# A METHOD OF MEASUREMENT OF THE ACTIVITY OF POTASSIUM AND SODIUM IONS WITHIN THE CELL

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To measure the activity of ions within the cell,a method which has been applied during the last few years is to employ glass electrodes to function as metals [3-7]. The intracellular measurement of the activity of sodium and potassium ions was first carried out by Hink [8, 9] who used an electrode made out of glasses $NaS_{11-18}$ and $NaS_{27-8}$ [4]. However, the external diameter of the tip of these electrodes was large, measuring on average 20-30$\mu$. Measurements were made of the giant muscle fibers of the crab and lobster, and of the giant axons of the squid. A. A. Lev and É. P. Buzhinskii [1] measured the activity of potassium ions within the frog muscle fiber using micro-electrodes which differed but little in their properties from the ordinary micro-electrodes used in electrophysiology.

We have made Na-selective micro-electrodes having an external tip diameter of 0.6-1 $\mu$, and we altered the arrangement for preparing K-selective micro-electrodes in a way which enabled us to reduce the tip diameter to a few tenths of a micron. We here describe the construction of Na- and K-selective micro-electrodes and a method for the measurement of the intracellular activity of sodium and potassium.

The selective micro-electrodes were drawn out from glass tubing made of $NaS_{11-18}$ [4, 5], and of a 4-component glass made by collaborators in the department of physical chemistry of Leningrad State University [3]; they used a normal horizontal or vertical electrode "forge." The micropipettes obtained were then sealed under microscopical control and filled by boiling in methyl alcohol. The filled electrodes were transferred into twice-distilled water which was changed several times, and placed in a 1 M solution of NaCl or KCl for several days to reduce and stabilize the potential of asymmetry.

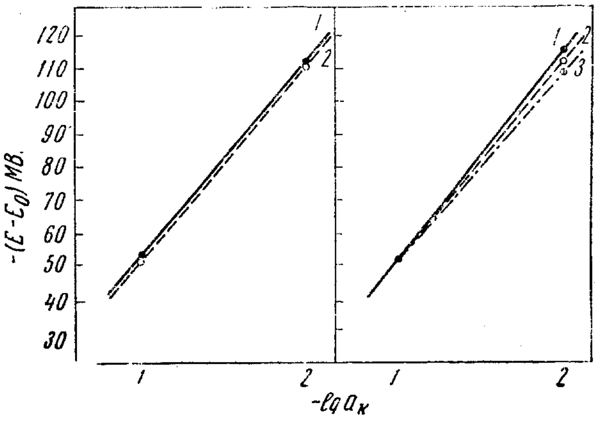

Fig. 1. Types of drift of potential in selective micro-electrodes.

Two methods of insulation of the micro-electrodes were used. The first was best applied to the Na-selective electrodes. Shortly before measurements were to be made the sealed and filled micro-electrode was introduced by means of a micromanipulator into an open micro-electrode of the usual non-selective glass having an external tip diameter of 1.5 -2$\mu$. The space between the 2 micro-electrodes in the upper and lower parts of the electrode were filled with phosphate cement.

To prepare K-selective micro-electrodes it was more convenient to use a different method of insulation. The tube of selective glass was placed within a wider tube of the ordinary more easily fusible glass (turning or neutral glass). The 2 tubes were fixed together by dental cement and stretched out in the micro-electrode forge. By appropriate choice of heating we obtained selective micro-electrodes covered over their whole length except

A Composition of Calibrating Solutions (in moles per 1000 g water)

| For Na˙ - selective micro-electrodes | For K˙ - selective micro-electrodes |
|---|---|
| NaCl 1.0 | KCl 1.0 |
| NaCl 0.1 +KCl 0.1 | KCl 0.1 +NaCl 0.01 |
| NaCl 0.1 +KCl 0.15 | KCl 0.1 +NaCl 0.05 |
| NaCl 0.01+KCl 0.1 | KCl 0.01+NaCl 0.01 |
| NaCl 0.01+KCl 0.15 | KCl 0.01+NaCl 0.05 |

for the tip by glass insulation. In the thin part of the electrode both tubes became joined together when drawn out. The diameter of the tip of such micro-electrodes could be reduced to a few tenths of a micron. We asserted a special control over selectivity only in the end part of the micro-electrode. We selected micro-electrodes having a length of non-insulated tip of 10-20 $\mu$ , which were then immersed in a fiber to a greater depth. The complete micro-electrodes were then filled as we have already described.

Complete selective microelectrodes had a resistance of the order ot 100-500 m Ω. The time taken for their potentials to develop was 1-10 sec. Certain selective micro-electrodes were rather thicker than the normal ones. Nevertheless they appeared to do negligible damage to a fiber; in any case the introduction of such electrodes into a fiber for a long period of up to 6 h had no noticeable influence on its resting potential or on the intracellular activity of the ions.

When the activity of sodium or potassium ions is being measured particular attention should be paid to the potentials of the micro-electrodes themselves which may be the cause of considerable error. We were able to reduce the potential of asymmetry of the selective micro-electrodes by washing them for several days in 1 M solutions of KCl and NaCl. In electrodes made out of soft 4-component glass having a high electrical conductivity the potentials of asymmetry were slight (of the order of a few millivolts). In Na-selective electrodes made from the very high-melting point glass $NaS_{11-18}$, the potentials of asymmetry reached a few hundreths of a volt, and often were variable. When we used selective micro-electrodes, their potentials of asymmetry were compensated by applying an opposite potential. The fluid contact potentials of the normal micro-electrodes which are used as contacts in the calomel electrodes were not compensated, and all that was done was to make a careful choice of these contacts in relation to their innate potentials. For the experiments we used electrodes whose potentials did not exceed 5mv, and discarded any electrodes with positive potentials.

