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SELECTIVE TRANSPORT OF IONS THROUGH BIMOLECULAR PHOSPHOLIPID MEMBRANES

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SUMMARY

1. The mechanism of ion transport through bimolecular phospholipid membranes in the presence of a number of lipid-soluble substances was studied.

2. Membrane conductance sharply increases on adding these substances to a lipid solution in heptane, or to aqueous solutions separated by a membrane, if transported ions are present in these solutions. The conductance increases linearly, or with the square of the concentration of carriers.

3. At constant concentration of the carrier, the bimolecular membrane conductance depends non-linearly on the concentration of the transported ion. Conductance reaches a maximum in the region of concentrations of transported ion corresponding to the binding of approximately one-half of the carriers on the membrane surface.

4. Current–voltage curves of bimolecular phospholipid membranes in the presence of the carriers are non-linear. In solutions of low buffer capacity effects connected with the diffusion overpotential near the membrane surface are observed.

5. In the presence of certain carriers the current–voltage curves show a region with negative resistance.

6. A potential difference arises from a transmembrane concentration gradient of penetrating ions or from the carrier-charged species. This potential difference has its maximum in the same region of concentration of transported ions as the conductance.

7. Direct passage of the charged form of the carrier through the membrane and the comparatively slow increase of conductance with the concentration of carrier indicate that the ions do not pass through the "pores" in bimolecular phospholipid membrane or by "relay-race" mechanism, but are transported by mobile carriers.

8. When an osmotic pressure gradient is created by sucrose in the presence of tetrachloro-2-trifluoromethylbenzimidazole, a potential difference arises which corresponds to the charged form being carried along by water flow.

9. The conductance of thick layers of non-polar solvent is changed insignificantly on adding the carrier to aqueous solution. This is connected with the rise of spacial charge. The presence in aqueous solutions of two carriers—one of which is charged

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Abbreviations: TTFB, tetrachloro-2-trifluoromethylbenzimidazole; FCCP, carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone; CCCP, carbonylcyanide-*m*-chlorophenylhydrazone.

positively and the second one negatively—results in a significant increase of conductance of such layers. This effect is also observed on bimolecular membranes.

10. An hypothesis is suggested whereby the selective transport of ions ($X^{\pm}$) through bimolecular phospholipid membranes is performed by charged ($C^{\pm}$) or uncharged (C) carriers. Ions are bound with the carrier by a chemical reaction on a membrane surface. The current through the membrane is carried by a charged form ($C^{\pm}$ or $CX^{\pm}$). The uncharged form (C or CX) diffuses along the concentration gradient. In some cases one carrier molecule and in other cases two molecules participate in the transport of one ion.

INTRODUCTION

A previous study[1] dealt with selective permeability of bimolecular membranes for H^+-ions. The present work is concerned with the mechanism of proton transport and that of certain other ions as well.

Bimolecular membranes were formed through a teflon aperture from a solution of phospholipids of bull brain in heptane as described earlier[1,2]. Current–voltage curves were recorded by non-polarizing electrodes on a polarograph of the type PO-5122 and vibron electrometer of the type 33B.

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

RESULTS

Bimolecular membrane conductance increase by different substances

A number of substances of protein nature[3], detergents[4], 2,4-dinitrophenol and other uncouplers of oxidative phosphorylation[1,5], as well as a number of antibiotics[6,7] were found to increase conductance of bimolecular membranes. The results of measuring membrane conductance in the presence of particular substances are shown in Table I. In order to compare the effect of these substances on artificial membranes and mitochondria the experiments (as in the earlier study[1,8]) were carried out in the mitochondria incubation medium. Substances are quite different in efficiency—as seen from the table. The experiments described in subsequent sections of the paper show that the conductance increase is connected with the increase of bimolecular membrane permeability for different ions. The ion essentially determining the membrane conductance is shown in the table. In most cases H^+ is the ion, for valinomycin it is K^+. In case of decylamine, picric acid, tetraphenylboron and dimethyldibenzylammonium chloride at pH 7.5 the bimolecular membrane conductance is essentially connected with the membrane permeability for these lipid-soluble ions. If the medium does not contain K^+ in the presence of more than 10^{-8} M valinomycin, the bimolecular phospholipid membranes form only on application of a large potential difference (400–600 mV). When the voltage is removed the membrane thickness quickly increases (the black regions disappear). The black membrane resistance in the absence of K^+ is large. This indicates that the bimolecular phospholipid membrane's permeability to Cl^-, $Tris^+$ and H^+ is low.

TABLE I

EFFECT OF DIFFERENT SUBSTANCES ON BIMOLECULAR MEMBRANE CONDUCTANCE

The concentrations of substances (in M) inducing the shown bimolecular membrane conductances are shown. Bimolecular membranes were formed (except the indicated cases) in the mitochondria incubation medium containing 0.25 M sucrose, 20 mM KCl, 10 mM potassium phosphate, 5 mM $MgCl_2$, 30 mM Tris–HCl (pH 7.5) and 2 mM EDTA. The dashes mean that higher concentrations, either do not act, or cannot be obtained because of the low solubility. Higher concentrations of gramicidin A were not studied. S.E. is not more than 30%.

