MODIFICATION OF ION TRANSPORT IN LIPID BILAYER MEMBRANES IN THE PRESENCE OF 2,4-DICHLOROPHENOXYACETIC ACID

I. Enhancement of Cationic Conductance and Changes of the Kinetics of Nonactin-Mediated Transport of Potassium

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ABSTRACT We have found that herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) has the ability to increase the rate of transport of positive ions of several kinds, and to inhibit transport of negatively charged tetraphenylborate ions in lipid bilayer membranes. It has been found that only the neutral form of 2,4-D is transport active, whereas the ionized form of 2,4-D does not modify transport of ions, and does not by itself permeate through lipid membranes. The results suggest that the enhancement of transport of positively charged ions such as tetraphenylarsonium$^+$ and nonactin-K$^+$ is dominated by the increase of the ion translocation rate constant. It has been shown that the enhancement of nonactin-mediated transport of K$^+$ by 2,4-D can be accounted for by a simple carrier model. We have observed that at 2,4-D concentration above 3×10^{-4} M the potassium ion transport in phosphatidylcholine-cholesterol as well as in cholesterol-free glycerolmonooleate membranes is enhanced to such a degree that, depending upon the concentration of potassium ions, it becomes limited by the rate of recombination of K$^+$ with nonactin, and/or by backdiffusion of unloaded nonactin molecules. Furthermore, the effect of 2,4-D is enhanced by ionic strength of aqueous solution. From the changes of kinetic parameters of nonactin-K$^+$ transport, as well as from the changes of membrane conductance due to tetraphenylarsonium$^+$ ions, we have estimated the changes of the electrical potential of the membrane interior. We have found that the potential of the interior of the membrane becomes more negative in the presence of 2,4-D, and that its change is proportional to the aqueous concentration of 2,4-D. The effect of 2,4-D on ion transport has been attributed to a layer of 2,4-D molecules absorbed within the interfacial region, and having a dipole moment directed toward the aqueous medium. The results of kinetic studies of nonactin-K$^+$ transport suggest that this layer is located on the hydrocarbon side of the interface.

INTRODUCTION

The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and other chlorinated phenoxy acids, widely used pesticides in agriculture, act as plant growth regulators (1) and as uncouplers of oxidative phosphorylation (2). 2,4-D is toxic to animals and humans (3). At large doses or prolonged exposure, severe degeneration of muscle tissue and redistribution of calcium in muscle can be detected (3,4). One manifestation of 2,4-D toxicity is myotonia, associated with repetitive nerve and muscle firing (5). The above physiological observations suggest that

2,4-D interferes with the process of redistribution of ions at the cellular level. The studies reported in this paper on the effect of 2,4-D on transport of electric charge in bimolecular lipid membranes pertain to our understanding of 2,4-D toxicity.

General Considerations Regarding the Mechanism of Electrical Conductivity of Lipid Membranes in the Presence of 2,4-D

Several likely mechanisms by which membrane conductivity can be altered by 2,4-D are: (*a*) Translocation of single 2,4-D anions. We have considered direct translocation of 2,4-D anions to be one of the possible transport models because chlorine substituents could render sufficiently high solubility of 2,4-D anions in membrane boundary regions. This mechanism of transport has been studied in detail with lipid soluble ions, and can be well characterized from the properties of current or voltage transients (6–19).

(*b*) "Uncoupler-type" of membrane electrical conductivity induced by 2,4-D. The ability of 2,4-D to uncouple oxidative phosphorylation in mitochondria (2) lends support to the hypothesis that the effect of 2,4-D on membrane electrical conductivity can be similar to that found for other uncouplers of oxidative phosphorylation (20–25). The typical feature of membrane conduction process is the coexistence of diffusion of the neutral uncoupler molecules across the membrane in one direction to the electrodiffusion of negatively charged uncoupler ion or dimer complex in the opposite direction. This mechanism is of great biological significance because substances of this type have the ability to dissipate hydrogen ion concentration gradients and electric potential differences across membranes.

(*c*) Mediated cation transport. Another conceivable mode of action of chlorinated phenoxy acids is the mediation of cation transport and cation-proton exchange, similar to that observed for nigericin (26). This hypothesis is based on common structural features and similarity in biological action. Nigericin and phenoxy acids both contain one carboxylic group, have hydrophobic and hydrophilic regions within the molecule, and uncouple oxidative phosphorylation (2, 27–29).

(*d*) Modification of membrane selectivity and changes of kinetics of carrier-mediated ion transport in the presence of 2,4-D. In addition to direct mechanisms of electrical conductivity induced by permeation of electrically charged 2,4-D molecules or its complexes, we must also consider indirect mechanism in which 2,4-D affects the kinetics of transfer of other membrane permeable ions. One possibility is that 2,4-D affects ion transit time by affecting membrane thickness or ion diffusion constant. These changes would influence the transport of negatively and positively charged ions more or less equally. The selectivity of membrane can be altered if the agent selectively influences either the density of membrane permeable ions at the membrane surface, or the probability of ion translocation across the membrane interior. For example, chaotropic ions (30), ions of dinitrophenol (31, 32), or of chlorinated phenols[1] adsorb at membrane surfaces and modify the density of permeable ions due to the formation of electrical double layer. Another mode of action of selectivity modifiers can be associated with the change of dipole potentials at the membrane/water interface. The ratios of cationic and anionic conductance of glyceride and phospholipid membranes have been related to the dipolar potential differences of the membrane interfacial regions (33–35). Szabo (36–38) and

[1]Hsu, K., R. Jayaweera, and P. Smejtek. Unpublished results.

others[2] (39, 40) have demonstrated that the increase of cholesterol content in glycerolmonooleate (GMO) membranes increases the permeability coefficient of negative ions, whereas the permeability coeffecient of positive ions decreases. Membrane conductivity changes observed in the presence of phloretin (41–43), as well as of some nitrophenols and acetophenons (41), have been also associated with the changes of interfacial dipolar potentials. The changes of distribution of electric potential within the membrane as a consequence of changes of interfacial dipole moment can also result in the change of kinetics of carrier-mediated transport (38–42).

The interference of pesticides with the carrier-mediated transport of ions is of considerable interest because this type of transport is widely utilized in biological membranes. The action of toxic substances on membranes is often very complex, even in the case of simple systems, such as artificial lipid bilayers. For example, tetrachloro-2-trifluromethyl-benzimidazole (TTFB) and other structurally related benzimidazoles not only induce electrical conductivity in membranes by themselves (24, 44, 45), but as Kuo et al. found (46, 47), they can block valinomycin-mediated transport of potassium as well.

PROCEDURES AND MATERIALS

The optically black lipid membranes were formed by the brush method on a 2-mm diameter hole in a wall of TFE (tetrafluorethylene resin) cell (Teflon, DuPont Co., Wilmington, Del.). Two types of membranes have been studied: egg lecithin/cholesterol/*n*-decane (PC-chol) and monoolein/*n*-decane (GMO). In PC-chol membranes the cholesterol mole fraction was 0.76; the total lipid content (i.e., lecithin + cholesterol) in the membrane-forming solution was 11.5 mg per milliliter. The GMO membranes were cholesterol-free; the composition of membrane forming solution was 25 mg per milliliter.

The aqueous solutions, except as noted below, contained KCl, buffer (phosphate, citrate, borate, ratios 0.002 M/0.002 M/0.0005 M), and 2,4-D; LiCl was used to adjust ionic strength. Tetraphenylarsonium ($TPhAs^+$) chloride, sodium tetraphenylborate ($TPhB^-$), and nonactin were first dissolved in ethanol, and then the ethanolic solution was added to the aqueous solution. The volume of ethanol in the final solution did not usually exceed 0.5%. The solutions containing tetraphenylborate were potassium-free to avoid precipitation of KTPhB. Nonactin was added to the membrane-forming solution to shorten the equilibration time of nonactin distribution between the aqueous medium and the membrane.

The TFE compartment of the measuring cell was made out of virgin material. It was boiled in ethanolic solution of sodium hydroxide for 5 min and then washed in deionized water before each experiment. Occasionally the cell was soaked in chromic acid. Cleanliness of the system was checked periodically by measuring background conductivity of membranes (order of 10^{-8} S/cm^2) in the absence of membrane modifiers. Despite this cleaning procedure, we experienced difficulties with nonactin contamination in the walls of Teflon cell. Although the nonactin impurity level was so low that it could not be detected in background conductivity tests, it interacted with 2,4-D sufficiently to have led to some erroneous conclusions (48). We subsequently found that nonactin diffused into Teflon is well protected from the environment, but that prolonged baking of the cell at 200–270°C effectively removes it.

The current-voltage characteristics of membranes were measured at least 10–15 min after the membranes become black, and after the conductivity stabilized. The temperature was 22 ± 1°C. For monitoring the conduction state of the membrane, a voltage pulse of 25 mV was periodically applied to the membrane, and the membrane current steps recorded as a function of time on a plotter. To minimize electrode polarization we used sintered Ag/AgCl electrodes (type 140; Annex Instruments, Santa Ana, Calif.) A separate pair of electrodes was used for each type of experiment to avoid cross-contamination.

[2]Pickar, A. D., and R. Benz. Accepted for publication in *J. Membr. Biol.*

The nonactin-K^+ and $TPhAs^+$ conductance was obtained from the steady-state current measurements (using model 135 electrometer, Princeton Applied Research, Corp. Princeton, N.J.). The absence of diffusion polarization was checked in a separate experiment, in which the time dependence of membrane current after application of membrane bias was measured, and the DC current magnitude monitored at various speed of stirring (up to 120 rpm). The solutions were stirred by means of TFE-coated stirring bars and rotating magnets. The diffusion polarization due to unstirred layers was found to be insignificant. At high membrane currents only a small electrode polarization was observed (up to several millivolts), for which the applied voltage was corrected. The $TPhB^-$ membrane conductance was obtained from the extrapolation of the relaxation current to the instant of application of a voltage step across the membrane (description of the setup is given in the following paper).

