Effect of Extracellular Alkali Metal Salts on the Electric Parameters of Human Erythrocytes in Normal and Pathological Conditions (Homozygous β-Thalassemia)

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The conductivity of human erythrocyte cells dispersed in various uni-univalent electrolyte solutions (NaCl, KCl, LiCl, CsCl; 0.15 M) have been measured in the frequency range from 10 KHz to 100 MHz at five temperatures between 5 and 45 °C. The results were analyzed in the light of the theory of conductivity polarization of a suspension of ellipsoidal particles covered with two confocal shells.

Differences in the electrical parameters of the membrane between normal and homozygous β-thalassemic cells have been evidentiated.

Introduction

In an our previous work [1], the conductivity properties of human red blood cells in physiological saline solution (0.15 M NaCl, pH = 7.4, 5 mM Na phosphate), have been studied in the frequency range from 10 KHz to 100 MHz at various temperatures between 5 and 45 °C.

The analysis was carried out both on normal and homozygous β-thalassemic cells and differences in the electrical parameters of the whole membrane, *i.e.*, the capacitance C_M and the conductance G_M per unit surface, have been evidentiated.

These differences, in connection with results obtained from electron spin resonance spectroscopy [2] indicate that the pathological membrane is less fluid and possesses a lower conductance than that of normal cell. This is probably due to a reduced ion transport influenced by a decreased concentration of the transmembraneous proteins or to different lipid-protein interactions.

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On the other hand, it is known [3, 4] that phospholipids interact with different ions leading to changes in the surface membrane charge [5] and resulting in an alteration of the transmembrane transport mechanism.

Various transport mechanisms in cell membrane have been studied and recently reviewed by Gunn [6], but these have been concerned preferentially with normal cells.

Since the exact alteration in the basic membrane components and the changes in the intracellular environment of thalassemic membrane have not yet completely determined, it is possible to suppose that different interactions with different ions may occur. In fact small ions have specific and selective transport mechanisms mainly induced by hydrophobic proteins which create cation conducting paths across the membrane.

It was therefore of particular interest to examine if changes in extracellular medium due to different ionic species may produce different effects on the physical state of the erythrocyte membrane with particular regard to β-thalassemic membrane.

This work is concerned with the influence of uni-univalent electrolytes (NaCl, CsCl, KCl, LiCl) at

physiological concentration, on the electrical properties of human erythrocyte membrane in normal and pathological homozygous β-thalassemic cells. The capacitance C_M and the conductance G_M per unit surface or, equally well, the dielectric constant ε_S and the conductivity σ_S of the hydrophylic part of the membrane including the polar-heads and the hydration layer on the ionizable groups of membrane proteins have been derived from conductivity measurements on aqueous suspensions (fractional volume $\Phi = 0.30$) in the frequency range from 10 KHz to 100 MHz at five temperatures between 5 and 45 °C.

Experimental

Fresh blood was drawn by venipuncture from normal and homozygous β-thalassemic donors. Separation of erythrocytes was obtained by centrifugation at 3.000 rpm for 10 min. Plasma and buffy coat were removed and the red cells were then washed three times in various isotonic phosphate-buffered saline containing different monovalent cations (5 mM NaH_2PO_4, pH = 7.4, 0.15 M NaCl, LiCl, KCl, CsCl respectively).

The suspensions were incubated at room temperature for 4 h to equilibrate the cells in the respective salt solutions and to avoid transients in the ion concentration.

The hematocrit (Hct) of these four final stock erythrocyte suspensions was about 50%. After the third wash, the cells were resuspended in the various buffers and the concentration was adjusted to the value corresponding to a fractional volume of dispersed phase $\Phi = 0.30$ with the aid of a Couther electronic particles counter.

The conductivities are obtained from impedance measurements of a cylindrical waveguide containing the sample excited beyond its cut-off frequency. The experimental error was estimated within 2%, over the whole frequency range. The temperature was varied from 5 to 45 °C within 0.1 °C. No evidence for alteration of the sample due to temperature was observed.

The erythrocyte cell is assumed to be an oblate spheroid of 0.8 μm in major axis and 2.4 μm in minor axis. The overall thickness of the membrane is assumed to be 50 Å.

In addition, the concentration of Li^+, K^+, Na^+ ions in the intracellular medium was measured by means of atomic absorption spectrometry with a Perkin Elmer Mod. 603 Flame Spectrophotometer.

