Biophys. Struct. Mechanism 2, 21—30 (1976)

Biophysics of Structure and Mechanism

pH-Sensitive Glass Microelectrodes and Intracellular pH Measurements

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Abstract. 1. Some properties of the open-tipped, uninsulated, pH-sensitive glass microelectrode were examined in several electrical experiments.

2. Based on these observations, technical and theoretical problems were considered for application to the pH measurement in small cells.

3. The intracellular pH, $(pH)_i$, of the epithelial cell in rat duodenum measured was approximately 7.0. A reduction in $(pH)_i$ was apparent (about 0.3) with the addition of 20 mM-glucose to the bathing fluid.

4. It was concluded that with certain limitations such uninsulated, open-tipped microelectrodes may be successfully utilized for intracellular pH measurements.

Key words: pH-Sensitive Glass — Uninsulated, Open-Tipped pH-Microelectrode — Tip Potential — Intracellular pH.

Introduction

Lavallée (1964) and Winship and Caflisch (1973) reported that open-tipped microelectrodes made of Corning 0150 pH-sensitive glass tubings were applicable to the measurement of intracellular pH. In the course of studying the nature of the tip potential (TP) of Ling-Gerard Type microelectrodes (Okada and Inouye, 1975a, b) we also investigated the feasibility of using microelectrodes made of 0150 glass. Based on our observations, technical and theoretical problems in applying such open-tipped pH-sensitive glass microelectrodes to the pH measurements in small cells are discussed.

Methods

Microelectrodes

Two types of glass microelectrodes of Ling-Gerard type were constructed in a pipette puller (Narishige, Tokyo) as described earlier (Okada and Inouye, 1975a). One was made of Pyrex capillaries of 2 mm outside diameter and 1 mm inside diameter (P-electrode), and the other of Corning 0150 capillaries of 1 mm outside diameter and 0.5 mm inside diameter (C-electrode), their tip diameters being 0.5 μm or less. Using the alcohol method (Tasaki *et al.*, 1954), C-electrodes were filled with 0.5 M KCl and then subjected to the potential and resistance measure-

ments with a system of reversible Ag-AgCl electrodes after aging for 7 ~ 14 days in the filling solution. Their resistances ranged from 100 to 1800 MΩ. P-electrodes for membrane potential measurements were filled with 3 M KCl by the glass fiber method (Tasaki *et al.*, 1968), their resistances and tip potentials ranged from 15 to 40 MΩ and 0 to − 5.0 mV, respectively. In order to make a comparison with the C-electrodes, P-electrodes were, sometimes, subjected to the measurements after aging for 7 days. Such a P-electrode had a smaller electrode resistance and a greater tip potential than a P-electrode without aging, as shown previously (Okada and Inouye, 1975a).

Measurements of Electrode Properties

The measurement technique of the electrode properties has been described in detail in the preceding paper (Okada and Inouye, 1975b). In brief, the microelectrode was connected to the high impedance input of the electrometer, the output of which was led to an oscilloscope and penrecorder. The tip potential (TP) was measured by the method described by Adrian (1956), with slight modification. The electrical resistance of the glass wall was measured using the arrangement described in the preceding paper (Okada and Inouye, 1975b); a thin layer of saline-agar was placed over the liquid paraffin (, air or CCl_4) in a small container, and saline with a pH of 7.3 was overlaid on the agar layer. The electrode tip was advanced through the agar layer into liquid paraffin (, air or CCl_4) under micrometer control and microscopic observation. Using such a method, we could also examine the "depth-effect" on pH-sensitivity described by Carter *et al.* (1967) and Winship and Caflisch (1973) by adding HCl or NaOH to the overlaid saline solution.