The experimental current-voltage data have been analyzed according to the following procedure. First, the specific membrane conductance, $G(V)$, was computed by using the area of the hole as the surface area of the membrane. Second, the zero voltage conductance, $G(0)$, was obtained from the best fit of the low voltage conductance data (typically up to 100 mV) to a polynomial of the second degree with respect to V. All kinetic information on the nonactin-mediated transport of K^+ was obtained from the voltage dependence of normalized membrane conductance, $G(V)/G(0)$, and the zero voltage conductance $G(0)$. The average number of membranes per one data point is seven; the error bar denotes the "N-1" SD.

Chromatographically pure egg lecithin was prepared by Dr. Kwan Hsu of our group (49), recrystallized cholesterol was a gift from Dr. D. McClure of the Chemistry Department, Portland State University, GMO was obtained from Applied Science Labs., Inc. (State College, Pa.), and *n*-decane, grade 99%+, from Aldrich Chemical Co. (Milwaukee, Ws.). Decane was purified in an alumina column. The tetraphenylarsonium chloride hydrate and sodium tetraphenylborate were obtained from Aldrich Chemical Co., 2,4-D was a gift from Dow Corning Co. (Midland, Mich.), and nonactin from the Squibb Institute of Medical Research (Princeton, N.J.). For the preparation of solutions we used inorganic chemicals of analytical grade, and deionized water from Millipore Q2 system (Millipore Corp., Bedford, Mass.)

RESULTS AND DISCUSSION

The results of experiments designed to find the effect of 2,4-D on membrane electrical conductivity have indicated that within experimental error and background membrane conductance (order to 10^{-8} S/cm^2), the ionized form of 2,4-D does not increase membrane conductivity by itself, and, therefore, does not appreciably permeate through lipid bilayer membranes.

2,4-D Induced Changes of Ionic Membrane Conductance

The primary effect of 2,4-D on ion transport in lipid bilayer membranes that has been found is that in the presence of lipid soluble ions, such as $TPhAs^+$ or $TPhB^-$, or the antibiotic nonactin (a carrier of potassium ions), the membrane conductance is changed by 2,4-D. The experimental results are shown in Fig. 1, where we plot the logarithm of zero voltage membrane conductance $G(0)$ versus the log of 2,4-D concentration. The results indicate that 2,4-D enhances transport of positive ions, and to a lesser degree inhibits the transport of negative ions. The effect is very strong; the enhancement of nonactin-K^+ transport is as large as four orders of magnitude.

pH Effect

The 2,4-D enhanced conductance due to nonactin-mediated transport of potassium was measured as a function of pH. The purpose of this experiment was to determine the

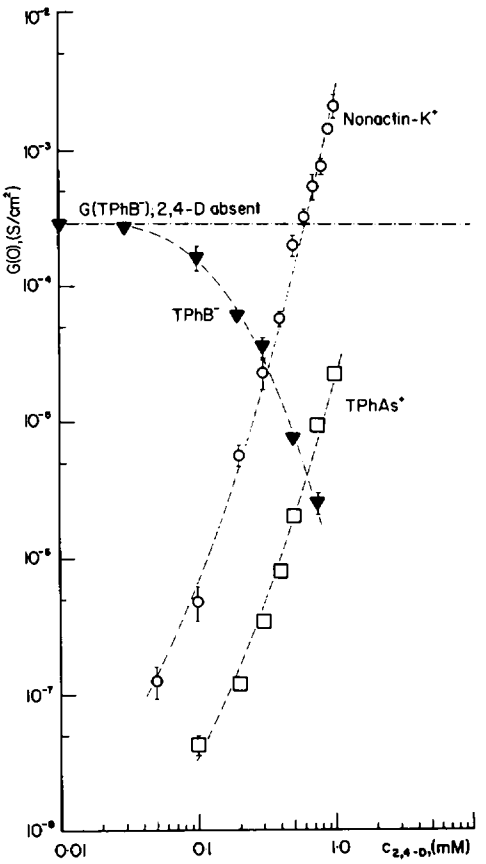


FIGURE 1 Effect of 2,4-D on the PC-chol membrane conductance due to nonactin, $TPhB^-$, and $TPhAs^+$. Experimental conditions: $c_{TPhAs^+} = 2 \times 10^{-3}$ M in 0.5 M LiCl; $c_{TPhB^-} = 1 \times 10^{-7}$ M in 0.5 M NaCl; $c_{nonactin}$ (aqueous solution) $= 1 \times 10^{-7}$ M, $c_{nonactin}$ (membrane solution) $= 3 \times 10^{-5}$ M, $c_{K^+} = 0.06$ M, ionic strength (LiCl + KCl) = 1 M, pH = 2 (buffered in all experiments).

relationship between the degree of conductivity enhancement and the degree of ionization of 2,4-D. As the results obtained in Fig. 2 on PC-chol membranes show, the enhancement is small at high pH. Under these conditions 2,4-D is predominantly present in the anionic form because its pK is between 2.6 and 2.8 (50, 51). The enhancement becomes more prominent as hydrogen ion concentration increases, i.e., with increasing concentration of neutral 2,4-D molecules. A similar study was done on membranes whose conductivity was induced by $TPhB^-$. In this case the opposite effect was observed; at high pH the $TPhB^-$ conductance in the presence of 2,4-D is high, but it decreases as the hydrogen ion concentration increases.

We have further verified that the observed pH dependence is not due to experimental artifacts because the membrane conductance in the absence of 2,4-D is pH independent. This result also suggests that the effect of expected membrane surface charge at low pH is insignificant, which is presumably due to high cholesterol content.

In sum, our results indicate that the enhancement of cationic conductance, and the suppression of the anionic conductance, are associated with the presence of neutral 2,4-D

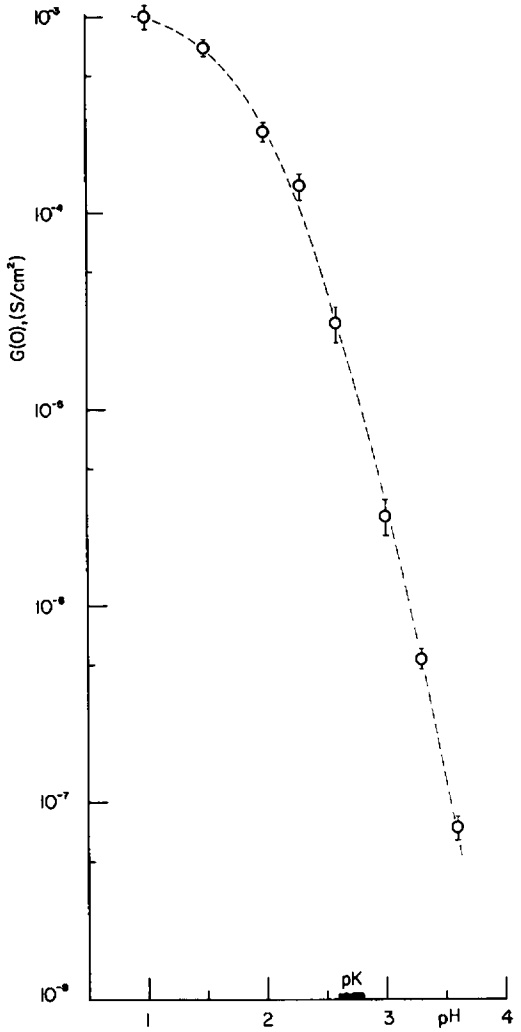


FIGURE 2 pH dependence of 2,4-D enhanced nonactin-K^+ conductance of PC-chol membranes. $c_{2,4\text{-D}} = 5 \times 10^{-4}$ M, c_{nonactin} (aqueous solution) $= 3 \times 10^{-7}$ M, c_{nonactin} (membrane solution) $= 1 \times 10^{-4}$ M, $c_{K^+} = 0.06$ M, ionic strength (LiCl + KCl) = 1 M.

molecules, and that the negatively charged 2,4-D molecules are inactive. The effect of 2,4-D can be understood in terms of the dipole hypothesis proposed to explain the effect of phloretin (41) and cholesterol (37) on ion transport in lipid bilayers. The observed changes of membrane conductivity can be associated with the changes of ion potential energy in membrane interior due to a layer of neutral 2,4-D molecules adsorbed at the membrane surface.

Changes in the Kinetics of Nonactin-Mediated K^+ Transport.

We have also observed that the enhancement of nonactin mediated transport of K^+ by 2,4-D is accompanied by changes of the voltage dependence of membrane conductance. These

effects are illustrated in Figs. 3 *a* and 3 *b*, where we show the dependence of zero voltage membrane conductance $G(0)$ on 2,4-D concentration, and the changes of the voltage dependence of the normalized membrane conductance $G(V)/G(0)$. In addition, we have found that in the presence of 2,4-D, the nonactin-mediated transport of K^+ becomes sensitive to ionic strength of the aqueous solution. Principal features of the ionic strength effect are depicted in Fig. 4. With the increase of ionic strength the membrane conductance increases (Fig. 4 *a*), and the first derivative of the conductance with respect to the applied voltage, dG/dV, monotonically decreases (Fig. 4 *b*). Furthermore, the effect of ionic strength appears to be a general one. In the presence of 2,4-D, both the $TPhAs^+$ and the nonactin-K^+ conductance increase, and the conductances changes are very similar (Fig. 4 *a*). This result is interesting because the mechanisms of ion transport across the membrane are very different. In addition to ion translocation across the membrane core, the transfer of potassium ion by nonactin involves recombination and dissociation of the ion and the carrier, whereas the transport of $TPhAs^+$ ion involves only adsorption at the membrane surface and diffusion across the membrane interior.

