

# Measurements of Electrical Potential Differences on Single Nephrons of the Perfused *Necturus* Kidney

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**ABSTRACT** Stable electrical potential differences can be measured by means of conventional glass microelectrodes across the cell membrane of renal tubule cells and across the epithelial wall of single tubules in the doubly perfused kidney of *Necturus*. These measurements have been carried out with amphibian Ringer's solution, and with solutions of altered ionic composition. The proximal tubule cell has been found to be electrically asymmetrical inasmuch as a smaller potential difference is maintained across the luminal cell membrane than across the peritubular cell boundary. The tubule lumen is always electrically negative with respect to the peritubular extracellular medium. Observations on the effectiveness of potassium ions in depolarizing single tubule cells indicate that the transmembrane potential is essentially an inverse function of the logarithm of the external potassium concentration. The behavior of the peritubular transmembrane potential resembles more closely an ideal potassium electrode than that of the luminal transmembrane potential. From these results, and the effects of various ionic substitutions on the electrical profile of the renal tubular epithelium, a thesis concerning the origin of the observed potential differences is presented. A sodium extrusion mechanism is considered to be located at the peritubular cell boundary, and reasons are given for the hypothesis that the electrical asymmetry across the proximal renal tubule cell could arise as a consequence of differences in the relative sodium and potassium permeability at the luminal and peritubular cell boundaries.

This paper describes the effects of changes in the ionic environment on electrical potential gradients maintained across proximal renal tubule cells of *Necturus maculosus*. Normally the cell interior is asymmetrically negative with respect to tubular and interstitial fluid. Since the tubular fluid itself is electro-negative with respect to interstitial fluid, the potential difference across the luminal cell membrane is less than that across the peritubular membrane (1-4). The effects of various ion species on these electrical potential gradients can be studied in the doubly perfused kidney of *Necturus*. Such measurements,

in conjunction with knowledge of respective ionic concentration gradients, provide information on permeability characteristics of the luminal and peritubular membranes and throw light on the origin of the observed potential differences. They also permit some conclusions concerning the role of each membrane in tubular transport processes.

An account of some of these experiments has been presented previously (5, 6).

#### EXPERIMENTAL METHODS

All experiments were performed at room temperature on adult *Necturi*, anesthetized with urethane according to Walker and Reisinger (7). Double perfusion of the left kidney was carried out following the techniques described by Cullis (8) and Höber (9). First, the aorta was exposed at midthoracic level and cannulated with a polyethylene catheter. Subsequently, two lateral abdominal incisions were made and the cephalad end of the anterior abdominal vein ligated. The lower part of the anterior abdominal wall was everted and the caudal part of the anterior abdominal vein also cannulated. Although it is possible to cannulate the renal portal vein directly at the lower kidney pole, most perfusions were done *via* the anterior abdominal vein which drains into the renal portal system. Perfusion was then begun from bottles placed above the kidney. The arterial vessel was kept 20 to 40 cm. above the kidney, the portal vessel at half this height. A small rotameter in each circuit assured a constant perfusion rate throughout the experiment (10). Lastly, the postcaval vein was cannulated rostral to the kidneys and mixed arterial and portal perfusate collected. In many experiments ureteral urine was also collected.

Some tests were performed during control perfusions to study the functional integrity of the perfused kidney preparation. These included (a) the degree of which inulin and creatinine are concentrated in the final urine, (b) the degree to which inulin is concentrated in samples of proximal tubule fluid, (c) the ability of the kidney to reduce the sodium and chloride concentration in the final urine below that of the perfusion fluid, and (d) the completeness of tubular epithelial impermeability to inulin and creatinine. The results of these studies indicate that the kidney functions adequately for a period of 4 hours. Its tubules reabsorb water from the glomerular filtrate as indicated by the degree to which inulin is concentrated in fluid samples collected from the proximal tubules, and in the final urine (11, 12). Mean ratios of 1.32 (standard error  $\pm 0.06$ , 6 urine samples) and of 1.25 (standard error  $\pm 0.07$ , 6 samples of proximal tubule fluid) were observed and are consistent with only minor reduction of water reabsorption. Also, the capacity to elaborate an osmotically dilute urine was only moderately diminished (13). Thus the ratio of the sodium concentration in urine/perfusion fluid averaged 0.20 (standard error  $\pm 0.03$ , 10 observations), and that of the chloride concentration was similar (0.22, 0.28, 0.31). The appearance of inulin or creatinine in the final urine was also tested for when these compounds were added to the portal perfusion circuit only. In *Necturus* both these substances enter the tubules exclusively by way of glomerular filtration (12). Any abnormal permeability of the tubular epithelium or an excessive contribution of portal fluid to the glomerular fil-

trate due to pressure imbalances between arterial and portal perfusion circuits would be revealed by either inulin or creatinine being excreted in significant amounts. We have found the highest concentration in ureteral urine not to exceed 5 per cent of the portal concentration (8 collection periods). Accordingly, no evidence for excessive leakiness of the renal tubule to these substances, typical for the poisoned or deteriorating kidney preparation (14), was found. These experiments confirm earlier studies (9) which had indicated that many renal functions in the perfused amphibian kidney are adequately maintained over several hours (9).

