Tip Potential and Fixed Charges on the Glass Wall of Microelectrode

LING-GERARD[1] type microelectrodes filled with 3 *M* KCl[2] have potentials, so-called tip potentials, across the tip boundary which cannot be accounted for by the normal liquid junction potential. An effective method of reducing the tip potential of glass microelectrodes would be of practical benefit. In this report, several filling methods are compared for the purpose of preparing microelectrodes of low tip potential.

Method. The glass tubing employed in these experiments was Pyrex capillaries of 2 mm outside diameter and 1 mm inside diameter, which were treated with H_2SO_4-dichromate mixture or 1% HCl solution, then washed in tap water and finally with distilled water. Micropipettes were made by a puller (Narishige, Tokyo), and pulling conditions were kept as constant as possible in order to minimize variation in the electrode properties. They were filled with 3 *M* KCl solution by 3 methods.

1. *Alcohol method*[3]. Electrodes were filled by boilling under reduced pressure and then the distilled water in the capillaries was replaced by 3 *M* KCl by immersing them in 3 *M* KCl solution usually for 7 days at 37 °C.

2. *Glass fibre method*[4]. The glass fibres employed in this method were washed with distilled water, acetic acid, ethyl alcohol and finally with ethyl ether.

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 eletrolyte solution was injected by means of a syringe which was pressed manually with a screw. With this method, filling of an electrode having tip resistance greater than about 15 *MΩ* was found to be difficult.

Therefore, to compare the 3 filling methods, the electrodes having a resistance of less than 15 *MΩ* were used in the present experiments. The tip potential (TP) was measured at 25 ± 1 °C by the method described by ADRIAN[5], with slight modification. Test solutions employed here were 0.01–3.0 *M* KCl solutions and Dulbecco's phosphate buffer saline (PBS)[6].

Results and discussion. As seen in Figure 1, the microelectrodes filled by the alcohol method had remarkably high TP values in comparison with those filled by the glass fibre method. The TP values of microelectrodes using the glass fibre method and the direct filling method under the same pulling condition seem to be almost of the same order of magnitude. As the electrodes filled by these 2 methods were utilized for measurements within 3 h after preparation, most of the TP of the electrodes filled by the alcohol method would appear to build up while the electrodes were immersed in 3 *M* KCl solution for 7 days at 37 °C. Indeed, as shown in Figure 2, a microelectrode filled by the alcohol method showed a remarkable increase in the TP values with the icrease of the storage period, while the resistance of this electrode gradually decreased. Moreover, the TP value of a microelectrode made by the glass fibre method also markedly increased after storing in 3 *M* KCl, while its resistance decreased. Such a result seems to be in contrast with the common view[5] that the TP values increased with their resistances. Further detailed studies, such as on the resistance of glass wall near the tip, are needed to elucidate this result. When the microelectrodes were filled with 3 *M* KCl solution adjusted to pH 2 with HCl, their TP values were remarkably low

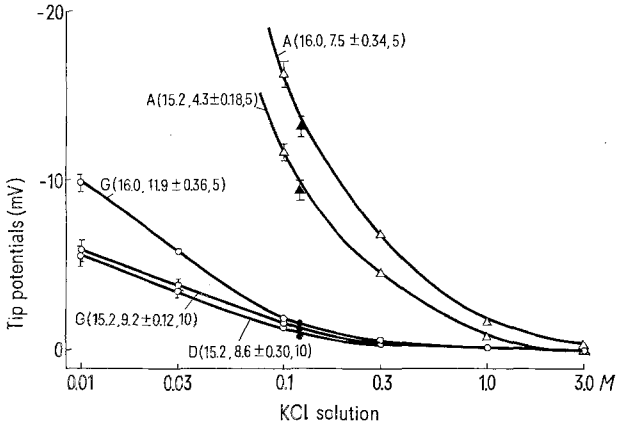

Fig. 1. Tip potentials of microelectrodes made by the glass fibre method and the direct filling method. ○ and △, Tip potential in 0.01 ~ 3.0 *M* KCl; ● and ▲, tip potential in PBS plotted at KCl of 0.12 *M*. A) Alcohol method. G) Glass fibre method. D) Direct filling method. The numbers in parentheses: (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.

