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Alteration of the passive electrical properties of lymphocyte membranes induced by GM1 and GM3 glycolipids

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The electrical conductivity of normal human lymphocyte suspensions has been measured in the frequency range from 10 kHz to 100 MHz, where a well-pronounced conductivity dispersion occurs, caused by the surface polarization at the interface between the cell membrane and the extracellular solution. We have investigated the alteration of the passive electrical properties of the cytoplasmatic cell membrane induced by two different gangliosides (GM1 and GM3) inserted, at various concentrations, into the outer leaflet of membrane double layer. The alterations observed in the dielectric parameters (the membrane conductivity and the membrane permittivity) derived on the basis of a 'double-shell' model, result in an overall increase of the ion permeation across the membrane and an enhanced polarizability of its hydrophilic region for both gangliosides investigated. The relevance of these alterations is discussed.

Introduction

There is growing current interest in the study of the electrical behavior of biological materials [1], such as biological tissues and cell suspensions and, in the last few years, the dielectric spectroscopy technique [2] has been extensively applied to investigate the passive electrical properties of a large variety of biological cell membranes [3,4].

Several drugs and molecules of biological relevance have been shown to have strong interactions with various components of cell membranes or with the whole membrane [5]. The preferential localization of these molecules in the membrane acyl-chain region or at the interface with the external medium, makes the cell's passive electrical properties a sensitive probe useful to study the alteration of the membrane structure induced by these molecules.

Gangliosides, which have been studied most extensively [6], are anionic glycolipids with a double-chain hydrophobic portion (ceramide) and a hydrophilic head with sialic-acid-containing oligosaccharide. Such molecules seem to have a primary role in the cell membrane recognition or as biotransductor of the cell membrane-mediated information. Furthermore, the gangliosides also seem to be able to influence the immunoactivity of other molecules, which are normal components of the cell membrane [7].

Some of these ganglioside activities can be viewed as connected to their individual properties, while other properties may be a consequence of modifications induced by gangliosides at the membrane surface. From this point of view, however, the knowledge of the structural and dynamic alterations that gangliosides can produce on the cell membrane structure, is quite poor.

The aim of this study was to investigate the structural effects induced by two different gangliosides on the normal resting human lymphocyte membrane under physiological conditions, through the measurements of the electrical conductivity and the electrical permittivity of the cytoplasmic membrane.

In particular, we have studied the influence of two different gangliosides, GM3 and GM1, of similar chemical structural sequence, the former of which is normally present in the cytoplasmic membrane of human lymphocyte cells, whereas the latter is not present at detectable concentrations. The oligosaccharide chains

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of these two gangliosides show different complexity and head group dimensions (the steric dimension is larger in GM1 than in GM3).

Since exogenous gangliosides are easily adsorbed onto biological cell membranes above the normal physiological concentration [8], it is possible, under appropriate incubation conditions, to increase the GM3 concentration or to insert GM1 molecules up to values of about 10^{-7} μg/cell.

This strategy allows us to investigate the interactions of different oligosaccharide chains (the hydrophilic part) with the outer layer of the cytoplasmic membrane, without relevant alteration of the hydrophobic layer of the lymphocyte membrane due to the insertion of the ceramide moiety of the two gangliosides.

In this article, we report some radiowave electrical conductivity measurements of human lymphocyte suspensions, which had previously been incubated with either GM1 or GM3 monosialogangliosides at different concentrations, from 1 to 10 μg/ml.

The alterations of the passive electrical properties of the cytoplasmic membrane have been discussed on the basis of a dielectric model which simulates the electrical behavior of a cell suspension. The model, which takes into account both the cytoplasmic and the nuclear membrane, is able to correlate some structural properties of the membrane, such as the ionic permeability, the fluidity or the dipole arrangement to the passive electrical properties of the bilayer as a function of the ganglioside concentration.

A preliminary report of this work has already appeared previously [9].

We observe an increase in the electrical conductivity of the cytoplasmic membrane induced by incubation with gangliosides, whereas the nuclear membrane seems to be unaffected. A similar behavior occurs to the membrane permittivity. The relevance of these alterations is discussed.

