

CONCENTRATION DEPENDENCE OF PERMEABILITY COEFFICIENTS TO AN ELECTROLYTE COMPONENT ACROSS BOVINE LENS CAPSULE IN VITRO

NORIAKI TAKEGUCHI *and* MASAYUKI NAKAGAKI

From the Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto, Japan.

ABSTRACT Theoretical and experimental studies have been made on permeability coefficients to various kinds of electrolyte across lens capsules that are dissected from bovine eyes and that are found to be positively fixed charged membranes from our experiments of membrane potentials. The differential permeability coefficient, P_m , is defined as $J_s = P_m(C_2 - C_1)$, where J_s is the flux of an electrolyte component in moles per sec across unit area of the lens capsule that separates two aqueous solutions of the same electrolyte at different concentrations, C_2 and C_1 . Various types of strong electrolytes were studied; KCl, NaCl, CaCl₂, MgSO₄, MgCl₂ and LaCl₃. It was found that at $C_2/C_1 = \text{constant}$, P_m decreases to zero as C_2 decreases and P_m increases to a limiting value, $(P_m)^\infty$, that is characteristic for the system of the salt used and the membrane as C_2 increases, despite of electrolytes. We assumed in theory that single ion activity coefficients of co-ion and gegen-ion are ideal, that the systems studied are in electric neutrality, that the fixed charge density of the membrane is independent of concentrations C_2 , and that Donnan equilibrium holds between the bulk solution and membrane surface. Although the concentration-dependent changes of P_m were quantitatively different depending on the type of electrolyte used, general agreement between theory and experiment was obtained over a wide range of concentrations except for the case of very dilute solutions.

INTRODUCTION

Since the lens is not supplied by blood vessels and is surrounded completely by a continuous membrane, the lens capsule, the degree of permeability of this membrane of necessity places certain limitations on the character and the amount of the substances that can enter the lens as nutriment or that can leave the lens as products of its metabolism.

As shown later from our experiments, this collagenous membrane taken from bovines has fixed *positive* charges in its membrane phase when it is suspended in some neutral electrolyte solutions equilibrated with laboratory air. Thus, the treatment of the diffusion process of ions that interact with fixed charges cannot be

treated by using Fick's law in its simplest form that is usually applied to the system of uncharged membranes. The study of electrically charged membranes has been greatly developed since the work of Michaelis (1928) because of their importance in biological systems. The first serious theoretical attempt to account for the selective permeability of charged membranes to ions is the fixed-charged theory of Teorell (1937) and Meyer and Sievers (1936). Subsequently, several workers have made such studies in the fields of ion exchange and biological membranes.

This article describes the concentration dependence of the permeability coefficients of various electrolytes across the anterior lens capsule, dissected from bovine eyeballs, and interprets the experimental results on the basis of the thermodynamics of irreversible processes.

Here, a short historical survey concerning the permeability, chemical contents and structural characteristics of the lens capsule will be given. Hess (1911) showed qualitatively that the lens capsule is normally permeable to all electrolytes and true solutes as well as to small and medium sized colloidal particles, such as hemoglobin. Friedenwald (1930 *a* and *b*) confirmed Hess' results and showed that for nonelectrolytes the permeability of the lens capsule decreases with increasing molecular weight of the diffusing substances and that from an experiment of electroosmosis the lens capsule carries net *negative* electrical charges when placed in physiologic sodium chloride solution. Nakagaki, Koga, and Iwata (1963 *a*, *b*, and *c*) showed that integral diffusion coefficients of phenoxazone compounds, such as xanthommatin, catalin, A-catalin, and C-catalin across the bovine lens capsule were obtained using Stokes diaphragm diffusion cell without mechanical stirring of the aqueous solutions and that the permeability coefficients of phenoxazone compounds have maximum values at its eyeball temperature. Pirie (1951) showed that ox and rabbit capsules contain a protein, physically and chemically similar to collagen, and a carbohydrate (10% of the capsular mass) consisting mainly of glucose with a small amount of galactose. His X-ray studies showed that the characteristic meridional spacing of 2.85 Å is present together with the equatorial spacing of 4.3 Å, but the longer spacings differ from those of typical collagen. Dische and Zelmenis (1965) showed that the collagenous protein of the lens capsule contains 15-19% (12.9-16.7 residues) hydroxyproline, if it is assumed that its nitrogen content is 18% as in other kinds of collagen. This is significantly higher than the hydroxyproline content, 11.8-14.2%, of any other collagen investigated thus far in vertebrates. As the collagenous protein in the lens capsule appears to be unorganized, the possibility was considered by these authors that the high content in hydroxyproline is characteristic for this type of collagen.

THE THEORETICALLY EXPECTED BEHAVIOR

Basic Equations in Membrane Phase

The system to be studied is isothermal and composed of a porous charged membrane separating two solutions of an electrolyte of different concentrations C_2 and C_1

(moles/l) both solutions being at the same hydrostatic pressure. The unidimensional flow of ions and water molecules is assumed to occur in the direction of membrane thickness. We take the space coordinates x in this direction. The origin of the x -axis is placed on the membrane surface that is in contact with the solution of higher concentration C_2 (i.e., $C_2 > C_1$). The value of x for the other membrane surface is denoted by Δx . The bulk solutions are stirred vigorously to keep their compositions uniform in it.

It is assumed that the contribution of the volume flow is neglected, although its contribution is not always small. Further we neglect the interaction between the free ions and solvent molecule. Under these assumptions, the unidimensional flow flux $(j_k)_c$ of ionic species k ($k = +, -$) relative to the frame of reference fixed to the membrane in the steady state at any point in the membrane phase can be written in term of the thermodynamics of irreversible processes (Fitts, 1962; Kobatake, Toyoshima and Takeguchi, 1966)

$$(j_+)_c = - (L_{++})_m \frac{d\bar{\mu}_+}{dx} - (L_{+-})_m \frac{d\bar{\mu}_-}{dx} \quad (1)$$

and

$$(j_-)_c = - (L_{-+})_m \frac{d\bar{\mu}_+}{dx} - (L_{--})_m \frac{d\bar{\mu}_-}{dx}. \quad (2)$$

Here, the subscripts $+$ and $-$ denote positive and negative ion species, respectively. $d\bar{\mu}_+/dx$ and $d\bar{\mu}_-/dx$ are the gradients of positive and negative electrochemical potentials, respectively, at a point considered. $(L_{ki})_m$ ($k, i = +, -$) are the phenomenological coefficients referred to the local center of mass and obey the Onsager reciprocal relations; $(L_{ki})_m = (L_{ik})_m$ ($i \rightleftharpoons k$). The ionizable groups are fixed on the polymeric chain so that they do not appear as a component in the equations given above.

