# EFFECT OF THE ANESTHETICS BENZYL ALCOHOL AND CHLOROFORM ON BILAYERS MADE FROM MONOLAYERS

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ABSTRACT The neutral anesthetics chloroform and benzyl alcohol, at concentrations that block the nerve impulse, greatly modify the transport parameters of positive and negative ions in lipid bilayers made from monolayers. Both chloroform and benzyl alcohol increase the membrane permeability to these ions and increase the translocation rate for tetraphenylborate. It was found that both anesthetics increase the membrane permeability to positive ions more markedly than to negative ions. It was also found that the membrane capacitance increases linearly with the concentration of benzyl alcohol. At 51 mM benzyl alcohol, the increase in capacitance is ~ 6%. Chloroform also increases the membrane capacitance; the increase in capacitance was found to be 6% at 18 mM chloroform. An analysis of the changes in the transport parameters of the lipophilic ions, together with the changes in membrane capacitance, suggests that benzyl alcohol and chloroform modify the dipole potential and dielectric constant of the membrane. Benzyl alcohol may also increase the "fluidity" of the lipid bilayer membranes. At 36 mM benzyl alcohol, the membrane permeability to acetamide increases by 38%.

## INTRODUCTION

Current theories of anesthesia state that neutral anesthetics, such as chloroform or benzyl alcohol, act at a hydrophobic site in the membrane (Seeman, 1972). However, it is not clear whether this site is located in the membrane proteins (Seeman, 1972; Boggs et al., 1976 *a, b*) or in the lipid matrix (Hubbell et al., 1970; Trudell, 1977; Mullins, 1975; Lee, 1976). The theory that states that the primary site of action of anesthetics is in the lipid matrix has received support from studies of the effect of anesthetics on the physical state of the hydrocarbon chains of phospholipids in model membrane systems. Thus, the action of anesthetics has been related to their ability to promote an increase in membrane fluidity (Trudell et al., 1973 *a,b*) or to their potency in increasing the pool size of melted lipids in the bilayer (Trudell et al., 1975; Vanderkooi et al., 1977). On the other hand, Miller and Pang (1976) and Pang and Miller (1978) have shown that some anesthetics, such as pentobarbital, are able to condense or to fluidize the lipid bilayer depending on its cholesterol content. Therefore, the ability of an anesthetic to disorder the membrane appears to be modulated by the membrane lipid composition. The main drawback of the fluidized lipid hypothesis is that nearly all the experiments have been made at anesthetic concentrations higher than those required to promote general anesthesia or block nerve conduction.

Haydon et al. (1977 *a, b*) have recently reported that the cutoff in anesthetic potency of *n*-alkanes is closely related to the decrease in adsorption of the alkane into planar lipid bilayer

membrane. On the basis of their findings, they have proposed that the blockage of the nerve impulse by *n*-alkanes in axons is due to their ability to increase the membrane thickness (and tension) that in turn will produce a destabilization of the voltage-dependent ionic channels. A similar hypothesis for the action of benzyl alcohol was proposed simultaneously by Ashcroft et al. (1977 *a, b*). The membranes used by Ashcroft et al. (1977 *a, b*) contain large amounts of solvent (cf., Haydon et al., 1977*b*). Since solvent may modify the benzyl alcohol adsorption parameters or conversely benzyl alcohol may modify the amount of solvent in the membranes, we decided to undertake a study of the action of benzyl alcohol and cholorform on membranes formed by the technique introduced by Montal and Mueller (1972). The facts that these membranes have a relatively large specific capacitance and exhibit a very small voltage-dependent capacitance (Benz et al., 1975; Alvarez and Latorre, 1978) indicate that they are practically devoid of solvent.

Studies of the membrane conductance induced by ionic probes are useful to elucidate the mode of action of anesthetics in lipid bilayer membranes. As was pointed out by McLaughlin et al. (1970); Szabo et al. (1972), and Szabo (1976), it is possible in principle to identify which membrane structural parameters are being affected by changes in membrane composition by using oppositely charged ionic probes. Lipophilic ions such as tetraphenylborate ($TPhB^-$) and tetraphenylarsonium ($TPhAs^+$) are excellent candidates as their chemical structure is almost identical, except for charge.

In this paper, we report the effect of benzyl alcohol and chloroform on the capacitance and on the transport parameters of $TPhB^-$, $TPhAs^+$, carbonylcyanide *m*-chlorophenylhydrazone ($CCCP^-$), the nonactin-$K^+$ complex, and on acetamide permeability in membranes made by apposition of two monolayers. We show that these anesthetics, at pharmacological concentrations, have a small effect on the membrane capacitance and on the acetamide permeability, but they have a marked effect on the transport parameters of positive and negative ion probes. Furthermore, an analysis of the changes in the conductances induced by these lipophilic ions suggests that the internal dipole potential and the dielectric constant of the membrane are affected by benzyl alcohol and chloroform.

## MATERIALS AND METHODS

### *Membrane Formation*

The membranes were formed at room temperature (20 ± 2°C), according to the technique described by Montal and Mueller (1972). The main departure from the classical Montal and Mueller procedure for forming membranes was that the aperture in the Teflon partition (Du Pont de Nemours & Co., Inc., Wilmington, Del.) separating the two aqueous phases was treated with 5% solution of squalene in pentane. The membranes were made from bacterial phosphatidylethanolamine (PE) (Supelco, Inc., Bellefonte, Pa.), or glycerolmonooleate (GMO) (Nu-Check Prep., Inc., Elysian, Minn.). The lipid was spread on the surface of the electrolyte solution using 10 $\mu$l of a solution containing 12.5 mg/ml of lipids in pentane.

When $TPhAs^+$ (Eastman Kodak Co., Rochester, N. Y.) was used, the membranes were made in the presence of the lipophilic cation. The final $TPhAs^+$ concentrations were $3.3 \times 10^{-6}$ M (GMO membranes) and $1.0 \times 10^{-3}$ M (PE membranes). $TPhB^-$ (Eastman Kodak Co.), $CCCP^-$ (Calbiochem La Jolla, Calif.) and nonactin (E. R. Squibb & Sons, Princeton, N. J.) were added to both compartments in concentrated ethanolic solutions after the membrane was formed. $TPhB^-$ final concentrations were $3.3 \times 10^{-7}$ M (GMO membranes) or $3.3 \times 10^{-8}$ M (PE membranes). $CCCP^-$ and nonactin final concentrations were $1 \times 10^{-6}$ and $2 \times 10^{-8}$ M, respectively. Control experiments showed

that ethanol at the concentrations used (<0.5% vol/vol) has no effect on membrane conductance or capacitance. The electrolyte solutions were symmetrical and consisted of 1 M NaCl or 1 M KCl (when nonactin was used) buffered at pH 6 with phosphate. The anesthetic concentration in the chamber was raised by withdrawl of a volume of electrolyte solution (0.15 or 0.30 ml) from both compartments and subsequent addition of the same volume of electrolyte solution containing the anesthetic (52 mM $CHCl_3$ solution or 192 mM benzyl alcohol solution). To avoid dilution of the ionic probes, the anesthetic solutions also contained the appropriate amount of $TPhB^-$, $TPhAs^+$ $CCCP^-$ or nonactin. To avoid evaporation of the chloroform, the chamber was sealed with a tight-fitting Lucite lid (Du Pont de Nemours & Co.). The concentration of the saturated solution of chloroform and the linearity of the dilutions were determined using gas chromatography (Yamamura et al., 1966).

### *Electrical Measurements*

The system for measuring the electrical properties of the bilayers has been described in detail by Alvarez and Latorre (1978). Essentially, it consists of a two-electrode "voltage-clamp." We used Ag/AgCl electrodes with a surface area >2 $cm^2$. The capacitance of the membranes was measured by applying 5-kHz, 10-mV, peak-to-peak, triangular voltage wave form. The amplitude of the charging current was measured with a digital multimeter (Hewlett-Packard Co., Palo Alto, Cal.; model 3476B). The system was calibrated before the experiment with a known capacitor. Capacitance measurements were made in the absence of the lipophilic ions as a function of anesthetic concentration in the electrolyte solution bathing the membrane. The area of the membrane was estimated from the area of the hole in the Teflon partition. It was calculated using the value of the diameter of the hole measured with a calibrated microscope having an overall magnification of 500×. The specific capacitance, the ratio of membrane capacitance to membrane area, was measured to an accuracy of 2%. The precision of the method when measuring changes in membrane capacitance is ~ 0.3%.

