# Electrical Potential Difference Measurements in Perfused Single Proximal Tubules of *Necturus* Kidney

GUILLERMO WHITTEMBURY and ERICH E. WINDHAGER

From the Biophysical Laboratory, Harvard Medical School, Boston. Dr. Whittembury's present address is Laboratorio de Biofisica, Facultad de Medicina, Lima, Peru. Dr. Windhager's present address is Department of Physiology, Cornell University Medical School, New York

ABSTRACT Transtubular and peritubular face electrical potential differences (P.D.) of the proximal tubules of the kidney of the amphibian *Necturus maculosus* have been measured *in situ*. These measurements have been carried out both under normal conditions, when the tubular fluid originates in the glomerular filtrate, and under conditions when the composition of the tubular fluid has been altered using the stopped flow microperfusion technique. Under normal conditions the transtubular potential difference is 20 mv. (lumen-negative) and the P.D. across the peritubular face is 74 mv. (cell-negative). The P.D. across the luminal face is thus 54 mv. (cell-negative). This electrical asymmetry is not influenced by replacing the normal tubular fluid by NaCl, NaCl + mannitol, or by alteration in the intraluminal pH from 7 to 4. On the other hand, replacement of Na by K or choline and the addition of small amounts of DNP to the perfusate diminish this asymmetry.

In 1938, Wilbrandt (1) first measured transtubular electrical potential differences in *Necturus* kidney. More recently, Giebisch (2) has measured peritubular face and transtubular potential differences using a technique by which the position of the electrode can be localized. He has also reported a number of potential difference measurements in kidneys perfused *via* both the aortic and portal circulations (3, 4).

The present series of measurements was initiated in late 1957 to complement studies on ion transport across the proximal tubule of *Necturus*. The experiments were designed to measure the potential difference across the whole proximal tubular wall *in situ*, both under normal conditions and during stopped flow microperfusion. Measurements were also made of potential differences across the peritubular face of the cell. This information can be

used to clarify the role of the cellular membranes in the transport processes. A brief account of these experiments has been presented previously (5, 6).

## EXPERIMENTAL METHOD

The technique was similar to that described by Giebisch (2). The exploring electrodes were micropipettes with tips smaller than 0.5 $\mu$, as described by Ling and Gerard (7), pulled from ~1 mm. O.D. pyrex tubes using the pipette puller of Alexander and Nastuk (8). They were filled by boiling in a 3 M KCl solution colored with chlorphenol red. The dye was first dissolved in a small amount of KOH solution, and then added to 3 M KCl to a final concentration of 0.1 per cent. The electrode was connected to an injection system containing a calomel electrode according to the design of Grundfest, Kao, and Altamirano (9). The criteria for acceptance of an electrode were: suitable shape (7), tip resistance between 3 and 30 megohms, and tip potential differences between 0 and −5 mv. The indifferent electrode was also a calomel electrode. It was connected to a Ringer agar bridge which ran to the peritoneal cavity of the animal, adjacent to the kidney. The recording instrument was a high impedance voltmeter, $10^{14}$ ohm input impedance, grid current less than $5 \times 10^{-14}$ amperes, manufactured by Keithley Instruments Inc., Cleveland, Ohio. The experiments were performed in a Faraday cage.

Before and after each impalement of the tissue, the tip potential and resistance were measured in a medium of composition similar to the animal's extracellular fluid. If a significant change was observed, the experiments were rejected.

The animals were anesthetized with tricaine methanesulfonate (Sandoz). After exposure of the ventral surface of the kidney, the agar bridge of the indifferent electrode was placed in the peritoneal space. The surface of the kidney was kept wet by intermittently dripping saline on it. The exploring electrode was then advanced with a micromanipulator, and a tubule impaled under a dissecting microscope. The following potential differences (P.D.'s) were measured: (*a*) peritubular face P.D., observed across the peritubular face when the exploring electrode is inside the cell; (*b*) transtubular P.D., observed across the whole tubular cell when the exploring electrode is in the tubular lumen. The luminal face P.D. may be determined from the difference between the two measured potentials.

