Transport of Picrate Anion against Its Concentration Gradient through a Dichloroethane Membrane

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The rate of picrate transport coupled to a diffusion of potassium ions through a bulk 1,2-dichloroethane membrane has been measured. The membrane, containing a potassium ionophore separated two aqueous phases, one containing picrate and potassium salt and the other containing picrate and lithium salt. The picrate anion was accumulated against the concentration gradient in the aqueous phase containing the lithium salt. The rate of picrate transport incrased with increasing ionophore concentration and in the absence of the ionophore, the transfer of picrate did not occur. The effect of potassium and lithium counter-ions such as chloride, nitrate and sulfate on the transport of picrate has been examined. The combination of potassium sulfate and lithium nitrate showed the highest rate of transport. In the potassium nitrate–lithium nitrate system, the transport of picrate was appreciably depressed by the transfer of nitrate ions across the membrane. The decrease in concentration of potassium ion in the aqueous phase brought about a lowering of the rate of picrate transport. The rate of picrate transport rose according to the following order of ionophore : dicyclohexyl-18-crown-6>dicyclohexyl-24-crown-8>dibenzo-18-crown-6>dibenzo-24-crown-8>valinomycin.

When the chemical potential difference of ions is imposed across an oil membrane, a lipophilic ion or a specific ion which binds to a transport carrier can be moved against the concentration gradient to one side of the membrane. The uphill transport of alkaline cations[1,2] and amino acids[3] through bulk organic liquid membranes has been reported and in these systems, the energy for transport was the chemical potential difference of the protons across the membrane. Kusaka *et al.*[4] have observed the accumulation of alanine and proline into phospholipid vesicles containing hydrohobic proteins as transport carriers, in response to a membrane potential introduced by the diffusion of potassium ions *via* valinomycin.

In the present paper, the uphill transport of picrate anion through the bulk 1,2-dichloroethane membrane has been investigated. In this system, the transport of picrate has been coupled to a diffusion of potassium ions *via* various potassium ionophores.

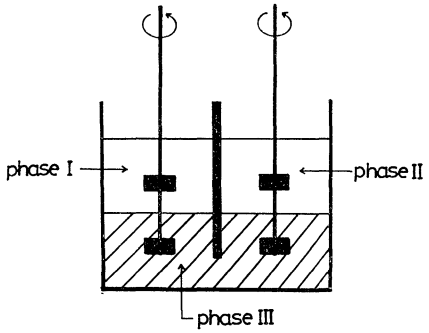

Fig. 1. Apparatus for measuring the transport of picrate. Initial composition of each phase;
phase I : aqueous solution (50 ml) containing 10^{-4} M K-picrate, 0.01 M Tris-H_2SO_4 buffer (pH 8.3) and 0.001—0.1 N K-salt,
phase II : aqueous solution (50 ml) containing 10^{-4} M K-picrate, 0.01 M Tris-H_2SO_4 buffer (pH 8.3) and 0.1 N Li-salt,
phase III : 1,2-dichloroethane (100 ml) containing 0—10^{-3} M ionophore.

Experimental

Apparatus. The apparatus used for measuring the transport of picarte is shown in Fig. 1. It is a modification of the apparatus described by Behr and Lehn.[3] The cell consisted of a cylindrical glass vessels (7 cm i.d.; height 7 cm) containing a central glass wall which separated the two aqueous phases (50 ml each). The dichloroethane phase (phase III, 100 ml) lay under these aqueous phases and bridged across the central separation. The aqueous phase I in the cell contained initially 10^{-4} M potassium picrate, a 0.01 M Tris-H_2SO_4 buffer of pH 8.3 and a 0.1 M potassium salt (the experiment on the effect of potassium ion concentration was different) while phase II contained 10^{-4} M potassium picrate, a 0.01 M Tris-H_2SO_4 buffer and a 0.1 M lithium salt. To the dichloroethane phase, an appropriate quantity of potassium ionophore was added. The cell was placed in a water bath adjusted to 25±0.1 °C. All three phases were agitated at 180 r.p.m. with a pair of glass stirrers.