TABLE I  
COMPOSITION OF PERFUSION FLUIDS

Bicarbonate-Ringer		Phosphate-Ringer		Potassium-Ringer	
NaCl	95.0 mm/l.	NaCl	95.0 mm/l.	K <sub>2</sub> HPO <sub>4</sub>	2.0 mm/l.
KCl	4.5 "	K <sub>2</sub> HPO <sub>4</sub>	2.0 "	KH <sub>2</sub> PO <sub>4</sub>	0.4 "
NaHCO <sub>3</sub>	10.0 "	KH <sub>2</sub> PO <sub>4</sub>	0.4 "	CaCl <sub>2</sub>	1.8 "
CaCl <sub>2</sub>	1.8 "	CaCl <sub>2</sub>	1.8 "	MgCl <sub>2</sub>	1.0 "
NaH <sub>2</sub> PO <sub>4</sub>	0.8 "	MgCl <sub>2</sub>	1.0 "	Glucose	2.2 "
MgCl <sub>2</sub>	1.0 "	Glucose	2.2 "	K <sub>2</sub> SO <sub>4</sub> -NaCl mixture (K <sup>+</sup> ) (Cl <sup>-</sup> ) = constant	
Glucose	2.2 "			Sucrose added to give 200 mOsm/l.	
1.5 per cent CO <sub>2</sub> - 96.5 per cent O <sub>2</sub>		100 per cent O <sub>2</sub>		100 per cent O <sub>2</sub>	
Sucrose-Ringer		Choline-Ringer		Sulfate-Ringer	
Sucrose	190.0 mm/l.	Choline chloride	95.0 mm/l.	Na <sub>2</sub> SO <sub>4</sub>	50.0 mm/l.
K <sub>2</sub> HPO <sub>4</sub>	2.0 "	K <sub>2</sub> HPO <sub>4</sub>	2.0 "	K <sub>2</sub> HPO <sub>4</sub>	2.0 "
KH <sub>2</sub> PO <sub>4</sub>	0.4 "	KH <sub>2</sub> PO <sub>4</sub>	0.4 "	KH <sub>2</sub> PO <sub>4</sub>	0.4 "
CaCl <sub>2</sub>	1.8 "	CaCl <sub>2</sub>	1.8 "	MgSO <sub>4</sub>	1.0 "
MgCl <sub>2</sub>	1.0 "	MgCl <sub>2</sub>	1.0 "	Ca-gluconate	1.8 "
Glucose	2.2 "	Glucose	2.2 "	Glucose	2.2 "
				Sucrose	87.0 "
100 per cent O <sub>2</sub>		100 per cent O <sub>2</sub>		100 per cent O <sub>2</sub>	
		2.0 gm. PVP or albumin added per 100 ml.			

The composition of various perfusion fluids is given in Table I. Bicarbonate or phosphate Ringer's was used for control experiments. All potassium Ringer's solutions had a composition such as to maintain constancy of the [K] [Cl] product. This serves to prevent transcellular shifts of these ions and of water during changes of the extracellular concentration of potassium (15-17). Sucrose, -choline, -and sulfate substitutions were used to study the effects of a lack of sodium and/or chloride ions on various electrical potential differences. In all experiments the composition of the perfusion fluids was changed in both the aortal and portal perfusion circuit. One hour was allowed to elapse after changing the composition of the perfusion fluid before any

electrical measurements were performed. The perfusion fluids contained a small amount of heparin, and polyvinylpyrrolidone or 20 per cent human plasma Albumin at a concentration of 2 gm. per cent. The kidney surface was covered with the respective perfusion fluids during all electrical measurements.

Two groups of experiments were carried out in which drugs were added to the perfusion fluid. In one of these ouabain was used at concentrations of  $1.4 \times 10^{-4}$  and  $1.4 \times 10^{-5}$  M/liter. In the second group  $\text{Hg}^{203}$ -labeled chlormerodrin (neohydrin) was added to the perfusion fluid to give concentrations ranging from 10 to 20  $\mu\text{g./ml}$ . Renal uptake of this mercurial diuretic was measured at the end of the experiment by radioassay according to methods previously described (18, 19). Specimens of lateral kidney sections were used since these are made up predominantly of proximal convoluted tubules. Ratios of mercury concentrations in tissue/plasma ranged from 8.75 to 20.4 (5 samples), values similar to those observed *in vivo* after pretreatment with  $\text{Hg}^{203}$ -labeled chlormerodrin (19).

Methods of measuring electrical potential differences across single renal tubule cells, and across the proximal tubular epithelium of single nephrons by micropipette electrodes, means of localization of the electrode tip within the kidney, and of recording equipment have been described previously (2). Electrodes were tested for tip potentials before use (20) and those having electrical asymmetries in excess of 6 mv. were discarded.

Most measurements of potential differences across the cell membrane of single tubule cells were done across the peritubular cell boundary by placing the indifferent electrode on the kidney surface. However, in some experiments the return lead was formed by a micropipette, filled with Ringer agar, which was placed into the tubule lumen. If the tip of the recording microelectrode is located intracellularly, the potential difference across the *luminal* cell membrane of single tubule cells can be measured directly. Thus, perfusion of single tubules *via* the glomerulus with perfusion fluids, to which a small amount of Evans blue ( $\text{T}_{1824}$ ) had been added to give an outline of the perfused nephron, made it possible to study directly the effects of ionic substitutions on the potential difference across the luminal cell membrane.

Estimates of intracellular potassium and sodium concentrations were made on 12 samples of lateral kidney specimens according to previously described techniques (2). Sodium and potassium concentrations in the postcaval effluent and in ureteral urine were measured by internal standard flame photometry. Chloride concentrations were determined using an Aminco-Cotlove automatic chloride titrator (21).

## EXPERIMENTAL RESULTS

### 1. *Electrical Potential Differences Measured across the Peritubular and Luminal Membrane of Single Proximal Tubule Cells*

The frequency distribution of potential differences measured across the peritubular cell membrane is presented in Fig. 1. Results obtained in experiments with control Ringer's solution are compared with those in which ionic substitutions or inhibitors were used. Under conditions of control perfusions a

mean electrical potential difference of 65 mv. (standard error  $\pm 0.43$ , cell inside negative) is obtained. This value is somewhat lower than that observed in the non-perfused *Necturus* kidney *in vivo*, in which values of  $-72$  (2) and  $-74$  (4) mv. have been reported. Somewhat lower mean values than these

