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## CHARGED AND NEUTRAL ION CARRIERS THROUGH BIMOLECULAR PHOSPHOLIPID MEMBRANES

E. A. LIBERMAN, V. P. TOPALY AND A. Y. SILBERSTEIN

*Institute of the Problems of Information Transmission, Academy of Sciences, Moscow, and Institute of Biophysics, U.S.S.R. Academy of Sciences, Pushchino-on-Oka (U.S.S.R.)*

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## SUMMARY

1. In order to clarify the influence of configuration of molecules on their motion within bimolecular phospholipid membranes the electrical properties of these membranes in the presence of "spherical" lipid-soluble molecules of 1,2-dicarbadodecaborane (barene) derivatives were investigated. Decachlorobarene in the pH region 2–13 is an effective carrier of  $H^+$  through bimolecular membranes, and as well as other proton carriers, is an uncoupler of oxidative phosphorylation. The mercury derivatives of barene are carriers of the halogen and rhodanide anions. Phenylldicarbaundecaborane anions easily penetrate bimolecular membranes.

2. In the presence of decachlorobarene at pH higher than the  $pK_2$  of decachlorobarene ( $pH > 12$ ), and at sufficiently high concentrations of  $I^-$ ,  $Br^-$  and  $Cl^-$  in the presence of dibarenylmercury, the bimolecular membranes have stationary current–voltage characteristics with negative resistance. This confirms the supposition that such characteristics should be observed when the two forms of ion carrier have a charge of the same sign.

3. The N-type current–voltage curves of bimolecular membranes in the presence of tetrachloro-2-trifluoromethylbenzimidazole, copper ions and hydroxyammonium may also be accounted for within the framework of this hypothesis.

4. On the curves of dependence of current on the time after the rapid change of the fixed membrane voltage the carrier current is observed which is followed by the time dependent current of penetrating ions. The ratio of these currents depends on the concentration of the carriers and penetrating ions, the initial membrane potential difference, and the value of the potential change. In the presence of a transmembrane concentration gradient of penetrating ions the time course of these currents also depends on the direction of the electric field applied and the charge sign of the carriers and ions carried. This dependence can be accounted for by the assumption that the direction of the field determines the side of the membrane to which the charged carriers are "pressed" before the change of the fixed voltage.

5. When the carriers are introduced only into the bimolecular membranes, the current–voltage curves change with time owing to carrier leaving the membrane for the solutions. This process is accelerated by the mixing of the water solutions. When only one solution is mixed, the time course of current–voltage curves depends on the value and direction of the electric field. Such experiments enable the charge sign of the carriers in the membrane to be determined. With decachlorobarene at  $pH > 12$  the

two forms of  $H^+$  carriers are negatively charged. The  $I^-$ ,  $Br^-$  and  $Cl^-$  carriers — the complexes of dibarenylmercury with these ions — are also negatively charged. With tetrachloro-2-trifluoromethylbenzimidazole, copper ions and hydroxyammonium (pH 6.0) the two forms of the carrier are positively charged. In the absence of penetrating ions, carriers of the dibarenylmercury type are not charged.

6. A hypothesis is suggested on the nature of the carriers which can be responsible for the properties of excitable cell membranes. An assumption is made that  $K^+$  and  $Na^+$  carriers of the valinomycin or gramicidin type can serve as excitability inducing substances if the molecules of these carriers contain one positive or two negative chemically linked charges, or if two or more similar molecules are linked together by flexible bonds which do not hinder the complexing with cations. Such an effect should also be observed if the mobile lipid-soluble molecules complex with two or more cations.

## INTRODUCTION

The present work is concerned with the investigation of the transport of protons and other ions through bimolecular phospholipid membranes by derivatives of 1,2-dicarbadoodecaborane (barene) (Fig. 1). These icosahedral molecules were recently predicted<sup>1</sup> and prepared<sup>2-4</sup>. It is of interest to know whether these relatively large lipid-soluble molecules penetrate the membranes as charged particles and serve as carriers of ions. Decachlorobarene, a C-H acid, was expected to be an effective proton carrier. As for the phenyldicarbaundecaborane anion it was hoped to observe its direct passage through the membrane<sup>5</sup>. The majority of the proton carriers studied earlier<sup>5-9</sup> are plane molecules. The penetrating tetrahedral ion tetraphenylboron contains four plane benzene rings. It could be that these molecules penetrate bimolecular membranes owing to their ability to be incorporated between two phospholipid molecules of the relatively rigid membrane and that the high permeability of the membrane is connected not only with a good solubility in a lipid phase and large dimensions of the particles, but also with the suitable configuration of the molecules. The icosahedral

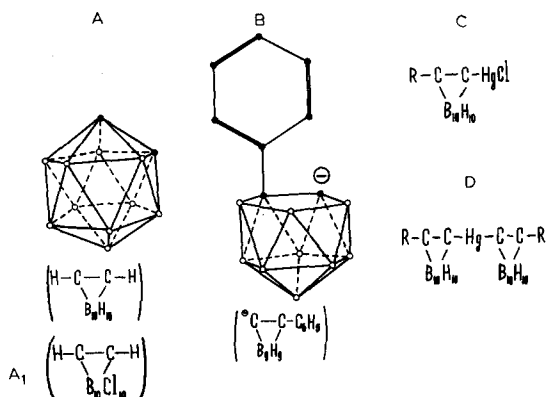


Fig. 1. Structure and chemical formula of 1, 2-dicarbadoodecaborane (barene) (A), decachlorobarene ( $A_1$ ), phenyldicarbaundecaborane anion (B) and mercury derivatives of barene (C and D). ●, carbon; ○, boron; R, alkyl, H or phenyl. The H atoms in A and B are not shown.

molecules allow this assumption to be checked. Furthermore, decachlorobarene is a dibasic acid. Therefore it could be expected that at high pH's ( $\text{pH} > \text{p}K_a$ ) decachlorobarene is a proton carrier of the type  $\text{T}^{2-}-\text{TH}^-$  (ref. 7). The current-voltage characteristics of bimolecular membranes with such a carrier should have a region with negative resistance<sup>9</sup>.

