ANALYSIS OF MEMBRANE PERMEABILITY COEFFICIENTS OF AMPHIBIAN SKIN BY MEANS OF ELECTRONIC DATA PROCESSING

I. A NEW CONSTANT FIELD EQUATION APPLICABLE TO AMPHIBIAN SKIN POTENTIAL

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Summary A new extended membrane equation applicable to carrier-mediated active transport is derived, as follows:

$$\Delta E = \frac{RT}{zF} \ln \frac{P_K C_K^o + P_{Na} C_{Na}^o + P_{Cl} C_{Cl}^i - \Phi}{P_K C_K^i + P_{Na} C_{Na}^i + P_{Cl} C_{Cl}^o - \Phi - \varphi},$$

where ΔE is membrane potential, φ and Φ correspond to active-transport flux and carrier-mediated flux respectively, and other notations have their usual meanings in membrane equations.

By means of electronic data processing, such an extended membrane equation is applicable to computation of the ion permeability coefficients of the skin of frog, toad, and newt using a least-square method. Applying the two-membrane theory of Ussing, the membrane potential of the amphibian skin is obtained as follows:

$$\Delta E = \frac{RT}{F} \ln \frac{C_{Na}^o + 0.103 C_K^o + 0.809 C_{Cl}^i - 62.1}{C_{Na}^i + 0.103 C_K^i + 0.809 C_{Cl}^o - 100.6} + 57 \text{ mV (frog)},$$

$$\Delta E = \frac{RT}{F} \ln \frac{C_{Na}^o + 0.205 C_K^o + 0.483 C_{Cl}^i - 37.0}{C_{Na}^i + 0.205 C_K^i + 0.483 C_{Cl}^o - 105.9} + 48 \text{ mV (toad)},$$

and

$$\Delta E = \frac{RT}{F} \ln \frac{C_{Na}^o + 0.328 C_K^o + 1.061 C_{Cl}^i - 82.5}{C_{Na}^i + 0.328 C_K^i + 1.061 C_{Cl}^o - 108.4} + 38 \text{ mV (newt)}.$$

These relations show a fairly good respective agreement with the observed potentials. Application of such an extended equation not only avoids the appearance of a physiologically meaningless negative permeability coefficient for some one of the ions, as is often obtained by applying the original Goldman equation, but also results in a fairly reasonable value for these coefficients.

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The central feature of the constant field equation (GOLDMAN, 1943; HODGKIN and KATZ, 1949) is that membrane permeability coefficients can be obtained from the transmembrane potential. The constant field equation has been presented as

$$\Delta E = \frac{RT}{zF} \ln \frac{P_K C_K^o + P_{Na} C_{Na}^o + P_{Cl} C_{Cl}^i}{P_K C_K^i + P_{Na} C_{Na}^i + P_{Cl} C_{Cl}^o}, \quad (1)$$

where R , T , and F have their usual thermodynamic meanings; z is the valence of ionic charge; P_K , P_{Na} , and P_{Cl} represent the membrane permeability for ions; C_K , C_{Na} and C_{Cl} represent the concentration; and the superscripts i and o stand for the bulk phase of the inner and outer side of the membrane. In the membrane phase, concentrations of these ions are known to be so low that the dilute solution approximation can be applied. They are also not so high in the bulk liquid phase of both sides under our experimental conditions.

Membrane Eq. (1) has been applied to data from a number of different natural membrane systems, and the relative permeabilities for the ions have been calculated successfully. Some dissatisfaction, however, has been expressed about the lack of quantitative application of the equation to the data. Using the data reported by HODGKIN *et al.* (1962) and by TASAKI (1968), for example, one of the present authors (SHINAGAWA, 1968) previously pointed out that the constant field equation was found to give a negative permeability coefficient for one of the ions. When a permeability coefficient is less than zero, the physiological meaning of the membrane permeability appears to be lost. Thus one might suspect that the constant field equation needs some modification. A modified equation applicable to active transport was previously reported by SHINAGAWA (1968, 1970).

In the present report, a new extended membrane equation applicable to carrier-mediated active transport will be presented. Then, using a linear least-square method by means of electronic data processing, ion permeability coefficients of the skin of frog, toad, and newt will be calculated from the extended membrane equation in order to demonstrate its applicability.

