CERTAIN CONCEPTS OF BIOLOGICAL MEMBRANE TRANSPORT

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Received on September 6, 1974

INTRODUCTION

The subject of ion transport across membranes is one of considerable current and future interest in various disciplines [1]. A number of vital processes in biology are considered to be functions of cell membranes. In complex biological organisms, the membrane acts as a physical boundary for each cell to maintain internal conditions, appropriate for carrying out its role as an individual unit for life processes. The current popular views about neuro-physiological basis of drug actions and effect of anaesthetics are based on the premise that ion transport across biological membranes are seriously affected by these agents.

The mechanism underlying the maintenance of resting potential and production of action potential is interpreted on the basis of ' ionic theory' which is widely accepted in the field of cell membrane physiology [2]. The basic concept of these theories is that the cytoplasm contains a high concentration of anions together with various kinds of amino acids that cannot permeate the cell membrane. On the other hand, the resting membrane is readily permeable to potassium and chloride ions and sparingly permeable to sodium ions. It is assumed that the movement of potassium and chloride ions across the resting membrane is caused by the electrochemical forces resulting from concentration differences of these ions in the extra and intracellular fluids and the difference in electrical potential. The ' ionic theory' proposes that the outward movement of sodium ions through cell membrane is caused by a sodium pump, pumping sodium ions from intracellular fluid as fast as they diffuse into the cytoplasm. Such a pump is assumed to be operated by the energy provided by chemical reactions associated with cellular metabolism. The concept of the pump and its ability to maintain the intracellular sodium concentration at about ten per cent of extracellular concentration is thus a phenomenological definition of ignorance about the mysterious action of cell membranes.

It would seem incredible that biological membranes could spontaneously and selectively transport molecules and ions to produce and maintain large concentration differences. The free energy change accompanying the transport of material against concentration gradient is positive. Since such processes are observed in nature in the field of biology, and second law of thermodynamics requires that for all spontaneously occurring processes, such free energy change should be negative, the paradox of active transport was conceived. The evident explanation, without abandonment of second law of thermodynamics, is that one should consider the total energetics of all spontaneous processes occurring in the system.

Another popular concept prevalent in physiology is that electrical excitability of nerve cells and muscle cells is due to time-dependent ion permeabilities across cell membranes, allowing the electrical potential difference to shift back and forth between two stationary states. To understand changes in membrane potentials and permeability properties of membranes to different ionic species quantitatively, it is important to study these aspects in as rigorous a manner as possible, taking into account knowledge available from physical chemistry and physics.

It would be most desirable to combine all available electro-chemical, thermodynamics, irreversible processes and molecular theory informations into a suitable complete picture of active biological membrane system. In principle, such a construction should be feasible, since biological membrane system consists of molecules, ions, and their interaction through intermolecular forces in non-equilibrium states [3].

COMMENTS ON ION TRANSPORT

Theoretically one approaches the problem of transport of ions across a diffusion barrier by resorting to the general diffusion theory. One has the choice of analysing the diffusion process by means of differential equations, in which one ignores the detailed kinetic theoretical aspects of the problem. Such approaches will be valid, when the ion size is very small, in comparison with the thickness of membranes. The other choice one has is to consider that diffusion proceeds by jumps of the molecules from one site to another. This basic random walk approach leads to difference equations.

It is evident that membranes must have a structure somewhat intermediate between solid and liquid states. Certain amount of rigidity is expected and required for the cell walls to act as a diffusion barrier. Certain amount of fluidity is also expected and required to fulfil transport functions. Pliability is necessary for the membrane to respond to constraints within reasonable time.

Since the early days of Faraday, the transport properties of electrolyte solutions have provided a challenging field for experimental and theoretical research. A major portion of existing theoretical attempts on ion transport across membranes is based on the Nernst-Planck equation. The molecular approach towards ion transport across membranes is based on the quasi-phenomenological relations of statistical theory of transport. Electrical interactions obey the coulomb potential. It is well known that the long range nature of coulombic forces, compared to non-coulombic interactions between molecules in condensed systems, give rise to many interesting effects in equilibrium and transport properties of systems of charged species. It is this aspect which gave rise to limiting square root concentration dependence term in observed properties of systems of electrolytes. The long range coulombic interactions cause non-randomness in the distribution of ions even in extremely dilute solutions, such that any particular ion is on the average surrounded by more unlikely charged ions. Thus, the position and velocity of any specified ion is intimately correlated with the position and velocities of all other ions.

Since the dielectric constant of water is about 78, and the dielectric constants of most lipids are of the order of 2, at normal temperatures, this intimate coupling between position and velocities of ions of a specified species, with the position and velocities of other kinds of ions should be stronger. More co-operative behavior between charged species should occur in a medium of low dielectric constant. Even the equations of electrodiffusion, based on Nernst-Planck equation, ignores coupling between fluxes of different species and the variation of mobility with position variable in an inhomogeneous diffusion barrier such as biological membranes as demanded by the properties of charged species and requirements of thermodynamics of irreversible processes.