The criteria given in detail by Giebisch (2) were used in determining the location of the electrode. Kidneys with a thick capsule were not used. Upon impalement, an abrupt change in P.D. was observed. When the investigator believed the tip to be inside a cell, the voltage was checked for stability; only those experiments in which a stable potential was recorded for 1 to 2 minutes were considered satisfactory. When conditions were especially favorable, a stable potential could be registered with a single impalement for as long as 5 to 10 minutes. If the voltage remained stable, further identification of the place of impalement was attempted by injecting the colored KCl solution through the micropipette. The formation of a very small colored zone around the tip and the absence of a tubular outline were considered to confirm intracellular electrode location. Experiments in which the impalement was smooth and clean and the pipette penetrated easily gave a high and steady potential. Lower,

but apparently stable, potentials were obtained when there was some difficulty in impalement and marked dimpling of the cell membrane occurred. Experiments in which the potential was not stable or excessive dimpling was observed were discarded.

Satisfactory transtubular potentials were more difficult to obtain because the electrode tip broke easily and the thick tubular wall (about 30 $\mu$) made penetration harder. It was necessary for the electrode tip to penetrate to the lumen without any perceptible backward movement and without touching the opposite wall. When excessive pushing and wiggling were necessary, smaller and unstable P.D.'s were obtained and discarded. Only in very favorable, rather transparent, kidneys could the localization of the tip be established by simple inspection. When a stable potential (in normal tubules, about $-20$ mv.) had been registered for 60 to 90 seconds after impalement, colored KCl solution was injected; the appearance of the tubular outline was taken as evidence of intratubular location of the electrode. In order to outline the tubule well, it was necessary that the microelectrode tip be large enough to permit injection of a significant amount of fluid, yet small enough to meet our usual criteria. As a result, it was necessary to discard a large number of exploring electrodes. The P.D.'s were stable for 2 to 3 minutes after impalement, though seldom longer. We have tried, by other means, to verify the presence of the microelectrode tip in the tubular lumen. In almost every case, injection of the colored KCl produced a drop in the P.D., as well as outlining the tubule; sometimes the sign reversed, the lumen becoming positive. In some circumstances, if the circulation of fluid in the tubular lumen was very active, the colored fluid would be washed out; after 1 to 2 minutes the P.D. became negative again, sometimes reaching $-20$ mv. If injection was again performed, the P.D. dropped again.

The P.D. across the glomerular membrane has not been investigated systematically; however, in 9 instances we have obtained values of: $-2$, 0, 0, $-1$, 0, $-2$, $-4$, 0, and $+2$ mv. There appears to be no gradation in the P.D. along the proximal tubule, the transtubular P.D. being independent of the segment of the tubule impaled.

## RESULTS

### *Potential Differences in Normal Tubules in situ*

Transtubular P.D. was measured in normal tubules, that is tubules in which the tubular fluid originated from the glomerulus. 86 satisfactory impalements carried out before a satisfactory system for injection was developed gave a transtubular P.D. of $-20 \pm 8$ mv. (standard deviation). The frequency distribution of these and subsequent data is shown in Table I. When the injection system became available in 1958, the scatter diminished markedly. The mean of all the subsequent measurements was $-20 \pm 4$ mv. (standard deviation), in good agreement with the value obtained by Giebisch (2). The P.D.'s measured across the peritubular face are also given in Table I. The mean value of $-74$ mv. and standard deviations of 8 mv. are essentially the

same as those obtained by Giebisch (2), in a series of 113 observations ($-72 \pm 7.2$ mv.). The luminal face P.D. is therefore $-54$ mv.

If the observed cell potentials are interpreted as diffusion potentials, they should be functions of the ionic concentrations across the membrane and of the relative permeability of the membrane to these ions (10, 11). The observed asymmetry in the potential would arise from different concentrations or from different permeabilities across the two faces of the cell or from a combination of the two.