Conductivity of a Spheroid Dispersion

The calculation of the conductivity of biological objects dispersed in aqueous media is based upon the model chosen to describe the biological cells.

Various levels of sophistication are possible, but, in general the representation of the molecular structure of a membrane reveals three regions of different characteristics, *i.e.*, the hydrophobic region which consists of aliphatic hydrocarbon chains, the internal and external polar-head layers and the hydration shell adjacent to the cell surfaces.

On the other hand, impedance measurements must be interpreted by means of an equivalent circuit which takes into account the above structural features of the membrane and in addition the bulk electrical properties of both suspending medium and cytoplasm.

It seems, therefore, reasonable to adopt for the electrical model of an erythrocyte cell an ellipsoidal particle randomly dispersed in an electrolyte solution and covered with two confocal cells.

The dispersed particle is assumed to be an oblate spheroid with semiaxes $a < b = c$, with the two shells corresponding to the hydrophobic membrane region and the polar-head regions with the two extracellular surface layers respectively.

More elaborated membrane models, similar to that proposed by Ashcroft *et al.* [9] have not considered here, since they involve a too large number of adjustable parameters.

The conductivity σ^* of a suspension of shelled ellipsoidal particles is given by [10]

$$\sigma^* = \sigma_m^* + \frac{\Phi}{3}\sigma_m^* \sum_{k=1,2,3} \frac{\bar{\sigma}_k^* - \sigma_m^*}{\sigma_m^* + (\bar{\sigma}_k^* - \sigma_m^*) A_{0k}} \tag{1}$$

with

$$\sigma_k^* = \sigma_\uparrow^* \frac{\sigma_\uparrow^* + (\bar{\sigma}_{\uparrow k}^* - \sigma_\uparrow^*) A_{1k} + \lambda (\bar{\sigma}_{\uparrow k}^* - \sigma_\uparrow^*)(1 - A_{0k})}{\sigma_\uparrow^* + (\bar{\sigma}_{\uparrow k}^* - \sigma_\uparrow^*) A_{1k} - \lambda (\bar{\sigma}_{\uparrow k}^* - \sigma_\uparrow^*) A_{0k}};$$

$$\bar{\sigma}_{\uparrow k}^* = \sigma_s^* \frac{\sigma_s^* + (\sigma_p^* - \sigma_s^*) A_{2k} + \mu (\sigma_p^* - \sigma_s^*)(1 - A_{1k})}{\sigma_s^* + (\sigma_p^* - \sigma_s^*) A_{2k} - \mu (\sigma_p^* - \sigma_s^*) A_{1k}}$$

where σ_m^*, σ_s^*, $\sigma_\uparrow^*$, σ_p^* are the complex conductivities of the aqueous phase, external shell, hydrophobic region of the membrane and internal medium respectively. Φ is the fractional volume of the dispersed phase.

The depolarization factors

$$A_{ij} = \frac{r_i q_i v_i}{2} \int_0^\infty \frac{d\xi}{(t_{ij}^2 + \xi)\sqrt{(\xi + r_i)^2 (\xi + q_i)^2 (\xi + v_i)^2}}$$

with $r_i = a,\ a_l,\ a_s;\ q_i = b,\ b_l,\ b_s;\ v_i = c,\ c_l,\ c_s$ for $i = 0,\ 1,\ 2$ and $t_{ij} = r_i,\ q_i,\ v_i$ for $j = 1,\ 2,\ 3$ respectively, are assumed to be

$$A_{i1} = A_{(i+1)1} \quad (i = 0, 1);$$
$$A_{i2} = A_{i3} \quad (i = 0, 1, 2)$$

since the thickness of the two confocal layers are negligible small compared with the cell dimensions.

The suffixes l and s refer to the axes of the particle covered with shells.

The parameters λ and μ are defined, for an oblate spheroid, as

$$\lambda = \frac{(a - d_l)(b - d_l)^2}{a b^2};$$
$$\mu = \frac{(a - d_l - d)(b - d_l - d)^2}{(a - d_l)(b - d_l)^2}$$

where d_l and d are the thickness of the two shells.

