The Antimony Microelectrode in Kidney Micropuncture*

GERHARD MALNIC and FRANCISCO LACAZ VIEIRA

Department of Physiology, Institute Ciências Biomédicas, University of São Paulo,
São Paulo, Brasil

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The use of pH-sensitive microelectrodes for the determination of tubular pH in amphibian nephrons dates from the early days of kidney micropuncture. Soon after this methodology was introduced by Richards and his coworkers, Montgomery and Pierce(1,2) published their papers on the determination of tubular pH in Necturus and in frogs by means of a quinhydrone microelectrode. In this microelectrode, the pH-sensitive element is a thin platinum wire covered by quinhydrone crystals, located inside a collection micropipette. Tubular fluid is aspirated into this pipette until it contacts the wire, and the voltage between this electrode and a reference electrode is measured and is proportional to the sample’s pH. Originally the pipete was filled with mercury. Later, other authors filled it with mineral oil equilibrated with CO₂, with which the tubular fluid had to equilibrate(3–5). Using this method, Gottschalk et al.(5) as well as Giebisch et al.(6) were able to show that, in the mammalian nephron, acidification starts in the proximal tubule, a different finding from that of Montgomery and Pierce, who had shown that, in the amphibian, acidification was a function only of the distal tubule.

Two basic mechanisms can be considered the cause of tubular acidification or bicarbonate reabsorption. One is the secretion of H+ ions into the tubular lumen by the tubular cell, and the other is the reabsorption of alkaline salts like sodium bicarbonate as such. In the first situation, bicarbonate would be decomposed into CO₂ and water through the formation and subsequent dehydration of carbonic acid in the tubular lumen. The observation by Walser and Mudge(7) that the hydrogen ion secretory rate in the mammalian nephron calculated upon assumption of the first mechanism would exceed the rate of uncatalyzed dehydration of carbonic acid at a concentration equal to that in plasma, in the tubular lumen, led to the expectation of a disequilibrium pH at this site unless this dehydration

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bevelled by grinding. Finally, a copper wire is soldered into their wide part for the connection to the measuring instrument (Fig. 1C).

 Theory: the antimony electrode is a metal/metal oxide electrode. Its active area, corresponding to some 20 to 100 \( \mu \text{m}^2 \), is constituted of metallic antimony covered by a thin layer of antimony trioxide (\( \text{Sb}_2\text{O}_3 \)) produced upon spontaneous oxidation by the oxygen of air. This oxide is very slightly soluble in water, which is one of the reasons for its use\(^{(17)} \). In contact with water the oxide is hydrated according to the following reaction\(^{(18)} \):

\[
\text{Sb}_2\text{O}_3 + 3 \text{H}_2\text{O} \rightleftharpoons 2 \text{Sb} (\text{OH})_3 \rightleftharpoons 2 \text{Sb}^{3+} + 6 \text{OH}^- \tag{1}
\]

At equilibrium, we have:

\[
a_{\text{Sb}} \cdot a_{\text{OH}}^3 = k_s \tag{2}
\]

where \( k_s \) is the solubility product of antimony hydroxide, and \( a_{\text{Sb}} \) and \( a_{\text{OH}} \) are the activities of the involved ions. In logarithmic form, we have:

\[
\ln a_{\text{Sb}} + 3 \ln a_{\text{OH}} = \ln k_s \tag{3}
\]

For the dissociation of water, we can write, similarly: \( \text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{OH}^- \), and

\[
a_{\text{H}} \cdot a_{\text{OH}} = k_w \tag{4}
\]

\[
\ln a_{\text{H}} + \ln a_{\text{OH}} = \ln k_w \tag{5}
\]

From relations (2) and (4), we get

\[
a_{\text{Sb}} = (k_s / k_w^3) \cdot a_{\text{H}}^3 \tag{6}
\]

The equilibrium between metallic antimony and its ions is given by the Nernst equation:

\[
E = E_o + \frac{RT}{3F} \cdot \ln a_{\text{Sb}} \tag{7}
\]

where \( E \) is the potential difference between metal and solution, and \( E_o \) the potential when \( a_{\text{Sb}} = 1 \).

