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Mechanism of Conductivity of Bimolecular Lipid Membranes in the Presence of Tetrachlorotrifluoromethylbenzimidazole

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Summary. The purpose of the work presented in this paper was to determine experimentally the actual mechanism of lipid bilayer conductivity in the presence of tetrachlorotrifluoromethylbenzimidazole (TTFB). The capacitance and conductance of lipid bilayers were measured with a current-clamp method, as a function of equal TTFB concentrations (10^{-7} to 5×10^{-5} M) in the two aqueous phases. The voltage across the membrane was measured as a function of time during rectangular current pulses. If the hydrogen buffer capacity of the solution is low, the voltage response to long current pulses has two components. The slow component is due to hydrogen ion concentration changes in the unstirred layers. This component disappears if the buffer capacity is made high enough. Membrane capacitance and conductance can be determined from the fast component of the voltage response. The conductance increases with the square of TTFB concentration (pH 2 to 7) and the capacitance is 0.4 $\mu F/cm^2$ for the range of concentration used. If solutions of low buffer capacity are used, shifts of hydrogen ion concentrations near the membrane give rise to a complicated dependence of the membrane potential on pH given a unit pH difference between the two aqueous solutions (protonic potential). This dependence can be explained if the membrane permeability for neutral uncoupler molecules (TH) is high enough. The membrane permeability coefficient is determined: $P_{TH}=10$ cm/sec. In other experiments the dependence of short-circuit current on pH difference between the two solutions was also measured, with the pH value on one side fixed in a given experiment. These complicated nonmonotonic dependences can be described using a mathematical equation with only two parameters: (1) the dissociation constant of TTFB in water (pK=5.2), and (2) the proportionality factor between short-circuit current and TTFB concentration squared. These data can be formally interpreted to mean that the membrane is permeable only to T_2H^- and TH, where T is TTFB anion and H is hydrogen ion. However, this model does not explain the high current values obtained because of the limited rate constant of T_2H^- formation in aqueous solution. An alternative model is proposed. It is shown that the T_2H^- is not formed in aqueous phase but rather within the membrane. The T_2H^- can be the intramembrane charge carrier if its life-time is long enough. If the average lifetime is short, current might be carried through the membrane by proton exchange between TH and T^-, when they collide. This mechanism could also account for the action of uncouplers of oxidative phosphorylation other than TTFB.

Despite the fact that in the last six years a good deal of experimental data on the conductivity of bimolecular lipid membranes (BLM) in the presence of uncouplers of oxidative phosphorylation was obtained, the mechanism of permeability has been ascertained in sufficient details only in one case. In 1971, Le Blanc [4] showed that the transfer of protons across the membrane in the presence of carbonylcyanidechlorophenylhydrazone is effected by a simple mechanism: under the influence of the electrical field the anion of the uncoupler crosses the membrane. The anion accepts a proton in the aqueous solution and forms a neutral molecule which returns across the membrane in accordance with the concentration gradient. As a result of this cycle, one hydrogen ion is transferred across the membrane. Unfortunately, this simple mechanism cannot explain the conductivity of membranes in the presence of all types of uncouplers. There is a large class of uncouplers that give rise to a quadratic dependence of conductance on the uncoupler concentration which does not come within Le Blanc's model. This model may be modified by the assumption that the current is transferred by an anion-dimer (T_2H^- instead of T^-). Such a model has been proposed by two groups of authors [2, 3]. As shown below, this model can give a formal explanation to all the experimental facts. Indeed if only a small fraction of the uncoupler forms dimers, the dimer concentration increases as a square of the uncoupler concentration and gives a bell-shaped dependence of conductance on pH with a maximum at the uncoupler pK.

The aim of this article is to ascertain the mechanism of BLM conductivity in the presence of tetrachloro-2-trifluoromethylbenzimidazole (TTFB), which is one of the most effective uncouplers.

Materials and Methods

Solutions

Bilayers were formed on a hole about 1 mm^2 in area in a Teflon cup, using a solution of total ox brain lipids in *n*-decane (20 mg/ml). The TTFB-containing aqueous solutions usually employed consisted of 0.1 to 1 M NaCl and 20 mM buffer (20 mM sodium citrate +20 mM sodium phosphate +20 mM boric acid). All the measurements were made at room temperature.

Electrodes

Ag-AgCl electrodes connected to each one of two aqueous solutions by agar-agar +3 M KCl bridges were used. The total resistance of this combination was 10^4 ohm or less.

Current Clamp

Voltage pulses of various durations and amplitude were applied from square-wave generator or batteries to the membrane through resistances of 10^6 to 10^9 ohm. The membrane potential was measured by a cathode follower with an input resistance of about 10^{10} ohm and recorded with an oscilloscope or a potentiometer.

Protonic Potential

Lipid films were formed in solutions of equal pH. After the bilayer formed, the pH of one of the solutions was changed by 0.2 to 0.3 pH units and measured with a glass pH-electrode. A grounded Ag-AgCl electrode was used as a reference electrode. The membrane potential was measured with an electrometer (± 1 mV accuracy).

Short-Circuit Current

This was measured with a sensitive microammeter. The potential drop on the input resistance of the ammeter was less than 1 mV. In each experiment, the pH of one solution was constant and pH of the other one was changed and measured with an accuracy of ± 0.01 pH unit. The reference electrode resistance was about 2×10^3 ohms. One film was used for each curve in Fig. 6.

