The Production and Testing of Double-Barreled pH Glass Microelectrodes for Measurement of Intratubular pH

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It has been possible to miniaturize the glass pH electrode to an extent that it can be used to measure the intraluminal pH of single mammalian nephrons(1). There are, however, a number of problems that arise from the miniaturization process. These include electrode insulation, the incorporation of a suitable reference electrode with the pH glass electrode, and the ultimate achievable miniaturization of the micro pH sensitive tip. There are certain criteria that must be satisfied before such electrodes can be used. The first arises from the problem of adequate insulation. Insulation must be such that only a small portion of the tip of the electrode that is entirely within the lumen of the nephron is responsive to hydrogen ion activity. The reference electrode should be an integral part of the pH electrode so that the voltage reading across the reference and pH electrode will not be affected by any transepithelial potentials that may exist within the nephron. The size of the tip of the electrode should be sufficiently small so as not to cause extensive damage to the nephron when the puncture is made. It is now possible to make a miniaturized pH electrode with an integral reference whose combined tip diameter is somewhat less than 1 μ.

Probably the most formidable problem in making the miniaturized pH electrode is to obtain adequate insulation of all but the extreme tip of the glass pH electrode. The types of insulating materials tried by us and the results obtained have been discussed previously(2). All organic insulating materials were unsuccessful; two methods of insulation proved satisfactory. The first was a staining technique in which some of the sodium in the surface of the glass pH capillary was exchanged with silver ions, thus obliterating its pH sensitivity(3). This method, although quite satisfactory for producing small numbers of electrodes, was

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found to be difficult to control in the production of the large numbers of electrodes usually needed for biologic studies. For this reason it seemed appropriate to concentrate on the second satisfactory method of insulation which involved coating the surface of the pH glass capillaries with a ceramic glaze or enamel prior to pulling the microtip(4). As shown in Fig. 1, when the glazed capillary is pulled into an electrode tip, a small portion of the pH-sensitive glass emerges from beneath the insulating glaze to form a pH-sensitive tip. The length of this tip ranges from 5-20 μ and is controlled by the diameter and wall thickness of the capillary, the thickness of the glaze, and the length of the taper produced by the electrode puller.

In order to produce an integral reference with the glass pH electrode, a small lead glass capillary was cemented to the previously glazed pH glass capillary. The two capillaries are pulled as a unit into a single double-barreled tip, with the pH side closed and the reference side open. This is accomplished by adjusting the outside diameters of the pH glass capillary and lead glass reference capillary so that there is a somewhat larger reference glass capillary than pH glass capillary. By trial and error it is possible to arrive at capillary dimensions that allow the pH side and reference side to end simultaneously and the pH side to pull closed while the reference side remains open. After pulling the microtip, both sides of the double-barreled electrode are filled under vacuum with distilled water. The reference side of the electrode is then filled by displacement with the reference solution. A Ag–AgCl electrode is placed in the reference side and sealed in with silastic rubber. Figure 2 is an illustration of the completed electrode.

Some peculiarities of the reference side of the double-barreled pH electrode assume particular importance when this type of electrode is used in renal nephron experiments. Originally the reference side of the electrode was filled with 3 M potassium chloride(5). It was found, however, that it was necessary to measure very carefully the relative tip potential between reference electrode and an indifferent calomel electrode in a variety of solutions before the completed double-barreled electrode was used for pH determination. When 3 M KCl was used, it was observed that frequently a large tip potential slowly developed in solutions where the primary anion in the solution was something other than chloride. This was particularly true in solutions containing phosphate, bicarbonate and sulfate.

