On the Henderson's Equation Concerning the Tip-potential of Microelectrode.

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In an experiment to study intracellular potential, the excised tissue such as nerve or muscle is placed in Ringer's solution and a glass capillary electrode of Ling and Gerard type (microelectrode) is widely used. A potential difference is frequently observed between the potassium chloride solution in the capillary and the Ringer's solution. It is a sort of liquid junction potential or diffusion potential. Oki and Kawasaki (1961), in this laboratory, reported a preliminary experiment about this potential and called it "tip potential". In the present paper, their experiment was carried out further and the applicability of Henderson's equation for its interpretation was discussed.

EXPERIMENTALS

The microelectrode was made by Katsuki-type puller. The diameter of the tip was less than 1 micron. It was filled with KCl solution by boiling under negative pressure. The cell for the potentiometry was constructed as follows;

Microelectrode | Test Solution | KCl-Agar | 3M·KCl | HgCl·Hg

The balancing circuit of a tube-potentiometer was the Du Bridge and Broum's one using Ux–54A (Toshiba) and its detail was reported previously (rf. 3). At the end of experiment, the tip was artificially broken in the test solution and a deflection of galvanometer was recorded. This was a zero-point for a potential calibration.

Figure 1 is a histogram of the measuerd potentials. The test solution is Ringer's. The frequency is greatest in the class ranging from 0 to 5 millivolts, i.e. there were many electrodes which had no tip potential. (Although it is preferable to select such electrodes in the practice to measure the intracellular potential, other electrodes which have some tip potential value are under the consideration in the present paper.) It was likely that the electrode with the smaller tip diameter had the greater electrical resistance. The potential value of the electrode having a high resistance, however, was not always great.

Figure 2 represents an experiment with one electrode when the concentration of the test solution is changed. Abscissa is a logarithmic scale of the concentration and ordinate, measured potential in millivolt. When the potential of the test solu-
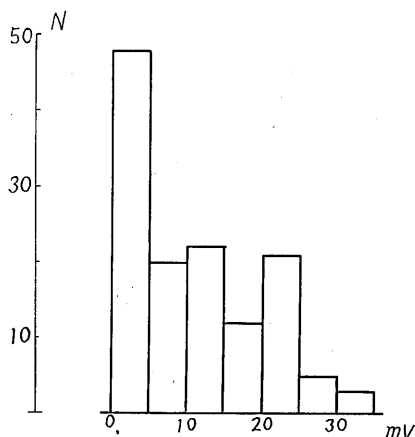
Fig. 1. A histogram of the liquid junction potential between the KCl solution in the glass-capillary electrode and Ringer's solution. (N = 131)
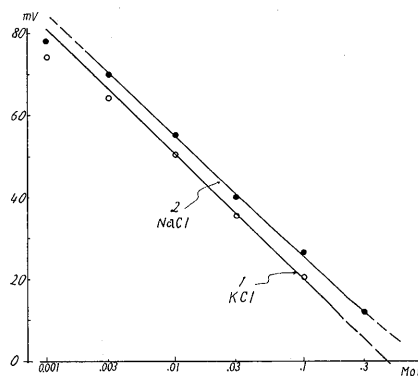

Fig. 2. Abscissa : Logarithm of the molal concentration of NaCl or KCl solution· Oridinate : tip potential in millivolt. Open circle or line 1 shows an experiment with KCl, and solid circle or line 2, with NaCl solution. Only one electrode is used in each. For a method to draw the lines, see in the text (discussion).

tion is higher than that of KCl solution in the microelectrode, the potential value is positive.   The test solution is KCl in line 1 (open circles) and, NaCl in line 2 (solid ones).   A method of drawing the curve is descussed below.

DISCUSSION

The Henderson's equation concerning the liquid junction potential (E) between the two different electrolyte solutions (Solution I and II), in which the ionic activities are c_1 and c_2, respectively, is

$$E=\frac{RT}{nF}\cdot\frac{(c_1u_1-c_1v_1)-(c_2u_2-c_2v_2)}{(c_1u_1+c_1v_1)-(c_2u_2+c_2v_2)}l_n\frac{c_1u_1+c_1v_1}{c_2u_2+c_2v_2} \qu{}\cdots\cdots\cdots\cdots\cdots(1)$$

where; u_1 and v_1 are mobilities of cathion and anion, respectively, in solution I; u_2 and v_2 are those in solution II: n, ionic valency: R, F and T, physical constants in ordinary meaning.

