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# Studies on the Origin of the Tip Potential of Glass Microelectrode

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**Abstract.** 1. Tip potential (TP) of glass microelectrodes filled with 3 M KCl increased remarkably with the increase in the storage period in 3 M KCl solution at 37° C, while the electrode resistances decreased gradually.

2. The electrical conductivity through the thin glass wall near the tip was found to increase in parallel with the TP increase.

3. The e.m.f. across the thin glass wall in the tip region was directly measured. This seems to contribute to the TP generation of the microelectrode when the conductivity of the glass wall is significantly high in the tip region.

4. Effects of the acid treatment of glass employed and the acidification of fillant electrolyte solution suggested that fixed negative charges on the glass wall play a fundamental role in the TP formation.

5. Based on these experimental results, it was concluded that not only the diffusion potential through the tip pore but also the interfacial potential through the thin glass wall near the tip contributes to the TP generation, and the contribution of the latter increases with a long exposure period of the electrodes to electrolyte solution.

6. In this connection, technical problems related to reduction of the tip potential were also discussed.

**Key words:** Glass-microelectrode — Tip Potential — Diffusion Potential — Interfacial Potential — Fixed Negative Charges.

## Introduction

The significance of direct measurements of the membrane potential by microelectrodes have become increasingly important in the investigation of the properties and mechanisms of cell membrane. Nastuk and Hodgkin (1950) showed that if the Ling-Gerard type microelectrodes (Ling and Gerard, 1949) were filled with 3 M KCl, their electrical resistances as well as the junctional potentials decreased. This type of microelectrode, however, often shows the intrinsic potential across the tip boundary, so-called tip potentials, which cannot be accounted for by the normal liquid junction potential. Adrian (1956) showed that if the tip diameter was larger than 5 μm, TP errors were negligible in the measured membrane potential, but such errors were marked with high TP microelectrodes which had very small tip diameters. The role of those potentials is, therefore, very important for the measurements of the membrane potentials in small cells.

To the present, these tip potentials have been often neglected in studies on excitable cells. The main problems were the frequency and relative changes in the membrane potential, such as those of spike generation. Since the absolute values of the membrane potentials come into question in the case of the inexcitable cells, the role of such tip potentials should be clearly defined (Okada *et al.*, 1973).

Tip potentials have long been considered as the result of contamination of proteins, dust etc., which produce different permeabilities of the tip to different ions (Adrian, 1956; Frank and Becker, 1964). Lavellée (1964) and Kawabata and Nakamura (1965) suggested that the tip potential was caused by the negative charges of the glass wall in the tip region. Bingley (1964), Agin and Holtzman (1966) and Agin (1969) suggested that TP originates from the interfacial potential through the thin glass film near the tip. Convincing experimental evidence for these postulations has, however, not been presented. Our own investigations concerning these points are outlined herein. A part of the present investigations has already been presented as a preliminary report (Okada and Inouye, 1975).

## Methods

### *Glass Tubings*

The glass tubings employed in our experiments were Pyrex 7740 capillaries with a 2 mm outside diameter and a 1 mm inside diameter. These were treated with $H_2SO_4$-dichromate mixture or 1% HCl solution, washed with tap water and finally with distilled water.

Micropipettes were made by a puller (Narishige, Tokyo), and pulling conditions (*e.g.* length of the glass tubings, heater current and so forth) were kept as constant as possible in order to minimize variation in the electrode properties.

### *Filling Methods*

The micropipettes were filled with filtered 3 M KCl solution. In some cases, pH of the filling KCl solution was adjusted to 2.0 with HCl. Three filling methods were used.

1. Alcohol method (Tasaki *et al.*, 1954): Electrodes were filled by a gentle boiling for 20 min in methanol under reduced pressure, and kept for 30 min at room temperature. They were then placed in distilled water for 7 hrs at 37° C. The distilled water in the capillary was replaced by 3 M KCl by immersing them in 3 M KCl solution usually for 7 days at 37° C.

2. Glass fiber method (Tasaki *et al.*, 1968): The glass fibers employed here were washed with distilled water, acetic acid, ethyl alcohol and finally with ethylether.

