Factors Governing the Transepithelial Potential Difference across the Proximal Tubule of the Rat Kidney

LINDSAY J. BARRATT, FLOYD C. RECTOR, JR., JUHA P. KOKKO, and DONALD W. SELDIN

From the Department of Internal Medicine, The University of Texas Southwestern Medical School, Dallas, Texas 75235

ABSTRACT Previous measurements of the transepithelial potential difference (PD) of the proximal tubule have yielded widely conflicting values (range − 20 to + 3 mV). In a recent study, Kokko has demonstrated that the PD of the in vitro perfused isolated proximal tubule of the rabbit varies in a predictable way from − 6 to + 3 mV, depending on the concentration of chloride, bicarbonate, glucose, and amino acids in the perfusing solution.

The present micropuncture study examines the effect of tubular fluid composition on the PD profile along the proximal tubule of the in vivo rat kidney. Low resistance measuring electrodes with large tips (3–5 μm OD) filled with 3 M KCl, were used to provide stable PD recordings. Experiments were performed to validate the use of these electrodes. Transepithelial PD measurements were made in immediate postglomerular segments identified by injection of dye into Bowman's space of accessible surface glomeruli and in randomly selected more distal segments of the proximal tubule. In the control state, the first loop was found to have a small but consistently negative PD which could be obliterated by an infusion of phloridzin. In contrast, the PD in later segments was consistently positive. Infusion of acetazolamide abolished the positive PD in the later segments. Acetazolamide and glucose infusion resulted in a negative PD which was abolished by the additional infusion of phloridzin.

These data provide evidence that glucose reabsorption is electrogenic and can account for the small negative PD normally present in the early proximal tubule. The positive PD in later segments appears to be a passive chloride diffusion potential. This positive potential is discussed as an important electrochemical driving force for significant passive reabsorption of sodium in the proximal tubule.

This work appeared in abstract form in *Clin. Res.* 1973. 21: 675.

Dr. Barratt's work was performed on a research fellowship from the National Kidney Foundation.

Received for publication 31 May 1973 and in revised form 13 September 1973.

INTRODUCTION

It has generally been accepted that net sodium reabsorption in the proximal tubule is entirely mediated by an active process which generates a negative transepithelial potential difference (PD). In 1966, Rector, Martinez-Maldonado, Brunner, and Seldin (1) proposed an alternative hypothesis, postulating that only the fraction of sodium reabsorbed in exchange for hydrogen (bicarbonate reabsorption) was active, while the remainder (NaCl) was reabsorbed passively. According to this view, the proximl tubule was considered to be impermeable to sodium bicarbonate, glucose, and amino acids but relatively permeable to NaCl.

Active reabsorption of bicarbonate, glucose, and amino acids could, at least theoretically, generate two important forces for the passive reabsorption of sodium chloride. First, if the reflection coefficient for NaCl was less than that for the impermeant solutes, an effective osmotic force would exist across the tubular epithelium which could promote the flow of a solution containing NaCl. Second, the passive diffusion of Cl^- down its concentration gradient, a process demonstrated to occur in the isolated perfused proximal tubule of the rabbit (2), would generate a positive transepithelial PD which would provide a favorable electrochemical gradient for passive outward movement of sodium.

This model would be critically dependent on the existence of a positive transepithelial PD and would therefore be inconsistent with the previously reported negative PD, particularly the − 20 mV values reported by us (3, 4) and others (5–9). Subsequent studies, especially by Frömter and associates Hegel and Wick, have

identified errors in the earlier measurements involving the failure to ensure the intraluminal position of the electrode tip (10, 11) and appropriately to correct for liquid junction potentials in the measuring system (12). Nevertheless, the magnitude and orientation of the proximal tubular potential in different preparations has remained controversial; a small positive potential reported by some (12), no potential by others (13), and a negative potential by still others (14–16). In a recent study from our laboratory, using the in vitro perfused isolated proximal tubule of the rabbit, Kokko (17) found that perfusion with a solution simulating glomerular ultrafiltrate resulted in a negative transepithelial PD of -6 mV (owing to active reabsorption of glucose and amino acids) whereas perfusion with a solution resembling late proximal tubular fluid resulted in a positive PD of $+3$ mV (because of passive outward Cl^- diffusion).

The present study was designed to explore the nature and significance of the transepithelial PD along the proximal tubule. First, the use of low resistance electrodes suitable for the measurement of small transepithlial PD was evaluated. Next, the potential profile as a function of tubular length was examined. Third, the role of glucose and bicarbonate reabsorption and chloride concentration gradients in the generation of the observed PD was assessed. The data obtained were then examined with respect to the model for active and passive sodium reabsorption previously proposed (1, 2).

METHODS

Studies were performed on a mutant strain of Wistar rats in which glomeruli were present on the kidney surface. 24 male and 5 female animals, with weights ranging from 120 to 250 g, were anesthetized with Inactin (Promonta, Germany) 100 mg/kg body wt intraperitoneally and were prepared for routine micropuncture of the left kidney. Rectal temperature was monitored throughout each experiment and maintained between 36–38°C by adjustments in temperature of a heated micropuncture table. After tracheostomy (PE 240 tubing) a polyethylene catheter (PE 50) was inserted into the left external jugular vein for intravenous infusions. A catheter of similar size was inserted into the left femoral artery for blood sampling and a third catheter was placed into the bladder via a suprapubic incision to allow constant urine drainage during the experiment. At this stage of the surgical preparation, an infusion of Ringers-bicarbonate solution (Na, 140; K, 5; Cl, 115; bicarbonate 30 meq/liter) was commenced at the rate of 0.02 ml/min. The infusion was continued at this rate throughout the remainder of the control period. The left kidney was exposed through a flank incision, placed in a Lucite cup, and then surrounded with cotton and 2% agar in Ringers-bicarbonate solution. The surface of the kidney was covered with warmed Ringers-bicarbonate solution.

Experimental procedures

Experiments were divided into three broad groups according to their purpose. Group A, validation of techniques; Group B, comparison of early vs. late proximal PD; and Group C, examination of factors generating the proximal PD.

Group A: validation of techniques. These experiments were designed to validate the electrical measuring system used in this study. In particular, the use of electrodes with relatively large tips and low tip resistances was assessed. The actual procedures employed are presented under Results.

