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Nigericin-Induced Charge Transfer Across Membranes

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Summary. The electric properties of the bilayer lecithin membranes have been studied in the presence of the antibiotic nigericin. When the antibiotic concentration is about 10^{-6} M the conductivity of the BLM is increased up to 10^{-7} ohm^{-1} cm^{-2}. The potassium ion concentration gradient gives rise to a transmembrane potential of the order of 40 mV per 10-fold concentration gradient with the side of the higher potassium concentration negative. The transmembrane potential produced by the hydrogen ion concentration gradient is a function of the potassium ion concentration which is equal on both sides of the membrane. For low potassium ion concentrations the hydrogen potential has the expected polarity with the solution having higher concentration of protons negative. For potassium ion concentrations exceeding 0.03 M the hydrogen potential has the reverse polarity. This unexpected result cannot be accounted for in terms of the available simple hypotheses about the charge transport mechanism for nigericin in BLM. In order to account for the experimental results obtained, a theoretical approach has been developed based on the assumption that charge is transported across the membrane by nigericin dimers. The theoretical predictions are in satisfactory agreement with the experimental results. The model also yields some predictions which may be verified in future experiments.

The group of ionophore antibiotics including nigericin, monensin, dianemycin and X-206 has been attracting considerable interest lately (Pressman, 1973). These compounds are polyethers, polyalcohols and monocarboxylic acids with molecular weights in the range from 700 to 1000. They are capable of extracting alkali cations from aqueous solutions into organic solutions and effecting exchange transport of potassium and hydrogen ions in various biological systems (Pressman, 1968). Nigericin is the most extensively studied representative of this group. At low concentrations (about 10^{-7} M) nigericin inhibits mitochondrial respiration and at higher concentrations ($10^{-6}-10^{-5}$ M) it uncouples oxidative phosphorylation (Graven, Estrada-O. & Lardy, 1966; Estrada-O, Graven & Lardy, 1967; Henderson, McGivan & Chappel, 1969). Nigericin effects trans-

membrane exchange of potassium for protons in mitochondria at concentrations as low as 10^{-7} M (Henderson & Chappel, 1967; Pressman, Harris, Jagger & Johnson, 1967). Harris and Pressman (1967) observed a similar exchange of potassium for protons in erythrocytes, whereas Packer (1967) and Shavit, Thore, Keister and San Pietro (1968) detected this exchange in chloroplasts. This indicates that nigericin can complex with potassium. Using X-ray diffraction methods Steinrauf, Czerwinsky and Pinkerton (1971) have demonstrated that dissociated nigericin type antibiotics form complexes with alkali metals. In the process the carboxyl group is deprotonated and the antibiotic forms a collar around the cations. The complex remains stable as a result of the interaction between the cation and the dipole parts of the antibiotic and as a result of the hydrogen bond between the opposite ends of the molecule with the deprotonated carboxyl group on one side and the hydroxyl group on the other. The complex so formed is a zwitter-ion (Pressman, 1973). Thus, nigericin can transport alkali ions in an electrically neutral form of zwitter-ions and protons in an electrically neutral nondissociated form.

The effect of nigericin on membranes was formerly assumed to induce nonelectrogenic exchange of equal amounts of different ions which have no influence on the electrical characteristics (Pressman, 1968). But Mueller and Rudin (1969) found that nigericin induces conductivity in the bilayer lipid membranes, though this effect is relatively weak as compared to other ionophores. Moreover, they detected transmembrane potential difference of the order of 30–40 mV for a 10-fold ion gradient in the presence of nigericin. They suggested that charge is transported across the membrane by a dissociated antibiotic form, namely by the nigericin anion. However, this assumption has not been supported by any experimental evidence.

Fergusson, Estrada-O and Lardy (1971) advanced a different hypothesis. By analyzing the difference between monensin and dianemycin on one hand and nigericin on the other, they suggested that in the formation of cyclic complexes of nigericin with alkali ions the hydrogen bond between the opposite ends of the molecule plays a lesser role than in other antibiotics because the carboxyl group ligands directly with the cation whereas the carboxyl group of monensin and dianemycin does not. Therefore, they believed that nigericin may form a positively charged complex as it can retain proton at the carboxyl group. Thus, charge may be transferred across the membrane by such a complex cation.

We have studied the electric properties of the bilayer lipid membranes in the presence of nigericin and analyzed the possible mechanisms for the electric charge transport.

Materials and Methods

The conductivity and transmembrane potential differences were measured by the conventional method (Lebedev & Boguslavsky, 1971). Membrane spreading was controlled by measuring the capacitance at 0.2 Hz. The initial conductivity did not exceed 10^{-8} ohm^{-1} cm^{-2}. Measurements were made with silver chloride electrodes contacting the solutions through KCl (0.1 M)-agar bridges. The potentials were measured with an electrometer with a recorder readout. Membranes were obtained from a mixture of lecithin (20 mg/ml) and cholesterol (15 mg/ml) dissolved in decane. Different buffer solutions were used in the experiments (*see* Figures). Nigericin was added only on one side. Special experiments have demonstrated that qualitatively the results were the same when nigericin was added on the other side. The potassium concentration and pH were shifted by adding HCl and KCl to the cell.

Results

The membrane conductivity increased when nigericin was introduced into solution. Fig. 1 shows the membrane conductivity as a function of nigericin concentration in a solution containing 10^{-2} M KCl, pH 5.8.

When nigericin was added on one side of the membrane with equal concentrations of potassium and hydrogen ions on both sides the transmembrane potential was zero.

For equal pH values but different potassium ion concentrations a potential difference appeared across the membrane. This transmembrane potential difference is shown in Fig. 2 as a function of logarithm of the ratio between the potassium ion concentrations on two sides of the membranes. The solution with higher potassium ion concentration was nega-

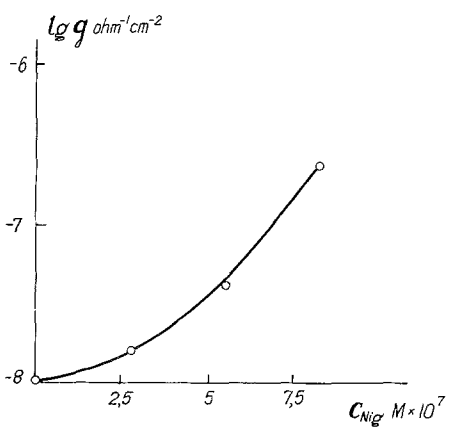

Fig. 1. Membrane conductivity *vs.* nigericin concentration added on one side of the membrane. The solution contained 0.01 M citric acid, 0.01 M NaCl, 0.01 M Na_2HPO_4, 0.01 M KCl. NaOH was added to a concentration of about 0.02 M to make pH 5.8