The general conclusion that one arrives at about the contribution of electrostatic forces to thermodynamic and transport properties in condensed systems is that the gradient of the thermodynamic potential which represents the driving force on ions is less than what it would be in an ideal solution of the same concentration gradient. The velocity of migration for a given driving force is also influenced by the electrostatic and hydrodynamic interactions between species in the system. In consideration of transport properties, so long as one can confine to stationary states, when fluxes can be regarded as proportional to forces, the proportionality constants are the defined phenomenological coefficients. In the spirit of linear phenomenological theory one has a system of linear equations [4],

$$-J_\alpha = \sum_{\sigma}^{n} \Omega_{\alpha\sigma} \nabla\mu_\sigma; \quad \alpha = 1, 2 \ldots n \tag{1}$$

which relate the velocities of different mobile species to gradients of chemical potentials. J_α denotes the flux of species α, expressed in moles per unit area per unit time.

The matrix Ω is symmetrical. The chemical potentials for ions of kind σ is defined by the relation,

$$\mu_\sigma = \mu_\sigma^\circ (T, P, C_1, C_2 \ldots C_n) + Z_\sigma e\psi \tag{2}$$

when there are n kinds of species in the system. $\mu_\sigma^0 (T, P, C_1 \ldots C_n)$ is the non-electrical part of thermodynamic potential. $Z_\sigma e$ is the electrical charge of an ion of kind σ and ψ is the electrostatic potential. In ionic solutions, the normal structure of so-called ion atmosphere is in equilibrium between electrical forces and thermal agitation of the molecules. These agencies are continually operating and will tend to restore equilibrium configuration after every disturbance.

In a membrane system at equilibrium, the electrical potential profile and concentration profile are in balance with each other, such that the flux caused by one force, say concentration gradient, is nullified by the flux caused by other force, electrical potential gradient. This is the basis of Nernst equilibrium potential relation,

$$0 = d \ln C_\alpha/dx + (Z_\alpha e/kT)(d\psi/dx) \tag{3}$$

which leads to

$$\psi(x) - \psi(0) = -(kT/Z_\alpha e) \ln \{C_\alpha(x)/C_\alpha(0)\} \tag{4}$$

where k is the Boltzmann constant, T is the temperature, $\psi(x)$ and $C_\alpha(x)$ are respectively the electrostatic potential and concentration of ionic species α at location x, in the inhomogeneous diffusion barrier. When the two terms in the right hand side of equation (3) do not equal under the influence of an external constraint, the system reaches a stationary state, resulting in a time independent flux.

The molecular equivalent of Nernst-Planck equation is [5]

$$-J_\alpha = (kT/\zeta_\alpha)(dC_\alpha/dx) + (Z_\alpha e C_\alpha/\zeta_\alpha)(d\psi/dx) \tag{5}$$

where ζ_α is the frictional coefficient of ion of kind α. Equation (5) is derivable from equation (1), by neglect of all coupling terms, $\Omega_{\alpha\beta}$ with $\alpha \neq \beta$, identification of $\Omega_{\alpha\alpha}$ with (C_α/ζ_α), and use of the limiting expression for chemical potential μ_α of ion α

$$\mu_\alpha(x) = \mu_\alpha{}^0(T, P) + kT \ln C_\alpha(x) + Z_\alpha e\psi(x) \tag{6}$$

The frictional coefficient ζ_α and the diffusion coefficient D_α of a molecule of kind α are related to each other by Einstein's relation,

$$\zeta_\alpha = (kT/D_\alpha) \tag{7}$$

The equation of continuity for species σ is given by the expression,

$$(\partial C_\sigma/\partial t) = \nabla \cdot J_\sigma + J_R \tag{8}$$

where J_σ is the matter flux. The quantity $(\partial C_\sigma/\partial t)$ represents time variation of concentration of species σ, which should vanish under conditions of stationary state, since there can be no explicit dependance on time t. The term J_R of equation (8) denotes the production or consumption of species σ, when σ participates in a chemical reaction occurring in the system. By convention, when species α is a reactant it is assumed consumed. In inhomogeneous diffusion barrier, the rate of the reaction is a function of position variable and is called the reaction rate profile. If α participates in a reaction of the type

$$\alpha + \beta \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \gamma \tag{9}$$

where k_1 and k_2 are rate constants, the rate of reaction is given by the expression,

$$J_R(x) = k_1 C_\alpha(x) C_\beta(x) - k_2 C_\gamma(x) \tag{10}$$

When $J_R = 0$ the reaction is said to be in equilibrium. In equation (9) it is expressed that α participates in a chemical reaction of association-dissociation type. One can propose some other chemical reaction in which α participates, for example, an enzyme catalyzed reaction. One has that for all reaction mechanisms that one can visualize, in which α participates under stationary state conditions,

$$\nabla \cdot J_\alpha + J_R(x) = 0. \tag{11}$$

The important point is that the flux of species α is a function of position variable x, in the membrane phase, when it participates in a chemical reaction which is not at equilirium at all locations in the membrane. One also has the conclusion that if a specified species σ does not participate in a chemical reaction occurring in the membrane phase, its flux is a constant, independent of position variable. However, the magnitude of the flux of the specified species σ, when it does not participate in a chemical reaction occurring in the membrane phase will be different than the flux that would be observed in the absence of such chemical reactions.