TABLE I

TRANSTUBULAR AND PERITUBULAR FACE P. D. FREQUENCY DISTRIBUTION

| Groups | No. of observations | | | | |
|---|---|---|---|---|---|
| | Without injection | Injection | | | |
| *mv.* | 1957 | 1958 | 1959 | 1960 | Total |
| *Transtubular* | | | | | |
| 0–3 | 2 | 1 | | | 1 |
| 4–7 | 2 | | | | |
| 8–12 | 12 | | | 5 | 5 |
| 13–17 | 11 | 3 | 1 | 11 | 15 |
| 18–22 | 33 | 19 | 39 | 45 | 103 |
| 23–27 | 13 | 3 | 11 | 9 | 23 |
| 28–32 | 6 | 1 | 3 | | 4 |
| 33–37 | 5 | | | | |
| 38–42 | 2 | | | | |
| *Peritubular face* | | | | | |
| 48–62 | | | 7 | 4 | 11 |
| 63–67 | | | 7 | 3 | 10 |
| 68–82 | | | 51 | 39 | 90 |
| 83–87 | | | 4 | 4 | 8 |
| 88–92 | | | 3 | 1 | 4 |

The studies of Walker *et al.* (12), Walker and Hudson (13), Bott (14, 15) and Giebisch (16) have shown that the tubular concentrations of Na, Cl, and H are normally equal to their concentrations in *Necturus* plasma. Recent experiments by Oken and Solomon (17) in this laboratory, show that the K concentration in the tubular fluid is about 1.8 times greater than that in serum. This observation would account for about 15 mv. of the observed 20 mv. asymmetry, on the basis of a Nernst equation for a K electrode ($\Delta\psi = \frac{RT}{zF} \ln [K]_{TF}/[K]_P = 58 \log 1.8 \backsimeq 15$ mv.). Differences in permeability are therefore necessary to account for the remaining 5 mv. asymmetry.

### *Potential Differences in Perfused Tubules*

The technique for stopped flow microperfusion previously described (18) allows replacement of the tubular fluid with a solution whose initial composition may be chosen at will, while the solution bathing the peritubular face of the cell remains the interstitial fluid of the animal. Peritubular face and transtubular P.D.'s were measured within 5 to 20 minutes of the initial perfusion. Table II gives the initial composition of the perfusion fluid together with the experimental results.

TABLE II

ELECTRICAL POTENTIAL DIFFERENCE IN SITU

| Initial perfusion fluid composition | Transtubular P.D. | No. of observations | Peritubular face P.D. | No. of observations | Lumina face P.D. |
|---|---|---|---|---|---|
| *mM/liter* | *−mv.** | | *−mv.* | | *−mv.* |
| Normal‡ | 20.0 ± 0.3 | 151 | 74 ± 1 | 123 | 54 |
| 100 NaCl (pH 7.5) | 21 ± 1 | 15 | 73 ± 3 | 10 | 52 |
| Mannitol substitution | | | | | |
| 50 NaCl + 93 mannitol | 17 ± 2 | 11 | | | |
| 10 NaCl + 168 mannitol | 19 ± 2 | 9 | | | |
| pH | | | | | |
| 97 NaCl (pH 5.5) | 19 ± 1 | 9 | 75 ± 3 | 6 | 56 |
| 97 NaCl (pH 4.5) | 19 ± 1 | 5 | | | |
| K substitution | | | | | |
| 90 NaCl + 10 KCl | 21 ± 1 | 6 | | | |
| 10 NaCl + 90 KCl | 11 ± 1 | 7 | | | |
| 100 KCl | 4 ± 1 | 18 | 30 ± 2 | 27 | 26 |
| 50 $K_2SO_4$ | 6 ± 1 | 9 | 24 ± 4 | 5 | 18 |
| Choline substitution | | | | | |
| 50 NaCl + 50 choline Cl | 9 ± 1 | 22 | 57 ± 1 | 7 | 48 |
| 10 NaCl + 90 choline Cl | 7 ± 4 | 30 | 43 ± 5 | 9 | 36 |
| 100 choline Cl | 0.0 ± 0.5 | 17 | 33 ± 2 | 20 | 33 |
| 50 $Na_2SO_4$ | 20 ± 1 | 15 | 48 ± 5 | 6 | 28 |
| 100 NaCl + DNP | 10 ± 1 | 16 | 34 ± 3 | 8 | 24 |
| 10 NaCl + 168 mannitol + DNP | 10 ± 2 | 9 | 36 ± 3 | 7 | 26 |

* Errors are standard errors of the mean.