To take into account the relatively high value of the fractional volume of the dispersed phase, Eq. (1) is used, rather than the ordinary Maxwell-Wagner mixture equation employed in ref. [10]. A similar procedure was adopted by Boned *et al.* [11] in the extending to high concentration the mixture equation for random distributed ellipsoidal particles. In the most general case, the medium inside the cell, the external medium and the two layers corresponding to the cell membrane display complex conductivities including both dielectric constant ε and *d.c.* conductivities σ, *i.e.*

$$\sigma_j^* = \sigma_j + i\omega\varepsilon_0\varepsilon_j, \quad j = m, s, l, p. \tag{2}$$

In Eq. (2), the dielectric constants of these materials at radiowave frequencies are real quantities since dipolar relaxation of the aqueous phase occurs at microwave frequencies and the orientational relaxation phenomena of the hydrophobic region of the bilayer occur at very low frequencies.

In Figs. 1 and 2 the conductivities of normal and pathological suspensions as a function of frequency, are shown at a temperature of 25 °C.

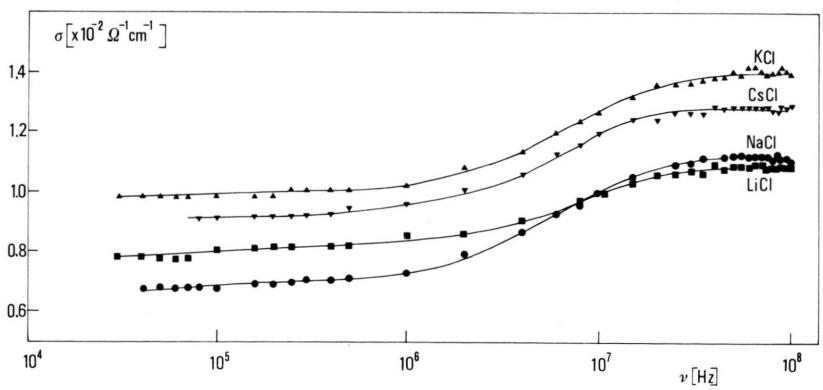

Fig. 1. Conductivity σ of normal erythrocyte cells in different electrolyte solutions as a function of frequency at a temperature of 25 °C. (●) LiCl; (■) NaCl; (▼) CsCl; (▲) KCl. Fractional volume of the dispersed phase $\Phi = 0.30$.

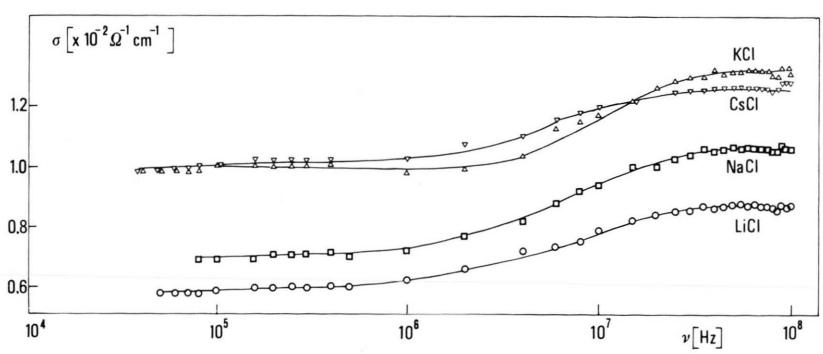

Fig. 2. Conductivity σ of homozygous β-thalassemic erythrocyte cells in different electrolyte solutions as a function of frequency at a temperature of 25 °C. (○) LiCl; (□) NaCl; (▽) CsCl; (△) KCl. Fractional volume of the dispersed phase $\Phi = 0.30$.