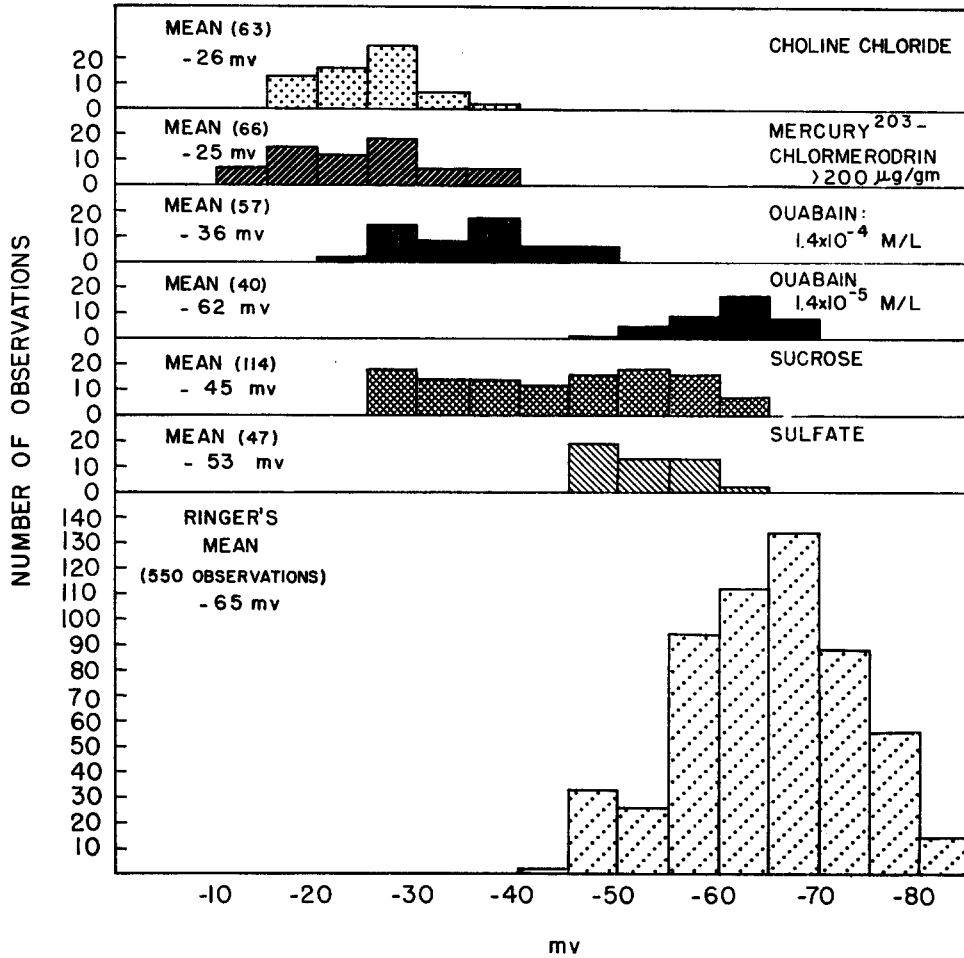


FIGURE 1. Frequency distribution of peritubular transmembrane potentials of single proximal renal tubule cells. Values of electrical potential differences are plotted, pooled in groups of 5 mv., against the number of observations.

have been observed in the whole excised *Necturus* kidney (22), incubated in amphibian Ringer's solution ( $-70$  mv.), and in *Necturus* kidney slices ( $-57$  mv.). It should be noted that a potassium concentration of 4.5 meq./liter was used in most of our perfusion experiments. This concentration is about 1 meq./liter higher than that normally found in *Necturus* plasma (2, 23, 24), and could

explain the lower potential difference in the perfused preparation as compared to *in vivo* observations, since the magnitude of the transmembrane potential of tubule cells is inversely related to the extracellular potassium concentration (see below).

In our series of control experiments (550 measurements) the absolute values of potential differences in successive measurements were unrelated to the time elapsed after beginning the perfusion. Also, the same results were observed when either bicarbonate or phosphate Ringer's solution was used.

Fig. 1 also contains results obtained during perfusion experiments with modified Ringer's solution. The replacement of sodium by choline has a strong depolarizing effect (mean potential difference:  $-26$  mv., standard error  $\pm 0.69$ ), an observation in agreement with that of Whittembury and Windhager (4). These results contrast findings on muscle and nerve in which substitution of sodium by choline frequently leads to hyperpolarization (25). This increase in polarization has been interpreted as being due to a smaller permeability of these cell membranes to choline than to sodium, effecting less shunting by this extracellular cation. However, a different situation obtains in the kidney in which *in vitro* studies have indicated a considerable permeability to choline (22, 26). The ability of positively charged choline ions to penetrate the cell membrane of tubule cells is qualitatively consistent with the observed reduction of intracellular negativity.

The addition of  $\text{Hg}^{203}$ -labeled chlormerodrin (neohydrin) to the perfusion fluid was also effective in depolarizing tubule cells (mean potential difference:  $-25$  mv., standard error  $\pm 0.99$ ; Hg concentration greater than  $200 \mu\text{g}/\text{gm}$ . wet tissue). A similar but smaller depolarization was observed in the non-perfused kidney when *Necturi* were pretreated with this mercurial compound (2). A fall in intracellular potassium concentration (27), inhibition of active sodium extrusion (27, 28), or increased sodium permeability of proximal tubule cells (29, 30) are known possible effects of organic mercurial diuretics such as chlormerodrin. At present, it cannot be decided whether one or a combination of these effects is responsible for the observed cell depolarization.

Ouabain depressed the transmembrane potential only in higher concentrations ( $1.4 \times 10^{-4}$  M/liter), resulting in a mean potential difference of  $-36$  mv. (standard error  $\pm 0.97$ ). In the lower concentration ( $1.4 \times 10^{-5}$  M/liter) it was without effect on cell polarization (mean potential difference:  $-61$  mv., standard error  $\pm 0.82$ ). Recently, cardiac glycosides have been shown to have strong inhibitory effects on renal tubular transfer of sodium and potassium ions (31, 32). It has also been demonstrated that low concentrations of these glycosides ( $10^{-6}$  M/liter) are without effect on the transmembrane potential of single muscle fibers (33), although they interfere with potassium uptake and sodium extrusion. This may be due to the insensitivity of the transmembrane potential to small changes in intracellular potassium concentration effected

by cardiac glycosides in the lower dose range (33). Whether a similar mechanism underlies the unchanged potential difference at the lower concentration of ouabain in the present study cannot be stated since no correlated data on intracellular potassium concentration and potential gradients are available.

A significant reduction of intracellular negativity was also observed when sucrose was substituted for sodium chloride (mean potential difference:  $-45$  mv., standard error  $\pm 1.08$ ), or when sulfate replaced chloride in the perfusion fluid (mean potential difference:  $-52$  mv., standard error  $\pm 0.65$ ). The latter observation is in agreement with the results of Whittombury and Windhager (4) who perfused single tubules with chloride-free sulfate Ringer's. Both the results resemble observations made on amphibian and mammalian muscle fibers (17, 34, 35) and will be dealt with subsequently.

