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THE THEORY OF TRANSPORT PHENOMENA IN BIOLOGICAL MEMBRANES

I. THE PASSIVE TRANSPORT AND RESTING POTENTIAL

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SUMMARY

The theoretical study of the passive transport of Na^+ and K^+ across biological membranes is based on the assumption that both kinetic and thermodynamic properties of membrane influence the flux of ions. Two models were investigated. Model A suggests the existence of two kinds of ion-exchange centers, one binding mainly Na^+ and the other mainly K^+. Model B suggests only one type of ion-exchange center with a different affinity to Na^+ and to K^+. Only Model A provides the equation which agrees with experimental data concerning the dependence of resting potential on concentration.

INTRODUCTION

It is well known that the concentration of K^+ inside cells is 10–20 times higher than in the surrounding medium. For Na^+ gradients of the same order but of opposite direction are observed. Experiments performed with tracers show that there exists an exchange of the ions between the cytoplasm and external medium. It means the absence of any impermeable partition concerning these ions.

The observed difference in potentials of the cytoplasm and external medium of the order 50–70 mV is determined by the existing concentration gradients of the ions (see *e.g.* the reviews of refs. 1 and 2).

These features of the cell can be explained on the basis of equilibrium thermodynamics if the cytoplasm possesses the property of specific ion binding. However, the measurements of activity and mobility of Na^+ and K^+ in axoplasm[3–5] show that K^+ is practically free and only 20–30 % of Na^+ is bound. Therefore, it can be suggested that the specific properties of the cell membrane, but not of the cytoplasm, determine the gradients of the ion concentrations. The resting potential occurs as the result of a non-equilibrium stationary process, not because of some unsymmetrical equilibrium ion binding. Such a concept is directly supported by the experiments with artificial medium inside the cells. In the work of BLOUNT AND LEVEDAHL[6] sea water passed through the vacuoles of the alga *Halicystis ovalis*. BAKER *et al.*[7–9] developed the method of replacement of the axoplasm of squid giant axons with artificial solutions. In the works of OIKAWA *et al.*[10] and of TASAKI *et al.*[11,12] the artificial solution passed through a selected part of the axon. It was shown that

the cell with artificial solution instead of cytoplasm possesses the ability to support the high internal concentration of K^+ and the low concentration of Na^+.

The diffusion along the gradient through permeable membrane determines the difference of electrostatic potentials between the solutions divided by this membrane. Using the "constant field" approximation suggested by GOLDMAN[13], HODGKIN AND KATZ[14] derived the formula for the resting potential, treated as diffusion potential

$$\Delta V = -\frac{RT}{F} \ln \frac{P_K c_K' + P_{Na} c_{Na}' + P_{Cl} c_{Cl}''}{P_K c_K'' + P_{Na} c_{Na}'' + P_{Cl} c_{Cl}'} \quad (1)$$

where c_i' and c_i'' are the concentrations of the ions of species i inside and outside the cell; $\Delta V = V'-V''$ is the difference of electrostatic potentials; P_i is the permeability coefficient of membrane for the ion i; F, the Faraday number; R, the gas constant; T, temperature in °K. If the Cl^- are in equilibrium, then

$$\Delta V = -\frac{RT}{F} \ln \frac{P_K c_K' + P_{Na} c_{Na}'}{P_K c_K'' + P_{Na} c_{Na}''} \quad (2)$$

EISENMAN AND CONTI[15] indicate that the cell membrane can be treated as an ion exchanger. In this case the diffusion equations give Eqn. 2 without the constant field approximation, and the constant field can be obtained only if the immovable ion-exchange centres are distributed homogeneously along the direction of ion motion.

Eqn. 2 can also be obtained using the expressions for the diffusional fluxes of the ions which were calculated by PARLIN AND EYRING[16] in their microscopic theory of ion diffusion across membranes.

It must be emphasized that in the experiments using artificial variations of the concentration of the solution at one side of the membrane, after the steady state is achieved, the concentration at the other side must also change. However, the fluxes are so small that during the lifetime of the sample the steady state cannot be achieved and the concentrations are practically constant during the measurements. Therefore the concentrations in Eqn. 2 are considered as not interdependent and independent of time.