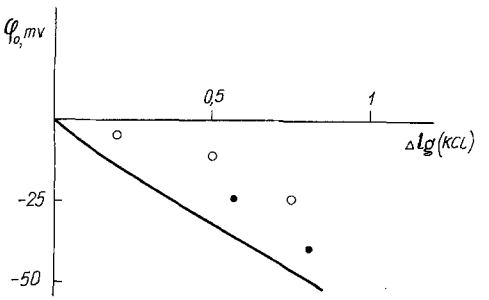

Fig. 2. Transmembrane potential *vs.* log of the ratio of the KCl concentrations on both sides of the membrane. The membranes were formed in the solution whose composition is described in Fig. 1. Nigericin was added to the solution on one side of the membrane (8×10^{-7} M). The dark circles are the potentials appearing when KCl was added on the same side as nigericin. The light circles denote the potentials appearing when KCl was added on the opposite side of the membrane. The curve was calculated according to Eq. (9)

tive. The potential difference was about 40 mV per 10-fold gradient of the potassium ions. As nigericin was added only on one side two methods were used to obtain the potassium gradient on the membrane: either KCl was added on the side containing nigericin or on the opposite side. From Fig. 2 it is clear that the potentials in these two cases do not differ appreciably. In the subsequent experiments HCl and KCl were added to the side containing no nigericin.

When there was a pH gradient across the membrane, the potential difference differed in magnitude and polarity depending on the potassium ion concentration. This dependence is shown in Fig. 3*a* for a pH difference of 2.3 units. The experimental results show three characteristic regions. For low potassium ion concentrations pH gradient gives rise to transmembrane potential differences with negative on the lower pH side. For intermediate potassium ion concentrations it assumes reverse polarity and for high potassium ion concentrations it is close to zero. The membrane conductivity was measured in the same experiment (Fig. 3*b*). It increases with increasing potassium ion concentration.

The transmembrane potential difference is plotted in Fig. 4 as a function of the pH gradient across the membrane. The potassium ion concentration was 0.1 M in both solutions corresponding to the maximum potential difference in Fig. 3*a*. The solution with higher pH value was found to be negative. For high pH gradients the transmembrane potential difference was unstable. The broken line shows the shift in the experimental points with time up to the moment when the membrane broke down.

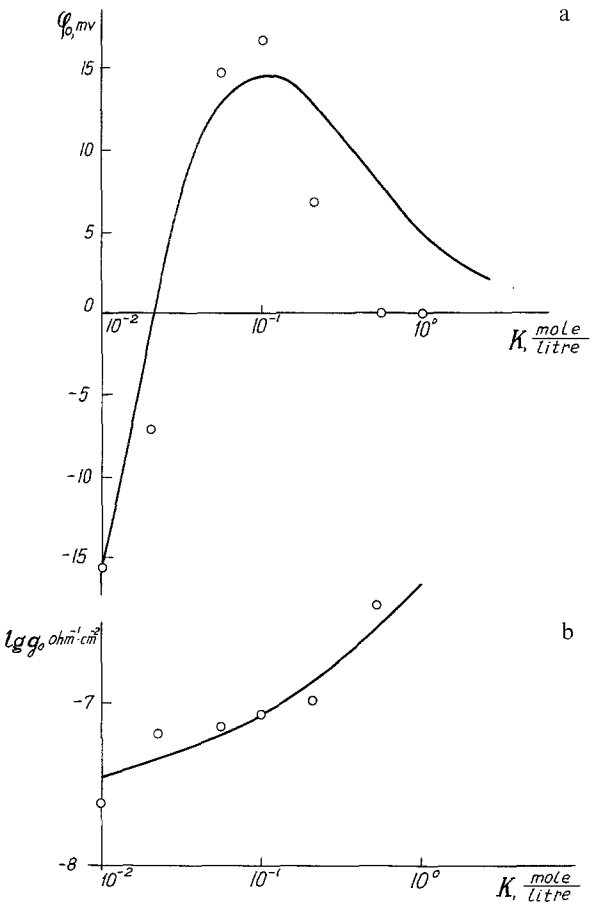

Fig. 3. Variation in the electrical properties of the membrane with KCl concentration in the solutions for the constant pH gradient of 2.3. The membranes were formed in the solution containing 0.1 M citric acid, 0.01 M Na_2HPO_4, 0.01 M NaCl, and KCl. NaOH was added to about 0.02 M to a pH of 5.8. KCl concentration is plotted along the x-axis. Nigericin (8×10^{-7} M) was added on one side of the membrane to the solution. Drops of HCl were added on the opposite side to give rise to pH gradient. (*a*) The transmembrane potential. The maximum potentials measured are shown. The curve is plotted according to Eq. (9). (*b*) The membrane conductivity. The curve is plotted according to Eq. (27)

Ion Transport Model

Let the total concentration of nigericin inside membrane be b.

The ionophore may be present in the ionized form T^-, as neutral molecules $L = HT$ or potassium complexes $R = KT$. At the membrane interface dissociation equilibrium exists between the forms T, L and R in the membrane and the ions H^+ and K^+ in the solution:

$$(H)\,[T] = \zeta_L [L] \qquad (K)\,[T] = \zeta_R [R]. \tag{1}$$

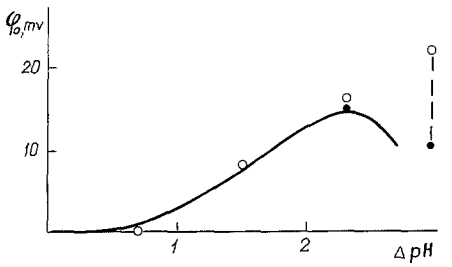

Fig. 4. Transmembrane potential *vs.* pH gradient. The membranes were formed in solution whose composition is described in Fig. 1. Nigericin (8×10^{-7} M) was added to the solution on one side of the membrane. Drops of HCl were added on the opposite side of the membrane to give rise to a pH gradient. The dotted line represents the virtual decrease of the potential up to the point of the membrane rupture. The solid curve was calculated according to Eq. (9)

The parentheses indicate concentration in the solution and the square brackets, concentrations in the membrane. In both cases the concentrations are taken close to the interface. The factors ξ_L and ξ_R are the dissociation constants for the respective complexes. It is convenient to introduce the dimensionless concentrations of hydrogen and potassium

$$(\bar{\mathrm{H}}) = \frac{(\mathrm{H})}{\zeta_L}; \quad (\bar{\mathrm{K}}) = \frac{(\mathrm{K})}{\zeta_R}. \tag{2}$$

Thus, we can write Eq. (1) in a simpler form as follows:

$$(\bar{\mathrm{H}})\,[T] = [L]; \quad (\bar{\mathrm{K}})\,[T] = [R]. \tag{3}$$

The concentrations of hydrogen and potassium in the solution will be denoted by H and K, respectively. When there are no concentration gradients in the nonstirred layers close to the membrane the parentheses in Eqs. (1) and (3) can be omitted.