Substance	*Concn. (M)*						*Ion transported*[*]
Conductance ($\Omega^{-1} \cdot cm^{-2}$)	$2 \cdot 10^{-8}$	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	
FCCP	$2 \cdot 10^{-8}$	$2 \cdot 10^{-7}$	$5.5 \cdot 10^{-6}$	$4.5 \cdot 10^{-5}$	$3.4 \cdot 10^{-4}$	—	H^+
Carboxylcyanide-*m*-chlorophenylhydrazone	$3 \cdot 10^{-7}$	$2.3 \cdot 10^{-6}$	$3.5 \cdot 10^{-5}$	—	—	—	H^+
TTFB	$5 \cdot 10^{-7}$	$1.7 \cdot 10^{-6}$	$6 \cdot 10^{-6}$	$1.7 \cdot 10^{-5}$	$3.5 \cdot 10^{-5}$	$1.3 \cdot 10^{-4}$	H^+
2,4-Dichlorophenol	$3.3 \cdot 10^{-5}$	$1.4 \cdot 10^{-4}$	10^{-3}	—	—	—	H^+
2,4,5-Trichlorophenol	$7 \cdot 10^{-6}$	$1.7 \cdot 10^{-5}$	$6 \cdot 10^{-5}$	$2.6 \cdot 10^{-4}$	—	—	H^+
Pentachlorophenol	$6 \cdot 10^{-6}$	$3 \cdot 10^{-5}$	—	—	—	—	H^+
m-Nitrophenol	$7 \cdot 10^{-4}$	$1.3 \cdot 10^{-3}$	$3 \cdot 10^{-3}$	—	—	—	H^+
2,4-Dinitrophenol	$3.5 \cdot 10^{-4}$	$1.4 \cdot 10^{-3}$	—	—	—	—	H^+
Picric acid	$1.5 \cdot 10^{-5}$	$7 \cdot 10^{-5}$	$8 \cdot 10^{-4}$	—	—	—	picrate ion and H^+
Decylamine	$3 \cdot 10^{-4}$	$7.5 \cdot 10^{-4}$	$2 \cdot 10^{-3}$	—	—	—	decylammonium ion and H^+
Acetoacetic ester	10^{-1}	$3 \cdot 10^{-1}$	—	—	—	—	—
Gramicidin A	$6 \cdot 10^{-11}$	$4.5 \cdot 10^{-10}$	$3.8 \cdot 10^{-9}$	$2.3 \cdot 10^{-8}$	$1.3 \cdot 10^{-7}$	—	K^+
Gramicidin A**	$4 \cdot 10^{-9}$	$8.5 \cdot 10^{-9}$	$2.3 \cdot 10^{-8}$	$6 \cdot 10^{-8}$	—	—	H^+
Valinomycin	$7 \cdot 10^{-10}$	$3 \cdot 10^{-9}$	$2.3 \cdot 10^{-8}$	$8 \cdot 10^{-8}$	$2 \cdot 10^{-7}$	$5 \cdot 10^{-7}$	K^+
I_2	$2.5 \cdot 10^{-5}$	$5 \cdot 10^{-5}$	$8 \cdot 10^{-5}$	$1.4 \cdot 10^{-4}$	$2.2 \cdot 10^{-4}$	$7 \cdot 10^{-4}$	I^-
I_2***	—	—	$4 \cdot 10^{-6}$	$2.2 \cdot 10^{-5}$	$6.5 \cdot 10^{-5}$	$1.4 \cdot 10^{-4}$	I^-
Dimethyldibenzylammonium chloride	$1.4 \cdot 10^{-4}$	10^{-3}	—	—	—	—	dimethyldibenzylammonium ion
Tetraphenylboron	$4 \cdot 10^{-8}$	$3 \cdot 10^{-7}$	$3 \cdot 10^{-6}$	$2.4 \cdot 10^{-5}$	—	—	tetraphenylborate ion

[*] In all cases the transported ions were found by the determination of the value and sign of the membrane potential difference under the concentration gradient of these ions. The H^+ and not OH^- transport was determined by the method described earlier[1].

** Bimolecular membranes were formed in 30 mM Tris–HCl (pH 7.5).

*** The mitochondria incubation medium contained 10 mM KI.

Dependence of bimolecular membrane conductance on the concentration of transported ions in solution

Fig. 1 illustrates the dependence of bimolecular membrane conductance on H^+ concentration in the presence of different "carriers" of hydrogen ions. All curves are seen to have a maximum at a H^+ concentration corresponding to the binding of about one-half of the "carriers" in the aqueous solution with H^+, *i.e.* at a pH equalling the p*K* of the "carrier". The second maximum on the 2,4-dinitrophenol curve is probably connected with the quinoid form present in the solution at high pH.