After membrane formation and addition of the lipophilic ion, the aqueous phases were stirred for 10 min to ensure equilibrium. The zero voltage conductances induced by $TPhAs^+$, $CCCP^-$, and nonactin-$K^+$ were measure from steady-state, current-voltage curves recorded directly on a *x-y* recorder. The rate of change of voltage was low enough to introduce negligible hysteresis. Since steady-state $TPhB^-$ transport is unstirred layer limited (LeBlanc, 1969), current measurements were made with a voltage pulse technique (Ketterer et al., 1971). For this anion, the initial current (or conductance) was estimated from the current extrapolated to zero time. To improve the signal:noise ratio of the $TPhB^-$-induced current transients, several current waveforms were added and stored in digital form with the help of a signal averager, model 1070 (Nicolet Instrument Corp., Madison, Wisc.).

The analog-to-digital conversion was done by an eight-bit transient recorder. The digitalized data from the curves were further analyzed with a Hewlett-Packard 9825A calculator coupled to a signal averager. The time constant of the current-measuring amplifier was 3 μs, limiting the effective time resolution of the system to ~30 μs.

### *Surface Potential Measurements*

PE or GMO monolayers were formed in a Teflon chamber divided in two compartments by a Teflon barrier that separated the surface of both compartments but allowed free contact of the subphases. The area of the compartments were 80 and 13 $cm^2$. Monolayers were formed in the larger compartment. Measurements of surface potential were made by using an air-ioinizing electrode of $^{210}Po$, which was suspended ~ 2 mm over the air/water interface, and a calomel electrode that was connected through a salt bridge to the film-free subphase. The subphase composition was 1 M NaCl, 20 mM phosphate, pH 6. A Keithley Electrometer (Keithley Instruments, Inc., Cleveland, Ohio; Model 602) was connected to both electrodes for the measurements of surface potential. Before spreading the lipid the surface was cleaned until a stable potential more negative than 500 mV was obtained. 5 μl of a 12.5 mg/ml solution of PE or GMO in pentane were then spread at the air/water interface and the new potential obtained was recorded. The concentration of benzyl alcohol was varied by withdrawl of subphase and subsequent addition of a 190-mM-benzyl alcohol, 1-M-NaCl, 20-mM-phosphate solution to the smaller compartment. The subphase was stirred with a magnetic bar.

### *Permeability Measurements*

The procedure for measuring the permeability coefficient ($P_d$) of acetamide was that described by Holz and Finkelstein (1970). [$^{14}$C]acetamide (New England Nuclear, Boston, Mass.) was added to one compartment only and 50-$\mu$l aliquots were taken from the "cold" compartment every 5 min.

## RESULTS

### *Specific Capacitance of PE and GMO Membranes*

The specific capacitance of PE and GMO membranes was found to be 0.68 ± 0.01 and 0.79 ± 0.01 $\mu$F/cm$^2$, respectively. The specific capacitance value obtained for GMO membrane is in good agreement with the value obtained by White (1978) for GMO-squalene bilayers formed with the pipette technique. The specific capacitance value for GMO membranes is independent of the area of the hole. Thus, if the membranes are formed in holes of areas of $2 \times 10^{-4}$ cm$^2$ and $1 \times 10^{-2}$ cm$^2$, the specific capacitance is 0.79 ± 0.01 and 0.80 ± 0.01 $\mu$F/cm$^2$, respectively.

The studies of Fettiplace et al. (1971) and White (1975) have shown that the membrane capacitance increases as the number of carbon atoms in the hydrocarbon solvent is increased. These results have been interpreted as a decrease in the molar fraction of solvent in the membrane. Inasmuch as squalene has 22 carbons, it is possible to predict from the above studies that the molar fraction of squalene in the bilayer must be very small indeed. The following characteristics of membranes formed by apposition of two monolayers and using squalene to pretreat the hole indicate that these membranes are virtually solvent-free. First, the large specific capacitance of our GMO membranes is in good agreement with the value reported by White (1978) of 0.7771 $\mu$F/cm$^2$ for GMO-squalene bilayers. Furthermore this value is ~0.1 $\mu$F/cm$^2$ larger than the so-called "solvent-depleted" bilayer made from the same lipid (Waldbillig and Szabo, 1978). Second, theoretical estimates of the geometric specific capacitance ($C_g$) of GMO solvent-free bilayer range from 0.75 to 0.81 $\mu$F/cm$^2$ (White, 1978). Third, $C_g$ is independent of applied potential for both GMO and PE bilayers. We have measured $C_g$ in the presence and in the absence of 150-mV DC bias with identical results. Finally, the work of Simon et al. (1977) also supports the notion that our membranes must contain a very small mole fraction of solvent. They showed that squalene is insoluble in dipalmitoyllecithin dispersions. We think that to give a theoretical value for PE is extremely difficult, because bacterial PE is a mixture of lipids containing acyl chains with different lengths.

### *Effect of Benzyl Alcohol and Chloroform on Membrane Capacitance*

Fig. 1 shows that benzyl alcohol increases the membrane capacitance of PE bilayers and that this increase in capacitance is a linear function of the anesthetic concentration in the aqueous phase.[1] At the nerve-blocking concentration (20 mM), the increase in capacitance is ~ 2% and at 51 mM, it is ~ 6%. After benzyl alcohol is added, the capacitance reaches a new value in ~

[1]Benz et al. (1975) showed that the membrane capacity of membranes made by apposition of two monolayers is a function of the applied hydrostatic pressure difference across the membrane. Membrane capacitance is at a minimum when the levels of the aqueous solution in both compartments are equal. Accordingly, all the measurements presented in Fig. 1 were performed at the minimum capacitance value.

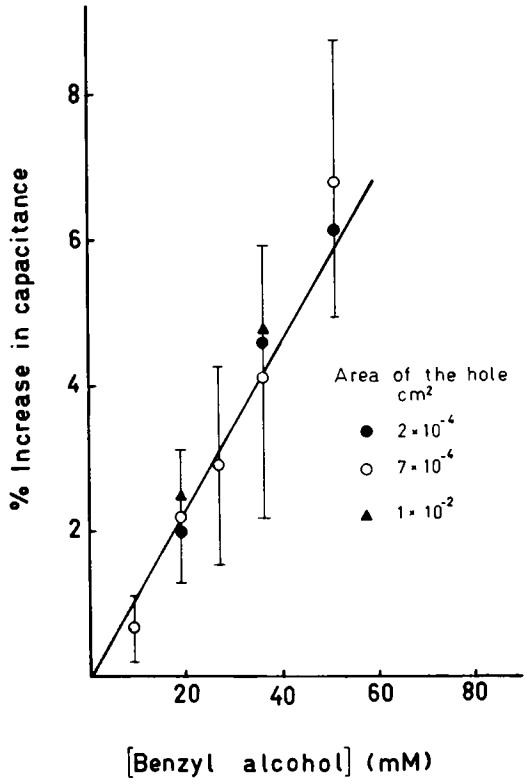


FIGURE 1 Effect of the benzyl alcohol concentration on the capacitance of PE membranes. The membranes were formed in 1 M NaCl, 10 mM phosphate pH 6. The area of the hole where the membranes were formed ranges from $2 \times 10^{-4}$ to $1 \times 10^{-2}$ $cm^2$. The bars represent the standard deviation of at least four membranes (see text for details.)

2 min and remains at that value for at least 1 h. Fig. 1 also shows that the magnitude of the effect of benzyl alcohol on membrane capacitance is independent of the area of the hole where the membranes are formed. Benzyl alcohol also increases the capacitance of GMO bilayers. The results obtained in this type of membrane are both quantitatively and qualitatively similar to those shown in Fig. 1.

