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Measurement of Tubular Fluid Bicarbonate Concentration by the Cuvette-Type Glass Micro pH Electrode*

DAVID Z. LEVINE†

Departments of Medicine and Physiology of the University of Ottawa Medical School, Ottawa, Ontario, Canada

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My comments will be confined to aspects of electrode construction or measuring technique that differ from those originally described by Uhlich, Baldamus, and Ullrich(1). I wish to indicate my deep gratitude to these investigators who kindly introduced me to their elegant method in 1968.

The high impedance Orion 801 amplifier with specially shielded cables was used for the pH electrode portion. The multichambered Plexiglas bath (Fig. 1) differs only in small details from that built by the Frankfurt group. Our chamber is perhaps simpler to construct because it is made of cylindrical shapes and we have not required sloping sides to control bubble accumulation. For reasons that are not entirely clear to me we usually get very quiet records despite the fact that we do not use a cage set-up.

The major modifications which we introduced involve sample handling, validation of the pCO_2 of the sample, and the question of so-called memory effects. In the following paragraphs I will review our experience relating to these issues and then comment on some of the theoretical points concerned with the calculation of tubular fluid bicarbonate or pH using this type of *in vitro* measuring system.

It was found that when the sample is introduced into the electrode, movement of its meniscus created both electronic noise and the danger of aspirating the entire sample thereby breaking contact with the external reference electrode. A constriction well up into the inert glass segment as shown in Figs. 2 and 3 stabilized the meniscus and minimized these difficulties.

Second, in our early trials serious discrepancies were found between the actual bicarbonate concentration of known micro sample standards, and the bicarbonate

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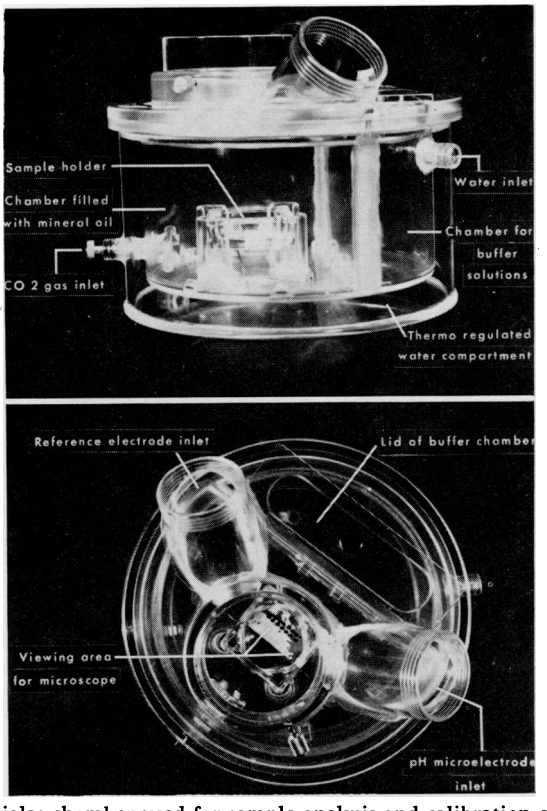

FIG. 1. Plexiglas chamber used for sample analysis and calibration of electrode.

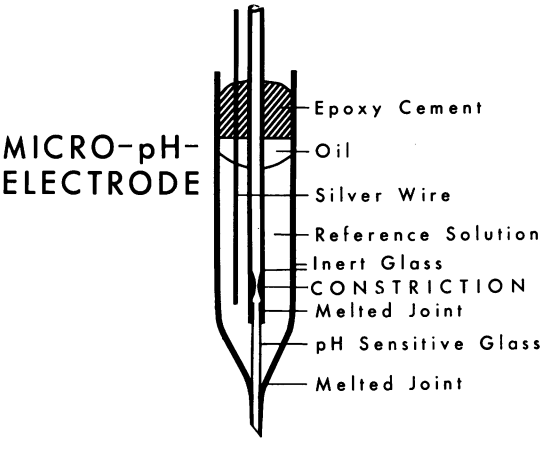

FIG. 2. Schematic drawing of micro pH electrode construction identical to that of Uhlich *et al.*(1) except for constriction.