Materials and Methods

Material and lymphocyte preparation

Peripheral blood lymphocytes from healthy donors were prepared from gradient centrifugation with Ficoll-Hypaque (Lymphocyte Separation Medium, Sigma, St. Louis, USA) following a standard separation procedure [10]. An analysis performed by a cell analyzer Technicon H1 System revealed a mean purity of lymphocyte preparations of about 86%. During all the conductivity measurements, the cell concentration was maintained constant at $1.5 \cdot 10^8$ cells/ml. Cell dimensions, before and after the treatment with gangliosides, were checked through accurate measurements performed by means of optical microscopy.

Cells ($1.5 \cdot 10^8$ cells/ml in phosphate-buffered saline solution, (pH 7.4)) were incubated with varying concentrations (1, 5 or 10 μg/ml) of either GM1 or GM3 (Sigma, St. Louis, USA) for 1 h at 37°C. The cells were then washed with phosphate-buffered saline solution (pH 7.4) with 10% serum, immediately before each conductivity measurement to remove the gangliosides not inserted in the membrane bilayer [11].

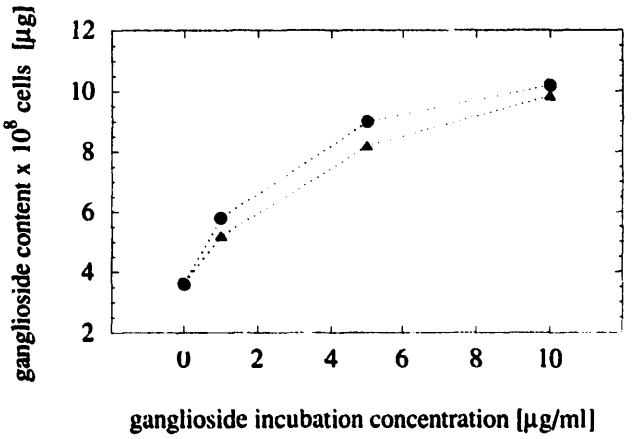

Fig. 1. The incorporated ganglioside concentration per lymphocyte cell as a function of the ganglioside concentration in the incubation medium. GM1 (circles), GM3 (triangles).

To measure the effective content of gangliosides incorporated within the cell membrane upon the incubation, the gangliosides were extracted from the cell suspension according to the method of Svennerholm and Fredman [12]. The quantitative determination of the lipid bound sialic acid was performed by means of colorimetric technique following the procedure adopted by Jourdian et al. [13] using resorcinol, which specifically stains sialic-acid-containing glycolipids in blue-purple. The absorbances were measured at 630 nm with a Perkin-Elmer Lambda 4B spectrophotometer. The concentration of the gangliosides incorporated into the lymphocyte bilayer for the different concentrations of gangliosides in the incubation solution, is reported in Fig. 1.

Method

The electrical conductivity of the cell suspensions was measured in the frequency range from 10 kHz to 100 MHz. using standard impedance measurements by means of two Impedance Analyzers Hewlett-Packard model 4192A (between 10 kHz to 10 MHz) and model 4193A (between 0.5 MHz to 100 MHz).

All measurements were carried out at a temperature of 37.0 ± 0.1°C, where the biochemical activity of the cell membrane might be significantly enhanced.

The experimental setup, the measuring cell and the calibration procedure based on measurements of standard liquids of known conductivities and dielectric constants have been described in detail elsewhere [14].

Theoretical background

The passive electrical properties of biological cell suspensions have been extensively studied in the last two decades and comprehensive reviews have appeared [3,4].

We will focus our interest on the conductivity dispersion which occurs typically in the radio frequency range as a consequence of a charge-accumulation at the interface between the low-conductivity cell membrane and the more highly conducting interior or exterior aqueous phases. This effect, known as the β-dispersion [1], makes it possible to extract, on the basis of appropriate dielectric cell suspension models, the electrical properties of the cell membrane by measuring, for instance, the electrical conductivity of the whole suspension.