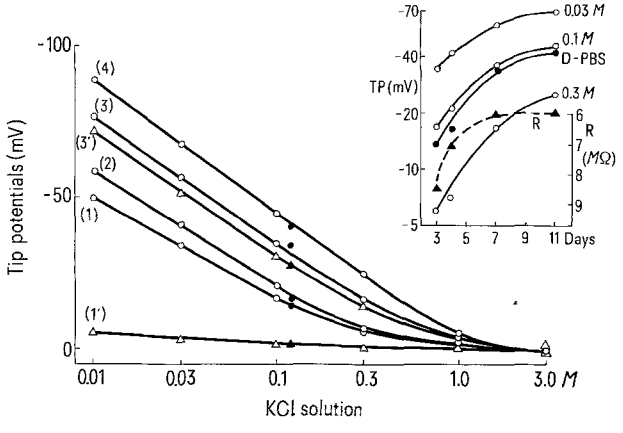

Fig. 2. Effect of duration of immersing in 3 *M* KCl at 37 °C on the TP values, (1) A microelectrode filled by the alcohol method, measured at the 3rd day of immersing in 3 *M* KCl. (2) The same electrode, measured at the 4th day. (3) The same electrode, measured at the 7th day. (4) The same electrode, measured at the 11th day. (1') Another microelectrode filled by the glass fibre method, measured immediately after preparation (the resistance: 15 *MΩ*). (3') The same electrode, measured at the 7th day of storing in 3 *M* KCl (7.5 *MΩ*). ○, Tip potential in 0.01 ~ 3.0 *M* KCl solutions; ●, tip potential in PBS plotted at KCl of 0.12 *M*. Insert: Changes in the electrode resistance (*MΩ* for cross marks) and the tip potential in the course of immersing in 3 *M* KCl.

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(Figure 3), as already noted by several investigators[7–9], and of nearly the same order of magnitude as those of the electrode filled by the glass fibre method (Figure 1). The effect of acid treatment of glass tubing on the TP values is also clearly seen in Figure 3.

Since many laboratories use capillaries and glass fibres straight form a shelf, some observations were made on the microelectrodes prepared by the glass fibre method without any acid treatment. The TP values of such electrodes observed in PBS showed considerable variations from −3.4 to 18.4 (means, around −9) mV. Such a high TP was markedly reduced by the use of acid-treated glass tubing and glass fibre or of acidified 3 *M* KCl. The TP values in the latter were around −2 mV (−0.3 ∼ −3.7 mV) and of nearly the same order of magnitude as those in the former (Figure 1).

All these results suggest that something happened to the tips 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. It has been suggested that the tip potential is the result of contamination[5,10]. In our experiments, the filling solution was freshly filtered, so that gross contamination can be ruled out. If the accumulation of miro-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 Figures 1 and 3, 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[11,12], for the acid treatment of glass as well as the acidification of the filling solution could to a certain extent counteract the formation of fixed negative charges.

Whatever the mechanism of generating the potential at the tip, the smaller the tip potential of an electrode, the more accurate is the membrane potential obtained. In view of the result shown in Figure 2, storing in 3 *M* KCl of the microelectrodes over 3 days should be avoided. Acidification of the filling solution up to pH 2 provides a simple means of preparing microelectrodes of low tip potential[7–9], if the high concentration of H^+ in the capillaries does not affect the membrane potential measurements. The glass fibre method is far superior to the alcohol method in order to obtain low tip potential. The direct filling method may be also applicable for microelectrodes of a little larger tip diameter, having the resistance of around 10 *MΩ*.

Zusammenfassung. Glasmikroelektroden, mit 3 *M* KCl gefüllt, wurden verschiedenartig präpariert und ihr Spitzenpotential mit 0.01 ∼ 3.0 *M* KCl gemessen. Die Resultate sprechen dafür, dass die negativen elektrischen Ladungen, an der Glaswand fixiert, eine entscheidende Rolle bei der Entstehung des Spitzenpotentials spielen.

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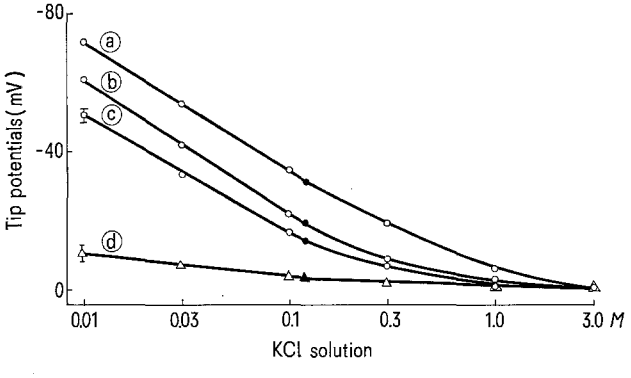

Fig. 3. Effect on the tip potential of acid treatment of glass tubing and acidification of filling solution. All electrodes tested were prepared under the same pulling condition (heater current, 16.0 A) and filled with the alcohol method. a) without any acid pre-treatment (electrode resistance was 7.2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

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Influence of Temperature on the Electric Activity of the Isolated Dog Retina

In the last few years, the influence of temperature on the function of isolated organs has been investigated to an increasing degree in order to get some information about kinetic and thermodynamic quantities of biological reactions. The temperature as a parameter of the retinal cell system has been examined many times, the measurements being carried out also on the isolated retina[1–5]. As corresponding experiments with the isolated dog retina have not yet been reported, the influence of temperature on the dog's retinal activity after light stimulation (ERG) was investigated. The dependence on temperature of the latency of restoration after a light stimulus was measured by means of double-stimuli.

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