We shall assume that the ion T^- and the molecule L may combine in the membrane forming dimer $M^- = TL^- = \mathrm{H}\,T_2^-$.[1] The equilibrium between these forms is described by the following equation:

$$[T]\,[L] = \zeta_M [M]. \tag{4}$$

Furthermore, assume that the potassium dimer $S^- = TR^- = \mathrm{K}\,T_2^-$ exists and its concentration is related to the concentrations of other species by the following equation:

$$\zeta_S [S] = [T]\,[R] = (\bar{\mathrm{K}})\,[T]^2. \tag{5}$$

1 A similar assumption was made recently by Celis *et al.* (1974) for antibiotic X-537 A.

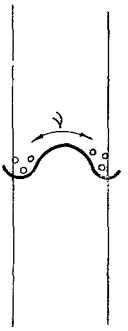

Fig. 5. Schematical representation of the membrane

We shall assume that in the membrane the species move by the Eyring hopping mechanism; that is, in the membrane the species are located in the potential wells near the surface (*see* Fig. 5) hopping between them with the rate constants v_T, v_L, v_M, v_R and v_S. In the subsequent text we shall show that the motion of the ions T^- can be neglected so that $v_T=0$. The potential φ applied to the membrane is assumed to change the hopping rates of the charged particles by a factor of $e^{\pm\psi/2}$ where $\psi=\beta\varphi$ and β is Faraday's constant divided by the gas constant and the absolute temperature. Under normal conditions $\beta=1/25\ \mathrm{mV}^{-1}$. The transmembrane potential difference is expressed with respect to the right-hand solution arbitrarily taken to be zero. The fluxes from left to right are regarded positive.

One of the equations characterizing the membrane is the equation of flux of matter. We shall assume that nigericin in the membrane works as an ion carrier. It means that nigericin molecules repeatedly shuttle inside the membrane before they cross its boundaries, so the net nigericin flux across membrane is negligible in comparison with the net ion fluxes. Therefore we can approximately assume that the total flux of all forms of nigericin across the membrane in the steady state is zero:

$$\begin{aligned} &v_L[L]_1 - v_L[L]_2 + 2v_M e^{-\frac{1}{2}\psi}[M]_1 - 2v_M e^{\frac{1}{2}\psi}[M]_2 \\ &\quad + v_R[R]_1 - v_R[R]_2 + 2v_S e^{-\frac{1}{2}\psi}[S]_1 - 2v_S e^{\frac{1}{2}\psi}[S]_2 = 0. \end{aligned} \tag{6}$$

The subscript 1 denotes the left-hand potential well and 2, the right-hand well. The concentration of ions T^- does not enter the equation as their mobility is assumed to be low. The factor 2 in the terms for dimers shows that each dimer contains two nigericin ions.

Another equation gives the relationship between the concentrations of the individual forms and the total amount of nigericin:

$$\begin{aligned} &[T]_1 + [L]_1 + [R]_1 + 2[M]_1 + 2[S]_1 + [T]_2 \\ &\quad + [L]_2 + [R]_2 + 2[M]_2 + 2[S]_2 = 2b. \end{aligned} \tag{7}$$

Eqs. (1)–(7) fully define the problem in steady state and in principle they can be used to determine all concentrations and, hence, all fluxes across the membrane for any potential. In particular, the steady-state electric current across the membrane is

$$J = -F\{v_M e^{-\frac{1}{2}\psi}[M]_1 - v_M e^{\frac{1}{2}\psi}[M]_2 + v_S e^{-\frac{1}{2}\psi}[S]_1 - v_S e^{\frac{1}{2}\psi}[S]_2\} \qquad (8)$$

where F is Faraday's constant. With the help of this equation we can find the transmembrane potential difference with an open external circuit in an asymmetrical system. For this the electric current must be taken to be zero and the resulting equation should be solved for the potential.

Our ion transport model is illustrated in Fig. 6. The lines represent the possible paths of the species across the membrane and the intersection points represent the formation or decomposition of the complexes. In terms of the carrier theory (Markin & Chizmadzhev, 1974) we may say that hydrogen and potassium ions are transferred by the collective transport with a small loop. The upper part of Fig. 6 shows the loop effecting the proton transport: the dimers HT_2^- move in one direction and the neutral molecules in the opposite direction. The small loop shown below carries out similar transport of potassium ions. However, these two loops are not independent; they are coupled via the common ion T^-. Though this ion itself does not move in the membrane it acts as a via media for redistributing concentrations of various forms of nigericin in the membrane. Therefore, both loops may strongly influence each other up to the point when the easiest pathway across the membrane may be composed of different parts belonging to both loops.

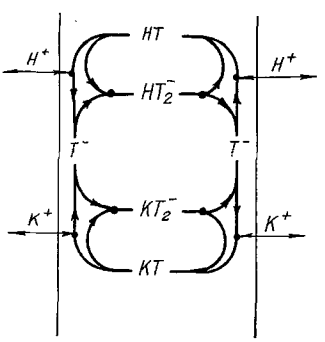

Fig. 6. The ion transport model involving nigericin. The lines with arrows indicate virtual mechanisms and pathways of the species motion in the membrane; the intersection points represent the reactions of decomposition and formation of complexes from the respective species

Electrical Properties of the Membrane

Solving the system of Eqs. (1)–(7) we find that the transmembrane potential is

$$\psi_0 = \ln \frac{(\bar{H}_1 + \gamma \bar{K}_1)(\bar{H}_2 + \alpha \bar{K}_2)^2}{(\bar{H}_2 + \gamma \bar{K}_2)(\bar{H}_1 + \alpha \bar{K}_1)^2} \tag{9}$$

where

$$\alpha = \frac{v_R}{v_L}; \qquad \gamma = \frac{\zeta_M v_S}{v_M \zeta_S}. \tag{10}$$

Eq. (9) contains two parameters α and γ besides the concentrations of hydrogen and potassium ions in the left-hand and right-hand solutions. The relation between these parameters determines the magnitude and polarity of the transmembrane potential.