Chloroform also increases the membrane capacitance (not shown). At the nerve-blocking concentration (5 mM) the capacitance increase is 2%, and at 18 mM it is ~ 6%.

*Effect of Benzyl Alcohol on the Conductances Induced by Anions and Cations*

Fig. 2 *a* shows the effect of benzyl alcohol on the conductances induced by $TPhB^-$, $TPhAs^+$, $CCCP^-$, and the nonactin-$K^+$ complex in PE membranes.[2] Fig. 2 *b* shows the effect of benzyl alcohol on the conductance induced by $TPhB^-$ and $TPhAs^+$ in GMO membranes. Benzyl alcohol increases the membrane conductance to all ion probes and in both types of membranes. After the addition of the anesthetic, the $TPhAs^+$ steady-state induced conductance reaches a constant value in ~ 3 min, whereas $TPhB^-$ instantaneous-induced conduc-

[2]The solid lines drawn in this and the following figures do not have any theoretical meanings.

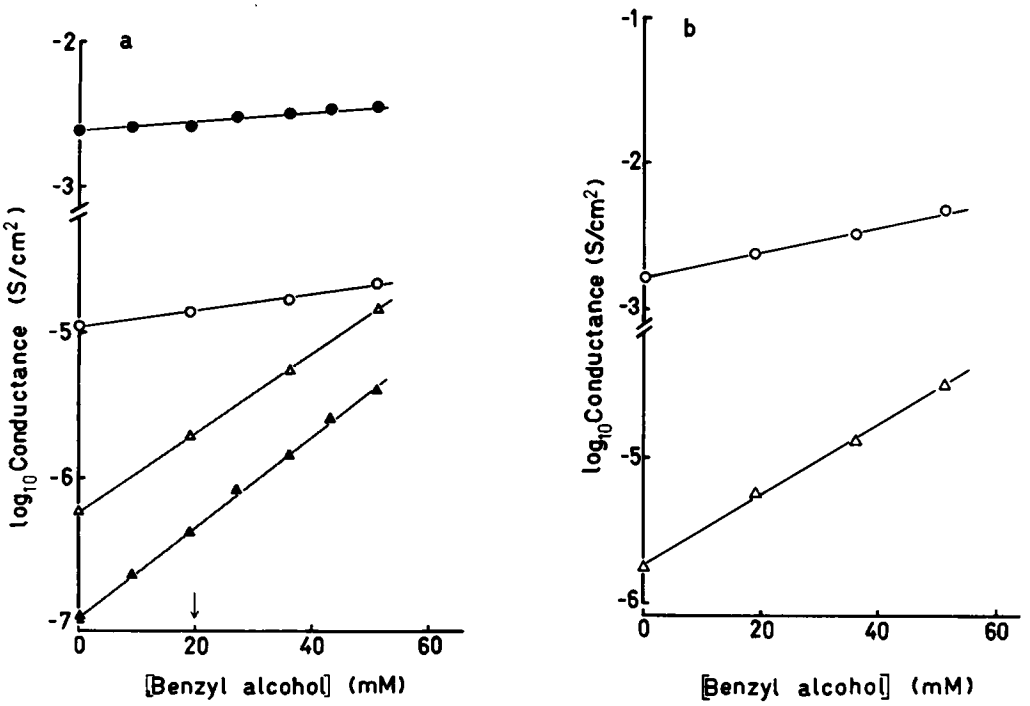


FIGURE 2 (*a*) Effect of benzyl alcohol on the $TPhB^-$ (●), $CCCP^-$ (○), nonactin $K^+$ (△), and $TPhAs^+$-induced (▲) conductance in PE membranes. The membranes were formed in 1 M NaCl, 10 mM phosphate pH 6, or 1 M KCl, 10 mM phosphate pH 6 when nonactin is used. 1 mM $TPhAs^+$ was present in the aqueous phase during the membrane formation when this lipophilic ion was used. $TPhB^-$, $CCCP^-$, and nonactin in ethanolic solution were added to a final concentration of $3.3 \times 10^{-8}$, $10^{-6}$, and $10^{-7}$ M, respectively, after the membrane formation. The membranes were left for 10–30 min in the presence of the lipophilic ion or carrier with continuous stirring before addition of the anesthetic. The measurements were performed 20 min after the addition of anesthetic. (*b*) Effect of benzyl alcohol on the $TPhB^-$ (○) and the $TPhAs^+$-induced (△) conductance in GMO membranes. The $TPhAs^+$ concentration was $3.3 \times 10^{-6}$ M. $TPhB^-$ was added to the aqueous phases to a final concentration of $3.3 \times 10^{-7}$ M. Other experimental conditions were similar to those of Fig. 2 *a*. Arrows indicate nerve-blocking concentration.

tance reaches a steady value only after 10 min. At the nerve blocking concentration, benzyl alcohol increases the conductance of PE membranes to $TPhAs^+$ fourfold and produces almost no change in the $TPhB^-$-induced conductance. Therefore, the results shown in Fig. 2*a* and *b* shows that although benzyl alcohol increases the membrane conductance for both ion probes, the absolute change in conductance as the concentration of the anesthetic increases is not equal for $TPhAs^+$ and $TPhB^-$.

Fig. 2 *a* shows that for PE membranes benzyl alcohol promotes similar changes in the conductance induced by $TPhAs^+$ and the nonactin-$K^+$ complex or in the conductance induced by $TPhB^-$ and $CCCP^-$. Statistically, however, the relative conductance (defined as the ratio between the conductance for an ionic probe in a membrane treated with benzyl alcohol and the conductance for the same probe of an untreated membrane) for $TPhAs^+$ is larger than that of the nonactin-$K^+$ complex at any given benzyl alcohol concentration by ~ 25–40%. The relative conductance for $CCCP^-$, on the other hand, is larger than that of $TPhB^-$ by ~ 20–30% at all benzyl alcohol concentrations (see Fig. 7).

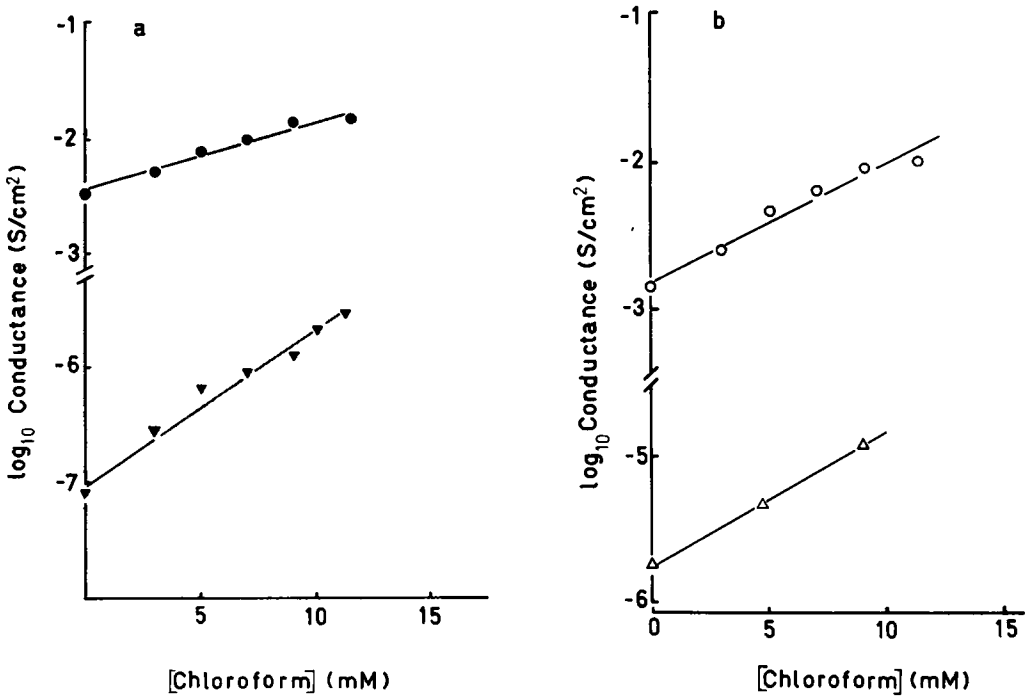


FIGURE 3 (*a*) Effect of chloroform on the $TPhB^-$ (●) and $TPhAs^+$-induced (▲) conductance in PE membranes. The measurements were performed 2 min after the addition of the anesthetic. Other experimental conditions were those of Fig. 2 *a*. (*b*) Effect of chlorofom on the $TPhB^-$ (○) and $TPhAs^+$-induced (△) conductance in GMO membranes. The measurements were performed 2 min after the addition of the anesthetic. Other experimental conditions were those of Fig. 2 *b*. Arrows indicate nerve-blocking concentration.