Here, we will summarize the relevant expressions of the two most widely used dielectric models, which consider the cell structure as due to a single external membrane (single-shell model) or due to a more composite structure, taking into account the nuclear envelope (double-shell model).

(A) Single-shell model. The shell suspension is described by a collection of spherical particles (the cytosol) of complex conductivity $\sigma_p^* = \sigma_p + i\omega\epsilon_0\epsilon_p$, coated with a shell (the cell membrane) of complex conductivity $\sigma_s^* = \sigma_s + i\omega\epsilon_0\epsilon_s$ and uniformly distributed in a continuous medium (the extracellular solution) of complex conductivity $\sigma_m^* = \sigma_m + i\omega\epsilon_0\epsilon_m$.

Here, ω is the angular frequency of the applied field, ϵ_0 the dielectric constant of free space and ϵ (assumed independent of frequency) the permittivity of the different media.

From an electrical point of view, the overall conductivity σ^* of the suspension can be written, on the basis of the classical works by Maxwell and later by Wagner [15], as follows

$$\frac{\sigma^* - \sigma_m^*}{\sigma^* + 2\sigma_m^*} = \phi \frac{\sigma_{eq}^* - \sigma_m^*}{\sigma_{eq}^* + 2\sigma_s^*} \tag{1}$$

where the equivalent conductivity σ_{eq}^* of the shelled particle is given by

$$\frac{\sigma_{eq}^* - \sigma_s^*}{\sigma_{eq}^* + 2\sigma_s^*} = \left(\frac{R}{R+d}\right)^3 \frac{\sigma_p^* - \sigma_s^*}{\sigma_p^* + 2\sigma_s^*} \tag{2}$$

Here, ϕ is the volume fraction of the dispersed phase, R the radius of the particle and d the thickness of the shell. The general solution of Eqns. 1 and 2, described in detail by Pauly and Schwan [16] and later by Hanai [17], predicts two distinct conductivity dispersions described by the relation

$$\sigma^* = \sigma_m^* \frac{A + i\omega B - \omega^2 C}{D + i\omega E - \omega^2 F}$$

where the quantities A, B, C, D, E and F, given in Ref. 17, define the relaxation parameters of each dispersion (the relaxation frequency and the conductivity increment) and depend on the values of the electrical phase parameters of the different media.

(B) The double-shell model. In this case, each cell is represented by two layers (the cytoplasmic and nuclear membranes respectively of complex conductivities $\sigma_s^* = \sigma_s + i\omega\epsilon_0\epsilon_s$ and $\sigma_n^* = \sigma_n + i\omega\epsilon_0\epsilon_n$), which define an intermembrane medium of conductivity $\sigma_i^* = \sigma_i + i\omega\epsilon_0\epsilon_i$ and an inner medium of conductivity $\sigma_p^* = \sigma_p + i\omega\epsilon_0\epsilon_p$. The thickness of the cytoplasmic and nuclear membranes are d and d_n respectively, the cell has radius R, whereas the inner medium has radius R_p.