Consider the properties of the transmembrane potential. If there is no potassium in the system then Eq. (9) is transformed into the Nernst formula

$$\psi_0 = -\ln \frac{H_1}{H_2}. \tag{11}$$

When the proton concentration on the left-hand side is greater than on the right the transmembrane potential is negative.

If the potassium concentrations in both solutions are the same the transmembrane potential is caused only by the difference in the proton concentrations. This potential will be called the "hydrogen" potential in contrast to the "potassium" potential caused by the difference in potassium ion concentration.

First consider the hydrogen potential for low proton concentration gradients: $\Delta H = H_1 - H_2$. We have

$$\psi_0 = \frac{\Delta \bar{H}}{\bar{H}_2 + \gamma \bar{K}} - \frac{2\Delta \bar{H}}{\bar{H}_2 + \alpha \bar{K}}. \tag{12}$$

The second term gives the "normal" or "Nernst" potential polarity and the first, the "anti-Nernst" polarity. It is this term that can lead to inversion of the transmembrane potential.

Inversion of the transmembrane potential depends on the relation between α and γ. This may be easily demonstrated by expressing Eq. (12) as

$$\psi_0 = \frac{(\alpha - 2\gamma)\bar{K} - \bar{H}_2}{(\bar{H}_2 + \gamma \bar{K})(\bar{H}_2 + \alpha \bar{K})} \Delta \bar{H}. \tag{13}$$

For the potential to be positive, we must have

$$\alpha > 2\gamma. \tag{14}$$

Then for sufficiently high K the potential will be positive and inversion occurs at the point

$$\bar{K}_{rev} = \frac{\bar{H}_2}{\alpha - 2\gamma}. \tag{15}$$

For high potassium ion concentrations the potential remains positive, at first rising with increasing potassium concentration, reaching maximum and then decreasing asymptotically to zero. However, Eq. (14) is valid for any concentration gradient. This may be readily seen from the general formula (9).

The potential inversion point is

$$\bar{K}_{rev} = \frac{\gamma(\bar{H}_1 + \bar{H}_2) + \sqrt{\gamma^2(\bar{H}_1 - \bar{H}_2)^2 + 4(\alpha - \gamma)^2 \bar{H}_1 \bar{H}_2}}{2\alpha(\alpha - 2\gamma)}. \tag{16}$$

The potential reaches maximum when the potassium ion concentration is

$$\bar{K}_{max} = \frac{\bar{H}_1 + \bar{H}_2}{2(\alpha - 2\gamma)} + \sqrt{\frac{(\bar{H}_1 + \bar{H}_2)^2}{4(\alpha - 2\gamma)^2} + \frac{(2\alpha - \gamma)\bar{H}_1 \bar{H}_2}{\alpha\gamma(\alpha - 2\gamma)}}. \tag{17}$$

The position of these characteristic points on the axis of potassium concentrations depends on the proton concentration – the farther they are to the right, the higher the proton concentration.

If Eq. (14) is not satisfied then there is no "anti-Nernst" region (i.e. where the transmembrane potential is positive). But the plot of the hydrogen potential *vs.* the potassium ion concentration may, nevertheless, have a maximum only in the "Nernst" region. Three characteristic plots of the hydrogen potential as a function of the potassium ion concentration are shown in Fig. 7. Curve 1 is obtained for

$$\alpha > 2\gamma. \tag{18}$$

It is here that the transmembrane potential inversion is observed. Curve 2 corresponds to the region

$$\frac{\gamma}{2} < \alpha < 2\gamma. \tag{19}$$

Under these conditions there is neither the transmembrane potential inversion nor maximum on the plot of ψ_0 as a function of K. Finally, for

$$\alpha < \frac{\gamma}{2} \tag{20}$$

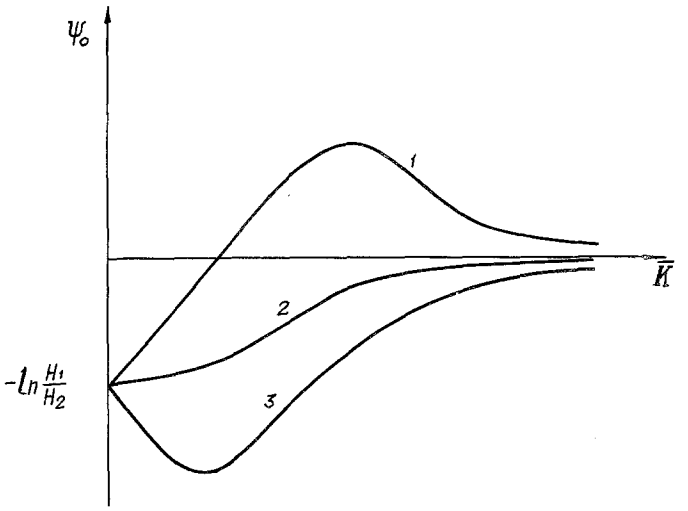

Fig. 7. Predicted variation of hydrogen potential with potassium ion concentration. Curves 1, 2 and 3 correspond to Eqs. (18), (19) and (20), respectively

we obtain curve 3; here the transmembrane potential remains negative throughout the region (in the "Nernst" region) but the curve has a maximum. The position of the maximum is given by Eq. (17) as before. The maximum potential is greater than that of the "Nernst" potential $\left|\ln\frac{H_1}{H_2}\right|$. This unusual result is a special feature of the collective transport model.

We have analyzed the hydrogen potential. However, the system is, in general, symmetrical with respect to potassium and hydrogen. All the effects found for the hydrogen potential should be applicable to the potassium potential. Eq. (9) corroborates this statement. For different potassium ion concentrations in solutions and equal proton concentrations on both sides of the membrane we may analyze the dependence of the transmembrane potential on the proton concentration. In this case we obtain the curves analogous to those shown in Fig. 8 but the conditions will be different. The curve of type 1 for the potassium potential inversion is obtained when Eq. (20) is satisfied. A steady curve of type 2 belonging completely to the "Nernst" region is obtained when Eq. (19) is satisfied. And, finally, the curve of type 3 is realized when Eq. (18) is satisfied.

A comparison of these conditions demonstrates that the same system cannot simultaneously exhibit reversion of both potassium potential and hydrogen potential. For given parameters the system may exhibit either the potassium potential inversion or the hydrogen potential inversion or none.

