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 HCO_3^- (KRB) and the bath contained KRB plus 6% albumin, net volume reabsorption (J_v, nl min^{-1} mm^{-1}) was -0.46 ± 0.03 (SEM); V_e, the spontaneous transepithelial potential difference, was -1.13 ± 0.05 mV, lumen negative. Both J_v and V_e were reduced to zero at 21°C or with 10^{-4} M ouabain, but J_v was not HCO_3^- dependent. Net Na^+ reabsorption, measured as the difference between $^{22}Na^+$ 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 $^{22}Na^+$ 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 V_e rose to +0.82 mV when tubules were perfused with 138.6 mM Cl^- solutions, V_e may become positive when tubular fluid Cl^- concentrations rise during volume reabsorption. The permeability coefficients P_{Na} and P_{Cl} computed from tracer fluxes were, respectively, 0.23×10^{-4} and 0.73×10^{-4} cm s^{-1}. A P_{Na}/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 P_{Na}/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-cm^2 (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 10^3 ohm-cm^2 (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 permeabile to Na^+ than to Cl^- (11, 14, 15, 18, 23).

In the amphibian proximal convoluted tubule, the transepithelial potential difference (V_e, mV) is approximately -15 mV, lumen negative (7, 18, 24). In the mammalian proximal convoluted tubule, V_e 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 V_e 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 V_e 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 $^{22}Na^+$ and $^{36}Cl^-$ 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 HCO_3^- 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% O_2–5% CO_2 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 $\times$ 10^{-4} cm. The internal diameter of the tubules was 22.3 $\pm$ 0.19 $\times$ 10^{-4} cm (SEM, 80 tubules). Three of these tubules had internal diameters less than 20 $\times$ 10^{-4} cm (respectively, 15, 18, and 19 $\times$ 10^{-4} cm), and none of the tubules had an internal diameter greater than 25 $\times$ 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 $\times$ 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 H_2O.

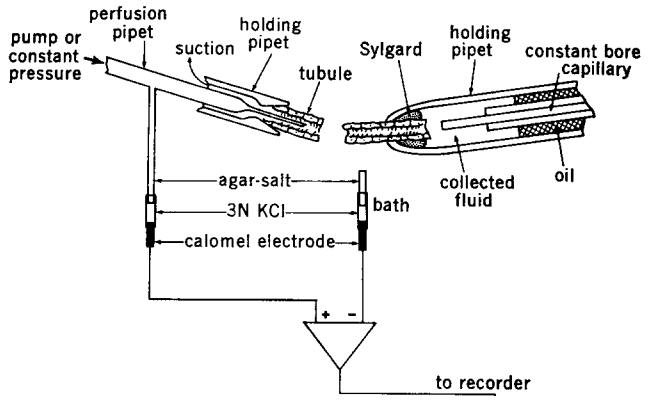

FIGURE 1. A schematic illustration of the apparatus for perfusing isolated renal tubules and the setup for measuring transepithelial potential differences.

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 $NaHCO_3$, 10 mM Na acetate, 5 mM KCl, 4 mM NaH_2PO_4, 1.8 mM $CaCl_2$, 1.0 mM $MgSO_4$, 8.3 mM glucose, and 5.0 mM L-alanine, adjusted to 300 mosmol·liter^{-1}, pH 7.4, and bubbled continuously with 95 %O_2 – 5 % CO_2. 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 % O_2–5 % CO_2.

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 $^{22}Na^+$ or $^{36}Cl^-$ 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 HCO_3^- was omitted from both the perfusing and bathing solutions (Fig. 2, Table IX), the system was gassed entirely with 100 % O_2 rather than 95 % O_2–5 % CO_2.

