J. Membrane Biol. 82, 15–23 (1984)

The Journal of
Membrane Biology

Effect of Gangliosides on Phospholipid Bilayers: A Study with the Lipophilic Ions Relaxation Method

Cesare Usai†, Mauro Robello‡, Franco Gambale†, and Carla Marchetti§

† Istituto di Cibernetica e Biofisica, Consiglio Nazionale delle Ricerche, 16032 Camogli, Genova, Italy, ‡ Istituto di Scienze Fisiche, Università di Genova, 16132 Genova, Italy, and § FIDIA Research Laboratories, 35031 Abano Terme, Padova, Italy

Summary. The presence of monosialoganglioside GM_1 in dioleoylphosphatidylcholine black lipid membranes modifies the transport properties of the hydrophobic ion tetraphenylborate and the kinetics of relaxation of this ion after the application of a voltage step. At zero applied voltage, the difference in the relaxation time constants between pure phospholipid and ganglioside-phospholipid mixed membranes is large. This difference may possibly rise from changes in the membranes fluidity since it has been found that the two types of membranes do not show appreciable difference in thickness. A uniform distribution of GM_1 in the membrane seems to be more probable than the presence of lateral phase separation phenomena. The partition coefficient of tetraphenylborate between the bathing NaCl solution and the membrane appears to depend on the ionic strength, which controls the screening effect of the Na^+ ions on the COO^- charged groups of the sialic acid of the ganglioside polar heads. Effects of dipolar potentials on the partition coefficient can be excluded, being the absorption plane of tetraphenylborate probably located outside the dipolar layer of the membrane.

Key Words gangliosides · lipid bilayer membranes · lipophilic ions · ion transport

Introduction

Gangliosides have been attracting great interest since the late thirties. In those years Klenk (1942) demonstrated the accumulation of glycosphingolipids containing sialic acid in the ganglion cells of the central nervous system in certain serious neurological disorders. Indeed they are present in most mammalian cells (generally located at the outer surface of the cytoplasmatic membrane), and particularly abundant in the nervous tissue.

The presence of the sialic acid in the oligosaccharide chain is the main distinctive feature of these membrane glycoconjugates.

The monosialoganglioside GM_1 contains one N-acetyl-neuraminic acid bound to the first galactosyl group (proximal galactose) of the oligosaccharide chain:

$$\text{Gal}\beta1\rightarrow3\text{GalNac}\beta1\rightarrow4\text{Gal}(3\leftarrow2\alpha\text{NeuAc})\beta1\rightarrow 4\text{Glc}\beta1\rightarrow1\text{Cer}.$$

The whole chemical structure of the GM_1 is shown in Fig. 1: the oligosaccharide moiety is linked to ceramide, as an N-acyl derivative of a sphingosine long-chain base.

In our case, 96.3% of the secondary hydrophobic chain consists of a saturated (18:0) fatty acid residue.

Much experimental evidence has been reported on the implication of gangliosides in biological processes involving recognition at the cell surface, synaptic function and neoplastic transformation (Hakomori & Jeanloz, 1970; Cuatrecasas, 1973*a*,*b*; Van Heyningen, 1974; Fishman & Brady, 1975, 1976; Wiegandt, 1975; Edelman, 1976; Porcellati, Ceccarelli & Tettamanti, 1976; Redwood & Polefka, 1976; Mullin et al., 1978).

In spite of the amount of data, a detailed knowledge of the role of gangliosides in the membrane organization has not yet been established. Model systems have proved to be a useful and popular tool to approach the problem (Maggio, Cumar & Caputto, 1978, 1980; Poss, Deleers & Ruysschaert, 1978; Tumanova, Badjinyan & Nalbandyan, 1978; Delmelle et al., 1980; Usai et al., 1983). In this paper we study the influence of the incorporation of GM_1 on the structure and properties of dioleoylphosphatidylcholine black lipid membranes. The hydrophobic ion tetraphenylborate has been used as a probe. In fact the study of the transport properties of hydrophobic ions across the membrane gives information about the structure of the bilayer (Benz &

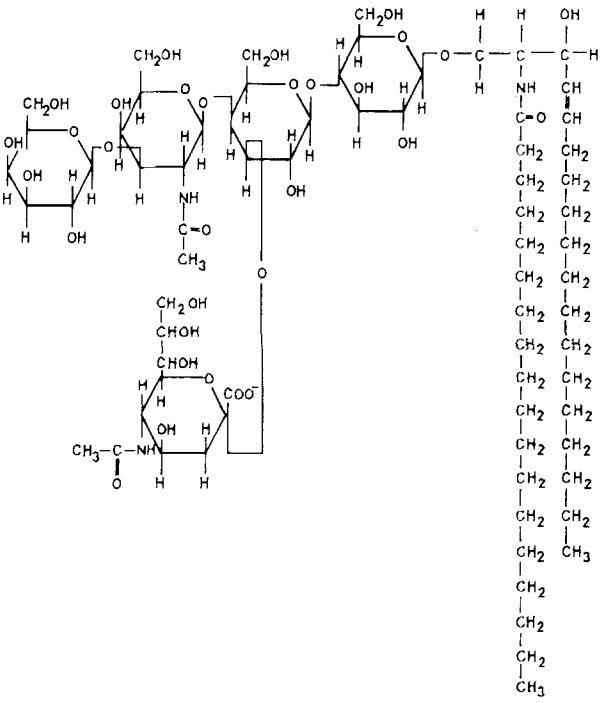

Fig. 1. Monosialoganglioside GM1 (N-Stearoyl). Chemical structure of GM_1

Läuger, 1977; Benz & Cross, 1978; Benz & Gisin, 1978; Pickar & Benz, 1978; Benz & Conti, 1981; Benz & Nonner, 1981).

Abbreviations

DOPC, dioleoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; NeuAc, N-Acetyl-Neuraminic Acid; TPhB, tetraphenylborate; GM_1, II^3NeuAc-GgOse$_4$Cer; DPH, 1,6-diphenyl-1,3,5-hexatriene.