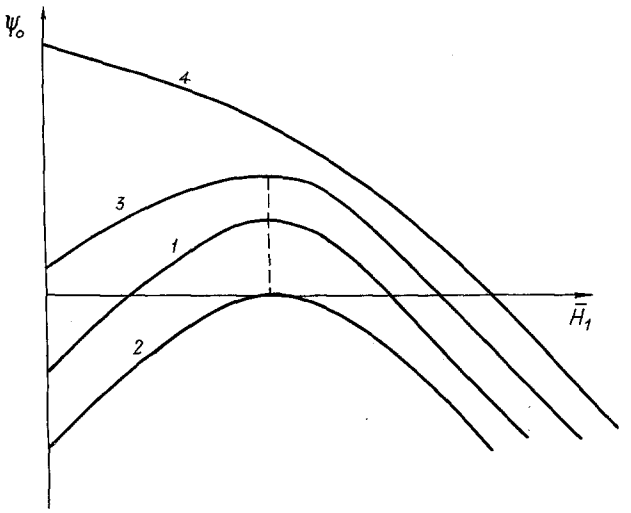

Fig. 8. Predicted variation of hydrogen potential with proton concentration gradient. The concentration is varied in the left-hand solution whereas the concentration in the right-hand solution is constant. Curves 1, 2 and 3 correspond to Eq. (18) and, also, curve 1 corresponds to small proton concentrations in the right-hand solution, curve 2 corresponds to the concentration described by Eq. (22) and curve 3 corresponds to the concentrations exceeding the value given by Eq. (23). Curve 4 is realized when Eq. (18) is not satisfied

Now consider the dependence of the hydrogen potential on the proton concentration H_1 in one solution for a given constant proton concentration H_2 in the other solution and for equal potassium concentrations. The shape of the curve is determined by the relation between α and γ. We shall assume that the parameters satisfy Eq. (18). The H_1 dependence of the potential is given by one of the curves 1, 2 or 3 in Fig. 8, plotted for three different concentrations of protons H_2 in the right-hand solution. For low H_2 we obtain curve 1 originating in the negative potential region, it intersects the x-axis and enters the positive potential region where it passes through the maximum and then it again intersects the x-axis returning to the negative potential region. This behavior of the transmembrane potential is not usual. The "normal" or "Nernst" curve would intersect the x-axis only once at the point $H_1 = H_2$ from above. The curve of this type is denoted in Fig. 8 by 4. In contrast to curve 4, curve 1 has two intersections with the x-axis – on the left at point $H_1 = H_2$ and on the right at point $\bar{H}_1 = \dfrac{\alpha(\alpha - 2\gamma)\bar{K} - \gamma\bar{H}_2}{\bar{H}_2 + \gamma\bar{K}}$.

If the parameter H_2 is increased, the transmembrane potential curve is somewhat deformed and lies below. The points of intersection with the

x-axis close up but the maximum remains as before at the point

$$\bar{H}_1 = (\alpha - 2\gamma)\,\bar{K}. \tag{21}$$

This downward shift of the curve with increasing H_2 continues until H_2 reaches

$$\bar{H}_2 = (\alpha - 2\gamma)\bar{K}. \tag{22}$$

In this case the intersection points coincide and thus the curve is tangential to the x-axis (curve 2 in Fig. 9). The whole curve in this case lies below the x-axis.

With further increase in $\bar{H}_2$ again two intersection points reappear and the curve shifts upward. This curve is similar to curve 1 in shape but its intersection points with the x-axis are opposite. When $\bar{H}_2$ exceeds

$$\bar{H}_2 = \frac{\alpha}{\gamma}(\alpha - 2\gamma)\bar{K} \tag{23}$$

the left intersection point vanishes and the curve begins in the positive potential range (curve 3). Furthermore, its maximum has the same abscissa.

These three curves (1, 2 and 3) are plotted for the case when Eq. (18) is satisfied. If the parameters do not satisfy this condition the transmembrane potential may be represented by curve 4 in Fig. 8. We have considered the hydrogen potential. In the general case the same results may be obtained for the potassium potential as well.

Now consider the membrane conductivity. Under asymmetrical conditions the conductivity for low currents is defined as the ratio between the current and the deviation of the transmembrane potential differe from the steady-state value given by Eq. (9):

$$g_0 = \left.\frac{dJ}{d\varphi}\right|_{\varphi=\varphi_0} \tag{24}$$

Solving the above system of equations we may find the conductivity of the membrane

$$g_0 = \frac{\beta F \frac{v_M}{\zeta_M} b^2 \sqrt{(\bar{H}_1 + \gamma\bar{K}_1)(\bar{H}_2 + \gamma\bar{K}_2)}\,(\bar{H}_1 + \alpha\bar{K}_1)(\bar{H}_2 + \alpha\bar{K}_2)}{W\left[W + \frac{4bv_M}{\zeta_M v_L}\sqrt{(\bar{H}_1 + \gamma\bar{K}_1)(\bar{H}_2 + \gamma\bar{K}_2)}\right]} \tag{25}$$

where

$$W = \tfrac{1}{2}(\bar{H}_2 + \alpha\bar{K}_2)(1 + \bar{H}_1 + \bar{K}_1) + \tfrac{1}{2}(\bar{H}_1 + \alpha\bar{K}_1)(1 + \bar{H}_2 + \bar{K}_2). \tag{26}$$

To simplify the results we have introduced an additional assumption in deriving this formula. The amount of dimers in the membrane is assumed to be less as compared to other forms of nigericin. It does not alter the qualitative results but gives comparatively simple formulas.

Under symmetrical conditions when $\bar{H}_1 = \bar{H}_2 = \bar{H}$ and $\bar{K}_1 = \bar{K}_2 = \bar{K}$ the expression for conductivity is:

$$g_0 = \frac{\beta F \frac{v_M}{\zeta_M} b^2}{(1+\bar{H}+\bar{K})\left[\frac{1+\bar{H}+\bar{K}}{\bar{H}+\gamma\bar{K}} + \frac{4bv_M}{\zeta_M v_L}\frac{1}{(\bar{H}+\alpha\bar{K})}\right]}. \tag{27}$$

Let us analyze the concentration dependences of the conductivity. Take $\alpha \gg \gamma \gg 1$ and $\bar{H} \gg 1$. If $\bar{H}$ is constant and $\bar{K}$ varies the conductivity will vary as shown by curve 1 in Fig. 9. In semi-logarithmic coordinates this curve has two plateaus and one maximum. The coordinates of the characteristic points are indicated in the plot. The relative heights of the plateaus depend on the relation between the parameters of the system. Unlike the transmembrane potential which depends on two parameters α and γ the conductivity variation with concentration depends on one more parameter $\frac{4bv_M}{\zeta_M v_L}$. If $\frac{4bv_M}{\zeta_M v_L} \ll 1$ the heights of the two plateaus of curve 1 may become the same; thus we obtain curve 2. However, both these curves have maximum near the point $\bar{K} = \bar{H}$. The maximum exists as a consequence of the assumption that α and γ are greater than unity. By definition it means that the potassium compounds of nigericin have higher mobility than the hydrogen compounds. By increasing the potassium concentration we trans-