Potential Measurement in Rat Small Intestine

Membrane potentials of the epithelial cells in rat duodenum were measured *in vitro* using the technique as described elsewhere (Okada *et al.*, 1975). As the control saline solution, a phosphate buffer saline (PBS) was used and contained (mM) NaCl, 127.0; KCl, 2.7; $CaCl_2$, 0.9; $MgCl_2$, 0.5; Na_2HPO_4, 8.0; KH_2PO_4, 1.5; mannitol, 20.0; and pH 7.3 ± 0.1. The mannitol in this solution was replaced with 20 mM glucose in the experiments on the effect of glucose.

Results and Discussion

Tip Potential and Its pH-Dependency

As shown in Fig. 1, TP of C-electrodes in KCl solution of a given concentration is apparently greater than that of P-electrodes after aging for the same period (7 days). When the external KCl concentration was varied (0.01 ~ 0.5 M) at constant pH (7.3 ± 0.1 with 5 mM Tris buffer), however, the former changed in parallel with the latter. As discussed in detail previously (Okada and Inouye, 1975a, b), TP of P-electrodes after several days aging in electrolyte solutions chiefly originates from the interfacial potential built up on the thin glass wall of the tip region, and magnitude largely depends upon the ionic strength of the bathing solution. These results suggest that an increase in fixed charges brought about by aging in an electrolyte solution can hardly be attributed to the type of glass used for preparing microelectrodes, Pyrex or 0150. The more positive TP of

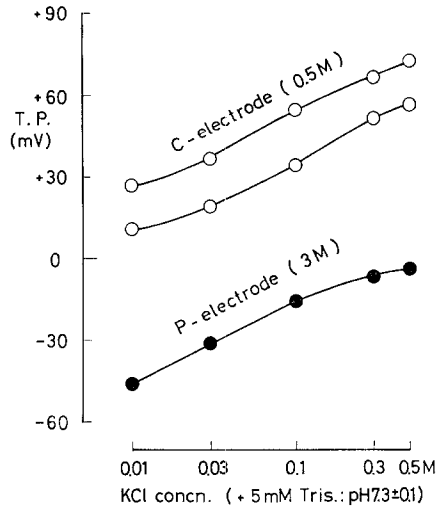

Fig. 1. Tip potentials of C- and P-electrodes measured in KCl solutions of various concentrations (+ 5 mM Tris-buffer; pH 7.3 ± 0.1) after 7 days of aging. ○: C-electrode filled with 0.5 M KCl, ●: P-electrode filled with 3 M KCl

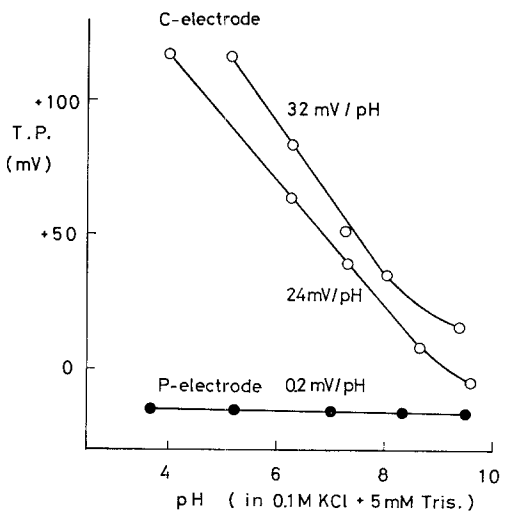

Fig. 2. Tip potentials of C- and P-electrodes measured in 0.1 M KCl solutions of various pH values (+ 5 mM Tris-buffer) after 7 days of aging. ○: C-electrode filled with 0.5 M KCl, ●: P-electrode filled with 3 M KCl

C-electrodes seems to reflect the effect of pH on the pH-sensitive part in their tip region. Indeed, when pH of the external solution was varied (5 mM Tris-buffer of pH 4 ~ 10) at a fixed KCl concentration, TP of C-electrodes increased in parallel with the decrease in pH, whereas TP of P-electrodes without aging did not change and even TP of P-electrodes after aging for the same period (7 days) was hardly affected by the pH changes (Fig. 2).