Group B: comparison of early vs. late proximal PD. There were 10 experiments in this group. Proximal tubular PD measurements were made in (*a*) immediate postglomerular loops and (*b*) randomly selected more distal loops of the proximal tubule. First loops were identified by injection of Ringers-bicarbonate solution, colored with 0.2% FD & C dye (Keystone Aniline & Chemical Co., Chicago, Ill.) into Bowman's space of accessible surface glomeruli. In some instances, it was possible to identify the first loop by its obvious connection with Bowman's capsule, and in such cases the PD measurement was performed before puncturing Bowman's capsule to verify the site of puncture. Ringers-bicarbonate solution was infused intravenously at 0.02 ml/min during these measurements.

Group C: examination of factors generating the proximal PD. The 17 experiments of this group were designed to evaluate the roles of glucose and bicarbonate reabsorption in the generation of the normal proximal transepithelial PD. Four different experimental procedures were employed.

Procedure 1. Administration of phloridzin during measurement of the PD in an early proximal loop. In 5 animals, the first proximal loop of a nephron was identified as described above. The measuring electrode was inserted into this loop and after a stable PD recording was achieved, 450 μg of phloridzin was injected intravenously while continuously monitoring the PD in the same tubular segment for 3–5 min after the injection. Glycosuria (detected by Combistix, Ames Co., Elkhart, Ind.) occurred in every experiment.

Procedure 2. Acetazolamide infusion. After control measurements in randomly selected proximal tubules, 12 animals were infused with acetazolamide, and further PD measurements made in random proximal segments. Six of these animals received an initial dose of acetazolamide of 20 mg/kg body wt, followed by a continuous infusion of 20 mg/kg body wt/h. The remaining six animals received half this dose, since the smaller dose was found to be much better tolerated while having a similar effect on PD measurements. During acetazolamide administration, the infusate was changed to 0.15 M sodium bicarbonate delivered at 0.04 ml/min to replace urinary losses of fluid and bicarbonate. Measurements of PD were commenced from 10–30 min after the initial dose of acetazolamide.

Procedure 3. Acetazolamide plus 10% glucose infusion. 7 of the 12 animals from procedure 2 were continued on to procedure 3. An infusion of 10% glucose at 0.1 ml/min was commenced in addition to the acetazolamide infusion. When glycosuria was detected, further PD measurements from randomly selected proximal tubules were made, beginning at times ranging from 14 to 90 min after the start of the glucose infusion. In three of these experiments, the glucose infusion rate was reduced to 0.075 ml/min after detection of glycosuria, whereas in the remaining four experiments, the infusion rate was maintained at 0.1 ml/min.

Procedure 4. Acetazolamide plus glucose phloridzin infusion. Four of the seven animals from procedure 3 were continued on to procedure 4. Phloridzin was administered in an initial dose of 450 μg followed by a constant infusion of 100 μg/kg/min. Acetazolamide and glucose infusions

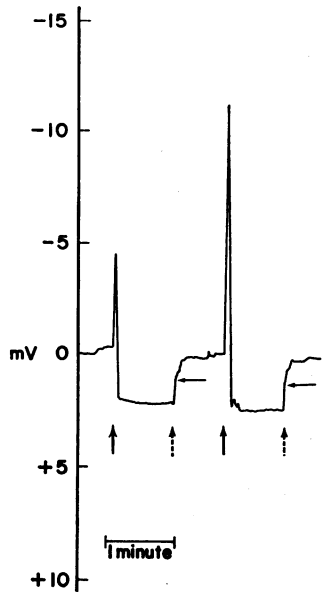

FIGURE 1 Recording of two successive measurements of transepithelial PD in randomly chosen proximal segments. Solid vertical arrows indicate tubular puncture while broken vertical arrows correspond to withdrawal of the electrode from the tubule. Horizontal arrows indicate the beginning of the "slow return phase" toward zero after withdrawal of the pipet from the tubule into the Ringers-bicarbonate solution covering the kidney. (See text)

were continued as for procedure 3. Further PD measurements were made from randomly selected proximal tubules commencing from 4 to 12 min after the initial dose of phloridzin. At the conclusion of these measurements, each of the four animals was terminally bled to allow determination of plasma electrolyte and glucose concentrations.

Electrical measuring system. Sharpened glass micropipets, with 3–5-μm tips (OD) filled with 3 M KCl and colored with either 0.4% lissamine green or 0.2% FD & C dye, were used as puncturing electrodes. These pipets were inserted into a Lucite chamber (also filled with 3 M KCl) and electrical contact was established with a Ag-AgCl electrode mounted in the chamber and connected to the input of a 31V Cary Vibrating Reed Electrometer (Cary Instruments, Monrovia, Calif.). The electrometer was connected to a Honeywell Elektronic 194 Recorder (Honeywell, Inc., Test Instruments Div., Denver, Col.). To permit the injection of KCl through the tip of the electrode, the Lucite chamber was equipped with a side arm which connected to a pressure injection apparatus filled with low viscosity silicon oil (Dow Corning 200 Dielectric fluid, Dow Corning Corp., Midland, Mich.). A reference calomel electrode, with a 3 M KCl bridge, made appropriate contact with the cut end of the rat's tail via a small container of Ringers-bicarbonate solution. The reference side of the circuit included; (*a*) a potentiometer which was used to set a suitable zero for the electrical system, and (*b*) a device by which a known voltage could be applied to allow calibration of the electrical system.

Method of measurement of transepithelial PD. With the measuring electrode in contact with the Ringers-bicarbonate solution covering the kidney surface, a nominal zero was set (needle of electrometer at center of electrometer dial) for the electrical system by means of the potentiometer on the reference side of the circuit. The pressure in the Lucite chamber of the electrode holder was adjusted via the fine pressure adjustment system attached to the side arm so that microscopically, a minute stream of colored 3 M KCl could be seen issuing from the tip of the measuring pipet into the Ringers-bicarbonate bath. The pipet was then lowered towards the kidney surface by means of a Leitz micromanipulator (E. Leitz, Inc., Rockleigh, N. J.) and a proximal tubule punctured along its longitudinal axis. Localization of the tip within the lumen was a simple procedure, since a rapid spontaneous flow of tubular fluid into the tip of the pipet occurred once this position had been achieved. This movement of fluid resulted from the higher pressure in the tubule compared with the Lucite chamber.

In a number of preliminary experiments, the validity of this localization technique was checked by increasing the pressure in the Lucite chamber and injecting a small amount of colored 3 M KCl solution from the electrode into the tubule after movement of tubular fluid into the pipet had occurred. This procedure resulted in the immediate development of a negative PD (mean approximately —10 mV) which was thought to represent a transepithelial potassium diffusion potential. In each instance the tubule was observed to fill with colored 3 M KCl solution, thus confirming the intraluminal position of the electrode tip. When the pressure in the Lucite chamber was reduced and the interface between the tubular fluid and 3 M KCl returned to its original position inside the tip of the pipet, a rapid return to the original PD reading occurred.