This change in magnitudes of flux of a species for a given boundary values of concentration difference arises due to altered resistance that the membrane diffusion barrier profers for transport of molecules of this specified species. This important point is made clear from the following expression from molecular theory for the resistance that a molecule of kind σ suffers during transport when it is located at x,

$$\zeta_\sigma (x) = \sum_\beta C_\beta (x) \, \zeta_{\sigma\beta} (x) \tag{12}$$

The parameters $\zeta_{\sigma\beta} (x)$ represents the contribution from molecules of kind β present in the system, through intermolecular forces, to the resistance suffered by a molecule of kind σ present at x[6]. Evidently this contribution is proportional to the number of molecules of kind β present. The summation is to be carried out over all species present in the system. Equation (12) represents the variation of the positional dependence of frictional coefficient of σ. If a reaction of the type presented in equation (9) occurs in the system and the reaction is not at equilibrium, then the concentrations of the three species α, β and γ vary with position. This variation is reflected in the variation of the frictional coefficient resistance for the transport of molecules of kind σ, which is specified not to participate in the reaction. Since the magnitude of flux of σ is determined by the forces responsible for its transport and the resistance, its flux is altered in the presence of chemical reaction. Whether this resistance represented by frictional coefficient is increased or decreased at a specified location x, depends on the concentrations of the species α, β and γ at this location and the strength of interaction parameters $\zeta_{\alpha\sigma}$, $\zeta_{\beta\sigma}$ and $\zeta_{\gamma\sigma}$. These parameters called partial frictional coefficients can be either positive or negative since from molecular theory, they are dependent on the pair potential energy of interaction weighted by probability functions. Thus, magnitude of flux and permeability coefficient P_σ for the species σ gets altered. Thus, one can understand that the diffusion barrier, which has been permeable for the species σ, can become more permeable or less permeable and even impermeable to the same species σ, in the presence of chemical reactions. Since equation of continuity demands that flux of the species σ should be independent of position, the collective behaviour of concentration gradients and electrical potential gradients should be such as to insure the constancy of fluxes of non-participating species.

The quantitative computation of these effects is feasible from our current knowledge of molecular theory. However, one is beset with the difficulty that experimental information about partial frictional coefficients are not available for quantitative comparison. One requires knowledge about concentration profiles of all species present in the system in addition to the type of chemcial reaction occurring in the system.

PERMEABILITY COEFFICIENT

The phenomenological definition of permeability coefficient is given by the relation,

$$(dq_\sigma/dt) = P_\sigma A\,(C_{\sigma 1} - C_{\sigma 2}) \tag{13}$$

where $C_{\sigma 1}$ and $C_{\sigma 2}$ are the stationary state concentrations of the substance σ in solutions on either side of the membrane phase. (dq_σ/dt) represents the rate at which substance σ crosses the membrane barrier of area A. The dimensions of P_σ is, thus, cm. sec^{-1}. The permeability coefficient P_σ is characteristic of the substance and the membrane in question. Since concentration of substance σ in the aqueous phase $C_{\sigma 1}$ need not equal the concentration in membrane phase at $x = 0$, $C_\sigma(0)$, one introduces the equilibrium partition coefficient K, for distribution of solute σ between lipid and water phases. Thus, permeability coefficients of two substances in the same membrane expresses the relative ease with which they are transported across the membrane.

EQUATIONS OF ELECTRODIFFUSION

For ionic species in multicomponent systems, the expression for chemical potential (*cf.* eq. 2) is written as [7]

$$\mu_\alpha(x) = \mu_\alpha{}^0(T, P) + \mu_\alpha(C_\alpha, C_\beta \ldots C_n) + Z_\alpha e\psi \tag{14}$$

where $\mu_\alpha{}^0(T, P)$ is the chemical potential (per molecule) in some defined standard state, and $\mu_\alpha(C, C \ldots C_n)$ is the composition dependent part of chemical potential. The precise evaluation of this composition dependent part of chemical potential is the essential problem of thermodynamics of mixtures. The variation of chemical potential of a specified component in a mixture due to variation of concentration of another species is an unknown function. The total electrostatic potential $\psi(x)$ of equation (4) should satisfy the Poisson equation, and includes contribution to potential at x due to presence of all charged species in the system and any externally applied electric potential. In the case of ideal solutions, the composition dependent part of chemical potential is by definition proportional to logarithm of its mole fraction. The logarithmic dependence on mole fraction arises from the relation between entropy and probability. Only for a very dilute solution, one can approximate this expression for ideal solution as

$$\mu_i = \mu_i^0 + kT \ln C_i \tag{15}$$

where C_i represents concentration of uncharged species i. When one assumes the validity of equation (15) and from (14) the Nernst–Planck equation is obtained. An improved correction due to Kirkwood [8] for the composition dependent part of chemical potential can be expressed in the form,

$$\mu_\alpha(C_\alpha, C_\beta \ldots C_n) = kT \ln C_\alpha + \sum_\beta C_\beta H_{\alpha\beta}^* \tag{16}$$

where $H_{\alpha\beta}^*$ are integrals over the potential energy of interactions between a molecule of kind α and a molecule of kind β. Substitution of equations (16) and (2) in (1) yields the fundamental relation for flux of an ionic species as a function of concentration and electrical potential profiles in an inhomogeneous barrier. The resulting Nernst–Planck type equation includes coupling between fluxes of different species.