Fig. 2 shows the same dependences of the transport through the bimolecular membranes for K^+ (valinomycin as "carrier")[6,7] and I^- (I_2 as "carrier")[9,10]. In this case there are also maxima on the conductance curves. These results are well explained if the ions are suggested to be transported through the bimolecular membranes by lipid-soluble carriers in all cases. Such a carrier can be charged, *e.g.* tetrachloro-2-trifluoromethylbenzimidazole, 2,4-dinitrophenol, 2,4,6-trinitrophenol or neutral: valinomycin and I_2. The carrier is dissolved in the membrane. At the membrane surfaces it is bound with the transported ion, and the carrier species, bound and unbound with the ion, diffuse through the membrane. At the membrane surfaces, chemical reaction equilibrium is established according to the law of mass action. The electric field on the membrane influences the transport of the charged form and the concentration of this form at the membrane surfaces is changed. The chemical reaction with the transported ion at the membrane surfaces leads to change in the concentra-

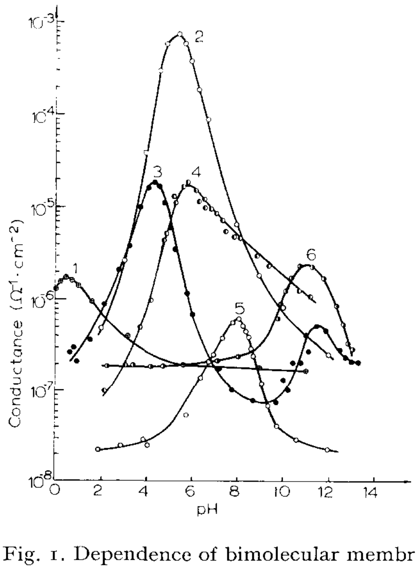


Fig. 1. Dependence of bimolecular membrane conductance on the pH of the aqueous solution in the presence of H^+ carriers: 1, 10^{-2} M picric acid; 2, $3\cdot10^{-5}$ M TTFB; 3, $2.5\cdot10^{-3}$ M 2,4-dinitrophenol; 4, $3\cdot10^{-5}$ M carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP); 5, $2\cdot10^{-3}$ M *m*-nitrophenol; 6, 10^{-3} M decylamine. The experiment was carried out in citrate–phosphate–borate buffer containing sodium (or potassium) citrate, sodium (or potassium) phosphate and boric acid. To change the pH, different quantities of concentrated HCl or NaOH were added. The concentration of each buffer component is 0.2 M (curves 1–3) and 0.02 M (curves 4–6).

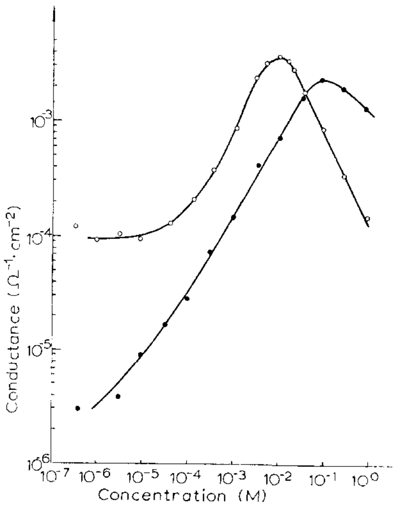


Fig. 2. Dependence of bimolecular membrane conductance, in the presence of 10^{-6} M valinomycin, on KCl concentration (medium: 30 mM Tris–HCl (pH 7.5)) (●—●), and in the presence of $5\cdot10^{-4}$ M I_2 on KI concentration (medium: 30 mM potassium acetate (pH 4.5)) (○—○).

tion of the uncharged form which diffuses through the bimolecular membrane along the concentration gradient. In addition, currents of charged and uncharged forms from the membrane to the solution, and *vice versa*, are induced by concentration gradients and the electric field. The curves of Figs. 1 and 2 would support such a concept of the transport process[11].

Current–voltage curves of bimolecular membranes in the presence of carriers

The above mechanism makes possible a qualitative explanation of non-linear current–voltage characteristics of bimolecular membranes observed in the presence of carriers. At low concentration of the transported ion (or in solutions with a low buffer capacity for H^+) the saturation curves shown in Fig. 3 are observed. In all cases, to maintain electroneutrality and equipotentiality, aqueous solutions contained a comparatively high concentration of non-penetrating ions. As is noticed when limiting the current through a metal electrode, the reduction of current can arise due to decrease of transported ion concentration in aqueous solution near the membrane surface. The current through the membrane, under a fixed constant potential difference between the solutions, decreases in time (Fig. 4). After switching off the voltage a potential, decreasing with time, is observed at the membrane. It is connected with a rise in the penetrating ion concentration gradient on the membrane. In solutions with high buffer capacity this phenomenon should not be observed, and in fact, it is not. The current–voltage curves are as shown in Fig. 5. Rapid rise of the current with voltage increase (Fig. 5, curve 1) can be explained by direct transport through the membrane of the unbound charged form of the tetrachloro-2-trifluoromethylbenzimidazole (TTFB), whose concentration in solution was higher than that of H^+. $TTFB^-$