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 ^{3}H 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-^{3}H 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 cm^3 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 HCO_3^- and the system was gassed with 95% O_2–5% CO_2, the salt bridges contained isotonic KRB and were equilibrated with the same solution, which was gassed with 95% O_2–5% CO_2. In those experiments where HCO_3^- was omitted from both the perfusing and bathing solutions and the system was gassed with 100% O_2, the salt bridges contained isotonic Krebs-Ringer buffer in which HCO_3^- 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 $\pm$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 $\pm$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_e was corrected for liquid junction potentials according to the Henderson equation (40) modified in terms of activities (39):

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

where $V_{1\to 2}$ 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 ith 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^{11} 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:

$$K_i = \frac{\dot{V}^{in} - \dot{V}^{out}}{A}\left[\frac{\ln\left({}^*C_i^{in}/{}^*C_i^{out}\right)}{\ln\left(\dot{V}^{in}/\dot{V}^{out}\right)} + \left(\frac{1+\sigma_i}{2}\right)\right], \quad (2)$$

where: $\dot{V}^{in}$ and $\dot{V}^{out}$ ($cm^3\ s^{-1}$) are, respectively, collection and perfusion rates; A (cm^2) is lumen surface area; ${}^*C_i^{in}$ and ${}^*C_i^{out}$ (cpm cm^{-3}) are the concentrations of the ith tracer ion in, respectively, perfusing fluid and collected fluid; and σ_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 ($J_i^{l\to b}$, eq $s^{-1}\ cm^{-2}$, Tables V–VII) were computed according to the expression:

$$J_i^{l\to b} = K_i \bar{C}_i, \quad (3)$$

where $\bar{C}_i$, 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), $\bar{C}_{Na}$ was taken to be the Na^+ concentration in perfusion fluid.

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, 2.5×10^{11} and 2.0×10^{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 ($J_i^{b\to l}$, eq $s^{-1}\ cm^{-2}$) were calculated neglecting the backflux component according to the expression:

$$J_i^{b\to l} = \frac{\dot{V}^{out}\ {}^*C_i^{out}}{AX_i^b}, \quad (4)$$

where X_i^b 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, cm s^{-1}) from the bath to lumen tracer fluxes and the expression (43):

$$P_i = \frac{-J_i^{b\to l}RT}{V_e Z_i^2 F}\left[\frac{1 - \exp\frac{Z_i F V_e}{RT}}{C_i^b}\right], \quad (5)$$

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

$$P_i = \frac{-J_i^{b\to l}}{C_i^b}. \quad (6)$$

The observed value for V_e 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 ± 3 mV to V_e, i.e., considerably in excess of the magnitudes of V_e 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 ± 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_v, nl min^{-1} mm^{-1}) and spontaneous transepithelial potential differences (V_e, mV) at 37°C for isotonic perfusing and bathing media. When the bath contained KRB plus 6%

TABLE I

TRANSPORT PROPERTIES OF PROXIMAL STRAIGHT TUBULES

Perfusing solution	Bath	J_v	V_e
		nl min⁻¹ mm⁻¹	*mV*
KRB	KRB + 6% albumin	−0.46±0.03 (480; 80)	−1.13±0.05 (396; 68)
KRB	rabbit serum	−0.48±0.03 (150; 24)	−1.33±0.10 (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_v) or observations (for V_e); the second term in parentheses is the number of tubules. A negative sign for J_v 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_v and V_e were, respectively, -0.46 ± 0.03 nl min^{-1} mm^{-1} and -1.13 ± 0.05 mV; and the values of J_v 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_v 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_v 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_v 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_v (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_v 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_v or V_e of smaller magnitude.

Two other characteristics of J_v 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_v measurements reported in proximal convoluted tubules were carried out on 0.5–1.5-mm long segments (6). Thus, although

TABLE II

EFFECT OF OUABAIN AND TEMPERATURE ON J_v AND V_e

T°	Ouabain	J_v	V_e
°C	*M*	*nl min⁻¹ mm⁻²*	*mV*
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 *P*<0.02	1.27±0.27 *P*<0.02
37	0	−0.46±0.04 (38; 8)	−0.96±0.10 (98; 18)
37	10^{-4}	−0.01±0.01 (25; 8)	+0.01±0.05 (122; 18)
Mean paired difference:		0.43±0.06 *P*<0.001	0 97±0.12 *P*<0.001

J_v and V_e 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.

TABLE III

EFFECT OF VARYING LUMINAL FLUID COMPOSITION ON J_v AND V_e

Perfusing solution	J_v	V_e
	nl min⁻¹ mm⁻¹	*mV*
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 *P*>0.2	0.11±0.28 *P*>0.5

J_v and V_e were measured with a complete KRB perfusing solution, and in the same tubule, when alanine and glucose in the perfusing solution were replaced isosmotically with urea. The results are expressed as described in Methods and in Table I.

the magnitude of J_v 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 J_v 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, V_e measurements indicate transepithelial potential differences in the vincinity 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 HCO_3^- 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 HCO_3^- concentration of collected fluid was 6.5 meq liter^{-1}, corresponding at 37°C to a pCO_2 of approximately 40 mm Hg. Thus, for complete hydration of tubular CO_2 to H_2CO_3 (45), the pH of collected fluid computed from the

TABLE IV

Cl^- CONCENTRATION OF COLLECTED FLUID

J_v	V_e	[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 J_v and V_e, 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.