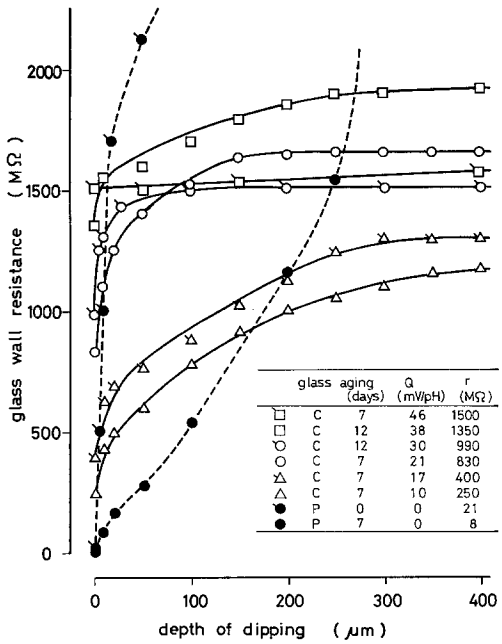

Fig. 3. Glass wall resistances of C-electrodes having different Q-values as well as P-electrodes. The aging periods, Q-values and electrode resistances without dipping (r) are listed in the insert

Linearity of the TP-pH plot in the range of pH $4 \sim 8$ (Fig. 2) shows that the pH-sensitivity of C-electrodes defined as $Q = -\Delta TP/\Delta pH$ is constant in the pH range of physiological interest. Moreover, the results shown in Fig. 1 suggest that the Q-value of each electrode remains almost constant in KCl of $0.01 \sim 0.5$ M, and presumably also in the other electrolyte solutions of the same ionic strength. As claimed by Lavallée (1964) and Winship and Caflisch (1973), such an open-tipped pH electrode appears to be applicable to the intracellular pH measurements.

Resistance and pH-Sensitivity of Partly Insulated Tip

Changes in the resistance and pH-sensitivity of the glass wall along the electrode shaft axis were examined. As long as the tip remained in saline or in the saline-agar layer, a microelectrode responded to acid or alkali addition and its resistance remained nearly constant, thus reflecting the electrode resistance as a whole. When the tip reached the paraffin, air or CCl_4 layer, however, the resistance (r) sharply increased. It becomes greater with the increase in the depth of dipping into paraffin (x), and then attained a nearly constant level 1000 to 1500 MΩ (Fig. 3). Response to acid and alkali addition was also lost at a certain depth, usually $x = 5 \sim 15$ μm.

Fig. 3 shows some examples of the r-x relation of C-electrodes having different Q-values. Also illustrated are those of the P-electrodes with and without aging. Of course, the P-electrodes were always practically pH-intensitive for all x's. It is worth noting here that the C-electrodes are roughly classified into 3 types according their characteristics; 1. Electrodes of $100 \sim 400$ MΩ, which give the r-x curve

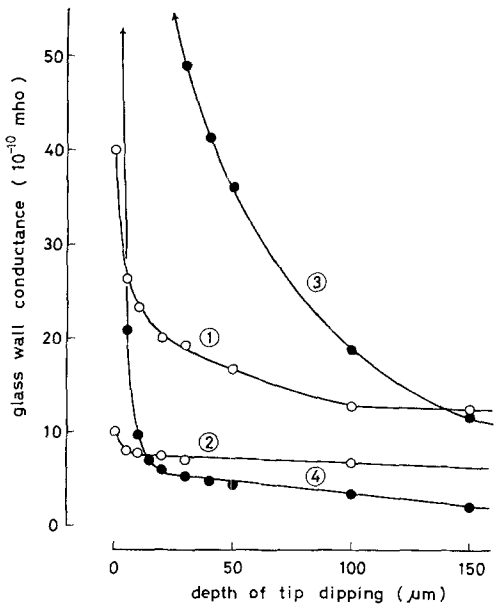

Fig. 4. Glass wall conductance of C-electrodes and P-electrodes. ①: C-electrode after 7 days of aging. $Q = 10$ (mV/pH), $r = 250$ (MΩ). ②: C-electrode after 12 days of aging. $Q = 30$, $r = 990$. ③: P-electrode after 7 days of aging. $Q = 0$, $r = 8$. ④: P-electrode without aging. $Q = 0$, $r = 21$

rising less steeply in the $x < 20$ μm and show a relatively low Q-value (less than 17). The Pyrex electrodes appear to be an extreme case of $Q \simeq 0$ in this category.