Theory

Detailed models for the transport of hydrophobic ions through lipid bilayer membranes have been proposed (Liberman & Topaly, 1968; LeBlanc, 1969; Ketterer, Neumcke & Läuger, 1971; Haydon & Hladky, 1972; DeLevie, Seidah & Larkin, 1974; Bruner, 1975; Benz, Läuger & Janko, 1976; Szabo, 1976; Läuger et al., 1981); therefore, according to the theoretical approach of Andersen and Fuchs (1975), we describe only the main points of the subject.

The transport of hydrophobic ions through lipid bilayers occurs in distinct steps: absorption (and desorption) of the ion at the interfaces between the aqueous phase and the membrane, and translocation across the inner potential barrier of the membrane. The application of a voltage jump V redistributes the ions between the interfaces. This results in a transient current $I(V,t)$ which decays exponentially with time:

$$I(V,t) = I(V,0)e^{-t/\tau(V)} \tag{1}$$

where $I(V,0)$ is the initial current and $\tau(V)$ the relaxation time constant. This time constant is strongly dependent upon the applied voltage:

$$\tau(V) = \tau(0)\exp\{\omega_d(eV/kT)^2\}/\cosh(\alpha eV/2kT) \tag{2}$$

where e is the elementary charge, k the Boltzman constant, T the absolute temperature, $\tau(0)$ the time constant in the limit of zero applied voltage, ω_d a parameter dependent on the membrane thickness d, and α the fraction of the applied voltage influencing the movement of tetraphenylborate.

The same parameters characterize the initial conductance-voltage relationship:

$$g(V,0)/g(0,0) = (2kT/\alpha eV)\{\sinh(\alpha eV/2kT)/\exp[\omega_d(eV/kT)^2]\} \tag{3}$$

where $g(V,0)$ is the initial conductance, and $g(0,0)$ is the extrapolation of $g(V,0)$ for $V \to 0$.

The zero voltage values, $\tau(0)$ and $g(0,0)$, are related to the rate constant of translocation k_i across the inner potential barrier, and to the partition coefficient β of tetraphenylborate between solution and membrane:

$$\tau(0) = 1/2k_i \tag{4}$$

$$g(0,0) = z^2\beta C_{\mathrm{TPhB}}k_i\alpha^2e^2/kT \tag{5}$$

being C_{TPhB} the tetraphenylborate concentration in the aqueous solution.

The electrical charge of the hydrophobic ions displaced across the membrane during a single current transient approaches $I(V,0)\tau$. The equivalent number of ions $N(V)$ depends on the applied voltage according to:

$$N(V) = N_{\mathrm{abs}}\tanh(\alpha eV/2kT) \tag{6}$$

where $2N_{\mathrm{abs}}$ is the total number of absorbed charges at the membrane-solution interfaces. The value of N_{abs} allows an independent determination of β, being:

$$\beta = N_{\mathrm{abs}}/C_{\mathrm{TPhB}}. \tag{7}$$

Materials and Methods

Optically black lipid membranes were formed either from pure dioleoylphosphatidylcholine, or from a mixture of this phospho-

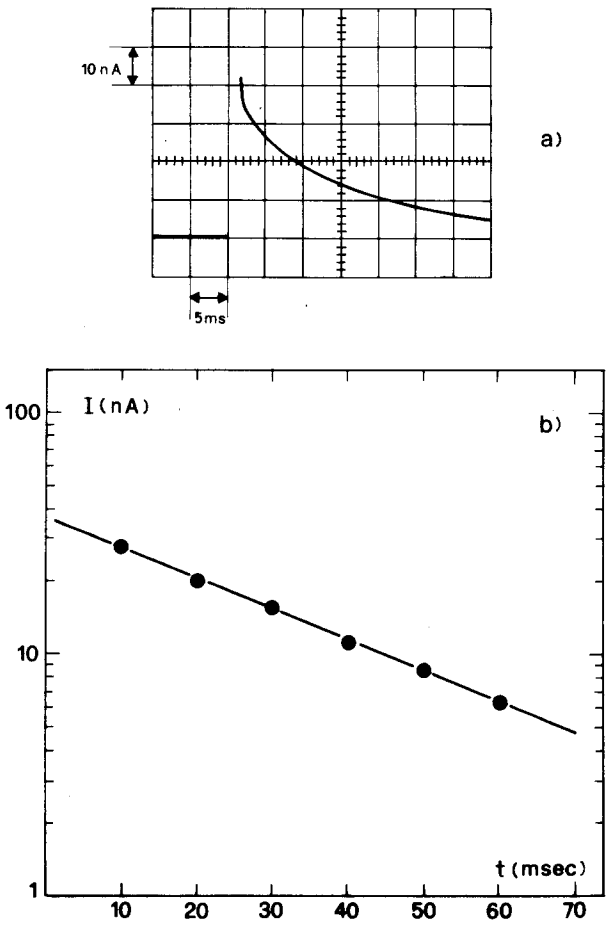

Fig. 2. Time course of the current flowing through the membrane after a 160-mV voltage step in linear (*a*), and semilogarithmic plot (*b*). Time constant of the curve $\tau = 17$ msec. $T = 25°C$. 2×10^{-8} M TPhB in 0.5 M NaCl solution. GM_1/DOPC (15.3% M/M) membranes, dissolved in *n*-decane (15 mg/ml)

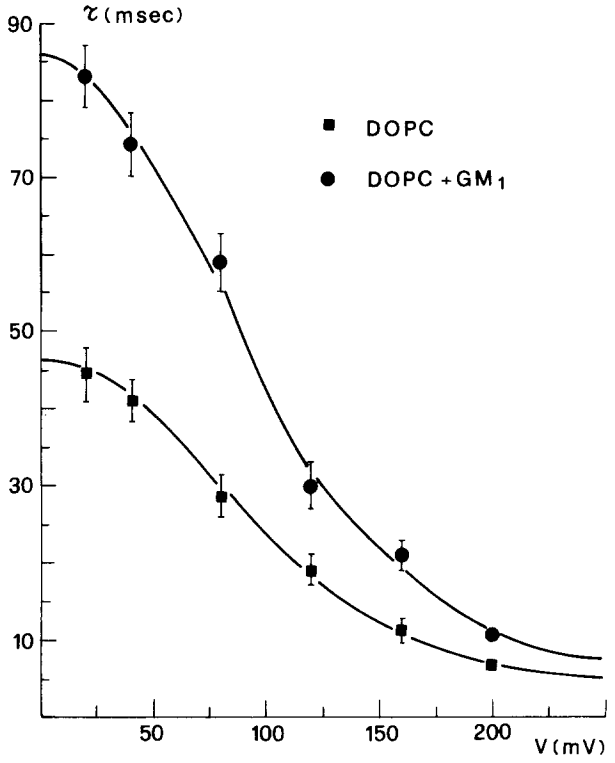

Fig. 3. Voltage dependence of the relaxation time constant for pure DOPC (■) and GM_1/DOPC (●) mixed membranes. NaCl 0.5 M plus 2×10^{-8} M TPhB. The experimental values of τ are fitted by Eq. (1)