The technique used in the present study for localizing the electrode tip in the tubular lumen was therefore confined to the observance of a rapid and spontaneous flow of tubular fluid into the pipet tip after puncture. Within a few seconds of this event, a stable PD recording was usually observed. After allowing a stable recording for at least 30 s, the pipet was withdrawn from the tubule back into the Ringers-bicarbonate solution. This resulted in an immediate deflection back towards, but not to, the original zero, followed by a slower return phase coinciding with the slow but spontaneous expression of tubular fluid out of the pipet (Fig. 1). The failure of the PD recording to return immediately to its prepuncture zero position was attributed to the presence of a small liquid junction potential established between the tubular fluid remaining in the tip of the pipet and the Ringers-bicarbonate solution in the bath surrounding the kidney. In three experiments, control measurements were made on 20 randomly selected tubules and the slow return phase which coincided with the elimination of this liquid junction was found to average + 0.8 mV. This slow return phase was not observed after puncture of immediate postglomerular loops or after punctures performed during acetazolamide administration. Presumably, in these instances, no significant liquid junction potential existed between the tubular fluid and the Ringers-bicarbonate solution, since the tubular fluid concentrations of chloride and bicarbonate would be similar to that of the Ringers-bicarbonate bath.

The postpuncture zero was read when a small stream of colored 3 M KCl was again visible beyond the tip of the pipet. Only those readings were accepted in which the prepuncture zero and postpuncture zero differed by less than 0.3 mV and in which the flow of KCl from the pipet tip postpuncture was spontaneous. If there was a small dis-

crepancy in the zero reading pre- and postpuncture (< 0.3 mV), this difference was averaged to determine the mean zero position.

Because of the magnitude of the PD measurements, (all less than 3 mV), small changes in tip potential were critical and required frequent assessment. It was recognized that a shift of the tubular fluid: 3 M KCl interface from the pipet tip into the pipet might in itself result in a change in PD if a significant tip potential existed prepuncture. This problems was avoided by frequently checking between punctures to be certain that no significant change in PD from the set zero position occurred when Ringers-bicarbonate solution (from the pool above the kidney surface) was aspirated into the tip of the pipet. If a PD deflection occurred with this maneuver (almost invariably in a positive direction), the measuring pipet was discarded and replaced. If no deviation from zero was detected, the pressure in the Lucite chamber was increased again to return the KCl to the pipet tip and further punctures were performed. In addition, if any foreign material was visible at the pipet tip between punctures and attempts to clear this by temporarily increasing the flow of KCl through the tip were unsuccessful, then the pipet was discarded and replaced.

Chemical analysis. Plasma sodium and potassium were measured with an IL Flame photometer (Instrumentation Laboratory, Inc., Lexington, Mass.). Plasma chloride was measured with a Cotlove Chloridometer and total CO_2 content of plasma was measured with a Natelson microgasometer. Blood glucose was measured by a Technicon Autoanalyzer (potassium ferricyanide method) (Technicon Instruments Corp., Tarrytown, N. Y.).

Statistical analysis. Differences between mean PD and zero for each period were determined using a Student *t* test. Differences in mean PD between experimental periods were determined using standard variance analysis (18). All values are expressed as the mean ±1 SEM.

RESULTS

Group A. Validation of techniques

The mean PD obtained from 195 measurements made on randomly selected proximal tubules from 25 experiments during a control period was + 1.6±0.04 mV (lumen positive). This value does not include measurements made on specifically chosen first proximal loops.

As noted under Methods, the measuring pipets used in this study possessed much larger tips (3–5 μm OD) than the traditional Ling-Gerard electrodes (< 1 μm). These larger electrodes had a low tip resistance that was found to be consistently 1 MΩ or less. Because of this low tip resistance, remarkably stable PD recordings could be obtained. The major disadvantage of the larger electrode theoretically relates to the trauma inflicted on the wall of the tubule during puncture, and subsequent shunting of the PD through the damaged area which represents a low resistance shunt pathway.

To investigate the possibility that significant shunting of PD might be occurring as a result of the use of the larger electrodes; in three animals the mean PD obtained with electrodes with 3–5-μm tips was compared with the mean PD obtained using much smaller electrodes with tips of external diameter less than 1 μm. The smaller tips were sharpened in a similar fashion to the larger tips and were just visible under the stereomicroscope used in the present study. True Ling-Gerard electrodes were not used since in preliminary experiments the PD recordings obtained were found to be highly unstable and unsatisfactory for accurate interpretation. Intraluminal localization of the smaller electrode tips was confirmed by expression of colored 3 M KCl solution from the electrode, after which the tubular fluid: 3 KCl interface was moved into the tapered portion of the electrode by reducing the pressure in the Lucite chamber.

In these three experiments, the mean PD measured in 24 randomly selected proximal tubules using the larger electrodes was + 1.8±0.07 mV, whereas the mean PD measured in 20 tubules using the smaller electrodes was + 1.9±0.10 mV. There was no significant difference between these two values. It seems reasonable to suppose that if shunting of PD does occur, the degree of shunting should be directly related to the size of the pipet used. The finding that no significant difference existed between PD measurements made with electrodes with 3–5-μm tips and electrodes with < 1-μm tips argues against, but admittedly does not exclude, the possibility that significant shunting of PD was occurring in our measurements.

The small magnitude of the PD measured in the present study also required that possible artifacts arising from liquid junction potentials in the measuring system be critically evaluated. To explore this problem, three experiments were performed where proximal PD measurements were made during a control period using both a Ringers-bicarbonate-filled electrode and a 3 M KCl-filled electrode. In these three experiments, a mean PD of + 1.3±0.06 mV was obtained from 10 randomly selected proximal tubules using the regular 3 M KCl electrode. When this electrode was replaced with one containing Ringers-bicarbonate solution, the mean random proximal PD was found to be + 0.5±0.9 mV ($n = 27$). The difference between these two values was highly significant ($P < 0.001$).

The junctions in the electrical measuring system during tubular puncture using 3 M KCl electrodes are as follows: Ag-AgCl: 3 M KCl: tubular fluid: epithelium: Ringers-bicarbonate: 3 M KCl: Hg-$HgCl_2$. Assuming that the small difference in ionic gradients between the 3 M KCl: tubular fluid and 3 M KCl: Ringers-bicarbonate junctions introduces no significant asymmetry into the measuring system, it is apparent that the measured PD represents the actual transepithelial PD and therefore requires no theoretical correction for liquid junction potentials.