THEORY

1. A membrane equation applicable to active transport.

It is well known that the Goldman-Hodgkin-Katz equation cannot explicitly represent active transport. One of the present authors (SHINAGAWA, 1970) has already attempted to extend Goldman's membrane equation, taking into account the flux by an active transport φ , as follows:

$$\Delta E = \frac{RT}{zF} \ln \frac{P_K C_K^o + P_{Na} C_{Na}^o + P_{Cl} C_{Cl}^i}{P_K C_K^i + P_{Na} C_{Na}^i + P_{Cl} C_{Cl}^o - \varphi}. \quad (2)$$

This equation can be derived as follows from the phenomenological equations of irreversible thermodynamics of the ion flux across membrane J (SHINAGAWA, 1964):

$$\left. \begin{aligned} J &= -P\Delta\lambda + P_r J_r \\ J_r &= Q_r J - Q\Delta \end{aligned} \right\}, \quad (3)$$

where J_r is the chemical reaction rate linking the ion flux; $\lambda = C \exp(zFE/RT)$, the electrochemical activity of the ion in the membrane phase; Δ represents the difference across, the membrane; A is the chemical affinity; P is the permeability constant; Q is the chemical coefficient; and P_r and Q_r are cross coefficients.

Ion flux is obtained as:

$$J = -\frac{P\Delta\lambda + P_r Q\Delta}{1 - P_r Q_r} = -p\Pi \left(C^o e^{-\frac{zF}{RT}\Delta E} - C^i \right) - \varphi\Pi, \quad (4)$$

where

$$\Pi = h \int_0^h e^{-\frac{zF}{RT}E(x)} dx,$$

and $p\Pi = P/(1 - P_r Q_r)$; $E(x)$ is the electric potential in the membrane of the thickness h . Under stationary conditions, i.e., $J = 0$, the following equation is readily deducible:

$$\Delta E = \frac{RT}{zF} \ln \frac{pC^o}{pC^i - \varphi}. \quad (5)$$

Thus, if one of the ions, Na^+ , K^+ , or Cl^- , links with the chemical reaction, the Goldman equation should be extended to Eq. (2). If the electrical potential is given according to the constant field, the reduced chemical reaction rate is obtained as

$$AQ/(1 - P_r Q_r) = \varphi \Delta E / (e^{-zF\Delta E/RT} - 1).$$

2. The constant field equation applicable to carrier-mediated active transport

In the case of carrier-mediated ion transport across a membrane, the carrier-ion complex may perhaps be electrically neutral. Such a non-ionic flux, J_{non} , linked with ion transport is expressed as:

$$J_{\text{non}} = -P_{\text{non}}\Pi\Delta\lambda, \quad (6)$$

where $\Delta\lambda \simeq \Delta C$, in the limit of electrical charge z_{non} being neraly zero

$$\Pi = h \int_0^h \left\{ 1 + z_{\text{non}} \frac{F\Delta E}{RT} x + 0(z_{\text{non}}^2) \right\} dx \simeq 1 - z_{\text{non}} \frac{F\Delta E}{2RT}, \quad (7)$$

$$\text{and} \quad J_{\text{non}} = -P_{\text{non}}(C_{\text{non}}^o - C_{\text{non}}^i) + P_{\text{non}} \frac{z_{\text{non}} F \Delta E}{2RT} (C_{\text{non}}^o - C_{\text{non}}^i). \quad (8)$$

The linkage with cation transport (represented by subscript l) is derived from the electric current as:

$$\begin{aligned} I = & -F \sum_l z_l P_l \frac{z_l F \Delta E / RT}{e^{-z_l F \Delta E / RT} - 1} (C_l^o e^{-z_l F \Delta E / RT} - C_l^i) \\ & + z_{\text{non}} F P_{\text{non}} \frac{z_{\text{non}} F \Delta E}{2RT} (C_{\text{non}}^o - C_{\text{non}}^i). \end{aligned} \quad (9)$$

Under the stationary condition $I = 0$, the constant field equation is give by:

$$\Delta E = \frac{RT}{zF} \ln \frac{\sum_i P_i C_i^o - \Phi}{\sum_i P_i C_i^i - \Phi} \quad (10)$$

and

$$\Phi = \frac{z_{\text{non}}^2}{2z^2} P_{\text{non}} (C_{\text{non}}^o - C_{\text{non}}^i), \quad (11)$$

where $|z_1|$ is assumed to be equal to z . Referring to Eqs. (2) and (10), an extended constant field equation applicable to carrier-mediated active transport is obtained as

$$\Delta E = \frac{RT}{zF} \ln \frac{P_K C_K^o + P_{Na} C_{Na}^o + P_{Cl} C_{Cl}^i - \Phi}{P_K C_K^i + P_{Na} C_{Na}^i + P_{Cl} C_{Cl}^o - \Phi - \varphi}. \quad (12)$$

In the present study, data on the membrane potential of amphibian skin were processed according to Eq. (12); permeability coefficients P , the carrier flux Φ , and active transport flux φ were computed.