Eqn. 2 well suits the experimental data concerning the concentration dependence of the resting potential only if the concentration of K^+ in the external medium is high enough[17] or if this concentration is sufficiently small within the cell[7]. If the external concentration is small, then its further decrease does not give the logarithmic rise of the resting potential. The value ΔV has some limiting value. The same is observed if the internal K^+ concentration is greatly increased.

It was shown by BAKER *et al.*[9] that the change of the potential with the concentration in the case of high internal K^+ concentrations occurs in such a manner that the value $P_K c_K'$ remains nearly constant, *i.e.* P_K decreases if c_K' increases. The authors suggest that an increase of the resting potential decreases the permeability.

The deviations of the experimental results from the theoretical Eqn. 2 can be explained by the derivation of this formula not taking into account some features of the transport mechanism. Many different models of transport have been proposed. In the paper of HILL AND KEDEM[18] twenty such models are collected and classified. The aim of our work is to choose a mechanism of transport which explains in the simplest way the observed dependence of the membrane permeability upon the

concentrations of ions in solutions. Such a designation of mechanism is necessary for the specification of the concept concerning the passive transport of ions.

Description of the model

Every derivation of Eqn. 2 is based on the assumption that the flux of ions across the membrane depends linearly on the concentration gradient. This means that the rate of motion is limited by free diffusion of the ions in the membrane substance. The membrane is treated microscopically as an energetical barrier for the ions whose height is different for ions of different species.

Let us suggest that the membrane can be saturated by ions: if many ions have entered the membrane already, the entrance of succeeding ions becomes more difficult. It is a kind of "negative cooperativity".

This suggestion is based on the low solubility of ions in fatty medium. The ions can occupy only some selected positions near the charged ion-exchange centers. These centers can move and the ions can move with them (the carrier model). The centers can be immovable but distributed in such a way that ions can 'jump' from one center to another (relay-race mechanism).

The dependence of the current of the potential is different in these two cases[15]. There are experimental indications in favor of the second model. We shall consider the ion-exchange centers immovable.

The driving force for the passive transport is the gradient of electrochemical potential. During the lifetime of the cell this gradient should drop, and the distribution of ions between the cell and external medium should tend to reach equilibrium, if the membrane did not contain some biochemical machine moving the ions opposite to the gradient of the electrochemical potential. We shall not discuss the work of the Na^+–K^+ pump in this paper. The mechanism of active transport is studied in another work[19].

The basic assumption in our calculations is that the motion of ions is limited by the membrane–solution frontier but not by diffusion in the membrane.

Two models of this kind can be suggested. The first model (Model A) contains the ion-exchange centers of two species: the first species binds mainly K^+, the second, Na^+. The second model (Model B): all centers belong to the same species, but their affinities to different ions differ and there exists a competition between the ions.

Investigation of Model A

Let n_i^0 be the total number of centers per unit volume of membrane possessing the ability to bind the ions of species i; n_i the number of these centers already occupied by ions i; q_{1i} the rate constant for the transition of the ion i from the intracellular medium into the membrane; q_{1i}', the rate constant for the opposite process; q_{2i}' and q_{2i} analogous rate constants for the external surface of the membrane. In the steady state the fluxes of the ions of given species moving per unit time across the internal and external membrane surfaces are equal:

$$I_i = q_{1i}c_i'(n_i^0 - n_i) - q_{1i}'n_i = q_{2i}n_i - q_{2i}'c_i''(n_i^0 - n_i) \tag{3}$$

or

$$I_i = n_i^0 \frac{q_{1i}q_{2i}c_i' - q_{1i}'q_{2i}'c_i''}{q_{1i}' + q_{2i} + q_{1i}c_i' + q_{2i}'c_i''} \tag{4}$$

Let us compare this formula with the expression obtained by HODGKIN AND KATZ[14] and by PARLIR AND EYRING[16].

$$I_i = \frac{P_i F^2 V \left[c_i' \exp\left(-\frac{FV}{RT}\right) - c_i''\right]}{RT\left[1 - \exp\left(-\frac{FV}{RT}\right)\right]} \tag{5}$$

Instead of permeability coefficient P_i, Eqn. 4 contains some effective value $P_i^{\text{eff.}}$, depending on concentrations:

$$P_i^{\text{eff.}} \sim \frac{q_{1i}' q_{2i}' n_i^0}{q_{1i} c_i' + q_{2i}' c_i'' + q_{1i}' + q_{2i}} \tag{6}$$

Eqn. 6 shows that in the case of low intracellular concentrations $P_i^{\text{eff.}}$ does not depend on c_i', and in the case of high concentrations $P_i^{\text{eff.}}$ decreases as $c_i'^{-1}$. Such a behavior of the permeability coefficient was considered in the work of BAKER *et al.*[9].