lipid and ganglioside GM_1, as a 15% (wt/vol) lipid solution in *n*-decane (BDH Chemicals Ltd., Poole, England, analytical grade). The binary mixture was obtained by dissolving the components in chloroform-methanol (2 : 1, vol/vol) in 15.3% GM_1/DOPC molar ratio. Then, the mixture was dried in nitrogen atmosphere to remove traces of solvent, and finally dissolved in *n*-decane. Single spot by thin-layer chromatography, synthetic dioleoylphosphatidylcholine was purchased from P-L Biochemicals Inc., Milwaukee, Wisconsin, and used without further purification. Ganglioside GM_1 from beef brain, over 98% pure, was provided by FIDIA Research Laboratories, Abano Terme, Italy.

Membranes containing solvent were prepared at 25°C according to the brush technique of Mueller et al. (1963). They were formed through a circular hole, 1.5 mm^2 area, made in a thin Teflon septum separating two symmetrical Teflon compartments containing 30 ml of unbuffered NaCl solution. During the experiments the ionic strength of the bathing solution was changed from 5×10^{-3} M to 5×10^{-1} M. Tetraphenylborate was purchased from Sigma Chemical Co., St. Louis, Missouri. It was first dissolved in stock ethanolic solution, and then diluted in aqueous solution just before the experiments. After membrane formation, adequate aliquots of tetraphenylborate solution were symmetrically added, under continuous stirring, to the bathing NaCl solution to a final concentration value of $10^{-8} \div 10^{-7}$ M.

The electrical measurements were performed under voltage-clamp conditions. Voltage jumps applied across the membrane were supplied by a function generator (Wavetek, model 116), through two silver/silver chloride electrodes having a surface area of 12 cm^2. The time constant of the electric circuit was below 300 μsec for membrane capacitances of about 6 nF. Faster circuits were unnecessary, the relaxation time constant of tetraphenylborate in dioleoylphosphatidylcholine being much slower. The electrical measuring system was tested with membrane equivalent RC circuits. The current transient, measured as voltage transient across an external series resistance (10 or 48 kΩ), was recorded on a storage oscilloscope (Tektronix, model 5103 N), and photographed for later analysis.

Experimental points were best fitted with the aid of a Hewlett-Packard HP85 microcomputer, and experimental errors resulted in 10% uncertainty on the values of the parameters.

Results

Figure 2(*a*) shows the time course of current through a GM_1/DOPC planar bilayer after the application of a voltage step of 160 mV. The concentration of tetraphenylborate is 2×10^{-8} M. Plotted in semilogarithmic scale (Fig. 2(*b*)), the curve gives a straight line with a slope of 17 msec, the time constant of the exponential.

The dependence of the relaxation time constant on the applied voltage is shown in Fig. 3 for pure

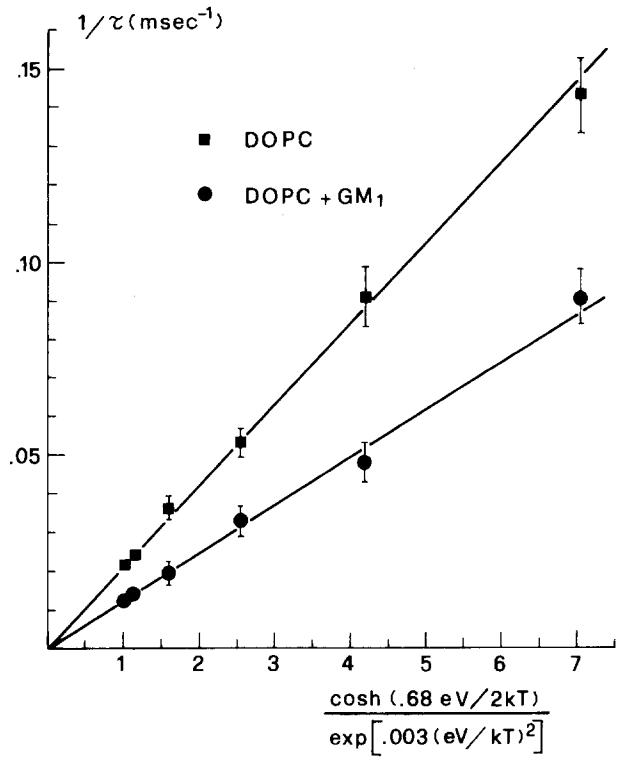

Fig. 4. Voltage dependence of the relaxation time constant. Same data of Fig. 3 are expressed as τ^{-1} versus $\cosh(0.68\ eV/2kT)/\exp\{0.003(eV/kT)^2\}$. Linear regression curves have correlation coefficients 0.996 (DOPC) and 0.999 (GM_1/DOPC)

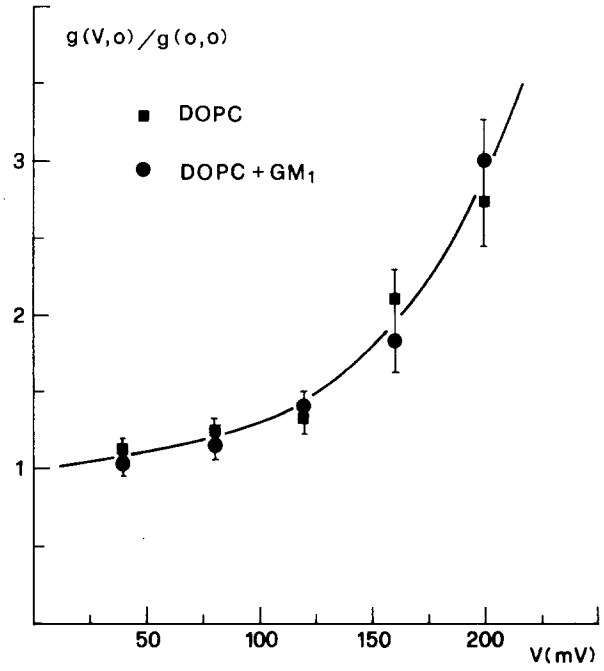

Fig. 5. Normalized initial conductance-voltage relationship for pure DOPC (■) and GM_1/DOPC (●) mixed membranes. Experimental conditions as in Figs. 3 and 4. The values of $g(V,0)/g(0,0)$ are fitted by Eq. (3)

