luminal and lateral-basal plasma membranes. Hopefully, experiments currently in progress may provide useful information concerning these questions.

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