Materials. The potassium ionophores, dicyclohexyl-18-crown-6 and dicyclohexyl-24-crown-8 were commercial products from Nihon Soda Co., dibenzo-18-crown-6 and dibenzo-24-crown-8 from Aldrich Chemical Co. and valinomycin from Sigma Chemical Co. All other chemicals were reagent grade and not subsequently purified.

Procedure. The dichloroethane used for phase III was pre-equilibrated with 10^{-4} M potassium picrate solution containing a Tris-H_2SO_4 buffer and potassium and lithium salts, whose concentrations were equal to those in the aqueous phases I and II. 1,2-Dichloroethane (100 ml) containing an appropriate quantity of ionophore was shaken with the above-mentioned picrate solution(250 ml) for *ca.* 6 h at 25 °C. During this treatment, the picrate solution was occasionally renewed until the picrate concentration in the aqueous solution became constant. After attainment of equilibrium, the picrate solution was removed, after which dichloroethane (100 ml) was placeed in the cell. Two kinds of aqueous solution used for phases I and II were subsequently poured into either side, as shown in Fig. 1. The transport of picrate was initiated by the addition of the aqueous solutions. The concentration of picrate in both the aqueous phases and the membrane potential were measured at regular time intervals, the determination being conducted on a Hitachi Model 100-10 spectrophotometer at 420 nm and that of the membrane potential using a Keithley Model

Table 1. Initial concentration of picrate in the 1,2-dichloroethane phase and concentration 7 h after the start of picrate transport for various membrane systems

K, Li salts Phase I-Phase II	Ionophre (Concn, M)	Picrate concentration (M) Initial	After 7 h
0.1 M KCl-0.1 M LiCl	Dicyclohexyl-18-crown-6 (10^{-5})	0.9×10^{-5}	0.8×10^{-5}
0.1 M KCl-0.1 M LiCl	Dicyclohexyl-18-crown-6 (10^{-4})	9.0×10^{-5}	7.2×10^{-5}
0.1 M KCl-0.1 M LiCl	Dicyclohexyl-18-crown-6 (10^{-3})	9.2×10^{-4}	8.2×10^{-4}
0.1 M KNO_3-0.1 M $LiNO_3$	Dicyclohexyl-18-crown-6 (10^{-4})	9.9×10^{-5}	9.3×10^{-5}
0.05 M K_2SO_4-0.05 M Li_2SO_4	Dicyclohexyl-18-crown-6 (10^{-4})	8.3×10^{-5}	6.1×10^{-5}
0.05 M K_2SO_4-0.1 M $LiNO_3$	Dicyclohexyl-18-crown-6 (10^{-4})	9.1×10^{-5}	6.6×10^{-5}
0.1 M KCl-0.05 M Li_2SO_4	Dicyclohexyl-18-crown-6 (10^{-4})	8.8×10^{-5}	6.5×10^{-5}
0.05 M KCl 0.05 M LiCl-0.1 M LiCl	Dicyclohexyl-18-crown-6 (10^{-4})	8.2×10^{-5}	7.1×10^{-5}
0.01 M KCl 0.09 M LiCl-0.1 M LiCl	Dicyclohexyl-18-crown-6 (10^{-4})	7.9×10^{-5}	6.8×10^{-5}
0.005 M KCl 0.095 M LiCl-0.1 M LiCl	Dicyclohexyl-18-crown-6 (10^{-4})	7.4×10^{-5}	6.8×10^{-5}
0.001 M KCl 0.099 M LiCl-0.1 M LiCl	Dicyclohexyl-18-crown-6 (10^{-4})	5.3×10^{-5}	5.2×10^{-5}
0.1 M KCl-0.1 M LiCl	Dicyclohexyl-24-crown-8 (10^{-4})	8.9×10^{-5}	7.4×10^{-5}
0.1 M KCl-0.1 M LiCl	Dibenzo-18-crown-6 (10^{-4})	2.6×10^{-5}	1.1×10^{-5}
0.1 M KCl-0.1 M LiCl	Dibenzo-24-crown-8 (10^{-4})	2.4×10^{-5}	2.1×10^{-5}
0.1 M KCl-0.1 M LiCl	Valinomycin (10^{-4})	8.9×10^{-5}	8.7×10^{-5}

610C or a Toadenpa Model PM-19A electrometer, with calomel electrodes connected to the aqueous phase, *via* salt bridges made of polyethylene tubing containing agar saturated with potassium chloride. The picrate concentrations in the dichloroethane phase before and after the measurement of picrate transport were also determined spectrophotometrically.