TABLE I

Summary of Mean Proximal Transepithelial PD from Random Tubular Punctures and First Loop Punctures in 10 Experiments

Experiment	Transepithelial PD	
	Random tubules	First loops
	mV	
2	+2.1 (4)	−0.5 (1)
4	+1.4 (12)	−1.0 (4)
5	+1.0 (5)	−0.5 (1)
6	+1.8 (9)	−0.5 (1)
7	+1.1 (8)	−0.6 (4)
8	+1.1 (8)	−1.0 (2)
10	+1.3 (7)	−0.9 (4)
11	+1.4 (10)	−1.0 (2)
15	+1.8 (6)	−0.5 (2)
19	+1.7 (8)	−1.0 (5)
Mean	+1.5	−0.8
SEM	±0.11	±0.07
n	10.0	10.0
P (Difference from 0)	<0.001	<0.001

n = number of experiments

Numbers in parentheses refer to number of proximal PD measurements in each experiment.

On the other hand, with a Ringers-bicarbonate-filled electrode, the following junctions are present during tubular puncture: Ag-AgCl : 3 M KCl : Ringers-bicarbonate : tubular fluid : epithelium : Ringers-bicarbonate : 3 M KCl : Hg-$HgCl_2$. Asymmetry is thus introduced by the presence of the Ringers-bicarbonate : tubular fluid junction, so that the measured PD should be different from the true transepithelial PD. The true transepithelial PD can be derived, however, by making a theoretical correction for the Ringers-bicarbonate : tubular fluid liquid junction potential, using the Henderson equation for a bi-ionic junction (19). By assuming; (*a*) that tubular fluid from randomly selected proximal segments contains Na, 140; Cl^-, 135; and HCO_3, 10 meq/liter; (*b*) that the composition of the Ringers-bicarbonate solution is Na, 140; Cl, 115; and HCO_3, 30 meq/liter; (*c*) that the activity coefficients of these ions are 1.0, and (*d*) that the relative free diffusional mobilities of these three ions (giving Cl^- an arbitrary value of unity) are Cl^-, 1.00; HCO_3, 0.58; Na, 0.67 (16), the calculated liquid junction PD is − 0.97 mV. Thus, the measured PD should differ from the true transepithelial PD by 0.97 mV. In fact, when this value is added to the PD measured with the Ringers-bicarbonate-filled electrode (+ 0.5 mV), a value of + 1.47 mV is obtained. This value agrees closely with the transepithelial PD of + 1.3 mV measured with the 3 M KCl-filled electrode.

Our experiments also permitted us to determine experimentally a value for the Ringers-bicarbonate : tubular fluid liquid junction potential. It was previously noted that after a proximal PD measurement in the control state with a 3 M KCl-filled electrode, withdrawal of the pipet from the tubular lumen into the Ringers-bicarbonate bath surrounding the kidney resulted in an immediate rapid deflection of the PD recording back toward zero, followed by a slower return phase, coinciding with the spontaneous expression of tubular fluid from the tip of the pipet into the bath (Fig. 1). This latter phase was attributed to the presence of a liquid junction potential established between the tubular fluid in the pipet tip and the surrounding Ringers-bicarbonate solution. This obviously represents the same junction as that discussed above, but with reversed polarity. The PD attributed to this junction was assessed in 20 punctures in three rats by specifically measuring the magnitude of the slow return phase after withdrawal of the pipet from the tubule after a transepithelial PD measurement, and was found to be 0.8 mV. This value of 0.8 mV, which represents an experimentally derived potential for the Ringers-bicarbonate : tubular fluid liquid junction is obviously comparable with that derived theoretically above (0.97 mV). The small difference between these two values probably reflects a minor discrepancy between the actual composition of the tubular fluid and the composition assumed in the theoretical calculation presented above.

When the experimentally derived PD for the Ringers-bicarbonate : tubular fluid liquid junction (0.8 mV) is added to the PD measured with the Ringers-bicarbonate electrode (0.5 mV), a value of + 1.3 mV is obtained for the transepithelial PD. This value is identical with that measured directly with the 3 M KCl electrode. The similarity of results obtained with electrodes filled with different solutions suggests that the small positive PD measured in randomly punctured proximal tubules is not the consequence of some unidentified artifactual liquid junction PD in the system.

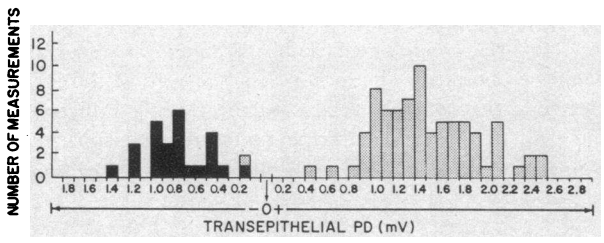

FIGURE 2 Comparison of transepithelial PD measured in first postglomerular segments (solid bars) and randomly chosen proximal segments (shaded bars). A clear-cut separation between the two groups of measurements is apparent.

TABLE II

Summary of Mean Proximal Transepithelial PD from Random Tubular Punctures during Control States (C), Acetazolamide (A), Acetazolamide + Glucose (A+G) and Acetazolamide + Glucose + Phloridzin (A+G+P) Infusion

Experiment	Transepithelial PD			
	C	A	A+G	A+G+P
			mV	
1	+1.4 (3)	0 (3)	—	—
3	+1.0 (9)	−0.1 (10)	—	—
6	+1.8 (9)	+0.1 (13)	—	—
9	+1.5 (14)	−0.2 (11)	—	—
12	+1.3 (6)	−0.1 (10)	—	—
13	+1.3 (10)	0 (6)	−0.8 (12)	—
14	+1.8 (11)	−0.1 (14)	−0.6 (13)	—
15	+1.8 (6)	+0.1 (10)	−0.6 (13)	—
16	+1.5 (5)	+0.1 (5)	−0.7 (6)	0 (7)
17	+2.0 (6)	+0.2 (7)	−0.6 (8)	+0.5 (11)
18	+1.2 (8)	+0.1 (9)	−0.5 (9)	+0.1 (10)
19	+1.7 (8)	0 (10)	−0.5 (8)	+0.3 (11)
Mean	+1.5	0	−0.6	+0.2
SEM	±0.08	±0.03	±0.03	±0.10
n	12	12	7	4
P (Difference from 0)	<0.001	NS	<0.001	NS

n = number of experiments

Numbers in parentheses refer to number of proximal PD measurements in each experiment.