2. Electrodes having resistances of 400 ~ 1200 MΩ, whose r-x curve rises quite sharply in $x = 0 \sim 10$ μm. These have a relatively high Q of 20 ~ 35 and a pH-sensitive region of less than 15 μm.

3. Electrodes of high electrode resistance (about 1500 MΩ or more) the tip potentials of which often fluctuate. These are usually pH-intensitive but occasionally are highly pH-sensitive ($Q > 40$). In the latter case, the pH-sensitivity extends over 50 μm or more from the extreme point of the tip. These electrodes are very breakable, probably because of the very thin and pH-sensitive glass wall over a wide range of the tip region. It is absolutely required that a coat of insulating material be applied (*e.g.* a glaze) to such an electrode, when intracellular pH measurements are made in small cells, in order that the pH sensitivity be confined only to the distal 15 μm or less of the tip. Such was also pointed out by Winship and Caflisch (1973).

The conductivity ($= 1/r$) – x plot reveals the changes in the specific resistance along the electrode axis as shown in Appendix of our preceding paper (Okada and Inouye, 1975b). Such a plotting typical of category 1 and 2 is shown in Fig. 4. It is quite evident that the electrode of category 2 shows a high conductivity only in the tip region (< 10 μm), just as a non-aged P-electrode, which is possibly a pH-sensitive region. On the other hand, the electrode of category 1 shows a higher conductivity through the glass wall than that of category 2, and this $(1/r) - x$

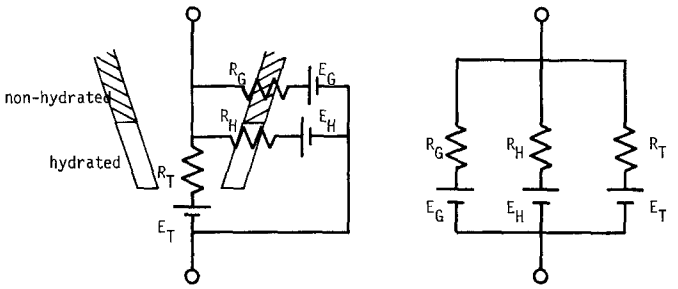

Fig. 5. Equivalent circuit model of C-electrode in the bulk solution. E_G, R_G: the e.m.f. and resistance across the glass wall of the pH-insensitive (non-hydrated) area. E_H, R_H: the e.m.f. and resistance across the glass wall of the pH-sensitive (well-hydrated) area. E_T, R_T: the e.m.f. and resistance through the tip pore

pattern is quite similar to that of an aged P-electrode. Such a high conductivity extending over 50 μm would result in a shunting effect upon the e.m.f. generated in the pH-sensitive tip region and a reduction of Q-values would ensue.

All these results suggest that uncoated, open-tipped pH-microelectrodes most suitable for intracellular pH measurements should be those belonging to category 2. They must have a relatively high sensitivity ($Q \geqq 20$) but the sensitive area should be confined to $5 \sim 15$ μm from the tip and strong enough to insert the cells.