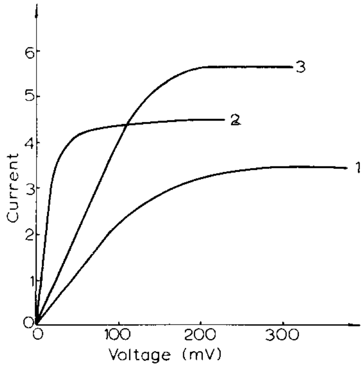


Fig. 3. Current–voltage curves of bimolecular membranes: 1, the membranes were obtained from lipid solution in heptane containing TTFB (1 mg/ml); the experiment was performed in water without buffer (pH 8.0); 2, 30 mM potassium acetate (pH 4.5), $5 \cdot 10^{-4}$ M I_2 and $3 \cdot 10^{-6}$ M KI; 3, 30 mM Tris–HCl (pH 8.0), 10^{-5} M valinomycin and 10^{-4} M KCl. The velocity of voltage changing equals 0.125 V/sec. The calibration of the current axis: Curve 1, 10^{-8} A; curve 2, $3 \cdot 10^{-8}$ A; curve 3, $5 \cdot 10^{-8}$ A.

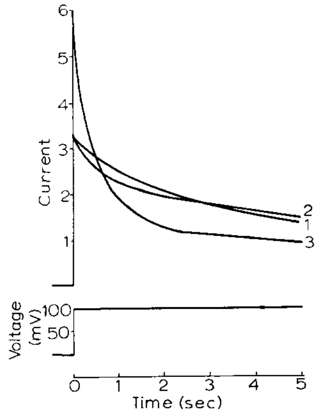


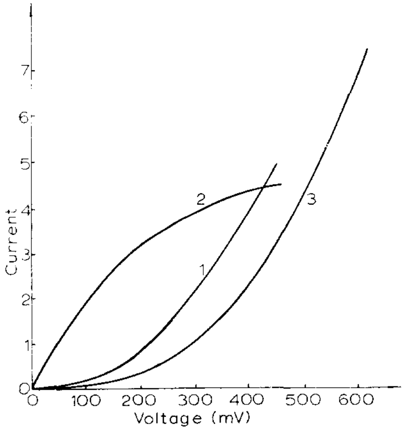
Fig. 4. Time dependence of bimolecular membrane current under fixed potential difference (100 mV) between solutions: 1, 0.1 M KCl, 10^{-4} M TTFB (pH 6.3); 2, 30 mM Tris–HCl (pH 8.0), $5 \cdot 10^{-6}$ M valinomycin and 10^{-5} M KCl; 3, 30 mM potassium acetate (pH 4.5), $5 \cdot 10^{-4}$ M I_2 and $5 \cdot 10^{-4}$ M KI. The calibration of the current axis: Curve 1, 10^{-7} A; curve 2, 10^{-8} A; curve 3, $5 \cdot 10^{-7}$ A. The moment that voltage switched on is taken as zero time.

transition from aqueous solution into membrane is connected with the change in ion free energy. It is easier for the ion to overcome this barrier at high potential difference between the aqueous solutions separated by the membrane. For the uncharged carrier, I_2, the same type of current–voltage curve (Fig. 5, curve 3) is observed at high concentration of I^- in solution. In this case a direct transport of I_3^- appears to occur. For the large carrier, valinomycin, current limitation due to slow diffusion of the charged form through the membrane must play a fundamental role. Actually, at high concentration of K^+ in solution the concentration of the valinomycin uncharged form in the membrane must be low. The shape of the current–voltage curve under these conditions is shown in Fig. 5 (curve 2). Current limitation cannot be explained by the change in K^+ concentration in the solution near the membrane, as neither change of current in time nor the appearance of a potential are observed. Direct transport of valinomycin bound with the K^+ through the membrane from one solution to another does not play an essential role, because, according to previous studies[12,13], the concentration of K^+–valinomycin complex in water is small.

Current–voltage curves with negative resistance

In solutions with a high concentration of TTFB and comparatively low concentration of H^+, N-type bimolecular membrane current–voltage curves are observed[1,14] (Fig. 6). Such curves allow one to obtain on bimolecular membranes the electric impulses similar to the nerve impulse of excitable membranes. One hopes that in the future such membranes can be used as generators and other non-linear elements.