MATERIALS, METHODS AND COMPUTATION

Abdominal skin of frog (*Rana nigromaculata*), toad (*Bufo vulgaris*), and newt (*Triturus pyrrhogaster*) was processed for skin potential measurement, altering the outside (epidermal side) ion concentrations against the inside (corium side) Ringer's solution bubbled by an air pump, by means of the membrane electrode method, which was previously reported by one of the authors (OKAMOTO, 1968b). Observed values are the mean of five experiments, fluctuation being less than 5 mV. The ultrastructure of these amphibian skins relating to their ion transport function was studied by means of electron microscopy and these observations have already been reported (OKAMOTO, 1968a; SHINAGAWA *et al.*, 1966, 1968).

The calculations were carried out using electronic computer systems I and II in the Data Processing Center of Kyoto University. The linear least square solutions were computed using a subroutine subprogram which was developed and registered at the scientific subroutine library of the center (SHINAGAWA). On application of USSING's (1958) two-membrane theory of frog skin, the potential is presented as follows:

$$\Delta E = E_1 + E_2,$$

$$E_1 = \frac{RT}{zF} \ln \frac{P_K C_K^o + P_{Na} C_{Na}^o + P_{Cl} C_{Cl}^i - \Phi}{P_K C_K^i + P_{Na} C_{Na}^i + P_{Cl} C_{Cl}^o - \Phi - \varphi},$$

and
$$E_2 = \frac{RT}{zF} \ln \frac{P_{2,K} C_K^i + P_{2,Na} C_{Na}^i + P_{2,Cl} C_{Cl}^R - \Phi_2}{P_{2,K} C_K^R + P_{2,Na} C_{Na}^R + P_{2,Cl} C_{Cl}^i - \Phi_2 - \varphi_2},$$

where the superscript R means inside Ringer's solution, the subscript 2 is the inner membrane, and E_1 and E_2 are the transmembrane potentials of the outer and inner

membranes, respectively. The superscript i represents bulk phase of the inner side of the outer membrane, extra-cellular fluid; $C_K^i = 2.6$ mM, $C_{Na}^i = 110$ mM, and $C_{Cl}^i = 77$ mM. Other ionic concentrations, such as intra-cellular fluid, were found to give negative permeabilities, $P < 0$. As it is difficult to calculate the potential of the inner (second) membrane E_2 by the least square method, computer experiments were undertaken. That is, the parameter on the inner membrane potential was varied in a wider range, and the minimum value of the sum of squares of errors (discrepancies between the observed potentials and the calculated ones) was obtained. Trial and error methods hitherto adopted for determining permeabilities do not yield adequate tests for adjustment of the membrane equation to the data. Thus, when the equation is not applicable to the data, use of the theory is not justified. Certain assumptions underlying the constant field theory may not seem strictly applicable to complicated membrane systems such as the skin of frogs. However, the electronic data processing system does provide a practical regression method which may be expected to justify the theory.

RESULTS

1. Frog skin potential

Observations on frog skin potentials altering external ion concentration were plotted in Figs. 1a, b, and c. Only Ringer's solution was used as internal solution. Figure 1a shows membrane potentials as a function of the sodium and potassium concentration. Solid lines were calculated from:

$$\Delta E = \frac{RT}{F} \ln \frac{C_{Na}^o + 0.103C_K^o + 0.809C_{Cl}^i - 62.1}{C_{Na}^i + 0.103C_K^i + 0.809C_{Cl}^i - 100.6} + E_2 \quad (13)$$

and $E_2 = 57$ mV, the equation being applicable to a range of sodium concentration of less than 40 mM. In the range of a higher sodium concentration (> 40 mM), P_{Na} becomes 0.552 instead of 1.0, and P_K is 0.092 (dotted lines in Fig. 1a). The interpolated equation of the whole range of sodium concentrations is obtained as:

$$\Delta E = \frac{RT}{F} \ln \frac{C_{Na}^o(1 - 0.0041C_{Na}^o) + 0.145C_K^o + 1.16C_{Cl}^i - 89.3}{C_{Na}^i(1 - 0.0041C_{Na}^o) + 0.145C_K^i + 1.16C_{Cl}^i - 100.4} + E_2. \quad (14)$$