DOPC and for GM_1/DOPC mixed membranes. Both sets of experimental data are best fitted by Eq. (2) with $\omega_d = 0.003$ and $\alpha = 0.68$. The values of $\tau(0)$ are 46 msec for DOPC and 86 msec for GM_1/DOPC membranes. It is possible to test these values by inserting the experimental data of Fig. 3 in a plot of τ^{-1} versus:

$$\cosh(0.68\ eV/2kT)/\exp\{0.003(eV/kT)^2\}.$$

The data and the related linear regression are shown in Fig. 4. The correlation coefficients are 0.996 for pure DOPC and 0.999 for GM_1/DOPC mixed membranes. For zero applied potential, the values of τ are again 46 and 86 msec, respectively.

An independent determination of ω_d and α can be obtained from the initial conductance-voltage relationship. Figure 5 shows the measured values of the normalized initial conductance for a DOPC and GM_1/DOPC membrane in the same experimental conditions as Figs. 3 and 4. Both series of data are best fitted by Eq. (3), within the experimental errors, for the values of ω_d and α previously found.

The values of ω_d as a function of membrane thickness d, have been tabulated by Andersen and Fuchs (1975) according to a model for ion transport within lipid bilayers where the potential energy barrier is assumed to be the image-force barrier. From electrical capacitance measurements, we calculated a thickness of about 40 Å for pure DOPC and GM_1/DOPC bilayers, regarding the membrane as a parallel plate capacitor. The values found for membrane thickness and for ω_d are consistent with those calculated by Andersen and Fuchs (1975) using the approximation of Haydon and Hladky (1972):

$$\omega_d \cong 0.003 \div 0.004 \text{ for } d = 30 \div 40 \text{ Å}.$$

Figure 6 shows the dependence of the number of tetraphenylborate ions moving through the GM_1/DOPC membrane on the applied potential. The aqueous phase contained 0.1 M NaCl plus 5×10^{-8} M TPhB. Experimental data are best fitted by Eq. (6) if $\alpha = 0.68$ and $N_{abs} = 180$ nC/cm^2. N_{abs} can be related to the partition coefficient of TPhB between water and membrane by Eq. (7). In the condition of Fig. 6 we have $\beta = 36 \times 10^{-3}$ cm.

The presence of a surface potential due to the COO^- charged group of the NeuAc in the GM_1 oligosaccharide chain affects the absorption of TPhB in the membrane. This makes N_{abs} depend upon the

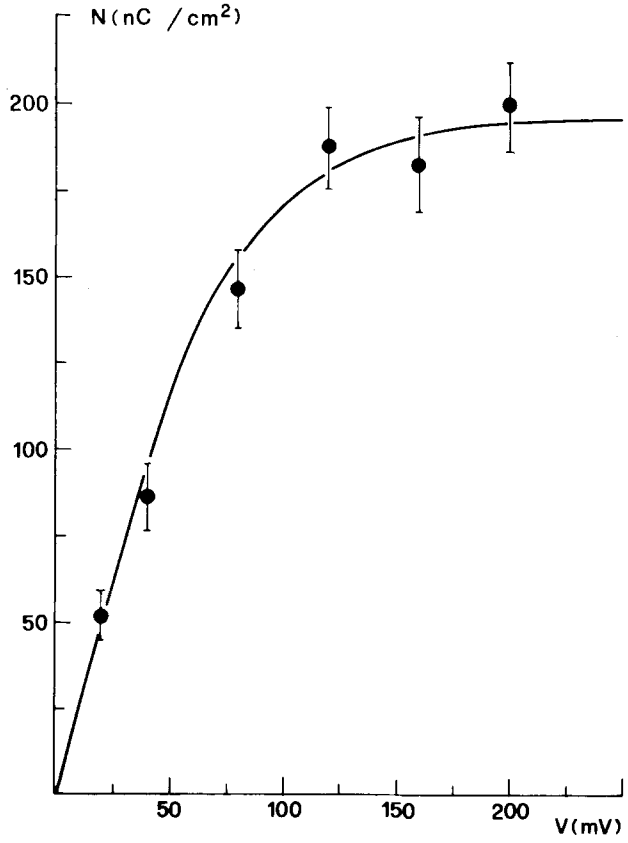

Fig. 6. Charge $N(V)$ moved across the membrane versus the applied potential in GM_1/DOPC Mixed membranes. The aqueous phase contained 0.1 M NaCl plus 5×10^{-8} M TPhB. Theoretical curve according to Eq. (6)

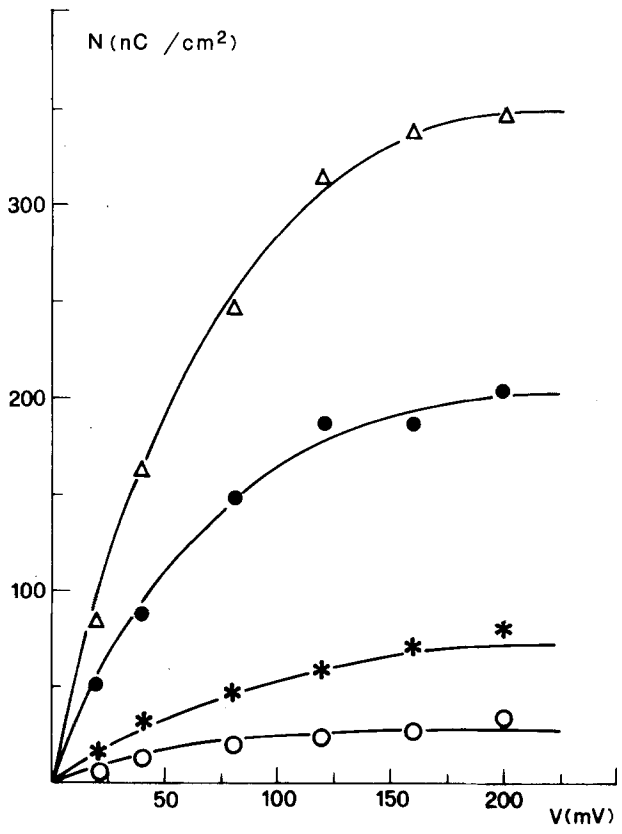

Fig. 7. Effect of different ionic strengths on the charge moved across the membrane. NaCl concentrations are: 5×10^{-3} M (○); 2×10^{-2} M (*); 10^{-1} M (●); 5×10^{-1} M (△). TPhB concentration = 5×10^{-8} M. Other experimental conditions as in Fig. 6. Error bars are omitted for sake of clarity