Bimolecular membranes were formed on a Teflon aperture from a solution of bull brain<sup>10</sup> in heptane as described earlier<sup>6</sup>. Sometimes this solution contained modifying substances. Current-voltage curves were recorded by Ag/AgCl electrodes, with agar-agar bridges containing potassium fluoride or acetate, on a polarograph of the Type PO-5122 and an electrometer of the Type Vibron 33B. The aqueous solutions of iodide contained 50 mM  $\text{Na}_2\text{S}_2\text{O}_3$  for elimination of iodine.

All relationships between current and time, and current and voltage, given in the paper apply to a bimolecular membrane with the area 0.65 mm<sup>2</sup>. All experiments were carried out at room temperature.

## RESULTS

### *Comparatively large spherical molecules penetrate bimolecular membranes and serve as ion carriers*

Fig. 2A shows the dependence of bimolecular membrane conductance on the concentration of some derivatives of barene. The membrane conductance begins to rise at very low concentrations. Comparatively large dimensions and practically spherical configuration of the molecules are not a hindrance for penetration through the bimolecular membranes, and the efficiencies of these substances are high. Figs. 2B and 3 show the dependence of bimolecular membrane conductance and membrane potential difference under a 10-fold concentration (or activity) gradient of penetrating ion ( $\Delta V/\Delta \log [\text{ion}]$ ) on penetrating ion concentration in the presence of barene derivatives. The data of Fig. 2B according to LIBERMAN *et al.*<sup>5,6</sup> indicate that decachlorobarene is an effective proton carrier. The maxima of the conductance and  $\Delta V/\Delta \text{pH}$  for decachloro-

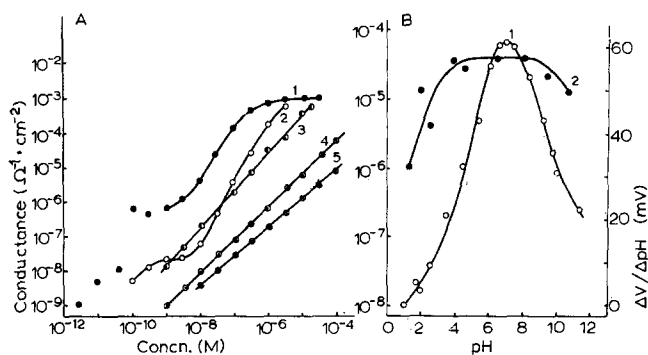


Fig. 2. A. Dependence of bimolecular membrane conductance under low voltage (20 mV) in 30 mM Tris-HCl (pH 7.5) on concentration of barene derivatives. 1, decachlorobarene; 2, dibarenylmercury; 3, methylbarenylmercury; 4, phenyldicarbaundecaborane anion; 5, hexachlorobarene. B. Dependence on pH of bimolecular membrane conductance under low voltage (Curve 1) and membrane potential difference under transmembrane pH difference equal to 1 (Curve 2). Aqueous medium: 20 mM potassium citrate, 20 mM potassium phosphate, 20 mM boric acid,  $3 \cdot 10^{-7}$  M decachlorobarene.

barene are at pH  $\sim 7$  corresponding to the  $pK_1$  ( $pK_1 = 7.0$ ). At pH  $\sim pK_2$  ( $> 12$ ) the conductance and  $\Delta V/\Delta \text{pH}$  have not maxima.

Methylbarenyl- and dibarenylmercury (Figs. 1C and 1D) are effective carriers of halogen anions as shown in Fig. 3.

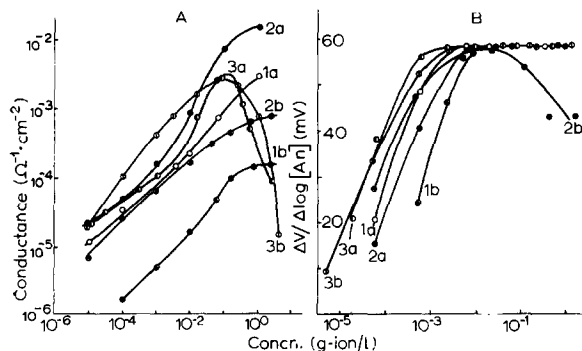


Fig. 3. A. Dependence of bimolecular membrane conductance on concentration of  $\text{Cl}^-$  (1),  $\text{Br}^-$  (2) and  $\text{I}^-$  (3). 1a, 2a and 3a, the water solution contains  $1 \cdot 10^{-5}$  M methylbarenylmercury; 1b, 2b, and 3b, bimolecular membranes are formed from phospholipid solution in heptane containing  $1 \cdot 10^{-3}$  M dibarenylmercury. The anions were added to aqueous solutions as potassium salts. B. Dependence of bimolecular membrane potential difference under 10-fold transmembrane concentration gradient of anions (An) on anion concentration. The symbols are those of A.