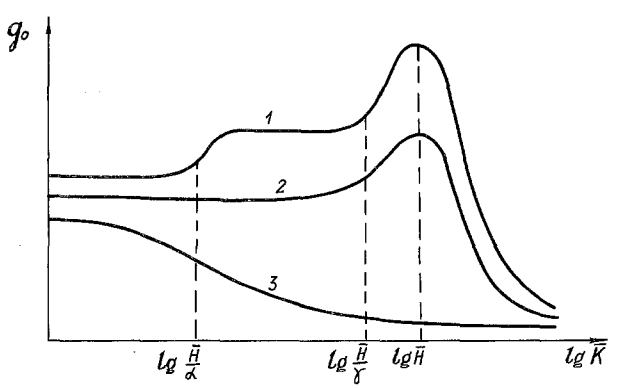

Fig. 9. Predicted variation of membrane conductivity with potassium ion concentration. *See text* for details

fer nigericin from the hydrogen forms into the potassium forms so that the conductivity increases reaching the maximum. With further increase in the potassium concentration in the membrane nigericin is lacking in ionized form and the conductivity decreases.

If the potassium compounds are less mobile than the hydrogen compounds, that is, if α and γ are considerably less than unity, then with variation of the potassium ion concentration the conductivity has no maximum and steadily decreases to zero (curve 3).

If ions of only one type are present in the system (hydrogen or potassium ions), Eq. (27) is transformed into the expression for the collective transport conductivity derived by Markin and Chizmadzhev (1974).

We have derived all the formulas required for analyzing the available experimental material. The next stage is to compare the theory with the experimental results and to determine parameters of the model. Naturally, we shall begin with those characteristics which require the least number of parameters for their description. The most important characteristic of this type is the dependence of the hydrogen potential on the concentration of potassium ions (Fig. 3*a*). We have only two fitting parameters α and γ for describing this dependence by Eq. (9). If we determine these parameters, for instance, from the coordinate of the maximum and inversion potentials, then the other properties of the curve, for example, its shape and amplitude are automatically obtained and cannot be varied arbitrarily. Accordingly, we have no free parameters for describing the dependence of the hydrogen potential on the pH gradient of the solutions and the dependence of the potassium potential on the transmembrane potassium concentration gradient. Therefore, even such fitting of the theoretical curves to the experimental points provides a sufficiently rigorous test of the applicability of the suggested model to this case. Thus, the following parameter values have been obtained:

$$\alpha = 3000, \qquad \gamma = 500. \tag{28}$$

Figs. 2–4 show the theoretical curves plotted for such parameter values. For constructing these curves we have taken $\xi_L = 10^{-10}$ moles/liter given by Pressman (1968) [according to Lutz, Früh and Simon (1971), $\xi_R = 10^{-6}$ moles/liter.[2]] The theoretical curves are satisfactorily close to the experimental points.

Now consider another experimental characteristic, namely, the dependence of the conductivity on the potassium concentration (Fig. 3*b*).

2 These constants were measured in rather different conditions. Unfortunately these are the only values available so we had to use them.

A comparison of the curve given by Eq. (25) with the experimental results demonstrates that $\frac{4bv_M}{\zeta_M v_L} \gg 10^7$. In this case there is a good agreement between the theoretical and experimental results. If we know the conductivity amplitude we can determine another parameter, namely, $bv_L = 2 \times 10^{14}$ moles $cm^{-2} sec^{-1}$.

Discussion

For analyzing our experimental results we have first considered the existing hypotheses on the possible mechanisms for the transport of charge across membranes induced by nigericin. There are only two such hypotheses. Mueller and Rudin (1969) suggested a mobile dissociated form of nigericin T^- whereas Fergusson, Estrada-O. and Lardy (1971) proposed the existence of the charged complex HKT^+. However, these hypotheses, either separately or together, cannot account for the transmembrane potential of variable polarity that we observed.

Then we tried to explain these results in terms of the induced ion transport across membranes. Today this theory in its present form is based on the models of carriers, relay-race and collective transport (Markin & Chizmadzhev, 1974). But neither the carrier model nor the relay-race model explains the transmembrane potential of variable polarity. This effect has been predicted theoretically only in the collective transport model though under entirely different conditions (Markin & Chizmadzhev, 1974). Thus, we were compelled to consider the collective transport model, or more exactly, a special case of it – the model of dimers.

In order to obtain the hydrogen potential of variable polarity it is sufficient to assume the existence of the mobile hydrogen dimer $M^- = HT_2^-$. If the main charge carriers are the dimers M^- and the membrane is permeable for the molecules $L = HT$ and $R = KT$ then the pH gradient between the solutions leads to the following results. At first, let us assume that there is no potassium in the system. Fig. 10*a* shows the concentration distribution (in an arbitrary scale). The proton concentration in the left-hand solution is greater than in the right-hand solution. When the external circuit is disconnected neither dimers M nor molecules L form flow across the membrane. (Naturally, this does not mean that the unidirectional fluxes are zero but we have in view only the resultant fluxes.) As the electric field does not affect the neutral molecules L, their concentration in the membrane must be constant. The equations of the dissociation equilibrium (1) suggest that the concentration of the ions T^- in the vicinity of the left-