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).

$^{22}Na^+$ and $^{36}Cl^-$ 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 $^{22}Na^+$ 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 $^{22}Na^+$ 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 J_v in these experiments.

Table VI compares values for unidirectional $^{22}Na^+$ fluxes in the same tubule before and after adding 10^{-4} ouabain to the bath. Several factors are relevant.

TABLE V

UNIDIRECTIONAL ^{22}Na FLUXES DURING ISOTONIC VOLUME REABSORPTION

$J_{Na}^{l\to b}$	$J_{Na}^{b\to l}$	Net $J_{Na}^{l\to b}$ (observed)	J_v	Net $J_{Na}^{l\to b}$ (predicted)
	eq s^{-1} cm^{-2} × 10^9		*nl min^{-1} mm^{-1}*	*eq s^{-1} cm^{-2} × 10^9*
4.86±0.26 (76; 11)	3.34±0.39 (14; 4)	1.52±0.12 ($P<0.02$)	−0.49±0.06	1.56

The experimental conditions were the same as those listed in Table 1 for an isotonic KRB plus 6% albumin bath. The observed net flux of sodium (net $J_{Na}^{l\to b}$) 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.

TABLE VI

EFFECT OF INHIBITION OF VOLUME REABSORPTION ON $J_{Na}^{l\to b}$

Ouabain	J_v	$J_{Na}^{l\to b}$
M	*nl min^{-1} mm^{-1}*	*eq s^{-1} cm^{-2} × 10^9*
0	−0.43	5.41±1.04 (20; 4)
10^{-4}	−0.05	4.20±0.95 (11; 4)
Mean paired difference:		1.20±0.30 ($P<0.05$)

The values of J_v are the mean values for individual tubules. The values of $J_{Na}^{l\to b}$ are expressed as described in Methods and Table I. $J_{Na}^{l\to b}$ was first measured under control conditions identical to those in Table V. Subsequently, $J_{Na}^{l\to b}$ was measured in the same tubule after net volume reabsorption had been inhibited by adding 10^{-4} M ouabain to the bath.

First, the mean paired difference between lumen to bath $^{22}Na^+$ fluxes before and after exposure to ouabain was 1.20 ± 0.30 × 10^{-9} eq s^{-1} cm^{-2} ($P <$ 0.05), in reasonable agreement with 1.52 ± 0.12 × 10^{-9} eq s^{-1} cm^{-2}, the net lumen to bath Na^+ flux computed from the difference between $J_{Na}^{l\to b}$ and $J_{Na}^{b\to l}$ (Table V). Second, the ratio $J_{Na}^{l\to b}/J_{Na}^{b\to l}$ 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 ± 0.03 × 10^{-4} cm s^{-1} (Table VIII). A comparable value of P_{Na}, 0.29 × 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, $J_{Na}^{l\to b}$ was 4.86 ± 0.26 × 10^{-9} eq s^{-1} (Table V); the comparable value reported for proximal convoluted tubules is 20.0–25.1 × 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 ± 1.45 × 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

$J_{Cl}^{l\to b}$	$J_{Cl}^{b\to l}$	$J_{Cl}^{l\to b} - J_{Cl}^{b\to l}$
	eq s^{-1} cm^{-2} × 10^9	
9.47±1.41 (25; 5)	8.28±0.33 (14; 4)	1.19±1.45 (P>0.5)

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

TABLE VIII

IONIC PERMEABILITY COEFFICIENTS IN PROXIMAL STRAIGHT TUBULES

P_{Na}	P_{Cl}
cm s^{-1} × 10^4	
0.23±0.03 (14; 4)	0.73±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 $^{22}Na^+$ fluxes in proximal convoluted tubules, 0.7–1.2 × 10^{-4} cm s^{-1}, is approximately fourfold greater than 0.23 ± 0.03 × 10^{-4} cm s^{-1}, the value of P_{Na} in the present experiments (Table VIII). Clearly, the ratio P_{Na}/P_{Cl} is lower in proximal straight tubules (Table VIII) than in proximal convoluted tubules (11, 14, 15, 18, 23) primarily because 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, HCO_3^- was replaced by 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_v and V_e at virtually zero: either the system was