Results

Effect of Ionophore Concentration. The picrate concentrations in both aqueous phases and the membrane potential against time curves for various concentration of dicyclohexyl-18-crown-6 are shown in Fig. 2. The polarity of the membrane potential was positive in phase II with respect to phase I. In these systems, phase I contained 0.1 M potassium chloride and phase II 0.1 M lithium chloride. The picrate concentration in phase I decreased with time [dotted line in Fig. 2 (a)], while that in phase II increased [solid line in Fig. 2 (a)]. Thus the picrate anion was transferred from phase I to phase II against the concentration gradient through the dichloroethane membrane. The increment of picrate in phase II however was larger than the decrement in phase I attributable to the liberation of picrate from the dichloroethane phase into phase II. The initial concentrations of picrate in the dichloroethane phase and the concentrations 7 h after the start of picrate transport are listed in Table 1, together with those for the other transport systems.

The rate of picrate transport increased with increasing ionophore concentration. In the absence of ionophore, the transfer of picrate was not observed. In this case, the membrane potential was about 25 mV.

Effect of Potatsium and Lithium Counter-ions. When the nitrates and sulfates of potassium and lithium were added in the aqueous phases in place of the chlorides, the transport of picrate was affected by the counter-ions, the effect of which is shown in Fig. 3.

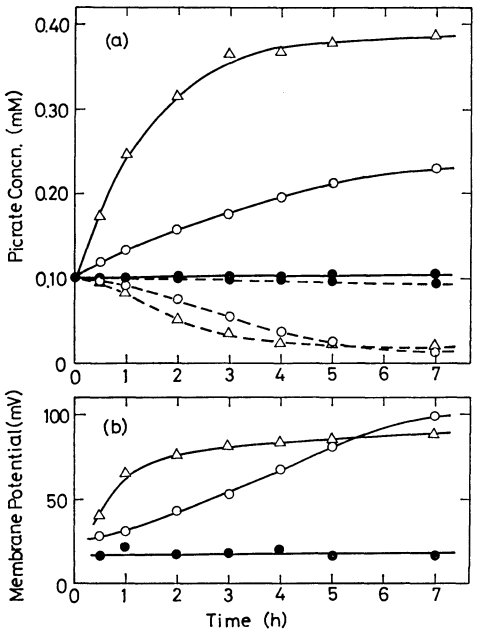

Fig. 2. Effect of ionophore concentration on the transport of picrate.
(a) Picrate concentrations in both aqueous phases against time curves.
The dotted and solid lines represent the picrate concentrations in phases I and II respectively.
(b) Membrane potential against time curves. Ionophore concentration;
● : 10^{-5} M, ○ : 10^{-4} M, △ : 10^{-3} M.
Ionophore; dicyclohexyl-18-crown-6, K, Li salts; phase I : 0.1 M KCl, phase II : 0.1 M LiCl.