The broken line in Fig. 1a was computed from Eq. (14). These calculated values agree fairly well with the observed potentials. Effects of other cations (Li^+ , NH_4^+ , Cs^+ , and Rb^+) are shown in Fig. 1b; application of the equation to the data is also fairly successful. However, the membrane potentials as a function of the anion concentration showed less satisfactory agreement with the computed values (Fig. 1c). As there does not appear to be published data concerning anion permeability coefficients, such an application, though not satisfactory, may be worth reporting. These ion permeability coefficients are presented in Table 1. Permeability coefficients of divalent ions including SO_4^{--} will be reported in separate papers.

Fig. 1. Membrane potential of frog skin as a function of concentration of the ions. The observed values are presented by plotting; lines drawn show the calculated values by means of an electronic computer system.
 (a) Effects of Na^+ and K^+ , (b) effects of other cations, and (c) effects of anions, counter cation being Na^+ .

Table 1. Ion permeability coefficients of frog skin computed from the extended membrane equation ($P_{Na} = 1$, when sodium concentration is less than 40 mM).

Ion	P_{ion}	ϕ	φ
K ⁺	0.103	62.1	38.5
Na ⁺ (> 40 mM)	0.552	62.5	0.59
Li ⁺	0.340	44.4	52.4
Rb ⁺ (< 100 mM)	0.112	64.0	36.9
Rb ⁺ (< 100 mM)	0.162	133.7	4.8
Cs ⁺	0.100	63.1	37.8
NH ₄ ⁺	0.096	66.0	35.7
Cl ⁻	0.809	62.1	38.5
Br ⁻	1.087	64.0	37.2
I ⁻	2.199	58.7	40.5
SCN ⁻	1.715	58.6	40.6
NO ₃ ⁻	0.450	66.4	35.7

2. Toad skin potential

The skin potentials of the toad are plotted in Figs. 2a, b, and c; the details were previously reported (OKAMOTO, 1968b). Curves in each figure were computed from Eq. (12) above. Application of these curves to the data is less satisfactory than those to the frog skin potential. When Eq. (1) is applied, however, negative permeability coefficients for one of the ions (P_{Cl}) is obtained by means of the least square method. Some modification of the original Goldman equation (1) such as Eq. (12) was deemed necessary.

Solid lines in Fig. 2a were calculated from:

$$\Delta E = \frac{RT}{F} \ln \frac{C_{Na}^o + 0.205C_K^o + 0.483C_{Cl}^i - 37.0}{C_{Na}^i + 0.205C_K^i + 0.483C_{Cl}^o - 105.9} + 48 \text{ mV}. \quad (15)$$

In the range of higher sodium (> 30 mM) and potassium concentrations (> 60 mM), P_{Na} becomes 0.231 and P_K , 0.098 (broken lines in Fig. 2a).

Computed ion permeability coefficients are presented in Table 2.

3. Newt skin potential

Newt skin potentials are plotted in Figs. 3a and b; curves in each figure were calculated from Eq. (12). Application of the equation to the data was found to be fairly good, whereas the negative permeability coefficient for Cl⁻ appeared when Eq. (1) was applied.

The solid lines in the figure were calculated from:

$$\Delta E = \frac{RT}{F} \ln \frac{C_{Na}^o + 0.328C_K^o + 1.061C_{Cl}^i - 82.5}{C_{Na}^i + 0.328C_K^i + 1.061C_{Cl}^o - 108.4} + 38 \text{ mV}. \quad (16)$$

in the range of higher sodium (> 30 mM) and potassium (> 40 mM) concentration, P_{Na} becomes 0.643 and P_K , 0.251 (broken lines in Fig. 3a). The ion permeability coefficients of the newt skin are tabulated in Table 3.

Fig. 2. Skin potential of the toad as a function of ion concentration. The plot presents the observed values; lines the computed ones. (a) Effects of sodium and potassium ion, (b) effects of other cations, and (c) effects of anions, counter cation being K^+ .

Table 2. Ion permeability coefficients of toad skin computed from the extended membrane equation ($P_{Na} = 1$, when sodium concentration is less than 30 mM).