The mechanism of transport of alkali ions by nonactin and other macrotetrolide antibiotics acting as ion carriers has been phenomenologically understood (52–56), which is why nonactin-K^+ complex is in the present case being used as a probe to detect changes of membrane properties caused by 2,4-D. However, the effect of ionic strength on the nonactin-induced membrane conduction in the presence of 2,4-D was unexpected, because for electrically neutral membranes the nonactin-mediated transport of K^+ is known to be insensitive to the ionic strength (54). We therefore did a set of control experiments without 2,4-D. The results are as follows: (*a*) the zero voltage conductance $G(0)$, and (*b*) the normalized membrane conductance $G(V)/G(0)$ are both independent of the ionic strength (Figs. 4 *a* and *c*). Although we have used membranes having different lipid content and very low pH, this result agrees with findings of other workers (52, 54) obtained under different conditions.

The effect of ionic strength can be qualitatively understood in the following way: as the concentration of electrolyte is increased, the concentration of free water molecules available for the solvation of 2,4-D decreases, which results in relatively lower energy state of 2,4-D in the membrane with respect to that in the aqueous solution, and greater partition of 2,4-D into the membrane. The effect of ionic strength depicted in Fig. 4 cannot originate from the screening of a possible positive charge at the membrane surface because (*a*) in the absence of 2,4-D there is no effect, and (*b*) the conductance increases exponentially with the ionic strength. Actually, as follows from the theory of diffuse double layer (57), the conductance, assuming that it is controlled by the membrane surface potential, would depend on the ionic strength, I_s, according to $G \propto \exp(-\text{const}/I_s^{1/2})$, a function that is incompatible with the experimental results.

The changes of voltage dependence of the nonactin-K^+ conductance with the increase of 2,4-D concentration and the ionic strength suggest that the kinetics of ion transport across the membrane interior change. To gain better insight into the effect of 2,4-D, we have studied the dependence of membrane conductance due to nonactin-K^+ complex on potassium ion concentration while maintaining the ionic strength constant by adding appropriate amounts of LiCl (nonactin is an inefficient carrier of Li^+ ions). Three 2,4-D concentrations were chosen:

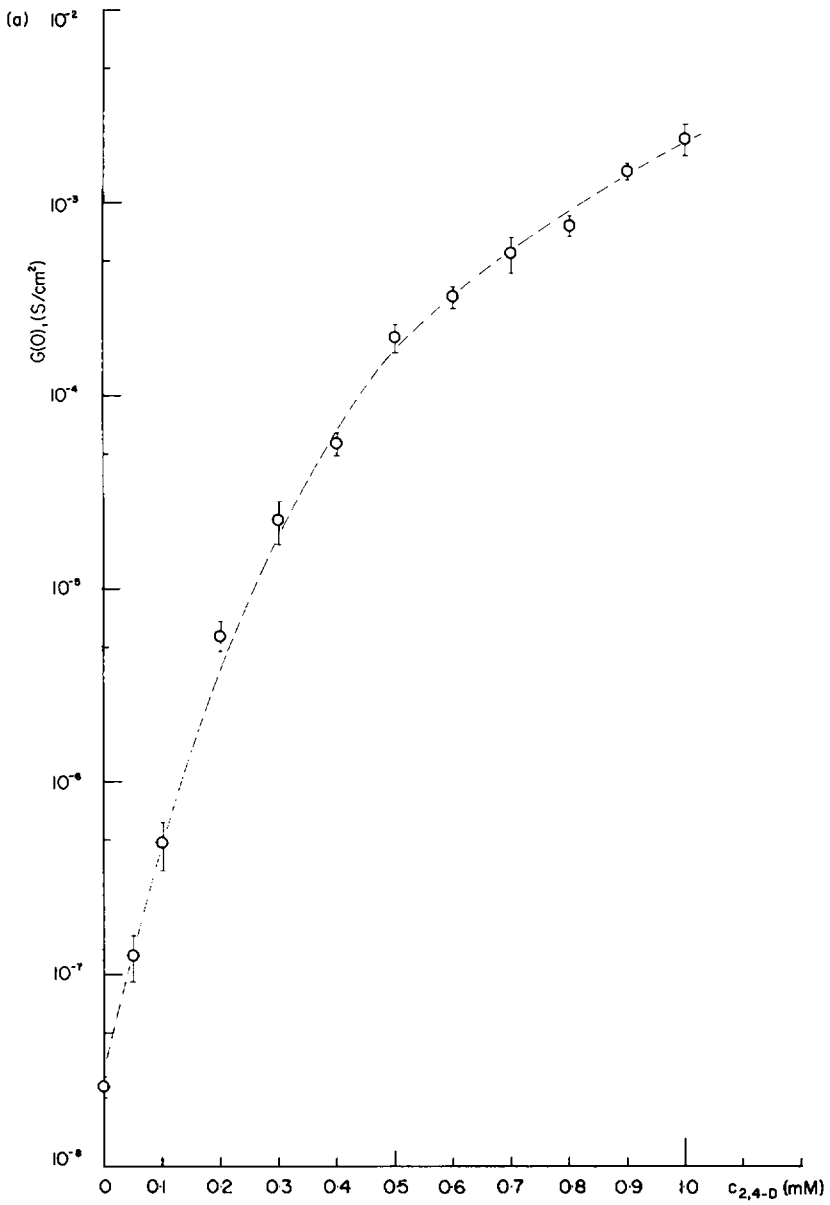
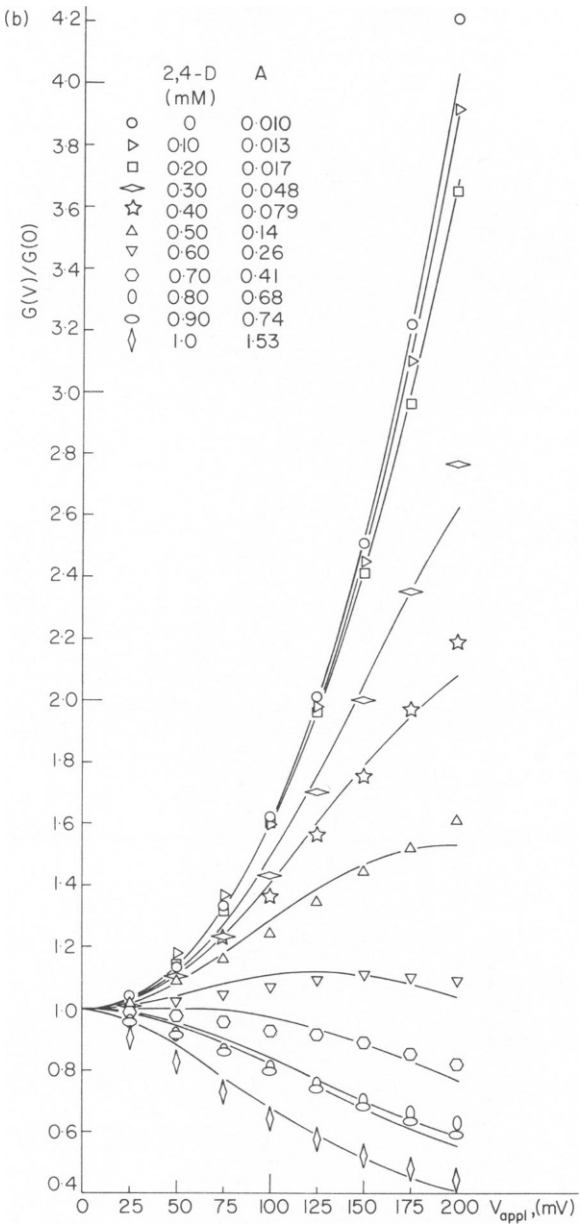
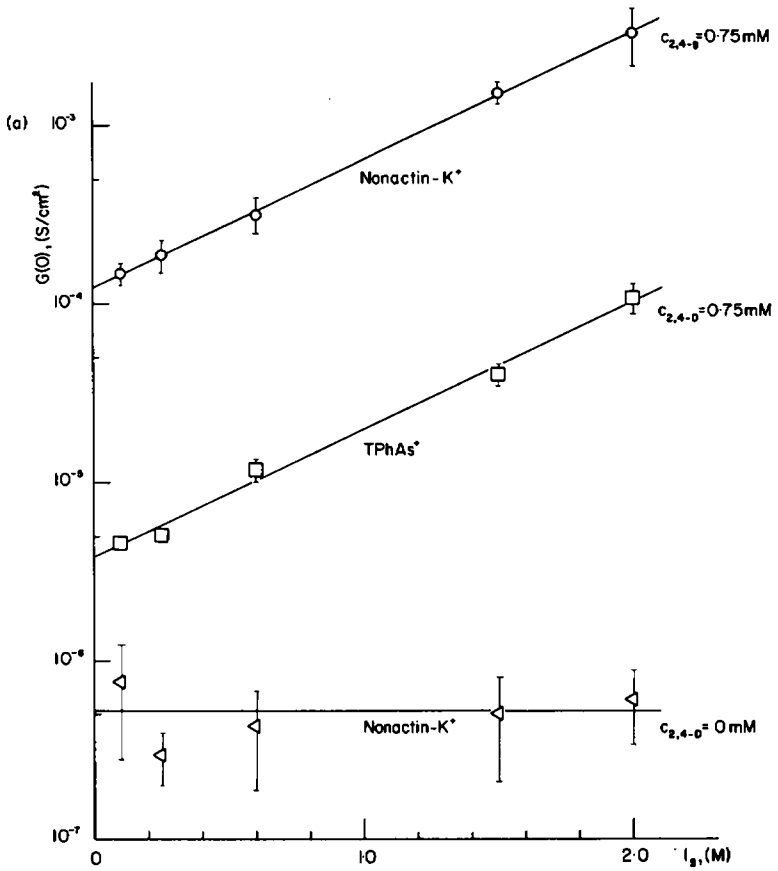
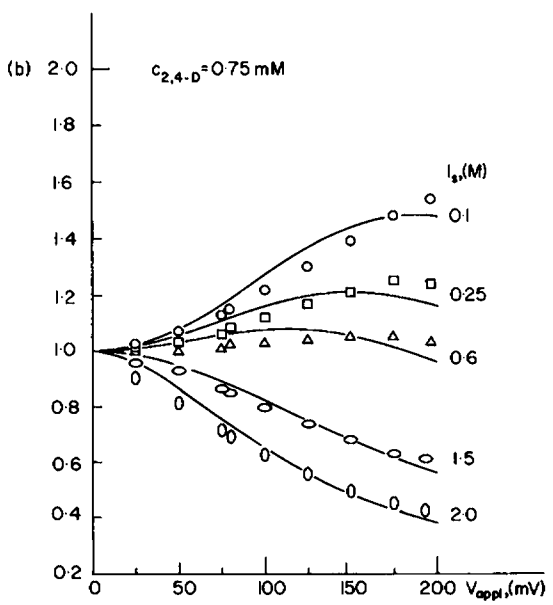