In looking for the dependence of the rest potential on the concentrations of the ions, Eqn. 4 can be used together with some form of a potential energy curve along the coordinate of motion of the ions $U(x)$.

As it has been suggested that the rate of this motion is limited by the situation at the membrane surface, potential energy can be described by two maxima at the surfaces and a minimum in the middle of the membrane. Let us express the potential energy in the form $U + zFV$, where zFV is the electrostatic part. For simplicity we assume that the curve $U(x)$ is symmetrical, and the asymmetry of potential energy is determined only by zFV (Fig. 1). This assumption does not change the principal results, but it diminishes the number of parameters involved in the theory. Let the electrostatic potential in the centrum of membrane be equal to half the sum of its values at the frontiers which are equal to the potentials in adjoining solutions. Such values are used in the constant field approximation. The concentrations in solution are relative, they are expressed as the ratio of the number of ions

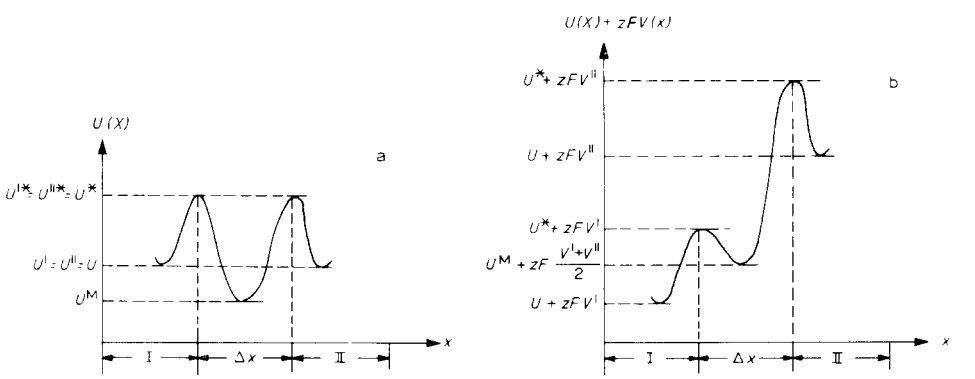

Fig. 1. The potential energy curve along the coordinate of motion. a. Symmetrical barriers at the membrane surfaces corresponding to the nonelectrostatic part of the potential. b. Potential energy containing the electrostatic part. I, intracellular solution; ΔX, width of membrane; II, external solution.

to the number of solvent molecules, in the same volume. The indices ′ and ″ correspond to quantities inside and outside the cell; index ^{m}, to quantities in the membrane; *, at the top of the energetical barrier.

According to the theory of rate processes[20], all the constants q_i can be expressed as the product of ΔX, the width of the barrier, and the absolute rate $(kT/h)\exp(-F^*/RT)$, where k is the Boltzmann's constant, h is Planck's constant, and F^* the free energy of activation. We get

$$\begin{aligned}
q_{1K} &= W \exp\left[-\frac{U_K'^* - U_K' + zF(V'^* - V')}{RT}\right] \\
q_{1K'} &= W \exp\left[-\frac{U_K'^* - U_K^m - zF(V'^* - V^m)}{RT}\right] \\
q_{2K} &= W \exp\left[-\frac{U_K'^* - U_K^m - zF(V''^* - V^m)}{RT}\right] \\
q_{2K'} &= W \exp\left[-\frac{U_K''^* - U_K'' + zF(V''^* - V'')}{RT}\right]
\end{aligned} \tag{7}$$

where $W = \Delta X(kT/h)$.