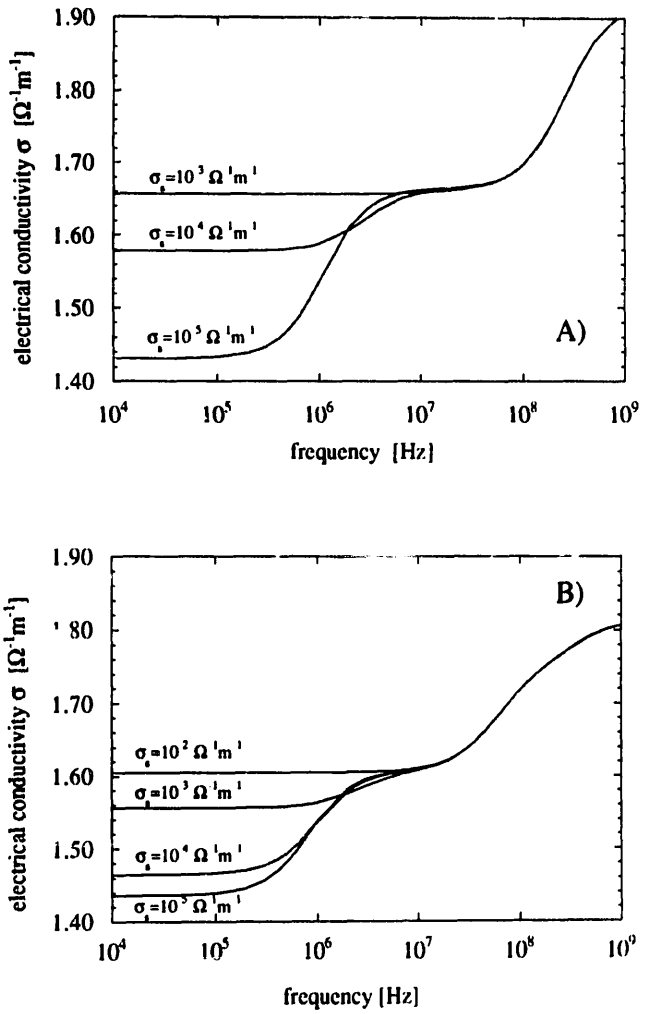

Fig. 2. The calculated conductivity of a cell suspension as a function of frequency for various values of the conductivity σ_s of the cytoplasmic membrane. (a) The curves are calculated from Eqn. 1 (single-shell model) with the following values of the phase parameters: $\sigma_s = 10$; $\sigma_p = 0.8\ \Omega^{-1}\ m^{-1}$; $\epsilon_m = 200$; $\sigma_m = 1.92\ \Omega^{-1}\ m^{-1}$; $\epsilon_m = 78.5$; $d = 50$ Å; $R = 5.64\ \mu m$; $\phi = 0.185$. (b) The curves are calculated from Eqn. 3 (double-shell model) with the following values of the phase parameters: $\epsilon_s = 10$; $\sigma_i = 0.5\ \Omega^{-1}\ m^{-1}$; $\epsilon_i = 200$; $\sigma_n = 10^{-3}\ \Omega^{-1}\ m^{-1}$; $\epsilon_n = 1$; $\sigma_p = 1.56$; $\epsilon_p = 100$; $\sigma_m = 1.92$; $\epsilon_m = 78.5$; $R_p = 1.0\ \mu m$; $d_n = 50$ Å; $R = 5.635\ \mu m$; $d = 50$ Å; $\phi = 0.185$.

Here, the equivalent conductivity of the stratified sphere, built step-by-step in analogy with the single-shell model, is given in Refs. 18, 19

$$\frac{\sigma_{eq}^* - \sigma_s^*}{\sigma_{eq}^* + 2\sigma_s^*} = \left(\frac{R}{R+d}\right)^3 \frac{\sigma_{ieq}^* - \sigma_s^*}{\sigma_{ieq}^* + 2\sigma_s^*} \tag{3}$$

where σ_{ieq}^* and σ_{neq}^* are written as

$$\frac{\sigma_{ieq}^* - \sigma_i^*}{\sigma_{ieq}^* + 2\sigma_i^*} = \left(\frac{R_n + d_n}{R}\right)^3 \frac{\sigma_{neq}^* - \sigma_i^*}{\sigma_{neq}^* + 2\sigma_s^*}$$

$$\frac{\sigma_{neq}^* - \sigma_n^*}{\sigma_{neq}^* + 2\sigma_n^*} = \left(\frac{R_p}{R_p + d_n}\right)^3 \frac{\sigma_p^* - \sigma_n^*}{\sigma_p^* + 2\sigma_n^*} \tag{4}$$

Substituting Eqns. 3 to 4 into Eqn. 1 yields the full description of the conductivity behavior of the cell suspension, depending on a set of morphometric- and electrical-phase-parameters which describe the different media used in the cell architecture.