FIGURE 3 (*a*) 2,4-D enhancement of nonactin-K^+ conductance of PC-chol membranes. Semilog plot of the 2,4-D concentration dependence of zero voltage conductance. Experimental conditions: $c_{nonactin}$ (aqueous solution) = 1×10^{-7} M, $c_{nonactin}$ (membrane solution) = 3×10^{-5} M, c_{K^+} = 0.06 M, ionic strength (LiCl + KCl) = 1 M, pH = 2 (buffered). (*b*) Voltage dependence of 2,4-D enhanced conductance. Plot of normalized membrane conductance $G(V)/G(0)$ versus V as a function of 2,4-D concentration. Each set of data points in this figure corresponds to one conductance point in Fig. 3 *a*. The solid curves were obtained from Eq. 5 and represent the prediction of the transport model. Experimental conditions: $c_{nonactin}$ (aqueous solution) = 1×10^{-7} M, $c_{nonactin}$ (membrane solution) = 3×10^{-5} M, c_{K^+} = 0.06 M, ionic strength (LiCl + KCl) = 1 M, pH = 2 (buffered).



0.35, 0.5, and 0.6 mM. The membrane conductance as a function of potassium ion concentration is shown in Fig. 5 *a*. With the exception of the case of highest 2,4-D concentration and high K^+ concentration, the membrane conductance linearly increases with the concentration of K^+ (Fig. 5 *a*). Thus the association reaction between K^+ and nonactin in the presence of 2,4-D remains to be of the first order. The results in Fig. 5, *b–d* indicate that the upward curvature of $G(V)/G(0)$ vs. V decreases both with increasing potassium ion and 2,4-D concentration. Again, the changes of dG/dV with the potassium ion concentration are


(a)
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
G(0), (S/cm²)
Nonactin-K⁺
$c_{2,4\text{-}D}$=0·75mM
TPhAs⁺
$c_{2,4\text{-}D}$=0·75mM
Nonactin-K⁺
$c_{2,4\text{-}D}$= 0mM
0
1·0
2·0
I_s,(M)


(b) 2·0
$c_{2,4\text{-}D}$=0·75 mM
1·8
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
I_s,(M)
0·1
0·25
0·6
1·5
2·0
0
50
100
150
200 V_{appl},(mV)

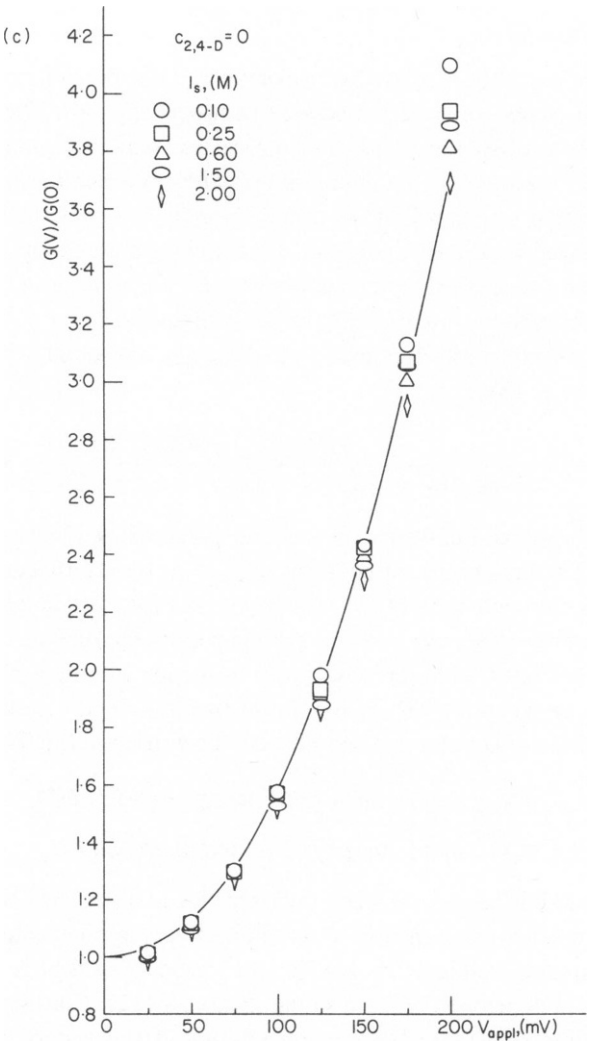


FIGURE 4 (*a*) Effect of ionic strength on the magnitude of membrane conductance associated with the transport of nonactin-K^+ and $TPhAs^+$ ions across the PC-chol membranes. $c_{2,4\text{-}D} = 7.5 \times 10^{-4}$ M, $c_{nonactin}$ (aqueous solution) $= 4.5 \times 10^{-8}$ M, $c_{K^+} = 0.1$ M, $c_{TPhAs^+} = 2 \times 10^{-3}$ M, pH = 2 (buffered). Control experiment: $c_{2,4\text{-}D} = 0$, $c_{nonactin}$ (aqueous solution) $= 3.5 \times 10^{-6}$ M, $c_{nonactin}$ (membrane solution) $= 1.9 \times 10^{-4}$ M, $c_{K^+} = 0.1$ M, pH = 2 (buffered). (*b*) Effect of ionic strength on voltage dependence of normalized membrane conductance associated with transport of nonactin-K^+ complex across PC-chol membranes. Each set of data points corresponds to one conductance point in Fig. 4 *a*. The solid curves were obtained from Eq. 5 and represent the prediction of the transport model. $c_{2,4\text{-}D} = 7.5 \times 10^{-4}$ M, $c_{nonactin}$ (aqueous solution) $= 4.5 \times 10^{-8}$ M, $c_{K^+} = 0.1$ M, pH = 2 (buffered). (*c*) Control experiment. A demonstration that in the absence of 2,4-D the effect of ionic strength on the voltage dependence of nonactin-K^+ conductance in PC-chol membranes is absent. $c_{2,4\text{-}D} = 0$, $c_{nonactin}$ (aqueous solution) $= 3.5 \times 10^{-6}$ M, $c_{nonactin}$ (membrane solution) $= 1.9 \times 10^{-4}$ M, $c_{K^+} = 0.1$ M, pH = 2 (buffered).

observed only when 2,4-D is present; in the absence of 2,4-D the voltage dependence of conductance is similar to that shown in Fig. 4 *c*.

The Carrier Model

The effect of 2,4-D on nonactin-mediated transport of K^+ can be understood in terms of the well established kinetic model of carrier-mediated transport (52–56). The transfer of K^+ ion across the membrane involves several distinct processes: the formation of the membrane permeable nonactin-K^+ complex at the membrane/water interface (a process characterized by the complex formation rate constant, k_R), the electrodiffusion of the complex across the membrane (characterized by the rate constant of complex translocation, k_{IS}), the release of the potassium ion from the carrier (rate constant of complex dissociation, k_D), and the back diffusion of the neutral carrier (rate constant of back diffusion, k_S). For equal concentration of potassium ions in both aqueous solutions, c_{K^+}, and membrane-bound carrier, the membrane current density is given by (58):

$$J_{IS} = (eN_S c_{K^+}) \frac{k'_R k'_{IS}/k'_D - k''_R k''_{IS}/k''_D}{1 + k'_{IS}/k'_D + k''_{IS}/k''_D + (k'_R k'_{IS}/k'_D + k''_R k''_{IS}/k''_D)c_{K^+}/2k_S}. \quad (1)$$

The single and double-primed quantities refer to the voltage dependent rate constants at the left and right sides of the membrane, respectively, and k'_{IS} is the translocation rate constant of nonactin-K^+ complex from left to right, whereas k''_{IS} is that for right to left. N_S is the number of adsorbed neutral carrier molecules per unit area of membrane surface.