Results and Discussion

The Voltage Response to a Current Pulse

To find out rate-limiting stages in charge transfer the current-clamp method was used. The time course of the membrane potential change induced by a short current pulse ($\tau_{\text{pulse}} \ll \tau_{\text{membrane}}$) is shown in Fig. 1. At the

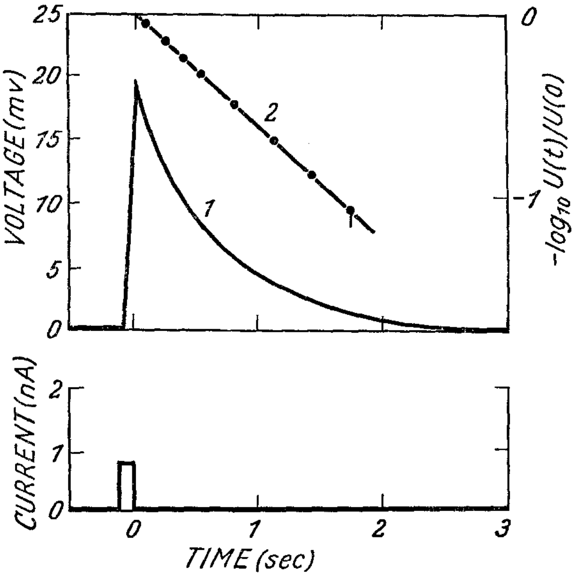

Fig. 1. The time course of membrane voltage in response to a rectangular current pulse The current pulse duration (0.1 sec) is much less than τ membrane. τ membrane was defined as the time constant of the exponential response. Solutions: 20 mM buffer, 2.5×10^{-7} M TTFB, pH 5.2. Membrane area 1.1 mm^2. Curve 1 in linear scale, curve 2-semilogarithmic scale. The time constant of the membrane is 0.7 sec. The membrane acts as an ideal RC-circuit of parallel $C_m = 4 \times 10^{-9}$ F and $R_m = 1.7 \times 10^8$ ohms

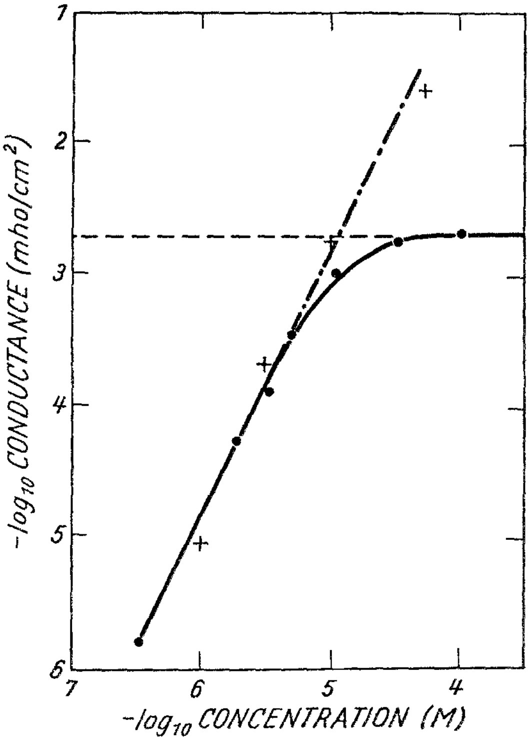

Fig. 2. The membrane conductance versus TTFB concentrations: (•) the direct current conductance at pH 5.6 from Eq. (5); (+) the conductance measured by a short-current pulse method at pH 5.2. Solutions: 1 M NaCl and 20 mM buffer. The dotted line corresponds to equation $g = 2 \times 10^7\,[C]^2$. The dashed line shows the equivalent conductance of the unstirred layers calculated from Eq. (2) at 20 mM buffer

end of the pulse the membrane potential (U_0) depends on the pulse duration (τ_{pulse}), its amplitude (I_0) and membrane capacitance (C_M): $U_0 = I_0 \cdot \tau_{\text{pulse}}/C_M$. The value of the capacitance was found to be $0.4 \pm 0.02\ \mu F/cm^2$ and is independent of the uncoupler concentration. The same value was found using a high frequency impedance method [5]. When the current pulse is switched off, the charged membrane capacitance discharges exponentially through the parallel membrane resistance. The time course of U on a semilogarithmic scale is a straight line (Fig. 1). The membrane time-constant τ_{membrane} is determined from the slope of this line. The membrane conductance (g_M) is defined as $g_M = C_M/\tau_{\text{membrane}}$.

At pH 5.2 and 20 mM buffer concentration the membrane conductance is proportional to the TTFB concentration squared. g_M values coincide with the membrane conductance measured with direct current up to 3×10^{-6} M TTFB but are higher when TTFB concentration becomes larger (Fig. 2).

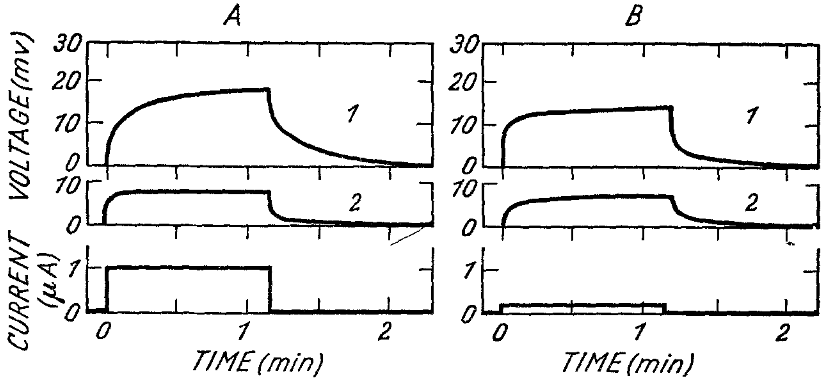

Fig. 3. The time course of membrane potential in a response to a long rectangular current pulse (pH 5.2, 1 M NaCl). The increase of the buffer capacity at constant TTFB concentration (5×10^{-5} M) causes a decrease of the amplitude of the slow component of the response. (*A*) 1 to 20 mM Na-citrate, 2 to 200 mM Na-citrate. The increase of the TTFB concentration at the constant buffer capacity (20 mM Na-citrate) causes a decrease in the amplitude of the fast component of the response but it has no effect on the slow one. (*B*) 1 to 1.10^{-5} M TTFB, 2 to 5×10^{-5} M TTFB