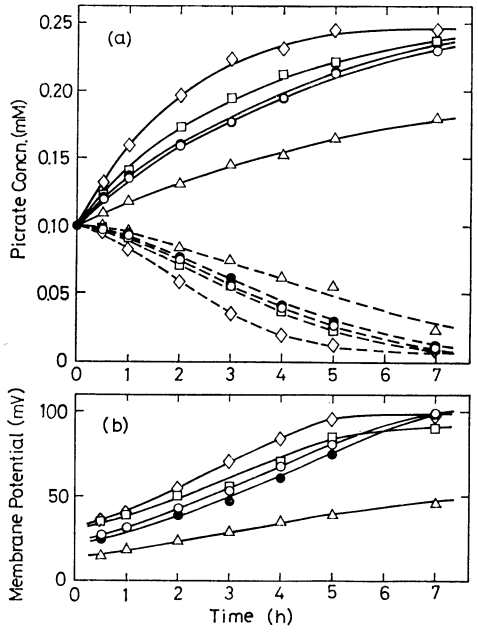

Fig. 3. Effect of potassium and lithium counter-ions on the transport of picrate.
The curves have the same meanings as in Fig. 2. K and Li salts in phases I and II; ○: 0.1 M KCl (I)–0.1 M LiCl (II), △: 0.1 M KNO_3 (I)–0.1 M $LiNO_3$ (II), □: 0.05 M K_2SO_4 (I)–0.05 M Li_2SO_4 (II), ◇: 0.05 M K_2SO_4 (I)–0.1 M $LiNO_3$ (II), ●: 0.1 M KCl (I)–0.05 M Li_2SO_4 (II). Ionophore; 10^{-4} M dicyclohexyl-18-crown-6.

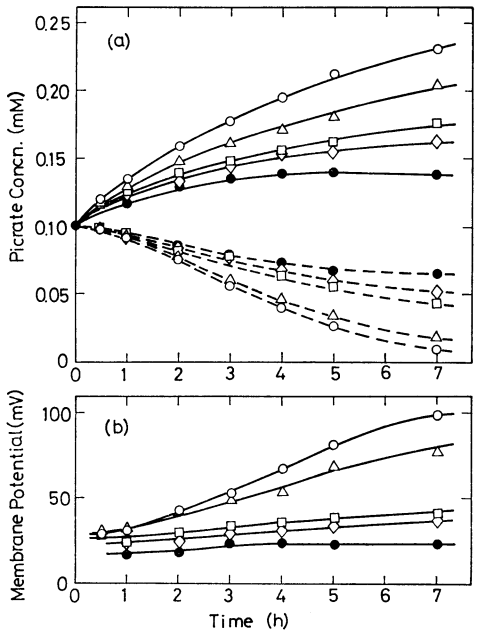

Fig. 4. Effect of potassium ion concentration on the transport of picrate.
The curves have the same meanings as in Fig. 2. K and Li salts in phases I and II; ○: 0.1 M KCl (I)–0.1 M LiCl (II), △: 0.05 M KCl, 0.05 M LiCl (I)–0.1 M LiCl (II), □: 0.01 M KCl, 0.09 M LiCl (I)–0.1 M LiCl (II), ◇: 0.005 M KCl, 0.095 M LiCl (I)–0.1 M LiCl (II), ●: 0.001 M KCl, 0.099 M LiCl (I)–0.1 M LiCl (II). Ionophore; 10^{-4} M dicyclohexyl-18-crown-6.

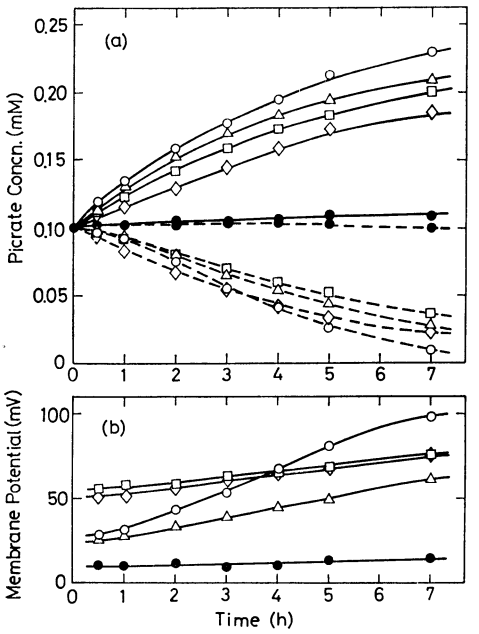

Fig. 5. Effect of ionophores on the transport of picrate.
The curves have the same meanings as in Fig. 2. Ionophore; ○: dicyclohexyl-18-crown-6, △: dicyclohexyl-24-crown-8, □: dibenzo-18-crown-6, ◇: dibenzo-24-crown-8, ●: valinomycin.
Ionophore concentration; 10^{-4} M, K, Li salts; phase I: 0.1 M KCl, phase II: 0.1 M LiCl.