3. Direct filling method: A very fine polyethylene tubing drawn by a microburner was inserted into a capillary up to the tip region and an electrolyte solution was injected by means of a syringe pressed manually with a screw.

### *Measurements of Tip Potential and Resistance*

Tip potentials (TP) were measured at 25 ± 1° C by Adrian's method (Adrian, 1956) with slight modification as shown in Fig. 1a. The resistance ($R_g$) and the potential ($E_g$) through the thin glass wall in the tip region were measured as a function of the distance from the extreme point of the tip ($x$) by the method as sketched in Fig. 1b; After the electrode resistance as a whole ($R$) was measured

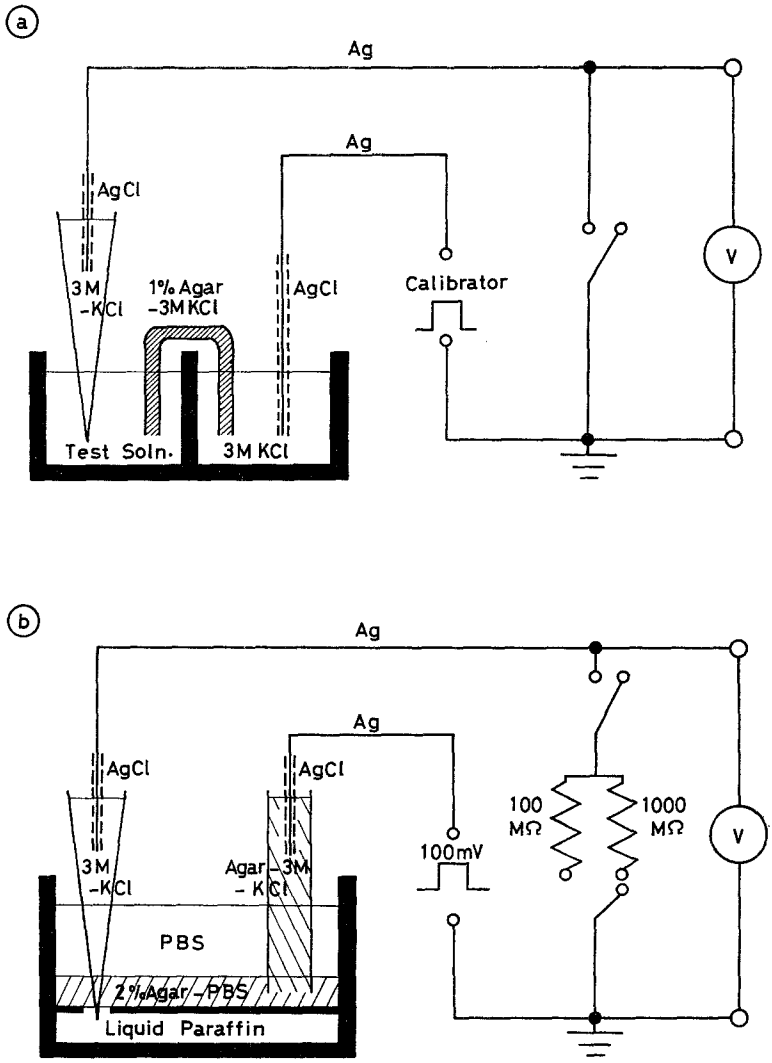


Fig. 1. Diagrams of the experimental arrangement for the measurement of a), tip potential in test solutions; and b), resistance and potential through the thin glass wall in the tip region

in the bulk solution (PBS), the electrode tip was then advanced through the agar layer into liquid paraffin (or $CCl_4$) to a certain depth (5 to 300 $\mu$m) under micrometer control and microscopic observations. When liquid paraffin serves as the insulator, the use of agar layer is necessary to prevent the float-up of paraffin.