One can understand the nature of this difference by studying the time course of the membrane potential in response to a long current pulse. Fig. 3 shows two components in the potential response with different characteristic times. The increase of buffer capacity leads to a decrease of the slow component and does not affect the fast one (Fig. 3A). The increase of TTFB concentration at constant buffer capacity decreases the fast component and has no effect on the slow one (Fig. 3B). All these data show that the slow component of the response is due to changes of hydrogen ion concentrations in the aqueous solutions close to the membrane. The characteristic diffusion time of buffer in unstirred layers of thickness l is about $l^2/D\simeq 10$ sec as it is found experimentally (Fig. 3). The contribution of aqueous diffusion processes to the measured resistance can be described by an equivalent diffusion resistance (R_D) in series with the membrane. R_D is estimated as the ratio of diffusion over voltage (φ_D) to the total flux of protons through the membrane (j_D) when φ_D tends to zero:

$$\varphi_D=\frac{RT}{F}\ln[(\mathrm{H})_{1b}/(\mathrm{H})_{2b}]\simeq\frac{RT}{F}\Delta\mathrm{H}/(\mathrm{H})$$

(here $(\mathrm{H})_{1b}$, $(\mathrm{H})_{2b}$ are proton concentrations in water near the two membrane boundaries, (H) is that in the bulk solutions, $\Delta\mathrm{H}=[(\mathrm{H})_{1b}-(\mathrm{H})_{2b}]\ll(\mathrm{H})$). The proton flux through the membrane is equal to the total flux of hydrogen ions (j_H), hydroxyl ions (j_OH) and the proton-bound buffer molecules (j_{BH}) through the unstirred layers:

$$j_O = j_H + j_{OH} + j_{BH} = D_H \frac{\Delta H}{2l} + D_{OH} \frac{\Delta OH}{2l} + D_B \frac{b \cdot \Delta pH}{2l}$$

where

$$D_H = 8 \times 10^{-5}\ \mathrm{cm^2/sec}, \quad D_{OH} = 4 \times 10^{-5}\ \mathrm{cm^2/sec}, \quad \text{and} \quad D_b \simeq 5 \times 10^{-6}\ \mathrm{cm^2/sec}$$

are, respectively, aqueous diffusion coefficients of H^+, OH^- and buffer, l is the thickness of the unstirred layers ($l \simeq 0.02$ cm), b is the buffer capacity of the aqueous solutions (for equimolar citrate-phosphate-borate buffer used $b \simeq 0.6$ (B), (B) is the buffer concentration)

$$R_D = \varphi_D / F \cdot j_8 = \frac{RT}{F^2} [D_H \cdot (H) + D_{OH} \cdot (OH) + D_B \cdot b \log_{10} e]^{-1} \cdot 2l. \qquad (1)$$

One can estimate the equivalent diffusion conductance in mho/cm² as

$$g = 1/R_D = 8(H) + 4.4(OH) + 0.17\, b. \qquad (2)$$

Using this simple formula one can choose an appropriate buffer concentration. In the absence of buffer at pH 6.0, $R_D = 10^5$ ohm cm². At low TTFB concentration the diffusion processes have no effect even without any buffer. Indeed in 10^{-7} M TTFB the voltage response to a long current pulse has only one time constant corresponding to a membrane capacitance of about 0.4 µF/cm². In 20 mM buffer, $R_D = 500$ ohm cm². As the TTFB concentration is increased the direct current conductance reaches this limit value (Fig. 2).

The concentration near the membrane is not changed during the short-current pulse. Thus, the conductance of the membrane itself is measured. This conductance is of about 0.01 mho/cm² at pH 5.2 and 5×10^{-6} M TTFB. The conductance of the unstirred layers can be made comparable with that of membrane in 5×10^{-5} M TTFB by increasing the buffer concentration to 0.2 M. In this case the direct current conductance of the membrane is about one-half of the value of the high frequency conductance. The change of conductance with frequency could be found by an impedance method at about 0.1 cps. Concentration changes near the membrane are out of phase with the applied alternating current. This phase lag results in the appearance of a large phenomenological capacitance in impedance measurements. In 0.2 M buffer, at pH 5.6 and with 5×10^{-5} M TTFB the capacitance should be about 10 µF/cm² at 1 cps. By increasing the frequency the capacitance can be reduced to the geometric value of 0.4 μF/cm^2 at 10^3 cps. Such frequency dispersion of capacitance as well as of conductance can be found only if the membrane conductance is comparable to or higher than the equivalent conductance of the diffusion layers. Therefore, if the buffer capacity is constant over the whole pH range used, the maximal dispersion of capacitance should be found at the pH value of maximal membrane conductance, i.e. at the pH equal to pK of the uncoupler.

Thus, in the presence of TTFB the membrane has a real capacitance of 0.4 μF/cm^2 throughout the frequency range used. This capacitance is the same as the capacitance of unmodified black film and apparently represents the geometric capacitance of the inner hydrophobic layer of the film. This capacitance discharges through the parallel resistance of the inner part of of the membrane (Fig. 1). Therefore, curve 2 in Fig. 2 shows the conductance of the inner part. The direct current conductance (curve 1 in Fig. 2) at low TTFB concentrations shows the total resistance of the inner layer and the membrane-water interfaces. Since these two curves coincide over a wide concentration range it must be that the limiting stage of the charge transfer is the inner part of the membrane.

Protonic Potential

Fig. 4 shows the dependences of membrane potential on pH given a unit pH difference between the two aqueous solutions ($\varphi_H = d\varphi/d\text{pH}$). The crosses on curve 1 are obtained with high buffer capacity. φ_H has the theoretical