In these systems, dicyclohexyl-18-crown-6 was used as an ionophore at a concentration in dichloroethane of 10^{-4} M.

In the potassium sulfate–lithium nitrate system, the highest rate of picrate transport was observed. On the contrary, a considerable decrease in the rate of picrate transport occurred in the potassium nitrate–lithium nitrate system.

Effect of Potassium Ion Concentration. The effect of varying the concentration of potassium chloride in phase I from 0.001 to 0.1 M on the transport of picrate is shown in Fig. 4. In this system, lithium chloride was added to compensate for the lack of chloride ion in phase I. The ionophore used was dicyclohexyl-18-crown-6 and the concentration in the dichloroethane phase was 10^{-4} M.

The rate of picrate transport decreased with decreasing potassium ion concentration. The change in membrane potential with time also decreased according to the reduction of the rate of picrate transport.

Effect of Ionophores. The picrate concentrations in the aqueous phases and the membrane potential against time curves for various ionophores are shown in Fig. 5. In these systems, phase I contained 0.1 M potassium chloride and phase II 0.1 M lithium chloride. The concentrations of ionophores in the dichloroethane phase were 10^{-4} M in all cases.

The rate of picrate transport increased according in the following order of ionophore; dicyclohexyl-18-crown-6 > dicyclohexyl-24-crown-8 > dibenzo-18-crown-6 > dibenzo-24-crown-8 > valinomycin. This order, with the exception of valinomycin, agreed with that for

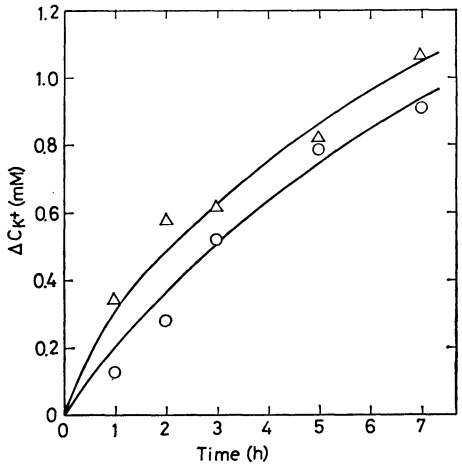

Fig. 6. Increment of potassium ion concentration (ΔC_{K^+}) in phase II against time curves.
○: 0.1 M KCl (phase I)–0.1 M LiCl (phase II) system, △: 0.1 M KCl (phase I)–0.05 M Li_2SO_4 (phase II) system. Ionophore; 10^{-4} M dicyclohexyl-18-crown-6.

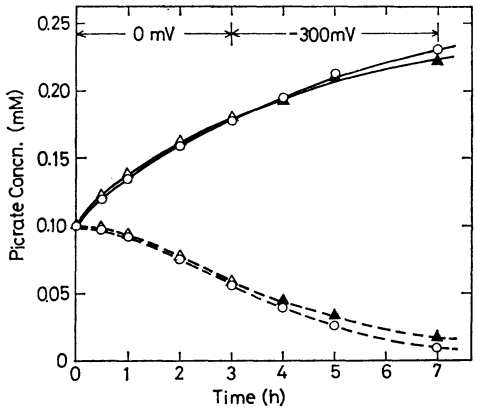

Fig. 7. Examination for the influence of membrane potential on the transport of picrate.
○: Usual transport, △: the potential difference between both aqueous phases was clamped at 0 mV, ▲: a potential difference of 300 mV was applied between both aqueous phases in the reverse direction of the membrane potential.
The dotted and solid lines represent the picrate concentrations in phases I and II respectively. Ionophore; 10^{-4} M dicyclohexyl-18-crown-6, K, Li salts; phase I : 0.1 M KCl, phase II : 0.1 M LiCl.

the permeability ratio of potassium ion to sodium ion across the liqiud membrane consisting of dioleyl phosphate and oleyl alcohol.[5]

Discussion

It has been reported that picrate is a lipophilic anion and that it permeates the phospholipid membrane electrophoretically.[6] In the present experiments, however, the transport of picrate through the dichloroethane membrane containing no ionophore was not observed: picrate was transferred only in the presence of potassium ion and potassium ionophore. The rate of picrate transport increased with increasing ionophore concentration suggesting that the picrate anion in phase I was dissolved in the dichloroethane phase forming an ion-pair with a complexed cation consisting of ionophore and potassium ion and then liberated into phase II by the dissociation of the ion-pair.