Transforming into decimal log and using relations (6) and (7), we obtain:

\[
E = E_o' + 2.303 \frac{RT}{F} \cdot \log a_{\text{H}}, \text{ or}
\]

\[
E = E_o' - 2.303 \frac{RT}{F} \cdot \text{pH} \tag{8}
\]

---

Fig. 1. Construction of Sb microelectrode: a, filling of glass capillary; b, drawing out by hand; c, finished microelectrode with microforge-drawn and bevelled tip, and with connecting wire.
giving a linear relation between $E$, the measured potential difference, and pH. In practice the constant $E'_0$ will include the value of the reference electrode which in our system is a calomel electrode.

*Characteristics of the Sb electrode:* the relation between buffer pH and voltage reading between the electrode and a reference calomel electrode is given in Table 1. It can be seen that the value of $V$/decade at $25^\circ$ is near the expected value of 59mV for most ranges, decreasing, however, in the more alkaline buffers. The electrode is affected by temperature changes, showing a temperature coefficient of 2.2 mV/°C between 20 and 40°C for buffers between 5.5 and 7.5(12). Therefore, the standard buffers used for calibration are kept at the temperature of the kidney (37°C) during the experiments.

It has been reported that the oxygen tension of the measured fluid can affect the readings by an antimony electrode. Within certain limits, it was reported to function as an oxygen electrode(19). However, El Wakkad indicates that this electrode is not a true oxygen electrode, but that the oxygen tension could be related to the formation of higher valence antimony oxides, which could affect the pH characteristics of the electrode(20). The reported effects of oxygen tension correspond in general to experimental situations where these variations are extreme, that is, where measurements with pure nitrogen and pure oxygen are compared to those in air(21). We have also been able to observe oxygen tension effects under such conditions. However, for the measurement of pH in the kidney cortical tubules the range of $pO_2$ values is rather limited. Measurements of oxygen tension in renal vein in 10 rats gave a value of $48.7 \pm 3.2$ mmHg. Therefore, the effect on pH readings of preequilibrium of buffers at $pO_2$ values ranging from 34 to 138 mmHg was studied and the results shown in Fig. 2. It is clear that in this range of oxygen tensions no significant changes were observed, indicating that pH measurements in the renal cortex should not be affected by the $pO_2$ values to be expected in this tissue. Accordingly, all gas mixtures used to preequilibrated perfusion fluids or to equilibrate fluid droplets for *in vitro* bicarbonate determinations contained oxygen in a proportion similar to that of air.

Since the early reports of the use of an antimony electrode, it has been observed that readings should be made without stirring the solution to be measured.

| pH  | mV       | mV/pH unit |
|-----|----------|------------|
| 4.09| 238 ± 4.7| 55.6       |
| 5.06| 292 ± 12.3| 54.0       |
| 6.04| 345 ± 9.7| 51.5       |
| 7.01| 395 ± 8.8| 38.7       |
| 8.25| 443 ± 9.4|            |
FIG. 2. Effect of preequilibration of buffer solutions at different oxygen tensions on readings of Sb electrode.

since its movement could introduce instability into the determination(14). Other authors, however, advocated vigorous(22) or moderate(23) stirring in order to obtain a more stable reading and rotating electrodes have been constructed to this end(24). Since in the renal tubules measurements have to be taken in the presence of fluid flow, it was necessary to test the effect of flow on measurements. This was done by inserting an electrode perpendicularly into a glass tube through which buffers or urine flowed at a measured rate varying from 0 to 60 mm/sec. No pH alterations were observed in buffer solutions and a maximum change of 0.04 pH was found for urine. The maximal flow velocity used in these model experiments is about 30 times greater than actual tubular flow velocities(25), indicating that under experimental conditions the stirring produced by the physiological flow velocity does not lead to significant alterations of pH. On the other hand, it could be argued that while the flow in our model is probably laminar, it could be turbulent in the renal tubule and therefore not strictly comparable to the model experiment. On the basis of the flow velocities and tubular diameter it is possible to calculate Reynolds numbers for the two situations. The Reynolds number is obtained by the relation

$$R = \frac{d \bar{v} \rho}{\eta}$$

where d is tube diameter, \(\bar{v}\) is the average velocity, \(\rho\) the density of the liquid, and \(\eta\) its viscosity; taking values for \(\rho\) and \(\eta\) equal to those for water, and using the diameter of 0.5 cm and a velocity of 0.5 cm/sec, a value of 250 is obtained for the “in vitro” experiment, well below the limit of 2100, above which tur-
bulent flow may appear (26). For the kidney tubule this number is less than 1, due to its small diameter, and it is certain that laminar flow obtains.