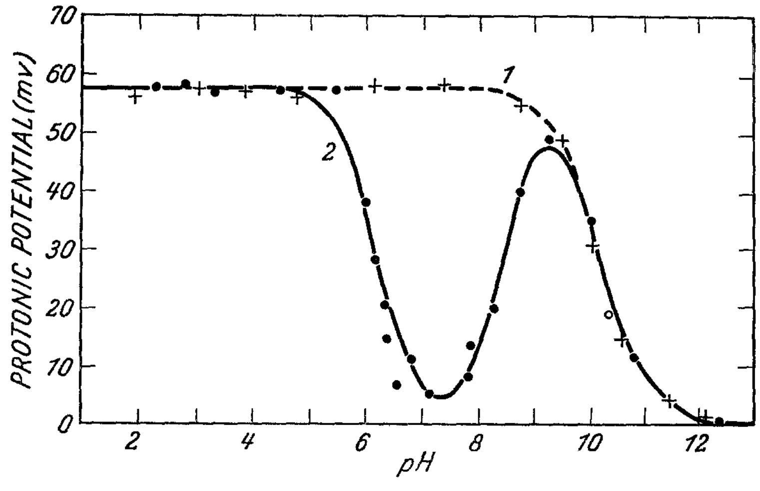

Fig. 4. The dependence of protonic potential on pH in solutions containing 3×10^{-5} M TTFB: (+) —200 mM buffer, (•) – unbuffered solutions. The smooth curves are calculated

value of 58 mV when the pH varies between 1 and 10, which indicates that in this wide range of pH the conductivity is effected by means of proton transfer. The decrease of the potential in the basic pH region is adequately explained by shunting of the pH-dependent conductance with anion TTFB (T^-) conductance. The concentration of this anion in the basic region is independent of pH. Hence, the conductance by anion should also be constant. Indeed in the basic region the direct current conductance decreases to a constant level of 10^{-7} mho/cm^2 in 3×10^{-5} M TTFB [6]. By means of the conductance value one can determine the membrane permeability coefficient for anion: $P_T = 2 \times 10^{-6}$ cm/sec.

In unbuffered solutions of the same TTFB concentration a drop of φ_H is observed in pH $\simeq 7.0$, i.e. in the region of minimal buffer capacity of water. As shown above, a high frequency conductance of the membrane is independent of buffer capacity. Therefore, in unbuffered solutions the conductance at pH 1 to 10 is also due to the proton transfer. The φ_H drop in the region of protonic conductance is adequately explained by the decrease of the difference in proton concentrations in aqueous solutions near the two boundaries of the membrane. As φ_H is zero current potential the decrease of the concentration difference can be caused only by a flux of a neutral uncoupler form across the membrane. The solid curve in Fig. 4 at pH $\leqq 9.0$ is calculated from $\varphi_H = \frac{RT}{F} \ln [(H)_{1b}/(H)_{2b}]$ where $(H)_1$ and $(H)_2$ are obtained from the diffusion equations for H^+, TH and T^- under condition $(TH)_{1b} = (TH)_{2b}$. The buffer capacity for curve 1 is assumed to be infinitely great, and for curve 2 it is found by titration of the aqueous solution. In the region of pH where the conductance is due solely to T^- the membrane potential depends on $l/P_{TH} \cdot D$ in the manner described by Le Blanc [4]. The permeability coefficient for the neutral molecules (P_{TH}) was obtained from this dependence: $P_{TH} \simeq 10$ cm/sec. Both calculated curves show satisfactory coincidence with the experimental points.

Uncoupler Concentrations Near the Membrane Separating Two Solutions of Different pH

We now consider the distribution of uncoupler concentrations near the membrane if P_{TH} is large and there is a large pH difference between two solutions. Consider two equipotential solutions with high buffer capacity of the same uncoupler concentrations but of different pH. They are separated by a bilayer. Let pH of the left solution be larger than pH of the right one (Fig. 5). In this case the TH-concentrations in the two aqueous solutions are different and a TH-flux from right to left takes place. Since P_{TH} is about

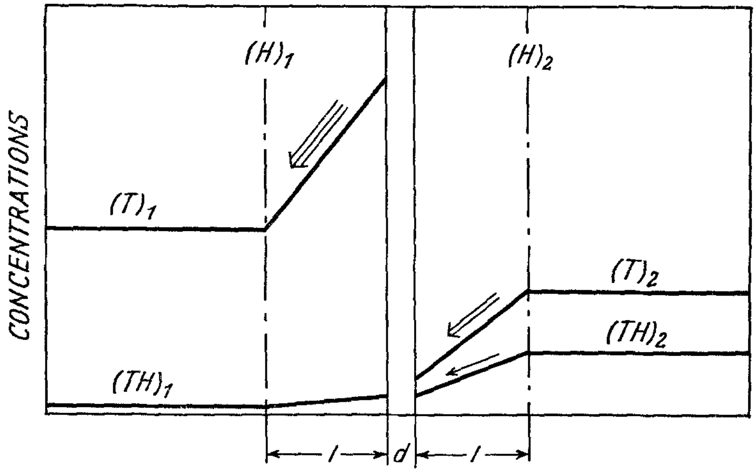

Fig. 5. The concentration profiles of TH- and T-forms and their fluxes in the unstirred layers. The profiles were calculated from Eqs. (4) and (6) given $P_{TH} \gg D/l$, $H_1/K = 0.05$ and $H_2/K = 0.5$. l, the thickness of the unstirred layers; d, the membrane thickness

five orders of magnitude higher than the equivalent diffusion layer permeability ($D/l \simeq 10^{-4}$ cm/sec) TH-concentrations near the two boundaries are equal in a wide range of pH values (pH ≤ 10) and the TH-concentration changes take place in diffusion layers. Let the reaction $T^- + H^+ \rightleftharpoons TH$ be fast so that the mass action law holds everywhere in the aqueous solutions. Then $(T) = (TH)/h$, where $h = (H)/K$, K is the dissociation constant of uncoupler in water. Under these conditions the T-concentrations near the membrane are also different from the corresponding concentrations in bulk solution. As a result the concentration difference near the two boundaries is larger than that between the two bulk solutions; hence

$$(T)_{1b} = (TH)_b/h_1; \tag{3}$$

$$(T)_{2b} = (TH)_b/h_2. \tag{4}$$

$(TH)_b$ is TH concentration just near the membrane boundaries. The concentrations of both forms of uncoupler near the membrane can be determined from the equations for the uncoupler flux in both diffusion layers:

$$j_C = D/l[C - (TH)_b - (T)_{1b}] = D/l[(TH)_b - (T)_{2b} - C] \tag{5}$$

where C is the total TTFB concentration.