The selection of the pair of Ag-AgCl electrodes was done in such a way that their potential difference in 3 M KCl was practically zero. These electrodes were led to the input of high-input impedance preamplifier (Nihon-Kohden Kogyo, MZ-3B), the output was led to an electronic recorder (Hitachi, QPD-53). When a known shunt resistance ($r_s = 100$ or 1000 M$\Omega$) was connected as shown in Fig. 1 b, the voltage measured during the application of a constant voltage pulse ($e = 100$ mV,

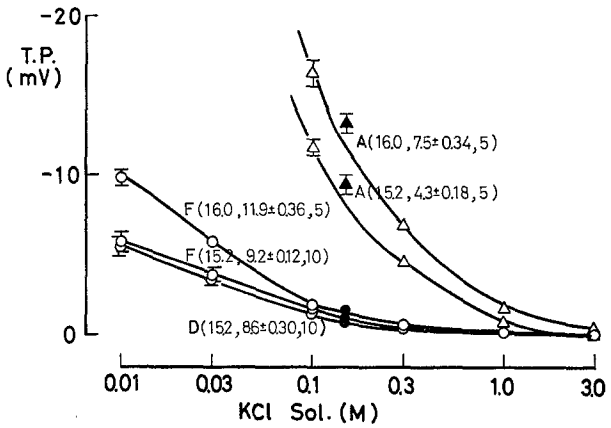


Fig. 2. Tip potentials of microelectrodes made by three filling methods. A), Alcohol method. F), Glass fiber method. D), Direct filling method. ○ and △, Tip potential in 0.01 ~ 3.0 M KCl; ● and ▲, tip potential in PBS plotted at KCl of 0.15 M. The number in parentheses: (heater current in Amp, electrode resistance ± S.E. in MΩ, number of the measured electrodes). The vertical bars represent standard errors on either side of average of 5 or 10 electrodes tested

duration 1 sec) was given as $v = e \cdot r_s/(r + r_s)$, where $r$ is the resistance to be measured. Using such a system, it was confirmed that the ohmic relation held good from $e = 10$ mV to $e = 100$ mV and that the high resistance up to 6000 MΩ could be determined with sufficient accuracy.

Test solutions employed here were 0.003 to 3.0 M KCl solutions and a phosphate buffer saline (PBS) which contained (mM) NaCl, 137.0; KCl, 2.7; $CaCl_2$, 0.9; $MgCl_2$, 0.5; $Na_2HPO_4$, 8.0; $KH_2PO_4$, 1.5; and pH 7.3 ± 0.1.

The electrodes prepared by the glass fiber method and the direct filling method were subjected to the measurements within 3 hrs after preparation.

## Results

### *Tip Potentials of Electrodes Made by Three Filling Methods*

To compare the three filling methods, the electrodes having resistances of less than 15 MΩ were prepared, since the filling of an electrode having tip resistance greater than about 15 MΩ was found to be difficult by the direct filling method. As seen in Fig. 2, the microelectrodes filled by the alcohol method (the electrodes of type A) had remarkably high TP values in comparison with those filled by the glass fiber method (F-electrodes) and by the direct filling method (D-electrodes). The TP and $R$ values of F and D-electrodes seem to be almost of the same order of magnitude under the same pulling condition. This fact suggests that the glass fibers near the tip region do not affect electrical properties of the microelectrode of type F. As the F and D-electrodes were utilized for measurements within 3 hrs after preparation, most of TP of the A-electrodes would appear to build up while the electrodes were immersed in 3 M KCl solution for 7 days at 37° C.

### *Effect of Duration of Storage in 3 M KCl*

As seen in Fig. 3a, a microelectrode showed a remarkable increase in the TP values with the increase of the storage period, while the resistance of this electrode