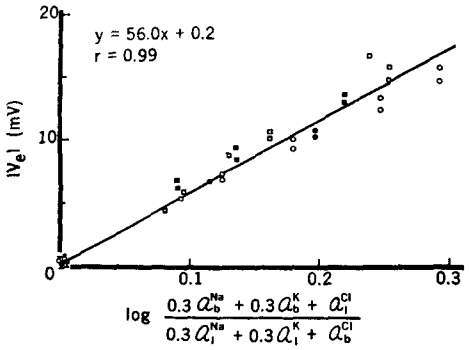

FIGURE 2. NaCl dilution potentials in proximal straight tubules. Cl^- replaced HCO_3^- in perfusing and bathing solutions. V_e was first measured, in a given tubule, when the NaCl concentrations in perfusing and bathing solutions were both 140 mM; the results, indicated in the figure as mean values ±SEM, 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_e, 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_e. 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):

$$V_e = 2.3\frac{RT}{F}\log\frac{\frac{P_{Na}}{P_{Cl}}a_b^{Na} + \frac{P_K}{P_{Cl}}a_b^{K} + a_l^{Cl}}{\frac{P_{Na}}{P_{Cl}}a_l^{Na} + \frac{P_K}{P_{Cl}}a_l^{K} + a_b^{Cl}}, \quad (7)$$

where Na^+, K^+, and Cl^- were assumed to be the major current-carrying species and a_b and a_l refer to ionic activities in, respectively, the bath and lumen. It is relevant to note again that the value of P_{Na} computed from $J_{Na}^{l\to b}$ at zero volume flow (Table VI) was 0.29×10^{-4} cm s^{-1}, while the value of P_{Na} computed from $J_{Na}^{b\to l}$ during isotonic volume reabsorption (Table V) was 0.23×10^{-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_v and V_e

In proximal convoluted tubules perfused in vivo, both J_v 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_v and V_e in proximal straight tubules is shown in Table IX. The mean paired differences between J_v 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_v was not altered when both HCO_3^- and CO_2 were omitted from perfusing and bathing media. Specifically, a change in J_v equal to or greater than 20% would have been detected within 95% confidence limits.

However, both the sign and magnitude of V_e were HCO_3^- dependent. When HCO_3^- was omitted from only the perfusing solutions, i.e., when the ionic

TABLE IX

EFFECT OF VARYING HCO_3^- IN PERFUSING AND BATHING SOLUTIONS ON J_v AND V_e

Perfusing solution		Bath			
$[Cl^-]$	$[HCO_3^-]$	$[Cl^-]$	$[HCO_3^-]$	J_v	V_e
	meq liter^{-1}			*nl min*$^{-1}$ *mm*$^{-1}$	*mV*
113.6	25	113.6	25	−0.38±0.12 (18; 3)	−0.63±0.09 (15; 3)
138.6	0	113.6	25	−0.44±0.03 (17; 3)	+0.82±0.10 (15; 3)
Mean paired difference:				0.06±0.10 $P>0.5$	1.45±0.01 $P<0.001$
113.6	25	113.6	25	−0.35±0.08 (20; 4)	−0.66±0.06 (15; 4)
138.6	0	138.6	0	−0.54±0.11 (20; 4)	−0.85±0.16 (15; 4)
Mean paired difference:				0.20±0.15 $P>0.1$	0.19±0.12 $P>0.2$
All tubules, including unpaired observations:					
138.6	0	138.6	0	−0.45±0.05 (50; 11)	−0.84±0.09 (55; 11)

J_v and V_e were measured, in a given tubule, under control conditions (Table I; KRB plus 6% albumin bath) and when Cl^- replaced HCO_3^- either in perfusing solution alone or in both the perfusing and bathing solutions. When bicarbonate was omitted from the perfusing solution alone, the system was gassed with 95% O_2-5% CO_2 ; when bicarbonate was omitted from both the perfusing and bathing solutions, the system was gassed with 100% O_2 . In some instances, unpaired observations were also carried out in a totally HCO_3^--free system. The results are expressed as described in Methods and in Table I.