### *Effect of Chloroform on the Conductances Induced by $TPhB^-$ and $TPhAs^+$*

Fig. 3 *a* and *b* shows the effect of chloroform on the $TPhB^-$-induced and $TPhAs^+$-induced conductances in PE and GMO membranes, respectively. In both types of membranes, increasing the concentration of chloroform increases the conductances induced by $TPhB^-$ and $TPhAs^+$. The values of conductance were obtained 2 min after the addition of the different chloroform concentrations shown in Fig. 3 *a* and *b*. We found that after 2 min with constant stirring of the solutions in both compartments, the $TPhAs^+$ steady-state induced conductance and the instantaneous $TPhB^-$-induced conductance reached a maximum value. At longer periods of time, the conductance promoted by both ion probes decreases, probably as a consequence of the evaporation of chloroform from the chamber. Seeman (1972) reported that a concentration of 5 mM chloroform is able to block nerve conduction. In PE membranes, the same concentration of anesthetic is able to promote a 9-fold increase in the $TPhAs^+$-induced conductance and a 2.5-fold increase in the $TPhB^-$-induced conductance (Fig. 3 *a*).

### *Chloroform and Benzyl Alcohol Modify the Time-Course of $TPhB^-$-Induced Current*

The time-course of the $TPhB^-$-induced current, in the absence and presence of 5 mM chloroform in PE membranes after a 10-mV step in potential was applied, is shown in Fig. 4*a*

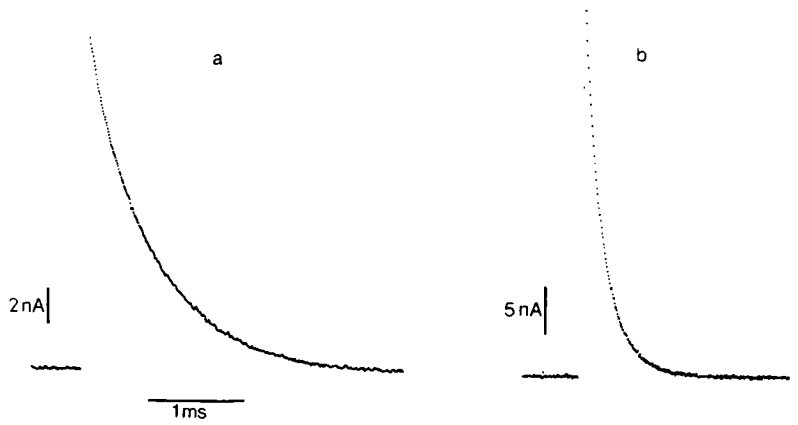


FIGURE 4 (*a*) $TPhB^-$-induced current of PE membranes after applying a 10-mV step in potential. Membrane formed in 1 M NaCl, 10 mM phosphate pH 6. $TPhB^-$ was added to the aqueous phases to a final concentration of $3.3 \times 10^{-8}$. No anesthetic added. (*b*) $TPhB^-$-induced current of PE membranes in the presence of 5 mM chloroform after applying 10-mV step in potential. Other experimental conditions were the same as Fig. 4 *a*.

and *b*, respectively. This concentration of chloroform promotes a 2.4-fold increase in the initial current and a decrease from 0.80 to 0.25 ms in the time constant for the current relaxation. For the two cases shown in Fig. 4, the steady-state current is almost negligible (<1% of the value of the instantaneous current), and the current relaxations follow an exponential time-course for at least four time constants. This indicates that $TPhB^-$ behaves like an ion almost perfectly trapped in the membrane; that the current relaxation represents the actual transport of $TPhB^-$ across the membrane interior; and that this is not due to diffusion polarization in the aqueous phases (e.g., Andersen and Fuchs, 1975). When log-linear regressions of the data shown in Fig. 4 *a* and *b* are performed, the correlation coefficients are 0.9998 and 0.9993, respectively. These correlation coefficients applied for at least four time constants.

Fig. 5 *a* shows the effect of different concentrations of chloroform on the time constant of the $TPhB^-$-induced current relaxation in PE and GMO membranes. In both cases, the time constants were determined by applying a potential step of 10 mV and recording the current decay with time. Fig. 5 *a* shows that increasing the chloroform concentration decreases the time constant of the $TPhB^-$-induced current relaxation. At 5 mM chloroform, the time constant decreases to 34% of its original value in both PE and GMO membranes.

Fig. 5 *b* shows that benzyl alcohol also promotes a decrease in the time constant of the $TPhB^-$-induced current relaxation. At 20 mM benzyl alcohol, the time constant decreases to ~40% of its original value in both PE and GMO membranes.[3]

As discussed by Szabo (1976) and Melnik et al. (1977), changes in membrane composition

[3]The decrease in the time constant of the $TPhB^-$-induced current relaxation as the anesthetic concentration is increased shown in Fig. 5 *a* and *b* is exponential. Because the translocation rate of $TPhB^-$ is determined by the height of the membrane energy barrier according to $1/\tau \propto \exp(-E/kT)$, where $E$ is the height of the membrane energy barrier, an exponential increase in $1/\tau$ is expected if both chloroform and benzyl alcohol are decreasing the value of $E$.

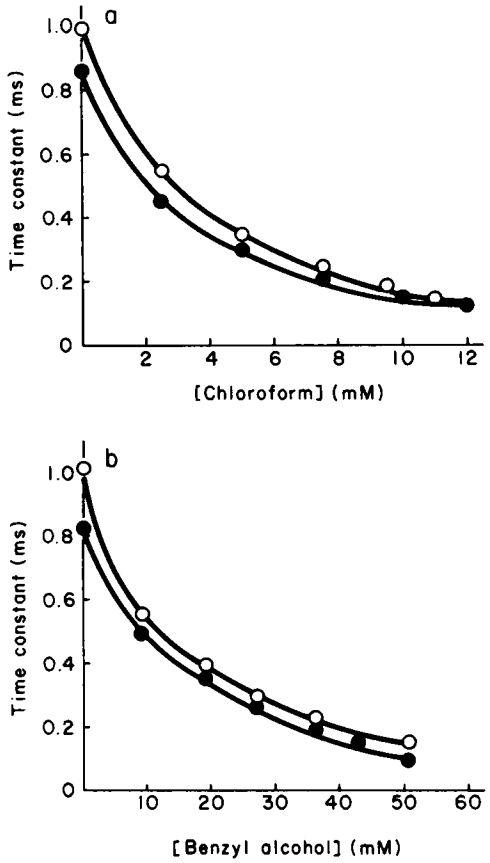


FIGURE 5 (*a*) Effect of chloroform on the time constant of the $TPhB^-$-induced current relaxation in PE (•) and GMO (○) membranes. The $TPhB^-$ concentration was $3.3 \times 10^{-8}$ M (PE membranes) and $3.3 \times 10^{-7}$ (GMO membranes). The membranes were formed in 1 M NaCl, 10 mM phosphate pH 6. (*b*) Effect of benzyl alcohol on the time constant of the $TPhB^-$-induced current relaxation of PE (•) and GMO (○) membranes. The experimental conditions were the same as Fig. 5 *a*.