Equivalent Circuit Analysis

There are no doubt many possible interpretations regarding the TP generation, however, the results reported hitherto (Bingley, 1964; Agin and Holtzman, 1966; Agin, 1969; Okada and Inouye, 1975a, b) strongly indicate that TP originates from one or more interfacial potentials between the glass itself and electrolyte solutions. In a simple approach to describe our system which generates a pH-sensitive TP, we adopted here the equivalent circuit diagram in Fig. 5, which is a modification of that proposed by Agin and Holtzman (1969), Lavallée and Szabo (1969), or Okada and Inouye (1975b). It is easy to derive TP of C-electrodes in a bulk solution from this model as

$$\mathrm{TP} = (R_T \cdot R_G \cdot E_H + R_H \cdot R_T \cdot E_G + R_H \cdot R_G \cdot E_T)/R, \tag{1}$$

where $R = R_H \cdot R_G + R_H \cdot R_T + R_T \cdot R_G$. Here, an e.m.f. of E_H appears across the glass wall in the pH-sensitive area with a resistance of R_H. The magnitude of E_H (but not R_H) depends on the difference in pH between the inside and the outside of the electrode. Since other E's and R's are practically insensitive to pH, the pH-sensitivity (Q) is given by

$$Q = -\frac{R_T \cdot R_G}{R} \frac{\Delta E_H}{\Delta \mathrm{pH}}. \tag{2}$$

So-called asymmetric potential may be included in E_H, but $\Delta E_H/\Delta$pH is theoretically expected to be around -60 mV/pH unit. When the tip of the electrode is completely insulated except for the pH-sensitive region, R_G would be extremely high, and so

$$Q \simeq \frac{60}{1 + R_H/R_T}.$$

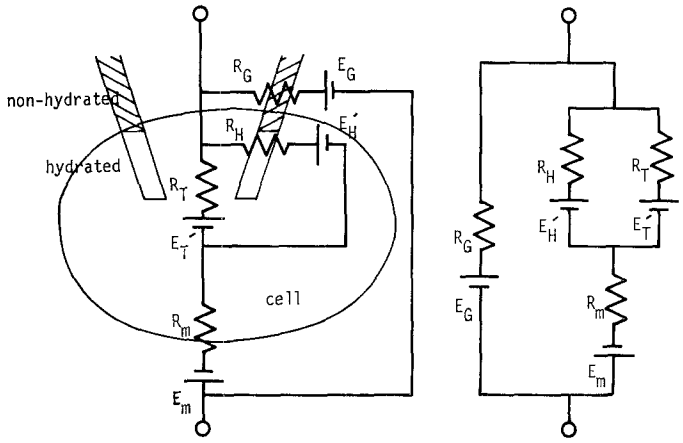

Fig. 6. A schematic diagram of C-electrode inserted into a cell. E_H': the e.m.f. across the glass wall of the pH-sensitive area in the cell fluid. E_T': the e.m.f. through the tip pore in the cell fluid. E_m: the membrane potential of the cell. R_m: the resistance of the cell membrane

In the open-tipped electrodes, however, R_T is finite and Q is always less than 60. If $R_H \simeq R_T$ as discussed below, the Q-value of such an insulated microelectrode is around 30 mV/pH unit. Winship and Caflisch (1973) reported about 45 mV/pH unit for the Q-value of their most suitable electrode. In this case, $R_H/R_T \simeq 1/3$ and R_H are not so different in the order of magnitude from R_T.

As described in the previous section, the Q-value of our electrode for intracellular measurements is $20 \sim 30$ mV/pH unit, so that

$$R_T \cdot R_G/R \simeq 1/3 \sim 1/2.$$

The results presented in Fig. 3 lead to

$$R_G \simeq 1000 \sim 1700\ \mathrm{M\Omega}$$

$$\frac{1}{R_G} + \frac{1}{R_H} + \frac{1}{R_T} = \frac{1}{\text{Electrode Resistance}} \simeq \frac{1}{400} \sim \frac{1}{1200}.$$

From these three relations it can be easily seen that these three resistances are in the order of magnitude of 10^3 MΩ and roughly $R_G \simeq R_H \simeq R_T$.