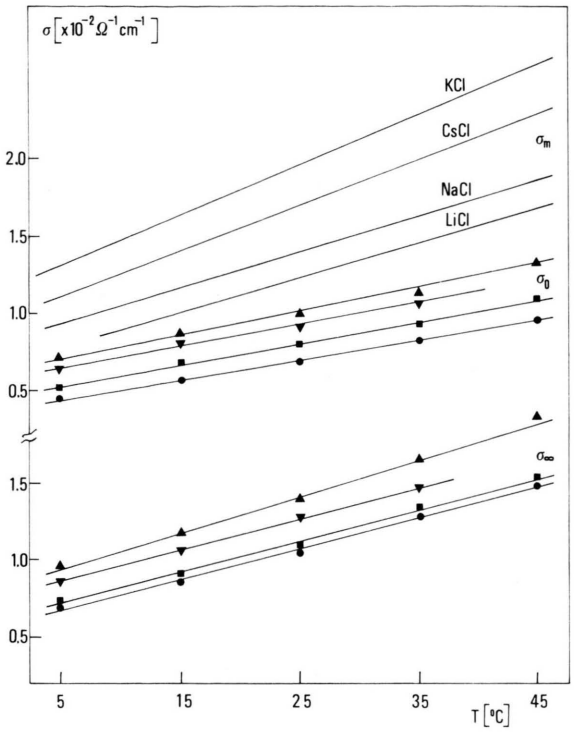

Fig. 3. Limiting parameters σ_0 and σ_∞ of the conductivity dispersion curve as a function of temperature for normal erythrocyte cells in different electrolyte solutions. (●) LiCl; (■) NaCl; (▼) CsCl; (▲) KCl. The measured conductivity σ_m of the different saline solutions are also reported.

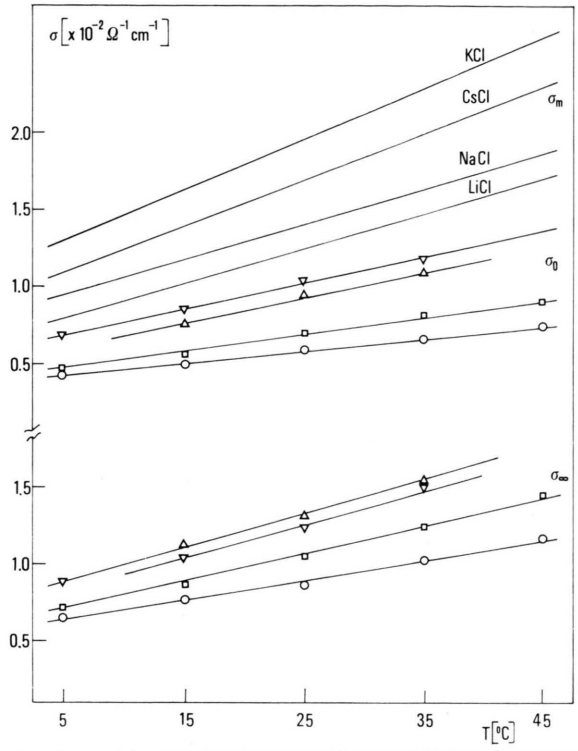

Fig. 4. Limiting parameters σ_0 and σ_∞ of the conductivity dispersion curve as a function of temperature for homozygous β-thalassemic erythrocyte cells in different electrolyte solutions. (○) LiCl; (□) NaCl; (▽) CsCl; (△) KCl. The measured conductivity σ_m of the different saline solutions are also reported.

The conductivity curves were analyzed in terms of a single Debye-type dispersion

$$\sigma = \sigma_0 + \frac{\omega^2 \tau^2 (\sigma_\infty - \sigma_0)}{1 + \omega^2 \tau^2}$$

where σ_0 and σ_∞ are the limiting values at low and high frequency respectively and τ is the relaxation time. In Figs. 3 and 4, σ_0 and σ_∞ are shown as a function of temperature.

Substituting of Eq. (2) in Eq. (1) yields the conductivity properties of the suspension as a function of frequency.

Results and Discussion

As pointed out elsewhere [1], the Maxwell-Wagner polarization effects due to the two bulk media (extracellular and intracellular medium) occur at very high frequencies ($\sim 10^3$ MHz) and consequently the observed conductivity dispersion must be due to polarizations confined in the membrane thickness and recognized as β dispersion.

In our model, the inner layer would be representative for the hydrophobic region and, as pointed out by Fettiplace *et al.* [12], this region would be expected to exhibit a dielectric constant of about 2.5, since this dielectric consists of aliphatic hydrocarbons essentially in liquid-crystalline phase and it has no strongly polarizable molecular components.

The conductivity is assumed of the order of $5 \times 10^{-7}\,\Omega^{-1}\,\mathrm{cm}^{-1}$.

It must be noted, however, that the determination of the membrane conductance is difficult to achieve, since this parameter is sensitive to the state of the cell and can also change by one order of magnitude.

The parameters σ_s, ε_s, σ_p are determined by fitting Eq. (1) to the measured conductivity values and are shown in Figs. 5 and 6 as a function of temperature.

The details of the fitting procedure and the influence of the required values of the other parameters on the conductivity profile have been already discussed [1, 13]. In particular, the dielectric constant of the suspending medium has been assumed equal to that of pure water, since its dielectric decrement is negligible, and the values reported by Asami *et al.* [13] for the dielectric constant of the intracellular medium of normal erythrocytes have been used.