The bimolecular membrane potential difference in the presence of a transmembrane concentration gradient of phenyldicarbaundecaborane anions is determined by the Nernst equation at concentrations higher than  $1 \cdot 10^{-8}$  M; hence the phenyldicarbaundecaborane is a very good penetrating anion.

The permeability of bimolecular membranes for phenyldicarbaundecaborane and for  $\text{H}^+$  and univalent anions in the presence of other barene derivatives speak in favour of a liquid structure of the membranes. This is in agreement with the data of LUZZATI *et al.*<sup>11</sup>.

*The substance inducing proton conductance of bimolecular membranes is an uncoupler of oxidative phosphorylation*

The data of Fig. 2B and refs. 6, 7, 12 suggest that decachlorobarene is an effective uncoupler of oxidative phosphorylation. The experiments with rat liver mitochondria confirmed this supposition. The rate of mitochondrial respiration was raised by 50% when the aqueous phase of the mitochondrial incubation mixture having the usual quantity of mitochondria contained about  $10^{-10}$  M decachlorobarene. This concentration of the latter raised the bimolecular membrane conductance by about  $1 \cdot 10^{-8} \Omega^{-1} \cdot \text{cm}^{-2}$ . Phenyldicarbaundecaborane anion penetrates the membranes of sonic mitochondrial particles and chromatophores of *Rhodospirillum rubrum*<sup>13</sup>. It is to be expected that barenyl derivatives of mercury will be effective uncouplers on "turned inside out" mitochondrial fragments and bacteria chromatophores in the presence of halogen and rhodanide anions.

*Bimolecular membrane current-voltage curves are of the N type when both forms of carrier have charges of the same sign*

Fig. 4 shows the stationary current-voltage characteristics of bimolecular mem-

branes in the presence of barene derivatives. In the presence of phenyldicarbaundecaborane (Fig. 4A, Curves 3 and 3a) and of tetraphenylboron<sup>9</sup> current-voltage curves with saturation are observed connected with the concentration gradient of phenyldicarbaundecaborane in the aqueous solutions at the membrane surfaces.

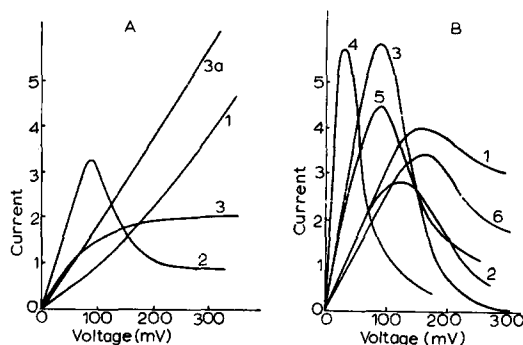
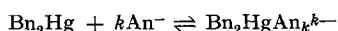
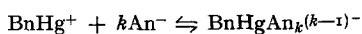


Fig. 4. Stationary current-voltage curves of bimolecular membranes in the presence of barene derivatives. A. 1, 20 mM potassium citrate, 20 mM potassium phosphate, 20 mM boric acid (pH 7.0),  $1 \cdot 10^{-5}$  M decachlorobarene; 2, 0.1 M KOH (pH 13.0),  $1 \cdot 10^{-5}$  M decachlorobarene; 3, 30 mM Tris-HCl (pH 7.5),  $3 \cdot 10^{-5}$  M caesium phenyldicarbaundecaborane; 3a, nonstationary characteristics of the same membrane recorded under high velocity of voltage change (8 V/sec). Calibration of the current axis: Curve 1,  $1 \cdot 10^{-6}$  A; Curve 2,  $2 \cdot 10^{-8}$  A; Curve 3,  $2 \cdot 10^{-9}$  A; Curve 3a,  $1 \cdot 10^{-8}$  A. B. 1, 2.5 M KCl; 2, 2.5 M KBr; 3, 0.25 M KI (with these three salts membranes were formed from phospholipid solution in heptane containing  $1 \cdot 10^{-3}$  M dibarenylmercury); 4,  $1 \cdot 10^{-2}$  M KI,  $1 \cdot 10^{-6}$  M dibarenylmercury (in water); 5, 0.1 M KI,  $1 \cdot 10^{-4}$  M dimethylbarenylmercury in the phospholipid solution; 6, 0.37 M KI,  $1 \cdot 10^{-5}$  M dimethylbarenylmercury (in water). Calibration of the current axis: Curve 1,  $3 \cdot 10^{-8}$  A; Curve 2,  $2 \cdot 10^{-7}$  A; Curve 3,  $1 \cdot 10^{-7}$  A; Curve 4,  $5 \cdot 10^{-8}$  A; Curve 5,  $2 \cdot 10^{-8}$  A; Curve 6,  $2 \cdot 10^{-7}$  A.