can alter the surface concentration of the permeant species and/or the rate at which they are translocated. Hydrophobic ions are expected to be located at the membrane interfaces. This is because the potential energy profile of a hydrophobic ion in the membrane may be represented by the sum of a position-dependent electrostatic term and a term that accounts for the hydrophobic interactions. When these two energy terms are added, the resulting potential profile shows two deep minima at the interfaces and a peak at the center of the membrane (Ketterer et al., 1971). Furthermore, as pointed out by Szabo (1976) and Melnik et al. (1977), the height of the membrane energy barrier will control the rate of translocation, and the depth of the potential energy minima at the membrane interfaces will control the adsorption coefficient of the hydrophobic ion. Accordingly, further insight into the action of chloroform and benzyl alcohol on ion transport across lipid bilayer membranes can be gained by using the above mentioned theoretical framework. Thus, the adsorption coefficient, $\kappa$ (for species adsorbed at both membrane surfaces), is given by the expression (Melnik et al., 1977)

TABLE I
EFFECT OF BENZYL ALCOHOL ON THE $TPhB^-$ TRANSPORT PARAMETERS

| Benzyl alcohol | PE | | GMO | |
|---|---|---|---|---|
| | $\kappa \times 10^2$ | $1/\tau$ | $\kappa \times 10^3$ | $1/\tau$ |
| *mM* | *cm* | $s^{-1}$ | *cm* | $s^{-1}$ |
| 0 | 5.66 | 588 | 5.42 | 499 |
| 9 | 3.38 | 1,225 | — | — |
| 19 | 2.44 | 1,893 | 2.82 | 1,012 |
| 27 | 1.82 | 2,631 | — | — |
| 36 | 1.60 | 3,401 | 2.34 | 1,773 |
| 51 | 1.34 | 5,155 | 2.12 | 3,247 |

$$\kappa = 4RT\tau G(0,0)/F^2C_a, \quad (1)$$

where $G(0,0)$ is the conductance at zero potential and at zero time, $C_a$ is the concentration of $TPhB^-$ in the aqueous phases, and $\tau$ is the time constant for the current relaxation.

Table I shows the effect of benzyl alcohol on the adsorption coefficient and the relaxation time for $TPhB^-$ in PE and GMO membranes. Increasing the benzyl alcohol concentration decreases the value for the time constant of the $TPhB^-$ induced current relaxation and decreases the adsorption coefficient. These types of effects are found in both PE and GMO membranes, although there are some quantitative differences between these two types of lipids. Thus, when the benzyl alcohol concentration is raised from 0 to 51 mM, the change in the adsorption coefficient for $TPhB^-$ is 4-fold in PE membranes and 2.5-fold in GMO membranes. The changes in the time constant are 8.7-fold and 6.5-fold in PE and GMO membranes, respectively, for the same change in benzyl alcohol concentration.

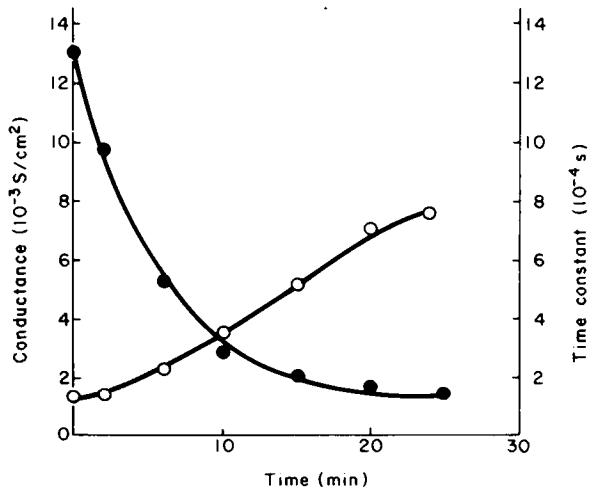


FIGURE 6 Reversibility of the effect of choloroform on the $TPhB^-$-induced conductance (●) and on the time constant of the $TPhB^-$-induced current relaxation (○) in PE membranes. At $t = 0$ the membrane was in the presence of 10 mM choloroform. The values of conductance and time constant in the absence of chloroform were $1.2 \times 10^{-3}$ S × cm$^{-2}$, and 0.80 ms, respectively. Membranes formed in 1 M NaCl, 10 mM phosphate pH 6. $TPhB^-$ was added to the aqueous phases to a final concentration of $1 \times 10^{-8}$M.

*Reversibility of the Effect of Chloroform on Ion Transport*

Fig. 6 shows that when the lid covering the membrane chamber is removed and the electrolyte solutions containing chloroform are stirred, the $TPhB^-$-induced conductance decreases and the time constant of the $TPhB^-$-induced current relaxation increases with time. Under conditions of continuous stirring, both parameters reached a value near that obtained in the absence of chloroform 25 min after the removal of the lid.

*Benzyl Alcohol Decreases the Surface Potential of PE Monolayers*

The surface potentials of saturated PE and GMO monolayers made from a solution of lipid in pentane were found to be 460 ± 20 and 320 ± 10 mV, respectively (air phase positive). Benzyl alcohol decreases the surface potential of PE monolayers. Thus, with 19, 36, and 51 mM benzyl alcohol in the subphase, the change in surface potential was 20 ± 10, 40 ± 13, and 60 ± 10 mV, respectively.

*Effect of Benzyl Alcohol on Nonelectrolyte Permeability*

The effect of benzyl alcohol on acetamide permeability was tested in PE membranes. The acetamide permeability of PE membranes is $(2.6 \pm 0.1) \times 10^{-5}$ cm/s. Benzyl alcohol induces a slight increase in the acetamide permeability. This increase was found to be 23% at 19 mM benzyl alcohol and 38% at 36 mM benzyl alcohol.

The value for the acetamide permeability coefficient obtained by us is 6.5-fold smaller than the value reported by Finkelstein (1976) in lecithin-decane membranes. However, the acetamide permeability obtained in PE membranes is approximately the same as that obtained in lecithin-cholesterol-decane membranes ($2 \times 10^{-5}$ cm/s; Finkelstein, 1976). Although this data suggests that PE membranes are more "tight" than lecithin-decane membranes, it is not possible at present to decide whether this difference arises from the difference in lipids or the presence of solvent in the latter membranes. Thus, Haydon et al. (1977*b*) have shown that cholesterol decreases the amount of solvent present in solvent-containing bilayers.

## DISCUSSION

*Estimation of the Dipole Potential Magnitude of PE and GMO Membranes*

The electrostatic potential difference between the membrane interior and the bulk aqueous phases is an important factor in determining membrane ion conductance (Liberman and Topaly, 1969; LeBlanc, 1970; Hladky and Haydon, 1973; Szabo, 1974). Moreover, because this potential seems to be affected by the anesthetics used in the present work (see below), it seems of interest to characterize it in both PE and GMO membranes.

The intrinsic membrane dipole potential is, in principle, not measurable; however, it can be estimated by comparing the permeabilities for positive and negative ions with similar chemical structure, such as tetraphenylborate and tetraphenylarsonium (e.g., Andersen and Fuchs, 1975). In PE membranes, the $TPhB^-$-induced conductance at a concentration of $3.3 \times 10^{-8}$ M is $3.3 \times 10^{-3}$ S/cm$^2$, and the $TPhAs^+$-induced conductance at a concentration of $10^{-5}$ M is $10^{-8}$ S/cm$^2$. Thus, the conductance ratio $G_{TPhB^-}:G_{TPhAs^+}$ is $10^8$ when equal

concentrations of $TPhB^-$ and $TPhAs^+$ are considered[4] (cf., LeBlanc, 1969; Andersen and Fuchs, 1975). This relative conductance found for PE membrane gives an electrostatic potential difference of 230 mV with the membrane interior positive. This value of the dipole potential was obtained assuming that the solvation energies of $TPhB^-$ and $TPhAs^+$ in the aqueous phase are the same. However, this assumption appears to be incorrect (for an opposite view, see Parker, 1969). Thus, Coetzee and Sharpe (1971) have estimated that the enthalpy of solvation of $TPhB^-$ is ~5 kcal/mol more negative than that of $TPhAs^+$. One would expect, then, that $TPhAs^+$ would be $\sim 10^4$-fold more permeable than $TPhB^-$ in the absence of any potential, if the solvation energies of both ions are taken into account (see, for example, Andersen and Fuchs, 1975). Considering this factor, the estimated difference in electrostatic potential is 345 mV.