In order to determine the relationship between the external potassium concentration and the peritubular membrane potential, the perfusion fluid was modified to contain various potassium concentrations. Usually, two levels of different potassium concentrations were used in each individual perfusion experiment. In Fig. 2 the membrane potential is plotted against the logarithm of external potassium concentration. Each point is the average potential difference of 11 to 550 individual measurements. Above 5 m.eq./liter, a straight line relationship obtains between peritubular transmembrane potential and the logarithm of external potassium concentration with a slope of 55 mv. per tenfold change in concentration. The dashed line indicates a slope of 58 mv./decade concentration change, which would obtain for an ideal potassium electrode. The closeness of the observed and theoretical slope indicates that the peritubular cell membrane of renal tubule cells is a cell boundary with dominant potassium electrode character. Below a concentration of 5 m.eq./liter the potential difference increases with falling external potassium concentration but deviates from the linear relationship, a behavior resembling that of nerve and muscle (25). The importance of this deviation in relation to certain permeability characteristics of the tubular cell membrane will be discussed later. Somewhat lower slopes, 48 and 51 mv./tenfold concentration change of extracellular potassium ions, were observed by others (22) in kidney slices and in excised *Necturus* kidney *in vitro*.

In another series of experiments single nephrons were perfused *via* the individual glomerulus with solutions containing potassium at different concentrations to study the relationship between extracellular potassium concentration and luminal membrane potential. The relationship between the membrane potential across the luminal cell boundary and the logarithm of intratubular potassium concentration is shown in Fig. 3. Again, each point represents the mean potential difference of 18 to 29 individual measurements. At the three higher potassium concentrations a straight line relationship between the two variables is observed, with a slope of 48 mv. per tenfold concentration change.

At the lowest potassium concentration a significant deviation from this relationship is seen. Again, the dashed line indicates a slope of 58 mv./tenfold concentration change. The observed dependency of the luminal transmembrane potential on the intratubular potassium concentration is characteristic for a cell membrane with a high degree of potassium selectivity.

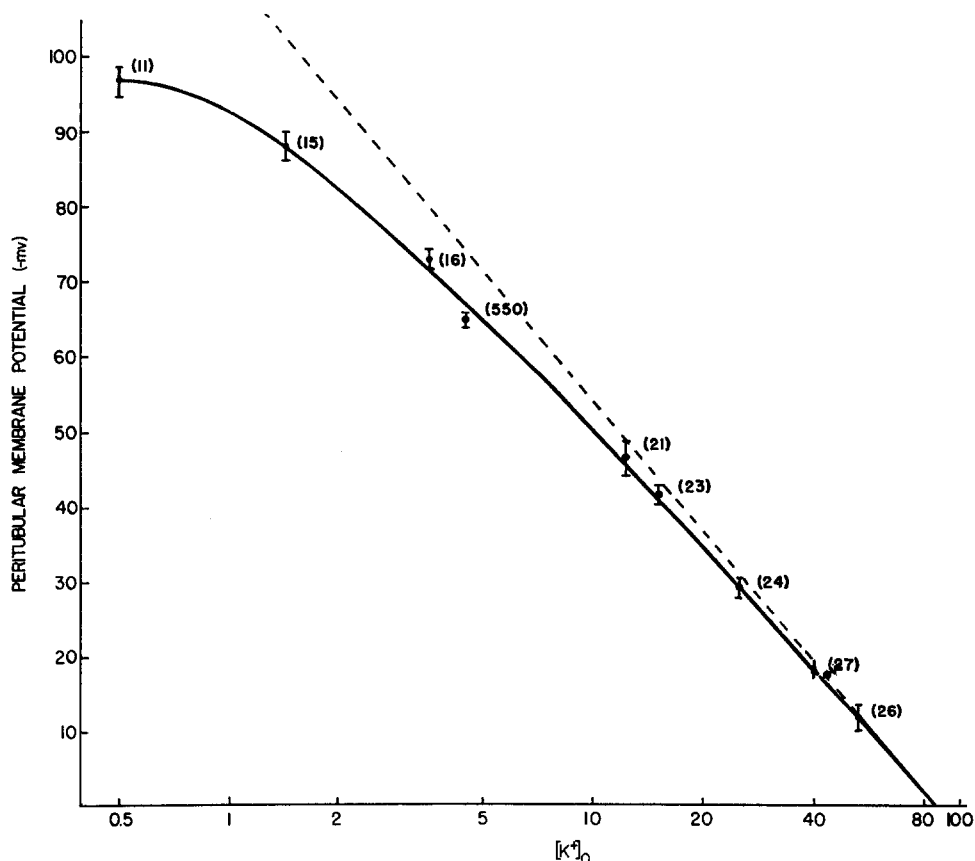


FIGURE 2. Relationship between peritubular potential differences and potassium concentration in perfusion fluid at constant product of potassium and chloride ions. The broken line indicates a slope of 58 mv./decade concentration difference. The height of the vertical line denotes the standard error of the mean, and the figures in parentheses indicate the number of observations.

The effectiveness of extracellularly applied potassium ions in depolarizing both the luminal and peritubular cell membrane indicates a considerable permeability of both cell boundaries to this ion. This is consistent with recent observations by Oken and Solomon (36) who found that perfusion of single tubules of *Necturus* with an initially potassium-free Ringer's solution leads to an increase of the potassium concentration of the collected perfusate above



that of plasma. These observations demonstrate a net transfer of potassium ions across both the peritubular and luminal cell membranes of proximal tubule cells.