As has already been said, we can take for the ions in solutions $U_i' = U_i'' = U_i$. Employing our assumption concerning the potential curve we have $U_i'^* = U_i''^* = U_i^*$ and $V'^* = V'$, $V''^* = V''$, $V' + V'' = 2V^m$. Let us denote

$$W \exp\left(-\frac{U_K^* - U_K}{RT}\right) = g_K \tag{8}$$

$$\exp\left[-\frac{zF(V' - V'')}{RT}\right] \equiv r \tag{9}$$

$$\exp\left(-\frac{U_K^m - U_K}{RT}\right) \equiv f_K \tag{10}$$

We get from Eqn. 7

$$q_{1K} = q_{2K'} = g_K; \qquad q_{1K'} = \frac{g_K}{f_K} r; \qquad q_{2K} = \frac{g_K}{f_K} r^{-1} \tag{11}$$

Putting Eqn. 11 into Eqn. 4, we get

$$I_K = \frac{n_K^0 g_K r(c_K' r^{-2} - c_K'')}{r + r^{-1} + f_K(c_K' + c_K'')} \tag{12}$$

The dimension of $c^0_K g_K$ is the same as that of the permeability coefficient P_K in Eqn. 5. A kinetic parameter has to be estimated from the data on the activation energy for the transport of ions across membrane. The parameter f_K is a thermodynamical quantity; it is the constant of equilibrium for the ions in solution and in the exchange centers of membrane.

For the flow of Na^+ we get the similar formula

$$I_{Na} = \frac{n_{Na}^0 g_{Na} r(c_{Na}' r^{-2} - c_{Na}'')}{r + r^{-1} + f_{Na}(c_{Na}' + c_{Na}'')} \tag{13}$$

The condition of electroneutrality of the total flux is

$$I_{Na} + I_K = 0 \tag{14}$$

Putting Eqns. 12 and 13 into Eqn. 14 we get the expression connecting the resting potential (included in r) with the concentrations of ions:

$$\frac{r^{-2}c_K' - c_K''}{r + r^{-1} + f_K(c_K' - c_K'')} = -\frac{n_{Na}{}^0 g_{Na}}{n_K{}^0 g_K}\left[\frac{r^{-2}c_{Na}' - c_{Na}''}{r + r^{-1} + f_{Na}(c_{Na}' - c_{Na}'')}\right] \tag{15}$$

As $c_K' - c_K'' \cong c_{Na}' - c_{Na}''$, we get Eqn. 2 from Eqn. 15 if $f_K = f_{Na}$ and $n^0{}_{Na}g_{Na}/n^0{}_K g_K = P_{Na}/P_K$. The equality $f_K = f_{Na}$ means that the equilibrium properties of the membrane do not depend on an exchange between K^+ and Na^+ and that the membrane can discriminate these ions only kinetically. That is the basic assumption of "diffusional" theories[14,16].

The logarithmic rise of the resting potential if $c_K' \to \infty$ or $c_K'' \to 0$ follows from Eqn. 2. However, Eqn. 15 gives the observed saturation of ΔV. Let us express c_K' and c_K'', assuming these concentrations independent:

$$c_K' = \frac{c_K''\left[1 - \frac{I_{Na}(r)}{rn_K{}^0 g_K} f_K\right] - \frac{I_{Na}(r)}{rn_K{}^0 g_K}(r + r^{-1})}{r^{-2} + \frac{I_{Na}(r)}{rn_K{}^0 g_K} f_K} \tag{16}$$

$$c_K'' = \frac{c_K'\left[r^{-2} + \frac{I_{Na}(r)}{rn_K{}^0 g_K} f_K\right] + \frac{I_{Na}(r)}{rn_K{}^0 g_K}(r + r^{-1})}{1 - \frac{I_{Na}(r)}{rn_K{}^0 g_K} f_K} \tag{17}$$

By definition (*cf.* for instance Eqn. 3) the flux I_i is positive if it is directed outwards from the cell. The passive flux of Na^+ is directed into the cell, therefore $I_{Na}(r) < 0$. The numerator of Eqn. 16 is positive and the denominator can become zero at some value of r which we shall designate r_1. As $c_K' > 0$, r_1 characterizes the limiting value of the resting potential at $c_K' \to \infty$. The form of Eqn. 17 shows that there exists a limiting value of the resting potential for $c_K'' \to 0$, determined by the value r_2 of the parameter r, making the numerator of Eqn. 17 zero. We have

$$r_1{}^{-2} + \frac{I_{Na}(r_1)}{r_1 n_K{}^0 g_K} f_K = 0 \tag{18}$$

$$r_2{}^{-2} + \frac{I_{Na}(r_2)}{r_2 n_K{}^0 g_K} f_K - \frac{1}{c_K'}\frac{I_{Na}(r_2)}{r_2 n_K{}^0 g_K}(r_2 + r_2{}^{-1}) = 0 \tag{19}$$