![Fig. 1. Emergence of pH-sensitive glass from beneath the ceramic glaze as the coated capillary is pulled into a tip. Figures 1–3 are reprinted by permission of Federation Proceeding (Ref. 2).](image-url)
The tendency for the tip potential to slowly become more negative in these solutions was increased when the resistance of the electrode was exceptionally high. Moreover, it was noted that the resistance of the electrode tended to increase as the tip potential became more negative. In order to obviate this problem, a variety of filling solutions were investigated. The range of KCl solution from 1.5 M to 3 M were tested and it was found that as the concentration of KCl was dropped below 3 M the tendency for the increase in tip potentials increased. It was therefore decided to alter the filling solution by adding a small amount of potassium nitrate to the potassium chloride solution as proposed by Grove-Rasmussen(6). The recommended ratio of 3 to 1:KCl to KNO₃ was not as useful as the solution we now use which is 2 M KCl and 0.5 M KNO₃. With this filling solution, even with high resistance reference electrodes (15 MΩ) the tendency for marked variation in the tip potential is exceedingly small. Nevertheless, it is necessary to test each electrode to be certain of no wide-variation in the tip potential of the reference side in the buffer solutions and physiologic solutions in which an electrode is to be used.

In actual practice after the electrode is correctly filled, it is subjected to a variety of tests(7). First, the pH sensitivity is tested in three known pH buffer solutions to establish the slope of the electrode and its linearity. At the same time the resistance of the pH side of the electrode is measured. It has been found that best electrode have a resistance of the pH side of between $5 \times 10^8$ and $10^9 \Omega$. The slope of the electrode should be at least 70% of the Nernst value for the temperature of the experiment. In general, electrodes with adequate resistance and good slope are linear over a pH range of from pH 4 to pH 10. We have recently found that some of the pH electrode tips apparently have a microopening which is not apparent from the resistance measurements or from the slope of the electrode or its linearity. This defect can only be discovered when pH of a known buffer solution which has a high ionic strength is measured. For this purpose we use a standard phosphate buffer to which has been added 200 mM of sodium chloride and 200 mM of potassium chloride. The pH of this final solution is determined by an appropriate macro pH instrument. In the event the micro pH electrode has

![Double-barreled micro pH electrode. The silastic rubber is used to seal the Ag–AgCl electrode into the reference sid.](Fig. 2)
a hole in its tip, it will give a falsely high pH reading for this buffer solution. On the other hand, if the tip is closed it will give the correct pH reading for this solution. Just what the consequences of using pH electrodes with a very small open tip are in biologic experiments has not been fully evaluated. Nevertheless, it seems important to circumvent this difficulty with the buffer test solution as outlined above.

As described above, the reference side of the pH electrode should be independently investigated with respect to its tip resistance and tip potential in various solutions. In the case of experiments where the tubule is to be perfused with a particular solution, it is obvious that this solution should be added to the group of solutions used to ascertain the stability of the tip potential of the reference side.

Finally, before using these electrodes in biologic experiments, it is necessary to determine accurately the length of the pH-sensitive tip of the microelectrode. This was originally accomplished in the kidney by an in vivo procedure in which a pH buffer was perfused through the lumen of a nephron; while the microelectrode was reading this pH, another pH buffer was overlayed on the surface of the kidney around the stem of the pH electrode(1). In the event no change in voltage reading was obtained when the second buffer was placed around the pH electrode, it was assumed that the electrode was adequately insulated. A more accurate and easier procedure for determining adequacy of insulation has been developed utilizing an in vitro test system(7) depicted in Fig. 3. A thin latex membrane is made and placed over a buffer which has been solidified with 3% agar. In this particular case the pH of the solidified buffer was 6. By means of microscopic visualization, the electrode is advanced through the membrane for a distance measured by a micrometer eyepiece. The distance advanced is usually 5–10 μ. The voltage of the electrode is then read for the agar buffer. In Step 2 a buffer is overlayed above the latex membrane surrounding the pH electrode. With a well-insulated electrode there should be no change in the voltage reading as the electrode should be reading the buffer agar only. Since it is possible that