The TH-form concentrations near the membrane are equal

$$(TH)_b = 2Ch_1 h_2/(2h_1 h_2 + h_1 + h_2). \tag{6}$$

At acid pH (h_1, $h_2 \gg 1$) the concentrations of both forms near the membrane are the same as those in bulk solution. The influence of the diffusion layers becomes apparent if h or h_2 is less than 1. In this case the uncoupler passes through the diffusion layers mainly as T^- because its concentration is higher than TH. Through the membrane the uncoupler passes as the neutral TH form. If $h_1 \ll 1$ and $h_2 \gg h_1$, then the concentrations of both forms near the membrane in the right solution is much less than in the bulk one. The uncoupler flux is $j_C = DC/l$. In the left solution the uncoupler passes mainly as T^-. Therefore,

$$(\mathrm{T})_{1b} \cong 2C \quad \text{and} \quad (\mathrm{TH})_b \simeq 2Ch_1. \tag{7}$$

It is clear that if h_1 tends to zero then $(\mathrm{TH})_b$ and $(\mathrm{T})_{2b}$ tend to zero also.

We should like to note that Eqs. (4) and (6) are obtained independently on any assumptions about the mechanism of charge transfer through the membrane. Their derivation is based on two assumptions only: (1) the reaction of uncoupler dissociation is fast enough, and (2) the membrane permeability to TH is so large that the aqueous TH concentrations near the two membrane interfaces are equal.

Steady-State Short-Circuit Currents

The dependence of short-circuit current (SCC) on the pH of the two solutions is shown in Fig. 6. A constant proton concentration H_1 is maintained in one solution. The curves in Fig. 6 show SCC plotted versus the pH value in the other solution (pH_2). In each of the three curves in Fig. 6 there is a maximum of SCC when $H_2 < H_1$ ($pH_2 > pH_1$) and a plateau when $H_2 \gg H_1$ ($pH_2 < pH_1$). The maximum and plateau values depend strongly on H_1.

As shown by Markin *et al.* [7], the SCC maximum cannot be explained in the frame of a monomolecular carrier model with a proton exchange at the interfaces. Let us now consider two models with an anion-uncoupler as the charge carrier through the whole membrane. Let the membrane be well permeable for TH and the anion flux be small enough throughout the pH range used so that it has no influence on the uncoupler concentrations near the membrane. If the charge is transferred by T^--anion as in Le Blanc's model [4], the SCC depends on H_2 monotonically. Indeed, according to Eqs. (4) and (6)

$$I = -Fj_T = F(1+j)(h_2-h_1)C\left[(1+h_1)(1+h_2)+\frac{\gamma}{2}(2h_1h_2+h_1+h_2)\right]^{-1}$$

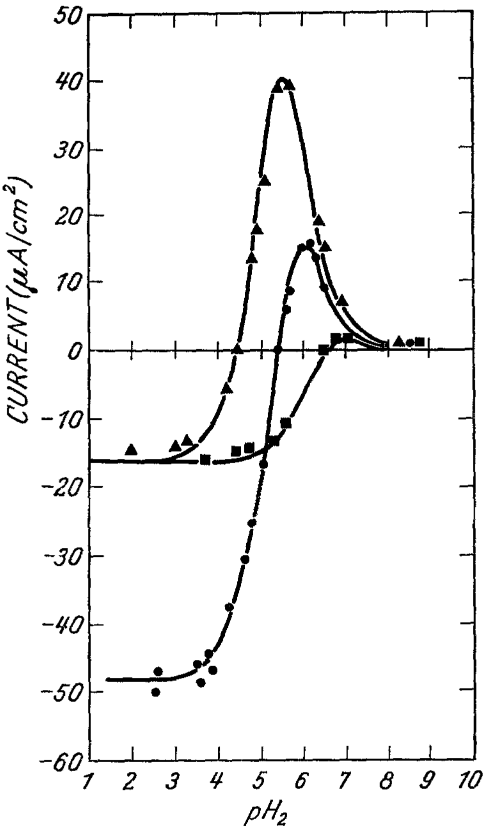

Fig. 6. The dependence of short-circuit current on the pH of one side of the membrane $(pH)_2$. pH on the other side (pH_1) is maintained constant; pH_1 value: ▲—4.5, ●—5.5, ■—6.5. Solutions: 7.5×10^{-6} M TTFB, 1 M NaCl, 20 mM buffer. The smooth curves are calculated from Eq. (8) using pK=5.2 and $P_2/K_2 = 2 \times 10^4$ cm/sec × M

where

$$\gamma = P_{\mathrm{TH}} \cdot 2l/D.$$

The monotonic change of this current is due to a monotonic increase of $(T)_{1b}$ and a monotonic $(T)_{2b}$ decrease when H_2 is decreased. Thus, the difference $[(T)_{1b} - (T)_{2b}]$ acquires the limiting value of $2C$.

If the current across the membrane is carried by the T_2H-dimer [2, 3], whose concentration is very much lower than the total uncoupler concentration, then the SCC value is

$$I = -F \cdot j_{\mathrm{T_2H}} = FP_2[(\mathrm{T_2H})_{2b} - (\mathrm{T_2H})_{1b}] = \left(\frac{FP_2}{K_2}\right) \frac{4C^2 h_1 h_2 (h_2 - h_1)}{(2h_1 h_2 + h_1 + h_2)^2} \qquad (8)$$

where P_2 is the membrane permeability coefficient to T_2H- form, K_2 is the equilibrium constant of the reaction $T^- + TH \underset{K''}{\overset{K'}{\rightleftharpoons}} T_2H^-$ $K_2 = k''/k'$ [the reaction is supposed to be sufficiently fast so that throughout the solution $K_2(T_2H) = (T)(TH)$]. Eq. (8) is derived directly from Eqs. (4), (6) and mass action law.