It is easy to see that for sufficiently high values of c_K' both equations give equal values for r_1 and r_2. It means that practically the same limiting value of the resting potential corresponds to high internal or low external concentrations of K^+.

Putting Eqn. 13 into Eqn. 18 we get the explicit expression for the limiting value of the resting potential:

$$r_1{}^3 - \frac{1}{f_K c_{Na}''}\frac{n_K{}^0 g_K}{n_{Na}{}^0 g_{Na}} r_1{}^2 - \frac{n_K{}^0 g_K f_{Na}}{n_{Na}{}^0 g_{Na} f_K}\left(\frac{c_{Na}'}{c_{Na}''} - 1\right) - \frac{c_{Na}'}{c_{Na}''} r_1 - \frac{n_K{}^0 g_K}{n_{Na}{}^0 g_{Na}}\frac{1}{f_K c_{Na}''} = 0 \tag{20}$$

This equation connects some quantities which in principle can be measured independently. If the permeability ratio $n_K^0 g_K / n_{Na}^0 g_{Na}$ and the equilibrium constants f_K and f_{Na} are known, the equation allows for an estimation of the theoretical value of the limiting resting potential.

As f_K and f_{Na} are not now known, but r_1 is a measured value, Eqn. 20 only allows some predictions concerning f_K. Let us write Eqn. 20 in the form

$$\frac{n_{Na}^0 g_{Na}}{n_K^0 g_K} f_K r_1 (r_1^2 c_{Na}'' - c_{Na}') - r_1^2 + 1 = f_{Na}(c_{Na}' - c_{Na}'') \tag{21}$$

and we get the condition for f_K,

$$f_K > \frac{n_K^0 g_K}{n_{Na}^0 g_{Na}} \frac{r_1^2 + 1}{r_1 (c_{Na}'' r_1^2 - c_{Na}')} \tag{22}$$

For the estimation of $n_K^0 g_K / n_{Na}^0 g_{Na}$ let us take $P_K / P_{Na} \sim 10$–100. The limiting value $\Delta V \simeq 60$ mV corresponds to $r_1 \simeq 3.3$. The concentration of Na^+ in sea water is 460 mM; it corresponds to $c_{Na}'' = 8$ mM. Let us take $c_{Na}''/c_{Na}' \simeq 10$. Putting these values into Eqn. 22 we get $f_K > 4 \cdot 10^2 - 4 \cdot 10^3$. The rough experimental estimation[21] shows that the concentration of K^+ in membrane is lower than in surrounding medium. Our estimation of f_K does not contradict those data, as the fundamental assumption followed states that the ions are not distributed homogeneously in the membrane volume but are condensed around the exchange centers, whose number in the membrane is limited. The "relay-race mechanism" of the transport can act if these centers are near one to another and the concentration of ions in their neighborhood can be higher than in solution.

Investigation of Model B

In this case the steady-state fluxes of ions are described by the following equations:

$$\begin{aligned} I_{Na} &= q_{1Na} c_{Na}' (n^0 - n_{Na}) - q_{1Na}' n_{Na} \\ &= q_{2Na} n_{Na} - q_{2Na}' c_{Na}'' (n^0 - n_{Na} - n_K) \\ I_K &= q_{1K} c_K' (n^0 - n_{Na} - n_K) - q_{1K}' n_K \\ &= q_{2K} n_K - q_{2K}' c_K'' (n^0 - n_{Na} - n_K) \end{aligned} \tag{23}$$

where n^0 is the concentration of the ion-exchange sites of the membrane maintaining the passive transport of the ions; q_{ij}, q_{ij}' are the rate constants for the crossing of energetical barriers at the ith surface of membrane by ions of the species j.