Group B. Comparison of early vs. late proximal PD

The results of 26 first loop PD measurements and 77 PD measurements in randomly selected more distal proximal segments in 10 rats in the control state are shown in Table I and Fig. 2. The mean first proximal loop PD was − 0.8±0.07 mV whereas the mean PD obtained in randomly selected tubules was + 1.5±0.11 mV. Both of these values differed significantly from zero ($P < 0.001$) and from each other ($P < 0.001$).

Group C. Examination of factors generating the proximal PD

Procedure 1. Administration of phloridzin during measurement of the PD in an early proximal loop. These experiments were designed to define the role of active glucose reabsorption in the generation of the negative PD found in the first postglomerular loops of the rat proximal tubule. Five animals received an intravenous injection of phloridzin during continuous monitoring of the PD in an early proximal loop. In each instance the small negative PD changed in a positive direction from a mean of − 0.8±0.11 to + 0.3±0.15 mV. This change was highly significant ($P < 0.001$).

Procedure 2. Acetazolamide infusion. This drug was administered to inhibit proximal bicarbonate reabsorption and prevent the development of significant chloride concentration gradients along the proximal tubule.

Table II compares the results of 95 random PD measurements made during the control period with 108 measurements after acetazolamide infusion in 12 rats. The mean control PD of + 1.5±0.08 mV decreased significantly to 0±0.03 mV ($P < 0.001$) after acetazolamide infusion. The mean PD during this period was the same in six rats infused with 20 mg/kg body wt/h acetazolamide (0±0.07 mV) as in six rats infused with 10 mg/kg body wt/h (+ 0.1±0.07 mV).

Procedure 3. Acetazolamide plus 10% glucose infusion. These experiments were performed to determine whether, in the absence of a significant chloride gradient, the later segments of the proximal tubule were able to reabsorb glucose actively and generate a negative PD comparable with that found in the earliest proximal loops under control conditions.

7 of the 12 experiments from procedure 2 were continued on to procedure 3. Table II illustrates the results of these seven experiments. The mean control PD fell from + 1.6±0.06 ($n = 54$) to + 0.1±0.07 mV ($n = 61$) after acetazolamide ($P < 0.001$). With the addition of a glucose infusion, the PD became negative with a mean value of − 0.6±0.06 mV ($n = 69$). This value differed significantly from zero ($P < 0.001$) and from the mean acetazolamide value ($P < 0.001$).

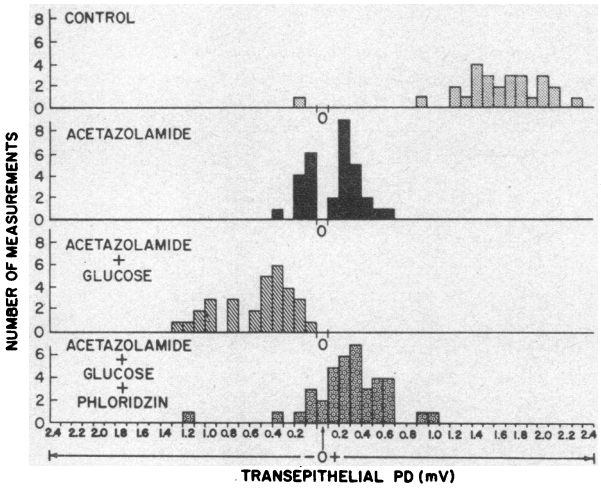

FIGURE 3 Distribution of transepithelial PD measurements made in randomly chosen proximal segments during control state, acetazolamide infusion, acetazolamide plus 10% glucose infusion, and acetazolamide plus 10% glucose plus phloridzin infusion.

Procedure 4. Acetazolamide and glucose and phloridzin infusion. In an attempt to confirm that active reabsorption of glucose was responsible for the generation of the negative PD observed during procedure 3, the drug phloridzin, a known inhibitor of glucose reabsorption in the proximal tubule (20), was subsequently administered, and further PD measurements were made in four of the seven animals from procedure 3. In these four animals the mean control PD was $+1.6\pm0.09$ mV ($n=27$). After acetazolamide, the mean PD fell to $+0.1\pm0.04$ mV ($n=31$) and subsequently became negative, -0.6 ± 0.06 mV ($n=31$) with the addition of a glucose infusion (procedure 3). Infusion of phloridzin resulted in a change in PD back to a mean value of $+0.2\pm0.06$ mV ($n=39$) which differed significantly from the value associated with procedure 3 ($P<0.001$) but did not differ significantly from the value associated with procedure 2 (acetazolamide infusion alone). The results of these four experiments are depicted in Table II and Fig. 3. At the conclusion of these four experiments, the following plasma electrolyte values were obtained: Na^+, 140 ± 2.4; K^+, 3.6 ± 0.13; Cl^-, 104 ± 1.7; HCO_3^-, 21.0 ± 0.8 meq/liter. The mean blood sugar level was 40 ± 73 mg/100 ml.

DISCUSSION

The present study indicates that the transepithelial PD in randomly selected proximal tubules has a mean value of $+1.6$ mV. Since this value is very small and differs in orientation from values reported by most other investigators (3–9, 14–16), it is essential that the electrical measuring system be stable and free of systematic artifacts. For this reason, the electrodes used in this study possessed large tips (3–5 μm OD) and hence had very low tip resistances (<1 MΩ). The use of larger electrodes for the measurement of transepithelial PD has recently been suggested by Frömter (12), while Laurence and Marsh (21) have validated their use in measurements of the PD in the distal tubule and collecting duct of the hamster.

These electrodes are particularly well suited to the detection of small potentials, since their low tip resistance permits remarkably stable recordings whereas their large tip size facilitates intraluminal localization and minimizes plugging of organic material from the tubular wall. Moreover, since the 3 M KCl: tubular fluid interface is shifted away from the tip and into the tapered portion of the electrode during PD measurements, the possibility of KCl leakage from the pipet tip (which would result in a negative potassium diffusion potential) is eliminated.

The single theoretical disadvantage of large electrodes concerns the recording of falsely low PD measurements owing to excessive trauma to tubular wall resulting in shunting of PD through the damaged area. Several lines of evidence argue against significant shunting. Our mean measured PD of $+1.6$ mV using large electrodes (3–5-μm tips) is not significantly different from our results with smaller electrodes (tips <1 μm) or from the value of 1.8 mV reported by Frömter (12) using Ling-Gerard electrodes. Moreover, the stability of the PD recording indicates that progressive shunting did not occur. Finally, the ready localization of the larger pipet tips reduces the necessity for manipulation after puncture so that tubular damage may be less than with the small Ling-Gerard electrodes which require extensive movement to establish that the pipette tip is in an intraluminal position.