Reducing or oxidizing agents can affect the performance of the antimony microelectrode (14). This was also found to be true for other substances, like acids, that could form complex salts with antimony (critic, tartaric, oxalic), some amino-acids and other metal cations like the cupric ion (17,21). Thus only the comparison of pH measured in fluid samples similar to those to be measured in actual experiments by glass and Sb electrodes could show if significant distortions of pH values were to be expected. As reported before there were no significant differences between such measurements in urine and plasma ultrafiltrate (12).

*Measurement of pH in micropuncture experiments.*

A schematical drawing of the various experimental designs that we have used with the antimony microelectrode is given in Fig. 3.

![Diagram of experimental uses of Sb microelectrodes.](image)

**Fig. 3.** Diagram of experimental uses of Sb microelectrodes. Upper diagram, "in situ" pH measurement. Sb, antimony electrode; LG, Ling-Gerard reference electrode; A, differential amplifier; DVM, digital voltmeter. Middle diagram, bicarbonate concentration determination. Lower diagram, set-up for continuous recording of tubular pH. P, polygraph.
In situ pH measurements: in the upper part of the figure the introduction of an antimony and a Ling-Gerard reference microelectrode into a cortical loop is shown. The electrodes are connected to a differential amplifier, for instance the Keithley mod. 604 differential electrometer amplifier, whose output is fed into a digital volt meter or into a recorder permitting the expansion of the voltage scale in the range of the used pH values. Differential measurements are necessary for distal tubular pH measurement, since a significant transtubular potential difference is normally found. In the case of the proximal tubule, we have frequently used a single ended measuring system with the antimony microelectrode connected to a Keithley mod. 602 electrometer, measuring the voltage against the rat's tail which is grounded via a calomel electrode. This is an advantage because although a good tip localization is easy to ascertain for the Sb electrode, this is not the case for the Ling-Gerard microelectrode in the proximal tubule. Since the transtubular PD in the proximal tubule is low, it does not interfere significantly where pH measurements(27). The electrode is calibrated in standard buffers after every impalement. It commonly maintains its pH sensitivity quite well and frequently one electrode can be used for an entire experiment.

Bicarbonate determination: this measurement is shown in the middle diagram of Fig. 3. The system is essentially similar to that of “in situ” determinations, but the electrodes are introduced into a droplet of collected tubular fluid maintained under mineral oil equilibrated with a gas mixture containing a known concentration of CO₂. The bicarbonate concentration of the tubular fluid sample can be calculated by the Henderson-Hasselbalch equation from the measured pH and the CO₂ concentration of the gas, or more precisely, determining the CO₂ concentration of the oil by measurement of the pH of an equilibrated droplet of known bicarbonate concentration and using this value for the tubular fluid samples. The comparison of such measurements with the “in situ” values determined at the collection site has indicated the existence of a disequilibrium pH in the normal distal tubule as well as in proximal and distal tubule of acetazolamide treated rats, as predicted by Walser and Mudge(7) and also found by Rector et al(11) with the aid of glass microelectrodes. More recently we have been able to study bicarbonate concentrations and segmental reabsorption rates, as well as “in situ” and disequilibrium pH, in a variety of acute and chronic alterations of acid-base equilibrium(28,29).