At acid, neutral and basic pH's up to 12 the bimolecular membrane current-voltage curves in the presence of decachlorobarene have no region with negative resistance (Fig. 4A, Curve 1). At these pH's the membrane current under the fixed voltage decreases with time if the aqueous solution has a low buffer capacity. In a medium with sufficiently high buffer capacity the membrane current under these conditions does not decrease. Experiments with the transmembrane buffer capacity gradient (for experimental procedure see ref. 6) showed  $H^+$  to be a penetrating ion whereas  $OH^-$  was not. At pH > 12, as was expected, N-type current-voltage curves were observed (Fig. 4A, Curve 2). In such pH's empty carrier is di-anion of the barene and carrier +  $H^+$  is the mono-anion. The idea thus seems to be confirmed that if both forms of carrier bound and unbound with the transported ion have charges of the same sign then the membrane current first increases with the voltage and subsequently decreases when the membrane voltage is sufficiently high, and practically all carriers are placed within the membrane near one of the membrane surfaces. This idea was proposed by HODGKIN *et al.*<sup>14</sup> to explain the transport of  $Na^+$  through excitable cell membranes. They supposed the  $Na^+$  carriers to be charged negatively. However, HODGKIN AND HUXLEY<sup>15</sup> later noted that under sudden depolarization the  $Na^+$  influx must be preceded by the opposite current connected with the motion of negatively charged carriers within the membrane into the cell. Such a current through the giant axon was not detected in the experiments with fixed voltage<sup>15</sup>.

Fig. 4B shows the stationary N-type current-voltage characteristics of bimolecular membranes in the presence of methylbarenyl- and dibarenylmercury in the aqueous solutions containing sufficiently high concentrations of halogen anions.

It is known that  $\text{Hg}^{2+}$  forms with halogen and other univalent anions ( $\text{An}^-$ ) stable complexes of the type:  $\text{HgAn}^+$ ,  $\text{HgAn}_2$ ,  $\text{HgAn}_3^-$  and  $\text{HgAn}_4^{2-}$ . In the molecules of mercury derivatives used in our experiments the sites of one or two anions are occupied by barenyls linked with mercury by strong bonds. The stability constants of the complexes formed by these derivatives with some univalent anions seem to be similar to those of the complexes formed by  $\text{Hg}^{2+}$  with these anions. However, unlike the latter complexes the former are highly soluble in the lipid phase of the bimolecular membrane. Transport of ions through membranes by these complexes can occur by means of the following reactions on the membrane surfaces:



where Bn denotes the barenyl moiety,  $\text{An}^-$  an unspecified anion, and  $k$  the number of anions.

In the presence of methylbarenylmercury the stationary current-voltage characteristics of bimolecular membranes are of the N type at high concentrations of  $\text{I}^-$  only. At low concentrations of  $\text{I}^-$  the neutral and positively charged forms of the carrier in the membrane seem to be predominant. The complexes  $\text{BnHgAn}_3^{2-}$ , when An is  $\text{F}^-$ ,  $\text{Cl}^-$  or  $\text{Br}^-$ , seem to be even less stable. At high concentrations of  $\text{I}^-$  the forms  $\text{BnHgI}_2^-$  and  $\text{BnHgI}_3^{2-}$  predominate and N-type current-voltage characteristics must be observed<sup>9</sup>.

Dibarenylmercury in the presence of halogen anions has only three forms, unlike methylbarenylmercury which has four. Therefore in the presence of dibarenylmercury and univalent anions the conditions in the bimolecular membrane are more favourable for the appearance of negative current-voltage characteristics. The experiments confirmed this expectation. As little as  $1 \cdot 10^{-4}$  M  $\text{I}^-$  is sufficient for developing the stationary N-type current-voltage curves. The characteristics are also of the N type in the presence of more than  $1 \cdot 10^{-2}$  M  $\text{Br}^-$  or more than  $3 \cdot 10^{-1}$  M  $\text{Cl}^-$ .  $\text{F}^-$  is transported through the bimolecular membrane very poorly.

Methyl and phenyl groups constitute a serious spatial hindrance for complexing of dimethylbarenylmercury  $[(\text{CH}_3\text{Bn})_2\text{Hg}]$  and diphenylbarenylmercury  $[(\text{C}_6\text{H}_5\text{Bn})_2\text{Hg}]$  with two halogen or other anions. These substances are mainly carriers of the type ( $\text{T} + \text{TAn}^-$ ) (ref. 7). That is why diphenylbarenylmercury, being an effective anion carrier, does not induce current-voltage characteristics of N type in the presence of any concentration of halogen anions. Dimethylbarenylmercury forms sufficiently stable complexes of the type  $\text{TAn}_2^{2-}$  with  $\text{I}^-$  only, and therefore induces such characteristics at high concentrations of  $\text{I}^-$  (Fig. 4B, Curve 5).

The rate of the entrance of methylbarenyl- and especially dibarenylmercury into the bimolecular membrane from aqueous solution is very low. In the presence of dibarenylmercury the resistance of "black" membranes decreases during 10–30 min. If the membranes are formed from a lipid solution in heptane containing dibarenylmercury the membrane resistance is established after 1–2 min and then increases very slowly. The time course of the current through a membrane with "polycharged"

carriers in the experiments with the fixed membrane potential can be accounted for by the motion of charged carriers within the membrane.

The curves show a decreasing region. The voltage at which this region occurs depends on the quantity of the neutral form of the carrier in the membrane. The current maximum on the curve of a bimolecular membrane just formed in water solution with a high concentration of  $I^-$  and dibarylmcury is at a low voltage (Fig. 4B, Curve 4) and then gradually shifts to a higher voltage. If a membrane is formed from a solution of phospholipid in heptane containing dibarylmcury then the current maximum on the current-voltage curve is at a high voltage after the formation of the membrane and in 1–2 min becomes established at a lower voltage (Fig. 4B, Curve 3).