The most unique feature of the present studies which has not been reported previously is the finding that the transepithelial PD varies along the length of the proximal tubule. Of the PD measurements made in first proximal loops, all 26 were found to be negative with a mean of -0.8 mV and a range from -0.2 to -1.4 mV (Table I, Fig. 2). In later proximal segments, 195 PD measurements were made. All but three of these had a positive value. The mean for the entire group was $+1.6$ mV with a range from -1.0 to $+2.5$ mV. We have thus demonstrated a consistent profile of PD values for the proximal tubule under normal conditions with the earliest segments consistently having a negative PD in contrast to the later segments which have a positive PD.

The different PD values obtained in the early and late proximal tubule may represent either differences in the intrinsic electrical properties of these different

segments, or compositional differences between the early and late proximal tubular fluid. In a recent study from our laboratory, Kokko (17) found that the PD in an in vitro perfused isolated rabbit proximal tubule was dependent on the composition of the perfusion fluid. When the perfusate simulated the ultrafiltrate of normal rabbit serum, the transepithelial PD was approximately − 6 mV. When glucose and amino acids were removed from this perfusate, the PD fell to approximately zero. Perfusion of the tubule with a fluid resembling late proximal tubular fluid (Cl, 143; HCO_3, 5 meq/liter, zero glucose, zero amino acids) resulted in a mean PD of + 3.2 mV. On the basis of these observations, Kokko suggested that active reabsorption of glucose and amino acids generated a negative PD, whereas, in the absence of these two substances, a high tubular fluid chloride concentration (relative to plasma) could generate a positive diffusion potential. Reabsorption of sodium bicarbonate was considered to be an active but nonelectrogenic process.

Extrapolating these results to the in vivo situation, it was predicted that the PD in the early and late proximal tubule should differ in magnitude and orientation. Close to the glomerulus, the tubular fluid should contain all the constituents of a plasma ultrafiltrate. Consequently, the active reabsorption of glucose and amino acids should generate a negative PD, assuming that the active reabsorption of bicarbonate proximal to the puncture site would be insufficient to generate a significant chloride concentration gradient. It has been demonstrated in previous micropuncture studies that the majority of bicarbonate (22–24), glucose (25), and amino acid (26) reabsorption transpires early in the proximal tubule leaving little of these substrates in the tubular fluid reaching the more distal portions. As a result of the reabsorption of these solutes with isosmotic amounts of water, the proximal tubular fluid chloride concentration has been shown to rise significantly above plasma concentration, achieving TF : P chloride ratios of approximately 1.3 (22–27). Thus, in the more distal portions of the proximal tubule, none of the substrates necessary for the generation of negative PD are present, and the high chloride concentration should generate a positive diffusion PD.

The results of the present in vivo study support these predictions in that the early proximal PD was found to be negative whereas the mean PD in randomly selected more distal segments was found to be significantly positive. However, for reasons that are not clear, the magnitude of the PD measurements we observed in vivo in the rat was considerably smaller than the magnitude of those measurements made by Kokko (17) in the in vitro perfused isolated rabbit tubule. In the present study, the mean PD measured in the first postglomerular loop was − 0.8 mV, significantly less than the − 6 mV recorded in the perfused rabbit tubule. The reason for this difference is unknown, but several possible explanations may be proposed. First, it is possible that this discrepancy represents a species difference. Second, it may reflect a difference between the in vivo and in vitro experimental situation, possibly some degree of shunting in vivo. Third, it is possible that in vivo the composition of the tubular fluid may have already been modified by reabsorptive processes proximal to the puncture site. Evidence for this suggestion is found in those experiments in which administration of phloridizin changed the first loop PD from − 0.8 to + 0.3 mV. The small positive PD remaining after administration of phloridzin suggests that a small concentration gradient for chloride had already been established proximal to the site of puncture. These data also suggest that the reabsorption of glucose, but not of amino acids, is the principal mechanism responsible for the generation of the negative PD in the early proximal tubule in vivo. It is possible that amino acid reabsorption is in fact electrogenic but cannot be detected because it is small in magnitude and obscured by the chloride diffusion potential.

The positive PD in the later portions of the proximal tubule appears to be the consequence of a passive chloride diffusion potential. Previous micropuncture studies from our laboratory (22, 28) have shown that under conditions similar to those in the present studies, tubular fluid chloride concentration rises significantly above that of plasma along the proximal tubule achieving TF : P chloride ratios of approximately 1.30. If the tubule were permeable only to chloride, this ratio could theoretically give rise to a transepithelial PD of + 7.0 mV, as predicted by the Nernst equation (29). However, since the tubule is also permeable to sodium, bicarbonate, and other ions, the chloride concentration gradient would give rise to a much smaller PD, depending on the relative permeability of the tubule to chloride, sodium, and bicarbonate. In support of the proposal that the positive PD in more distal portions of the proximal tubule represents a passive chloride diffusion potential, administration of acetazolamide, which has been demonstrated in previous micropuncture studies (24, 27) to inhibit, bicarbonate reabsorption and prevent a rise in tubular fluid chloride concentration along the proximal tubule, was found in the present study to reduce the mean proximal PD from + 1.5 to 0 mV (Table II, Fig. 3).

The more distal portions of the proximal tubule do not appear to differ intrinsically from the earlier portions since they were also able to generate a negative PD when actively reabsorbing glucose in the absence of a significant transepithelial chloride concentration gradient. This was demonstrated by those experiments in which

10% glucose was infused in association with acetazolamide. The mean random tubular PD of 0 mV associated with acetazolamide infusion alone changed significantly to − 0.6 mV when an infusion of 10% glucose was superimposed (Table II, Fig. 3). This appeared to be an effect specifically related to active glucose reabsorption since the subsequent administration of phloridzin promptly returned the PD to approximately zero again (Table II, Fig. 3).

Two possible mechanisms might explain the generation of a negative transepithelial PD during glucose reabsorption. The first mechanism, based on an earlier hypothesis by Crane (30, 31), suggests that sodium enters the cell across the luminal membrane via a carrier mechanism that requires the presence of glucose. The sodium is subsequently actively transported out of the cell and thus gives rise to a negative transepithelial PD. The second possibility is that glucose reabsorption, by generating local osmotic gradients, pulls a solution of sodium chloride through negatively charged pores and thus generates a streaming potential. In a recent microperfusion study of the proximal tubule of the Japanese newt, Maruyama and Hoshi (32) found that the addition of glucose to the luminal perfusing solution generated a negative transepithelial PD by a mechanism which depolarized the luminal cell membrane. This finding constitutes evidence against streaming potentials and suggests that glucose generates a negative PD by its effect on the entry of sodium into the cell.