ionic strength of the bathing solution because of the screening effect of the Na^+ ions on the negative surface charge. In Fig. 7 we show such an effect of NaCl solutions at different ionic strength but equal TPhB concentration (5×10^{-8} M) on $N(V)$. The action of the ionic strength of the NaCl solution on N_{abs} may be predicted by the Gouy-Chapman theory (Läuger & Neumcke, 1973; McLaughlin, 1977) if the partition coefficient of TPhB is affected only via changes in surface potential. Therefore, assuming that the activity coefficient of the lipophilic ion in aqueous solution is independent of the ionic strength (Benz et al., 1976), we have:

$$N_{abs}(C_{NaCl}) = N_{abs}(\infty)\{\alpha/(8\varepsilon_o\varepsilon RTC_{NaCl})^{1/2} + [(\sigma^2/8\varepsilon_o\varepsilon RTC_{NaCl}) + 1]^{1/2}\}^{-2z} \quad (8)$$

where σ is the number of surface elementary charges per unit area, $N_{abs}(\infty)$ the limiting value of N_{abs} at infinite ionic strength, C_{NaCl} and ε, respectively, the concentration and the dielectric constant of the NaCl bathing solution, z the valency of the hydrophobic ion, ε_o the permittivity of free space, and R the gas constant. Figure 8 shows the dependence of N_{abs} on the ionic strength of the solution at a TPhB concentration of 5×10^{-8} M. The experimental data are best fitted by Eq. (8) if $N_{abs}(\infty) = 780$ nC/cm², and $\sigma = 2 \times 10^{-3}$ elementary charges/Å².

Diffusion polarization in the aqueous phase and ion-ion interactions in the bilayer become very significant at high TPhB concentrations. In these conditions, N_{abs} is no longer a linear function of C_{TPhB}, and the transient current shows a nonexponential decay (Anderson & Fuchs, 1975; Bruner, 1975; Andersen et al., 1978). Equation (6), therefore, cannot be used to calculate N_{abs}. That is not the case for the value of TPhB concentration (5×10^{-8} M) chosen for our experiments. In Fig. 9 N_{abs} and τ are plotted versus TPhB concentration for GM_1/DOPC membranes: τ does not depend on C_{TPhB} for values below 10^{-7} M, and N_{abs} shows linear dependence in the same range.

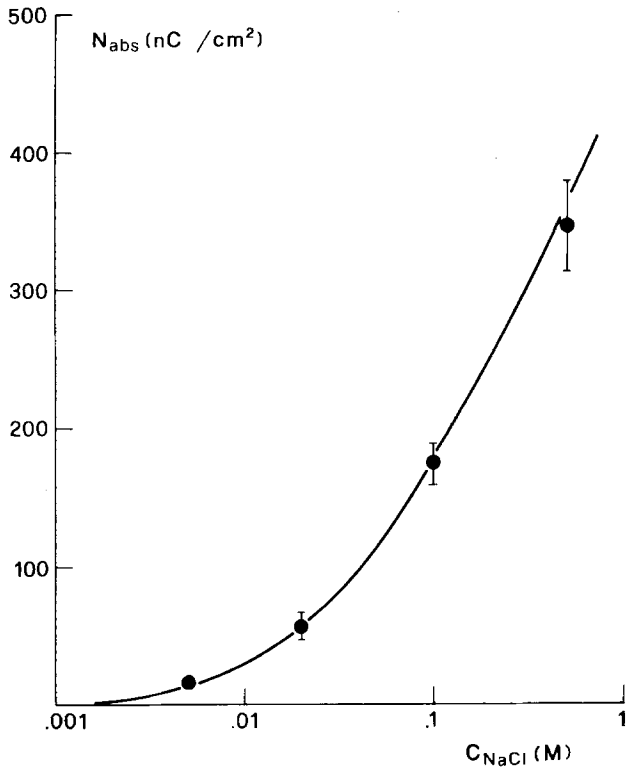

Fig. 8. Number of TPhB ions absorbed into the GM_1/DOPC mixed membranes versus NaCl concentration. TPhB concentration = 5×10^{-8} M. Experimental data are fitted by Eq. (8)

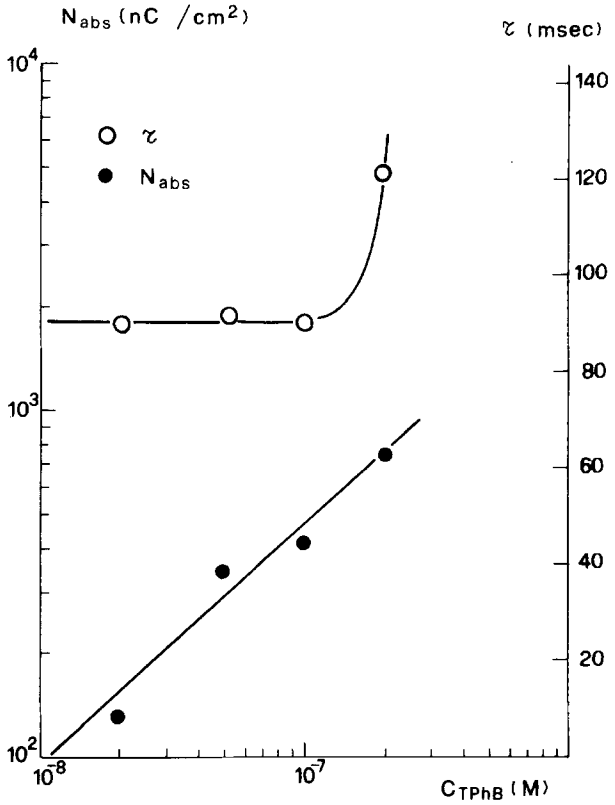

Fig. 9. Number of TPhB ions absorbed into the membrane (●) and relaxation time constant (○) versus TPhB concentration for GM_1/DOPC bilayers in the same experimental conditions as the previous Figures. The experimental points are fitted by eye in the full lines

Discussion

The experimental results show that the relaxation of tetraphenylborate in GM_1/DOPC differs from the relaxation in pure DOPC membranes.