The overall membrane thickness in assumed to be 50 Å and the hydrophobic layer of about 20 Å.

Fig. 5 shows the conductivity σ_p of internal medium of erythrocyte cells as derived from the fitting procedure.

Two characteristics attract attention:

- The first is that σ_p for all the samples dispersed in the various electrolytes is well below the values of the corresponding saline solution. Moreover, this value is about a half or lower than the value to be expected from the salt content of the cytoplasm. This situation was previously observed by Pauly and Schwan [14] for erythrocytes in physiological saline solution. These authors measured the conductivity of thightly packed erythrocytes (Hct = 98.2%) and a value of about 5.2×10^{-3} $\Omega^{-1}\,cm^{-1}$ was obtained at 25 °C, whereas, the addition of the contributions of the different ionic species present inside the cell would result in an internal conductivity of about 14.5×10^{-3} $\Omega^{-1}\,cm^{-1}$. This feature, observed in various biological systems [15, 17] appears to be due to a reduction of the ionic mobility caused by electrical or hydrodynamic interactions with the various cell components.
- Secondly, in normal red blood cells, it was found that, of the four alkaline ions, only Li^+ has significantly effect on the conductivity of the internal medium, whereas Na^+, K^+, Cs^+ appear to influence, in the whole temperature range, the internal conductivity in the same way.

On the contrary, in pathological erythrocytes, σ_p assumes approximately the same value, within the experimental uncertainties, for all the samples but, as can be seen in Fig. 5, σ_p lies on an average level lower than that of normal cells.

To justify these fenomenological findings, we have carried out measurements of ion concentration

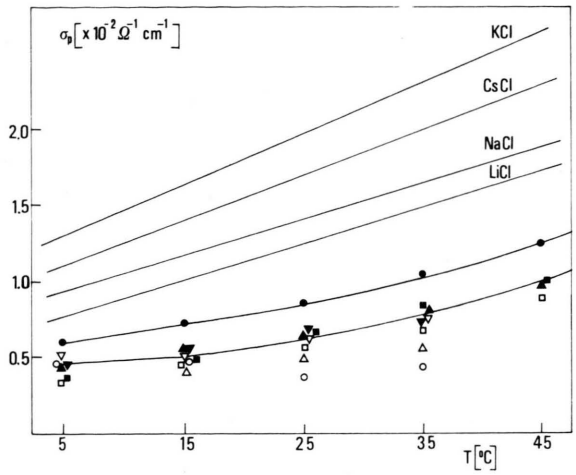

Fig. 5. Conductivity σ_p of the internal medium for normal and homozygous β-thalassemic erythrocyte cells as a function of temperature. For the symbols see the legend in Figs. 1 and 2. The conductivity of the various saline solutions are also reported for comparison.

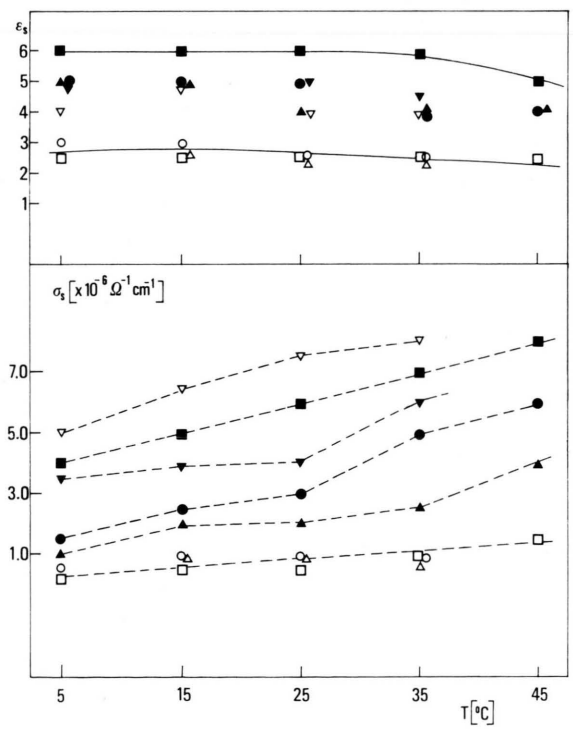

Fig. 6. The dielectric constant ε_s and the conductivity σ_s of the hydrophylic layer of normal and homozygous β-thalassemic membrane as a function of temperature derived from the fitting procedure (see text). For symbols see the legends of Figs. 1 and 2. The lines are drawn for visual purpose only.

inside erythrocyte cells by means of atomic absorption spectrometry.