Our results suggest that in the presence of glucose, the entire length of the proximal tubule has the capacity for electrogenic sodium transport. Since sodium bicarbonate reabsorption is considered to be a nonelectrogenic process, these results suggest that there is a component of active sodium transport which is independent of hydrogen secretion but dependent on glucose reabsorption and which can effect net sodium chloride reabsorption. This electrogenic sodium transport appears to be specifically dependent on glucose reabsorption since administration of phloridzin eliminated the small negative PD associated with glucose plus acetazolamide infusion (Table II, Fig. 3). The fact that glucose reabsorption is normally completed in the first 15–20% of the proximal tubule when the TF/P inulin ratio has risen only to a minor extent suggest that glucose-dependent active sodium reabsorption is small in amount and limited to the early proximal tubule.

The failure to demonstrate an electrogenic sodium transport system in the more distal segments of the proximal tubule in the absence of glucose raises the question as to the mechanism responsible for isotonic sodium chloride reabsorption in this area. Two forces might account for passive reabsorption of sodium chloride. First, the diffusion of chloride down its concentration gradient generating a positive PD serves as an electrical driving force for sodium reabsorption. Second, the effective osmotic force generated across the tubular epithelium by differences in the reflection coefficients of NaCl, $NaHCO_3$, and glucose would promote water reabsorption and, along with it, some NaCl reabsorption.

It is not clear, however, whether these forces could account for the observed rates of sodium chloride reabsorption. It can be estimated from free-flow micropuncture studies performed under conditions similar to those in the present experiments that the magnitude of isotonic NaCl reabsorption in the later part of the proximal tubule is approximately 2 nl mm^{-1} min^{-1} or 0.3 neq mm^{-1} min^{-1} for both Na^+ and Cl^-.

The electrochemical gradients for Na^+ and Cl^- are unequal. Since there is no concentration gradient for sodium, the electrochemical driving force can be accounted for solely by the PD of + 1.6 mV. Using the published value for sodium permeability in the rat proximal tubule of 15×10^{-7} cm^2 s^{-1} (33), passive diffusion could account for only one-third of the observed rate of sodium reabsorption, or 0.1 neq mm^{-1} min^{-1}. By contrast, passive outward diffusion of chloride is driven by a large chemical concentration gradient of approximately 25 meq/liter, which is opposed by the very small PD. Using the published value for chloride permeability of 10×10^{-7} cm^2 s^{-1} (33), passive movement down its concentration gradient could account for about two-thirds of the observed rate of chloride reabsorption, or 0.2 neq mm^{-1} min^{-1}.

The second force for passive reabsorption, the effective osmotic pressure, affects sodium and chloride equally (by promoting the bulk flow of fluid). Using the published value of 0.7 for the NaCl reflection coefficient (34), it is estimated that bulk flow accounts for approximately one-third of the observed rate of Na and Cl reabsorption.

Thus, the sum of the electrochemical and osmotic driving forces can account for all of the Cl^- reabsorption but only two-thirds of the sodium reabsorption. This disparity may be due to inaccuracies in the various permeability coefficients utilized in the calculations. On the other hand, the disparity might be attributable to still a third process.

It is possible that about one-third of the chloride movement is associated with a commensurate inward diffusion of bicarbonate down its steep electrochemical gradient. Microperfusion studies by Bank and Aynedjian (35) clearly demonstrated that bicarbonate readily diffused into HCO_3^--free fluids used to perfuse that rat proximal tubule. It is attractive to suggest as a hypothesis that the inward leak of HCO_3^- permits the re-

maining one-third of Na^+ to be reabsorbed via an active process coupled to H^+ secretion. This would not be inconsistent with the positive PD since sodium bicarbonate reabsorption appears to be nonelectrogenic. Such a theory is supported by the finding (36) that the reabsorption of the shrinking drop of saline is inhibited 30–40% by acetazolamide. Moreover, Bank and Aynedjian (35) have demonstrated that when the proximal tubule is perfused with a HCO_3^--free solution, HCO_3^- accumulates in the tubular fluid only after hydrogen secretion is inhibited by acetazolamide.

We would suggest, therefore, that the reabsorption of the glomerular filtrate in the proximal tubule is a biphasic process. Early in the proximal tubule, glucose, amino acids, and $NaHCO_3$ are reabsorbed actively along with isosmotic amounts of water. As a consequence, the concentration of Cl^- in the residual tubular fluid is raised above that of plasma and the more distal portions of the proximal tubule are presented with essentially isomotic sodium chloride solution (with zero or minimal concentrations of glucose, amino acids, and sodium bicarbonate). Of the total sodium reabsorption in this portion of the tubule, approximately one-third appears to be due to passive diffusion driven by a positive PD, approximately one-third occurs by bulk flow of solution driven by an effective osmotic pressure across the tubule, and approximately one-third occurs by active Na^+-H^+ exchange driven by the inward passive diffusion of bicarbonate. It is of interest that the contribution of these various processes estimated from free-flow micropuncture experiments is in agreement with similar estimates recently made by Ullrich, Sauer, and Fromter (33) using stationary microperfusion techniques.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance of Kay Williams in preparing the manuscript.

This work was supported in part by U. S. Public Health Service Grant 1 PO1 HE 11662.