Bimolecular membrane current–voltage characteristics with negative resistance were observed earlier by MUELLER AND RUDIN[3] in the presence of unidentified



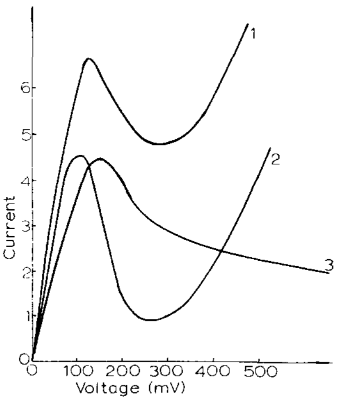
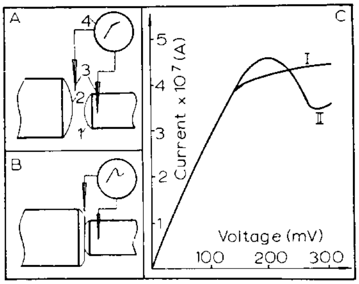


Fig. 5. Current–voltage curves of bimolecular membranes at high concentration of penetrating ion (or high buffer capacity): 1, 0.2 M potassium citrate, 0.2 M potassium phosphate, 0.2 M boric acid (pH 7.8), 10^{-4} M TTFB; 2, 0.15 M KCl, $5 \cdot 10^{-7}$ M valinomycin; 3, 30 mM potassium acetate (pH 4.5), $3 \cdot 10^{-2}$ M KI and $5 \cdot 10^{-6}$ M I_2. The velocity of voltage change equals 0.125 V/sec. The calibration of the current axis: Curve 1, 10^{-7} A; curve 2, 10^{-7} A; curve 3, $3 \cdot 10^{-6}$ A.

Fig. 6. Bimolecular membrane current–voltage curves with negative resistance: 1, 30 mM potassium acetate (pH 4.5), 10^{-3} M KI and $8 \cdot 10^{-4}$ M I_2; 2, 0.1 M KCl, 10^{-5} M $CuSO_4$, 10^{-4} M TTFB, pH 8.8; 3, 30 mM Tris–HCl (pH 7.5), 10^{-4} M tetraphenylboron. The velocity of voltage changing equals 0.125 V/sec. The calibration of the current axis: Curve 1, $5 \cdot 10^{-7}$ A; curve 2, $2 \cdot 10^{-8}$ A; curve 3, $2 \cdot 10^{-8}$ A.

protein material. In the presence of carriers they usually appear for bimolecular membranes in cases where the limitation of current connected with the diffusion of the penetrating ion from solution to the membrane is essential (Fig. 6, curves 1, 3). Such characteristics are not observed in buffer solutions of high capacity. To test the suggestion that such characteristics are connected with the processes in the near-membrane region we used an arrangement of two bimolecular membranes. The current–voltage characteristics of one membrane were recorded; the second membrane was then brought up to the first one so that a narrow gap of aqueous solution of about 100 Å thickness was left between them. Bringing together the membranes turns the steady characteristics into a falling curve or results in the commencement of a falling region at a lower voltage (Fig. 7).



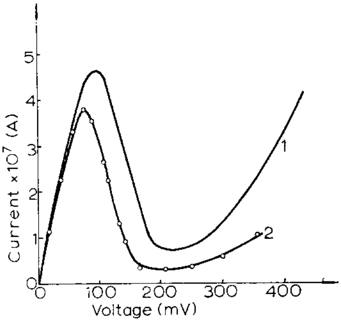


Fig. 7. Installation diagram and current–voltage curves in 0.1 M KCl, 10^{-4} M TTFB at pH 7.0 before and after bringing together the two membranes. A, Bimolecular membranes 1 and 2 are formed on the endings of two polyethylene tubes (see ref. 15). The current–voltage characteristics of the membrane 1 are recorded by the electrodes 3 on the polarograph 4. B, The membranes are brought together. C, The curve 1 is recorded under the conditions illustrated by diagram A, the curve II involving negative resistance is recorded under the conditions of the diagram B. The effect is reversible.

Fig. 8. Current–voltage curves of the bimolecular membranes obtained from lipid solution in heptane containing TTFB (1 mg/ml) in 0.1 M KCl, 10^{-5} M $CuSO_4$ and 30 mM hydroxylamine (pH 5.5): ———, the curve recorded under the velocity of voltage change equal to 0.125 V/sec; O—O, stationary curve plotted on the points resulting from voltage fixation for 2 min.

The current–voltage curves of bimolecular membranes in the presence of I_2, tetraphenylboron and TTFB are not stationary and the voltage at which a falling region on the curve occurs depends on the concentration of the penetrating ion.

However, the characteristics involving negative resistance cannot be explained only by near-membrane processes. Under some conditions such characteristics are weakly dependent upon the velocity of voltage change. The falling region on the current–voltage curves of bimolecular membranes obtained in aqueous solutions containing TTFB at pH 9–10 appears upon adding Cu^{2+} and is deepened with increasing amounts of Cu^{2+} and Cu^{+} concentration (Fig. 6, curve 2). In the presence of hydroxylamine the curves (Fig. 8) have a negative resistance region at pH 4–11. At pH 5–7 the aqueous solution of hydroxylamine has high buffer capacity for H^+. These facts indicate that a falling characteristic of bimolecular membranes in the presence of TTFB and Cu^{2+} is not due to change of near-membrane pH on passage of electric current through membrane.