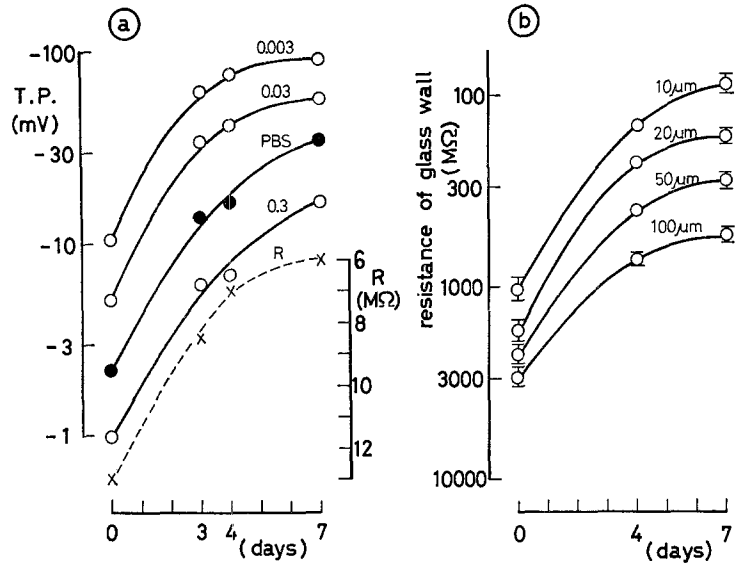


Fig. 3. Effect of duration of immersion in 3 M KCl solution at 37° C. a), Examples of the TP values in 0.3~ 0.003 M KCl solutions and in PBS, as well as the electrode resistances in PBS; b), the resistances of glass wall in PBS when the tip was partly insulated. (Numerals attached: The depth of dipping into the liquid paraffin in μm. Vertical bars: Standard errors on either side of an average of 7 electrodes tested.)

decreased gradually. Such a result seems to be in contrast with the common view (Adrian, 1956) that the TP values increased with their resistances. For elucidation, measurements of the resistance through the thin glass wall near the tip ($R_g$) were carried out. An example of these results is illustrated in Fig. 4a, in which $R_g$ was plotted against the length of glass dipped into liquid paraffin ($x$ in μm). It shows that $R_g$ of the electrode of type F is significantly greater than that of A, while $R_g$ of the latter decreases with an increase in the period of immersion in 3 M KCl (Fig. 3b). This finding may be related to the decrease in the electrode resistance with the increase in the storage period. The conductance of the glass wall, $G_g = R_g^{-1}$, was plotted against $x$ (Fig. 4b); the slopes of these curves reflect the reciprocal of the specific resistance as shown in Appendix. Fig. 4b shows that the specific resistance of the glass wall of the microelectrode freshly prepared increases very steeply along the electrode axis in the narrow tip region (0~ 15 μm). In contrast with this result, the specific resistance of the glass wall of the microelectrode immersed in 3 M KCl for a long time increases less steeply in a wide tip region (0~ 100 μm). This fact suggests that the extensive hydration of glass matrix occurred in a wide range from the extreme point of the tip on exposure to KCl solution. Such a fact seems to result in fairly high conductivity of the Pyrex membrane, as suggested by Agin and Holtzman (1966). Such a high conductivity of the thin glass wall near the tip would contribute to the TP formation, if the boundary potential difference through the thin glass wall in the tip region ($E_g$) is adequate.

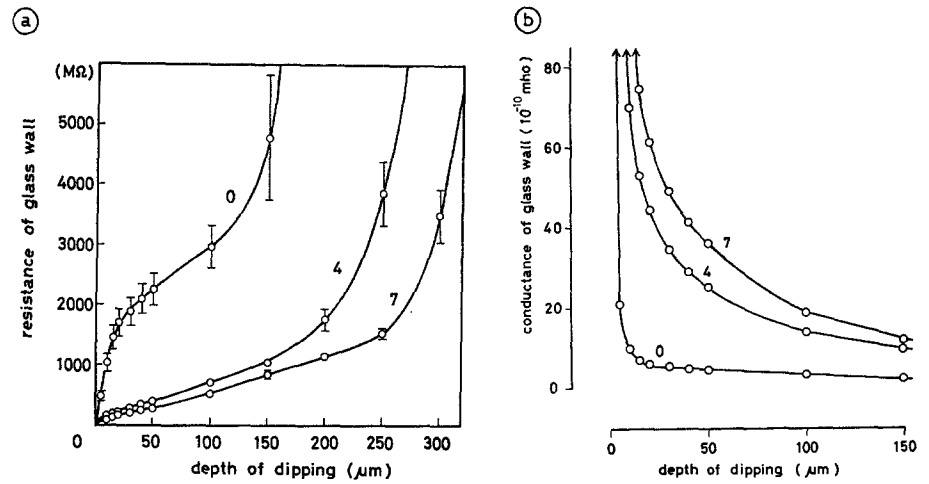


Fig. 4. Resistances (a) and conductances (b) of the F and A-type electrodes with insulated tip as a function of the depth of dipping the tips. (0), Microelectrodes of type F, measured within 3 hrs after preparation. (4) and (7), Microelectrodes of type A measured at the 4th and 7th day after immersion in 3 M KCl, respectively. The vertical bars represent standard errors on either side of an average of 7 electrodes tested