The conductance ratio, $G_{TPhB^-}:G_{TPhAs^+}$, is $10^4$ in GMO membranes (see Results). Making the same considerations as for the case of PE membranes, the estimated dipole potential is 230 mV. Therefore, the interior of GMO membranes is less positive than the interior of PE membranes by ~120 mV.

The surface potential of PE and GMO monolayers found by us, 460 and 320 mV, respectively, are in good agreement with the values of 500 and 320 mV reported by Hladky (1974). Although the dipole potential calculated from measurements of lipophilic ion-induced conductance are substantially lower than those obtained in monolayers formed at the air/water interface, both results indicated that the dipole potential of PE and GMO membranes is large and positive when the aqueous phase is taken as reference.

### *Possible Membrane Structural Parameters Modified by Benzyl Alcohol and Chloroform*

The data presented in the Results section can be analyzed quantitatively if we consider that the conductance, $G$, of a given ion, in the limit of zero applied potential, is given by (Szabo et al., 1972)

$$G = \frac{F^2}{d} uC_a \exp\left[-(\mu_m^0 - \mu_a^0)/RT\right] \exp\left(-z_i F\psi/RT\right), \qquad (2)$$

where $d$ is the membrane thickness, $u$ is the mobility of the permeant species within the hydrocarbon region of the membrane, $C_a$ is the concentration of the permeant species in the aqueous phases, $\mu_m^0$ and $\mu_a^0$ are the standard chemical potentials of the permeant species in the center of the membrane and in the aqueous phases, respectively, $\psi$ is the electrostatic potential in the membrane relative to that of the aqueous phases, $z_i$ is the valence of the ion, and $R$, and $T$, and $F$ have their usual meanings.

In the absence of any electrostatic potential ($\psi = 0$) we define $G$ as the intrinsic conductance of the membrane.

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[4]In our calculation of the internal dipole potential, we have implicitly assumed that the conductances induced by $TPhB^-$ and $TPhAs^+$ are linear functions of the lipophilic ion concentration. In PE membranes made by apposition of two monolayers, the $TPhB^-$ conductance increases linearly with the $TPhB^-$ concentration up to $3 \times 10^{-7}$ M (Melnik et al., 1977). The $TPhAs^+$-induced conductance in PE membranes increases linearly with the $TPhAs^+$ concentration in the range $10^{-5} - 5 \times 10^{-4}$. This is the reason why we have used the $TPhAs^+$ concentration of $10^{-5}$ M in our calculation of the dipole potential instead of 1 mM used throughout the Results section. The latter figure would have given us a slight overestimation of the dipole potential.

It follows from Eq. 2 that the relative conductance, $\bar{G}$, defined as the ratio between the conductance for an ionic probe of the modified membrane ($G_{mod}$) (i.e., after the anesthetic is added to the aqueous phases) and the conductance for the same probe of an unmodified membrane ($G_{ref}$) is given by

$$\bar{G} = \frac{G_{mod}}{G_{ref}} = \frac{\bar{u}}{\bar{d}} \exp(-\Delta\Delta\mu^0/RT) \exp(-z_i F\Delta\psi/RT), \quad (3)$$

where $\bar{u}$ is the relative mobility of the probe in the membrane, $\bar{d}$ is the relative membrane thickness, $\Delta\Delta\mu^0 = (\Delta\mu^0{}_{mod} - \Delta\mu^0{}_{ref})$ is the change in the chemical free energy of partition ($\Delta\mu_m$), and $\Delta\psi = (\psi_{mod} - \psi_{ref})$ is the change in electrostatic potential of the membrane. The changes promoted by benzyl alcohol on the conductance induced by nonactin-$K^+$ and $TPhAs^+$ (PE membranes) follow a different pattern than those changes promoted by benzyl alcohol on the conductance induced by $CCCP^-$ and $TPhB^-$. Since the chemical structures of the two cationic and that of the two anionic probes are widely different, we will assume that specific interactions are negligible, and that the conductance changes are due to changes in nonspecific interactions (i.e., changes in mobility, thickness, dielectric constant, and interfacial potentials). If we assume identical changes in mobility, in the term $\exp(-\Delta\Delta\mu^0/RT)$ and $\bar{d}$ for both anionic and cationic probes promoted by the addition of the anesthetic, from Eq. 3, we have (Szabo, 1974):

$$\left(\frac{\bar{G}_+}{\bar{G}_-}\right)^{-1/2} = \exp(F\Delta\psi/RT) \quad (4)$$

$$(\bar{G}_+ \times \bar{G}_-)^{1/2} = \frac{\bar{u}}{\bar{d}} \exp(-\Delta\Delta\mu^0/RT). \quad (5)$$

Eq. 4 allows us to obtain the contribution to the overall conductance of the electrostatic potential term that affects in the same magnitude but in the opposite directions the membrane conductance for negative and positive probes. Eq. 5, on the other hand, allows us to obtain the contribution of those membrane structural parameters that affect the membrane conductance for positive and negative probes in the same magnitude and direction.

Fig. 7 *a* shows the plot of relative conductance for $TPhAs^+$, nonactin-$K^+$, $CCCP^-$, and $TPhB^-$ of PE membranes as a function of the benzyl alcohol concentration in the bathing solution. The calculated changes in intrinsic conductance (Eq. 5) and electrostatic potential (Eq. 4), using the data for $TPhB^-$ and $TPhAs^+$, are plotted in Fig. 7 *b*. Fig. 7 *b* shows that benzyl alcohol promotes a concentration-dependent change in both the intrinsic conductance and the electrostatic potential of the membrane. At 20 mM — the nerve blocking concentration — benzyl alcohol induces a 2.2-fold increase in the intrinsic conductance and a surface potential drop of ~15 mV. Because the experiments were done at high ionic strength and because both PE and GMO membranes are neutral at pH 6 (McLaughlin et al., 1970; McLaughlin et al., 1971), changes in double layer potential are improbable, and the calculated change in membrane surface potential must be ascribed to changes in the dipole potential of PE membranes.

Table I shows that the $TPhB^-$ adsorption coefficient changes 4.2-fold when the concentration of benzyl alcohol is changed from 0 to 51 mM. If $TPhB^-$ in PE membranes "sees" all the

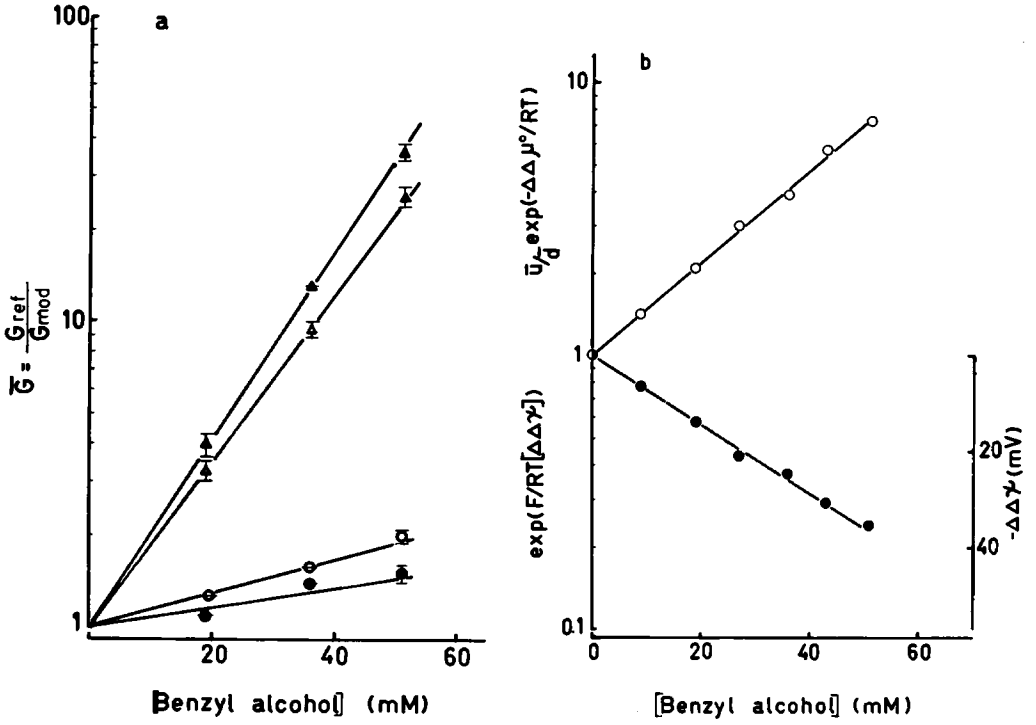


FIGURE 7 (*a*) Calculated values of relative conductance, $\bar{G}$, for $TPhB^-$ (●), $CCCP^-$ (○), non-$K^+$ (△), and $TPhAs^+$ (▲) as a function of the benzyl alcohol concentration in the aqueous phase. Each point is the average of at least five membranes. Bars represent the standard deviation. (*b*) Calculated values of intrinsic conductance (○) and dipole potential changes (●) as a function of the benzyl alcohol concentration in the aqueous phases. See text for details.