Using Onsager reciprocal relation, we can now write the electric current density I and the flow flux J_s of electrolyte component as

$$\begin{aligned} I/F &= Z_+(j_+)_c + Z_-(j_-)_c \\ &= - [Z_+(L_{++})_m + Z_-(L_{+-})_m] \frac{d\bar{\mu}_+}{dx} - [Z_+(L_{+-})_m + Z_-(L_{--})_m] \frac{d\bar{\mu}_-}{dx} \end{aligned} \quad (3)$$

and

$$\begin{aligned} \nu J_s &= (j_+)_c + (j_-)_c \\ &= - [(L_{++})_m + (L_{+-})_m] \frac{d\bar{\mu}_+}{dx} - [(L_{+-})_m + (L_{--})_m] \frac{d\bar{\mu}_-}{dx} \end{aligned} \quad (4)$$

where ν is the total number of ions in one electrolyte molecule. F is the Faraday

constant. Z_+ and Z_- are the valencies of positive and negative ions, respectively. Assuming that the electrochemical potential $\bar{\mu}_k$ can be written as the sum of a chemical potential μ_k and a electrical term $Z_k F \psi$, we have

$$\bar{\mu}_k = \mu_k + Z_k F \psi = \mu_k^\circ + RT \ln a_k + Z_k F \psi \quad (5)$$

where μ_k° is the standard chemical potential, a_k is the activity of species of k and ψ is the electric potential. As usual, defining the effective mobilities l_+^* and l_-^* of positive and negative ions in the x -direction by

$$(L_{++})_m Z_+ + (L_{+-})_m Z_- = l_+^* C_+^* Z_+$$

and

$$(L_{+-})_m Z_+ + (L_{--})_m Z_- = l_-^* C_-^* Z_- \quad (6)$$

and taking account of equation 5, we get for equation 3 and equation 4,

$$I/F = - l_+^* C_+^* Z_+ \left(RT \frac{d \ln a_+}{dx} + F Z_+ \frac{d \psi}{dx} \right) - l_-^* C_-^* Z_- \left(RT \frac{d \ln a_-}{dx} + F Z_- \frac{d \psi}{dx} \right) \quad (7)$$

and

$$\nu J_s = - l_+^* C_+^* \left(RT \frac{d \ln a_+}{dx} + F Z_+ \frac{d \psi}{dx} \right) - l_-^* C_-^* \left(RT \frac{d \ln a_-}{dx} + F Z_- \frac{d \psi}{dx} \right) - (L_{+-})_m (2 - Z_-/Z_+ - Z_+/Z_-) RT \frac{d \ln a_{\pm}}{dx}. \quad (8)$$

Here C_+^* and C_-^* are the *effective mobile* positive and negative ion concentrations, respectively. a_{\pm} is the mean activity that satisfies the relation

$$(Z_+ - Z_-) d \ln a_{\pm} = Z_+ d \ln a_- - Z_- d \ln a_+. \quad (9)$$

Flow flux J_s expressed in equation 8 is composed of three terms. The first and second terms in equation 8 represent the flux of positive and negative ions, respectively, that obey the identity

$$\text{flux} = (\text{effective mobility}) \times (\text{mobile ion concentration}) \times (\text{force}).$$

The third term in equation 8 shows the contribution to flow flux that arises from the interaction between positive and negative ions. In any case of Z_+ and Z_- , the third term in equation 8 cannot cancel out. If we assume this third term is negligibly small

compared with the first and second terms in equation 8 as a first approximation, we get

$$\nu J_s = - l_+^* C_+^* \left(RT \frac{d \ln a_+}{dx} + F Z_+ \frac{d\psi}{dx} \right) - l_-^* C_-^* \left(RT \frac{d \ln a_-}{dx} + F Z_- \frac{d\psi}{dx} \right). \quad (10)$$

Two assumptions for the ionic mobility in the membrane are made in our paper. Let ϕ be the ratio of the ionic mobility in the membrane to the ionic mobility in the bulk solution at the same concentration. First, the effective ionic mobility in the x -direction, l_k^* ($k = +, -$), equals to the quantity of the ionic mobility in the membrane divided by the tortuosity of the membrane. This assumption is equivalent to stating

$$l_k^* = l_k \phi / \epsilon \quad (11)$$

where ϵ is the tortuosity of the membrane and l_k is the ionic mobility in the bulk solution. Second, ϕ / ϵ and l_k are independent of the bulk concentration with which the membrane is in contact. Since no electric field is applied externally across the membrane in the system considered, no electric charge is transported from one face of the membrane to the other. This means $I = 0$. Putting $I = 0$ into equation 7, we get

$$F \frac{d\psi}{dx} = \frac{-RT}{l_+^* C_+^* Z_+^2 + l_-^* C_-^* Z_-^2} \left(l_+^* C_+^* Z_+ \frac{d \ln a_+}{dx} + l_-^* C_-^* Z_- \frac{d \ln a_-}{dx} \right). \quad (12)$$

To obtain the expression for the flow flux that satisfies the condition $I = 0$, we insert equation 12 into equation 10.

$$\nu J_s = \frac{-RT l_+^* l_-^* C_+^* C_-^*}{l_+^* C_+^* Z_+^2 + l_-^* C_-^* Z_-^2} \left(Z_+ \frac{d \ln a_-}{dx} - Z_- \frac{d \ln a_+}{dx} \right) \times (Z_+ - Z_-). \quad (13)$$

equation 12 and equation 13 are the basic equations for membrane potentials and the flow flux of solute in the membrane in case of $I = 0$, respectively.