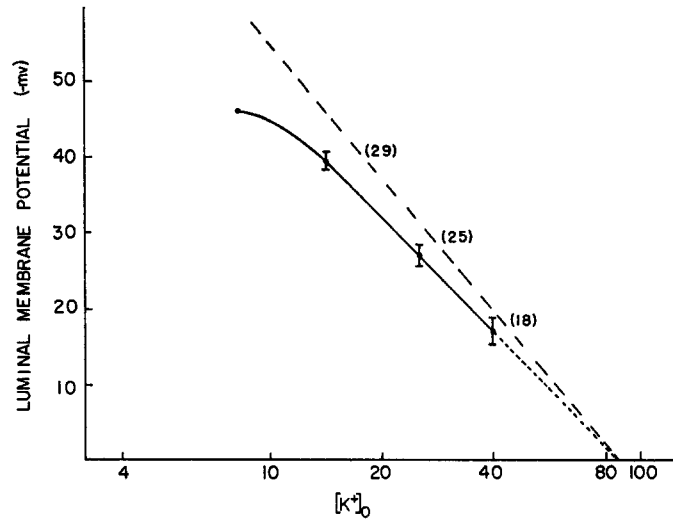


FIGURE 3. Relationship between luminal potential difference and potassium concentration in perfusion fluid at constant product of potassium and chloride ions. The broken line indicates a slope of 58 mv./decade concentration difference. The point at the lowest potassium concentration is the difference between the mean peritubular and transtubular potential difference. The corresponding potassium concentration is 1.8 times that in the control perfusion fluid. The height of the vertical line denotes the standard error of the mean, and the figures in parentheses indicate the number of observations.

## 2. *Electrical Potential Differences Measured across the Proximal Renal Tubular Wall of Single Nephrons*

The frequency distribution of proximal transtubular potential differences during various perfusion experiments is summarized in Fig. 4. In all instances, the tubule lumen was negative with respect to the peritubular extracellular medium. During control experiments the mean potential difference was  $-19$  mv. (standard error  $\pm 0.57$ ). These values are in good agreement with observations made by others (3, 4) on single perfused proximal tubules of *Necturus*, and measurements made in this laboratory on non-perfused tubules *in vivo*. In both instances, a mean potential difference of  $-20$  mv. was observed. Some experiments were performed to examine the effects of a lack of sodium or sodium chloride in the perfusion fluid on the transtubular potential difference. Reduction of the sodium concentration is known to diminish the electrical potential difference across the isolated frog skin, a preparation in which the sodium ion is intimately involved in the generation of the observed electrical

asymmetry (41, 44). In the perfused kidney preparation both the substitution of sucrose for sodium chloride, or of choline for sodium, was found to reduce the transtubular potential difference. This is indicated by a mean potential difference of  $-7.2$  mv. (standard error  $\pm 0.37$ ) for sucrose, and of  $-3.8$  mv. (standard error  $\pm 0.14$ ) for choline perfusions. Similar observations were made by Whittembury and Windhager (4) who found that the electrical asymmetry

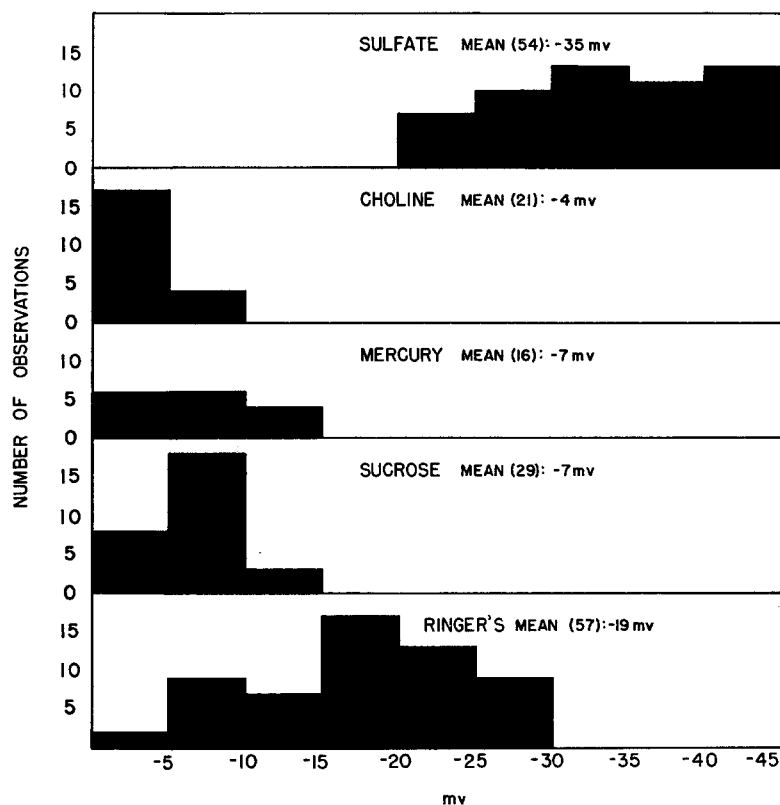


FIGURE 4. Frequency distribution of transtubular potential differences measured across single proximal renal tubules. Values of electrical potential differences are plotted, pooled in groups of 5 mv., against the number of observations.

across the proximal tubular epithelium is abolished when the tubule lumen is perfused with a choline chloride solution.

A reduction of the transtubular potential difference was also observed in experiments in which  $\text{Hg}^{203}$ -labeled chlormerodrin (neohydrin) was added to the perfusion fluid (mean:  $-7$  mv., standard error  $\pm 1.12$ ). Substitution of sulfate for chloride ions in the perfusion fluid increased the potential difference (mean:  $-35$  mv., standard error  $\pm 1.09$ ). This latter effect is qualitatively

similar to observations made on the frog skin (43), and thought to arise as a consequence of the presence of an ion species less permeant than chloride and effecting less electrical shunting.

It is certain that ionic substitutions, carried out on both sides of the tubular epithelium by perfusion of the whole kidney, are more effective in changing the ionic environment than tubular perfusion alone. Thus it has been shown that the sodium concentration of the collected perfusate is about 30 m.eq./liter when the perfusion fluid initially contained 10 m.eq./liter (4). This change in the composition of the perfusion fluid may explain why no change of the transtubular potential difference was found by others (4) when the tubule lumen alone was perfused with a solution initially containing 10 m.eq./liter of sodium and 90 m.eq./liter mannitol. Also, only a small reduction in potential difference across the frog skin was observed when the sodium concentration of the outside solution was lowered to one-tenth of its initial value (45). A change of the original perfusion fluid may account for the inability of a sodium sulfate solution to increase the transtubular potential difference when applied to the tubule lumen alone (4). It is possible that the initial composition of these perfusion fluids was changed enough during the time which elapsed between the filling of the tubule lumen and making the potential measurements to render them ineffective.