By using the Nernst-Planck solution of the electrodiffusion equation (12), and treating the membrane as an image potential barrier for the diffusing ions, the voltage dependent rate constants of translocation of the charged complex can be written in the following way:

$$k'_{IS} = k_{ISO} \exp[-\omega(eV/kT)^2] \exp(-\beta eV/2kT),$$
$$k''_{IS} = k_{ISO} \exp[-\omega(eV/kT)^2] \exp(\beta eV/2kT). \quad (2)$$

V is the applied potential difference between the right side and the left side of the membrane, and k_{ISO} is the translocation rate constant in the limit of zero applied voltage. Parameter β is the fraction of the applied voltage that is effective in ion translocation (12); ω is a small constant whose value is dependent on membrane thickness (12). Consistent with the barrier model is the assumption that $(1 - \beta)/2$ is the fraction of the applied potential difference between the recombination plane and the aqueous solution, so that the recombination and dissociation rate constant are voltage dependent as well:

$$k'_R = k_{RO} \exp[-(1 - \beta)eV/4kT], \quad k''_R = k_{RO} \exp[(1 - \beta)eV/4kT],$$
$$k'_D = k_{DO} \exp[(1 - \beta)eV/4kT], \quad k''_D = k_{DO} \exp[-(1 - \beta)eV/4kT]. \quad (3)$$

The value of parameter β is not a priori known, but there are good arguments that it should be close to unity (12, footnote 2). Hladky's results for nonactin and trinactin complexes in GMO/hexadecane membranes suggest that β is between 0.8 and 0.9 (55, 56); these references should also be consulted for more detailed discussion of the problem of voltage dependent rate constants. To reduce the number of unknown parameters, we assume $\beta = 1$, and set $k'_R = k''_R = k_R$ and $k'_D = k''_D = k_D$. The membrane barrier distortion parameter ω has

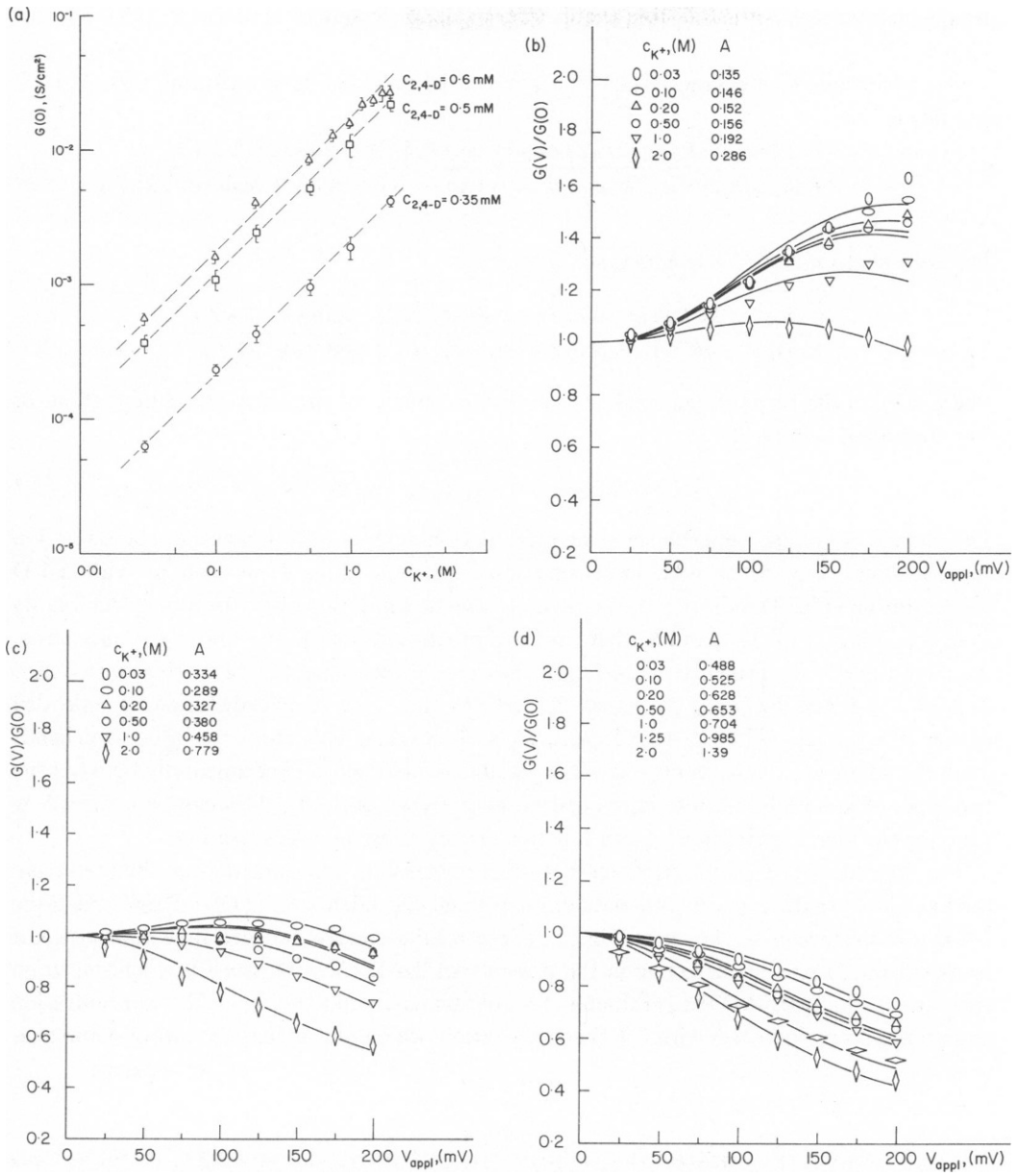


FIGURE 5 (*a*) Dependence of 2,4-D enhanced nonactin-K^+ conductance on potassium ion concentration in PC-chol membranes. $c_{nonactin}$ (aqueous solution) = 4.5×10^{-8} M, $c_{nonactin}$ (membrane solution) = 1.4×10^{-5} M, pH = 2 (buffered), ionic strength (LiCl + KCl) = 2 M. (*b–d*) Effect of potassium ion concentration on voltage dependence of normalized membrane conductance of PC-chol membranes at three concentrations of 2,4-D. The solid curves were obtained according to Eq. 5 and represent the prediction of the transport model. $c_{nonactin}$ (aqueous solution) = 4.5×10^{-8} M, $c_{nonactin}$ (membrane solution) = 1.4×10^{-5} M, pH = 2 (buffered), ionic strength (LiCl + KCl) = 2 M. (*b*) $c_{2,4\text{-}D}$ = 0.35 mM; (*c*) $c_{2,4\text{-}D}$ = 0.50 mM; (*d*) $c_{2,4\text{-}D}$ = 0.60 mM.

been set to 0.005 (see Table I in reference 12) because the thickness of PC/cholesterol/*n*-decane membranes, evaluated from the specific capacitance data of Hanai et al. (59), is about 3.2 nm.

The membrane conductance $G(V) = J_{IS}/V$ can be obtained by substituting Eqs. 2 and 3 into Eq. 1.

$$G(V) = \frac{1}{V}\frac{(2ek_RN_Sk_{ISO}c_{K^+}/k_D)\exp[-\omega(eV/kT)^2]\sinh(eV/2kT)}{1 + (2k_{ISO}/k_D + k_{ISO}k_Rc_{K^+}/k_Sk_D)\exp[-\omega(eV/kT)^2]\cosh(eV/2kT)}. \quad (4)$$

The normalized membrane conductance is given by

$$\frac{G(V)}{G(0)} = \frac{2kT}{eV}\frac{(1 + A)\exp[-\omega(eV/kT)^2]\sinh(eV/2kT)}{1 + A\exp[-\omega(eV/kT)^2]\cosh(eV/2kT)}, \quad (5)$$

where $G(0)$ is the zero voltage conductance, and parameter A involving two combinations of rate constants, is equal to

$$A = 2k_{ISO}/k_D + k_{ISO}k_Rc_{K^+}/k_Sk_D. \quad (6)$$

The change of voltage dependence of membrane conductance with increasing potassium ion concentration (Fig. 5) or with increasing ionic strength (Fig. 4) as well as with 2,4-D concentration (Fig. 3) indicates the presence of kinetic limitation of ion transport. As follows from the model (Eqs. 1, 4, and 6) there are two processes limiting the flow of K^+ ions across the membrane in the presence of 2,4-D: (*a*) slow rate of recombination of carriers with ions if $2k_{ISO}/k_D \gtrsim 1$ and $k_{ISO}k_Rc_{K^+}/k_Sk_D \ll 1$, and (*b*) slow rate of backdiffusion of unloaded carrier if $k_{ISO}k_Rc_{K^+}/k_Sk_D \gtrsim 1$ and $2k_{ISO}/k_D \ll 1$. Because only the latter process depends upon the potassium ion concentration, it is possible to distinguish experimentally between the two types of kinetic limitations developed in the presence of 2,4-D. This can be achieved by studying the kinetic parameter A as a function of potassium ion concentration.

The dependence of kinetic parameter A on the potassium concentration as obtained from the fit of Eq. 5 to the experimental data of the voltage dependence of $G(V)/G(0)$ for the three 2,4-D concentrations is shown in Fig. 6. These results clearly indicate that both types of transport limitations are effective in the presence of 2,4-D. At low potassium concentration (between 0.03 and 0.5 M) the parameter A is approximately independent of K^+ concentration and progressively increases with 2,4-D concentration, which means that the rate of complexa-

TABLE I

CHANGE OF KINETIC PARAMETERS OF NONACTIN-K^+ IN PC-CHOL MEMBRANES WITH 2,4-D CONCENTRATION AS OBTAINED FROM THE POTASSIUM CONCENTRATION DEPENDENCE (IONIC STRENGTH = [2.0 M])

$c_{2,4\text{-D}}$	$2k_{ISO}/k_D$	$k_{ISO}k_R/k_Dk_S$	k_R/k_S
mM		M^{-1}	M^{-1}
0.35	0.13 ± 0.01	0.07 ± 0.01	1.08 ± 0.17
0.50	0.28 ± 0.02	0.24 ± 0.02	1.71 ± 0.19
0.60	0.45 ± 0.05	0.46 ± 0.05	2.04 ± 0.32