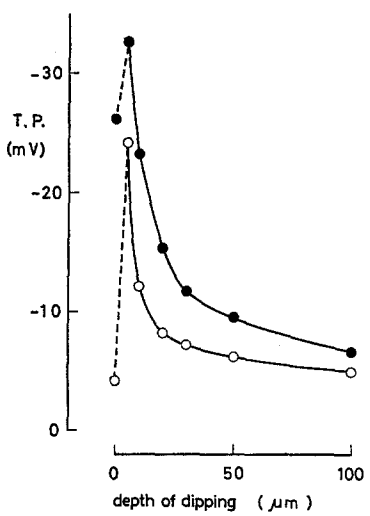


Fig. 5. Tip potential in PBS of electrodes with insulated tip as a function of the depth of dipping the tip. ○, Potentials measured immediately after preparation; ● potentials measured after 7 days of immersion

We then attempted to estimate the magnitude of $E_g$ by direct measurements of the tip potential of electrode, the tip of which was partly insulated as described above. An example of $E_g$-$x$ relation is presented in Fig. 5. It should be noted here that in agreement with the findings of Caillé and Gagné (1971) $E_g$ at $x = 5$ μm was always found to be greater than TP ($E$) under the usual conditions irrespective of

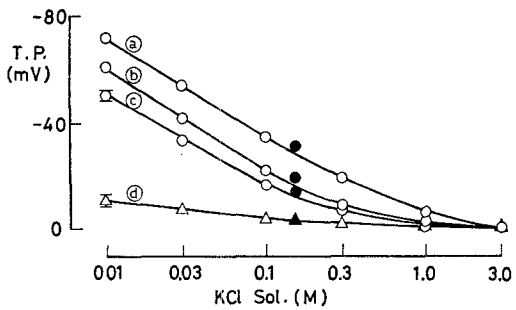


Fig. 6. Effect on the tip potential of acid treatment of glass tubing and acidification of filling solution. All electrodes were prepared under the same pulling condition (heater current, 16.0 A) and filled with the alcohol method on the same time. a), without any acid pre-treatment (electrode resistance was 7.2 ± 0.11 MΩ); b), pre-treated with 1 % HCl for 32 hrs (7.2 ± 0.34 MΩ); c), pre-treated with $H_2SO_4$-dichromate mixture for 72 hrs (7.5 ± 0.34 MΩ); d), pre-treated with $H_2SO_4$-dichromatic mixture for 72 hrs and filled with 3 M KCl adjusted to pH 2 with HCl (14.6 ± 0.83 MΩ). ○ and △, Tip potential in 0.01 ~ 3.0 M KCl; ● and ▲, tip potential in PBS plotted at KCl of 0.15 M. The vertical bars represent standard errors on either side of average of 5 electrodes tested

the type of electrode, F for A. $E_g$ was about − 30 mV for the type A measured at 7th day and around − 20 mV for F, the ratio of $E/E_g$ being 0.75 ± 0.03 ($n = 7$) for the former and 0.15 ± 0.02 ($n = 7$) for the latter.

Under the present experimental conditions for $R_g$ and $E_g$-measurements, it is difficult to completely exclude a possibility that the thin layer of electrolyte solution seeps in together with the advancing tip between the glass wall and the liquid paraffin, and that the wetted outer surface of the tip carries the current. If the conductivity of such a layer is greater than (or comparable to) the conductivity through the glass wall, the measured TP values should be reflected on the measured $E_g$ values. In spite of the large difference in TP values between the type A electrode measured at 7th day and the type F measured immediately, however, the difference in $E_g$ values was not so large. It might be said, therefore, that the conductivity of the wetted outer layer of the tip did not contribute so much and the measured $R_g$ and $E_g$ values would mainly show the resistance and the potential through the glass wall.