The effectiveness of various ionic substitutions in the perfused kidney was tested by analyses of the composition of the postcaval effluent during perfusions with sodium- and/or chloride-free solutions. We have observed that disappearance of these ion species is not accomplished within a time period ranging from 1 to 4 hours. Thus, in a representative experiment with choline chloride as main osmotic constituent, the sodium concentration after 1 hour was still 12 m.eq./liter, and after 3 hours 5.15 m.eq./liter. In an experiment in which sucrose was substituted for sodium chloride, the sodium concentration after 1 hour was 6.0 m.eq./liter, and after 3 hours 5.0 m.eq./liter. During the same time period the chloride concentration fell to 7.55 and 6.14 m.eq./liter, respectively. Similar values, ranging from 3.0 to 8.6 m.eq./liter for sodium or chloride, were observed in all instances. Despite the fact that the postcaval vein does not only drain the renal vascular bed and, accordingly, the collected fluid need not be a precise reflection of the renal perfusate, it is unlikely that complete renal washout of an ion species is accomplished. Thus, although perfusion of the whole *Necturus* kidney is probably more effective in changing the ionic environment than perfusion of single tubules *in vivo*, nevertheless, caution must be applied in attempts to correlate quantitatively observed changes in electrical potential differences with the initial concentration of various ion species in the perfusion fluids.

## DISCUSSION

A consideration of the origin of the observed electrical potential differences makes it necessary to analyze the electrolyte composition and ionic transport function of the proximal tubular epithelium in *Necturus*.

Evidence obtained by micropuncture studies in *Necturus* indicates that as far as sodium, chloride, and hydrogen ions are concerned, no concentration gradients are established across the proximal tubular wall (11, 13, 37, 38). Several lines of evidence show conclusively that sodium movement constitutes an active transport (39, 40). The reabsorptive movement of chloride out of the tubule lumen can be accounted for quantitatively by the observed electrical asymmetry and thus represents, by Ussing's definition (41), a passive process (42). While earlier studies indicated a potassium concentration in the tubule lumen equal to that in *Necturus* plasma (37), more recent experiments have shown that in the perfused and non-perfused proximal tubule of *Necturus*, the potassium concentration may be as high as 1.8 times that found in plasma (24, 36). We have assumed that this condition applies also for the perfused *Necturus* kidney.

Data on the electrolyte composition of the proximal renal tubule cells in *Necturus* indicate a concentration of 108 m.eq./liter for potassium, and one of 37 m.eq./liter for sodium and 32 m.eq./liter for chloride (22). We have found similar values of 90 m.eq./liter (standard error  $\pm 3.06$ ) for potassium, and 40 m.eq./liter (standard error  $\pm 2.30$ ) for sodium in the cell water of samples of the perfused *Necturus* kidney (12 specimens). It should be recognized that these specimens, although composed predominantly of proximal tubules, do not represent an entirely homogeneous population of tubule cells. This causes some uncertainties as to whether the above data on electrolyte concentrations accurately reflect those of the cell water of proximal tubule cells.

Nevertheless, the data on electrolyte movement and ion composition indicate a cell region of relatively low sodium and high potassium content between the tubular and peritubular fluid compartments, both of which are characterized by high sodium and low potassium concentrations. In the following a concept of the origin of the observed electrical asymmetry across the proximal tubule cell will be presented which seems compatible with most observations. Fig. 5 shows a cell model incorporating the essential permeability and transport characteristics.

One point of interest is the relationship between transtubular sodium movement in conjunction with the electrical profile of the proximal tubular epithelium. The proximal tubular epithelium normally pumps sodium ions out of the tubule lumen (3, 39, 40). Furthermore, the concentration of sodium in the tubular lumen and the peritubular fluid is equal, and exceeds that of the cell interior by more than twofold. Also, as pointed out previously, the cell

interior constitutes a region of electronegativity with respect to both the tubule lumen and the peritubular surroundings. Accordingly, sodium ions move down an electrochemical potential gradient from the lumen to the cell interior. No expenditure of metabolic energy would be needed for such passive

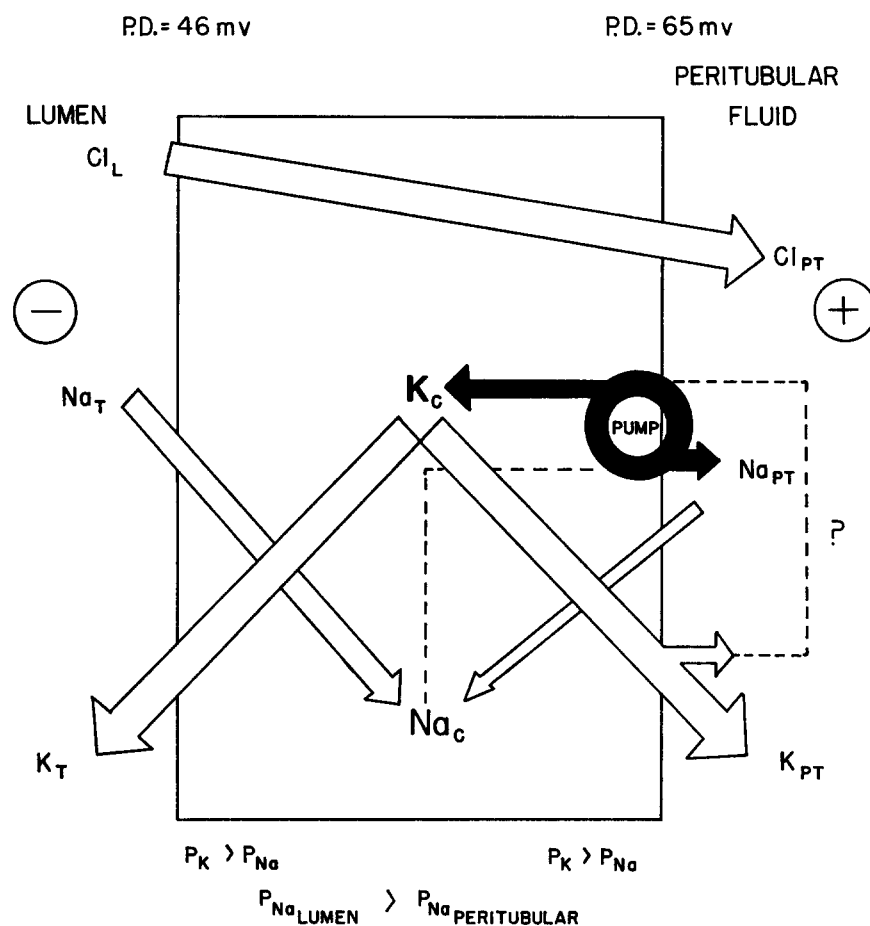


FIGURE 5. Model of proximal renal tubular cell, illustrating hypothesis of the origin of renal tubular potential differences. The thickness of the oblique arrows, not drawn to exact proportions, indicates the relative permeabilities for potassium and sodium at the luminal and peritubular cell membrane. Chloride ions move passively along the electrical potential gradient. Subscripts "L" and "PT" designate concentration of various ion species in the tubular and peritubular fluid, respectively.