Assumptions for C_+^ , C_-^* , a_+ , and a_- in the Membrane Phase*

Let's assume that the activities of positive and negative ions are equal to the effective mobile positive and negative ion concentrations in the membrane, respectively.

$$a_+ = C_+^* \quad \text{and} \quad a_- = C_-^*. \quad (14)$$

Activity coefficients in the membrane γ_+ and γ_- , are defined as

$$a_+ = \gamma_+ \bar{C}_+ \quad \text{and} \quad a_- = \gamma_- \bar{C}_- \quad (15)$$

where \bar{C}_+ and \bar{C}_- are the analytical ion concentrations of positive and negative ions, respectively, in the membrane. Furthermore, we assume that all of co-ions and of gegen-ions can move freely i.e.,

$$\gamma_+ = \gamma_- = 1 \quad \text{or} \quad a_+ = \bar{C}_+ \quad \text{and} \quad a_- = \bar{C}_-. \quad (16)$$

For convenience, we consider a membrane that is ionized positively with a charge density θ (moles/l, $\theta > 0$). (When the membrane has fixed negative charges, θ , in following equations must be replaced by $-\theta$. ($\theta > 0$)) Then, the requirement that the electric neutrality must be realized in any element of the membrane gives the relation

$$Z_+ \bar{C}_+ + Z_- \bar{C}_- + \theta = 0. \quad (17)$$

Under the assumption that the volume concentration of fixed ions in the membrane, θ , does not vary with the bulk concentration with which it is in contact, we get

$$Z_+ d\bar{C}_+ = -Z_- d\bar{C}_-. \quad (18)$$

Introducing of equations 11, 14, 16, 17, and 18 into equations 12 and 13 and rearrangement, leads to

$$\frac{F}{RT} \frac{d\psi}{dx} = \frac{-(l_+ - l_-)}{(l_+ Z_+ - l_- Z_-) \{C_+ - (l_- Z_- \theta / Z_+) / (l_+ Z_+ - l_- Z_-)\}} \frac{d\bar{C}_+}{dx} \quad (19)$$

and

$$vJ_s = -D_o \frac{\phi}{\epsilon} \left(\frac{Z_+ - Z_-}{-Z_-} \right) \cdot \left[1 - \frac{(l_+ - l_-) Z_- \theta}{(Z_+ - Z_-)(l_+ Z_+ - l_- Z_-) \{C_+ - (l_- Z_- \theta / Z_+) / (l_+ Z_+ - l_- Z_-)\}} \right] \frac{d\bar{C}_+}{dx} \quad (20)$$

where

$$D_o = RT l_+ l_- (Z_+ - Z_-) / (l_+ Z_+ - l_- Z_-). \quad (21)$$

D_o , called Haskell equation or Nernst limiting equation, is assumed to be independent of concentration. For electrolytes in free aqueous solution the diffusion coefficient expressed in equation 21 does not vary greatly with concentration up to about 1 mole. Integrating equation 19 and equation 20 over the thickness of the membrane under the condition that J_s is constant along the x -direction in stationary state, we get

$$\frac{F}{RT} (\Delta\psi) \text{ in membrane} = \frac{-(l_+ - l_-)}{(l_+ Z_+ - l_- Z_-)} \ln \left(\frac{\bar{C}_+^{\text{II}} l_+ Z_+^2 + \bar{C}_-^{\text{II}} l_- Z_-^2}{\bar{C}_+^{\text{I}} l_+ Z_+^2 + \bar{C}_-^{\text{I}} l_- Z_-^2} \right) \quad (22)$$

and

$$\nu(J_s) \text{ in membrane} = -D_0 \left(\frac{\phi}{\epsilon \Delta x} \right) \left(\frac{Z_+ - Z_-}{-Z_-} \right) \left[(\bar{C}_+^{\text{II}} - \bar{C}_+^{\text{I}}) - \frac{(l_+ - l_-)Z_- \theta}{(Z_+ - Z_-)(l_+ Z_+ - l_- Z_-)} \ln \frac{\bar{C}_+^{\text{II}} l_+ Z_+^2 + \bar{C}_+^{\text{I}} l_- Z_-^2}{\bar{C}_+^{\text{I}} l_+ Z_+^2 + \bar{C}_+^{\text{II}} l_- Z_-^2} \right] \quad (23)$$

where \bar{C}_+^{II} and \bar{C}_+^{I} are the positive ion concentrations in the membrane at $x = 0$ and $x = \Delta x$, respectively, and equation 17 has been taken into account.

Concentrations and the Membrane Potential in Phase Boundary

From the principle of continuity of matter, it is shown that

$$S_a \times (J_s)_{\text{in membrane}} = (J_s)_{\text{phase boundary}} \quad (24)$$

where S_a is the effective area available for diffusion per unit geometrical membrane area. S_a relates to the swelling properties of the membrane and it is assumed to be constant independent of bulk electrolyte solution in this paper. Total membrane potential is the sum of phase boundary potential and diffusion potential

$$\Delta\psi = (\Delta\psi)_{\text{phase boundary}} + (\Delta\psi)_{\text{in membrane}} \quad (25)$$

