TIP POTENTIAL OF THE MICROELECTRODE AND THE INTRACELLULAR POTENTIAL OF *NITELLA FLEXILIS**

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The glass capillary electrode (microelectrode) of Ling and Gerard type[1] which has been widely used in electro-physiological experiments must have a juction potential at the tip. Indeed, R. H. Adrian[2] studied this potential and reported that it could not be neglected when the tip diameter was less than 2 micra. This is worthy to note in the measurement of membrane resting potential.

Although the Nitella-cell used here was large enough ($3 \sim 7$cm $\times$ $0.4 \sim 0.6$mm.) and did not require a microelectrode with such a small tip for its intracellular potential measurement, it was easier to insert a sharp tip into the cell-interior. Moreover, other electro-physiologically excitable cells are very small and may be investigated in the future. Hence, a preliminary research was carried out to study the tip junction potential (abbrebiated as; t. p.) of the microelectrode and the intracellular potential of Nitella.

MATERIALS AND METHODS

Nitella was obtained from the Botanical Institute, Faculty of Science, Kyoto University, and stocked in an artificial pond-water which contained, in millimolar, 0.025 KCl, 0.05 KNO_3, 0.2 NaCl, 0.25 $CaCl_2$, 0.25 $Ca(NO_3)_2$, 0.1 $MgSO_4$, and a trace of KH_2PO_4(3). An internodal cell was dissected before the experiment and immersed in 0.1 millimolar KCl solution for 24 hours. The vessel made of perspex-glass (5×5cm$^2 \times$ 1cm) was separated into two compartments. One was filled with 3 molar KCl-agar, in which an Ag·AgCl electrode was embedded to serve as the indifferent electrode. In the other, a Nitella-cell or the test solution was placed and a 3 molar KCl-agar-bridge was used between the two compartments for electrical connection. The well-known "Du Bridge and Braun's circuit" shown in **Fig. 1** was used for the potential measurement and satisfactory lineality was obtained between

* The essential part is reproduced from "*Yamaguchi-Igaku*": Vol. 9, pp. 760 & 1341, 1960.

applied voltage and deflection of galvanometer.

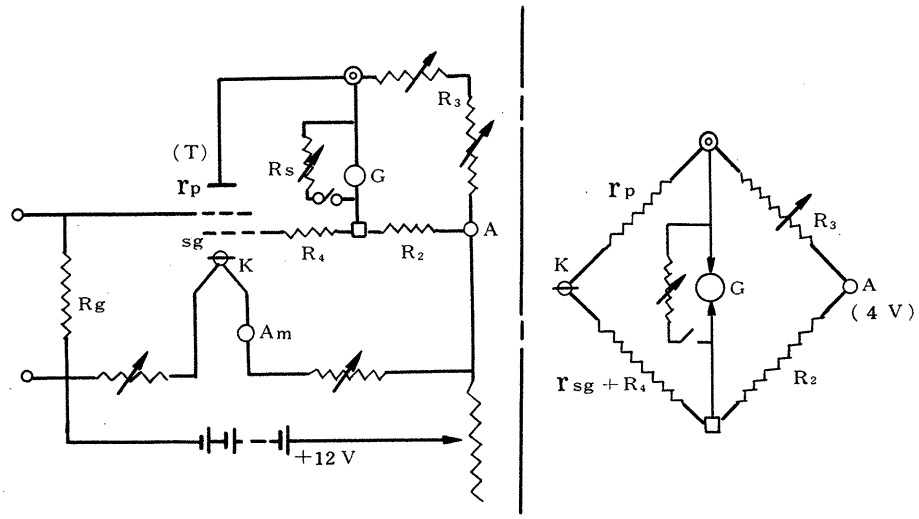

Fig. 1 Left: Circuit for potential measurement. Right: Essential part from the left. (Symbols in both parts correspond each other.)
Rg: high resistance tube (10^{18} ohm, Matzuda 1018), Rs: Ayrton's shunt for galvanometer (50 K. ohm). (T): vacuum tube for small potential measurement.
R_2, R_3, R_4: 2, 100+0.05, 7 K. ohms respectively. Other resistances: for adequate voltage-supply.

In this equipment, the t. p. could be measured by a deflection of galvanometer when the tip was dipped into the solution and the preliminary short-circuit at the input stage was disconnected. At the end of experiment, the tip was artificially broken in the test solution and the deflection was observed once more. This deflection had to be coincident with the beginning one where the input stage was short-circuited. In this case, the deflection might be assumed as a zero-point for the calibration of potential difference, because the t.p. of the microelectrode with a large tip-diameter was negligibly small as described above[2].

RESULTS AND DISCUSSION

I. *Some character of tip potential*

It has been accepted that the difference in ionic mobility between K^+ and Cl^- is a determining factor for the production of t.p.. Therefore, its value must be affected by the electrically charged matters existing at the tip; i.e., in almost all the excitable cells which are used in electro-physiological research, the concentrations of K^+, Cl^-, and charged organic matters in the intracellular fluid are different from those in the extracellular fluid.

Relationship between the t.p. and the concentration of KCl in the test solution was studied and the results are shown in **Fig. 2.**

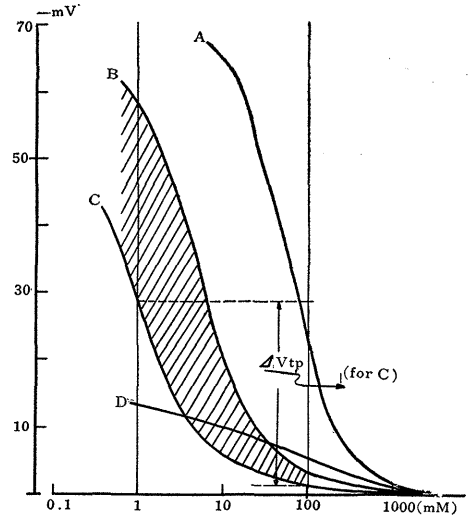

Fig. 2 Relationship between tip junction potential (ordinate) and KCl concentration in the external solution (abscissa). Each curve shows the results with one microelectrode. Curves A and D were the exceptional cases. The t.p.s of most of the electrodes were in the shaded area.