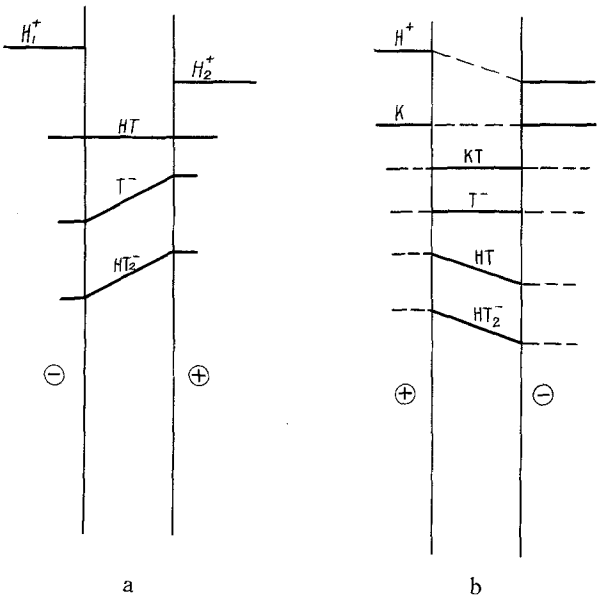

Fig. 10. Distribution of the species in the membrane and the transmembrane potential: (*a*) In the absence of potassium; (*b*) in the presence of potassium

hand boundary is less than that in the vicinity of the right-hand since the proton concentration there is greater and the concentration of the molecules L is constant everywhere. As the concentration of the complexes M is proportional to the product of the concentrations of T and L, on "multiplying" the respective curves we readily obtain the distribution of dimers in the membrane. As may be seen from the plot the dimer concentration at the left-hand boundary is less than that at the right-hand boundary. Thus, when the external circuit is disconnected the concentration gradient of the charged particles M^- should be observed in the membrane. In the absence of electric current this gradient is compensated by the spontaneous transmembrane potential difference with the negative pole in the left-hand solution and the positive pole in the right-hand solution.

Let us now introduce potassium into the system. It gives rise to potassium complex $R = \mathrm{K}T$ in the membrane in addition to the compounds already mentioned. This new species will change the concentration distribution. This change will depend on the mobility of the complex R. In order to obtain the most pronounced effect let us assume its mobility is far greater than the mobility of the molecule L. Thus, there will be practically no gradient of the R complex concentration in the membrane. This fact is represented by the horizontal line in Fig. 10*b*. The concentrations of the potassium ions and of the complexes R determine the concen-

tration of the ions T^- (*see* Eq. 1) which is also constant. If we know distributions of T^- and H^+ we may readily find the distribution of the molecules L and then of the dimers M^-. As may be seen from the plot the concentration of the dimers M^- is greater at the left-hand interface than at the right-hand interface. When the external circuit is disconnected the concentration gradient of the dimers M^- is compensated by the transmembrane potential difference with the negative pole in the right-hand solution and the positive pole in the left-hand solution. Thus, introduction of potassium into the system leads to repolarization of the membrane.

However, the existence of the dimer M^- is not adequate to give a full account of the experimental results. Potassium in the system may equalize the T^- concentration and subsequently repolarize the membrane only if it is present in sufficiently great quantities. On further increasing the potassium concentration the transmembrane potential should reach its saturation level and cease to vary. However, the experiments demonstrate that after repolarization of the membrane (potential inversion) the potential passes its maximum and decreases to zero with further increase in the potassium concentration (Fig. 3*a*). This behavior of the potential may be explained by assuming that the membrane is shunted by some other ions. Indeed, this effect may be attributed to the nigericin ions T^-. However, we have to show that these ions are capable of shunting the membrane so that this shunting is highly effective at high potassium concentrations and practically absent for intermediate concentrations.

Addition of potassium leads to decreased concentrations of the ions T^- and the molecules L. The concentration of the dimers M^- in the membrane which is proportional to the product of concentrations of T^- and L has an even higher rate of decrease. Hence, the membrane conductivity with respect to the dimers M^- generating the transmembrane potential decreases faster than the conductivity with respect to the shunting ions T^-. The relative contribution of the shunt increases with increasing potassium concentration and the transmembrane potential may disappear. Thus, the behavior of the transmembrane potential as a function of the potassium concentration may be accounted for. But in this case the total membrane conductivity should decrease with increasing potassium concentration. However, the experimental results are contrary – the membrane conductivity increases with growing potassium concentration (Fig. 3*b*). Hence, the ions T^- cannot shunt the membrane!

Therefore we have to assume that nigericin forms some other ion in the membrane. Such shunting ion might be the potassium complex with nondissociated nigericin KHT^+ suggested by Fergusson *et al.* (1971).

It is hard to disprove this mechanism based only on the general considerations. Therefore, we have analyzed this model (*see* Appendix) demonstrating that its predictions are in contradiction with the experimental results.

Thus, we have to assume that the potassium dimer KT_2^- is the shunting species. The above analysis supports this assumption.

Thus, the electric characteristics of the membrane show that the potassium and hydrogen dimers of nigericin can transport charge across the membrane. Of course, these observations are not sufficient to prove our conclusion finally. Various investigations, particularly, the electric properties of membranes in the presence of nigericin are needed to verify this hypothesis. For instance, it may be of interest to verify the theoretical prediction that the potassium potential reversion cannot occur in the system with reversion of the hydrogen potential. It is also of importance to study in more detail the dependence of the membrane conductivity on the concentration of the ions transported since the theory predicts a characteristic shape of the curve with the maximum.

Analyzing the electrical properties of the membrane we concluded that nigericin inside the membrane commutes between its interfaces carrying ions from one solution to another. This kind of motion was called the small loop (Markin & Chizmadzhev, 1974). But it is by no means a fundamental assumption. Similarly, we may consider another case where nigericin enters into the solution through the interfaces so that its motion is limited not by membrane interfaces but the adjacent nonstirred solution layers. The results will be the same since they may be derived directly from the above equations.

The experimental data suggest that the dimers are considerably more effective in transporting charges across the membrane than the nigericin anions and that permeability for the potassium compounds is considerably higher than permeability for the hydrogen compounds. Let us compare these with the data available on the properties of ionophores and nigericin. The effective transmembrane exchange of the ions K^+ and H^+ by nigericin in the electrically neutral form demonstrates that the permeability of the membrane for the neutral form of molecule is considerably higher than permeability for the negatively charged ion.

The charged molecules are known to have higher permeability when they complex with an electrically neutral hydrophobic molecule. This evidently explains the fact that some protonophores carry charge across the membrane in the form of dimers THT^- (McLaughlin, 1972).

Many protonophores are capable of complexing with complicated hydrophobic molecules (Liberman, Topaly & Topaly, 1971), thereby

sharply increasing their efficiency as charge carriers across membranes. It seems that a similar situation is true for the nigericin dimers.

X-ray diffraction studies of nigericin and its metal complexes (Steinrauf *et al.*, 1971) have demonstrated that potassium complexes of nigericin have cyclic structure in contrast to the nondissociated form of the antibiotic which is linear in configuration. This structural difference accounts for different mobilities of the hydrogen and potassium complexes and dimers. Thus, the assumptions stipulated in the formal model do not contradict the data on the properties of nigericin and other ionophores.