In the case that the solute flow is considered to be very low, we assume as a first approximation that Donnan equilibrium holds between the membrane phase and the bulk phase of solution. When the assumption of γ_+ and γ_- expressed in equation 16 are valid and the osmotic pressure difference between two phases is neglected, it follows from Donnan equilibrium

$$(C_+^{\circ}/\bar{C}_+)^{z_-} = (C_-^{\circ}/\bar{C}_-)^{z_+} \quad (26)$$

where \bar{C}_+ and \bar{C}_- are the positive and negative ion concentrations in the membrane at $x = 0$ or $x = \Delta x$. C_+° and C_-° are the positive and negative ion concentrations in bulk electrolyte solution and satisfy the following relation

$$C_+^{\circ} = -Z_- C \quad \text{and} \quad C_-^{\circ} = Z_+ C \quad \text{if} \quad Z_+ \approx -Z_-$$

or

$$C_+^{\circ} = C_-^{\circ} = C \quad \text{if} \quad Z_+ = -Z_- = Z \quad (27)$$

where C is the bulk electrolyte concentration. Concentrations at phase boundary, \bar{C}_+^{II} and \bar{C}_+^{I} , can be calculated by solving quadratic, cubic and biquadratic equations

that are derived from equations 17, 26, and 27:

$$\bar{C}_+^2 + (\theta/Z)\bar{C}_+ - C_k^2 = 0 \quad \text{if } Z_+ = -Z_- = Z \quad (28)$$

$$\bar{C}_+^3 + \theta\bar{C}_+^2 + (\theta^2/4)\bar{C}_+ - C_k^3 = 0 \quad \text{if } Z_+ = 2 \quad \text{and} \quad Z_- = -1 \quad (29)$$

and

$$\bar{C}_+^4 + \theta\bar{C}_+^3 + (\theta^2/3)\bar{C}_+^2 + (\theta^3/27)\bar{C}_+ - C_k^4 = 0 \quad \text{if } Z_+ = 3 \quad \text{and} \quad Z_- = -1 \quad (30)$$

where $k = 2$ at $x = 0$ and $k = 1$ at $x = \Delta x$.

For the phase boundary potential, using Donnan equilibrium, we obtain

$$(\Delta\psi)_{\text{phase boundary}} = \frac{RT}{FZ_+} \ln \frac{C_+^o}{\bar{C}_+} = \frac{RT}{FZ_-} \ln \frac{C_-^o}{\bar{C}_-}. \quad (31)$$

Expression for the Permeability Coefficient P_m

The permeability coefficient, P_m , the limiting permeability coefficient at the limit of sufficiently large C , $(P_m)^\infty$, and the reduced permeability, $(P_m)_r$, are defined as

$$(J_s)_{\text{phase boundary}} = P_m(C_2 - C_1) \quad (32)$$

$$(P_m)^\infty = S_a D_o \phi / \epsilon \Delta x \quad (33)$$

and

$$(P_m)_r = P_m / (P_m)^\infty \quad (34)$$

Fig. 1 shows $(P_m)_r$ for various electrolytes as a function of $\log(C_2/\theta)$, calculated from equations 23, 24, 28, 29, 30, 32, 33, and 34, when the ratio of C_2/C_1 is kept constant at 2.

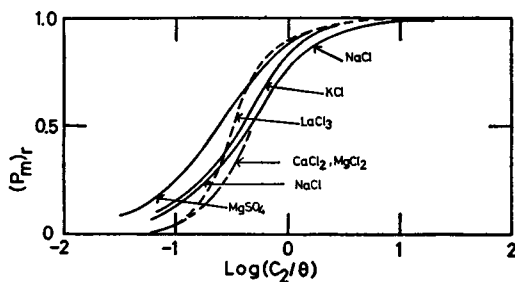


FIGURE 1 Theoretical curves of $(P_m)_r$ as a function of $\log(C_2/\theta)$ for various types of salt used at $C_2/C_1 = 2$.

MATERIALS AND METHODS

The eyeball was taken, immediately after slaughter, from the body of a gelded ox 3-4 yr old, of an improved cross between an original Japanese breed and a Brown Swiss or Devon. The bovine lens were dissected from the posterior part of the eyeball. With sharp scalpel, several shallow nicks were made in the posterior capsule by positioning its anterior surface against the lip of a Petri dish. The anterior capsule was removed from the lens by peeling forward with a smooth pulling motion. In this article only the anterior capsules were used as membranes. The typical circular anterior lens capsules 10-13 mm in diameter have thicknesses ranging from 0.03 to 0.1 mm. The thickness of the membrane, Δx , was measured by a dial lens gauge that could be read with an accuracy of ± 0.001 mm after a run of measurements. Before a run of diffusion measurement it was suspended in water and stirred for several days with many changes of water, in order to remove attached epithelial cells and lens fibres. Such treatment did not alter the appearance of the lens capsule, but would presumably remove any soluble substances present. The treatment was also employed by A. Pirie (1951) for studying the composition of ox lens capsule.

The salts used were analytical grade reagents and were subjected to no purification except drying. The water was distilled and deionized with a mixed bed ion exchange column. Carbon dioxide dissolved in it was not degassed. The pH of aqueous solutions in equilibrium with laboratory air is about 5.8.

Permeability Coefficients

Fig. 2 shows a schematic diagram of an apparatus used for measuring permeability coefficients. The apparatus consists of two equal reservoirs (45.0 ml) separated by a membrane. The concentrations of the same diffusing substances differ in its two chambers i.e., $C_2 \neq C_1$. In a series of these experiments, we set $C_2 = 2 C_1$. The diameter of the exposed portion of the membrane was 0.80 cm. Leakages around the edges of the membrane were prevented by placing silicon rubber gaskets on both sides of the membrane. The anterior capsule was set so that the solute flow could occur from the front surface of the lens capsule to the inner surface across the membrane. Bulk solutions were stirred by a pair of magnetic bars with rates of stirring sufficient to obtain maximum permeability coefficients. All measurements were made at $25 \pm 0.01^\circ\text{C}$. After introducing solutions, they were stirred for about two hours prior to each run. Fresh solutions were again introduced to commence the run. Diffusion experiments were done from the smaller to higher concentrations in a run of measurements, since it needs longer time to equilibrate the new test solution when the concentration in the membrane is higher than the test solution. Flow flux was determined by measuring the change of the concentration in bulk solution with time. Its change was indicated by the change of the conductivity in solution 2. The conductivity was measured by a universal

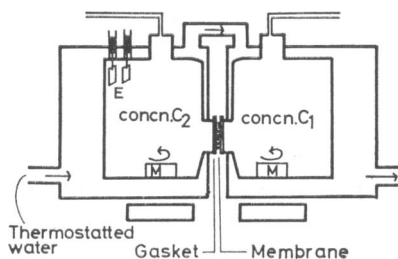


FIGURE 2 Schematic diagram of the glass used for the measurement of membrane permeabilities. *E*, platinized-platinum electrodes. *M*, magnetic stirrers.