Under conditions of stationary states, the sum of forces over all species should balance, which is expressed by the Gibbs–Duhem relation,

$$\sum_\sigma C_\sigma(x)\,\mu_\sigma'(x) = 0 \tag{17}$$

The gradient of electrochemical potential of a charged species α in an inhomogeneous system is given by the expression,

$$(d\mu_\alpha/dx) = kT(d \ln C_\alpha/dx) + Z_\alpha e\psi'(x) + \sum_\beta C_\beta' H_{\alpha\beta}^* \tag{18}$$

An expression for the same quantity obtained from molecular theory is

$$(d\mu_\alpha/dx) = -(J_\alpha \zeta_\alpha/C_\alpha) + \sum_\beta J_\beta \zeta_{\alpha\beta} \tag{19}$$

One may verify that equation (19) satisfies equation (17) when summed over all species present in the system. From equations (18) and (19), one identifies that [9]

$$\begin{aligned} -J_\alpha &= (kT/\zeta_\alpha)\,C_\alpha' + (Z_\alpha e C_\alpha/\zeta_\alpha)\,\psi' \\ J_\alpha \zeta_{\alpha\alpha} &= H_{\alpha\alpha}^* C_\alpha' \\ J_\beta \zeta_{\alpha\beta} &= H_{\alpha\beta}^* C_\beta' \end{aligned} \tag{20}$$

In equation (20), the plausible assumption that the molecular integrals $H_{\alpha\sigma}^*$ and partial frictional coefficients $\zeta_{\alpha\sigma}$ may be regarded as position independent in the inhomogeneous diffusion barrier has been employed. Thus, one observes the origin of Nernst-Planck equation and the manner in which coupling between fluxes of different species should be taken into account. The expression for the flux of a charged species α, is thus given by

$$- J_\alpha \zeta_\alpha = kTC_\alpha' + Z_\alpha e C_\alpha \psi' + \sum_\sigma J_\sigma \zeta_{\alpha\sigma} C_\alpha \tag{21}$$

Equivalently,

$$C_\alpha' + (Z_\alpha e C_\alpha / kT)\, \psi' = - (J_\alpha \zeta_\alpha / kT) - (kT)^{-1} \sum_\sigma H_{\alpha\sigma}^* C_\alpha C_\sigma' \tag{22}$$

Equation (22) yields a formal expression for the relation between concentration and electrical potential profile as

$$\begin{aligned}
C_\alpha(x) &= C_\alpha(0) \exp\,[- Z_\alpha e \triangle\psi / kT] \\
&\qquad - (kT)^{-1} \exp\,\{- Z_\alpha e \psi(x)/kT\}\, [G_\alpha(x) + \sum_\sigma F_{\alpha\sigma}(x)] \\
G_\alpha(x) &= \int_0^x J_\alpha \zeta_\alpha \exp\,\{Z_\alpha e \psi(x)/kT\}\, dx \\
F_{\alpha\sigma}(x) &= \int_1^x H_{\alpha\sigma}^*\, C_\alpha(x)\, C_\sigma'(x) \exp\,\{Z_\alpha e \psi(x)/kT\}\, dx \\
&= \int_1^x J_\sigma \zeta_{\alpha\sigma} C_\alpha(x) \exp\,\{Z_\alpha e \psi(x)/kT\}\, dx \\
\triangle\psi &= \psi(x) - \psi(0)
\end{aligned} \tag{23}$$

The expression for permeability coefficient of ionic species in the membrane, P_α, is given by

$$\begin{aligned}
P_\alpha &= J_\alpha K_\alpha / [C_\alpha(h) - C_\alpha(0)] \\
&= J_\alpha K_\alpha / \{C_\alpha(0)\, [\theta_\alpha \exp\,\{- Z_\alpha e \triangle\psi / kT\} - 1]\} \\
K_\alpha &= C_\alpha(0)/C_{\alpha 1} = C_\alpha(h)/C_{\alpha 2} \\
\theta_\alpha &= 1 - [C_\alpha(0)\, kT]^{-1} \exp\,(- Z_\alpha e \psi(0)/kT)\, \{G_\alpha(h) + \sum_\sigma F_{\alpha\sigma}(h)\}
\end{aligned} \tag{24}$$

where h is the thickness of diffusion barrier. When there is no chemical reactions occuring in the membrane phase, the expression (12) with the assumptions that fluxes of all species and partial frictional coefficients are independent of x, may be substituted in the intergrals of $G_\alpha(x)$ and $F_{\alpha\sigma}(x)$.

Thus, one obtains an equation for permeability of α in the membrane involving only $C_\alpha(x)$ and $\psi(x)$.

Equation (24) expresses the variation of permeability of ionic species across biological membranes as a function of concentrations and electric potentials. Recall that this is the fundamental concept of 'ionic theory' in nerve excitation and cell physiology. The implication of equation (24) is that the diffusion barrier, which is permeable to a specified ionic species α under certain circumstances, can become impermeable for the same species under different values of difference in electric potentials, concentration of other species and existence of occurrence of chemical reactions. Under these conditions, the system is capable of maintaining difference in concentration of the specified ionic species under conditions of stationary states. This is a characteristic of active transport systems [10].

AN EXAMPLE

Consider a system of three permeable ions, α, β and γ transporting across a diffusion barrier of thickness h, and participating in ion-pair formation reactions of the kind

$$\begin{aligned} &\alpha + \gamma \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \delta \\ &\beta + \gamma \underset{k_4}{\overset{k_3}{\rightleftharpoons}} \epsilon \end{aligned} \tag{25}$$

where k_1, k_2, k_3 and k_4 are assumed position independent rate constants. Imposing that $Z_\alpha = Z_\beta = -Z_\gamma = 1$, the resulting ion-pairs are electrically neutral. The two reactions rate profiles

$$\begin{aligned} J_{R1}(x) &= k_1 C_\alpha(x)\, C_\gamma(x) - k_2 C_\delta(x) \\ J_{R2}(x) &= k_3 C_\beta(x)\, C_\gamma(x) - k_4 C_\epsilon(x) \end{aligned} \tag{26}$$

and the five equations of continuity

$$(\partial C_\sigma / \partial t) = \pm J_R + (dJ_\sigma / dx) \tag{27}$$

determine the concentration profiles of this system. The electrical potential profile is given by Poisson equation. From equations of continuity, one has for stationary states that

$$\begin{aligned} -J_\alpha' &= J_\delta' = J_{R1}(x) \\ -J_\beta' &= J_\epsilon' = J_{R2}(x) \\ -J_\gamma &= [J_{R1}(x) + J_{R2}(x)] \end{aligned} \tag{28}$$