The difference in $\tau(0)$ may be explained by modifications of either the bilayer thickness, or fluidity. We shall examine the two possibilities.

As far as the former is concerned, the occurrence of the same value for α and ω_d in both types of bilayers is indicative of no variations in membrane thickness. It might be argued that the influence of ω_d on Eqs. (2) and (3) is not too strong, so that fairly different values of ω_d do not affect significantly the fitting of experimental data. Let us assume, however, that the difference in $\tau(0)$ between pure DOPC and GM_1/DOPC mixed membranes is the effect of a variation in bilayer thickness. Then, we may calculate the thickness of a GM_1/DOPC membrane from one of a pure DOPC (Parsegian, 1969; Benz & Läuger, 1977; Benz & Gisin, 1978) using the following equation:

$$\tau\dagger(0)/\tau(0) = k_i/k_i\dagger \simeq \exp\{-[(e^2/4\pi\varepsilon_o\varepsilon_m kT) \ln(2\varepsilon_w/(\varepsilon_w + \varepsilon_m))](1/d\dagger - 1/d)\}$$

where $\tau\dagger(0)$ and $\tau(0)$ are the time constants in membranes of thickness $d\dagger$ and d, $k_i\dagger$ and k_i the respective translocation rate constants, ε_w and ε_m the dielectric constants of water and membrane. Assuming $d \simeq 40$ Å for the thickness of pure DOPC bilayers, we obtain $d\dagger \simeq 50$ Å for GM_1/DOPC mixed membranes. The corresponding value of ω_d is 0.0054 (Andersen & Fuchs, 1975). This value is too large to fit our experimental points with Eqs. (2) and (3), in spite of their weak dependence on ω_d. Furthermore, in a previous paper (Usai et al., 1983) we showed that the capacitance of a DOPC bilayer is not modified by the incorporation of the monosialoganglioside GM_1, disialoganglioside GD_{1a}, and trisialoganglioside GT. Possibly, the small differences in the composition of hydrophobic moieties of DOPC and gangliosides do not modify the membrane thickness, also owing to the presence of a certain percentage of *n*-decane. Finally, Cornell and Separovic (1983) showed that in phospholipid membranes the major change in dimension which occurs with variation in acyl chain length is in the area occupied per molecule, rather than in the bilayer

thickness. Therefore, we conclude that no modification of DOPC bilayer thickness occurs with the incorporation of GM_1.

Several elements may change the fluidity of DOPC membranes: larger size of the GM_1 polar head, electrostatic interactions among the COO^- charged groups of the sialic acids, or introduction of saturated fatty acid chains (18 : 0) in the unsaturated (18 : 1) hydrophobic tails of DOPC.

Uchida et al. (1981) studied DPPC/ganglioside mixed dispersion by time-resolved fluorescence measurements of DPH. They found that the molecular arrangement or microheterogeneity of the hydrocarbon region surrounding DPH changes depending on DPPC/ganglioside ratio and on temperature, and suggested that, above the phase transition temperature of DPPC, the hydrocarbon region in DPPC/ganglioside mixed dispersions is more ordered than in pure DPPC, presumably due to a close molecular packing of the two different types of molecule. Furthermore, the increase in $\tau(0)$ observed when unsaturation of the fatty acid chain decreases, has been reported for the hydrophobic ion dipicrylamine in phosphatidylcholine membranes with C_{18} fatty acid residues differently unsaturated, and for the valinomycin-Rb^+ complex in a series of monoglyceride membranes (Benz, Fröhlich & Läuger, 1976; Benz & Läuger, 1977; Benz & Gisin, 1978). This effect on the fluidity probably implies that the GM_1 is uniformly distributed in the membrane even though, in principle, formation of ganglioside clusters cannot be excluded. It is well known (Sharom & Grant, 1978; Delmelle et al., 1980) that gangliosides incorporated in phospholipid bilayers tend to interact and organize themselves in clusters depending on membrane fluidity, concentration and transition temperature of gangliosides (25°C for GM_1). Sinha and Smejtek (1983), studying the effect of 3-phenylindole on lipophilic ion transport, demonstrated that the presence of clusters is not detectable by relaxation measurements, the deviation from exponential decay being very slight. Nevertheless, the formation of GM_1 aggregates seems quite improbable for two reasons. Delmelle et al. (1980) using electron spin resonance techniques on mixed liposomes of gangliosides and dipalmitoylphosphatidylcholine have in fact shown that, above the temperature of phase transition for the phospholipid, the gangliosides are not clustered but randomly distributed in the membrane. In our case, the temperature we used for the experiments was much higher than the transition temperature of the DOPC (−22°C, Bach & Chapman, 1980). Moreover Gambale et al. (1982) have found that some glycolipid/DOPC mixed bilayers show a marked increase in membrane ionic conductance by increasing the glycolipid/phospholipid molar ratio, therefore suggesting that the effect may be due to the presence of lateral phase separation of the components. In the present case, measurements at different concentrations of GM_1 in DOPC membranes did not show any appreciable variations in ionic conductance.

The effects of surface potential have been described by Eq. (8).

The value of the surface charge density found experimentally, $\sigma = 2 \times 10^{-8}$ elementary charge/Å^2, is in very good agreement with the expected value of $\sigma = 2.2 \times 10^{-3}\ e^-/$Å^2 for a mixed membrane of 15.3% GM_1/DOPC molar ratio (Usai et al., 1983).