*Effect of Acid Treatment of the Glass Tubing and the Filling Solution*

In order to observe the effect of acid treatment of the glass tubing on TP, the microelectrodes of type A were prepared at the same time from the glass tubings without or with acid pretreatment under the same pulling conditions. As shown in Fig. 6, the effect of acidification of the glass tubing on the TP values is clear. When the microelectrodes were filled with 3 M KCl solution adjusted to pH 2 with HCl, their TP values were remarkably low (Fig. 6), as already noted by several investigators (Williams *et al.*, 1971; Wann and Goldsmith, 1972; Riemer *et al.*, 1974), and of nearly the same order of magnitude as those of the electrode prepared by the glass fiber method under the same pulling conditions (Fig. 2).

All these results suggest that the properties of the tips changed while the electrodes were immersed in the neutral 3 M KCl solution, and that this could be

prevented to a considerable extent by the acid treatment of glass, and/or the acidification of filling solution.

## Discussion

It has been suggested that the tip potential is the result of contamination (Adrian, 1956; Frank and Becker, 1964). In our experiments, the filling solution was freshly filtered, so that gross contamination was ruled out. If the accumulation of micro-dirt causes the TP formation, the fluctuation of the TP values observed with different electrodes would be expected to be much greater than that seen in Figs. 2 and 6, and the effect of acid treatment shown above would be difficult to explain. Our results are in general consistent with the view that fixed negative charges on the glass wall will bring about tip potential, as already suggested (Lavallée, 1964; Kawabata and Nakamura, 1965), for the acid treatment of glass as well as the acidification of the filling solution could counteract to a certain extent the formation of fixed negative charges.

Some authors (Adrian, 1956; Kawabata and Nakamura, 1965) maintain that TP is the result of diffusion potential modified by fixed charges inside of electrode tip. Indeed, as described above, the negative charges on the glass wall in the tip region appear to play a fundamental role in TP formation. On the other hand, based on the view that the electrical resistance of thin glass wall near the tip should fall considerably on exposure to water, Agin and Holtzman (1966) and Agin (1969) claimed that TP originates from the interfacial potential between glass and electrolyte solution. Our direct measurements of the resistance of the thin glass wall show that the conductance in this portion increased considerably on exposure to KCl solutions, and the interfacial potential, if it is present, should contribute to the TP formation. Indeed, Fig. 3 shows that the increase in TP and the decrease in the electrode resistance on exposure to KCl solution are closely related with the decrease in $R_g$ near the electrode tip. Moreover, an interfacial potential through the thin glass wall near the tip was actually present and to a considerable degree as shown in Fig. 5. Such an interfacial potential ($E_g$) could be derived from the fixed negative charges on this thin glass wall. Assuming the negative charge density is homogeneous over a whole portion of glass surface, $E_g$ between 3 M (inside) and C M KCl (outside) solutions could be expressed by the Gouy-Chapman theory (Gouy, 1910; Chapman, 1913) as follows.

$$E_g = \frac{2\,RT}{F} \cdot \left\{ \sinh^{-1}\left( \sqrt{\frac{1000\,\pi}{2\,\varepsilon RT}} \cdot \frac{\sigma}{\sqrt{3}} \right) - \sinh^{-1}\left( \sqrt{\frac{1000\,\pi}{2\,\varepsilon RT}} \cdot \frac{\sigma}{\sqrt{C}} \right) \right\}$$

$$\simeq -25.7 \left\{ \log \frac{3}{C} + 2 \log \frac{1 + \left(1 + \dfrac{C}{0.0895\,\sigma^2}\right)^{1/2}}{1 + \left(1 + \dfrac{3}{0.0895\,\sigma^2}\right)^{1/2}} \right\} \qquad (1)$$

where $R$, $T$ and $F$ are the physical constants in ordinary meaning, $\varepsilon$ is the dielectric constant ( $\simeq 80$) and $\sigma$ is the absolute value of the fixed charge density ($\mu$ coul/cm$^2$). It is apparent from Eq. (1), the $E_g$ tends to $-25.7$ log 3/C for high $\sigma$. Thus it seems feasible that a strongly hydrated microelectrode gives rise to a value of around $-30$ mV for $E_g$ in PBS whose ionic strength is around 0.15. According to Eq. (1), the limit of $E_g$ in 0.03 to 0.003 M KCl solutions is around $-50 \sim -75$ mV,