Since the pH-sensitive region is confined to the cell dimension, a diagram of a pH-sensitive microelectrode inserted into a cell could be approximately described as in Fig. 6, in which the e.m.f. of E_m, the so-called membrane potential, and the resistance of R_m across the cell membrane should be included. Since the value of R_m for most cells is some ten MΩ, it can be safely assumed as $R_m \ll R_H$, R_T and R_G. Thus the potential difference measured in this system is approximated to

$$\mathrm{PD} = \frac{1}{R}\left(R_T \cdot R_G \cdot E_H' + R_H \cdot R_T \cdot E_G + R_H \cdot R_G \cdot E_T'\right) + E_m\left(1 - \frac{R_H \cdot R_T}{R}\right). \quad (3)$$

Here, E_H' depends on intracellular pH, $(\mathrm{pH})_i$, and its deviation from pH of the bathing solution, $(\mathrm{pH})_0$, is given by

$$\Delta\mathrm{pH} = (\mathrm{pH})_i - (\mathrm{pH})_0 = (E_H' - E_H)/Q. \quad (4)$$

On the other hand, E'_T, the potential difference at the pore of tip in the cell fluid, would hardly differ from E_T in the bathing fluid, because this potential would be probably derived from the diffusion potential (Okada and Inouye, 1975b), and the solution inside of an electrode is a KCl solution of high concentration (0.5 M). Using Eqs. (1)$\sim$(4), the membrane potential measured with the pH-sensitive microelectrode, E'_m, can be written as

$$E'_m = -Q \cdot \Delta\mathrm{pH} + E_m (1 - R_H \cdot R_T/R).$$

Putting the difference between the membrane potentials obtained with C- and P-electrodes as ΔMP ($= E'_m - E_m$), therefore,

$$(\mathrm{pH})_i - (\mathrm{pH})_0 = -\Delta\mathrm{MP}/Q - (R_H \cdot R_T/R) \cdot E_m/Q. \tag{5}$$

The first term in the right hand of this equation can be evaluated by the measurement of E_m with Pyrex microelectrodes, but the second one remains unknown unless the values of R_H, R_G and R_T are estimated by other independent measurement(s). The second term disappears only when $R_G \to \infty$ is achieved by an appropriate insulation. Even in the case of uninsulated electrodes, change in $(\mathrm{pH})_i$ could be accurately estimated by evaluating the changes in ΔMP from the following equation, if E_m remains fairly constant.

$$\delta(\mathrm{pH})_i = \delta(\mathrm{pH})_0 - \delta(\Delta\mathrm{MP})/Q \tag{6}$$

Of course, an approximate estimation of $(\mathrm{pH})_i$ might be achieved from the following equation obtained by putting $R_H \cdot R_T/R \simeq 1/3$ into Eq. (5), because $R_H \simeq R_T \simeq R_G$ for our pH-sensitive microelectrodes.

$$(\mathrm{pH})_i \simeq (\mathrm{pH})_0 - \Delta\mathrm{MP}/Q - E_m/3Q \tag{7}$$

Application of pH-Sensitive Microelectrodes to Intracellular Measurements

Based on the foregoing discussions, we attempted to apply the pH-sensitive Corning-glass microelectrodes to $(\mathrm{pH})_i$ measurement of duodenal epithelial cells of rats. The dimension of the duodenal epithelial cells was around 15 μm $\times$ 50 μm in the histological section. The membrane resistance (R_m) of the cell in physiological saline was found to be 24.3 $\pm$ 2.2 MΩ ($n = 47$) (Okada and Irimajiri, unpublished observation). Therefore the above discussions are quite plausible at least as far as these particular cells are concerned.