Kinetic measurements: one of the important characteristics of the antimony microelectrode is its rapid response to pH changes as can be seen in Fig. 4, which shows a pH curve obtained by the experimental design given on the lower part of Fig. 3. A cortical tubule is perfused with a double barrelled micropipette; a column of an alkaline buffer solution is injected, isolated by oil, and maintained in contact with the antimony microelectrode system. The pH of this fluid column is monitored continuously, and recorded on a polygraph channel. The perfusion solution contains sodium in equilibrium concentration and raffinose in order to reduce volume changes during the measurements. Chloride is substituted for totally or in part, by bicarbonate or phosphate. In Fig. 4, it can be noted that after starting the perfusion with alkaline buffer the new pH level is reached
almost instantaneously. The approach of the recording to the new level, obtained from graphs such as that in Fig. 4, is of an approximately exponential nature, as shown on Fig. 5. In this representative example the new equilibrium value is approached with a half-time of 0.21 seconds, sufficiently fast for an undistorted evaluation of the actual pH changes going on in the tubular lumen. As shown in Fig. 4, after blocking the tubule with oil the pH first falls rapidly, then progressively slower, and reaches a steady-state after a period of ½ to 1 minute, depending on the experimental situation. We have described in detail how such perfusion experiments can be used to evaluate the rate of change of bicarbonate concentrations in the tubular lumen(9). The measured pH values are transformed into bicarbonate concentrations by the Henderson-Hasselbalch equation, assuming equilibrium with the rat's pCO₂. When the bicarbonate concentrations are plotted on a semilog graph paper, as shown on Fig. 6, an exponential concentration decrease can be noted in the latter part of the graph.

![Graph](image)

**Fig. 4.** Recording of pH variation during tubular perfusion with bicarbonate containing solution. At right, values of standard buffer solutions. From Giebisch, G., and Malnic, G., Proc. 4th Int. Congr. Nephrol. 1 (181-194), Karger: Basel, 1970.

![Graph](image)

**Fig. 5.** Velocity of response of Sb microelectrode to a change of pH. Voltage deflection in mm at new equilibrium minus mm at time t plotted against seconds.
where equilibrium with the rat's pCO₂ can be reasonably expected. The observed half-time of bicarbonate concentration decline is most likely related to the re-absorption rate of this ion. On the other hand, the bicarbonate concentrations calculated for the early part of the curve are certainly artifactually high since equilibrium with the rat's pCO₂ should not yet have occurred and since, in this series of experiments, the perfusion fluid was preequilibrated with air. From this part of the graph the rate of equilibration of the perfuson fluid with the blood CO₂ or H₂CO₃ concentration can be calculated. The carbonic acid concentrations at every moment are obtained from the measured pH and the bicarbonate concentration as extrapolated from the slower exponential toward zero time. A graph obtained in this way is shown in the lower insert of Fig. 6. It can be seen that the carbonic acid concentration of the perfusate column approaches exponentially the plasmatic concentration of this acid, with a half-time of 1.85 seconds in this case. This approach was shown to probably depend upon the rate of CO₂ diffusion into the column, and not on its rate of hydration since the latter as calculated from in vitro rate constants, is about one order of magnitude greater than the observed rate of carbonic acid concentration increase(9). It is thus possible to obtain by this technique a measure of the per-
meability of the tubular wall to CO₂, and also of the rate of bicarbonate re-absorption if a sodium bicarbonate containing fluid is used. It should be stressed that such measurements are possible because the response time of the antimony microelectrode is considerably faster than the observed physiological pH changes within the tubular lumen.

Recently, we performed experiments where the injected buffer solution is alkaline sodium phosphate and where the rate of titratable acidity formation in the tubular lumen is obtained(30). These methods have permitted the study of the mechanisms of tubular acidification in a variety of experimental conditions. *Titratable acidity in tubular fluid samples:* Recently, Solomon and Alpert(31) and Karlmark(32) have used the Sb microelectrode for the measurement of titratable acidity of fluid samples collected from renal tubules. One microelectrode can be used both for pH measurement and intermittent titration; alternatively, two electrodes may be employed, one for each of these functions. This procedure is based on the electrode reaction discussed above whereby, upon passage of current, OH⁻ ions are liberated and can be used to titrate an acid sample toward a more alkaline pH. The current used for this titration is stored on a condensor bank and the total charge used is measured at the end of the titration. The same procedure has also been used to measure the concentration of ammonium ions in tubular fluid samples, adding formaldehyde after the titratable acidity measurement and titrating again the H⁺ ions liberated from the ammonium ions back to plasma pH(32).

**Pitfalls in the use of the antimony microelectrode:** For a good function of the electrode the continuity of the metal down to its very tip is essential. Sometimes small air bubbles or cracks prevent this continuity by opening the measuring circuit; this factor should be checked by microscope during the preparation of each electrode.