According to Eq. (8) the dependence of SCC on h_2 shows a maximum at any value of h_1. The current fall, as h_2 tends to zero (basic pH_2), is due to the decrease of dimer concentrations near the membrane. Indeed, these concentrations are proportional to the product $(T)_b \cdot (TH)_b$, but as h_2 tends to zero, the premembrane anion concentrations tend to finite values ($(T)_{1b}$ tends to zero, $(T)_{2b}$ tends to $2C$) and $(TH)_b$ tends to zero. Hence, both T_2H and the current tend to zero. The solid curves in Fig. 6 are calculated from Eq. (8) using the parameter values: $P_2/K_2 = 2 \times 10^4$ cm/sec × M and pK = 5.2. Thus, Eq. (8) not only predicts qualitatively the appearance of maxima in the curves but also yields a good fit of the calculated curves to the quite complex experimental dependence of the SCC on pH_1 and pH_2. Only two parameters had to be determined *viz* P_2/K_2 and K. The maximum value of the current was used to define P_2/K_2. The magnitude of K was found using Eq. (8) as pH tends to zero. In this case SCC has a maximum value at $H_2 = K_2$. The measurements showed that at $pH_1 = 2.0$ the SCC maximum corresponds to $pH_2 = 5.5 \pm 0.1$, i.e. $pK = 5.2 \pm 0.1$.

It was shown previously [2, 3] that a dimer model with a very high P_{TH} can explain the experimental dependencies of the conductance upon the uncoupler concentration, the protonic potential (over a wide range of pH) and the bell shape of the conductance versus pH curve. In Fig. 7 membrane conductance as a function of pH is plotted for 2.5×10^{-6} M TTFB, showing the maximum at pH 5.2. The solid curve is calculated from

$$g = \frac{F^2}{RT} \cdot \frac{P_2}{K_2} \cdot \frac{C^2 h}{(1+h)^2} \tag{9}$$

for the P_2/K_2 and pK values determined from the SCC. Thus, all the experimental data concerning the electrical properties of BLM treated with TTFB are well described in the formal sense by a model which assumes membrane permeability to the TH and T_2H forms.

However, the high conductivity of the membrane with TTFB requires a high enough dimer concentration in water and a high rate of its production. An optical density of an aqueous solution of TTFB (pH 5.2) is proportional to the TTFB concentration to 3×10^{-5} M with an accuracy of 10%. Hence, the dimer concentration in the aqueous solution is not greater than 3×10^{-6} M.

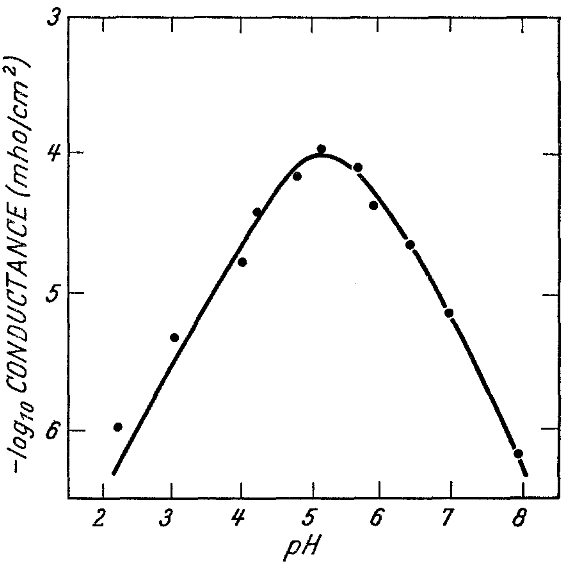

Fig. 7. The dependence of zero current conductance on pH. Solutions: 2.5×10^{-6} M TTFB, 1 M NaCl, 20 mM buffer. The solid curve is calculated from Eq. (9) using pK=5.2 and $P_2/K_2=2\times10^4$ cm/sec × M

The rate constant k' cannot be greater than 10^{11} M^{-1} sec^{-1} because of a diffusion limitation. We shall now evaluate a maximum current of dimers. The stationary distribution of the dimer concentration in water may be found from the diffusion equation if the chemical reaction of dimer formation is taken into account:

$$\frac{d}{dt}(\mathrm{T_2H})=D_2\frac{d^2}{dx^2}(\mathrm{T_2H})+k'(\mathrm{T})(\mathrm{TH})-k''(\mathrm{T_2H})=0. \tag{10}$$

The boundary conditions are

$$D_2\frac{d}{dt}(\mathrm{T_2H})|_{x=0}=j=-I/F$$

$$(\mathrm{T_2H})|_{x=\pm\infty}=(\mathrm{T_2H})_0=C^2h/k(1+h)^2.$$

D_2 is the dimer diffusion coefficient in water. It is assumed that $(\mathrm{T})=C/(1+h)$, $(\mathrm{TH})=Ch/(1+h)$ throughout the solutions. (If this is not so we shall get a lower estimate for the current.) The solution of the diffusion equation is

$$(\mathrm{T_2H})_x=(\mathrm{T_2H})_0-j\frac{\lambda}{D_2}\exp(-x/\lambda) \tag{11}$$

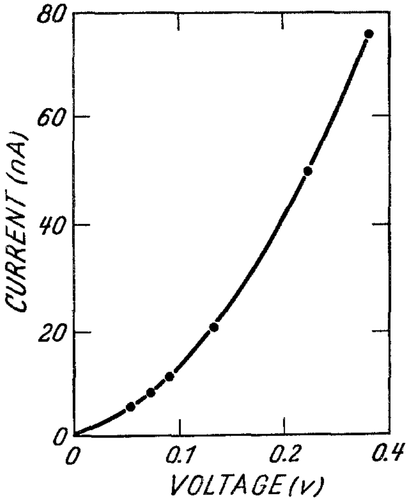

Fig. 8. The voltage-current relationship of a membrane in the presence of 2.5 to 5 × 10^{-6} M TTFB. Solutions are the same as in Fig. 7; pH 7.0

where

$$\lambda = \sqrt{D_2/k''}.$$

The limiting current will be observed at a zero dimer concentration near one of the boundaries;

$$I_{\mathrm{lim}} = F j_{\mathrm{lim}} = F(\mathrm{T_2H})_0 D_2/\lambda = \frac{FC}{1+h} [(\mathrm{T_2H})_0 D_2 k' h]^{\frac{1}{2}}. \tag{12}$$

Fig. 8 shows the voltage-current characteristics of the membrane at pH 7.0 and 2.5×10^{-6} M TTFB. At 280 mV the current is equal to 7.5×10^{-6} Amps/cm^2 which is far from the limiting value since the first derivative of the characteristic is positive. However, according to Eq. (12) the current is to be less than 4×10^{-7} Amps/cm^2.