REFERENCES

1. Rector, F. C., Jr., M. Martinez-Maldonado, F. P. Brunner, and D. W. Seldin. 1966. Evidence for passive reabsorption of NaCl in proximal tubule of rat kidney. *J. Clin. Invest.* **45**: 1060. (Abstr.)
2. Kokko, J. P., F. C. Rector, Jr., and D. W. Seldin. 1970. Mechanism of salt and water reabsorption in proximal convoluted tubule (PCT). Proceedings of the American Society of Nephrology. **4**: 42. (Abstr.)
3. Clapp, J. R., F. C. Rector, Jr., and D. W. Seldin. 1962. Effect of unreabsorbed anions on proximal and distal transtubular potentials in rats. *Am. J. Physiol.* **202**: 781.
4. Bloomer, H. A., F. C. Rector, Jr., and D. W. Seldin. 1963. The mechanism of potassium reabsorption in the proximal tubule of the rat. *J. Clin. Invest.* **42**: 277.
5. Solomon, S. 1957. Transtubular potential differences of rat kidney. *J. Cell. Comp. Physiol.* **49**: 351.
6. Bank, N. 1962. Relationship between electrical and hydrogen ion gradients across rat proximal tubule. *Am. J. Physiol.* **203**: 577.
7. Kashgarian, M., H. Stöckle, C. W. Gottschalk, and K. J. Ullrich. 1963. Transtubular electrochemical potentials of sodium and chloride in proximal and distal renal tubules of rats during antidiuresis and water diuresis (diabetes insipidus). *Pfluegers Arch. Gesamte Physiol. Menschen Tiere.* **277**: 89.
8. Malnic, G., R. M. Klose, and G. Giebisch. 1964. Micropuncture study of renal potassium excretion in the rat. *Am. J. Physiol.* **206**: 674.
9. Marsh, D. J., and S. Solomon. 1964. Relationship of electrical potential differences to net ion fluxes in rat proximal tubules. *Nature (Lond.).* **201**: 714.
10. Frömter, E., and U. Hegel. 1966. Transtubulare potentialdifferenzen an proximalen und distalen Tubuli der Rattenniere. *Pfluegers Arch. Gesamte Physiol. Menschen Tiere.* **291**: 107.
11. Frömter, E., T. Wick, and U. Hegel. 1967. Untersuchungen uber die Ausspritzmethode zur Lokalisation der Mikroelectrodenspitze bei Potentialnessungen am proximalen Konvolut der Rattenniere. *Pfluegers Arch. Gesamte Physiol. Menschen Tiere.* **294**: 265.
12. Frömter, E. 1972. Progress in microelectrode techniques for kidney tubules. *Yale J. Biol. Med.* **45**: 414.
13. Maude, D. L. 1969. Mechanism of NaCl transport by mammalian proximal tubule. *Fed. Proc.* **28**: 589.
14. Burg, M. B. 1969. Transport characteristics of isolated perfused proximal tubules. *In:* Renal Transport and Diuretics, International Symposium Feldafing. K. Thurau and H. Johrmärker, editors. Springer-Verlag kG, Berlin. 109.
15. de Mello, G. B., and G. Malnic. 1970. Algumas caracteristicas electrofysiológicas de túbulos proximais de rins de rato. *Proc. Congr. Soc. Brasileira Paro Progr. Ciencia, 22nd.* 326.
16. Boulpaep, E. L., and J. F. Seely. 1971. Electrophysiology of proximal and distal tubules in the autoperfused dog kidney. *Am. J. Physiol.* **221**: 1084.
17. Kokko, J. P. 1973. Proximal tubule potential difference. Dependence on glucose, HCO_3, and amino acids. *J. Clin. Invest.* **52**: 1362.
18. Snedecor, G. W. 1956. Statistical Methods. The Iowa State College Press, Ames, Iowa. 5th edition.
19. Barry, P. H., and J. M. Diamond. 1970. Junction potentials, electrode standard potentials, and other problems in interpreting electrical properties of membranes. *J. Membrane Biol.* **3**: 93.
20. Chan, S. S., and W. D. Lotspeich. 1962. Comparative effects of phloridzin and phloretin on glucose transport in the cat kidney. *Am. J. Physiol.* **203**: 975.
21. Laurence, R., and D. J. Marsh. 1971. Effect of diuretic states on hamster collecting duct electrical potential differences. *Am. J. Physiol.* **220**: 1610.
22. Rector, F. C., Jr. 1964. Micropuncture studies on the mechanism of urinary acidification. Renal Metabolism and Epidemiology of Some Renal Disease. Proceedings 15th Annual Conference on the Kidney. J. Metcoff, editor. National Kidney Foundation. 1st edition. 9.
23. Malnic, G., F. Lacaz Vieira, and H. Enokibara. 1965. Effect of furosemide on chloride and water excretion in single nephrons of the kidney of the rat. *Nature (Lond.).* **208**: 80.

24. Malnic, G., M. Mello Aires, and F. Lacaz Vieira. 1970. Chloride excretion in nephrons of rat kidney during alterations of acid-base equilibrium. *Am. J. Physiol.* **218**: 20.
25. Frohnert, P. P., B. Hohmann, R. Zwiebel, and K. Baumann. 1970. Free flow micropuncture studies of glucose transport in the rat nephron. *Pfluegers Arch. Gesamte Physiol. Menschen Tiere.* **315**: 66.
26. Weise, M., G. M. Eisenbach, and H. Stolte. 1971. Mikropunktionsuntersuchungen über die Resorption freier Aminosäuren im Einzelnephron der Säugetierniere. *Symp. Ges. Nephrol. 7th 1971.* 161.
27. Kunau, R. T., Jr. 1972. The influence of the carbonic anhydrase inhibitor, Benzolamide (CL-11,366), on the reabsorption of chloride, sodium, and bicarbonate in the proximal tubule of the rat. *J. Clin. Invest.* **51**: 294.
28. Rector, F. C., Jr., H. A. Bloomer, and D. W. Seldin. 1964. Effect of potassium deficiency on the reabsorption of bicarbonate in the proximal tubule of the rat kidney. *J. Clin. Invest.* **43**: 1976.
29. Wilde, W. S. 1945. The chloride equilibrium in muscle. *Am. J. Physiol.* **143**: 666.
30. Crane, R. K. 1962. Hypothesis for mechanism of intestinal active transport of sugar. *Fed. Proc.* **21**: 891.
31. Crane, R. K. 1968. Absorption of sugars. *Handb. Physiol.* **3** (Sect. 6): 1323.
32. Maruyama, T., and T. Hoshi. 1972. The effect of D-glucose on the electrical potential profile across the proximal tubule of newt kidney. *Biochim. Biophys. Acta.* **282**: 214.
33. Ullrich, K. J., F. Sauer, and E. Frömter. 1972. Transport parameters for sodium, chloride and bicarbonate in the proximal tubules of the rat kidney. Recent Advances in Renal Physiology. International Symposium on Renal Handling of Sodium, Brestenberg, 1971. H. Wirz and F. Spinelli, editors. AG. S. Karger, Basel.
34. Bauman, K., E. Frömter, and K. Ullrich. 1967. Passiver Stofftransport durch Epithelzellschicht von Harnkanalchen. *Ber. Bunsenges. Phys. Chem.* **71**: 834.
35. Bank, N., and H. S. Aynedjian. 1967. A microperfusion study of bicarbonate accumulation in the proximal tubule of the rat kidney. *J. Clin. Invest.* **46**: 95.
36. Radtke, H. W., G. Rumrich, E. Kinne-Saffran, and K. J. Ullrich. 1972. Dual action of acetazolamide and furosemide on proximal volume reabsorption in the rat kidney. *Kidney Int.* **1**: 100.