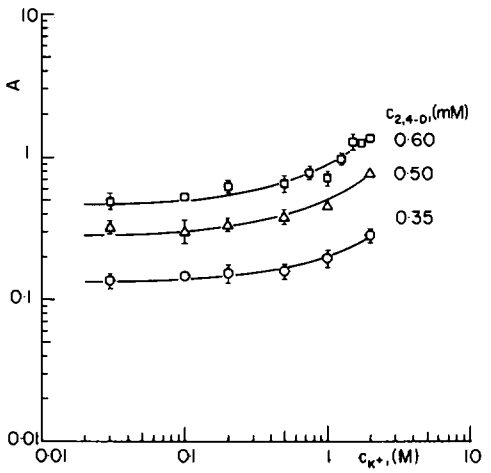


FIGURE 6 Dependence of kinetic parameter A for nonactin mediated transport of K^+ in PC-chol membranes on the potassium ion concentration for three concentration levels of 2,4-D. The solid curves represent the fit of Eq. 6 for the parameters given in Table I.

tion on the positively biased membrane side becomes limited by the magnitude of the recombination rate constant k_R. At higher potassium ion concentration (above 0.5 M), the value of parameter A further increases, and thus an additional limiting step becomes significant. In this case, as follows from the model, the limitation can be attributed to the depletion of unloaded ion carriers at the positively biased membrane surface. One can apply linear regression analysis (Eq. 6) to the experimental dependence of parameter A on the concentration of K^+ and obtain two sets of combinations of rate constants: $2k_{ISO}/k_D$ and $k_{ISO}k_R/k_Sk_D$. Their values are given in Table I. Note that both combinations of rate constants change with the concentration of 2,4-D by about the same factor (Fig. 6), which indicates that 2,4-D changes primarily the ratio k_{ISO}/k_D, because this ratio is present in both terms of parameter A. The results also suggest that within the above 2,4-D concentration range the ratio of k_R/k_S is not very sensitive to 2,4-D; it changes by less than a factor of two. According to our adopted model, the conductance of the membrane is also proportional to k_{ISO}/k_D (Eq. 4). Thus, the increase of zero voltage membrane conductance and the changes of the voltage dependence of the conductance in the presence of 2,4-D originate from the change of the ratio k_{ISO}/k_D.

It is of interest to examine the question of the effect of 2,4-D on the ion-carrier recombination process at the membrane surface because it has been found that it can be modified by the presence of membrane additives, such as sterols (38–40). From the model (Eqs. 4–6) and from the numerical values of the rate constant combinations presented in Table I, it follows that at sufficiently low potassium concentration the second limiting process is inefficient. In that case the second term in the parameter A can be neglected, and the following approximations hold:

$$A \approx 2k_{ISO}/k_D \qquad (7\,a)$$

$$G(0) \approx (e^2c_{K^+}/2kT) \cdot Ak_RN_S/(1 + A). \qquad (7\,b)$$

TABLE II

EFFECTS OF 2,4-D CONCENTRATION ON KINETIC CHARACTERISTICS OF NONACTIN-K^+ TRANSPORT IN PC-CHOL MEMBRANES, (IONIC STRENGTH = 1.0 M)

$c_{2,4\text{-D}}$	$G(0)$	$A \approx 2k_{ISO}/k_D$	$k_R N_S$
mM	S/cm^2		*cm/s*
0	$(0.26 \pm 0.04) \times 10^{-7}$	0.01 ± 0.01	$(0.23 \pm 0.12) \times 10^{-7}$
0.05	$(0.13 \pm 0.03) \times 10^{-6}$	0.02 ± 0.01	$(0.67 \pm 0.39) \times 10^{-7}$
0.10	$(0.45 \pm 0.15) \times 10^{-6}$	0.01 ± 0.01	$(0.31 \pm 0.19) \times 10^{-6}$
0.20	$(0.57 \pm 0.10) \times 10^{-5}$	0.02 ± 0.01	$(0.30 \pm 0.12) \times 10^{-5}$
0.30	$(0.23 \pm 0.06) \times 10^{-4}$	0.05 ± 0.01	$(0.44 \pm 0.13) \times 10^{-5}$
0.40	$(0.57 \pm 0.07) \times 10^{-4}$	0.08 ± 0.01	$(0.69 \pm 0.14) \times 10^{-5}$
0.50	$(0.20 \pm 0.04) \times 10^{-3}$	0.14 ± 0.01	$(0.15 \pm 0.03) \times 10^{-4}$
0.60	$(0.33 \pm 0.04) \times 10^{-3}$	0.26 ± 0.04	$(0.14 \pm 0.02) \times 10^{-4}$
0.70	$(0.55 \pm 0.11) \times 10^{-3}$	0.41 ± 0.07	$(0.16 \pm 0.04) \times 10^{-4}$
0.80	$(0.76 \pm 0.10) \times 10^{-3}$	0.68 ± 0.04	$(0.17 \pm 0.02) \times 10^{-4}$
0.90	$(0.15 \pm 0.02) \times 10^{-2}$	0.74 ± 0.13	$(0.30 \pm 0.04) \times 10^{-4}$
1.00	$(0.21 \pm 0.04) \times 10^{-2}$	1.53 ± 0.28	$(0.31 \pm 0.06) \times 10^{-4}$

The experiment on the effect of 2,4-D on membrane conductance, whose results are shown in Fig. 3, was done at sufficiently low potassium concentration so that the above approximations are applicable. Thus it is possible to evaluate the effect of 2,4-D on the product of the recombination rate constant and the surface density of nonactin, $k_R N_S$, by using the data on zero voltage conductance and parameter A in combination with Eqs. 7 *a* and *b*. In Table II we compared[3] $G(0)$, $2k_{ISO}/k_D$ and $k_R N_S$ as a function of 2,4-D concentration. The results suggest that a low 2,4-D concentrations ($c_{2,4\text{-D}} < 0.3$ mM) the enhancement of potassium ion transport by 2,4-D is primarily due to the increase (100-fold) of the product $k_R N_S$. Both k_R and N_S are expected to be sensitive to 2,4-D-induced changes of the membrane surface. Another membrane modifier, cholesterol, was found to increase partition of another ion carrier, valinomycin, into the membrane and to decrease its recombination rate constant with Rb^+ ions (39). Unfortunately, steady-state measurements alone do not make it possible to separate the increase of conductance due to the increase of k_R from that of N_S. Also, within this 2,4-D concentration range the ratio of rate constants k_{ISO}/k_D remains small. Its value is about 7×10^{-3}, which compares favorably with 4×10^{-2} for GMO membranes obtained by Hladky (55, 56) because the nonactin-K^+ conductance in GMO membranes is in general much greater than that in PC (54) and in our PC-chol membranes. At high 2,4-D concentrations ($c_{2,4\text{-D}} > 0.3$ mM) the product $k_R N_S$ approaches saturation. However, the increase of membrane conductance in this range is primarily due to the increase of the ratio k_{ISO}/k_D. Because the difference in conductance as well as in the ratio of k_{ISO}/k_D of monoolein and phospholipid membranes can be related to the dipolar potential differences at the membrane boundary (34, 35), the increase of k_{ISO}/k_D in the presence of 2,4-D suggests the possibility that the effect of 2,4-D on membranes can be also of dipolar nature.

[3]The ratios k_{ISO}/k_D obtained from the data in Fig. 3 and those from the potassium concentration dependence as given in Table I are different because different ionic strength was employed in those experiments.

Cholesterol-Free membranes

It is conceivable, although not very likely, that the observed effect of 2,4-D on ion transport in PC-chol membranes is associated with the exclusion of cholesterol from the membrane into the membrane torus. It is known that cholesterol inhibits the transport of positive ions and enhances the transport of negative ions (36–39). Thus, lowering the cholesterol content in the membrane would result in the effect we have observed. To verify this possibility, we have studied the effect of 2,4-D on nonactin-mediated K^+ transport in cholesterol-free GMO membranes. If the presence of cholesterol in the membrane were critical, the conductance effect of 2,4-D would be absent. In contrast to this expectation, we have found that the conductance characteristics of GMO membranes changed in a manner similar to those found for PC-chol membranes (see Fig. 3). The membrane conductance $G(0)$ increased, and simultaneously the slope dG/dV decreased in the presence of 2,4-D (data not shown).

The conductance data obtained on GMO membranes were analyzed in the same manner as those for PC-chol membranes, except that ω was set equal to 0.007 because the thickness of GMO/*n*-decane membranes, estimated from the specific capacitance data in reference 39, is about 4.8 nm. In Fig. 7 we have compared the 2,4-D concentration dependence of parameter A obtained on PC-chol and GMO membranes under identical conditions with the 2,4-D concentration dependence of $TPhAs^+$ conductance. First, we find that $A_{GMO} > A_{PC\text{-}chol}$ and, second, the value of kinetic parameter A increases exponentially with 2,4-D concentration by about the same factor for both types of membranes. This result contradicts the cholesterol exclusion hypothesis and strongly suggests that the effect of 2,4-D on cation transport is associated with the change of the ion translocation rate constant, and that the effect is almost independent of the structure of polar heads. Because at low K^+ concentration $A \approx 2k_{ISO}/k_D$, we find that in the absence of 2,4-D the value of k_{ISO}/k_D of GMO/*n*-decane membranes is about 0.02 ± 0.006. This is smaller but comparable to 0.04 found for nonactin-K^+ transport in GMO/hexadecane membranes by Hladky (55, 56). Our result is compatible with the observation that GMO/*n*-decane membranes are thicker than GMO/hexadecane membranes (39).

The results of studies of the effect of ionic strength on membrane conductance at high 2,4-D concentration also point toward the possibility that at moderate and high 2,4-D concentration the effect is dominated by the increase of the positive ion translocation rate constant. Using the data from Fig. 4 and arguments similar to those employed in the analysis of 2,4-D concentration dependence, we find that the kinetic parameter A also increases with the ionic strength. The similarity between the ionic strength dependence of the parameter $A_{non\text{-}K^+}$, which is proportional to k_{ISO}/k_D, with that of $TPhAs^+$ conductance $G(0)$, which is also proportional to the rate constant of translocation across the membrane (Fig. 8), also supports the hypothesis that 2,4-D increases the probability of translocation of positive ions across the membrane interior.