### *Current of penetrating ions and carriers through membrane*

#### *Influence of concentration of penetrating ions in water solution*

The results of fixed potential experiments with bimolecular membranes in the presence of decachlorobarene and dibarylmcury are shown in Fig. 5. With decachlorobarene the penetrating ion is  $H^+$ . The carriers are negatively charged. There is a transmembrane pH difference. The hydrogen potential difference on the membrane

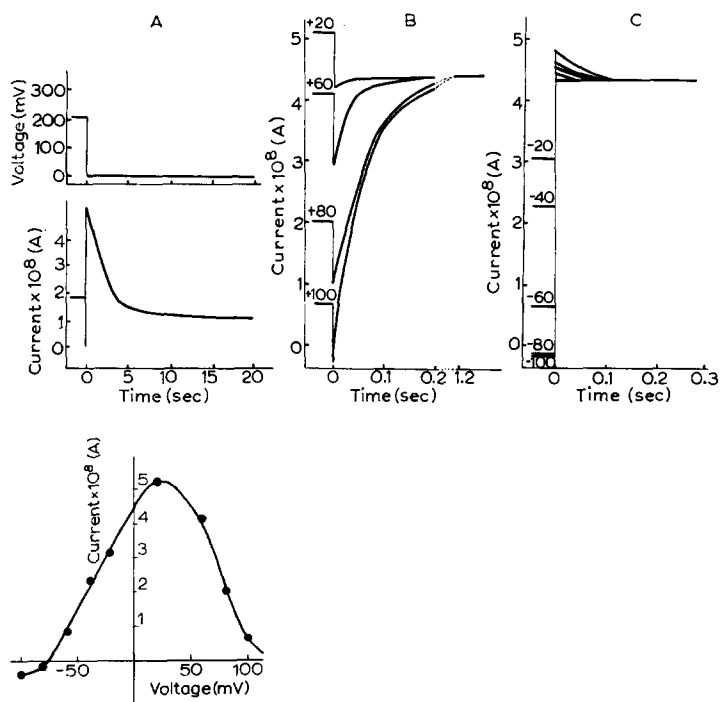


Fig. 5. Time dependence of bimolecular membrane current during rapid change of fixed potential difference. A.  $1 \cdot 10^{-6}$  M decachlorobarene,  $pH_{out}$  13.5,  $pH_{in}$  12.5. The equilibrium membrane potential difference =  $-40$  mV. In the upper part of the figure the voltage change with time is plotted. B and C. Bimolecular membranes are formed from phospholipid solution in heptane containing  $1 \cdot 10^{-4}$  M dibarylmcury. Concentration of the KI in the outer compartment 0.1 M; in the inner compartment 5 mM. The equilibrium membrane potential difference =  $-75$  mV. At the left of the curves the voltage in mV is indicated before the fixation of zero potential difference. The fixed potential difference is changed at zero time. The potential of the outer compartment is taken as 0. D. Stationary current-voltage curve for the same membrane as in B and C.

equals 30–40 mV (*minus* in the “inner” compartment). After sudden “depolarization” of the membrane the large “outer” current appears which changes into a hydrogen current along the transmembrane  $H^+$  concentration gradient (Fig. 5A). Therefore during the transport of cations by negatively charged carriers the large outer current is observed which HODGKIN AND HUXLEY<sup>16</sup> expected to detect. Unfortunately the conditions of the experiments with decachlorobarene ( $pH > 12$ ) do not correspond to those in the experiments on the giant axon of *Loligo*: the  $H^+$  concentration in water is very low, and the rate of water dissociation is unsatisfactory for keeping the  $H^+$  concentration constant in near-membrane water solutions. The rate of water dissociation can assure a stationary membrane  $H^+$  current of about  $1 \cdot 10^{-7}$  A/cm<sup>2</sup> but in the presence of  $1 \cdot 10^{-5}$  M decachlorobarene the current has a value of about  $1 \cdot 10^{-6}$  A/cm<sup>2</sup>.

Methylbarenyl- and dibarenylmercury in the presence of high concentrations of  $I^-$  seem to form negatively charged carriers of anions. Under these conditions the effects of ion “exhaustion” occur in the aqueous solutions near the membrane. A sudden change of membrane voltage in the linear region of the current–voltage curve (up to about 50 mV) results in a change of membrane current without capacity jumps. A change of voltage in the region of more than 100 mV results in a capacity current which seems to be connected with the motion of the carriers within the membrane. The time course of the membrane current when the voltage is suddenly changed in the presence of a transmembrane gradient of  $I^-$  is shown in Figs. 5B and 5C. The stationary current–voltage characteristic of the membrane is shown in Fig. 5D.

When negatively charged carriers transport the anions the outer current of carriers under the conditions described above is expected to be low and to become the large and comparatively slowly increasing current of penetrating ions. Indeed, carriers under the conditions of “resting potential” are “pressed” to that membrane surface near which the concentration of the penetrating ions is lower. As a result the concentration of the carrier bound with the ion ( $TI^{2-}$ ) within the bimolecular membrane is comparatively low. If the carrier concentration is low and the carriers move rapidly within the membrane then the initial (outer) current will be low. After fixation of zero potential difference on the membrane the concentration of the  $TI^{2-}$  form gradually increases. As a result the inner current of the anions will increase in time.