changes in dipole potential induced by benzyl alcohol, the change in the adsorption coefficient calculated from the dipole potential data shown in Fig. 7 *b* is 4.2-fold, in good agreement with the data shown in Table I. It can be argued that the decrease in the $TPhB^-$ adsorption coefficient is due to a displacement of this ion from the membrane produced by the adsorption of benzyl alcohol. There are at least two independent evidences that make such displacement unlikely. First, the good agreement between the dipole potential changes measured with ion probes and the changes in $TPhB^-$ adsorption coefficient. Second, benzyl alcohol produces a decrease in the dipole potential of PE monolayers that agrees reasonably well with the values obtained using ion probes. Benzyl alcohol also induces a decrease in the dipole potential of phosphatidylcholine monolayers.[5]

We can further analyze the changes in intrinsic conductance of the membrane induced by a given anesthetic by taking into account the different factors that contribute to it: the mobility of the ionic probe in the membrane, the thickness of the membrane, and the chemical-free energy of partition of the ionic probe.

Since $TPhAs^+$ and $TPhB^-$ are ions with a hydrophobic surface, the chemical-free energy

[5]Simon, S. Personal communication.

of partition for these ions depends on the magnitude of the hydrophobic energy barrier and the image force energy barrier in the membrane. The image force energy depends on the dielectric constant and the thickness of the membrane (Parsegian, 1969; Neumcke and Läuger, 1969).

*Mobility*

The intrinsic conductance (Eq. 5) depends, among other factors, on the mobility of the ion probe in the membrane. It is possible, therefore, that some or all of the observed increase in intrinsic conductance can be due to a decrease in the membrane viscosity promoted by benzyl alcohol. We think that this possibility seems unlikely for at least three reasons. First, in PE membranes and at 19 mM benzyl alcohol the acetamide permeability increases only by 23%, whereas at the same concentration, benzyl alcohol promotes 220% increase in the intrinsic conductance. Second, changes in microviscosity of cell membranes and model systems due to the presence of anesthetics have also been estimated from changes in rotational rates measured with fluorescent probes and/or changes in the magnetic resonance spectrum of some molecules that are able to partition in the membrane. Metcalfe (1970) measured the relaxation time of the aromatic proton of benzyl alcohol in erythrocyte membranes. He found an increase in probe mobility of 29% at the benzyl alcohol nerve-blocking concentration. Moreover, the same author reported no change in aromatic proton relaxation in erythrocyte lipid vesicles when the concentration of this anesthetic was increased between 15 and 150 mM. We think that this last result is more relevant for our results, since our membranes are made of pure lipids. Finally, it can be argued that the ion probes used by us are larger than benzyl alcohol or acetamide, and therefore, these ions may experience larger mobility changes. The fact that benzyl alcohol promotes a smaller change in $\overline{G}$ for the nonactin-$K^+$ complex than in $\overline{G}$ for $TPhAs^+$, and that benzyl alcohol promotes a larger change in $\overline{G}$ for $CCCP^-$ than in $\overline{G}$ for $TPhB^-$ speaks against this possibility. Thus, the nonactin-$K^+$ complex is larger than $TPhAs^+$, and $CCCP^-$ (of dimensions comparable to those of benzyl alcohol) is smaller than $TPhB^-$ (see below).

*Area, Thickness, and Dielectric Constant of the Membrane*

At least two independent measurements can give information related to changes in area, thickness, and dielectric constant of the membrane produced by benzyl alcohol: first, measurements of the geometric capacitance of the membrane, $C_g$, *given by the equation*

$$C_g = \frac{\epsilon_0 \epsilon^m A}{d}, \tag{6}$$

where $\epsilon_0$ is the permittivity of the free space, $\epsilon^m$ is the dielectric constant of the membrane,[6] $A$ is the membrane area, and $d$ is the thickness of the membrane; and second, measurements of the conductance induced by positive and negative probes.

Our results in PE bilayers show that $C_g$ increases linearly with the benzyl alcohol concentration in the aqueous phases (see Fig. 1). Recently, Ebihara et al. (1979) have found

[6]Strictly speaking, for polar substances such as benzyl alcohol or water, the term permittivity is more correct than dielectric constant (Grant et al., 1978).

an effect of benzyl alcohol on the capacitance of "solventless" PE bilayers similar to the one reported here. These results differ from those reported by Ashcroft et al. (1977*a*, *b*). Ashcroft et al.'s results in lecithin-tetradecane membranes show a decrease in membrane capacitance as the concentration of benzyl alcohol is increased. They have interpreted this decrease in capacitance as an increase in membrane thickness. The difference between the results of Ashcroft et al. and our results can be explained by the large amount of solvent present in lecithin-tetradecane membranes (Haydon et al, 1977*b*) used by Ashcroft et al. that may modify the benzyl alcohol adsorption parameters. Therefore, extrapolations of the effect of anesthetics on solvent-containing membranes to anesthesia in biological systems seem questionable.

According to Eq. 6, the increase in membrane capacitance promoted by benzyl alcohol could be accounted for by a decrease in $d$, an increase in $\epsilon^m$ or the membrane area, or by changes in all of these parameters simultaneously.

General and local anesthetics decrease the surface tension of lipid monolayers at the air/water interface (Seeman, 1972; Ueda et al. 1975) and induce expansion in vesicles and erythrocyte membranes (Seeman, 1975). If anesthetics expand planar lipid bilayers made from monolayers, this expansion must be at the expense of a reduction of the total area occupied by the plateau border. Since the relation perimeter/area is inversely proportional to the membrane radius, we should expect that the effect of benzyl alcohol on membrane capacitance would decrease when the membrane radius is increased if this effect is due to an increase in membrane area. We have done experiments in which the membrane area was changed by a factor of ~50 (i.e., from $2.3 \times 10^{-4}$ to $1 \times 10^{-2}$ $cm^{-2}$). We found that the relative increase in membrane capacitance induced by benzyl alcohol is the same regardless of the membrane area (e.g., Fig. 1). Therefore, we conclude that there is not a significant increase in the area of bilayers made from monolayers due to the addition of benzyl alcohol to the aqueous phase.

Ebihara et al. (1979), using x-ray diffraction, found that the bilayer thickness of egg phosphatidylcholine and dipalmitoyllecithin multilamellar vesicles above the transition temperature does not change within experimental error in the presence of 75 mM benzyl alcohol. Moreover, Turner and Oldfield (1979), using high field deuterium nuclear magnetic resonance, have recently found that benzyl alcohol, at the nerve-blocking concentration, does not modify the thickness of dimiristoyl phosphatidylcholine vesicles above the transition temperature. At a much higher concentration of benzyl alcohol (~300 mM), they found a small (~1 Å) decrease in membrane thickness.

Thus, both the results obtained by Ebihara et al. and Turner and Oldfield agree that benzyl alcohol at local anesthetic concentrations does not modify the thickness of lipid bilayers above the transition temperature. This fact has also been found for other general anesthetics (Franks and Lieb, 1978). If the results obtained by Ebihara et al. (1979) and Turner and Oldfield (1979) are extrapolated to the results reported in this paper, we can conclude that changes in membrane thickness in the range 0-51 mM benzyl alcohol must be very small. Even in the worst case, a change in membrane thickness of 2 Å at 51 mM benzyl alcohol would explain the capacitance results, but would not explain the changes in intrinsic conductance as discussed below.