In the case of a neutral carrier transporting cations (valinomycin and gramicidin) the falling region on the bimolecular membrane current–voltage curves appears or deepens after the addition of a negatively charged carrier, *e.g.* *m*-nitrophenol, to the solution. If in the membrane there are two carriers (one +, another —), and if their concentrations are not very high, the carriers will be moved by the electric field to different sides of the membrane. In this case, not only the concentration of the carriers in the middle part of the membrane, but also the potential difference in the membrane region where the resistance is high, will become lower.

The mechanism of the appearance of the negative resistance region on bimolecular membrane current–voltage characteristics in the presence of other ion carriers is probably partly connected with the effects described above. However, it is not yet clear whether there are two types of charged carrier for I_2 and tetraphenylboron (Fig. 6).

Fig. 9 shows the dependence of short-circuit current on pH differences in buffer solutions containing TTFB. On one side of the bimolecular membrane the pH is constant and equals 5.8. The short-circuit current is not a steadily increasing function of the transmembrane pH difference, though the potential difference under these conditions does increase steadily.

Dependence of bimolecular membrane potential difference on penetrating ion and carrier ion concentration gradient

Fig. 10 shows the dependence of membrane potential difference under a tenfold transmembrane concentration gradient of penetrating ion ($\Delta V/\Delta \lg[A^-]$, A = penetrating ion) on the concentration of this ion in solution. These curves indicate the high selectivity of the carriers studied. They have maxima which are found at the same concentrations as those in Fig. 1. Table II indicates a rather good correlation of the

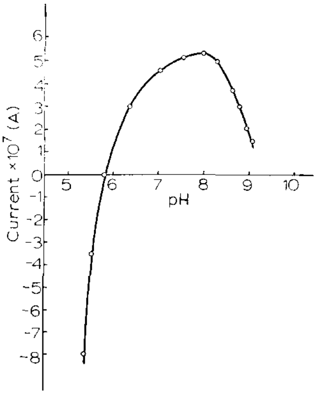


Fig. 9. Dependence of short-circuit bimolecular membrane current in 0.2 M potassium citrate, 0.2 M potassium phosphate, 0.2 M boric acid, 10^{-4} M TTFB on pH difference. On one side of the membrane the pH is constant and equals 5.8; on the other side it is changed.

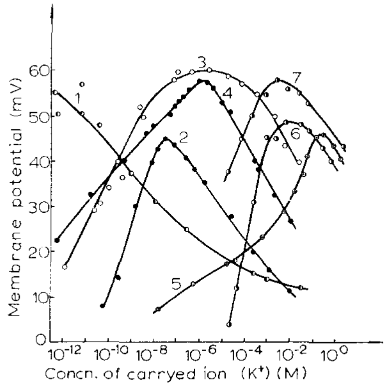


Fig. 10. Dependence of bimolecular membrane potential difference under tenfold transmembrane concentration gradient of penetrating ion (A) on the concentration of A, 1, 1 mM decylamine; 2, 2 mM *m*-nitrophenol; 3, $3 \cdot 10^{-5}$ M TTFB; 4, $3 \cdot 10^{-5}$ M FCCP; 5, 10 mM picric acid (medium is citrate–phosphate–borate buffer (see legend to Fig. 1) and A is H^+); 6, 30 mM potassium acetate (pH 4.5), 0.5 mM I_2, A is I^-; 7, 30 mM Tris–HCl (pH 7.5), 10^{-5} M valinomycin.

positions of the maxima in Figs. 1 and 10, and of the equilibrium constants of the respective carrier complexes.

These results and those given above can be explained not only by the hypothesis of the carriers advanced earlier[11] but also, suggesting that in the membrane there are "pores" selectively permeable for ions or chains of immobile carriers with a "relay-race" mechanism. However, a very slow increase of conductance with increase of carrier concentration in solution militates against the latter suggestion (Fig. 11).

TABLE II

CORRELATION OF STABILITY CONSTANTS OF ION–CARRIER COMPLEXES AND MAXIMA IN FIGS. 1 AND 10

All stability constants are shown for aqueous solution except equilibrium valinomycin + $K^+ \leftrightarrows$ valinomycin–K^+, whose constant is determined in ethanol[13]. K_{I_2} is the constant of the equilibrium $I_2 + I^- \leftrightarrows I_3^-$. The rest of the constants are the usual acid or base dissociation constants.