concentration calculated from the pCO_2 of the chamber and the measured pH of the sample. Although making bicarbonate standards is not without problems, we soon realized that our difficulties lay in the false assumption that the standards were 100% equilibrated with the tank of CO_2 gas which we had meticulously analyzed ourselves. Indeed, there is no *a priori* way of determining adequate tonometry of the oil in our partly open Plexiglas bath. Although it is true that brisk tonometry for long periods of time produces full equilibration, practically this presents two serious difficulties. Vigorous tonometry significantly increases the noise level and thereby obscures accuracy. Also, the greater the rate of bubbling the more difficult it is to maintain adequate vision because of the tendency of bubbles to collect on the under surface of the Plexiglas. These difficulties have been circumvented by tonometerizing at some steady but low rate of bubbling and then operationally determining the pCO_2 of the samples' environment by measuring the pH of known bicarbonate micro standards. This is done during the measuring run; in this way changes in ambient pCO_2 can readily be appreciated. Provided the bubbling rate is constant a steady state can always be reached whereby pCO_2 changes less than 1 or 2 mm Hg during an hour period. Clearly, if it can be established that the electrode does not drift significantly, by measuring its response to phosphate buffer, and if we know the pCO_2 is validly determined, then the bicarbonate concentration of the unknown sample can be calculated. We have designed a windshield-wiper device which removes the few bubbles that accumulate even at low tonometry rates.

Our last and most vexing electrode problem was the observation that severe "memory effects" existed when we went from samples of high bicarbonate concentration to low values. Rinsing of the pH glass segment with large sample volumes was not successful. This meant that contamination, at least in the usual sense, was not responsible. Fortuitously, it was discovered that repeated traversing of the pH-sensitive glass with the oil-sample meniscus before closing the circuit regularly removed this memory phenomenon.

Figure 4 shows a sample record in which repeated determinations of differing and known bicarbonate concentrations are accurately carried out using this pro-

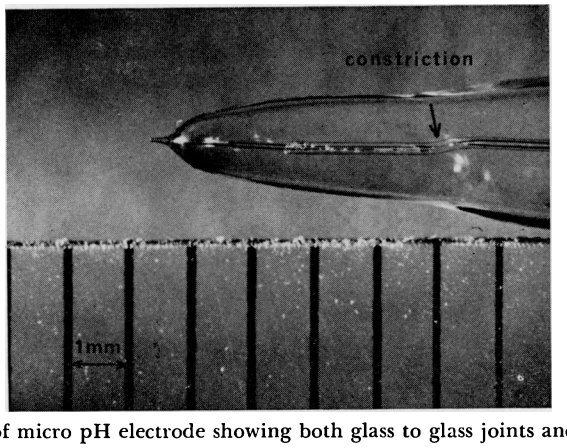

FIG. 3. Photo of micro pH electrode showing both glass to glass joints and constriction.

cedure. Figures 5 and 6 show records of actual sample runs and demonstrate that drift is quite small, pCO_2 is well maintained and that resolution of better than 0.12 mV is not unusual.

Finally, it would be appropriate to consider other assumptions that are implicit in these measurements. We assume that the pCO_2 of the tubular fluid and the epithelium of the exposed kidney during micropuncture is identical to that of blood. Since proximal tubular fluid is virtually devoid of buffer substances (the concentrations of ammonium and phosphate are very low) then the bicarbonate concentration will not change when the pCO_2 of the sample is set at a

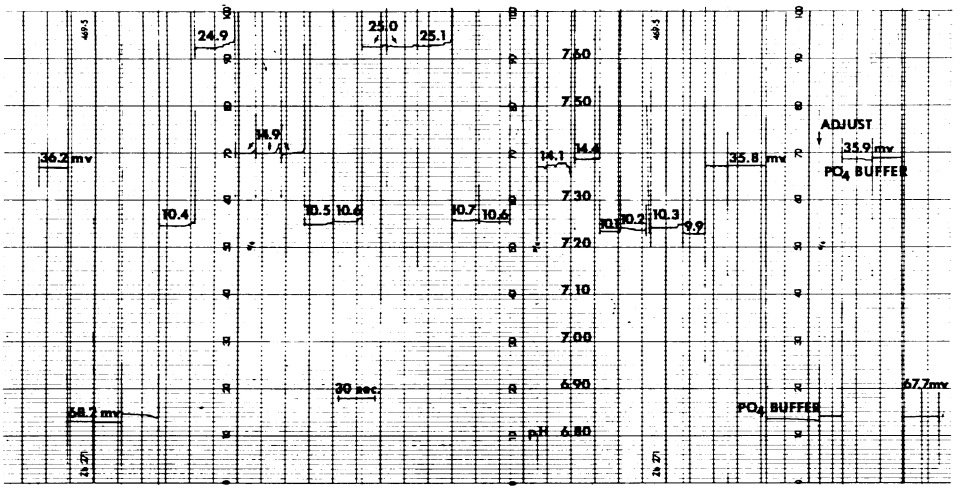

FIG. 4. Actual electrode recording demonstrating absence of "memory" effects. Vertical lines indicate separate samples (i.e., there are 18 separate samples on this record). Measured values of bicarbonate standards of 10,15 25 mEq/l.

