Tip Potentials in Microelectrodes Filled by Boiling under Reduced Pressure

In practice, there exists a junction potential between the fluid filling a LING-GERARD glass microelectrode[1] and the aqueous medium with which its tip is in contact[2,3]. Interest in this potential difference (p.d.) arises from the error which its presence may cause in measurement of the direct current (d.c.) level of transmembrane potentials. Thus ADRIAN[4] and KÜCHLER[5] have demonstrated that the resting potential of frog sartorius muscle fibers is significantly underestimated when impalements are made with micropipettes having 'tip potentials' more negative than -5 mV when in contact with normal Ringer's solution.

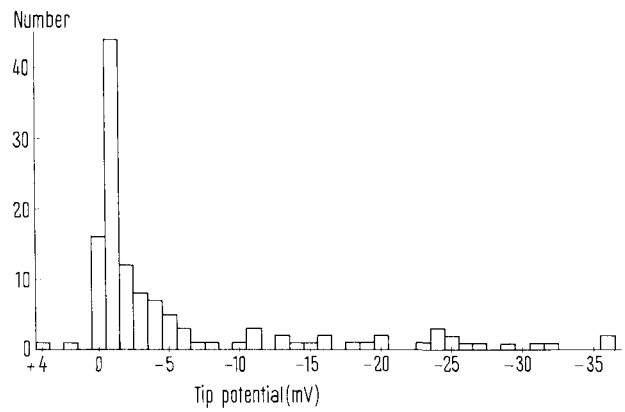
Of the methods described for filling micropipettes[6,7] the simplest is boiling in the desired solution. This has the advantages of allowing a large number of pipettes to be filled simultaneously, and of requiring only a few minutes. Disadvantages are the possibility of damage to tips from thermal or mechanical trauma, and the higher incidence of large tip potentials than with gentler techniques[2]. Modification of the method to filling by boiling under reduced pressure and gradual release of suction reduces traumatic damage to the tips. Since the advantages cited are of considerable practical importance, quantitative information concerning tip potentials in micropipettes filled by this modified technique is desirable. In addition, data relating to the growth of tip potentials with time after filling, and with storage temperature, is also of obvious practical significance.

Microelectrodes were prepared from unselected, commercially available borosilicate glass tubing (2 mm outside diameter, 0.5 mm wall, Corning 234440). After being cut into convenient lengths, the tubing was cleaned in hot dichromate-H_2SO_4, washed vigorously in tap water and boiled several times in distilled water. Cleaned glass was oven dried and stored in closed containers. A vertical puller, similar to one described by FRANK and BECKER[7] was employed. A coil consisting of $1^1/_2$ turns of nichrome wire (18 gauge) was heated by 16 A alternating current (a.c.). The weak pull (mass of solenoid plunger and moveable carriage) was 325 g, and the heavy pull, applied after the softened glass has been extended 4 mm, was 725–1200 g. Electrodes were mounted tips down on a conical acrylic plastic holder (capacity, 25 pipettes). The holder was inserted into a 500 ml lipless beaker containing filtered 3 *M* KCl. A circular cap, attached to the pipette holder, made an airtight seal against the rim of the beaker. This assembly was placed in a water bath (75 ± 5 °C), and after a few minutes the beaker was evacuated by a water faucet aspirator (partial vacuum ca. 10 mm Hg). After the KCl had boiled vigorously for 10 min, the suction was released gradually, and the volume restored with distilled water. Filled electrodes were transferred to square plastic Petri dishes, where they were mounted horizontally on a strip of plasticene, covered with 3 *M* KCl. The KCl in the filling beaker was filtered under suction through a very dense paper (S and S 576) each time a new batch of electrodes was prepared.

Microelectrode properties were measured using a symmetrical system consisting of a pair of Ag,AgCl electrodes connected to a differential cathode follower input (grid current less than 10^{-11} A). The micropipette was mounted on a probe filled with agar-Ringer's solution and containing a coil of chlorided silver wire. The tip of the micropipette dipped into a bath containing Ringer's solution. A second probe containing a coil of chlorided silver wire in agar-Ringer's solution, contacted directly the Ringer's solution in the bath. Recorded were (a) the asymmetry potential with both probes immersed in the Ringer's solution in the bath, (b) the degree of unbalance introduced when a microelectrode was connected to one probe, (c) the resistance of the microelectrode, and (d) the change in voltage after breaking off the final 1–2 mm of the microelectrode. Resistance was evaluated by applying a positive-going rectangular wave of 5 msec duration and 50 mV amplitude through the second probe from a low impedance generator, and measuring the reduction in amplitude of the recorded rectangular wave after shunting the microelectrode to ground with a 10 or 20 MΩ resistor. Tip potentials were measured to the nearest 0.5 mV and resistances to the nearest 0.5 MΩ. Tip potentials measured by the change in the asymmetry potential, procedure (b), never differed from the readings obtained by breakage, procedure (d), by more than 0.5 mV.