Carrier	$K(M)$	$Cg_{max}(M)$*	$C(\Delta V/\Delta lg[A])_{max}(M)$**	A
FCCP	$2\cdot10^{-6}$	$(2 \pm 1)\cdot10^{-6}$	$(1.6 \pm 0.5)\cdot10^{-6}$	H^+
TTFB	$2\cdot10^{-6}$	$(3 \pm 1.5)\cdot10^{-6}$	$(2.5 \pm 2.0)\cdot10^{-6}$	H^+
Pentachlorophenol	10^{-5}	$(1 \pm 0.5)\cdot10^{-5}$	$(3 \pm 1.5)\cdot10^{-5}$	H^+
m-Nitrophenol	10^{-8}	$(1 \pm 0.5)\cdot10^{-8}$	$(3 \pm 2)\cdot10^{-8}$	H^+
2,4-Dinitrophenol	10^{-4}	$(8 \pm 4)\cdot10^{-5}$	$(1 \pm 0.5)\cdot10^{-4}$	H^+
	—	$(2.5 \pm 1.5)\cdot10^{-11}$	$(1.6 \pm 0.5)\cdot10^{-11}$	—
Picric acid	$2\cdot10^{-1}$	$(3 \pm 1.5)\cdot10^{-1}$	$(2 \pm 1)\cdot10^{-1}$	H^+
Decylamine	$2\cdot10^{-11}$	$(1 \pm 0.5)\cdot10^{-11}$	—	H^+
Valinomycin	$1.9\cdot10^{-4}$	$(1 \pm 0.5)\cdot10^{-1}$	$(3 \pm 1.5)\cdot10^{-3}$	K^+
I_2	$1.5\cdot10^{-3}$	$(1 \pm 0.5)\cdot10^{-2}$	$(1 \pm 0.5)\cdot10^{-2}$	I^-

* Cg_{max}, concentration of penetrating ion [A] at which the bimolecular membrane conductance is maximal.

** $C(\Delta V/\Delta lg[A])_{max}$, concentration at which the bimolecular membrane potential difference under tenfold transmembrane concentration gradient of penetrating ion is maximal.

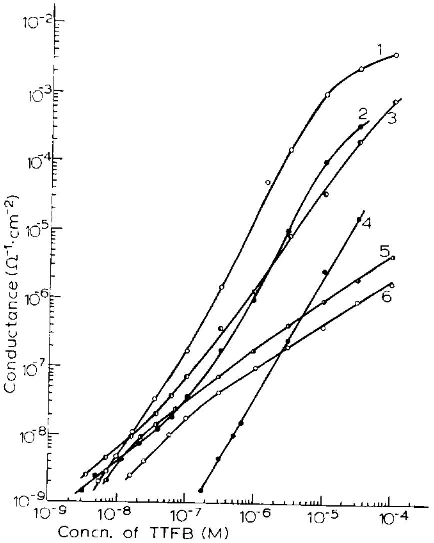


Fig. 11. Dependence of bimolecular membrane conductance on TTFB concentration in 20 mM potassium citrate, 20 mM potassium phosphate and 20 mM boric acid at different pH: 1, pH 5.6; 2, pH 3.4; 3, pH 7.3; 4, pH 1.2; 5, pH 9.2; 6, pH 11.1.

The experiments show that 1–2 molecules of the carrier participate in the transport of one ion. Furthermore, in solutions with low concentrations of H^+, the $TTFB^-$ concentration gradient produces the bimolecular membrane potential difference. The *minus* sign is observed in this case in a solution with low concentration of TTFB. This phenomenon indicates the direct passage of negatively charged carriers through the membrane. At pH 11 and $3 \cdot 10^{-5}$ M TTFB $R_{H^+} \approx R_{TTFB^-}$ (R = resistance). Thus the permeability of the bimolecular membranes to $TTFB^-$ under these conditions is less than that to H^+ by a factor of approx. 10^6–10^7. No direct passage of $TTFB^+$ at all pH's down to pH 1 is observed (ΔV under 10-fold concentration gradient of TTFB is less than 3 mV).

Direct passage of the carrier ion is especially well observed in case of picric acid. Permeabilities of bimolecular membranes to H^+ and picrate ions are approximately equal. At pH 7.5, ΔV on the membrane with a 10-fold concentration gradient of picrate ion is more than 40 mV if the picrate ion concentration is more than 10^{-5} M.

Osmoelectric experiments

The osmotic pressure gradient on the membrane was obtained by adding sucrose to one of the solutions separated by the bimolecular membrane. The membrane potential difference induced by osmotic water flow (ΔV_{osm}) in the presence of different carriers and the dependence of this potential difference on the concentration of penetrating ions in aqueous solution were recorded. In most cases ΔV_{osm} was hardly measurable. However, when it had a marked value (in the case of TTFB) there was a dependence not only of the value but also of the sign of the potential on the concentration of penetrating H^+ (Table III). If the ions would pass through charged or uncharged pores in the membrane such an effect cannot be expected. The negative sign of the potential in sucrose solution at high pH can be explained by the transportation of negatively charged carrier in the water flow. The membrane permeability to H^+, and not to OH^-, under these conditions, was demonstrated earlier[1].