The induction of current flow through the electrode can lead to polarization or even to electrolysis with dissolution of the electrode tip accompanied by the liberation of gas. This was observed when the heating system of the rat operating table was left in function during the measurements; the described phenomena occurred when the heating system was activated. To prevent this occurrence, which produces an effective opening of the measuring circuit, a water-filled table was used and the electrical heating turned off during the measurements. The water reservoir maintained the necessary constancy of the temperature long enough, until the heating could be turned on again. The oil bathing the kidney was heated by a remotely located water circulating system.

Another difficulty we have sometimes encountered concerns high tip potentials of the Ling-Gerard reference electrodes; these can lead to unpredictable junction potentials in different solutions, for instance in the buffers which will be added to the pH voltage. We have reduced these potentials by using low-resistance glass microelectrodes (3 to 5 M) freshly filled with 3M KCl; the storage of these electrodes for more than two or three days frequently leads to increased tip potentials as well as high electrode resistance.
From the preceding discussion it is apparent that the antimony microelectrode is a valuable tool for renal tubular pH measurements. This is due to the simplicity of its manufacture, its perfect insulation and its low resistance, which reduces electrical interference and thus increases electrode stability. Theoretical aspects of electrode function, as well as its construction and characteristics, are described. Oxygen tension does not affect the readings within the physiological range of tensions expected in the renal cortex, only values as extreme as pure nitrogen or oxygen leading to significant alterations. Stirring of the measured fluid, which may affect pH readings, does not interfere with “in vivo” tubular fluid measurements at the physiological flow rates, as verified in model experiments. Readings in plasma ultrafiltrate and urine were within ± 0.15 units of values determined with macro glass electrodes. This microelectrode has been used for the determination of “in situ” pH along the nephron of rat kidney, and also for the “in vitro” measurement of bicarbonate concentrations in tubular fluid samples preequilibrated with a known pCO₂. The occurrence of a disequilibrium pH was observed in several experimental situations. Furthermore, this electrode responds very rapidly to pH changes, reaching a new equilibrium value with a half-time of a few tenths of a second. This permits continuous monitoring of rapid intratubular pH changes. Thus, an evaluation of the kinetics of CO₂ transfer across the tubular epithelium and of the rate of bicarbonate reabsorption and hydrogen ion secretion in cortical segments is made possible.

REFERENCES

1. Montgomery, H., and Pierce, J. A. The site of acidification of the urine within the renal tubule in amphibia. Amer. J. Physiol. 118, 144-152 (1937).
2. Pierce, J. A. and Montgomery, H. A microquinhydrone electrode: its application to the determination of the pH of glomerular urine of Necturus. J. Biol. Chem. 110, 763-775 (1935).
3. Bank, N. Relationship between electrical and hydrogen ion gradients across rat proximal tubule. Amer. J. Physiol. 203, 577-582 (1962).
4. Clapp, J. R., Watson, J. F., and Berliner, R. W. Osmolality, bicarbonate concentration, and water reabsorption in proximal tubule of the dog nephron. Amer. J. Physiol. 205, 273-280 (1963).
5. Gottschalk, C. W., Lassiter, W. E., and Mylle, M. Localization of urine acidification in the mammalian kidney. Amer. J. Physiol. 198, 581-585 (1960).
6. Giebisch, G., Windhager, E. E., and Pitts, R. F. Mechanism of urinary acidification, in “Biology of Pyelonephritis,” pp. 277-287. Little, Brown, Boston, 1960.
7. Walser, M., and Mudge, G. H. Renal excretory mechanisms, in “Mineral Metabolism,” (C. L. Comar and F. Bronner, Eds.), Vol. 1 A, pp. 287-336. Academic, New York, 1960.
8. Gibbons, B. H., and Edsall, J. T. Rate of hydration of carbon dioxide and dehydration at 25°C. J. Biol. Chem. 238, 3501-3507 (1963).
9. Malnic, G., and Mello Aires, M. Kinetic study of bicarbonate reabsorption in proximal tubule of the rat. Amer. J. Physiol. 220, 1759-1767 (1971).
10. Carter, N. W., Rector, F. C., Campion, D. S., and Seldin, D. W. Measurement of intracellular pH of skeletal muscle with pH-sensitive glass microelectrodes. J. Clin. Invest. 46, 920-933 (1967).
11. Rector, F. C., Carter, N. W., and Seldin, D. W. The mechanism of bicarbonate reabsorption in the proximal and distal tubules of the kidney. J. Clin. Invest. 44, 278-290 (1965).
12. Vieira, F. L., and Malnic, G. Hydrogen ion secretion by rat renal cortical tubules as studied by an antimony microelectrode. *Amer. J. Physiol.* 214, 710–718 (1968).