In the very dilute solution, the ionic activity can be considered to be the same as the concentration.   However, the KCl solution in the microelectrode is not dilute and we do not know the exact value of this activity. (In the handbook of physical-chemistry, it is described that the activity coefficient is 0.57 (25°C) in 3M·KCl solution.   It is doubtful, in the present experiment, that the concentration of KCl solution in the elecrtode is always 3.0 molal.   The figure, 0.57, is not compatible in the following calculation. It seems likely that the ionic activity is variable, case by case.) Therefore, the KCl solution was used firstly as the test solution.

The liquid jonction potenrial between the same electrolyte solution is given by the Nernst's equation; i.e.

$$E = \frac{RT}{nF} \cdot \frac{u-v}{u+v} \ln \frac{c_1}{c_2} \quad \dots\dots\dots\dots\dots (2)$$

Equation (2) can be derived from equation (1). (Because; $u_1 = u_2 \equiv u$ and $v_1 = v_2 \equiv v$) It can be seen, from equation (2), that the potential has a linear relation to the logarithm of c_2 when both the ionic mobilities and c_1 are constant. The slope of this straight line is determined by the term, $(u-v)/(u+v)$. However, in the case where we consider the mobility of K-ion to be almost the same as that of Cl-ion, such a potential as is seen in Fig. 2 can not be resulted. It seems likely that there is some disturbing effects upon the ionic mobility.

Generally speaking, a glass-wall of capillary has a negative charge. When a diameter of the glass-capillary is very small, it is reasonable to consider that the negative charge would decrease the mobility of the anion diffusing through the capillary. Therefore, following assumption is possible in equation (2):

$$v = au, \quad 0 < a < 1$$

$$E = 57.7 \times \frac{1-a}{1+a} \log \frac{c_1}{c_2} \quad \dots\dots\dots\dots\dots (3)$$

$$(E: mV, \ 18°C)$$

Practically, as in the Fig. 2, the measured potentials are plotted against the logarithm of KCl-concentration tested (c_2). A straight line has to be obtain in the dilute range of c_2, as stated above. It is easy to draw the straight line by the least mean-square method. From its slope, "a" can be calculated ($a = 0.31$ in Fig. 2). Furthermore, the intersecting point on the abscissa gives approximate value of c_1 (0.46). These figures and the ionic mobility from the textbook are introduced to equation (1), together with an assumption that the mobility of Cl-ion in NaCl solution are decreased by the same degree at the boundary: i.e.

$$u_1 \equiv u \quad u_2 = \frac{43.5}{64.6} \quad u \doteqdot 0.67u \quad v_1 = v_2 = 0.31u$$

$$c_1 = 0.46 \quad c_2 \equiv x \quad y \equiv E(mV, \ 18°C)$$

$$y = 57.7 \times \frac{0.317 - 0.36x}{0.603 - 0.98x} (\log 0.603 - \log x) \quad \dots\dots\dots\dots\dots (4)$$

Substituting an arbitral figure to x in equation (4), the corresponding y is calculated; and the relationship between the tip potential of one microelectrode and the concentration of the tested NaCl solution can be drawn graphically. This is the line 2 in Fig. 2. An agreement of the experimental results, which are shown by the solid

circles, with the calculated ones is satisfactory. It is regrettable, however, that a general relationship between the tip potential and the concetration of some electrolyte solution cannot be formulated; because each microelectrode has a different charge at its tip and/so the decreasing grade of the ionic mobility or ionic activity cannot be anticipated.

SUMMARY

    A liquid junction potential between the KCl solution in the glass-capillary electrode and Ringer's-, NaCl- and KCl solution was measured with a tube-potentiometer. A negative charge of the glass-wall decreases the mobility of Cl-ion at the tip of the electrode and generates the liquid junction potential. The decreasing grade of the mobility and ionic activity can be estimated from the measured potential between the KCl solutions inside and outside the capillary. From these estimation, the potential can be calculated by the Henderson's equation.

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