It was demonstrated that the microelectrode with a tip diameter of less than 2 micra had, in reality, a junction potential at its tip. However, the potential value of each electrode was not identical in the test solutions having the same KCl concentration, the potential of the first electrode (B in the figure) being -58mV, the second (C), -29mV, the third (D), -13mV and so on, in the 1.0 millimolar KCl solution. The factors responsible for this difference could not be elucidated, but it was certain that high t.p. values did not always correspond to the smallness of the tip-diameter and/or high electrical resistance. If, however, the experiment concerning only one microelectrode were taken, it could be said qualitatively that the lower was the concentration of KCl in the test solution the greater was the value of the t.p.

In another experiment, it was found that the t.p. was lowered in the KCl solution containing a protein. The degree of this lowering was about 15% in the solution which contained 1% bovine plasma albumin.

Here again, two facts must be noted: ① The concentration of KCl in the intracellular fluid is higher than that in the extracellular, and ② The extracellular fluid ordinarily used for the excised tissue in physiological experiments does not contain protein.

In this experiment, the generation of potential difference may be shown in the following scheme:

1) When the electrode tip is in the test solution;

(Indifferent electrode) (Microelectrode)
Ag·AgCl : 3M·KCl | test solution || 3M·KCl : AgCl·Ag
(J) $(T.P.)_0$

2) When the tip is in the cell-interior;

(Ind. electrode) (Microelectrode)
Ag·AgCl : 3M·KCl | extracell. sol. || intracell. fluid || 3M·KCl : AgCl·Ag
(J) (M.P.) $(T.P.)_i$

The position of existing potential is indicated by the vertical line. Two dotted lines in both schemes denote the physical polarization potential and may be canceled out in the potential measurement. (J) is the junction potential caused by the KCl-agar-bridge and may be neglected because of its small value. (M. P.) is the intracellular potential to be measured. $(T.P.)_0$ and $(T.P.)_i$ indicate t.p.s when the tip is placed in the extracellular- and in the intracellular fluid, respectively. $(T.P.)_0$ is not equal to $(T.P.)_i$ because, as stated above, the KCl concentrations are different in these two cases.

Assuming that the concentration of K^+ in the cell-interior of Nitella is about 140 millimols[4], the potential difference between $(T.P.)_0$ and $(T.P.)_i$ was evaluated as shown in Fig. 2 by the symbol, ΔV_{tp}. The measured intracellular potential was corrected referring this difference.

II. *Intracellular potential of Nitella.*

The concentration of KCl in the extracellular fluid has a marked effect on the intracellular potential of an irritable cell. This is a well-known fact for the muscle and nerve of warm- and cold-blooded animals, and its applicability to Nitella-cell was recently recognized[5, 6].

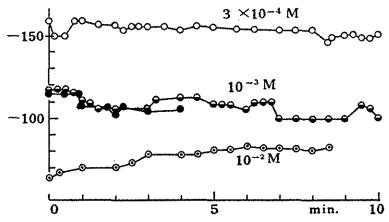

Fig. 3 Time courses of the intracellular potentials of Nitella-cell after the insertion of a microelectrode. The KCl concentration in the extracellular solution was indicated on each curve. (See the text for details.)

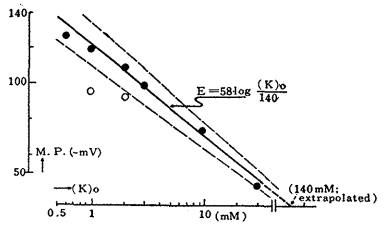

Fig. 4 Relationship between the intracellular potential (ordinate) and KCl concentration in the extracellular solution (abscissa). The solid line was drawn by the equation indicated and the range of 10% deviation was shown by the broken lines.

Figure 3 shows time-courses of the established intracellular potentials after the insertion of a microelectrode into the Nitella-cell. The curves indicate 4 experi-

ments in which extracellular solution contained 0.3, 1.0, 1.0, and 10 millimolar KCl, respectively. It may be seen that the measured potential-level was maintained for about 10 minutes and that its value was smaller in the outer solution of higher KCl concentrations than in that of lower KCl.

Experimental results concerning the relationship between the intracellular potential and the KCl concentration in outer solution were statistically treated and **Fig. 4** was drawn. The applicability of the following formula was also tested:

$$E = \frac{RT}{F} \cdot 1n \frac{(K)_0}{(K)i}$$

where; E represent intracellular potential (negative); $(K)_0$, K^+ concentration in outer solution; (K)i, K^+ concentration in intracellular fluid; R, T, F are physical constants.

Generally speaking, the insertion of a microelectrode into the cell-interior was not so difficult, and the established potential was stable for at least 30 minutes. The linear relationship between E and $(K)_0$ was ascertained in the range where the KCl concentration in the extracellular fluid was between 0.5 and 30 millimols.

SUMMARY

1. Tip junction potential of the microelectrode widely used in electro-physiological research, and intracellular potential of *Nitella flexilis* were studied by using an appropriate circuit (shown in figure 1).

2. Some electrodes had a high tip potential and were not suitable. Others with relatively low potentials might be used in the experiments with adequate correction.

3. A linear relationship was demonstrated between the intracellular potential of *Nitella* and the logarithm of K^+ concentration in the extracellular solution.

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