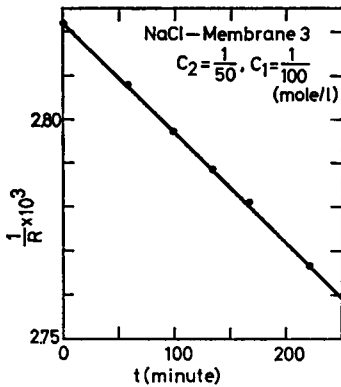


FIGURE 3 Plots of inverse resistance of the solution 2, $1/R$, against time, t , for a system of lens capsule 3 and NaCl; $C_2 = 1/50$ and $C_1 = 1/100$ (mole/l).

bridge (Yokogawa-Hewlett-Packard Co. 4255 A Type). Because of the large volume of the chamber of the small area of the exposed portion of the membrane, and of the small difference in the two bulk concentrations across the membrane, very small concentration changes could be detected and its changes were limited to less than 2% during every experiment. Thus, concentration gradients across the membrane could be considered constant during the runs within the experimental error. By way of an example, Fig. 3 shows the inverse resistance of the solution 2 vs. time plot for a system of NaCl and lens capsule 3. Because no curvature could be discerned in any of the plots with this procedure, the flow of solute per second across unit area of the membrane (J_s)_{phase boundary} turns out to be constant during the run. Permeability coefficients P_m defined in equation 26 were calculated from (J_s)_{phase boundary}, C_2 and C_1 .

Membrane Potential

The membrane potential arising between two solutions of an electrolyte of different concentrations was conducted by saturated KCl bridges to calomel electrodes and was measured by a vibrating reed electrometer (Toshiba Co., Tokyo, UFE-26301 Type). The caps of the salt bridges were carefully ground so that the test solutions might not be contaminated due to the leakage of KCl. The bulk solutions were vigorously stirred to obtain the maximum membrane potential. After conditioning the membrane with test solutions, fresh test solutions were again introduced to commence measurements as in case of permeability coefficients. All measurements were made in duplicate or triplicate at 25°C.

RESULTS AND DISCUSSION

Fig. 4 shows the membrane potential of a lens capsule that is dissected from the lens and immediately set in the cell without washing it as a function of time measured from the beginning of the conditioning of the membrane with KCl solutions; $C_2 = 0.001$ and $C_1 = 0.0005$ (moles/l). Plus sign of membrane potentials indicates that the value of electrical potential in solution C_2 is positively higher than the value of electrical potential in solution C_1 . Solutions were not stirred for the conditioning in this case, but solutions were vigorously stirred and fresh test solutions were introduced in the cell as in the other cases whenever membrane potentials were measured. From Fig. 4 it is estimated that about 40 min were necessary for all of ions

that should exist in the membrane after sufficient times to penetrate into the membrane from the bulk solution, that the initial lens capsule with the epithelium had the perfect permselectivity at the concentration examined so that the membrane potential becomes the ideal value i.e., $(RT/F) \ln (C_2/C_1) = 17.5 \text{ mv}$ at 25°C , that the isolation of the epithelium from the lens capsule decreased the membrane poten-

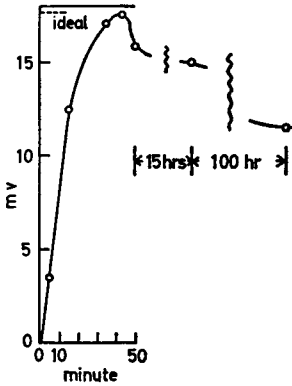


FIGURE 4 Membrane potentials of a lens capsule that is set in the cell immediately after the dissecting from the lens without washing off the epithelium for the system of KCl; $C_2 = 0.001$ and $C_1 = 0.0005$. Time scale is taken from the time of the beginning of the conditioning of the membrane with the KCl solutions.

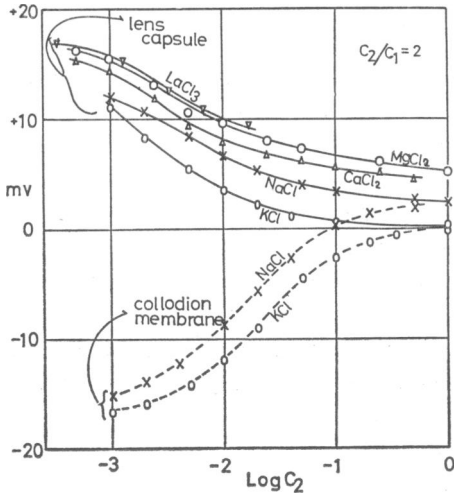


FIGURE 5 Solid lines show typical membrane potentials for systems of NaCl, KCl, CaCl₂, MgCl₂, and LaCl₃ and lens capsules at $C_2/C_1 = 2$. Broken lines show membrane potentials for systems of NaCl and KCl and collodion membranes that have fixed negative charges at $C_2/C_1 = 2$ taken from Fig. 5 in the paper by Kobatake et al. (1965).

tial, and that the lens capsule without the epithelium had lower permselectivity than the initial lens capsule with the epithelium. In this system, the final membrane potential of the lens capsule after the isolation of the epithelium was 11.5 mv.