TABLE III

DEPENDENCE OF OSMOELECTRIC POTENTIAL DIFFERENCE (ΔV_{osm}) IN THE PRESENCE OF $5 \cdot 10^{-5}$ M TTFB ON pH

0.1 M sucrose is added on one side of bimolecular membranes. Sign of the potential is shown in sucrose solution.

pH	1.0	3.0	5.0	7.0	9.0	11.0
ΔV_{osm} (mV)	+10 ± 2	+ 5 ± 2	0 ± 2	−10 ± 1	−25 ± 5	−25 ± 5

It is necessary to indicate that if the solution in the near-membrane region is poorly mixed a negative potential in sucrose at alkaline pH's and a positive one at acid pH's can result from buffer dilution by water flow, by a change of the near-membrane pH towards that of pure water. In this case the potential difference must decrease when mixed. Yet no such effect of the mixing of sucrose solution on ΔV_{osm} was found. Unfortunately one cannot be sure that the rate of solution mixing by a magnetic mixer (25 rev./sec) is sufficient, while at a higher rate the membranes were destroyed.

Ion transport through thick layers of non-polar solvent

All carriers listed above sharply increase the bimolecular membrane conductance for ions. However, acting one by one they are of low efficiency in thick layers of non-polar solvent. This phenomenon appears to be concerned with the fact that the charged carrier cannot pass through a thick layer because of the initiation of spacial charges. One could expect that when these carriers are used, *e.g.* the neutral one for cations and the neutral one for anions, high conductance of thick layers of non-polar solvents would result. Experiments on thick layers of heptane confirmed this suggestion.

Two carriers are also more efficient on bimolecular membranes. This fact is illustrated by Table IV.

TABLE IV

DEPENDENCE OF BIMOLECULAR MEMBRANE CONDUCTANCE (IN $\Omega^{-1} \cdot \text{cm}^{-2}$) ON THE CONCENTRATION RATIO OF PICRIC ACID AND DECYLAMINE IN AQUEOUS SOLUTION

Experiment was carried out in 20 mM sodium citrate, 20 mM sodium phosphate, 20 mM boric acid (pH 3.0).

Picric acid (mM)		*Conductance ($\Omega^{-1} \cdot cm^{-2}$)*		
	Decylamine (mM)	*0*	*0.25*	*2.0*
0		$(3 \pm 1) \cdot 10^{-9}$	$(1.0 \pm 0.2) \cdot 10^{-7}$	$(5.0 \pm 1.0) \cdot 10^{-7}$
0.25		$(2.0 \pm 0.4) \cdot 10^{-6}$	$(7.5 \pm 0.5) \cdot 10^{-5}$	$(2.5 \pm 0.5) \cdot 10^{-4}$

DISCUSSION

The results described above show that charged and uncharged carriers can selectively carry ions through bimolecular membranes, the carrier forms bound with the ion being neutral or charged. Extremely high selective permeability of the membranes for ions in the presence of carriers seems to be connected with the fact that the mobility of the carriers, especially comparatively large ones, depends little on what ion is transported. Thus, unlike the situation with ion-exchangers and glasses, the difference in the permeability for ions is essentially determined by the difference in the stability constants of ion–carrier complexes.

All ion carriers are lipid-soluble substances. But this is not all: the ability for ion formation in a non-polar solvent is required. Molecules which change their electron orbital configuration on forming ions, in such a way that the ion becomes more "lipophilic" than the neutral form of the carrier, are suggested to be more effective. In this connection it can be noted that the absorption spectrum of the charged form of TTFB in heptane differs more from that in aqueous solution than the corresponding 2,4-dinitrophenol spectra[8].

Molecules that mask polar groups during ionization in a non-polar solvent, and reveal non-polar ones, must be good carriers. The change in valinomycin configuration after K^+ binding is evident by the fact that, in the presence of valinomycin in K^+-free solution, phospholipids form only the thick membranes. The addition of K^+ to the solution leads to the formation of the bimolecular membranes.

It is not excluded that there are special carriers for transporting ions in the cell membranes as HODGKIN AND HUXLEY[16] suggested. An attempt to discover the maximum conductance of the type shown in Figs. 1 and 2 at high K^+ concentration on both sides of cell membrane would be of interest. However, in experiments with hypertonic solutions of potassium acetate carried out on frog muscle fibers by LIBERMAN AND CHAYLAKHYAN, such a maximum was not observed. The final answer can surely be obtained only on perfused fibers.

The results presented in this paper show that current–voltage characteristics with negative resistance and impulse generation can be largely related to diffusion processes not only in the membrane but in near-membrane regions as well. It would be interesting therefore to study the role of the gap between the excitable membrane of neurons and Schwann cells and the gaps in the endoplasmic reticulum of muscle fibers.

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