We get the concentrations of ions in membrane from the following system of equations:

$$\begin{aligned} (n^0 - n_{Na} - n_K)(q_{1Na} c_{Na}' + q_{2Na}' c_{Na}'') &= n_{Na}(q_{1Na}' + q_{2Na}) \\ (n^0 - n_{Na} - n_K)(q_{1K} c_K' + q_{2K}' c_K'') &= n_K (q_{1K}' + q_{2K}) \end{aligned} \tag{24}$$

Excluding n_K and n_{Na} from Eqn. 23 and using Eqn. 24, we get

$$I_K = \frac{n^0}{q_{1K}' + q_{2K}} \left[\frac{q_{1K} q_{2K} c_K' - q_{1K}' q_{2K} c_K''}{1 + \frac{q_{1K} c_K' - q_{2K}' c_K''}{q_{1K}' + q_{2K}} + \frac{q_{1Na} c_{Na}' + q_{2Na}' c_{Na}''}{q_{1Na}' + q_{2Na}}} \right] \tag{25}$$

Comparison of Eqns. 25 and 5 shows that in this case the effective permeability depends not only on the concentrations of the ions of given species, but also on the concentrations of the competitive ions. $P_K^{eff.}$ and $P_{Na}^{eff.}$ depend equally on the concentrations; they can differ only by a constant factor. Therefore the calculation of $\Delta\Gamma'$ with the use of Model B gives a result different from that in the case of Model A. As a matter of fact, the difference of potentials depends on one parameter, *i.e.* on the permeability ratio. If the effective permeabilities for K^+ and Na^+ depend on the concentration distribution in an identical manner, then the dependence of the potential difference on the concentrations must be the same as in the case of permeabilities which do not depend on concentrations. Therefore Model B must lead to Eqn. 3 for the resting potential. Let us show that this is true.

We can introduce in Model B the effective number of sites accessible to the ions of each species. This number is determined by parameters q and by the distribution of concentrations. It can be seen that Eqn. 4 is transformed into Eqn. 25 if we put instead of n_K^0 the effective number of exchange centers corresponding to K^+, equal to

$$n_K^{0\,eff.} = n^0 \frac{1 + Q_K}{1 - Q_K + Q_{Na}} \tag{26}$$

where

$$Q_i = \frac{q_{1i}c_i' + q_{2i}'c_i''}{q_{1i}' + q_{2i}} \tag{27}$$

The value Q_i is the ratio of probabilities of binding of the free ion of the species i to the free site in the membrane and of the removal of this ion from the membrane. This ratio increases with the affinity of the ion to the exchange centers. If the affinity of Na^+ is much smaller than the affinity of K^+, then $Q_{Na} \ll Q_K$ and $n_K^{0\,eff.} \cong n^0$. It means that Model B leads to the same expression for the flux of K^+ as Model A. The larger portion of exchange sites belongs to K^+.

Now instead of n_{Na}^0/n_K^0 we put the ratio of effective values $n_{Na}^{0\,eff.}/n_K^{0\,eff.}$ taken from Eqns. 26 and 27 into Eqn. 15 connecting the resting potential with concentration. We get the equation

$$r^2c_K' + c_K'' = \alpha(r^2c_{Na}' + c_{Na}'') \tag{28}$$

where α is a quantity which does not depend on concentrations. Putting $\alpha = P_{Na}/P_K$ and r from Eqn. 9 into Eqn. 28 we get Eqn. 2.

CONCLUSIONS

Thus experimental examination of Eqn. 2 allows one to distinguish the Models A and B. The experimental data obtained by Baker *et al.*[7,9] and TASAKI *et al.*[12] show that the resting potential cannot exceed some limiting value if we change the concentrations of K^+ in the intracellular and surrounding solutions. This result is explained only by Model A. Model B cannot account for this effect of "saturation".

The establishing of the passive permeability mechanism of biological membranes is also important, as it is the beginning of the theoretical study of the generation of nerve impulse in axons. The generation must be considered as the change of K^+

and Na^- currents in definite order resulting from the additional difference of potentials at the membrane of axon. It is known[22] that switching off the mechanism of the active transport does not influence the ability of membrane to produce the spike. It means that the excitation changes the passive fluxes of Na^+ and K^-. The calculation of the external field action on these fluxes is the next problem of the theory of transport processes in biological membranes.

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