The results on membrane potentials across bovine lens capsules in electrolyte solutions used (equilibrated with air and kept at pH 5.8) demonstrate the presence of fixed positive charges within the capsules. In Fig. 5, curves expressed by solid lines show experimental results of membrane potentials for systems of KCl, NaCl,

MgCl₂, CaCl₂ and LaCl₃ and lens capsules at $C_2/C_1 = 2$, and curves expressed by broken lines show typical data taken from Fig. 5 in the paper by Kobatake, Takeguchi, Toyoshima, and Fujita (1965) for systems of KCl and NaCl and collodion membranes that have fixed negative charges at $C_2/C_1 = 2$. It is shown from equations 22, 25, and 31 that, for MgCl₂, CaCl₂, and LaCl₃ as well as NaCl and KCl, the values of membrane potentials of positively fixed charged membranes have positive values that tend to $(RT/F) \ln (C_2/C_1)$ and values of membrane potentials of negatively fixed charged membranes have negative values that tend to $(-RT/F) \ln (C_2/C_1)$ when concentrations of bulk electrolyte solutions are sufficiently dilute. From the results of membrane potentials, bovine lens capsules in the electrolyte solutions equilibrated with laboratory air (pH of its solutions is about 5.8) are proved to have fixed positive charges. This observation on the sign of fixed charges is in contradiction to the one of Friedenwald obtained from the experiment of electroosmosis (1930 *a*, also in our introduction). The experiment of the water flow across the membrane must be done carefully, with strict measures to keep the temperature of the test solutions constant to within $\pm 0.01^\circ\text{C}$ (For example, Toyoshima, Kobatake, and Fujita (1967 *a*). The experimental method of electroosmosis used by Friedenwald, did not satisfy the conditions required for strict temperature control of the solutions. Therefore, the possibility is considered by the authors that Friedenwald's result on the sign of fixed charges was not accurate.

The stability of physico-chemical properties, e.g., membrane potential, permeability coefficients and mechanical permeabilities of water, of lens capsules without epithelia in water or in salt solutions used was good for a few weeks. The data on the stability of lens capsules in our study were entirely in accord with those of Friedenwald (1930 *a*).

Experimental values of $(P_m)_r$ of NaCl for membranes 2, 4, and 7, (indicated ●, ○, and ⊖, respectively, in Fig. 6) fit the dotted theoretical curves with $\theta = 1.32 \times 10^{-3}$, 2.00×10^{-3} , and 2.64×10^{-3} from left to right. Experimental values of $(P_m)_r$ of KCl for membrane 1 (indicated ● in Fig. 7) and those of $(P_m)_r$ of MgSO₄ for membrane 7 (indicated ○ in Fig. 8) fit well the dotted theoretical curves with $\theta = 1.82 \times 10^{-3}$ and 5.04×10^{-3} , respectively. In Fig. 9, experimental data of

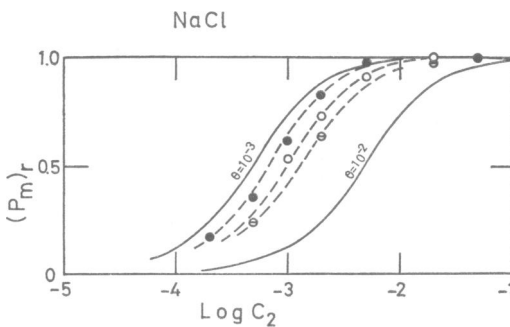


FIGURE 6 Plots of $(P_m)_r$ against $\log C_2$ for systems of membrane 2, 4, and 7 indicated ●, ○, and ⊖, respectively, with NaCl at $C_2/C_1 = 2$. Solid and dotted lines represent theoretical curves with various values of θ from left to right; $\theta = 10^{-3}$, 1.32×10^{-3} , 2.00×10^{-3} , 2.64×10^{-3} , and 10^{-2} .

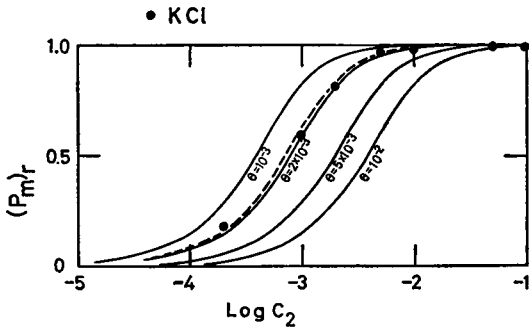


FIGURE 7 Plots of $(P_m)_r$ against $\log C_2$ for a system of membrane 1 and KCl at $C_2/C_1 = 2$ indicated \bullet . Solid and dotted lines represent theoretical curves with various values of θ from left to right; $\theta = 10^{-3}$, 1.82×10^{-3} , 2×10^{-3} , 5×10^{-3} , and 10^{-2} .

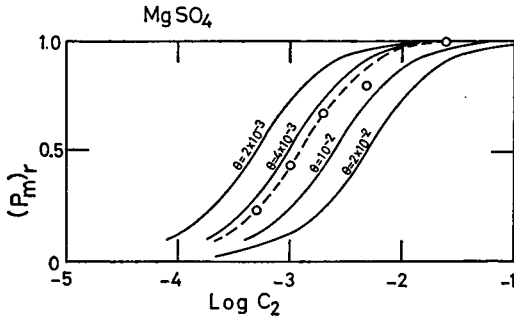


FIGURE 8 Plots of $(P_m)_r$ against $\log C_2$ for a system of membrane 7 and $MgSO_4$ at $C_2/C_1 = 2$ indicated \circ . Solid and dotted lines represent theoretical curves with various values of θ from left to right; $\theta = 2 \times 10^{-3}$, 4×10^{-3} , 5.04×10^{-3} , 10^{-2} , and 2×10^{-2} .

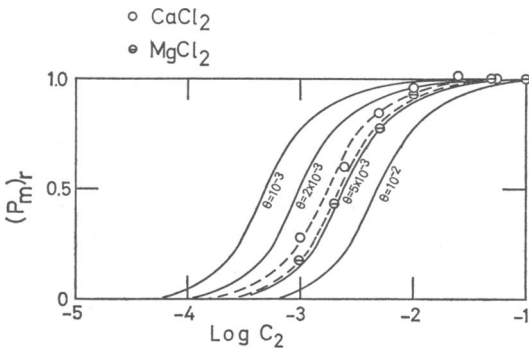


FIGURE 9 Plots of $(P_m)_r$ against $\log C_2$ for two systems of membrane 3 with $CaCl_2$ and of membrane 5 with $MgCl_2$ at $C_2/C_1 = 2$. \circ $CaCl_2$ and \ominus $MgCl_2$. Solid and dotted lines represent theoretical curves with various values of θ from left to right; $\theta = 10^{-3}$, 2×10^{-3} , 3.64×10^{-3} , 4.47×10^{-3} , 5×10^{-3} , and 10^{-2} .