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**Fig. 3.** The *in vitro* test system. In Step 1, the electrode tip is advanced into the agar-hardened buffer a measured distance. In Step 2, pH 7.4 buffer is overlayed on the latex membrane. In Step 3 a potential of 90 mV is applied across the latex membrane.
biologic potentials have an effect when imposed across the pH electrode, a third test is made in this in vitro system. In Step 3 the effect of a potential across the latex membrane on the voltage reading is ascertained. Again, in a well-insulated electrode there should be no change in the voltage reading for the pH electrode since it should still read the appropriate value for the buffered agar. To clarify this important procedure, the results of a test on an adequately and an inadequately insulated electrode are shown in Table 1. The electrodes had been calibrated in standard buffer solution prior to being subjected to the in vitro test. Both electrodes were advanced into the solidified pH 6 buffer. The insulated electrode was advanced to a depth of only 10 μ. Both electrodes gave a voltage reading for the agar buffer pH 6 quite in keeping with readings previously obtained for the standard buffered pH 6. However, when the latex membrane was overlayed with pH 7.4 buffer the uninsulated electrode registered a marked shift in the voltage reading thus indicating that this electrode was inadequately insulated above 42 μ from its tip. In contrast, the adequately insulated electrode showed no voltage change when the pH 7.4 buffer was overlayed on the membrane surface. Finally, when an electrical potential was imposed across the latex membrane, the adequately insulated electrode again showed no voltage change whereas the inadequately insulated electrode showed a marked voltage change. This technique permits us to accurately ascertain the length of the pH-sensitive tip of the micro pH electrode and thus use only adequately insulated electrodes in biologic experiments.

It has been previously determined that these electrodes are capable of accurately reading pH of biologic fluids containing high protein(2,7). In particular it has been shown that they can produce blood pH readings comparable to those obtained by a commercial macro pH system. In addition, the pH values of homogenates of various tissues can be accurately read by these electrodes.

Because of the high external resistance of the circuit, in making measurements with these micro pH electrodes, it is necessary to use a high quality electrometer with a high input impedance (10¹⁰ Ω). In practice a Cary vibrating reed electrometer was used to read the potential across the reference and pH sides of the electrode system. It is possible to use the reference side of the electrode as a Ling-type electrode to measure a transepithelial potential simultaneously with the pH measurement. This requires an additional electrometer in the circuit. A diagram of such an arrangement has been previously published(7). In order to obtain stability of the voltage measurements made with micro pH electrodes, it is helpful, and sometimes necessary, to conduct the experiments in a shielded or Faraday-type cage. In rat experiments, it is generally necessary to perfuse the surface of the exposed kidney with warmed oil in an effort to maintain the surface temperature of the kidney at a normal value. The pH buffers used to calibrate the micro pH electrode must therefore be kept at this same temperature. This is necessary because a mathematical correction for temperature difference between buffers and the unknown biologic fluid is exceedingly difficult due to the high temperature coefficient of pH glass. Although mineral oil may be used to perfuse the surface of the kidney, since it is sometimes useful to siliconize the
| Depth of penetration μ | Electrometer reading mV | Apparent pH of agar buffer | Depth of penetration μ | Electrometer reading mV | Apparent pH of agar buffer |
|------------------------|-------------------------|----------------------------|------------------------|-------------------------|----------------------------|
| Buffer pH 6.0          | —                       | + 157                      | —                       | + 155                   | 55 mV/pH unit              |
| Buffer pH 7.4          | —                       | + 76                       | 58 mV/pH unit           |                         |                            |
| Agar buffer pH 6.0     | 10                      | + 155                      | 6.04                    | 42                      | + 137                      | 5.96                      |
| Overlaid buffer pH 7.4 | 10                      | + 155                      | 6.04                    | 42                      | + 86                       | 7.24                      |
| Transmembrane potential of 90 mV imposed | 10 | + 155 | 6.04 | 42 | + 101 | 6.97 |

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outside surface of the micro pH electrode, it is necessary to use some oil other than mineral oil which condenses with the siliconized surface of the pH glass. Warmed castor oil has proved a satisfactory substitute for mineral oil and has no adverse effect on the micro pH electrode or the biologic preparation.

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