The results obtained with the C-electrodes of $Q \geqq 20$ mV/pH are summarized in Table 1, in which the non-corrected $(\mathrm{pH})_i$ is computed from $\Delta\mathrm{pH} = \Delta\mathrm{MP}/Q$, and the corrected one by Eq. (7). Taking into consideration the lack of data concerning the absolute magnitude, $(\mathrm{pH})_i$ obtained with different microelectrodes is fairly constant irrespective of variation in the Q-value. The non-corrected $(\mathrm{pH})_i$ value, 6.3, is nearly equal the value in rat skeletal muscle reported by Carter *et al.* (1967). Recently, Paillard (1972) has pointed out that such a low $(\mathrm{pH})_i$ value would be an artifact because the membrane potentials recorded by the pH-microelectrodes are partly shunted. Indeed he obtained the low $(\mathrm{pH})_i$ values in rat and crab muscles using non-insulated pH-microelectrodes. The corrected $(\mathrm{pH})_i$ value, 7.0, is almost identical with the $(\mathrm{pH})_i$ values of various cells reported by many investigators (Caldwell, 1954; Kostyuk and Sorokina, 1961; Carter, 1961; Lavallée, 1964;

Table 1. Measurements of $(pH)_i$ in duodenal epithelial cells (see text for explanation)

No.	1	2	3	4
Q (mV/pH)	32	27.5	24	20
E_m' ± SE (mV) (n)	−22.7 ± 1.3 (10)	−25.7 ± 1.1 (13)	−22.5 ± 0.4 (5)	−27.7 ± 2.5 (5)
E_m ± SE (mV) (n)	−53.9 ± 1.3 (21)	−53.2 ± 1.7 (10)	−52.5 ± 1.6 (12)	−50.5 ± 1.1 (5)
$\Delta MP/Q$	0.98	1.00	1.25	1.14
$\Delta MP/Q + E_m/3\,Q$	0.41	0.36	0.52	0.30
$(pH)_0$	7.30	7.43	7.40	7.40
$(pH)_i$ (non-corrected)	6.3	6.4	6.2	6.3
$(pH)_i$ [corrected by Eq. (7)]	6.9	7.1	6.9	7.1

Table 2. Effect of 20 mM-glucose on the $(pH)i$ value (see text for explanation)

No.	3		4	
Q (mV/pH)	24		20	
condition	Control	Glucose	Control	Glucose
E_m' ± SE (mV) (n)	−22.5 ± 0.4 (5)	−20.0 ± 1.8 (4)	−27.7 ± 2.5 (5)	−23.7 ± 1.8 (7)
E_m ± SE (mV) (n)	−52.5 ± 1.6 (12)	−51.1 ± 1.5 (6)	−50.5 ± 1.1 (5)	−50.2 ± 1.8 (8)
$\Delta MP/Q$	1.25	1.30	1.14	1.33
$(pH)_0$	7.40	7.23	7.40	7.23
$\delta(pH)_i$ [— Eq. (6)]	0.2		0.4	
$\Delta MP/Q + E_m/3\,Q$	0.52	0.59	0.30	0.49
$(pH)_i$ (corrected)	6.9	6.6	7.1	6.7
$\delta(pH)_i$	0.3		0.4	

Paillard, 1972; Thomas, 1974). Thus the use of Eq. (7) appears reasonable, at least for our pH-sensitive microelectrodes.

The change in pH_i in the presence of external glucose (20 mM) is presented in Table 2. Glucose added to the bathing solution causes a slight depolarization of duodenal epithelial cells, but it is so slight that Eq. (6) holds. The values of decrease in $(pH)_i$ thus estimated are listed in Table 2 together with the values estimated by the corrected $(pH)_i$ values. The addition of glucose brought about a decrease of about 0.3 in $(pH)_i$. This may possibly be related to the increased glycolysis evoked by the increased intracellular sugar.

The results presented here suggest that our analysis on the tip potential provides a basis for applying pH-sensitive glass microelectrodes to intracellular pH measurement. The uninsulated, open tipped microelectrodes can be applied to

measure the intracellular pH value of the small cell as the tip is sharp, and construction is quite feasible as sealing and insulating procedures are not required.

Acknowledgements. Thanks are due to M. Ohara, Kyoto University, for assistance with the manuscript and to Miss T. Wada for secretarial service.

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Received July 24, 1975/Accepted October 14, 1975