The rate of picrate transport depended on various factors such as the concentration of ionophore and potassium ion and the type of potassium and lithium counter-ions and ionophore. To investigate the mechanism of this transport system, the change in potassium ion concentration in phase II with time was measured by means of flame analysis, the results of which for the potassium chloride–lithium chloride and potassium chloride–lithium sulfate systems are shown in Fig. 6. The change in chloride ion concentration in phase II for the potassium chloride–lithium sulfate system was also determined by a titrimetric method. It was found that increment of potassium ion concentration in phase II was approximately 10^{-3} M for 7 h and that for chloride ion concentration also approximately 10^{-3} M indicating that approximately 1% of the potassium chloride in phase I was transferred to phase II across the membrane. Using the assumption of electroneutrality, the number of moles of potassium ions diffusing into phase II must equal the sum of those of chloride and picrate ions transferred into phase II. The effect of potassium and lithium counter-ions on the transport of picrate can be explained by the difference in permeability across the membrane. To obtain the order of permeability for the counter-ions, the membrane potential of the dichloroethane membrane in the absence of picrate and Tris-H_2SO_4 buffer was measured. The membrane potentials for the potassium chloride–lithium chloride, potassium nitrate–lithium nitrate, potassium sulfate–lithium sulfate and potassium sulfate–lithium nitrate systems were found to be approximately 70, 50, 80, and 160 mV, respectively. The polarity of the potential was positive in phase II with respect to phase I. Using the relationship between the membrane potential and permeability coefficient derived by Hodgkin and Katz,[7] the following order of permeability for the counter-ions was established: $NO_3 > Cl > SO_4$— in fact, the rate of picrate transport decreased according to the same order for the potassium counter-ions. In the case of the potassium nitrate–lithium nitrate system, the liberation of picrate from the ion-pair at the phase II-dichloroethane interface was depressed by the co-transport of nitrate with potassium ion, whereas, in the case of the potassium sulfate–lithium nitrate system, the transfer of nitrate from phase II to phase I facilitated the transport of picrate.

In general , the membrane potential has been shown to be important in transport across the membrane. In the present experiments, the membrane potential gradually increased with time attaining a steady value. To examine the role of the membrane potential, the potential difference between phases I and II was set 0 mV by a short circuit method.[8] No difference in the results was observed , as shown in Fig. 7. Furthermore, only a slight decrease in the rate of picrate

transport occurred, even when a potential difference of 300 mV was applied between both the aqueous phases in the reverse direction of the membrane potential (Fig. 7), *i.e.* the transport of picrate is largely unaffected by the membrane potential. The explanation for this is that the transport of picrate occurs only by the diffusion of potassium ions across the membrane and therefore, the decrease in concentration gradient of the potassium ion across the membrane brings about a lowering of the rate of picrate transport, as seen from Fig. 4.

The effect of ionophores on the transport of picrate is associated with the permeability ratio of the potassium ion to the lithium ion for the dichloroethane membrane. This permeability ratio depends on the ionic selectivity of ionophore, the mobility of the potassium– and lithium–ionophore complexes in the dichloroethane membrane and the partition coefficient of the complexes.[9] It is thought that the behavior of the above-mentioned crown compounds for the dichloroethane membrane system is similar to that for the dioleyl phosphate–oleyl alcohol membrane system previously described.[5] In that system, the oleyl phosphate–oleyl alcohol membrane in the presence of valynomycin showed the highest permeability ratio of potassium ion to sodium ion. In the present experiment, the rate of picrate transport was extremely small in the presence of valinomycin and bearing in mind that the membrane potential is very samll in the presence of valinomycin, this may be attributed to a lowering of the potassium-ion selectivity of valinomycin, based on the interaction between valinomycin and picrate.

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