Solutions of these reaction rate profiles are in general difficult. However, if one invokes the plausible assumption that concentrations of neutral ion-pairs, C_δ and C_ϵ, are independent of position variable, and that reaction rates and concentration profiles are analytic functions of position variable such that they may be expanded in a Taylor series, the evaluation of reaction rate profiles become simplified [11]. When the concentrations at the boundaries are $C_\alpha(0) = 8 \times 10^{-2}$ moles per litre, $C_\beta(0) = 2 \times 10^{-2}$, $C_\gamma(0) = 3 \times 10^{-2}$ $C_\alpha(h) = 1 \times 10^{-2}$, $C_\beta(h) = 10 \times 10^{-2}$ and $C_\gamma(h) = 5 \times 10^{-2}$, and the rate constants are $k_1 = 2 \times 10^4$, $k_3 = 4 \times 10^4$ mole^{-1} cm^3 sec^{-1}, $k_2 = 3$ sec^{-1} and $k_4 = 2$ sec^{-1}, the reaction rate profiles can be computed as

$$
\begin{aligned}
J_{R1}(x) &= S_0 + S_1 x + S_2 x^2 \\
J_{R2}(x) &= T_0 + T_1 x + T_2 x^2 + T_3 x^3 \\
S_0 &= 1\cdot433 \times 10^{-5} \text{ mole cm}^{-3} \text{ sec}^{-1} \\
S_1 &= -\,10 \text{ mole cm}^{-4} \text{ sec}^{-1} \\
S_2 &= -\,2\cdot8 \times 10^{7} \text{ mole cm}^{-5} \text{ sec}^{-1} \\
T_0 &= 5\cdot6 \times 10^{-5} \\
T_1 &= 16 \\
T_2 &= 9\cdot6 \times 10^{7} \\
T_3 &= 6\cdot4 \times 10^{13}
\end{aligned}
\tag{29}
$$

In obtaining equation (29), it is assumed that membrane thickness is 1×10^{-6} cm, and that higher order Taylor expansion coefficients are of decreasing importance. The conditions that the reactions are confined to take place within the membrane phase, yielding position independent values of concentrations of ion-pairs is utilized to terminate the Taylor series with finite terms. The concentrations of ion-pairs satisfying the boundary values of concentrations and rate constants are, respectively, $C_\delta = 1\cdot122 \times 10^{-5}$ mole cm^{-3} and $C_\epsilon = 4 \times 10^{-5}$ mole cm^{-3}. The concentration profiles of the three ionic species consistent with the above assumed values are

$$
\begin{aligned}
C_\alpha(x) &= a_0 + a_1 x \\
C_\beta(x) &= b_0 + b_1 x + b_2 x^2 \\
C_\gamma(x) &= c_0 + c_1 x \\
a_1 &= -\,70 \text{ mole cm}^{-4}, \quad b_1 = 0, \quad c_1 = 20 \text{ mole cm}^{-4} \\
b_2 &= 8 \times 10^{7} \text{ mole cm}^{-5}
\end{aligned}
\tag{30}
$$

Thus, for the system under consideration, the reaction rate profiles $J_{R1}(x)$ and $J_{R2}(x)$ consistent with boundary values of reactants and the condition that

$$\int_0^h J_{R1}(x)\,dx = 0 = \int_0^h J_{R2}(x)\,dx \tag{31}$$

and the imposed condition that concentration of neutral ion-pairs are position independent are satisfactorily computed. Inclusion of higher order Taylor expansion coefficients are necessary to satisfy the conditions mentioned above, for different values of rate constants and boundary concentrations of the reactants. It is possible to evaluate the concentration and reaction rate profiles in these cases, except that the computation may be numerically tedious.

INFLUENCE OF CHEMICAL REACTIONS ON FLUX OF A NON-PARTICIPATING SPECIES

Consider the system described in preceding section, with the additional stipulation that in addition to the three ionic species α, β and γ, an inert species η undergoes transport across the same membrane. The four fluxes are computable in principle from the four simultaneous relations,

$$\begin{aligned} -J_\sigma &= (kT\ C_\sigma'/\zeta_\sigma) + (Z_\sigma e C_\sigma/\zeta_\sigma)\,\psi' \\ &\quad + (C_\sigma/\zeta_\sigma)\,\{J_\sigma\zeta_{\alpha\sigma} + J_\beta\zeta_{\beta\sigma} + J_\gamma\zeta_{\gamma\sigma} + J_\eta\zeta_{\eta\sigma}\} \\ \sigma &= \alpha,\ \beta \text{ or } \gamma \\ -J_\eta\zeta_\eta &= kTC_\eta' + C_\eta\,\{J_\alpha\zeta_{\alpha\eta} + J_\beta\zeta_{\beta\eta} + J_\gamma\zeta_{\gamma\eta} + J_\eta\zeta_{\eta\eta}\} \end{aligned} \tag{32}$$

When the reactions specified in equations (25) do not occur in the membrane system, the fluxes J_α, J_β, J_γ and J_η are constants independent of position variable, x, in the system. Whether the reactions occur or not, the flux of species η, which does not participate in the reactions, is independent of position variable. Its magnitude will be different, however, in the presence of fluxes of other species and chemical reactions occurring between these species. Quantitative computation of this variation of flux and permeability of such inert species is the vexing problem of biological transport.