$(P_m)_r$ of $MgCl_2$ for membrane 5 (indicated \ominus) and of $CaCl_2$ for membrane 3 (indicated \circ) fit well the dotted theoretical curves with $\theta = 4.47 \times 10^{-3}$ and 3.64×10^{-3} , respectively. In Fig. 10, experimental data of $(P_m)_r$ of $LaCl_3$ for membrane 4 (indicated \bullet) fit the dotted theoretical curve with $\theta = 6.31 \times 10^{-3}$. There is a general fair agreement between calculated and experimental fluxes.

In Table I, values of diffusion coefficients in bulk solutions, D_o (cm^2/sec) used for calculations, of fixed charge density, θ (mole/l), of limiting permeability coefficients, $(P_m)^\infty$ (cm/sec), of membrane thickness, Δx (cm), and of $S_a\phi/\epsilon$ are listed for

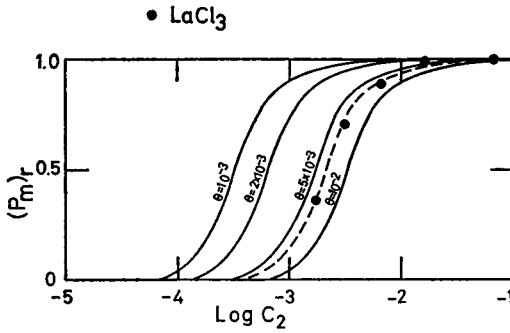


FIGURE 10 Plots of $(P_m)_r$ against $\log C_2$ for a system of membrane 4 and LaCl_3 at $C_2/C_1 = 2$ indicated \bullet . Solid and dotted lines represent theoretical curves with various values of θ ; $\theta = 10^{-2}$, 2×10^{-3} , 5×10^{-3} , 6.31×10^{-3} , and 10^{-2} .

TABLE I
VALUES OF PARAMETERS FOR SYSTEMS OF
LENS CAPSULES AND ELECTROLYTES

Chemical	Membrane No.	$D_o \times 10^5$	$\theta \times 10^3$	$(P_m)^\infty \times 10^4$	$\Delta x \times 10^3$	$S_a \phi / \epsilon$
		cm ² /sec	mole/l	cm/sec	cm	
KCl	1	1.90	1.8 ₂	4.9 ₉	6.6 ₁	0.17 ₄
KCl	7	1.90	2.3 ₀	4.7 ₄	12. ₅	0.39 ₅
NaCl	2	1.56	1.3 ₂	4.8 ₉	7.3 ₈	0.23 ₁
NaCl	3	1.56	1.2 ₁	4.7 ₇	10. ₀	0.30 ₇
NaCl	4	1.56	2.0 ₀	5.2 ₃	7.3 ₂	0.23 ₇
NaCl	7	1.56	2.6 ₄	3.5 ₃	12. ₅	0.28 ₁
MgSO ₄	7	0.75	5.0 ₄	3.2 ₅	12. ₅	0.54 ₁
MgCl ₂	5	1.14	4.4 ₇	4.9 ₄	9.3 ₃	0.40 ₄
CaCl ₂	3	1.20	3.6 ₄	4.1 ₄	10. ₀	0.34 ₇
LaCl ₃	4	1.09	6.3 ₁	2.2 ₇	7.3 ₂	0.15 ₂

systems of various salts and lens capsules. Here, $(P_m)^\infty$ is equal to the limiting value of P_m at the limit of sufficiently high concentrations of bulk solutions. θ is calculated by superposing the experimental $(P_m)_r \sim \log C_2$ curve on the theoretical $(P_m)_r \sim \log (C_2/\theta)$ curve in Fig. 1. Δx is measured by the method written in *Materials and Methods* of this paper. $S_a \phi / \epsilon$ is equal to $(P_m)^\infty \Delta x / D_o$. The magnitude of θ for lens capsules increases roughly in the order; KCl, NaCl < CaCl₂, MgCl₂ < MgSO₄ < LaCl₃, where LaCl₃ having the greatest θ . A rough estimate of the order of $S_a \phi / \epsilon$ is LaCl₃ < NaCl, KCl < CaCl₂, MgCl₂ < MgSO₄, where LaCl₃ having the least value of $S_a \phi / \epsilon$. If we assume the change of ϕ / ϵ on the kinds of salt is relatively small, above order of $S_a \phi / \epsilon$ shows roughly the order of swelling properties in the salt solutions. It is a future object of study why the magnitude of θ and $S_a \phi / \epsilon$ should change from electrolyte to electrolyte. In our treatment, S_a has been assumed to be independent of bulk concentrations. Our preliminary experiments of water transport showed that mechanical permeabilities of water across the lens capsule are independent of bulk electrolyte concentrations in the range of con-

concentrations of $2 \times 10^{-3} \sim 1$ mole/l and that values of mechanical permeability for concentrations $< 10^{-3}$ molar are slightly greater than those for concentrations $> 2 \times 10^{-3}$ molar. Thus S_a is constant over all but the lowest concentrations, and depends on the kind of salt used.

It is noted that the dependences of $(P_m)_r$ on concentrations are different according to the types of salt and that they are expressed mostly by the first term in brackets of equation 23 in all types of salt. The second term in brackets of equation 23 that exist in case of $l_+ \approx l_-$ represents the smaller part of dependences of permeability coefficients on concentrations. For example, the magnitude of the second term in case of NaCl is shown graphically to be equal to the difference between the two curves of KCl and NaCl at same C_2/θ in Fig. 1 since the second term is null in case of KCl ($l_K = l_{Cl}$). And the difference turns out to be relatively small in comparison with the first term. If we neglect the second term in case of $l_+ \approx l_-$ and roughly fit the experimental data on the theoretical curve of $(P_m)_r$ calculated only from the first term, we obtain apparent values of θ equal to 1.25 times the real value of θ in the case of NaCl, $MgSO_4$, or $LaCl_3$ and 1.64 times the real value in the case of $MgCl_2$ or $CaCl_2$.