Fig. 2 shows the theoretical frequency-dependence of the conductivity of a cell suspension derived from the single-shell or the double-shell model (Eqns. 2 and 3, respectively) for various values of the conductivity of the cytoplasmic membrane. The other parameters involved are listed in the caption to the figure.

As can be seen, the presence of the nuclear membrane inside the lymphocyte cell, i.e., a multilamellar structure of the cell, allows an additional polarization process to occur, which causes the broadening of the relaxation spectrum.

Results and Discussion

Fig. 3 shows a typical conductivity spectrum in the frequency range from 10^4 to 10^8 Hz at the temperature of 37°C for a suspension of human lymphocytes incubated for 1 h with GM3 ganglioside at different concentrations, from 1 μg/ml to 10 μg/ml. The conductivity spectrum of the unaffected control lymphocyte suspension is also shown for comparison.

Similar spectra, with the same main features, were observed for lymphocyte suspensions treated, under the same experimental conditions, with GM1 ganglioside.

In the attempt to correlate the measured conductivity behavior with the alterations of the passive electrical properties of the cell membranes induced by incubation with gangliosides at different concentrations, the cell morphology must be known.

The distribution of cell size measured flow cytometrically is shown in the hystograms of Fig. 4, both for normal control cells and for cells incubated with GM1 and GM3 gangliosides. As can be seen, the presence of

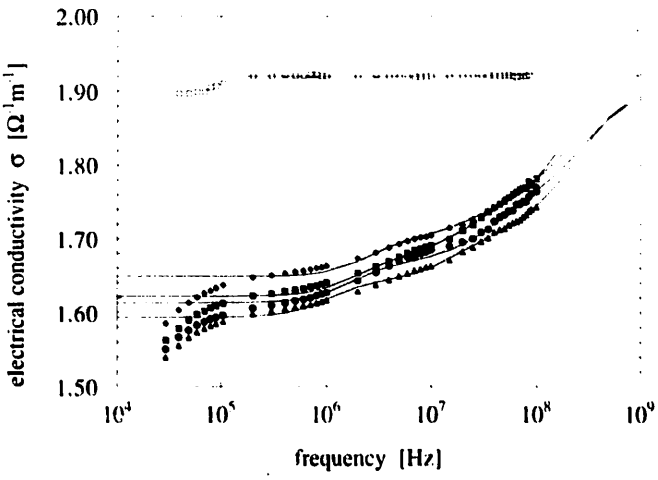

Fig. 3. The measured electrical conductivity of lymphocyte cell suspension as a function of frequency at a temperature of 37°C. The cell suspension was incubated with GM3 at different concentrations for 1 h. (▲) Control suspension; (●), 1 μg/ml; (■), 5 μg/ml; (◆), 10 μg/ml. The full lines are the calculated values according to the double-shell model with parameters derived from the fitting of Eqn. 3 to the experimental data. The conductivity of the extracellular medium is also shown (□) and the full line represents the expected behavior as a function of frequency, assuming a Debye-type relaxation function.

gangliosides, up to 10 μg/ml, does not produce any relevant alteration in the overall average cell volume.

In the dielectric model we adopted, the lymphocyte suspension is represented as a collection of spherical particles with an average diameter of 5.5 μm. The thickness of the cytoplasmic membrane is assumed to be 75 Å and the same value has been considered for the nuclear envelope, that contains about 70% of the total cell volume.

The electrical-phase parameters relevant to the single-shell or to the double-shell model have been determined by fitting the theoretical curve to the observed conductivity dispersion data to five parameters (the conductivity and the permittivity of cytoplasmic membrane (σ_s and ϵ_s), the analogous quantities of the nuclear membrane (σ_n, ϵ_n) and the conductivity of the inner medium σ_p).

The other parameters in Eqn. 3 have been directly measured or have been assumed on the basis of data from literature. In particular, the conductivity of the external medium σ_m has been measured in the supernatant by centrifuging the cell suspension. It must be noted, however, that the measured conductivity of the external medium does not show any detectable difference between the control supernatant and the supernatant of the samples treated with ganglioside. This further confirms that the observed conductivity variations in the treated cell suspensions are just related to the gangliosides incorporated on the cell bilayers.