2,4-D Induced Changes of Electric Potential in the Membrane

The translocation rate constant for ion diffusing across the membrane interior, k_i, whether it is k_{ISO} for nonactin-mediated potassium transport, or k_i for the direct transport of $TPhAs^+$ ions, is determined, among other factors, by the ion potential energy barrier $W(x)$ of the

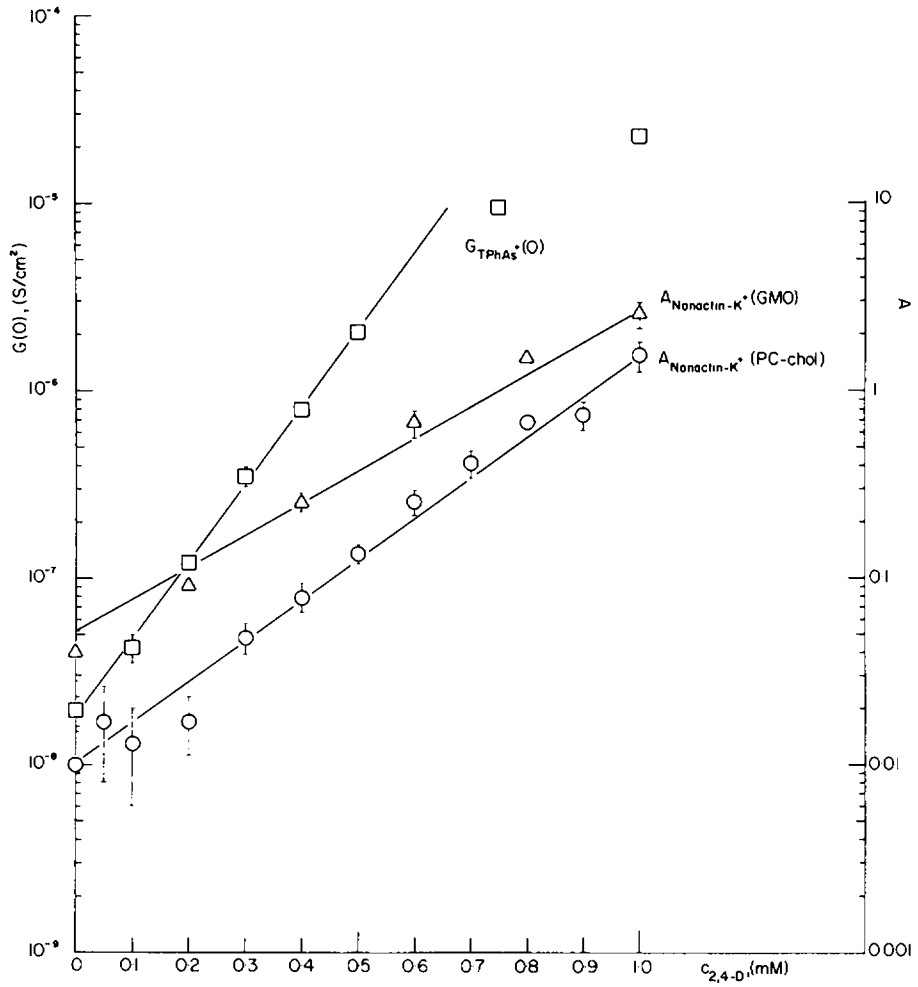


FIGURE 7 A comparison of 2,4-D concentration dependence of $TPhAs^+$ conductance with that of kinetic parameter $A \propto k_{ISO}/k_D$ for PC-chol and GMO membranes. Experimental conditions: PC-chol membranes: $c_{TPhAs^+} = 2 \times 10^{-3}$ M, in $c_{LiCl} = 0.5$ M. $c_{nonactin}$ (aqueous solution) $= 1.1 \times 10^{-7}$ M, $c_{nonactin}$ (membrane solution) $= 3.2 \times 10^{-5}$ M, $c_{K^+} = 0.06$ M, ionic strength (LiCl + KCl) = 1 M, pH = 2 (buffered).

membrane interior. In general, the rate constant k_i is related to the ion potential energy barrier $W(x)$ according to

$$k_i \propto D_m \Big/ \int_{\eta}^{d-\eta} \exp\left[W(x)/kT\right] dx,$$

where η and $d - \eta$ are the locations of the ion potential energy wells within the membrane boundary regions (12), and D_m is the ion diffusion coefficient in the membrane interior. The ion potential energy barrier $W(x)$ has a very broad maximum in the central region of the membrane, and it can be shown (37) that it is the height of this barrier, ϕ, measured with

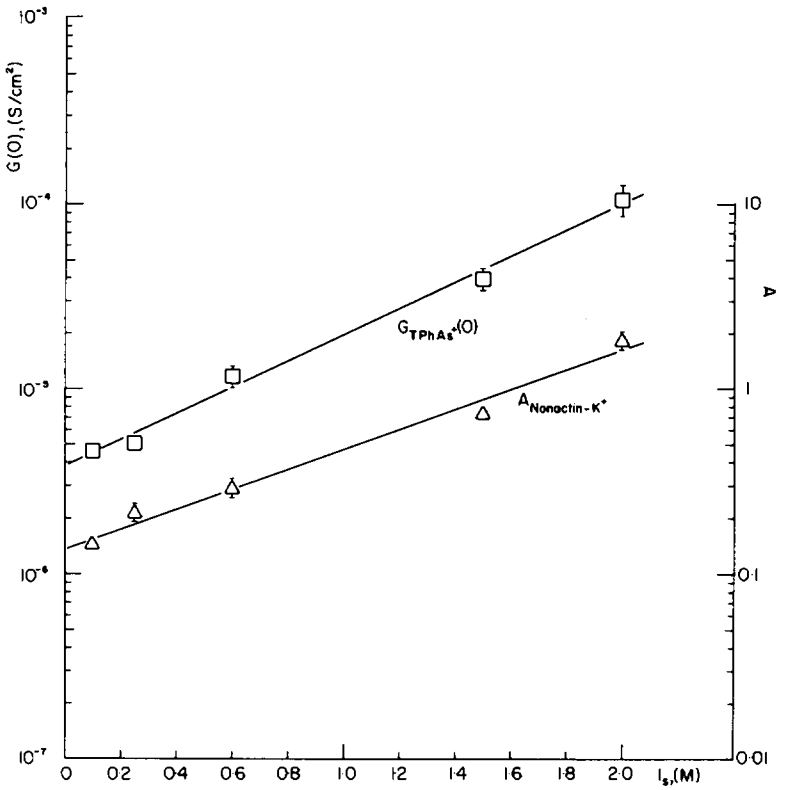


FIGURE 8 A comparison of ionic strength dependence of $TPhAs^+$ conductance with that of parameter A $\propto k_{iso}/k_D$ at fixed 2,4-D concentration. Experimental conditions: PC-chol membranes: $c_{2,4\text{-D}} = 7.5 \times 10^{-4}$ M, c_{nonactin} (aqueous solution) $= 4.5 \times 10^{-8}$ M; $c_{TPhAs^+} = 2 \times 10^{-3}$ M; $c_{K^+} = 0.1$ M, pH = 2 (buffered).

respect to the ion potential energy of the well, or the adsorption-reaction plane, that primarily determines the magnitude of the ion translocation rate constant k_i. Because the experimental data on membrane conductance (Fig. 1) indicate that the effect of 2,4-D is asymmetric with respect to the sign of the electric charge of the transported ion, it is reasonable to assume that 2,4-D changes the electrostatic potential of the membrane interior.

Using the changes of $TPhAs^+$ conductance and the changes of parameter A of nonactin-K^+ transport, one can estimate the changes of the electric potential of the membrane interior induced by 2,4-D. The electric potential difference between the central region of the membrane core and the bulk aqueous solution, ψ, has two components (see inset in Fig. 9): the potential difference between the adsorption-reaction plane and the aqueous solution, θ, and the potential difference between the center of the membrane and the adsorption-reaction plane, ϕ. In other words $\psi = \theta + \phi$. In general, the changes of the $TPhAs^+$ conductance depend on both the changes of the density of adsorbed $TPhAs^+$ ions, which can be accounted by $\Delta\theta$, as well as on the changes of the translocation rate constant, which can be attributed to $\Delta\phi$. Within this framework, the zero voltage conductance of 2,4-D-modified membrane can

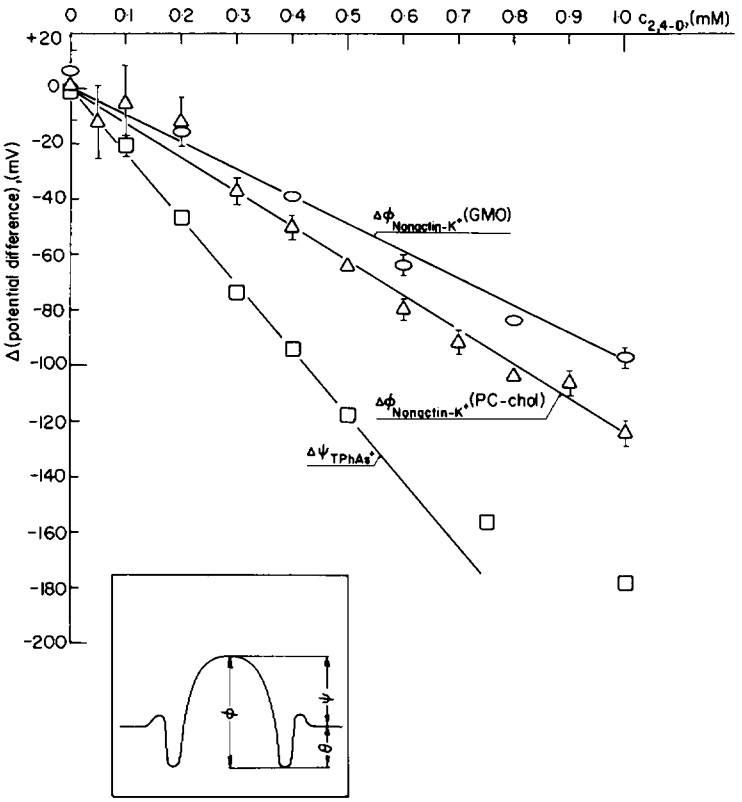


FIGURE 9 Change of electric potential difference between membrane interior and the aqueous solution ($\Delta\psi_{\text{TPhAs}^+}$, PC-chol membranes) and between the membrane interior and the reaction plane ($\Delta\phi_{\text{non-K}^+}$, PC-chol and GMO membranes) as a function of 2,4-D concentration. The potential difference changes have been estimated from the changes of membrane conductance ($\Delta\psi_{\text{TPhAs}^+}$) and from the changes of the kinetic parameter A ($\Delta\phi_{\text{non-K}^+}$) given in Fig. 7.