The agreement is not as good for the number of absorbed charges at infinite ionic strength: the value of the partition coefficient corresponding to the fitted value $N_{abs}(\infty) = 780$ nC/cm^2 is $\beta(\infty) = 0.16$ cm, about twice as much as the value, $\beta = 0.09$ cm, observed with the DOPC uncharged membranes. This discrepancy might be ascribed to dipolar effects not considered in Eq. (8). The surface potential of a monolayer of charged lipid can be expressed (Gaines, 1966; Maggio et al., 1978) as:

$$V = V_{dip} + \psi_o = (0.12\pi/A)\mu_\perp + \psi_o \qquad (9)$$

where A is the molecular area in nm^2, ψ_o is the contribution to surface potential of the surface charge distribution ($\psi_o = 0$ for uncharged membranes) and V_{dip} the contribution of the overall dipole moment $\mu_\perp$ in the direction perpendicular to the membrane interface. The incorporation in a lipid bilayer of another lipid with different dipole moment modifies the surface potential via a change in dipolar potential ΔV_{dip}. Szabo (1976) showed that the change of the dipole potential of a bilayer may affect the partition coefficient of the hydrophobic ion if the absorption plane is located in the dipolar layer of the membrane. The dependence of β on ΔV_{dip} (Benz & Cross, 1978; Benz & Gisin, 1978) can be given by:

$$\beta = \beta_o \exp(-F\delta\Delta V_{dip}/RT) \qquad (10)$$

where ΔV_{dip} is the difference in dipolar potential, δ is the molar ratio of the two lipids, β_o the partition coefficient for $\delta = 0$ and F the Faraday constant. Using the value $\mu_\perp = 50$ mD for GM_1 (Maggio et al., 1978) and $V_{dip} = 440$ mV for lecithin (Haydon, 1975), from Eqs. (9) and (10) we obtain $\beta = 7 \times 10^{-3}$ cm, a value very different from that found experimentally, $\beta(\infty) = 0.16$ cm. Therefore the change in dipolar potential of a DOPC bilayer induced by the incorporation of GM_1 does not affect the absorption

of tetraphenylborate, because the absorption plane of the lipophilic ion is probably located outside the dipolar layer of the mixed membrane.

The difference between the value of $\beta(\infty)$ and β for uncharged DOPC membranes is therefore due to other reasons. Size and packing of polar heads could be one of them. Benz and Läuger (1977), Benz and Gisin (1978) and Pickar and Benz (1978) showed the influence of the unsaturation degree of the fatty-acid chain on the partition coefficients of various lipophilic ions and ion carriers. In their experiments these authors found a decrease of β by increasing the number of double bonds of the hydrophobic tail, without changing the nature of the polar head. Since β should not be affected by membrane fluidity, its alteration may possibly be due to differences in packing of the polar heads. Another reason could be that the assumption made to fit Eq. (8) is oversimplified: possibly, variations in the activity coefficient of TPhB with ionic strength have been improperly overlooked. Benz and Läuger (1977) reported a similar discrepancy for the hydrophobic ion dipicrylamine in charged phosphatidylserine membranes.

The authors thank Dr. Alfredo Gorio for fruitful comments on the manuscript.

References

Andersen, O.S., Feldberg, S., Nakadomari, H., Levy, S., McLaughlin, S. 1978. Electrostatic interactions among hydrophobic ions in lipid bilayer membranes. *Biophys. J.* **21:**35–70

Andersen, O.S., Fuchs, M. 1975. Potential energy barriers to ion transport within lipid bilayers. Studies with tetraphenylborate. *Biophys. J.* **15:**795–930

Bach, D., Chapman, D. 1980. Calorimetric studies of biomembranes and their molecular components. *In:* Biological Microcalorimetry. A.E. Beezer, editor. pp. 275–309. Academic, London

Benz, R., Conti, F. 1981. Structure of the squid axon membrane as derived from charge-pulse relaxation studies in the presence of absorbed lipophilic ions. *J. Membrane Biol.* **59:**91–104

Benz, R., Cross, D. 1978. Influence of sterols on ion transport through lipid bilayer membranes. *Biochim. Biophys. Acta* **506:**265–280

Benz, R., Frölich, D., Läuger, P. 1976. Influence of membrane structure on the kinetics of carrier-mediated ion transport through lipid bilayers. *Biochim. Biophys. Acta* **464:**465–481

Benz, R., Gisin, B.F. 1978. Influence of membrane structure on ion transport through lipid bilayer membranes. *J. Membrane Biol.* **40:**293–314

Benz, R., Läuger, P. 1977. Transport kinetics of dipicrylamine through lipid bilayer membranes. Effects of membrane structure. *Biochim. Biophys. Acta* **468:**245–258

Benz, R., Läuger, P., Janko, K. 1976. Transport kinetics of hydrophobic ions in lipid bilayer membranes. Charge pulse relaxation studies. *Biochim. Biophys. Acta* **455:**701–720

Benz, R., Nonner, W. 1981. Structure of the axolemma of frog myelinated nerve: Relaxation experiments with a lipophilic probe ion. *J. Membrane Biol.* **59:**127–134

Bruner, L.J. 1975. The interaction of hydrophobic ions with lipid bilayer membranes. *J. Membrane Biol.* **22:**125–141

Cornell, B.A., Separovic, F. 1983. Membrane thickness and acyl chain length. *Biochim. Biophys. Acta* **733:**189–193

Cuatrecasas, P. 1973*a*. Interaction of *Vibrio cholerae* enterotoxin with cell membranes. *Biochemistry* **12:**3547–3557

Cuatrecasas, P. 1973*b*. Gangliosides and membrane receptors for cholera toxin. *Biochemistry* **12:**3558–3566

DeLevie, R., Seidah, N.G., Larkin, D. 1974. Tetraphenylborate adsorption into an artificial ultrathin membrane. *Electroanal. Chem. Interf. Electrochem.* **48:**153–159

Delmelle, M., Dufrane, S.P., Brasseur, R., Ruysschaert, J.M. 1980. Clustering of gangliosides in phospholipid bilayers. *FEBS Lett.* **121:**11–14

Edelman, G.M. 1976. Surface modulation in cell recognition and cell growth. *Science* **192:**218–226

Fishman, P.H., Brady, R.O. 1975. Modification of membrane glycolipids by oncogenic agents. *In:* Modification of Lipid Metabolism. E.G. Perkins and L.A. Witting, editors. pp. 105–117. Academic, New York