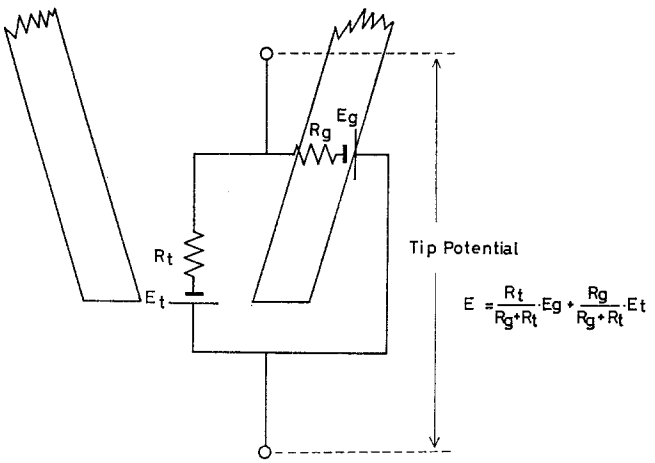


Fig. 7. Equivalent circuit model illustrating origin of microelectrode tip potential. $R_t$, resistance at the tip pore; $R_g$, resistance of glass wall near the tip; $E_t$, diffusion potential through the tip pore; $E_g$, boundary potential through the thin glass wall near the tip

but we found a TP value of $-60 \sim -100$ mV for the A-electrode (cf. Fig. 3a) Therefore TP could not be attributed to $E_g$ given by Eq. (1) alone, hence a contribution of a potential of some other origin appeared to be generated at the tip pore ($E_t$), as already claimed (Adrian, 1956; Kawabata and Nakamura, 1965; Lavallée and Szabo, 1969). The TP formed at the tip could be expressed as shown in Fig. 7, and the following relation derived.

$$\frac{E}{E_g} = \frac{R_t}{R_t + R_g} + \frac{R_g}{R_t + R_g} \cdot \frac{E_t}{E_g}. \tag{2}$$

Here $E_t$ might be assumed as being mainly, if not solely, diffusion potential and expected to be considerably low in so far as 3 M KCl is used as the filling solution. As a rough approximation, therefore, the relation of $R_t/(R_t + R_g) \simeq E/E_g$ could be applied at least for the electrodes of type A. Taking account of the $E/E_g$ values obtained in our experiments, $R_g$ of the A-electrode could be regarded as of the same order of magnitude as $R_t$, while $R_g$ of the freshly prepared F-electrodes is expected to be 6 or more times higher than $R_t$. Thus the present experiments appear to suggest that, when freshly prepared, TP of glass microelectrodes might be chiefly, if not solely, diffusion potential modified by the presence of fixed negative charges on the glass wall near the tip, but contribution of interfacial potential across the thin glass wall region increases on long exposure to water, resulting in a high TP.

In view of the result shown in Fig. 3, storing in 3 M KCl of the microelectrodes over 3 days should be avoided in order to obtain an electrode with a low TP. Acidification of the filling solution up to pH 2 provides a simple means of preparing microelectrodes of low tip potential (Fig. 6), if the high concentration of $H^+$ in the capillaries does not affect the membrane potential measurements. The glass fiber method is far superior to the alcohol method in order to obtain low tip potential.

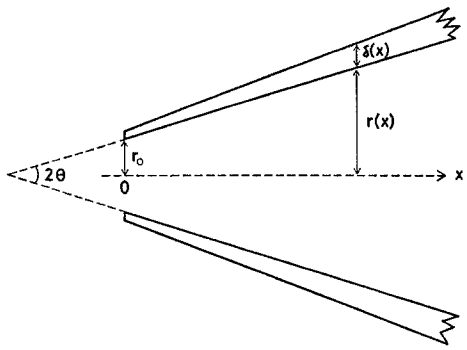


Fig. 8. Schematic glass microelectrode tip (see text for explanation)

The direct filling method may be also applicable for a microelectrode with a slightly larger tip diameter, having the resistance of around 10 MΩ.