An example of analysis of the conductivity data with the two models given above is shown in Fig. 5. It can

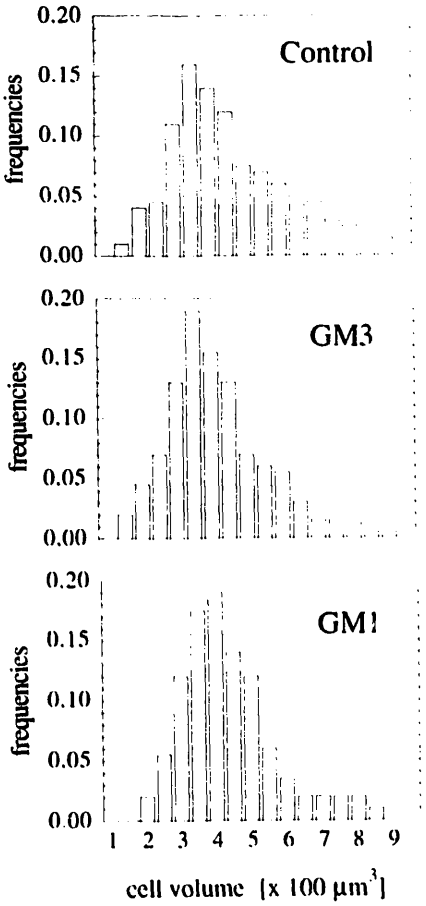

Fig. 4. Histograms of the cell volume measured by means of optical microscopy after the incubation with 10 μg/ml GM1 and 10 μg/ml GM3. Control cell suspension is also shown for comparison. Control $r_p = 460 \pm 160$ μm^3; GM1 $r_p = 435 \pm 130$ μm^3; GM3 $r_p = 412 \pm 150$ μm^3. The quoted uncertainties represent the standard deviations of the distribution.

be observed that the fitting based on the double-shell model, appreciably improved with respect to that based on the single-shell model. The improvement is well

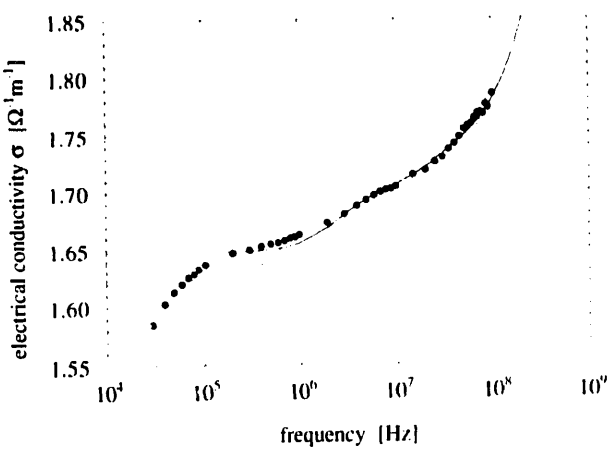

Fig. 5. The electrical conductivity of lymphocyte suspension incubated with GM3 (10 μg/ml for 1 h) as a function of frequency. The calculated values according to the double shell model (full line) and to the single-shell model (dotted line) are also shown.