A full description of the time course of the membrane current in the presence of mobile carriers is possible on the basis of the model described earlier<sup>16</sup>. For this purpose the redistribution of the charges within the membrane, and the influence of this redistribution on the rates of the reactions at membrane surfaces, should be taken into account. The contributions of different processes in the membrane capacity current may have different values and directions. Therefore the observed capacity currents of the carriers can be low. Indeed, at sufficiently high carrier concentrations the capacity currents of the bimolecular membranes and of giant axons<sup>17</sup> are very low.

*On the mechanism of N-type membrane current–voltage characteristics in the presence of tetrachloro-2-trifluoromethylbenzimidazole*

As we had no suitable positively charged ion carriers we had to use complex carrier tetrachloro-2-trifluoromethylbenzimidazole–copper ions in the presence of which the bimolecular membrane stationary current–voltage characteristics have a region with negative resistance under the conditions in which the buffer capacity and concentration of transported ions are sufficiently high<sup>5</sup>. In the absence of copper ions reac-



ting with tetrachloro-2-trifluoromethylbenzimidazole, forming different complexes which are proton carriers, N-type membrane characteristics are not observed. The stationary current-voltage characteristics of bimolecular membranes of such type appear in the presence of  $\text{CuSO}_4$  (ref. 18). In the present work we did not intend to make a full examination of the chemistry of the carriers which are formed by tetrachloro-2-trifluoromethylbenzimidazole and copper ions at different pH's and which ions they transport. The pH region 5.5–9.0 was studied. At these pH's in the presence of hydroxyammonium N-type curves are observed<sup>5</sup>. Evidence presented below shows that at pH 5.5–6.5 proton and hydroxyammonium cations are the transported ions. At pH 7.5–9.0 the proton is mainly transported. If the concentration of hydroxyammonium is sufficiently high then the near-membrane concentrations of hydroxyammonium ions and  $\text{H}^+$  are maintained constant owing to the high buffer capacity of the aqueous solution. Under these conditions a concentration change of the transported ions near the membrane surfaces does not play an important role, and currents connected with the motion of the charged carriers within the membrane can be noted. All this is demonstrated in Fig. 6A.

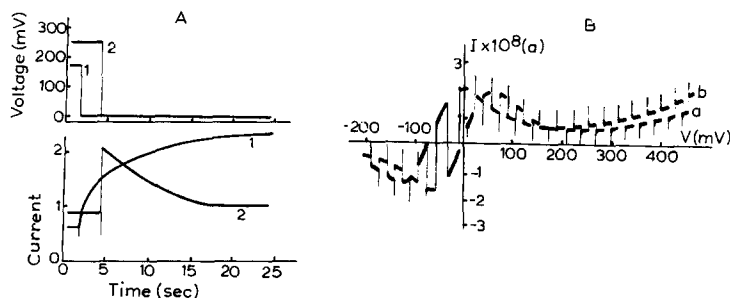


Fig. 6. A. Time dependence of bimolecular membrane current during rapid change of fixed potential difference in 0.1 M hydroxyammonium chloride,  $1 \cdot 10^{-3}$  M  $\text{CuSO}_4$ . The membranes are formed from phospholipid solution containing  $1 \cdot 10^{-3}$  g/ml tetrachloro-2-trifluoromethylbenzimidazole. Curve 1,  $\text{pH}_{\text{out}}$  6.5;  $\text{pH}_{\text{in}}$  5.5; the equilibrium potential =  $-40$  mV. Curve 2,  $\text{pH}_{\text{out}}$  9.0;  $\text{pH}_{\text{in}}$  8.0; the equilibrium membrane potential =  $-50$  mV. In the upper part of the figure the voltage change in time is plotted. The calibration of the current axis: Curve 1,  $1 \cdot 10^{-7}$  A; Curve 2,  $2 \cdot 10^{-8}$  A. B. Demonstration of the capacity membrane current. The rectangular voltage pulses (75 mV) during the linearly changing voltage  $V$ . The voltage on the membrane for Curve a equals  $V$ , for Curve b,  $-(V + 75)$  mV. The aqueous solution was 3.5 mM hydroxyammonium chloride (pH 6.0),  $2 \cdot 10^{-6}$  M tetrachloro-2-trifluoromethylbenzimidazole,  $2 \cdot 10^{-6}$  M  $\text{CuSO}_4$ . The membrane was formed from phospholipid solution in heptane containing 0.3 mg/ml tetrachloro-2-trifluoromethylbenzimidazole.

The mode of membrane current change with time after the "depolarization" indicates that at pH 5.5–6.5 both forms of  $\text{H}^+$  and hydroxyammonium carriers are positively charged (Fig. 6A, Curve 1). Indeed, after the relatively low capacity current the slow increase of penetrating ion current is observed. Therefore one may suppose that the carriers in the polarized membrane (170 mV) were "pressed" to the membrane surface near which the concentration of  $\text{H}^+$  and hydroxyammonium is lower. One might expect that at higher pH the  $\text{H}^+$  carriers will be negatively charged because of dissociation of tetrachloro-2-trifluoromethylbenzimidazole. Curve 2 in Fig. 6A confirms this. After the membrane depolarization the capacity membrane current is followed by the current of penetrating ions which rapidly reaches the maximal value and then decreases slowly.

In Fig. 6B the results of rapid change of membrane voltage at different points of the stationary N-type curve are shown. Though current in the linear part of the curve can be higher than that in the region of current minima a rapid change of the voltage leads to a rapid change of membrane current without the transient processes observed in the presence of "exhaustion" in near-membrane water solutions. This "exhaustion" depends mainly on the value of the membrane current but not on the value of the membrane potential difference. In the falling region of the curve after the voltage jump, transient processes are observed which might be expected if the charged carriers move within the membrane (Fig. 6B).