Dependences of permeability coefficients on concentration for an *uni-univalent* electrolyte—a collodion membrane system, the ratio of bulk concentrations C_2/C_1 being kept constant, were studied by Manegold and Viets (1931) and recently by Toyoshima, Kobatake, and Fujita (1967 *b*). In their experiments and our experiments including multivalent electrolyte systems, P_m decreases to zero as the concentration of bulk solutions decreases and P_m increases to a limiting value, $(P_m)^\infty$, as the concentration of bulk solution increases, despite the use of various kinds of salt. Both in theory and in practise, the systems studied give S-shaped $P_m \sim \log C_2$ or $(P_m)_r \sim \log C_2$ relation, C_2/C_1 being kept constant. Thus, the systems studied do not obey Fick's law that requires P_m and $(P_m)_r$ to be independent of the concentration of bulk solutions and always to be equal to constant and 1, respectively. It is easily shown from Fig. 1 that if the fixed charge density of the membrane θ is very small, for example $\theta = 10^{-5}$, $(P_m)_r$ is apparently constant in the range of bulk concentrations higher than $C_2 = 10^{-4}$, and that Fick's law for ionic species of solute is perfectly materialized in the nonionized membrane system when the water flow across the membrane is zero.

The assumption for activity coefficients used in this paper i.e. $\gamma_+ = \gamma_- = 1$, seems to be oversimplified. In fact, it is well known that activity coefficients depend on concentrations of bulk solutions (for example, Hills et al., 1961). In their permeability study, Mackie and Meares (1955 *a* and *b*) used the experimental equations for activity coefficients that depend on concentrations and depend greatly on types of salt. Toyoshima, Kobatake, and Fujita (1967 *b*) used the equation for activity coefficients of uni-univalent electrolyte that was derived from the additivity rule commonly used in the field of polyelectrolyte solution and that depends on con-

centrations. Their final results that represent dependencies of permeability coefficients on concentrations for uni-univalent electrolytes nearly accord with our results. The additivity rule is known to hold if only one kind of univalent gegen ion exist and the interaction between fixed charges and gegen ions is purely electrostatic except for the effect of the Bjerrum bond. Yuasa, Kobatake, and Fujita (1968) showed experimentally that the additivity rule does not hold exactly for the system of the collodion membrane and an uni-univalent electrolyte. At this stage, it is expected that the additivity rule would not materialize for the system of multivalent electrolyte and the lens capsule membrane. Therefore, the agreement between results obtained and theory may be more than happenstance when we apply ideality of ionic behavior, without using concentration-dependent activity coefficients (for example, additivity rule), to bio-membranes with Donnan equilibrium and electroneutrality.

One of the authors (N. T.) wishes to express his sincere thanks to Professor Y. Kobatake of Osaka University for his encouragement.

Received for publication 15 August 1968 and in revised form 9 December 1968.

REFERENCES

- DISCHE, Z., and G. ZELMENIS. 1965. *Invest. Opth.* 4:174.
- FITTS, D. D. 1962. In *Nonequilibrium Thermodynamics*. McGraw-Hill Book Co., Inc., New York. p. 45.
- FRIEDENWALD, J. S. 1930 a. *Arch. Opth.* 3:182.
- FRIEDENWALD, J. S. 1930 b. *Arch. Opth.* 4:350.
- HESS, C. 1911. In *Graefe-Saemisch Handbuch der Gesamten Augenheilkunde*. Verlag von Wilhelm Engelmann, Leipzig, East Germany. Aulf. 3, pp. 35-49.
- HILLS, G. J., P. W. M. JACOBS, and N. LAKSHMINARAYANAIAH. 1961. *Proc. Roy. Soc. A* 262:257.
- KOBATAKE, Y., N. TAKEGUCHI, Y. TOYOSHIMA, and H. FUJITA. 1965. *J. Phys. Chem.* 69:3981.
- KOBATAKE, Y., Y. TOYOSHIMA, and N. TAKEGUCHI. 1966. *J. Phys. Chem.* 70:1187.
- MACKIE, J. S., and P. MEARES. 1955 a. *Proc. Roy. Soc. A* 232:498.
- MACKIE, J. S., and P. MEARES. 1955 b. *Proc. Roy. Soc. A* 232:510.
- MANEGOLD, E., and K. VIETS. 1931. *Kolloid Z.* 56:7.
- MEYER, K. H., and J. F. SIEVERS. 1936. *Helv. Chim. Acta.* 19:649.
- MICHALIS, L. 1928. In *Colloid Symp. Monogr.* H. B. Weiser, editor. Chemical Catalog Co, New York. 5:135.
- NAKAGAKI, M., N. KOGA, and S. IWATA. 1963 a. *Nippon Yakugaku Zashi.* 83:275.
- NAKAGAKI, M., N. KOGA, and S. IWATA. 1963 b. *Nippon Yakugaku Zashi.* 83:279.
- NAKAGAKI, M., N. KOGA, and S. IWATA. 1963 c. *Nippon Yakugaku Zashi.* 83:365.
- PIRIE, A. 1951. *Biochem. J.* 48:368.
- TEORELL, T. 1937. *Trans. Faraday Soc.* 33:1053.
- TOYOSHIMA, Y., Y. KOBATAKE, and H. FUJITA. 1967 a. *Trans. Faraday Soc.* 63:2828.
- TOYOSHIMA, Y., Y. KOBATAKE, and H. FUJITA. 1967 b. *Trans. Faraday Soc.* 63:2814.
- YUASA, M., Y. KOBATAKE, and H. FUJITA. 1968. *J. Phys. Chem.* 72:2871.