We must note that the accuracy of the measurement of activity does not influence the absolute value of the asymmetry potential, but its stability. The drift of the asymmetry potential was usually 0.1-1 mv/h. Electrodes showing high drift were discarded. In most micro-electrodes, in the region pNa and pK 0-2 the potentials were stable, and variation with time was found only when the activity of the ions was low. Thus, in such electrodes, the slope of the calibration curves altered (Fig. 1). Therefore, during long experiments we recalibrated the electrodes every hour.

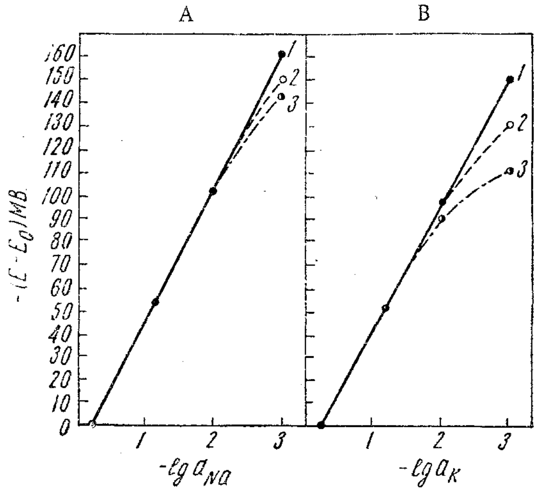

Fig. 2. Calibration curve for the Na-selective (A) and K-selective (B) micro-electrodes.

For measurement and recording of the EMF we used a recording electronic potentiometer ÉPP-09 M2. To the input of the potentiometer we connected a 2-tube amplifier having a high input resistance of $10^{-12}$ ohm, a low output resistance of about 500 ohms, and a gain of approximately unity. In the amplifier we used an ÉM-4 electrometer tube; the instrument enabled the value of the EMF to be measured to an accuracy of ±0.25 mV. A more detailed electrometer circuit has been described previously [2].

The selective electrode was an ordinary micro-electrode filled with 3 M KCl, and it was introduced into the same muscle fiber. To facilitate entry both electrodes were placed in a special holder and by means of a micromanipulator their tips were arranged to be strictly parallel to the length of the muscle fiber, and then both were implanted in it. A chlorided silver wire was lowered into the selective electrode; the normal micro-electrode was joined by means of an agar bridge (1% agar and 2.5 M KCl solution) to a calomel electrode. Both the selective and the calomel electrodes were connected by 2 coaxial cables to the input of the amplifier. The 2nd calomel electrode was placed in Ringer and connected through a low-resistance calibrator (calibrating potential 50 or 100 mV) to ground. The normal micro-electrode was then used to measure the resting potential between these 2 electrodes and a 2nd calomel electrode, and it was also used as an electrometric switch to the 1st calomel electrode.

After the micro-electrodes had been introduced into the fiber we recorded the following quantities related to the difference of potentials between the selective and the ordinary micro-electrode, and the difference of potential between the normal micro-electrode and the extracellular calomel electrode. We measured first the activity of the intracellular ions, then the resting potential Also, as a control, we measured the difference of potentials between the selective and extracellular calomel electrode which was immersed in Ringer. This difference represents the sum of the potential of the field and the potential which corresponds to the activity of the measured ion.

The experiments were begun by calibrating the selective electrodes in pure and mixed solutions of KCl and NaCl (see table).

Calibration was carried out in order to obtain a graph showing the relationship between the potential of the selective micro-electrode and the activity of the corresponding ion (Fig. 2). To determine the activity of the potassium and sodium ion within the cell from the graph we found the activity corresponding to a given potential.

Na-selective micro-electrodes are very stable chemically and mechanically. They can be used for 6-8 months. The useful life of a K-selective micro-electrode is not more than 20-30 days; if kept longer they become brittle and gradually lose their potassium function.

In biological media containing up to 0.15 M sodium a micro-electrode out of glass $NaS_{11-18}$ may measure its activity to an accuracty of approximately 0.2% provided the potassium ion concentration does not exceed 0.03 M [2]. Because the selectivity of a 4-component glass is not great ($K_{K/Na} \sim 0.3$) [3], micro-electrodes prepared from it can be used only for investigations of activity within the cells where the concentration of potassium ions is quite high (up to 0.15 M), and where the sodium ion concentration is low. In Ringer the potassium ion activity cannot be determined by means of this electrode.

## SUMMARY

Micro-electrodes of selective glass were used to measure potassium and sodium intracellular ionic activity. The diameter of the electrode tip measured only a few tenths of a micron. The usual non-selective glass was used for the micro-electrode insulation.

For biological electrolyte concentrations the selective micro electrode potential is directly proportional to the logarithm of the corresponding ion activity, and is expressed by Miko'skii's equation.

The slope of the graph of the potential in millivolts against log activity was less than the theoretical value of 58 mV, being 52.2±1.05 mv for the Na-selective electrodes. To measure and record the EMF an amplifier with an electrometer input circuit and an electronic recording potentiometer were used. The selective electrode and an ordinary micro-electrode filled with 3 M KCl solution were connected with the calomel electrode and introduced simultaneously into the same cell. The resistance of the micro-electrodes was 100-500 mΩ.

Measurement of their potential took 1-10 sec.

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