Ion	P_{ion}	ϕ	φ
K^+ (< 60 mM)	0.205	37.0	68.9
K^+ (> 60 mM)	0.098	34.4	0.21
Na^+ (> 30 mM)	0.231	34.4	0.21
Li^+	0.570	1.0	96.1
Cs^+ (< 44 mM)	0.239	43.2	64.9
Cs^+ (> 44 mM)	0.123	38.7	1.22
NH_4^+ (< 44 mM)	0.203	39.5	66.9
NH_4^+ (> 44 mM)	0.086	36.5	0.59
Cl^-	0.483	37.0	68.9
F^-	0.048	35.2	36.4
Br^-	0.078	34.5	39.1
I^-	0.032	33.5	37.8
HCO_3^-	0.663	27.4	38.3
HPO_4^-	0.696	27.4	38.2
SCN^-	0.532	43.2	41.6

Fig. 3. Skin potential of newt as a function of ion concentrations. The plot presents the observed values; lines the computed ones. (a) Effects of sodium and potassium ion, and (b) effects of other cations, counter anion being Cl^- .

DISCUSSION

We failed to determine the permeability coefficients of the inside membrane of amphibian skin, since the skin potential progressively fell, showing hardly any

Table 3. Ion permeability coefficients of newt skin computed from the extended membrane equation ($P_{Na} = 1$, when the sodium concentration is less than 30 mM).

Ion	P_{ion}	ϕ	φ
K^+ (<40 mM)	0.328	82.5	25.9
K^+ (>40 mM)	0.251	82.6	1.35
Na^+ (>30 mM)	0.643	82.6	1.35
Li^+	0.423	46.1	52.8
Cs	0.319	81.8	25.9
Rb^+ (<60 mM)	0.319	81.1	25.7
Rb^+ (>60 mM)	0.288	85.3	2.23
Cl^-	1.061	82.5	25.9

tendency to recover when the inside solution was replaced by electrolyte solutions other than the standard Ringer's solution. A possible membrane equation for the inner membrane of the frog skin could be as follows:

$$E_2 = \frac{RT}{F} \ln \frac{0.02C_{Na}^i + C_K^i + 0.1C_{Cl}^R - 374.2}{0.02C_{Na}^R + C_K^R + 0.1C_{Cl}^i - 47.8}. \quad (17)$$

Potentials of the inside membrane of the amphibian skin (E_2) are close to the usual difference in potentials across the cell membrane, so that the inner membrane seemed to be the cell membrane in the epidermis.

Active transport potential ΔE_{act} can be defined as:

$$\Delta E_{act} = \Delta E_{obs} - \Delta E', \quad (18)$$

where ΔE_{obs} is the observed potential difference across the membrane, and $\Delta E'$ is the potential difference calculated from Eq. (12), without ϕ and φ . The active transport potential of the frog skin in 40 mM NaCl outside solution becomes 7.26 mV from Eq. (18). That of the toad skin in 25 mM NaCl outside solution is 24.8 mV, and that of the newt skin in 40 mM NaCl is 1.7 mV.

The mean value of the potential difference across the frog skin bathing in Ringer's solution was observed to be 52 mV. The calculated value of the potential is 47 mV from Eq. (12), and $\Delta E' = 51.4$ mV. Then, the active transport potential came close to zero when the frog skin was bathed in Ringer's solution. Decrease of the potential difference across amphibian skin in the range of higher sodium concentrations has been hitherto attributed to penetration of the ion into the epithelial tissue. Therefore, we calculated the degree of penetration from the data on skin potentials by means of the least square method. The calculation (details of which will be reported elsewhere) showed that the increase of sodium concentration in the intra-epithelial fluid was less than 10^{-9} M. Decrease of the skin potential in the bathing solution of higher salt concentration may be due to decrease of the cation permeability coefficients and of the active transport potential.

Permeability coefficients of the frog skin in this report are close to those reported by Iro (1962) from the original Goldman equation ($P_K/P_{Na} = 0.067$

and $P_{Cl}/P_{Na} = 0.6$). The extended membrane equation Eq. (12), can also act as the active transport potential and flux. A negative permeability coefficient of monovalent ions was nonexistent for toad and newt skin. Such was not the case for some of the ions when the Goldman equation was applied. Concerning divalent ions, however, circumstances appear to be far from simple: for example, when Eq. (12) is formally applied to the toad skin potential, a quite small but negative P value is obtained for the SO_4 ion ($P_{so_4} = -0.003$). Permeability coefficients of divalent ions including SO_4 will be discussed in succeeding papers, in connection with detailed discussions of active transport potential.

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