downhill movement.<sup>1</sup> On the other hand, net transfer from the cell interior into the peritubular fluid occurs against an electrochemical potential gradient

<sup>1</sup> However, processes which are independent of an applied electrical field such as exchange diffusion, linked, or non-linked sodium-potassium exchange cannot be excluded from the available data (44).

and necessitates an active extrusion mechanism at this site. Such a localization of the sodium pump is also an integral part of cell schemes presented by Pitts (46), Whittembury *et al.* (3, 22), and others (47, 48). At present it cannot be stated whether this sodium extrusion mechanism is linked with potassium uptake into the tubule cell. Two points are relevant. First, the potassium concentration in the cell interior significantly exceeds that to be expected from the magnitude of the electrical potential gradient (see below). This indicates an accumulation of potassium in excess of that to be expected from a purely passive distribution. This finding alone is not conclusive as to whether linked sodium-potassium transfer is present, but it is analogous to that in cell systems where such active potassium uptake has been demonstrated by flux measurements to be coupled with sodium extrusion (49–51). Second, ouabain inhibits fluid reabsorption from the proximal tubule in *Necturus* (52) and other species (31, 32), a process known to depend on transtubular net movement of sodium ions (39). At the same concentration this cardiac glycoside has been found to reduce cell negativity, an observation consistent with loss of intracellular potassium ions. Again, these observations do not establish a linked sodium-potassium transfer but are consistent with a coupled pump mechanism. Another pertinent observation is the finding that ouabain inhibits potassium influx into renal cortical tissue (53), and is known to interfere with linked sodium-potassium exchange in other tissues (33, 54–56). Thus, as far as the renal tubular epithelium is concerned, evidence for a linked peritubular sodium-potassium ion exchange is, at present, indirect and mainly by analogy with other biological membranes. More direct experiments will be necessary to clarify this point.

As a further point of interest, it should be noted that both the luminal and peritubular cell membranes show considerable potassium selectivity and, accordingly, behave predominantly as potassium electrodes. However, the magnitude of the potential difference across the two cell boundaries differs. A mean potential difference of 46 mv. is observed across the luminal, one of 65 mv. is measured across the peritubular cell membrane. Consideration of the peritubular concentration gradient for potassium ions indicates a mean ratio of 20 between cell water and peritubular interstitial fluid when data from specimens of the perfused kidney are taken. According to the Nernst equation, a membrane potential of  $-77$  mv. would be expected at a temperature of  $25^{\circ}\text{C}$ ., assuming equal activity coefficients inside and outside of the cell, and no contribution by other ion species to the diffusion potential at this site. The mean potential difference found was  $-65$  mv., a value significantly less than the potassium equilibrium potential. If a substantial fraction of intracellular potassium ions is not bound, the most likely explanation for this discrepancy is an active uptake of potassium ions across the peritubular cell membrane by a metabolic process. First, such a condition would maintain the intracellular

potassium concentration at a higher level than that demanded by electrochemical equilibrium with the extracellular medium. Furthermore, such inward directed, carrier-mediated potassium movement would oppose the diffusion gradient of potassium ions and lower the electrical potential gradient. Finally, this interpretation is also consistent with observations on kidney slices in which active uptake of potassium ions into renal cells has been demonstrated. (27).

Since the variation of the peritubular membrane potential with the logarithm of the external potassium concentration is less than the theoretical 58 mv./decade concentration change, it is also likely that the peritubular cell membrane is slightly permeable to other ion species. Such incomplete potassium selectivity has been found in many other tissues (25), and could account for the observed deviation, particularly if extracellular sodium ions were permitted to leak into the cell interior along their respective electrochemical potential gradients.

When similar considerations are applied to the luminal cell membrane, permeability characteristics of the same nature, although with essential quantitative modifications, are indicated. The observed potential difference ( $-46$  mv.) is lower than the potassium equilibrium potential, more so than at the peritubular cell membrane, and the slope of the luminal transmembrane potential as function of intratubular potassium concentration is less than that measured across the peritubular cell boundary. Both these findings are consistent with an even higher sodium permeability of the luminal cell membrane as compared with the peritubular cell membrane. Such higher sodium permeability would be the basis for a more effective sodium diffusion potential opposing the potential difference generated by the concentration gradient of potassium ions. The observation that substitution by sucrose for sodium chloride in the perfusion fluid reduces the observed electrical asymmetry across the tubular epithelium, is also consistent with the thesis that sodium ions contribute to the lowering of the potential difference across the luminal cell membrane.

According to this view, the potential difference across the proximal tubular epithelium would be generated by two potassium diffusion potentials in series. The following points should be considered in this connection. First, the potassium diffusion potentials are of opposite sign, since the potassium concentration in the cell water is high with respect to *both* the tubule lumen and the peritubular fluid. Hence, a concentration gradient obtains from a cell region of high potassium content to both luminal and peritubular regions of low potassium level. Second, the potassium diffusion potentials are of unequal magnitude. This is due to the fact that recent evidence indicates that the potassium concentration in the tubule lumen exceeds that in plasma (36), and may be 1.8 times that in the peritubular fluid. No data are available for the

perfused *Necturus* kidney but we have assumed that it behaves in this respect similarly to the non-perfused kidney *in vivo*. Third, both potassium diffusion potentials are reduced, to a different degree, by passive leakage of sodium ions into the cell interior. A higher degree of leakage of sodium ions at the luminal cell boundary, as indicated by the evidence presented above, reduces the magnitude of the potassium diffusion potential to a greater extent than at the peritubular cell membrane, and contributes to intratubular negativity. Finally, it should be emphasized that in the present treatment it is postulated that the relative potassium and chloride permeabilities are equal at the luminal and peritubular cell boundaries. This is a simplifying but unproved assumption.