‡ The tubular lumen contained normal physiological fluid filtered by the glomerulus.

### *Potential Differences with Normal and Mannitol-Substituted Fluids*

When the normal glomerular filtrate is replaced with a perfusion solution containing 100 mM NaCl there is no difference in either the transtubular or

peritubular face P.D. Analysis of the tubular fluid at the end of a 20 minute perfusion period shows the Na and K concentrations to have normal values (17, 19). When Giebisch (3, 4) perfused both aortic and portal circulations in *Necturus* kidneys with a fluid similar to the extracellular fluid of the animal, the transtubular P.D. was −19 mv. No significant difference was observed in the transtubular P.D. when NaCl was replaced by mannitol. The Na concentration of the perfusion fluid at the end of 20 minutes is about 30 mM (19) when the perfusion fluid contains 10 mM NaCl initially. This rise in Na concentration is in accord with previous results (20) which have shown a 35 mM NaCl gradient to be the largest gradient that may be maintained across the tubule. The transtubular P.D. expected for a perfusion fluid containing 30 mM NaCl may be calculated from Equation 13 in the succeeding paper (21) making use of the observation of Oken and Solomon (17) that the tubular K concentration comes to about 5.6 mM when the initial perfusion fluid contains NaCl at a concentration of either 65 or 100 mM. This calculated P.D., −9 mv., is significantly lower than the observed value of −20 mv. The disagreement may be accounted for by either of the following assumptions: (*a*) the permeability of the luminal face of the cell to Na is not constant, but increases as the luminal Na concentration decreases; or (*b*) the luminal Cl concentration has not come to equilibrium with the cellular Cl concentration so that the application of Equation 13 to the present problem is not justified. Meyer and Bernfeld (22) and Linderholm (23) also found relatively small changes in P.D. across the frog skin when the NaCl concentration in contact with the outside face was diminished from 100 mM to 10 mM. In Giebisch's (3, 4) experiments, perfusion of both faces of the cell with sucrose diminished the transtubular P.D. to −7 mv. and the peritubular face P.D. to −45 mv.

*Influence of pH on Potential Difference*

The H ion concentration was varied by a factor of $10^3$ by adding 3 mM $PO_4$ buffer to a perfusion fluid containing 97 mM of NaCl. No significant change was observed in the P.D.'s. Since H ion concentrations are usually many orders of magnitude lower than the concentrations of other ions, the P.D. would be expected to change only if the luminal face of the cell had a very large and selective permeability to H or if the Na pump were sensitive to pH. Neither seems to be the case in our preparation in which, as discussed in the succeeding paper (21), the Na pump appears to be at the peritubular face of the cell. In the case of the frog skin (22, 24), however, pH does have a marked effect on the P.D. across the inward facing membrane, where the ion pump is located (25) (equivalent to the peritubular face in our preparation).

*Potential Differences with K-Substituted Fluids*

When Na in the perfusion fluid is replaced by K, it is necessary to maintain a constant [K] [Cl] product in order to avoid Cl shifts across the cell membrane. This precaution was observed in the perfusion with $K_2SO_4$ (100 m.eq. K/liter). In these experiments the observed luminal face P.D. of $-17.5$ mv. is compatible with a luminal K concentration of some 60 mM. Such a low K concentration seems possible if there is a net flux of Na and water into the lumen, as has been observed in isosmolar perfusions with low concentrations of Na. The loss of K from the lumen could also cause the intracellular K concentration to diminish. In other perfusions in this series, the [K] [Cl] product was not kept constant. The luminal face P.D.'s were then found to lie between the new K "equilibrium" P.D. and the normal P.D., as would be the case if the Cl equilibrium P.D. were close to the normal P.D.