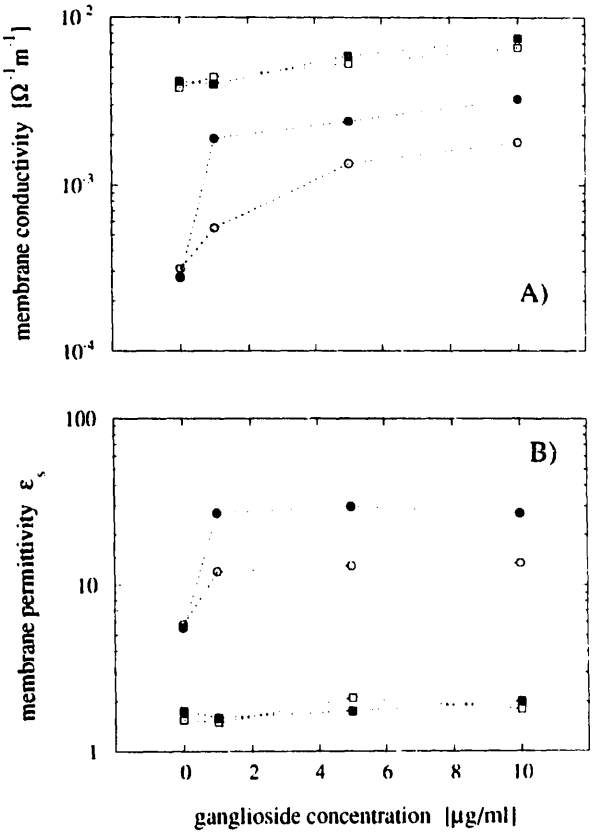

Fig. 6. (a) The membrane conductivity σ_s of the cytoplasmic membrane (circles) and of the nuclear membrane (squares) derived from the fitting procedure based on the double-shell model as a function of the ganglioside concentration. Open symbols, cells incubated with GM3; full symbols, cells incubated with GM1. (b) The same as for the membrane permittivity ϵ_s of the cytoplasmic membrane (circles) and of the nuclear membrane (squares). Open symbols, cells treated with GM3; full symbols, cells treated with GM1.

above the expected one for the increasing of the free parameters involved.

In the light of these observations, we tried to discuss the membrane electrical parameters on the basis of the double-shell model only. Fig. 6 summarizes the results of different experiments obtained for the membrane electrical parameters as a function of the ganglioside concentration.

The main alteration we observed concerns with the cytoplasmic membrane, whose conductivity increases for example from $2 \cdot 10^{-4}$ Ω^{-1} m^{-1} in control cells, to $2 \cdot 10^{-3}$ Ω^{-1} m^{-1} and up to $5.5 \cdot 10^{-4}$ Ω^{-1} m^{-1} in cells incubated, respectively, with GM1 and GM3 at the concentration of 1 μg/ml. These values increase further, but more gradually, as the ganglioside concentration is increased, indicating a saturation effect.

A similar behavior emerges from inspection of the permittivity data, since the permittivity of cytoplasmic membrane increases from 4.5 in control cells, to 28 for GM1 incubated cells or to 12 for GM3 incubated cells.

A first comment is required. The surface of cell membrane is highly structured, whereas both the mod-

els we adopted are based on smooth spherical particles. Consequently, as pointed out by Hu et al. [20], the parameters deduced from the above fitting procedure must be considered as 'effective' values, which characterize the average properties, including the surface structure and the composition of the adjacent media. Despite this fact, the alterations induced by gangliosides on the cytoplasmatic membrane are marked.

Fig. 6 evidences some differences between GM1 and GM3 induced alterations. As pointed out by Uchida et al. [21] on the basis of fluorescence anisotropy measurements and by Bertoli et al. [22] by means of E.P.R. studies, GM1 ganglioside inserted into artificial membranes causes a decrease in the overall membrane fluidity and the membrane itself becomes more rigid. This effect is more evident with GM1 and should increase with homologous gangliosides, as the GM3 ganglioside, mainly depending on the structural features of the polar head groups.

Since the ganglioside incorporation occurs, to a first approximation, without any relevant alteration of the bilayer structure, this suggests, as noted above, that only the outer face of the bilayer is involved. The interactions should occur preferably between the polar head groups of the ganglioside and the charged hydrophilic part of the bilayer. In this context, gangliosides with different oligosaccharide chains should produce different alterations of the passive electrical properties of the membrane.

As pointed out by Tettamanti et al. [23], the effect induced by exogenous gangliosides on in vitro incubation of various cells depends critically on the physical form of the gangliosides in the incubation medium. It is well known that gangliosides tend to form micellar aggregates, with large aggregation number, when the concentration reaches values of about 10^{-6} to 10^{-5} M [24]. In this investigation, the ganglioside concentration employed varied from $6.4 \cdot 10^{-7}$ M to $6.4 \cdot 10^{-6}$ M for GM1 and from $8.0 \cdot 10^{-7}$ M to $8.0 \cdot 10^{-6}$ M for GM3.