Thus, it is impossible to explain the high value of membrane conductivity assuming that dimer-$\mathrm{T_2H^-}$ formed in water is the charge carrier. One may suppose that the dimer is formed in the membrane and transfers the charge across the hydrophobic region. Across the boundary the charge may be transferred either by hydrogen ions via the heterogeneous reaction:

$$\mathrm{T^-_{membrane}} + \mathrm{H^+_{water}} \rightleftharpoons \mathrm{TH_{membrane}}$$

or by $\mathrm{T^-}$ which exchange at the boundaries with TH. These two possibilities cannot be distinguished by our experiments, since the boundary resistance is not significant.

Another mechanism may be suggested for charge transfer within the membrane which shows equally close correspondence to the known experimental data.

Mobile Relay-Race Mechanism

Consider a membrane in which the TH- and T^--forms move under the action of electrical and concentration gradients. Suppose, as before, that P_{TH} is so high that the concentrations of TH-molecules in water near the membrane interfaces are equal. Suppose also that the mobility of T^--ions in the membrane interior is much lower then that of TH. The charge transfer within the membrane would then take place mainly by proton exchange between TH and T^- when they collide. This exchange results in a movement of the proton a distance δ of which is on the order of the uncoupler molecular dimension. Suppose this transition is of a single barrier type. Then the relay-race flux of the protons is equal to the product of the number of the collisions and of the probability of a proton transition along the electric field at a collision. The probability is proportional to $\exp(FE\,\delta/2\,RT)$ for a movement along the field and to $\exp(-FE\,\delta/2\,RT)$ for the opposite direction. Here $E=\varphi/d$ is the field intensity which we assume constant throughout the membrane (the big potential jumps at the membrane boundaries are not considered since the boundary resistance is small as was shown above). The proton flux from a plane X to a plane $x+\delta$ inside the membrane is:

$$j_H=\nu([\mathrm{TH}]_x[\mathrm{T}]_{x+\delta}\exp(FE\delta/2RT)-[\mathrm{TH}]_{x+\delta}[\mathrm{T}]_x\exp(-FE\delta/2RT) \qquad (13)$$

where [TH] and [T] are the concentrations in the membrane, ν is a coefficient which depends on the mobility of both forms, on the effective collision cross-section and on the height of the barrier. Let us suppose δ to be small in comparison with the membrane thickness d. Expanding Eq. (13) in a series and neglecting all terms higher than second order we obtain a differential equation for the relay-race flux

$$j_H=\delta\nu\left([\mathrm{TH}]\frac{d}{dx}[\mathrm{T}]-[\mathrm{T}]\frac{d}{dx}[\mathrm{TH}]+[\mathrm{TH}][\mathrm{T}]F\varphi/RT\right). \qquad (14)$$

The fluxes of TH and T^- are given by the diffusion equations:

$$j_{TH}=-\frac{RT}{F}U_{TH}\frac{d}{dx}[\mathrm{TH}]; \quad j_T=-\frac{RT}{F}U_T\left(\frac{d}{dx}[\mathrm{T}]+[\mathrm{T}]F\varphi/RTd\right) \qquad (15)$$

where U_{TH} and U_T are the mobility of the corresponding particles in the membrane. In the steady state, the total fluxes of the proton and uncoupler are constant with respect to X:

$$j_{\mathrm{H}}+j_{\mathrm{TH}}=\mathrm{const}_1; \qquad j_{\mathrm{T}}+j_{\mathrm{TH}}=\mathrm{const}_2. \tag{16}$$

Since P_{TH} is very high, the TH concentration is also constant with respect to X. The distribution of T along the X-axis may be determined from Eq. (14). According to Eq. (16), $(j_{\mathrm{H}}-j_{\mathrm{T}})$ is also constant and as long as the relay j_{H} is by assumption much greater than the carrier flux j_{T}, one may consider that j_{H} is constant with sufficient accuracy. Then from Eq. (14) follows

$$j_{\mathrm{H}}=\delta v[\mathrm{TH}]\left(\frac{d}{dt}[\mathrm{T}]+[\mathrm{T}]F\varphi/RTd\right). \tag{17}$$

The solution of this equation with the boundary conditions

$$[\mathrm{T}]_1=K^-(\mathrm{TH})_b/h_1 \text{ at } x=0; \quad [\mathrm{T}]_2=K^-(\mathrm{TH})_b/h_2 \text{ at } x=d; \quad [\mathrm{TH}]=K^0(\mathrm{TH})_b$$

is

$$j_{\mathrm{H}}=\frac{F\varphi}{RT}\delta vK^-K^0(\mathrm{TH})_b^2[h_1-h_2\exp(-F\varphi/RT)]/h_1h_2[1-\exp(-F\varphi/RT)] \tag{18}$$

where K^- and K^0 are the partition coefficients of T^- and TH between the membrane and water. The premembrane TH-concentration $(\mathrm{TH})_b$ is determined by Eq. (6). The voltage-dependence of the current given by Eq. (18) is the same as in the Goldman constant field equation but the concentration dependence is different. Consider the short-circuit current through the membrane in this model. From Eqs. (6) and (18) when $\varphi=0$

$$j_{\mathrm{H}}=4\delta vK^-K^0C^2h_1h_2(h_1-h_2)/(2h_1h_2+h_1+h_2)^2. \tag{19}$$

This expression coincides with Eq. (8) for the dimer model and therefore describes the experimental data well if $\delta\, v\, K^-K^0=2\times10^4$ cm/sec × M and pK = 5.2. The conductance is

$$g=\frac{F^2}{RT}\delta vK^-K^0(\mathrm{TH})(\mathrm{T}). \tag{20}$$

Here, (TH) and (T) are the concentrations in the aqueous solutions. It is easily seen that the conductance of the relay model is bell-shaped as a function of pH, with the maximum at the pK of the uncoupler, and is quadratically dependent on its concentration [1]. Thus, the mobile relay-race and the dimer models provide the same functional dependences of g on C, g on pH, I_0 on H_1 and H_2 and φ_{H} on pH. These models therefore cannot be distinguished on the basis of available measurements.