Side by side with the charge transport across the membrane in the presence of nigericin, intensive transmembrane exchange of protons for potassium takes place. The exchange fluxes seem to exceed greatly the flux die to the external electric field. If the exchange fluxes are assumed to be mainly due to the monomeric nigericin complexes the above theory demonstrates that the exchange fluxes are given by

$$j_{\text{exch}} = \frac{2bv_L v_R(\bar{H}_1\bar{K}_2 - \bar{H}_2\bar{K}_1)}{(v_L\bar{H}_1 + v_R\bar{K}_1)(1+\bar{K}_2+\bar{H}_2)+(v_L\bar{H}_2+v_R\bar{K}_2)(1+\bar{K}_1+\bar{H}_1)}. \tag{29}$$

The exchange transport varies the hydrogen and potassium ion concentrations in two solutions separated by the membrane. As may be seen from Eq. (29) the exchange of potassium for protons ceases when

$$\frac{H_1}{H_2} = \frac{K_1}{K_2}. \tag{30}$$

Pressman (1968) made a similar conclusion.

However, the above condition does not mean that all types of transport cease in the membrane. Firstly, nigericin will continue to carry out the transmembrane exchange of protons for protons and potassium ions for potassium ions. Secondly, electric current may flow in the membrane. Indeed, when Eq. (30) is satisfied the transmembrane potential of the disconnected circuit given by Eq. (9) is

$$\psi_0 = \ln\frac{H_2}{H_1} = \ln\frac{K_2}{K_1}. \tag{31}$$

If the membrane potential is different from the potential (31) due to some factors this will give rise to electric current in the membrane caused by the presence of nigericin. This electric current vanishes only when the transmembrane potential coincides with the value of Eq. (31).

Appendix

Consider another version of the ion transport model across the membrane allowing for reversion of the transmembrane potential. The reversion of the transmembrane potential caused by the hydrogen ion gradient with potassium addition necessitates charge transport across the membrane by the dimer HT_2^-. Moreover, one more charged species must move in the membrane which should be capable of shunting the membrane and "dumping" the potential when potassium is added. Here we shall analyze the hypothesis put forward by Fergusson *et al.* (1971) suggesting that the positively charged complex of nondissociated nigericin with potassium KHT^+ exists in the membrane.

Thus, assume that in the membrane the complex $W = KHT^+$ may exist along with the dissociated nigericin T^-, the neutral molecule $L = HT$, the potassium complex $R = KT$ and the hydrogen dimer $M = HT_2^-$. The equations for the steady-state problem are:

$$T_1 + L_1 + R_1 + W_1 + 2M_1 + T_2 + L_2 + R_2 + W_2 + 2M_2 = 2b.$$

$$v_L L_1 - v_L L_2 + v_R R_1 - v_R R_2 + 2v_M M_1 e^{-\frac{1}{2}\psi} - 2v_M M_2 e^{\frac{1}{2}\psi} + v_W W_1 e^{\frac{1}{2}\psi} - v_W W_2 e^{-\frac{1}{2}\psi} = 0.$$

$$J = F(-M_1 v_M e^{-\frac{1}{2}\psi} + M_2 v_M e^{\frac{1}{2}\psi} + v_W W_1 e^{\frac{1}{2}\psi} - v_W W_2 e^{-\frac{1}{2}\psi}).$$

The transmembrane potential for small gradients is given by

$$\psi_0 = \frac{-v_L v_M LM - v_R v_W RW - 3v_M v_W MW + v_R v_M RM}{(v_L L + v_R R)(v_M M + v_W W) + 9v_M v_W MW} \cdot \frac{\Delta H}{H} - \frac{2v_R v_M RM + v_L v_W LW + 6v_M v_W MW}{(v_L L + v_R R)(v_M M + v_W W) + 9v_M v_W MW} \cdot \frac{\Delta K}{K}.$$

This expression consists of two terms – the "hydrogen" term and the "potassium" term. They are proportional to the concentration gradients of the respective ions. The potassium term is of the "Nernst" polarity, that is, its negative pole is in the solution with higher potassium concentration. The hydrogen term is more complex since its numerator includes both positive and negative terms. Hence, the hydrogen potential may change its sign.

Now consider the conditions corresponding to our experiment. The potassium ion concentrations in both solutions are assumed to be equal and to vary simultaneously. Then the above expression demonstrates that for low potassium ion concentrations the transmembrane potential is of the "Nernst" polarity, that is, its negative pole is in the solution with

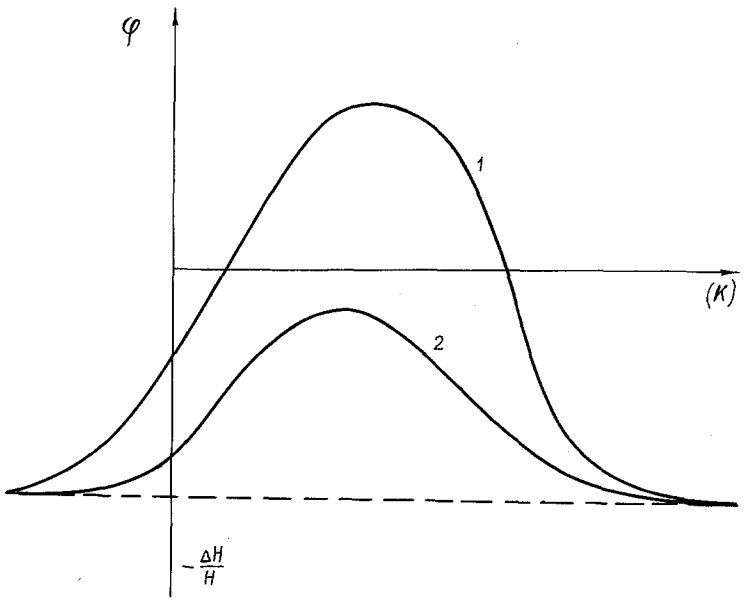

Fig. 11. Hydrogen potential *vs.* potassium ion concentration in the model with the shunting complex KHT^+. Curves 1 and 2 correspond to different parameters of the model

higher hydrogen concentration. If the nigericin concentration is sufficiently high, then addition of potassium to solutions may lead to repolarization of the membrane – the transmembrane potential curve may intersect the x-axis and enter the "anti-Nernst" region (Fig. 11, curve 1). Further, the curve passes its maximum and drops to the x-axis. But significant differences may arise from the preceding case. When the potassium ion concentration is increased further, repolarization of the membrane occurs once more. The potential returns to the "Nernst" region and finally approaches the saturation Nernst value of $-\frac{\Delta H}{H}$. Curve 2 shown in Fig. 11 is realized for other values of the system's parameters.

Thus, this model does not agree with the experimental results for high potassium ion concentrations.

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