It has been pointed that the smaller the tip potential of an electrode, the more accurate is the membrane potential obtained (Adrian, 1956; Hülser and Webb, 1973; Riemer *et al.*, 1974), whatever the mechanism of the TP formation. Indeed, the diffusion potential at the tip pore in the bathing solution would be different from the one in the cell fluid. Therefore this part of TP should be kept as small as possible. But the interfacial potential of TP, if it is the Gouy-Chapman potential, in the surrounding saline would be nearly equal to the one in the cell fluid because the ionic strength in both fluids is probably almost equal. If so, the errors brought about by this part of TP could be properly corrected. Indeed, the TP values in PBS (plotted at KCl of 0.15 M in Figs. 2 and 6) were not so different from the TP values in 0.15 M KCl.

Caillé and Gagné (1971) showed that the glass wall resistance near the tip increased steeply at $x = 0 \sim 5\,\mu m$. Based on this observation, they concluded that the microelectrode freshly prepared is applicable to electrophysiological measurements of cells larger than 15 μm. We could not reproduce these results with $CCl_4$ alone, probably because of rippling at the interface between saline and $CCl_4$. When we used the agar layer, an accurate measurement at $x = 0 \sim 5\,\mu m$ could not be done. Our results obtained on the microelectrode without aging are, however, consistent with those reported by Caillé and Gagné (1971), whereas the results on the microelectrode after 7 days of aging are greatly different, as shown in Fig. 4. Such results suggest that the microelectrodes stored for a long time in a solution should not be used for small cells, because the membrane potential measured may be electrically shunted through the high conductive glass wall outside of the cell.

## Appendix

*Estimation of the Specific Resistance Change of the Glass Wall in the Tip Region*

The electrical conductance ($G$) of the open-tipped glass microelectrodes is easily calculated, if simplifying assumptions are made. Let us assume the electrode to be uniformly conical with an included angle $2\theta$ (Fig. 8). Designating the axial coordinate $x$ with the origin at the tip end, the radius in the conical, $r(x)$, is given by $(r_0 + x \cdot \tan\theta)$ where $r_0$ is the radius of the tip pore. Putting the thickness of the glass wall at $x$ as $\delta(x)$, the ratio of $k = r(x)/\delta(x)$ is assumed

constant, because Bils and Lavallée (1964) showed by electron microscopic observations that the ratio of inner and outer diameter of the glass microelectrode remained the same as that of the capillary prior to pulling. As illustrated in Fig. 7, $G$ is composed of two components in parallel; the conductance through the glass wall ($G_g = 1/R_g$) and that through the fillant electrolyte at the pore ($G_t = 1/R_t$), both being mainly confined within the tip region. $G_t$ is approximated by

$$G_t = \pi r_0 \cdot \tan\theta/\gamma$$

as shown by Snell (1969), where $\gamma$ is a constant specific resistance ($\Omega$-cm) of the fillant electrolyte. If we now assume a specific resistance, $\varrho(x)$, of glass wall as a function of $x$ depending on the extent of hydration of thin glass wall,

$$G_g = \int_0^l \frac{2\pi \cdot r(x)}{\delta(x) \cdot \varrho(x)}\, dx = k' \int_0^l \frac{dx}{\varrho(x)}$$

where $k'$ is a constant ($= 2\pi/k$) and $l$ is a length of the glass micropipette.

If dipping the electrode tip into liquid paraffin (or $CCl_4$) accomplishes a complete insulation of the soaked tip region ($= x\,\mu m$), the electrode conductance observed with the method shown in Fig. 1 b, $G_g(x)$, would be expressed by the following equation.

$$G_g(x) = k' \int_x^l \frac{dx}{\varrho(x)}.$$

Thus it is obvious that

$$\frac{d\,G_g(x)}{dx} = -\frac{k'}{\varrho(x)}.$$

Using this equation, therefore, we can estimate the $\varrho(x)$ value from the slope of $G_g(x) - x$ plot.

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