The requirement from equations of continuity yields,

$$0 = kT\,[C_\eta'' - (\zeta_\eta' C_\eta'/\zeta_\eta)] + [C_\eta' - (C_\eta\zeta_\eta'/\zeta_\eta)]\,\sum_\sigma J_\sigma\zeta_{\sigma\eta} \tag{33}$$

Equation (33) expresses the manner in which concentration profile of η adjusts to variation in frictional coefficients with position, intensity of interactions with other transporting species, such that its flux is independent of x, under conditions of stationary states. Equation (33) is valid when there is no chemical reactions in membrane phase. When the reactions specified in equations (25) occur in the membrane phase, one obtains in place of equation (33), another equation of constraint,

$$0 = \big(\text{Terms in right hand side of equation (33)}\big) \\ - (C_\eta/\zeta_\eta)\,[J_{R1}\,(\zeta_{\alpha\eta} + \zeta_{\gamma\eta}) + J_{R2}\,(\zeta_{\beta\eta} + \zeta_{\gamma\eta})] \tag{34}$$

The influence of chemical reaction on fluxes of inert species are thus computable from knowledge of partial frictional coefficients concentration profiles and electrical potential profiles of all species in the presence and in the absence of chemical reactions. An example in which all such required inputs for solutions of the set of equations (32) is provided in this communication. Since flux of the inert species η is independent of position variable it suffices to compute the flux at any convenient location in membrane phase, where all required information for solution of equation (32) are available.

For the purpose of numerical computations, assume that the diffusion coefficients $D_\alpha(0) = 5 \times 10^{-7}$, $D_\beta(0) = 6 \times 10^{-7}$, $D_\gamma\ (0) = 10 \times 10^{-7}$ and $D_\eta(0) = 8 \times 10^{-7}$ (expressed in $\text{cm}^2\,\text{sec}^{\ 1}$). The frictional coefficients of the four species at 300° K, are (expressed in $\text{ergs cm}^{-2}\,\text{sec}$), $\zeta_\alpha\ (0) = 82{\cdot}825 \times 10^{-9}$, $\zeta_\beta(0) = 69{\cdot}023 \times 10^{-9}$, $\zeta_\gamma(0) = 41{\cdot}4132 \times 10^{-9}$ and $\zeta_\eta(0) = 51{\cdot}7666 \times 10^{-9}$. Defining the contributions to frictional coefficients of species σ, from molecules of the membrane system other than α, β, γ and η, as $A_\sigma = \sum_j' C_j \zeta_{j\sigma}$, and assuming these values as $A_\alpha(0) = 40{\cdot}825 \times 10^{-9}$, $A_\beta(0) = 39{\cdot}023 \times 10^{-9}$, $A_\gamma(0) = 21{\cdot}4132 \times 10^{-9}$ and $A_\eta(0) = 31{\cdot}767 \times 10^{-9}$ one has four equations relating the partial frictional coefficients which may be expressed as

$$\begin{vmatrix} \zeta_{\alpha\alpha} & \zeta_{\alpha\beta} & \zeta_{\alpha\gamma} & \zeta_{\alpha\eta} \\ \zeta_{\alpha\beta} & \zeta_{\beta\beta} & \zeta_{\beta\gamma} & \zeta_{\beta\eta} \\ \zeta_{\alpha\gamma} & \zeta_{\beta\gamma} & \zeta_{\gamma\gamma} & \zeta_{\gamma\eta} \\ \zeta_{\alpha\eta} & \zeta_{\beta\eta} & \zeta_{\gamma\eta} & \zeta_{\eta\eta} \end{vmatrix} \begin{vmatrix} C_\alpha(0) \\ C_\beta(0) \\ C_\gamma(0) \\ C_\eta(0) \end{vmatrix} = \begin{vmatrix} 40 \\ 30 \\ 20 \\ 20 \end{vmatrix} \times 10^{-9} \tag{35}$$

where it is assumed that $C_\eta(0) = 3 \times 10^{-5}\,\text{mole cm}^{-3}$. The assumed values of A_σ are significant fraction of $\zeta_\sigma(0)$. Insisting that $\zeta_{\alpha\alpha}$, $\zeta_{\alpha\beta}$, $\zeta_{\beta\beta}$, $\zeta_{\gamma\gamma}$, and $\zeta_{\eta\eta}$ are positive definite and that $\zeta_{\alpha\gamma}$ and $\zeta_{\beta\gamma}$ are negative definite, one has that the values, $\zeta_{\alpha\alpha} = \zeta_{\beta\beta} = 4 \times 10^{-4}$, $\zeta_{\alpha\beta} = 5{\cdot}74 \times 10^{-4}$, $\zeta_{\alpha\gamma} = -1{\cdot}5 \times 10^{-4}$, $\zeta_{\beta\gamma} = -8{\cdot}976 \times 10^{-4}$, $\zeta_{\gamma\gamma} = 17{\cdot}784 \times 10^{-4}$, $\zeta_{\eta\eta} = 4{\cdot}333 \times 10^{-4}$, $\zeta_{\alpha\eta} = \zeta_{\beta\eta} = 1 \times 10^{-4}$ and $\zeta_{\gamma\eta} = -1 \times 10^{-4}$ satisfy the set of equations (35).