Evidently under these conditions the region of the current-voltage curve with negative resistance has this property because tetrachloro-2-trifluoromethylbenzimidazole and copper ion form complexes, that is ion carriers whose two forms have charges of the same sign.

*The charge sign of carriers having both forms with charges of the same sign*

For the determination of the charge sign of the ion carriers within the membranes, solutions of phospholipid in heptane containing tetrachloro-2-trifluoromethylbenzimidazole, methylbarynyl- or dibarynylmercury were used for formation of bimolecular membranes. Membranes were obtained in the water solutions containing no carriers. Under these conditions the bimolecular membrane stationary current-voltage characteristics of N type are observed<sup>5</sup>. The resistance of such membranes increases in time as the result of an outflow of carriers from the membranes into the water solutions. The outflow of carriers from membranes is accelerated when the solutions are vigorously mixed. If sufficiently large potential difference is maintained on the membrane then both forms of the carriers will be drawn towards one side of the membrane. That is why the mixing of water solutions should affect the current-voltage characteristics in a manner that depends on which water solution is mixed. If the water solution is mixed near that membrane surface to which carriers are "drawn" then the membrane current at a large potential difference should increase while mixing, since direct passage of the carriers through the membrane might be an essential component of the minimal current and on the other hand the membrane conductance at low voltage ( $g_0$ ) should decrease. The mixing of solution near the second membrane surface can result in the decreasing of  $I_{\min}$ , and  $g_0$  changes slightly. At pH 5.0–6.5 water solutions containing  $1 \cdot 10^{-5}$  M  $\text{CuSO}_4$  (ref. 18) and 10 mM hydroxyammonium chloride<sup>5</sup>, when hydroxyammonium is a main transported ion, the mixing of the solution near the membrane surface with the negative pole of voltage results in a considerable increase in  $I_{\min}$  and a decrease in  $g_0$  (Fig. 7A). The mixing of the water solution near the positively charged membrane surface does not influence  $I_{\min}$  or  $g_0$ . Therefore the hydroxyammonium ion carriers under these conditions are positively charged. At pH about 6 the complex of tetrachloro-2-trifluoromethylbenzimidazole with copper ions is expected to have one or two positive charges. Such experiments at pH 7.5–9.0 permit us to conclude that  $\text{H}^+$  carriers in this region of pH are negatively charged.

Similar experiments with methylbarynyl- and dibarynylmercury in the solutions containing  $\text{I}^-$  show that the two forms of carrier are negatively charged (Fig. 7B). In the absence of transported ions the effect of mixing of one water solution does not depend on the membrane voltage when membranes contain dibarynylmercury. Hence, carriers (dibarynylmercury) are not charged.

Under low membrane voltages ( $< 50$  mV) the influence of voltage sign on the effect of mixing of one water solution is low. However, in each carrier form with the same charge sign the mixing of one water solution at zero membrane voltage is more effective than under the high voltage which draws the carriers to the opposite membrane surface.

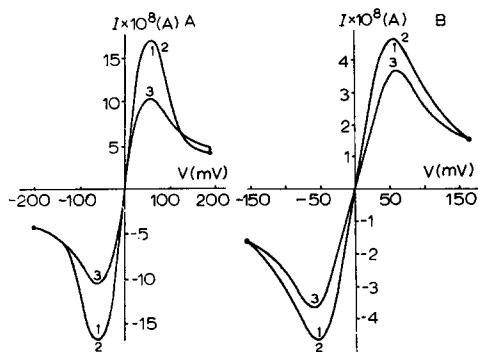


Fig. 7. The determination of the charge sign of carriers within bimolecular membranes. The outer solution is mixed for 10 sec under the membrane voltage indicated by the points on the curves. The potential of the outer solution is taken as 0. Abscissa, potential of the inner solution. A. The bimolecular membrane was formed from phospholipid solution containing tetrachloro-2-trifluoromethylbenzimidazole (2 mg/ml). The aqueous solutions contained 50 mM KCl, 10 mM hydroxyammonium chloride (pH 6.0),  $1 \cdot 10^{-5}$  M  $\text{CuSO}_4$ . Curve 1, characteristics before the mixing; Curve 2 (coincides with Curve 1), after mixing under  $-200$  mV; Curve 3, after mixing under  $+200$  mV. Such change of curves shows the charge of both carrier forms to be positive. B. The bimolecular membrane was formed from phospholipid solution containing  $2 \cdot 10^{-4}$  M dibarenylmercury. The aqueous solutions contained 0.1 M KI. Curve 1, characteristics before mixing; Curve 2 (coincides with Curve 1), after mixing under  $+160$  mV; Curve 3, after mixing under  $-160$  mV. Both forms of carrier were negatively charged.

At a sufficiently high concentration of the neutral form of the carrier the method described is poorly sensitive for the determination of the sign of the charged carrier form. This seems to be connected with the high rate of passage of the neutral form through the membrane surface compared with that of the charged form of the carriers studied.

#### DISCUSSION

The experimental results obtained in this work provide further corroboration of the hypothesis suggesting that ion transport through bimolecular membranes takes place by means of mobile carriers but not through pores in the membrane. An especially strong argument in favour of mobility of carriers within membranes, in our opinion, is the appearance of N-type bimolecular membrane current-voltage characteristics when both forms of ion carrier have the same sign of charge as with decachlorobarene or mercury derivatives of barene. The theoretical current-voltage curves obtained on the basis of the hypothesis of mobile charged ion carriers are also of N type<sup>19</sup>.