be related to that of the untreated, or the reference, membrane (superscript ref), by the Boltzmann factor:

$$G_{\text{TPhAs}^+}(0) \approx G^{\text{ref}}_{\text{TPhAs}^+}(0) \cdot \exp(-e\Delta\psi/kT). \tag{8}$$

Thus, from the $TPhAs^+$ conductance data one can estimate the changes of the height of the ion potential energy barrier between the center of the membrane and the aqueous solution. The change of $TPhAs^+$ conductance is a measure of both the change of the $TPhAs^+$ partition coefficient and the translocation rate constant.

For the nonaction-K^+ transport, all the previous evidence suggests that the changes of parameter A are dominated by the changes of the translocation rate constant k_{ISO}. These in turn reflect the changes of the potential difference between the center of the membrane and the reaction plane, $\Delta\phi$:

$$A \approx A^{\text{ref}} \exp(-e\Delta\phi/kT). \tag{9}$$

In Fig. 9 we illustrate the changes of the potential differences $\Delta\psi$ and $\Delta\phi$ as a function of 2,4-D concentration obtained from weighted fit of Eqs. 8 and 9 to the data in Fig. 7. These potential difference changes are approximately proportional to the concentration of 2,4-D, and at high 2,4-D concentration they correspond to several kT. It is interesting to note that the potential difference changes, $\Delta\phi$, in both PC-chol and GMO membranes are almost identical.

In terms of the adopted dipole hypothesis (41, 37), we postulate that the decrease of the electric potential of the membrane interior originates from a layer of 2,4-D molecules adsorbed within the membrane interfacial region. To account for the change of electric potential of the membrane interior, the molecular dipole moments have to be oriented toward the aqueous solution, as depicted in Fig. 10. By treating the dipole layer as two sheets of electric charge, the potential difference across the layer is equal to

$$V_{dip} = N_{2,4\text{-D}} \cdot p_{\perp}/\epsilon\epsilon_0 = Kc_{2,4\text{-D}} \cdot p_{\perp}/\epsilon\epsilon_0, \tag{10}$$

where $N_{2,4\text{-D}}$ is the surface density of oriented 2,4-D molecules, $p_{\perp}$ is the component of 2,4-D dipole moment normal to the surface, K the partition coefficient of 2,4-D between the membrane surface and aqueous solution, $\epsilon\epsilon_0$ is the permittivity. Equating V_{dip} to the potential difference $\Delta\psi$ or $\Delta\phi$, and $p_{\perp}$ to the dipole moment of 2,4-D molecule, which is 3.33 Debye (60), one can estimate $N_{2,4\text{-D}}/\epsilon$. For electric potential change of 100 mV, $N_{2,4\text{-D}}/\epsilon = 7.97 \times 10^{16}$ m^2, which for effective dielectric constant of the boundary region $\epsilon = 10$ corresponds to separation between 2,4-D molecules of 1.1 nm.

The increase of both the $TPhAs^+$ conductance and the kinetic parameter $A_{non\text{-}K^+}$ with the ionic strength can be also attributed to the increase of the ion translocation rate constant. Because the effect of ionic strength is qualitatively similar to that of 2,4-D concentration (compare Figs. 3 and 4), we assume that the partition coefficient of 2,4-D between the membrane interface and the aqueous solution increases with the ionic strength

$$K = K_0 + K_1 I_s, \tag{11}$$

where I_s represents the ionic strength; K_0 is the distribution coefficient between the

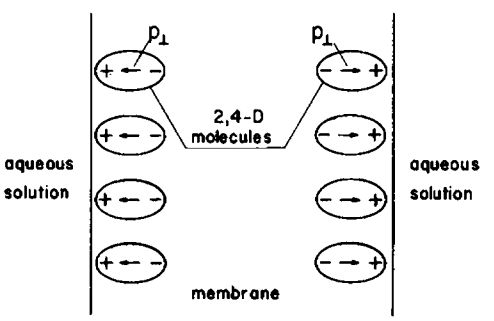


FIGURE 10 A diagram depicting the dipole hypothesis used to interpret the effect of 2,4-D on ion transport. The neutral 2,4-D molecules are assumed to be adsorbed in the membrane and oriented in such a way so that their dipole moments are directed toward the aqueous solution. This results in lower potential energy of positive ions and higher potential energy of negative ions in the membrane interior, which causes changes in membrane permeability in opposite direction for ions having opposite electric charge.

membrane surface and water, whereas K_1 accounts for "salting out" effect of the electrolyte. It then follows that the dependence of $TPhAs^+$ conductance on 2,4-D concentration and the ionic strength is equal to

$$G_{TPhAs^+}(0) = G^{ref}_{TPhAs^+}(0) \cdot \exp\left[(b^G_0 + b^G_1 I_s)(c_{2,4\text{-D}} - c^{ref}_{2,4\text{-D}})\right],$$

and similarly, the kinetic parameter A of nonactin-K^+ transport is given by

$$A_{non\text{-}K^+} = A^{ref}_{non\text{-}K^+} \cdot \exp\left[(b^A_0 + b^A_1 I_s)(c_{2,4\text{-D}} - c^{ref}_{2,4\text{-D}})\right].$$

These two equations describe both the effect of 2,4-D and ionic strength. In the framework of the dipole hypothesis, the terms b_0 and $b_1 I_s$ are proportional to the partition coefficients of 2,4-D, K_0 and $K_1 I_s$, the fraction of the dipolar potential difference that affects the transport process in question, and to the other characteristics of the dipolar layer (Eq. 10).

To obtain more detailed information on the effect of 2,4-D on the membrane boundary region it is necessary to measure directly the distribution of the membrane permeable species between the aqueous medium and the membrane surface, as well as the changes of the translocation rate constant as a function of 2,4-D concentration and ionic strength. This can be achieved by current relaxation studies with tetraphenylborate ions, which is the subject of the subsequent paper.

Biological Implications

Biological significance of the pesticide-induced change of the rate of transport of positive and negative ions in lipid membranes is at the present time unknown. Because the effect is rather weak within the biologically significant pH range, it appears more likely that 2,4-D induced permeatoxicity is associated with some metabolic product. In recent years the metabolism of 2,4-D has been studied rather extensively in plants and plant tissue cultures (61–67). It has been shown that 2,4-D is metabolized into amino acid conjugates and ring hydroxylated derivatives. The hydroxylated metabolities have been found physiologically inactive, and their appearance is associated with detoxification processes (63, 67). In contrast, the amino acid conjugates possess the growth stimulation activity, especially the less polar conjugates such as leucine, isoleucine, valine, alanine, and methionine (64, 66). It remains to be seen whether the physiological activity of these substances can be correlated with their ability to modify ion transport in lipid bilayers.

CONCLUSIONS

In this work we have studied the effect of 2,4-D on the direct- and carrier-mediated transport of ions in lipid bilayer membranes, and proposed a model for the action of 2,4-D on ion transport. The major conclusions drawn from our results are as follows:

(*a*) The ionized form of 2,4-D does not permeate through lipid membranes.

(*b*) 2,4-D increases the rate of transport of positively charged lipid soluble ions, and inhibits the transport of negatively charged tetraphenylborate ions.

(*c*) Only the neutral molecule of 2,4-D affects the transport of ions across lipid membranes.

(*d*) 2,4-D changes the kinetics of nonactin-mediated potassium ion transport, and the

kinetic effects can be understood in terms of a simple carrier transport model. We have shown that one of the major effects of 2,4-D is associated with the increase of the rate constant of translocation of the loaded carrier as compared to its dissociation rate constant. At high 2,4-D concentration the rate of electric field driven potassium ion transport across the membrane is sufficiently high to become limited at low potassium concentration by the rate of recombination of nonactin with K^+ ions, and at high potassium concentration by the rate of backdiffusion of unloaded nonactin molecules.

(*e*) The rate of transport of tetraphenylarsonium$^+$ ions across the membrane, as well as the ratio of k_{ISO}/k_D of nonactin-K^+ exponentially increases with the concentration of 2,4-D and ionic strength. From these data it has been possible to determine the changes of the electric potential of the membrane interior, which are negative and as large as 140–180 mV.

(*f*) The action of 2,4-D on ion transport in lipid membranes is consistent with the hypothesis that the dipole moment of 2,4-D molecules, absorbed within the membrane interfacial region, is directed toward the aqueous medium. The results of kinetic studies of nonactin-mediated transport of potassium suggest that the layer of 2,4-D molecules is predominantly located below the plane of recombination of nonactin and K^+ ions, that is, on the hydrocarbon side of the interface.

(*g*) We have found that modification of ionic permeability of lipid membranes by 2,4-D is rather small at biological pH. Thus it is very likely that permeatoxicity of 2,4-D is associated with some metabolic product of 2,4-D, rather than with 2,4-D molecule itself.

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