Ignoring the coupling terms and electrical potential terms, assuming Fick's law and linear concentration profiles, for the specified boundary concentrations, and diffusion coefficients, the flux of the four species at location $x = 0$, are computed as

$$
\begin{aligned}
J_\alpha &= -\,0{\cdot}35 \times 10^{-4} \text{ moles cm}^{-2}\text{ sec}^{-1} \\
J_\beta &= +\,0{\cdot}48 \times 10^{-4} \\
J_\gamma &= +\,0{\cdot}20 \times 10^{-4} \\
J_\eta &= +\,0{\cdot}40 \times 10^{-4}
\end{aligned}
\tag{36}
$$

The negative sign associated with the magnitude of flux indicate that flux occurs in a direction of increasing x, *i.e.*, from left hand side to right hand side, while the positive sign denotes that flux has a direction from right to left.

Using the assumed values of partial frictional coefficients when the gradient of electrical potential at $x = 0$, $\psi'(0)$ equals zero, the computed fluxes of four species using the set of four equations (32) in the presence of chemical reactions (denoted by asteris) and in the absence of chemical reactions (denoted by superzero) are listed in Table I. One concludes from the values listed in Table I, that inclusion of coupling terms affects the magnitude and direction of fluxes of species rather seriously.

TABLE I

Computed values of fluxes at $x = 0$, *using equations* (32) *when* $\psi'(0)$ *equals zero*

$$
\begin{aligned}
J_\alpha^0 &= -\;\;7{\cdot}8139 \times 10^{-4} & J_\alpha^* &= -\;2{\cdot}8375 \times 10^{-4} \\
J_\beta^0 &= -\;\;4{\cdot}8638 \times 10^{-4} & J_\beta^* &= -\;1{\cdot}6010 \times 10^{-4} \\
J_\gamma^0 &= \;\;13{\cdot}1947 \times 10^{-4} & J_\gamma^* &= \;\;3{\cdot}6059 \times 10^{-4} \\
J_\eta^0 &= -\;\;1{\cdot}5762 \times 10^{-4} & J_\eta^* &= -\;0{\cdot}1952 \times 10^{-4}
\end{aligned}
$$

$$
\begin{aligned}
kTC_\alpha'(0) &= -\,2{\cdot}8989 \times 10^{-12} \text{ ergs mole cm}^{-4} \\
kTC_\beta'(0) &= 3{\cdot}3130 \times 10^{-12} \\
kTC_\gamma'(0) &= 0{\cdot}8283 \times 10^{-12} \\
kTC_\eta'(0) &= 1{\cdot}6565 \times 10^{-12} \\
kTC_\beta'^*(0) &= 0
\end{aligned}
$$

The flux of species α has the same direction as dictated by its concentration gradient. However, its magnitude ($\times 10^4$) has increased from a value of $-\,0{\cdot}35$ given by Fick's law, to $-\,7{\cdot}8$ when coupling terms are included in the absence of chemical reaction and to $-\,2{\cdot}8$ in the presence of chemical reactions. The flux of the inert species η, $(J_\eta \times 10^4)$, has changed both its magnitude and direction from a value of 0·40 to −1·57 in the absence of chemical reaction and to a value of 0·2 in the presence of chemical reactions in which this species does not participate. The direction of flux of η is evidently in a direction opposed to that prescribed by its concentration difference across the membrane barrier. This is active transport. Thus for this system one will conclude that both η and β are actively transported while α and γ are passively transported. However, these values listed in Table I, are based on validity of assumed values of partial frictional coefficients and restricted to the assumed case when $\psi'(0)$ equals zero.

EVALUATION OF ELECTRICAL POTENTIAL GRADIENT

In order to compute the fluxes of the four species using the set of four equations (32), one needs the values of $\psi'(0)$ in the presence and absence of chemical reactions. Substitution of equation (18) in (17) and integration once of the resultant yields,

$$\begin{aligned} kT\,[C_\alpha(x) + C_\beta(x) + C_\gamma(x) + C_\eta(x)] &+ \tfrac{1}{2}\sum_{\sigma} H_{\sigma\sigma} C_\sigma(x)^2 \\ &+ \sum_{\sigma}\sum_{\substack{\delta \\ \sigma \neq \delta}} H_{\sigma\delta} C_\sigma(x)\, C_\delta(x) \\ &= (\epsilon/8\pi)\,\{\psi'(x)\}^2 + \text{constant}. \end{aligned} \tag{37}$$

Evaluation of the value of expression (37) at $x = 0$, and subtraction yields,

$$\begin{aligned} kT \sum_{\sigma} \{C_\sigma(x) - C_\sigma(0)\} &+ \tfrac{1}{2}\sum_{\sigma} H_{\sigma\sigma}\{C_\sigma(x)^2 - C_\sigma(0)^2\} \\ &+ \sum_{\sigma}\sum_{\substack{\delta \\ \sigma \neq \delta}} H_{\sigma\delta}\{C_\sigma(x)\, C_\delta(x) - C_\sigma(0)\, C_\delta(0)\} \\ &= (\epsilon/8\pi)\,[\psi'(x)^2 - \psi'(0)^2] \\ \psi'(x) &= \psi'(0) - (4\pi e/\epsilon)\{I_\alpha(x) + I_\beta(x) - I_\gamma(x)\} \\ I_\sigma(x) &= \int_0^x C_\sigma(x)\, dx. \end{aligned} \tag{38}$$