It follows from these considerations that the determinants of the transtubular potential difference are: (a) the concentration gradient of potassium ions across the proximal tubular epithelium, and (b) the degree of inequality of the sodium permeability at the luminal and peritubular cell boundaries.

An estimate of the relative sodium permeabilities of the luminal and peritubular cell membranes can be made if the provision of a non-electrogenic ion pump at the peritubular cell membrane, passive distribution of chloride ions, and steady-state conditions are granted. Hodgkin and Katz (57) have presented a modification of the Goldman equation which is applicable to the present situation.

$$\Delta\Psi = \frac{RT}{F} \cdot \ln \frac{[K]_i + b[Na]_i}{[K]_o + b[Na]_o}$$

in which  $\Delta\Psi$  is the electrical potential difference,  $b$  the permeability coefficient of sodium relative to potassium, and  $[Na]_i$  and  $[K]_i$  and  $[Na]_o$  and  $[K]_o$ , the sodium and potassium concentrations inside and outside of the tubule cell. Equality of activity coefficients is assumed.

The experimental points of Fig. 3 which describe the behavior of the peritubular cell membrane, can be fitted by an equation with the coefficient of 0.03 for  $b$  in the concentration range from 4.0 to 50.0 m.eq./liter  $K_o$ . The value of  $b$  of the experimental points of Fig. 4, characterizing the luminal cell membrane, can be fitted by the above equation if  $b$  is given a value of 0.1. A value of 90 m.eq./liter and 40 m.eq./liter, respectively, was used for the intracellular potassium and sodium concentrations in the above equation. Recently, the permeability coefficient of sodium relative to potassium has been estimated from flux measurements *in vitro* in *Necturus* kidney slices by Whittembury *et al.* (22). The values found ranged between 0.04 and 0.09 and are, accordingly, in general agreement with the relative permeability coefficients of sodium derived by the approach outlined above. This supports the view that differences in sodium permeability of the estimated magnitude could account



for the observed modifications of the potassium diffusion potentials at the luminal and peritubular cell membrane.

The role of chloride ions in the proximal tubular cell system is not entirely clear. Evidence obtained from bidirectional flux measurements across single proximal tubules in *Necturus* indicates that the observed flux asymmetry can quantitatively be accounted for by the magnitude and direction of the observed transtubular potential difference (5, 6, 42). This would imply passive distribution according to the electrochemical potential gradient across both the luminal and peritubular cell membranes. Since the concentration of chloride ions in the tubular lumen and the peritubular fluid is equal, a smaller electrochemical gradient opposes chloride diffusion at the luminal cell boundary. This would permit leakage of chloride ions into the cell interior, provided that intracellular chloride ions are in electrochemical equilibrium across the peritubular cell membrane and, consequently, are kept at such a low intracellular level that the transluminal concentration gradient exceeds the opposing electrical potential difference. A passive distribution of chloride ions across the peritubular cell membrane would be given by

$$\Delta\Psi = \frac{-RT}{F} \ln \frac{[\text{Cl}]_o}{[\text{Cl}]_i}$$

In the perfused kidney, a mean potential difference of  $-65$  mv. would, at an extracellular chloride concentration of 100 m.Eq./liter, correspond to a chloride concentration of 7.75 m.Eq./liter cell water. This value is much less than the measured intracellular chloride concentration in *Necturus* kidney of 32 m.Eq./liter (22). Although we have not measured the intracellular chloride concentration in the perfused *Necturus* kidney, measurements inherently difficult due to uncertainties in estimating the extracellular fluid volume, we suspect it to be considerably in excess of the calculated value. An heterogeneity of renal cell population as suggested by Conway *et al.* (58), or some compartmentalization of the intracellular fluid (22), could account for the discrepancy between calculated and observed chloride concentrations. Both these hypotheses necessitate a region of high chloride content which would result in an inordinately high *mean* chloride concentration as measured in tissue specimens.

In spite of these uncertainties with respect to the behavior of chloride ions there are several lines of evidence which support the notion of a passive distribution of this ion species. Conway *et al.* (58) have shown that part of the frog kidney *in vitro* behaves in such a way that the product of the potassium and chloride concentrations in kidney cell water approximates, at higher potassium concentration, the product of these ions in the incubating medium. This is a situation analogous to muscle (15, 17, 59) in which the following

relationship holds:

$$\frac{[K]_o}{[K]_i} = \frac{[Cl]_i}{[Cl]_o} = e \cdot \frac{F\Psi}{RT}$$

Two consequences arise from such a relationship: first, a loss of potassium ions would be expected from kidney cells were the product  $[K]_o \cdot [Cl]_o$  reduced in the perfusion fluid. Also, the reduction of the product of  $[K]_o \cdot [Cl]_o$  would be expected to lead to a fall of the membrane potential of tubule cells until the intracellular chloride concentration had declined to approach a ratio in the neighborhood of a new potassium equilibrium potential. Both these predictions are fulfilled. Thus the potassium concentration in the postcaval effluent increased significantly in experiments with chloride-free perfusion fluids. This resulted in a mean increase of 3.88 m.eq./liter in experiments with sucrose-Ringer's (14 one hour collections), and of 1.16 m.eq./liter in perfusions with sulfate-Ringer's (7 one hour collections). The smaller potassium loss observed during perfusion with sulfate-Ringer's may be caused by the fact that kidney tissue is permeable to this ion (26). As a consequence, sulfate may partially substitute for chloride in the relationship  $[K]_i \cdot [Cl]_i = [K]_o \cdot [Cl]_o$ . As far as the transmembrane potential of tubule cells is concerned under conditions of perfusion with chloride-free solutions, we have observed a significant depolarization under both experimental conditions. Again, perfusion with sucrose-Ringer's was more effective than with sulfate-Ringer's (see Fig. 1). The experimental arrangement was not designed to study the electrical transients and further experiments are indicated to test these under conditions of altered chloride and potassium concentrations in order to gain information on the relative chloride and potassium conductances.

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