Development of tip potentials in microelectrodes upon storage

Time (days)	Temperature (°C)	Median tip potential (mV)	Median resistance (MΩ)	*n*
1	22	− 2.5	13.0	15
2	22	− 2.0	11.0	25
3	22	− 5.0	11.5	16
6	22	− 14.0	15.0	17
9	22	− 18.0	10.0	11
3	6	− 2.0	12.0	10
14	6	− 3.0	10.0	11
240	6	− 39.5	15.0	10



Histogram showing the distribution of tip potentials of 126 microelectrodes measured within 2 h of drawing and filling with 3 *M* KCl. The class interval is 1.0 mV and the median tip potential is − 2.5 mV. The arithmetic mean is − 6.4 mV.

1 G. LING and R. W. GERARD, J. cell. comp. Physiol. *34*, 383 (1949).
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3 J. DEL CASTILLO and B. KATZ, J. Physiol., Lond. *128*, 396 (1955).
4 R. H. ADRIAN, J. Physiol., Lond. *133*, 631 (1956).
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6 D. W. KENNARD, in *Electronic Apparatus for Biological Research* (Ed., P. E. K. DONALDSON; Butterworth Scientific Publications, London 1958), p. 534.
7 K. FRANK and M. C. BECKER, in *Physical Techniques in Biological Research* (Ed., W. L. NASTUK; Academic Press, New York and London 1964), vol. V, part A, p. 22.

The results are summarized in the accompanying Figure and Table. The Figure is a histogram of the tip potentials of 126 microelectrodes measured within 2 h of filling. Included are the values from 13 different batches of pipettes. In none of the batches was the median tip potential greater than – 5 mV, although in several batches the mean tip potential was greater than – 5 mV due to the occurrence of relatively few pipettes which had large tip potentials. For the entire series the mean tip potential was – 6.4 mV and the median – 2.5 mV; perhaps a more practical statistic is that 75% of the freshly prepared microelectrodes had tip potentials less negative than – 5 mV.

The effect of micropipette storage at room temperature and at 6 °C on the development of tip potentials is given in the Table. It appears that microelectrodes prepared and stored in the manner described change little at room temperature for 48 h. Thereafter the microelectrodes develop increasingly larger tip potentials, and on the 3rd or 4th day the median tip potential exceeds – 5 mV. In contrast microelectrodes stored at 6 °C do not alter appreciably in tip potentials for at least 2 weeks. Cold storage, however, does not prevent the eventual development of tip potentials, for microelectrodes stored for 8 months in the cold had a median tip potential of – 39.5 mV (range – 30.5 to – 46.0 mV).

On the basis of these results it appears that the method of filling micropipettes with 3*M* KCl by boiling under reduced pressure not only offers the advantages of speed and of processing large numbers simultaneously, but it also yields micropipettes with small tip potentials. $^3/_4$ of the pipettes had tip potentials less negative than – 5 mV, and the median tip potential did not alter for 48 h at room temperature, or for 2 weeks at 6 °C[8].

Zusammenfassung. Glasmikroelektroden, mit 3*M* KCl-Lösung gefüllt durch Kochen in Wasserstrahlvakuum, zeigen einen Zentralwert für das Potential an der Elektrodenspitze (tip-potential) von – 2,5 mV; 75% geben entsprechende Werte niedriger als – 5 mV. Lagerung der Elektroden bei 6 °C lässt das «tip-potential» mindestens 2 Wochen unverändert, während bei 22 °C sein Zentralwert nach 3–4 Tagen grösser als – 5 mV wird.

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[8] Supported in part by USPHS Grant No. NB 03663.

ADDENDUM

E. DE VITO and J. A. SANTOMÉ: Disc Electrophoresis of Proteins in the Presence of Sodium Dodecyl Sulphate, Experientia *22*, fasc. 2, p. 124 (1966). In this paper it was inadvertently omitted to state that in the five pore gel the percentage of acrylamide was 10% for bovine growth hormone, 13% for insulin and 7.5% for aldolase.

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Pour tous renseignements, s'adresser au Secrétaire Général, Prof. GUY EMSCHWILLER, Société de Chimie Physique, 10, rue Vauquelin, Paris 5ème.