*Potential Differences with Choline-Substituted Fluids*

When Na was replaced by choline, the changes in the P.D. were similar to the changes induced by K. Giebisch (3, 4) has obtained similar results. Analysis of tubular fluid (initial composition Na, 3 mM; choline, 100 mM) at the end of the usual 20 minute perfusion time showed a Na concentration of about 30 mM (19). At the same time some 10 per cent of the tubular water had been absorbed, which indicated that choline had disappeared from the tubule. This finding has been confirmed in preliminary experiments by Herrera (26) using $C^{14}$-choline which gave a final choline concentration of about 65 mM. His results are compatible with the finding in the succeeding paper (21), that the tubule is almost as permeable to choline as to K. Mammalian kidney cells have also been shown to be permeable to choline (27, 28). The similarity in the behavior of choline and KCl is emphasized by the observation that the asymmetry between the two faces of the cell almost disappears when the perfusion fluid contains 100 mM choline Cl or KCl.

*Potential Differences with $Na_2SO_4$*

If the kidney were absolutely impermeable to $SO_4$, it would be possible to suppress the anion conductance by substituting $SO_4$ for Cl. However, kidney tissue is not impermeable to $SO_4$ (27), and the transtubular P.D. with $Na_2SO_4$ is no different from that with NaCl. Since Giebisch (3, 4), in doubly perfused tubules, has found a transtubular P.D. of 35 mv., $SO_4$ is required on both sides of the membrane to produce this effect. Our peritubular face P.D. is lower than normal, in good agreement with Giebisch. A similar depolarization has been observed in muscle by Hodgkin and Horowicz (29) who have shown

that a sudden change in extracellular Cl concentration causes the cell to depolarize, whereupon K moves out of the cell until a new K equilibrium is reestablished.

*Influence of DNP on Potential Differences*

Both the transtubular and the peritubular face P.D. fell to about half the normal value when the perfusion fluid contained $2 \times 10^{-4}$ M dinitrophenol. Giebisch (30) has observed a fall in the peritubular face P.D. in kidneys perfused with a fluid containing ouabain. The results of these experiments are in accord with the findings of Schatzmann, Windhager, and Solomon (31), that water movement, which depends on the Na pump, is inhibited by DNP and ouabain.

## DISCUSSION

The cells of the *Necturus* proximal tubule are very different from other cells in which a Na pump plays a prominent role. They are particularly permeable to choline and $SO_4$, so that two of the investigative tools normally employed in studies on muscle and nerve do not give unequivocal results. Furthermore, they also differ from other epithelial cells; in frog skin, for example, the outer face behaves like a Na electrode and the inner face behaves like a K electrode. In contrast, both faces in the *Necturus* proximal tubule cell behave predominantly as K electrodes, as has been shown by Giebisch (30). These unusual permeability properties cause the concentrations of the ions in the tubule cell to be extremely labile, so that the cell is especially sensitive to changes in external environment. Even in the present situation in which the peritubular face of the cell was constantly exposed to the normal interstitial fluid, the ion content of the cell varied rapidly with changes in the composition of the luminal fluid, as evidenced by the shifts in peritubular potential difference shown in Table II. In consequence, we have made use of a second approach which, as seen in the subsequent paper, appears to clarify many of the points under discussion.

These experiments were initiated by Dr. Hans J. Schatzmann at the end of his stay in the laboratory in 1957, and his warm enthusiasm for this problem was a particular stimulus during our subsequent work.

This research has been supported in part by the Life Insurance Medical Research Fund and by the National Science Foundation.

Dr. Whittembury was a Rockefeller Foundation Fellow.

Dr. Windhager was a Life Insurance Medical Research Fellow.

*Received for publication, July 12, 1960.*

## BIBLIOGRAPHY

1. WILBRANDT, W., *J. Cell. and Comp. Physiol.*, 1938, **11**, 425.
2. GIEBISCH, G., *J. Cell. and Comp. Physiol.*, 1958, **51**, 221.