The double-shell model suggests the possibility that the interactions involve also the nuclear membrane to be investigated. As can be seen in Fig. 6, the parameters of the nuclear envelope do not change appreciably upon the ganglioside incubation. The electrical conductivity, deduced from the fitting procedure is of the order of $5 \cdot 10^{-3}$ Ω^{-1} m^{-1} and it is of particular interest to compare this value with the one expected for the nuclear envelope.

The nuclear membrane of lymphocytes is a complex structure where the transport of biological material occurs throughout aqueous pores large enough to offer a negligible constrain to the movement of small inorganic ions. The conductance of a single nuclear pore, having a diameter of about 9 nm and a length of about 80 nm which contains a solution of conductivity 10^{-2} Ω^{-1} cm^{-1} [25], is of the order of 800 pS.

If the pore density of human lymphocytes is of the order of the $(6–7) \cdot 10^{12}$ pore/m^2 [26], the conductivity of the nuclear membrane should be as high as $2 \cdot 10^{-3}$ Ω^{-1} m^{-1}. This value is in reasonable agreement with that estimated from our conductivity measurements. This fact can be considered a relevant feature for the discussion of the experimental data within the double-shell model approach.

The extent to which the cytoplasmic membrane alteration is involved, following the ganglioside enrichment, must be further examined.

The possible molecular actions of gangliosides at molecular level are largely unknown. Nevertheless, the changes observed both in the cytoplasmic membrane conductivity and permittivity indicate an increase of the ionic permeation (shown by the increase of σ_s) and an enhanced electrical polarizability (shown by an increase of ϵ_s) of the overall membrane structure.

If the ionic transport across the cytoplasmic membrane is simply due to the existence of conducting pathways embedded in the less conductive hydrocarbon bilayer, the membrane conductivity σ_s can be written, to a first approximation, as

$$\sigma_s = N_{pore}\sigma_{pore}\pi\left(\frac{D}{2}\right)^2 + \sigma_{sc}\left[1 - N_{pore}\pi\left(\frac{D}{2}\right)^2\right] \quad (5)$$

where σ_{pore} is the conductivity of the conducting pore of area S_{pore}, N_{pore} is the number concentration (pores per unit surface) and σ_{sc} is the conductivity of the hydrocarbon bilayer.

The observed increase of the cytoplasmic membrane conductivity may be attributed, from a macroscopic point of view, to an increase of the activated transmembrane pores, whose concentration, according to Eqn. 5, should increase from $3.1 \cdot 10^{12}$ pore/m^2 in control cells, to $3.1 \cdot 10^{13}$ pore/m^2 in GM1-treated cells and to $8.5 \cdot 10^{12}$ pore/m^2 in GM3-treated cells. These values reflect reasonably well the increase of the ganglioside density in the lymphocyte membrane following the incubation (Fig. 1). The increase of the ganglioside concentration implies a proportional increase in the overall membrane conductivities.

A tentative explanation of the cell-membrane permeability increase could be given in terms of the clustering process which gangliosides undergo when their concentration is higher than a critical value [27], as in our treated samples.

In such clusters, particularly when their formation is supported by ions or proteins, the gangliosides are packed in a more effective way, because of a less active ganglioside-ganglioside repulsive interaction, as it is confirmed by the reduction of the ceramide and oligosaccharide mobility [22]. The ganglioside intra-cluster packing, hence, could be more effective than the phospholipid matrix packing. So, the cell membrane needs

to rearrange its structure with consequent formation of channels and increasing its permeability.

Finally, the increase of the permittivity of the cytoplasmic membrane in presence of GM1 and GM3 molecules must be associated to an increase of the membrane polarizability. This could be due to the polar component of the gangliosides. In fact, GM3, with a smaller head group area than GM1, induces a less marked increase of the membrane permittivity.

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