composition of the perfusing solutions resembled that of fluid obtained from late superficial proximal convolutions (9, 10), V_e reversed polarity and was $+0.82 \pm 0.10$. When HCO_3^- was excluded from both perfusing and bathing solutions, V_e 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 HCO_3^--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_t 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_t 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 H_2O, as noted by others (27). When perfusion was stopped completely, V_t rose to +0.9 mV, in agreement with the value of V_t when HCO_3^- was omitted from perfusing but

TABLE X

EFFECT OF FLOW RATE AND PERFUSION PRESSURE ON V_t

Perfusion pressure	Perfusion rate	V_t
cm H_2O	*nl min^{-1}*	*mV*
15	~30	−0.9
1	~0.5–1.0	−0.9
0	0	+0.9
15	~30	−1.0

This table lists a representative experiment showing the effects on V_t 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_t 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 HCO_3^-: 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_v and V_t 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_v and V_t 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 HCO_3^-), V_e became positive in sign. Thus, in the in vivo superficial mammalian nephron, it may be that V_e 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 HCO_3^- requirement for volume reabsorption. A comparison of Tables I and IX shows that J_v was unaffected by removal of HCO_3^- 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 HCO_3^- is omitted from luminal and peritubular capillary fluids (11, 12). The lack of an appreciable HCO_3^- 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 HCO_3^- 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 HCO_3^- 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 HCO_3^-, 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 Ussing's flux ratio equation:

$$\frac{J^{l\to b}_{Na}}{J^{b\to l}_{Na}} = \frac{\bar{a}^{l}_{Na}}{\bar{a}^{b}_{Na}} \exp \frac{zF\bar{V}_e}{RT}, \qquad (8)$$

where $\bar{a}^{l}_{Na}$ and $\bar{V}_e$ 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_e, 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_e. 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), 0.29×10^{-4} cm s^{-1}, was not appreciably different from 0.23×10^{-4} cm s^{-1} (Table VIII), the value for P_{Na} computed from bath to lumen $^{22}Na^+$ 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 $\overline{V}_e$ for calculating the Na^+ flux ratio, we assume that: V_e in Table I (-1.13 mV) represented transepithelial potential differences at the perfusing ends of tubules; V_e during HCO_3^- exclusion from the perfusing solution (Table IX, $+0.82$ mV) or at zero perfusion rate (Table X, $+0.9$ mV) reflected V_e at the collecting ends of tubules; and $\overline{V}_e$ can be approximated by the arithmetic mean of V_e at perfusing and collecting ends. Under these conditions, $\overline{V}_e$ was -0.16 m 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_e 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_e 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_e 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_e may be described empirically by a number of different expressions.

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

$$V_e = 2.3 \frac{RT}{F} \frac{P_{Na}/P_{Cl} - 1}{P_{Na}/P_{Cl} + 1} \log \frac{a_l^{NaCl}}{a_b^{NaCl}}, \quad (9)$$

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_e 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

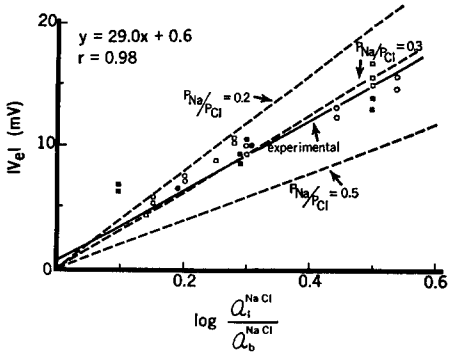

FIGURE 3. The data from Fig. 2 were plotted according to Eq. 9. The solid line was drawn from the indicated values of slope and intercept, which was computed from a regression analysis of the data (correlation coefficient = 0.98). The dotted lines were drawn according to Eq. 9 for the P_{Na}/P_{Cl} ratios indicated in the figure. For convenience, the V_e values at a zero salt gradient were omitted from the figure, but were used in calculating the regression line.

magnitudes of J_v and V_e. 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_e might have depended in part on the Cl^- concentration ratio between luminal and lateral intercellular space fluids. Thus, the positive sign of V_e 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_e 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 transepithelial 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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