Notice that unlike the dimer model the relay model does not require a great mobility of the anion T^-. A high conductance may be obtained even at zero mobility of the T^- form. At the same time we should emphasize that formation of the T_2H^--complex is an essential feature in both models. The dimer in the membrane is in dynamic equilibrium with the TH- and T^--forms. If the lifetime of the dimer is long compared with the mean time membrane crossing, the charge is carried by the dimer. In the other limiting case, where the lifetime of the dimer is short compared to the membrane crossing time, the charge is moved by the relay mechanism.

Other Uncouplers Inducing BLM Conductivity by the Same Mechanism

Besides TTFB we studied a number of 2-trifluoromethylbenzimidazole (TFB) derivates: monobrom-, monochlor-, mononitro-, and dinitro-TFB.

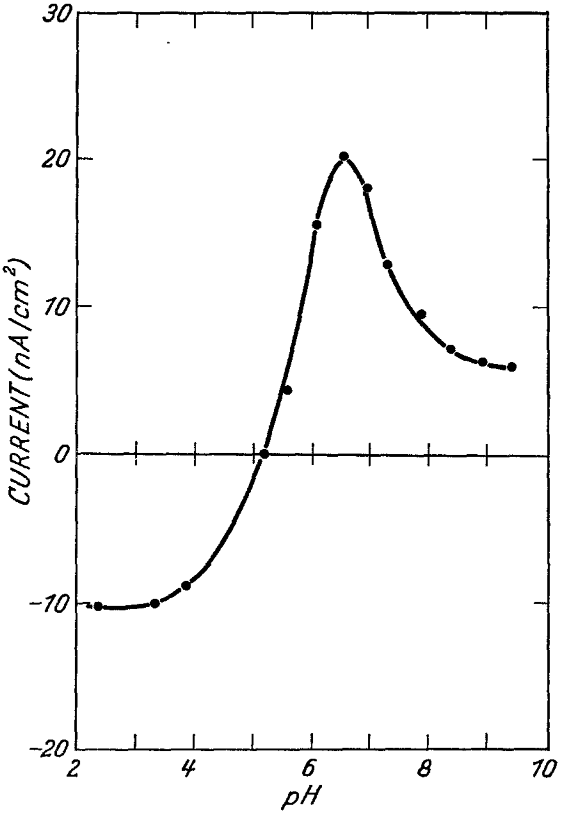

Fig. 9. The dependence of zero current conductance on pH for a membrane in the presence of 5,6-dinitro-TFB. Solutions: 1 M NaCl, 20 mM buffer, 3×10^{-4} M uncoupler (upper curve) and 3×10^{-5} M (lower curve). The curves are calculated from Eq. (9) using pK=6.0 and $P_2/K_2 = 2.7$ cm/sec×M

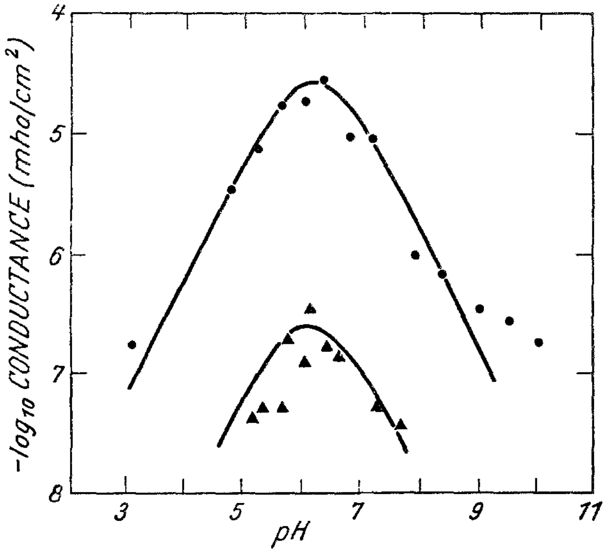

Fig. 10. $(pH)_2$-dependence of short-circuit current at $(pH)_1 = 5.1$ in solutions, containing 3×10^{-5} M 5,6-dinitro-TFB, 1 M NaCl, 20 mM buffer. The curve is drawn by eye through data points

pK of all these substances is greater than that of TTFB, and the maximal membrane conductivity drops with the increase of the pK of a modifier. The investigation by means of the current-clamp method shows that the membrane has a single rate-limiting stage with a parallel capacitance of about 0.4 μF/cm^2. For all the four uncouplers the dependence of conductance on an uncoupler concentration was found to be quadratic at pH = pK (*see*, for example, Fig. 9). For one of the derivatives (dinitro-TFB) with a relatively high permeability we succeeded in finding the pH dependence of SCC on pH (Fig. 10). It proved to be nonmonotonic just as for TTFB. All the data make it possible to conclude that the whole class of TFB derivatives provides conductivity by a single mechanism as described above for TTFB.

Phenol derivatives constitute another class of substances, which act the same way. It has been established [6] that a membrane in the presence of 2,4-dinitrophenol shows a nonmonotonic dependence of short-circuit current on pH. Our investigation reveals that membranes modified by a number of phenols (2,4-dinitrophenol, trichlorophenol, pentachlorophenol) respond to a current pulse by the same way as that in the presence of TTFB. McLaughlin [8] has shown that in the presence of 2,4-DNP the membrane conductance may be proportional to the uncoupler concentration squared. Thus, the dimer model of conductivity is characteristic not only of uncouplers of the benzimidazole type. Apparently, a great number of substances which can penetrate the membrane and induce conductance may function similarly.

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