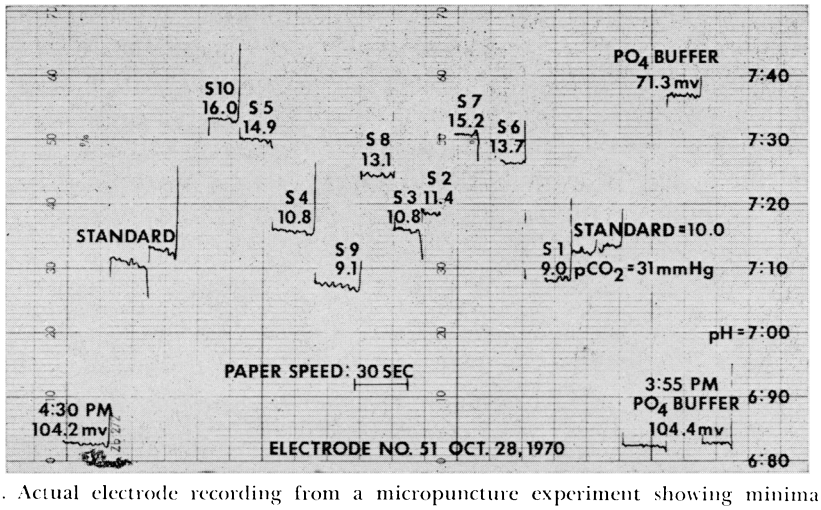

FIG. 5. Actual electrode recording from a micropuncture experiment showing minimal drift and stable pCO_2. Bicarbonate concentration of samples(S) in mEq/l.

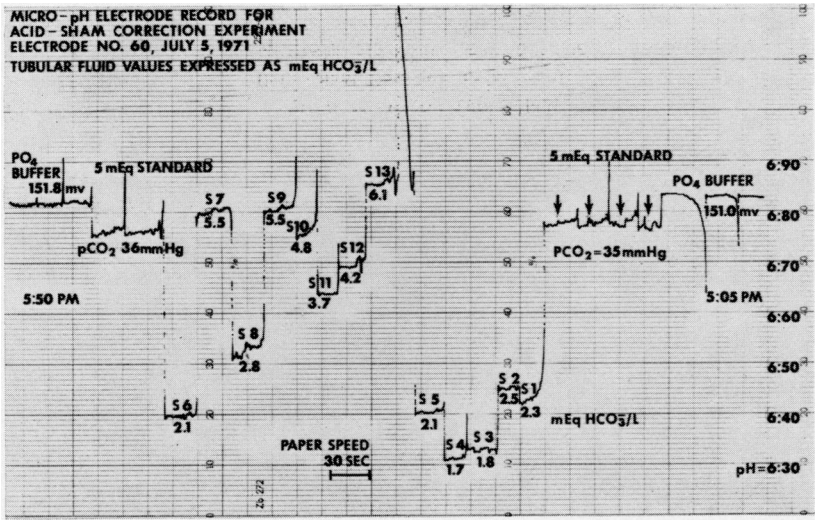

FIG. 6. Actual electrode recording from an ammonium chloride acidosis experiment in which bicarbonate concentrations are very low.

new level as an artifact of its *in vitro* measurement by the microelectrode. However, if one wishes to quantitate the biological effect of a given pCO_2 on the nephron's epithelium, or to calculate accurately the intratubular pH (by knowing the bicarbonate concentration of the sample and the blood pCO_2) then the issue of the identity of blood pCO_2 and that of the punctured nephrons is critical. In general it is assumed that proximal tubular fluid has CO_2-solubility and pK characteristics of a 300-mOsm saline solution at 37°, that its pCO_2 is that of arterial blood, and that no disequilibrium pH exists in the proximal tubule. If this is the case, proximal tubular fluid pH can be derived from arterial pCO_2 and *in vitro* bicarbonate measurements. However, in a given experimental setting it is obvious that if there is a tendency for a disequilibrium pH to develop, or if tubular-blood pCO_2 differences exist, or if the pH changes, such a calculation of intratubular pH would be invalid. Finally, since distal tubular fluid can vary significantly in its solute concentration, obviously calculation of distal tubular fluid bicarbonate concentration is not acceptable unless at least the Na concentration of the sample is determined and appropriate pK corrections are made. At present, no other means of circumventing this error, which may be appreciable, are available.

REFERENCES

1. Uhlich, E., Baldamus, C. A., and Ullrich, K. J., 1968. Behaviour of CO_2-pressure and bicarbonate in the countercurrent system of renal medulla. *Pflugers Arch.* **303**, 31.