Since changes in the intrinsic conductance can be accounted for neither by changes in the

membrane viscosity nor by changes in the relative thickness, $\bar{d}$, it seems likely that benzyl alcohol modifies the image force barrier. Accordingly, if two membranes of thickness $d_1$ and $d_2$ and dielectric constant $\epsilon_1^m$ and $\epsilon_2^m$ are considered, the difference in peak potential energy $\Delta W(\epsilon^m, d)$ is given by (Parsegian, 1969):

$$\Delta W(\epsilon^m,d) = \frac{(z_i e_0)^2}{8\pi\epsilon_0 r}\left(\frac{1}{\epsilon_2^m} - \frac{1}{\epsilon_1^m}\right) - \frac{(z_i e_0)^2}{4\pi\epsilon_0}\left(\frac{1}{d_2\epsilon_2^m} - \frac{1}{d_1\epsilon_1^m}\right) \ell n 2, \tag{7}$$

where $e_0$ is the electronic charge and $r$ is the radius of the lipophilic ion. It is clear from Eq. 6 and 7 that $\Delta W(\epsilon^m,d)$ and $C_g$ both depend on the thickness and dielectric constant of the membrane, but the effect of variation of these parameters by the anesthetic agents will affect the membrane capacitance and the intrinsic conductance in a quantitatively different manner, since for a given ionic probe:

$$\frac{G_{\text{mod}}}{G_{\text{ref}}} \propto \exp - [W(\epsilon^m,d)/kT], \tag{8}$$

and therefore small changes in $d$ or $\epsilon^m$ will promote large changes in membrane intrinsic conductance. Benz and Läuger (1977) have found a reasonable agreement between the theoretical predictions of this model and the translocation of the lipophilic ion, dipicrylamine. Dilger and McLaughlin (1979) have also presented some evidence with the uncoupler 4, 5, 6, 7-tetrachloro-2-trifluoromethyl-benzimidazole (TTFB) that suggests that this model is applicable to lipid bilayers. The results seen in Figs. 1 and 7 show that whereas $C_g$ has changed only 2.3% at 20 mM benzyl alcohol, the intrinsic conductance has changed 220%, in reasonable agreement with the above analysis. Moreover, changes in the membrane thickness alone do not explain quantitatively the changes in intrinsic conductance. For example, assuming that all the change in $C_g$ promoted by benzyl alcohol is given by a change in $d$, at 20 mM benzyl alcohol, the change in the intrinsic conductance calculated through Eq. 7 is 1.2-fold. However, for the same benzyl alcohol concentration, we found experimentally a 2.2-fold change in intrinsic conductance (see Fig. 7). This discrepancy is more marked at higher concentrations of benzyl alcohol. On the other hand, if all the change in capacitance is attributed to a change in the dielectric constant of the membrane, the change in the intrinsic conductance calculated through Eq. 7 is 1.9-fold when the concentration of benzyl alcohol is 20 mM, in good agreement with the experimental results.[7] Eqs. 7 and 8 predict that a change in the dielectric constant of the membrane will promote a larger $\Delta W$ (and, therefore, a larger change in $\bar{G}$) as the radius of the ionic probe decreases. This is, in fact, observed in the case of $TPhAs^+$ and the nonactin-$K^+$ complex (Fig. 7 *a*). If the radii of both ions are taken into account ($r_{TPhAS^+}$ = 4.5 Å and $r_{non\text{-}K^+}$ = 6 Å; Millero, 1971; Simon and Morf, 1973), the difference in relative conductances for both probes are well explained.

At present, the same analysis cannot be done for $CCCP^-$ and $TPhB^-$ because, to our knowledge, the Born radius for $CCCP^-$ is not known. The differences are well explained, nevertheless, if radii of 3.5 and 4.5 Å are ascribed to $CCCP^-$ and $TPhB^-$, respectively.

[7]To account for the data of intrinsic conductance change, the dielectric constant of the membrane has to change from 2.13 (Ashcroft et al., 1977*b*) to 2.18 at the nerve-blocking concentration of benzyl alcohol.

Therefore, changes in the dielectric constant of the membrane alone can account for the changes in capacitance and intrinsic conductance simultaneously.

Although the data presented in Fig. 2 *a*, showing the effect of chloroform on the conductance induced by lipophilic ions, are not true equilibrium values (see Results), a semiquantitative analysis can be done using the same treatment as for the benzyl alcohol data (Fig. 7). The estimated decrease in the dipole potential at the nerve-blocking concentration of chloroform is ~16 mV, and the increase in the intrinsic conductance is ~4.4-fold. Although no values of the effect of chloroform on the thickness of bilayer membranes have been reported, the dependence of the order parameter of spin-labeled probes (Boggs et al., 1976*b*) with the chloroform concentration, together with the effect of chloroform on the membrane capacitance and on the conductance induced by $TPhB^-$ and $TPhAs^+$ found here strongly suggest that the effect of chloroform on the bilayer structural parameters may be similar to that of benzyl alcohol.

### *Possible Interpretations of the Changes Induced by Benzyl Alcohol on the Dipole Potential and Dielectric Constant*

The model used to analyze the results does not give any information about the changes at the molecular level. What follows gives some possible molecular interpretations that could account for our results.

DIPOLE POTENTIAL The data presented in Results showed that benzyl alcohol induces a concentration-dependent decrease in the dipole potential of PE membranes. This fact is also supported by the direct evidence of a decrease in dipole potential of PE monolayers promoted by benzyl alcohol. Colley and Metcalfe (1972) have given clear evidence that benzyl alcohol is mainly located at the aqueous/membrane interface, therefore, the decrease in dipole potential promoted by the anesthetic can be because: (*a*) the benzyl alcohol molecules adsorb at the interface with their dipole moment oppositely to that of the lipid (i.e., the negative pole towards the membrane interior); (*b*) benzyl alcohol molecules interact with lipid molecules; or (*c*) both effects act simultaneously. Further studies are needed to determine what is the actual cause.

DIELECTRIC CONSTANT The inclusion of polar molecules in the membrane interior will produce changes in the relative permittivity of the membrane.

It is known that the partition of benzyl alcohol between aqueous phase and lipid bilayers is favorable to the lipid bilayer and represented by a partition coefficient of ~14 (Colley and Metcalfe, 1972; Ebihara et al., 1979). From the data of partition of benzyl alcohol in the interfacial and hydrocarbon region of the membrane (Colley and Metcalfe, 1972) one can conclude that above the transition temperature of the lipids an important fraction of the benzyl alcohol molecules are located in the membrane interior. Assuming ideal mixtures of benzyl alcohol and the hydrocarbon chains of the phospholipids, at a molar ratio of hydrocarbon chains:benzyl alcohol of ~20, there are enough molecules of benzyl alcohol in the bilayer interior to account for the changes in capacitance and intrinsic conductance of PE bilayers.

The above discussion implies, as was already pointed out by Colley and Metcalfe, that benzyl alcohol appears to have two sites of adsorption: one at the interface, which might be responsible for the observed changes in dipole potential; the other in the bilayer interior, which

may be responsible for the observed changes in permittivity or dielectric constant of PE bilayers.

### *Some Pharmacological Implications*

Andersen et al. (1975, 1978) and Szabo (1977) have pointed out that the characteristics of the $TPhB^-$ transport across lipid bilayer are very similar to the gating currents present in nerve membranes (Armstrong and Bezanilla, 1973; Keynes and Rojas, 1973).

Our results have shown that the effect of chloroform and benzyl alcohol on the $TPhB^-$ transport through the membrane is to enhance the translocation rate of this lipophilic ion (see Fig. 5) in agreement with the changes in structural parameters of the bilayers described above. If these changes were promoted in an excitable membrane, they would be sensed by any membrane-bound charge or dipole (Lundström, 1977). The opening and closing of the sodium channel in nerve is controlled by charged or permanent dipole structures that move in the membrane electric field (Armstrong and Bezanilla, 1973). We expect, therefore, that anesthetics will increase the rate of transition between closed and open states of excitable channels with the subsequent alterations in membrane excitability.

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