13. Ives, D. J. G., and Janz, G. J. “Reference Electrodes: Theory and Practice,” pp. 336–356. Academic, New York, 1961.

14. Stock, J. T., Purdy, W. C., and Garcia, L. M. The antimony–antimony oxide electrode. *Chem. Rev.* 58, 611–626 (1958).

15. Haggard, H. W., and Greenberg, L. A. An antimony electrode for continuous recording of the acidity of human gastric contents. *Science* 93, 479–480 (1941).

16. Thompson, F. C., and Brudevold, F. A micro antimony electrode designed for intraoral pH measurements in man and small experimental animals. *J. Dent. Res.* 33, 849–853 (1954).

17. Perley, G. A. Antimony electrode for industrial hydrogen ion measurements. *Ind. Eng. Chem.*,* Anal. Ed.* 11, 316–318 (1939).

18. Kortum, G., and Bockris, J. O. M. “Textbook of Electrochemistry,” p. 294. Elsvier, New York, 1951.

19. Tourky, R., and Mousa, A. A. Studies on some metal electrodes. III. Does the antimony electrode behave simply as a metal-metal oxide electrode in air? *J. Chem. Soc. London* 752–755 (1948).

20. El Wakkad, S. E. S. The electrochemical behaviour of the antimony electrode. *J. Chem. Soc., London*, 2894–2896 (1950).

21. Perley, G. A. Characteristics of the antimony electrode. *J. Chem. Soc., Anal. Ed.* 11, 319–322 (1939).

22. Britton, H. T. S. and Robinson, R. A. The use of the antimony–antimony oxide electrode in the determination of the concentration of hydrogen ions and in potentiometric titrations. *J. Chem. Soc. London* 458–473 (1931).

23. Gysinck, T. Use of the antimony electrode for determining the degree of acidity. *Chem. Abstr.* 27, 2325 (1933).

24. Hooghoudt, S. B. The antimony electrode as an indicator of hydrogen-ion concentration in soil suspensions. *Veslag. Landbouw. Onderzoek.* 35, 162–208 (1930).

25. Thurau, K., and Deetjen, P. Kinematographische Untersuchungen am Warmblueternephrion. *Nachr. Akad. Wiss. Goettingen, Math. Phys. Kl.* 2, 27–37 (1961).

26. Barrow, G. M. “Physical Chemistry,” pp. 542–543. McGraw-Hill, New York, 1966.

27. Froemter, E., and Hegel, U. Transstubulaere Potentialdifferenzen an proximalen und distalen Tubuli der Rattenniere. *Pflugers Arch.* 291, 107–120 (1966).

28. Malnic, G., Mello-Aires, M., and Giebisch, G. Micropuncture study of renal tubular hydrogen ion transport during alterations of acid-base equilibrium. 222, 147–158 (1972).

29. Mello-Aires, M., and Malnic, G. Micropuncture study of tubular acidification during hypochloremic alkalosis in the rat. *Pflugers Arch.* 331, 13–24 (1972).

30. Malnic, G., Mello-Aires, M., de Mello, G. B., and Giebisch, G. Acidification of phosphate buffer in cortical tubules of rat kidney. *Pflugers Arch.* 331, 275–278 (1972).

31. Solomon, S. and Alpert, H. A method for determining titratable acidity in nanoliter samples of biological fluids. *Anal. Biochem.* 32, 291–296 (1969).

32. Karlmark, B. An ultramicro method for the separate titration of hydrogen and ammonium ions. *Pflugers Arch.* 323, 361–365 (1971).