Therefore,

$$\begin{aligned} \psi'(x)^2 - \psi'(0)^2 \\ = -(8\pi e/\epsilon)\,\psi'(0) \sum_{\sigma} I_\sigma(x)\, Z_\sigma + (16\pi^2 e^2/\epsilon^2)\{\sum_{\sigma} I_\sigma(x)\, Z_\sigma\}^2 \end{aligned} \tag{39}$$

Thus, one obtains,

$$kT \sum_{\sigma} \triangle C_{\sigma}(h) + (H_{\sigma\delta} \text{ terms})$$

$$= - e\psi'(0) \sum_{\sigma} I_{\sigma}(h) Z_{\sigma} + (2\pi e^{2}/\epsilon) \{ \sum_{\sigma} I_{\sigma}(h) Z_{\sigma} \}^{2} \qquad (40)$$

where for the system under consideration, $kT \sum_{\sigma} \triangle C_{\sigma}(h)$ equals $17 \cdot 4656 \times 10^{5}$ ergs cm^{-3}, $\sum_{\sigma} I_{\sigma}(h) Z_{\sigma} = 9 \cdot 5 \times 10^{-11}$ moles cm^{-2} in the absence of chemical reactions. When contributions from unknown $H_{\sigma\delta}$ terms are ignored, one obtains,

$$e\psi'(0) = 4 \cdot 14815 \times 10^{-5} \, \mathrm{esu}^{2} \, \mathrm{cm}^{-2} \qquad (41)$$

when there is no chemical reactions.

One observes that $Z_{\alpha} e C_{\alpha} \psi'(0)$ is of the order of $3 \cdot 3171 \times 10^{-9}$ esu^{2} mole cm^{-5}, while $kT \; C_{\alpha}'$ term equals $- 2 \cdot 8989 \times 10^{12}$ ergs mole cm^{-4}. Since (esu^{2}/ergs) has dimensions of cm, one observes that the contributions from gradient of electric potential terms dominates over contributions from concentration gradient terms, when $H_{\sigma\delta}$ terms are ignored. In the presence of chemical reactions, the value of $(2\pi e^{2}/\epsilon) \{ \sum_{\sigma} I_{\sigma}(h) Z_{\sigma} \}^{2}$ assumes a value of $4 \cdot 56859 \times 10^{7}$, while $kT \sum_{\sigma} \triangle C_{\sigma}(h)$ term equals $0 \cdot 1746 \times 10^{7}$. The value of $e\psi'(0)$ in the presence of chemical reactions is computed to equal

$$e\psi'(0)^{*} = 1 \cdot 750349 \times 10^{-6} \, \mathrm{esu}^{2} \, \mathrm{cm}^{-2} \qquad (42)$$

The computed values of fluxes of four species using equations (32) and the above values of $e\psi'(0)$ both in the presence and absence of chemical reactions is listed in Table II.

TABLE II

Computed values of fluxes at x = 0, *using equations* (32) *and the values of* $e\psi'(0)$ *presented in equations* (41) *and* (42)

$J_{\alpha}^{0} = + \; 0 \cdot 3121 \times 10^{-2}$	$J_{\alpha}^{*} = + 0 \cdot 14335 \times 10^{-3}$
$J_{\beta}^{0} = 3 \cdot 2917 \times 10^{-2}$	$J_{\beta}^{*} = 1 \cdot 3676 \times 10^{-3}$
$J_{\gamma}^{0} = - \; 15 \cdot 4289 \times 10^{-2}$	$J_{\gamma}^{*} = - 6 \cdot 4433 \times 10^{-3}$
$J_{\eta}^{0} = 1 \cdot 4686 \times 10^{-2}$	$J_{\eta}^{*} = 0 \cdot 5728 \times 10^{-3}$

DISCUSSION

The main objective of this communication is to provide a review of the interesting problems of biological membrane transport and a theoretical basis for the postulates of physiology that permeabilities of ionic and neutral species are dependent on numerous factors such as electrical potential gradients, concentration profiles and chemical reactions occurring in the membrane phase. The influence of chemical reactions on the direction and magnitude of flux of a non-participating species is analysed on the basis of equations of continuity and a numerical example of this paper.

The manner in which quantitative computation of fluxes of all species in question in the presence and in the absence of chemical reactions can be accomplished is illustrated by the numerical example. Assuming that concentration gradients of ion-pair molecules in the membrane phase can be ignored, the concentration profiles of charged species participating in the chemical reactions (25) for specified boundary concentrations, rate constants are satisfactorily evaluated by the procedure indicated in the paper. Having thus a well-specified system, the influence of chemical reaction, concentration profiles of these species on the flux of a non-participating species is presented by the partial frictional coefficient formalism. Critically, the numerical estimation of $\psi'(0)$ suffers from lack of knowledge of the magnitudes of contributions from $H_{\sigma\delta}$ terms. In order to compromise with our current status of knowledge, computed fluxes when $\psi'(0)$ equals zero and when $H_{\sigma\delta}$ terms can be ignored are presented in this paper.

One need not accept the estimated values of partial frictional coefficients presented in the numerical example. Given the existence of partial frictional coefficients, and variation of diffusion coefficients with position, the manner in which a phenomenon like active transport can arise in a biological inhomogeneous membrane phase is illustrated by the computed fluxes.

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