Fishman, P.H., Brady, R.O. 1976. Biosynthesis and function of gangliosides. *Science* **194:**906–915

Gaines, G.L. 1966. Insoluble monolayers at liquid–gas interfaces. *In:* Interscience Monographs on Physical Chemistry. I. Prigogine, editor. J. Wiley, New York

Gambale, F., Robello, M., Usai, C., Marchetti, C. 1982. Properties of ionic transport through phospholipid-glycolipid artificial bilayers. *Biochim. Biophys. Acta* **693:**165–172

Hakomori, S.I., Jeanloz, R.W. 1970. Glycolipids as membrane antigens. *In:* Blood and Tissue Antigens. D. Aminof, editor. pp. 149–161. Academic, New York

Haydon, D.A. 1975. Functions of the lipid in bilayer ion permeability. *Ann. N.Y. Acad. Sci.* **264:**2–16

Haydon, D.A., Hladky, S.B. 1972. Ion transport across thin lipid membranes: A critical discussion of mechanisms in selected systems. *Q. Rev. Biophys.* **5:**187–282

Ketterer, B., Neumcke, B., Läuger, P. 1971. Transport mechanism of hydrophobic ions through lipid bilayer membranes. *J. Membrane Biol.* **5:**225–245

Klenk, E. 1942. Uber die Ganglioside, eine neue gruppe von zuckererhaltigen gehirnlipoiden. *Hoppe-Seyler's Z. Physiol. Chem.* **273:**76–88

Läuger, P., Benz, R., Stark, G., Bamberg, E., Jordan, P.C., Fahr, A., Brock, W. 1981. Relaxation studies of ion transport systems in lipid bilayer membranes. *Q. Rev. Biophys.* **14:**513–598

Läuger, P., Neumcke, B. 1973. Theoretical analysis of ion conductance in lipid bilayer membranes. *In:* Membranes. A Series of Advances. G. Eisenman, editor. Vol. 2, pp. 1–59. M. Dekker, New York

LeBlanc, O.H., Jr. 1969. Tetraphenylborate conductance through lipid bilayer membranes. *Biochim. Biophys. Acta* **193:**350–360

Liberman, E.A., Topaly, V.P. 1968. Selective transport of ions through bimolecular phospholipid membranes. *Biochim. Biophys. Acta* **163:**125–136

Maggio, B., Cumar, F.A., Caputto, R. 1978. Surface behaviour of gangliosides and related glycosphingolipids. *Biochem. J.* **171:**559–565

Maggio, B., Cumar, F.A., Caputto, R. 1980. Configuration and interactions of the polar head group in gangliosides. *Biochem. J.* **189:**435–440

McLaughlin, S. 1977. Electrostatic potentials at membrane-solution interfaces. *Curr. Top. Membr. Transp.* **9**:71–144

Mueller, P., Rudin, D.O., Tien, H.T., Wescott, W.C. 1963. Methods for the formation of single bimolecular lipid membranes in aqueous solution. *J. Phys. Chem.* **67**:534–535

Mullin, B.R., Pacuszka, T., Lee, G., Kohn, L.D., Brady, R.O., Fishman, P.H. 1978. Thyroid gangliosides with high affinity for thyrotropin: Potential role in thyroid regulation. *Science* **199**:77–79

Parsegian, A. 1969. Energy of an ion crossing a low dielectric membrane: Solution of four relevant electrostatic problems. *Nature (London)* **221**:844–845

Pickar, A.D., Benz, R. 1978. Transport of oppositely charged lipophilic probe ions in lipid bilayer membranes having various structures. *J. Membrane Biol.* **44**:353–376

Porcellati, G., Ceccarelli, B., Tettamanti, R.O., editors. 1976. Ganglioside function. Biochemical and pharmacological implications. *Adv. Exp. Med. Biol.* Vol. 71. Plenum, New York

Poss, A., Deleers, M., Ruysschaert, J.M. 1978. Evidence for a specific interaction between GT ganglioside incorporated into bilayer membranes and thyrotropin. *FEBS Lett.* **86**:160–162

Redwood, W.R., Polefka, T.G. 1976. Lectin receptor interaction in liposomes. II. Interaction of wheat germ agglutinin with phosphatidylcholine liposomes containing incorporated monosialoganglioside. *Biochim. Biophys. Acta* **455**:631–643

Sharon, F.J., Grant, C.W.M. 1978. A model for ganglioside behavior in cell membranes. *Biochim. Biophys. Acta* **507**:280–293

Sinha, B.A., Smejtek, P. 1983. Effect of 3-phenylindole on lipophilic ion and carrier-mediated ion transport across bilayer lipid membranes. *J. Membrane Biol.* **71**:119–130

Szabo, G. 1976. The influence of dipole potentials on the magnitude and the kinetics of ion transport in lipid bilayer membranes. *In:* Extreme Environment: Mechanism of Microbial Adaptation. M.R. Heinrich, editor. pp. 321–348. Academic, New York

Tumanova, R.Y., Badjinyan, S.A., Nalbandyan, R.M. 1978. Resistance of planar bilayers of gangliosides. *Biochem. Biophys. Res. Commun.* **84**:520–526

Uchida, T., Nagai, Y., Kawasaki, Y., Wakayama, N. 1981. Fluorospectroscopic studies of various ganglioside and ganglioside-lecithin dispersions. Steady-state and time-resolved fluorescence measurements with 1,6-diphenyl-1,3,5-hexatriene. *Biochemistry* **20**:162–169

Usai, C., Marchetti, C., Gambale, F., Robello, M., Gorio, A. 1983. Capacitance-voltage relationship in phospholipid bilayers containing gangliosides. *FEBS Lett.* **153**:315–319

Van Heyningen, W.E. 1974. Gangliosides as membrane receptors for tetanus toxin, cholera toxin and serotonin. *Nature (London)* **249**:415–417

Wiegandt, H. 1975. Glycoproteins and glycolipids of the cell surface. *In:* Biomembranes, Lipids, Proteins and Receptors. R.M. Burton and F.S. Esters, editors. pp. 51–64. NATO Advanced Study Institute, Bi-Science, Webster Groves, Mo.

Received 3 January 1984; revised 3 May 1984