3. GIEBISCH, G., Nierensymposium, Göttingen, August, 1959. Aktiver Ionentransport und Lokalisation des Stofftransports in der Niere, (K. Kramer and K. J. Ullrich, editors), Stuttgart, Georg Thieme, 1960, p. 49.
4. GIEBISCH, G., *Circulation*, 1960, **21,** 879.
5. WHITTEMBURY, G., Fourth Annual Meeting of the Biophysical Society, Philadelphia, 1960.
6. WHITTEMBURY, G., *J. Gen. Physiol.*, 1960, **43,** No. 5, suppl., 43.
7. LING, G., and GERARD, R. W., *J. Cell. and Comp. Physiol.*, 1949, **34,** 383.
8. ALEXANDER, J. T., and NASTUK, W. L., *Rev. Scient. Instr.*, 1953, **24,** 528.
9. GRUNDFEST, H., KAO, C. Y., and ALTAMIRANO, M., *J. Gen. Physiol.*, 1954, **38,** 245.
10. GOLDMAN, D. E., *J. Gen. Physiol.*, 1943, **27,** 37.
11. HODGKIN, A. L., and KATZ, B., *J. Physiol.*, 1949, **108,** 37.
12. WALKER, A. M., HUDSON, C. L., FINDLEY, T., JR., and RICHARDS, A. N., *Am. J. Physiol.*, 1937, **118,** 121.
13. WALKER, A. M., and HUDSON, C. L., *Am. J. Physiol.*, 1937, **118,** 130.
14. BOTT, P. A., Renal Function, Transactions of the Fifth Conference, New York, Josiah Macy Foundation, 1953, 42.
15. BOTT, P. A., *in* Proceedings of the Eighth Annual Conference on the Nephrotic Syndrome, (J. Metcoff, editor), New York, National Nephrosis Foundation Inc., 1957, 39.
16. GIEBISCH, G., *Am. J. Physiol.*, 1956, **185,** 171.
17. OKEN, D. E., and SOLOMON, A. K., *J. Clin. Inv.*, 1960, **39,** 1015, and personal communication.
18. SHIPP, J. C., HANENSON, I. B., WINDHAGER, E. E., SCHATZMANN, H. J., WHITTEMBURY, G., YOSHIMURA, H., and SOLOMON, A. K., *Am. J. Physiol.*, 1958, **195,** 563.
19. OKEN, D. E., and WHITTEMBURY, G., unpublished observation.
20. WINDHAGER, E. E., WHITTEMBURY, G., OKEN, D. E., SCHATZMANN, H. J., and SOLOMON, A. K., *Am. J. Physiol.*, 1959, **197,** 313.
21. WHITTEMBURY, G., SUGINO, N., and SOLOMON, A. K., *J. Gen. Physiol.*, 1961, **44,** 689.
22. MEYER, K. H., and BERNFELD, P., *J. Gen. Physiol.*, 1946, **29,** 353.
23. LINDERHOLM, H., *Acta Physiol. Scand.*, 1952, **27,** suppl. 97, 1.
24. SCHOFFENIELS, E., *Arch. internat. physiol.*, 1955, **63,** 513.
25. KOEFOED-JOHNSEN, V., and USSING, H. H., *Acta Physiol. Scand.*, 1958, **42,** 298.
26. HERRERA, F., personal communication.
27. MAIZELS, M., and REMINGTON, M., *J. Physiol.*, 1958, **143,** 275.
28. WHITTAM, R., and ROBINSON, J. R., *Biochem. J.*, 1958, **70,** 16P.
29. HODGKIN, A. L., and HOROWICZ, P., *J. Physiol.*, 1959, **148,** 127.
30. GIEBISCH, G., *J. Gen. Physiol.*, 1961, **44,** 659.
31. SCHATZMANN, H. J., WINDHAGER, E. E., and SOLOMON, A. K., *Am. J. Physiol.*, 1958, **195,** 570.