In the presence of tetrachloro-2-trifluoromethylbenzimidazole, copper ions and hydroxyammonium, the composition of water solutions, the outer electromotive force source and the resistance may be so chosen that bimolecular membranes generate electric impulses and rhythmic oscillations.

One may suppose that the mechanism of charged carriers is exhibited in excitable cell membranes. One of the possibilities may be the following.

(1)  $\text{Na}^+$  is transported through the membrane by positively charged lipid-soluble carriers. The concentration of the carriers within the membrane is comparatively low, but the mobility and rate constant of heterogeneous association of the carrier with  $\text{Na}^+$ , as well as the rate constant of the dissociation of this complex, are high. These carriers, unlike those known at present, selectively bind  $\text{Na}^+$  but not  $\text{K}^+$ . Molecules of valinomycin and gramicidin A type with chemically bound positively charged groups (*i.e.* tetraalkylammonium) could possibly serve as models of such carriers at least as far as the charge is concerned.

(2)  $\text{K}^+$  is transported through the membrane by negatively charged lipid-soluble carriers. The concentration of these carriers is higher than that of  $\text{Na}^+$  and the rate constants of heterogeneous association and dissociation are lower than those of  $\text{Na}^+$  carriers. The molecules of the same antibiotics with two or more chemically bound negatively charged groups can serve as models of such carriers.

(3) The charge of any carrier form is higher than unity.

(4) At rest the  $\text{Na}^+$  carriers are drawn by the electric field to the inner membrane surface, and  $\text{K}^+$  carriers to the outer surface.

(5) Under membrane depolarization,  $\text{Na}^+$  current increases more quickly than that of  $\text{K}^+$  owing to the difference in the values of the rate constants of association and dissociation of corresponding complexes.

(6) The inactivation of positively charged  $\text{Na}^+$  carriers occurs owing to reversible binding with negatively charged  $\text{K}^+$  carriers, the forms with ions being bound more effectively.

(7) After repolarization the gradual decomposition of the complex carrier- $\text{Na}$ -carrier- $\text{K}$  occurs as the electric field draws the  $\text{Na}^+$  carriers towards one side of the membrane and  $\text{K}^+$  carriers towards the other.

For explaining the effects connected with the role of  $\text{Ca}^{2+}$ , the influence of  $\text{Ca}^{2+}$  on membrane  $\text{K}^+$  conductance in the presence of nactines<sup>20</sup> and gramicidin A<sup>21</sup> should be taken into consideration.

The maximal bimolecular membrane conductance at certain concentrations of transported ions<sup>5,9</sup>, the dependence of bimolecular membrane capacity on frequency<sup>22</sup>, and other properties of bimolecular membranes in the presence of substances influencing the membrane conductance, are well described not only by the model of mobile carriers<sup>16,19</sup> but also by the model of charged pores<sup>23</sup>. In the latter model the chain of carriers is believed to be arranged stationarily across the membrane, and ions jump under the influence of the electric field from one carrier to the next ion-free carrier. Such a mathematical model<sup>23</sup>, with suitable selection of the parameters, is in good agreement with the experimental data, the coincidence sometimes being better than in the model of mobile carriers. However, the charge increase of "pore" links does not result in the appearance of N-type current-voltage characteristics, unlike the model of mobile carriers which predicts such curves observed in the experiment. Certainly, a complex model can be examined in which the "pore" is broken by the electric field, but it will actually be a version of the mobile carrier hypothesis with additional parameters. The experimental distinction between these two modifications is not clear at present, and the development of such a mathematical version is unlikely. It is not impossible that such a model can describe the situation in the excitable cell

membranes where the carrier currents are very low compared with the ionic currents. This obstacle, which upsets the splendidly simple and elegant hypothesis of HODGKIN *et al.*<sup>14</sup>, may also apply to the version elaborated above.

The experiments with dibarenylmercury permit us to suppose that the cation carriers in the excitable cell membranes are neutral in the absence of transported cations, but capable of forming positively charged complexes with one or more cations. In our opinion, such a property should be common, for example, to complex molecules containing valinomycin or gramicidin A type molecules linked together by flexible bonds. Every molecule of such type should complex with  $K^+$ , for example. The addition of one  $K^+$  gives it one positive charge. On acquiring more  $K^+$  it becomes a positively "polycharged" complex. The molecular configuration of alamethicin, inducing N-type membrane current-voltage characteristics in the presence of  $Na^+$  (ref. 24), seems to permit its complexing with one, two and three cations. One may assume that a complex consisting of two chemically bound valinomycin molecules will effectively react with  $Ca^{2+}$  and can serve as a  $Ca^{2+}$  carrier. Such a structure of the carriers within the cell membrane explains the absence of carrier membrane currents in aqueous solutions with low concentrations of penetrating ions<sup>17</sup>.

When one water solution contains KBr only, and the second solution KI and some other penetrating ions, the bimolecular membrane with dibarenylmercury is able to generate an electric impulse and a rhythmic oscillation after the voltage shift. The time parameters of these impulses are close to those of the nerve impulses and rhythmic oscillations of the nerve cell membranes. Therefore it is not impossible that  $K^+$  and  $Na^+$  in the excitable cell membranes are transported by one carrier only.

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