For normal samples in physiological saline solution (0.15 M NaCl), the Na^+ and K^+ concentrations yield values of 2.5×10^{-2} M and 8.2×10^{-2} M respectively, which are in agreement with the literature data.

For the cells dispersed in other electrolyte solutions, *i.e.*, 0.15 M KCl, CsCl, the concentration of Na^+ and K^+ ions in the intracellular medium vary within about a factor two or three.

These results are in agreement with the fact that the conductivity σ_p is approximately the same for the samples, when dispersed in NaCl, KCl, CsCl.

On the contrary, the internal medium of normal cells dispersed in LiCl possesses the same concentration of K^+ and Na^+ ions, but a very high concentration of Li^+ ions is present ($\sim 2 \times 10^{-2}$ M). This relevant increment of Li^+ ion concentration justifies the observed increment in the σ_p conductivity.

The absence of such a kind of effect in the case of pathological cells dispersed in the same lithium solution may be due to a different transparence of the cellular membrane to Li^+ ions. This probably could be due to an alteration of the specific pathways through the membrane, which govern the transport mechanism of this small charged solute.

The other two parameters derived from the fitting procedure, *i.e.*, ε_s and σ_s, describe the electrical properties of the more external layer of the membrane which consists, in our model, of both polar head groups of the phospholipids and the hydration of the various dissociable groups of polyelectrolytes anchored to the membrane.

As above pointed out, the analysis of the experimental data was carried out assuming for ε_l and σ_l values of the order of 2.5 and $5 \times 10^{-7}\ \Omega^{-1}\,cm^{-1}$ respectively, independent, for normal and pathological cells, of the electrolyte environment.

This was done on the indication coming from electron spin resonance measurements performed on the erythrocyte membrane of red blood cells dispersed in the same electrolyte solutions and labelled with two different probes, 5NSA and 16NSA, (5- or 16-nitroxide stearic acid) with the nitroxy groups located at the 5th and 16th position, exploring different regions across the membrane.

The results of these measurements [18] support the idea that the hydrophobic region of the lipid bilayer is not altered by the ionic species, since the coupling constant a'_N, related to the local polarity of the environment, remains unchanged, in the limit of the experimental errors, in the presence of the various ions.

On the other hand the small difference of a'_N observed between normal and pathological cells does not justify a change in the values assumed for ε_l and σ_l.

The values of ε_s and σ_s as a function of temperature are shown in Fig. 6.

For normal samples, the conductivity σ_s depends on the ionic species giving further support to selective permeability behaviour of the cell membrane.

In our membrane model, σ_s assumes values of the order of $1 \div 10 \times 10^{-6}\ \Omega^{-1}\,cm^{-1}$ and takes into account the hydrophylic layer with the hydration shell.

The low value of the overall membrane conductivity in comparison with those of the external and internal media produces the observed pronounced conductivity increments.

On the other hand, the assumption of a membrane-near space with a marked decrease of the ionic mobility has been previously proposed by Wolf *et al.* [19] to explain measurements of conductivity in human red blood cell suspensions.

In fact, small ions are able to penetrate between polyelectrolyte segments and inside the polar head region, but their mobility is reduced by electrostatic interactions with charged ionized groups.

Some observations can be drawn from an inspection of Fig. 6. The dielectric constant ε_s of the outer shells of the membrane assumes a value of about 6 for the sample dispersed in NaCl and a somewhat lower value, about 5, for the samples in KCl, LiCl, CsCl, with a moderate reduction as the temperature is increased.

These values correspond to a specific capacitance of about 1.8 and 1.4 $\mu F/cm^2$ respectively. These values seem to be appreciably larger than those previously reported [1] but, it must be noted that in this work a more elaborate membrane model has been adopted and these capacitance values refer to the polar head group layers only.

The lower value of ε_s observed in pathological membrane, for all the samples investigated, corresponds to a specific capacitance which approaches that of a lipid bilayer membrane ($0.4 \div 0.6\ \mu F/cm^2$).

This fact suggests, as already pointed out [1, 12] a different interaction of hydrocarbon chains with non-polar residues of proteins or, more probably a reduced presence, in the pathological state, of the transmembraneous proteins immersed in the hydrocarbon region of the cell membrane.

It must be observed, moreover, that the value of ε_s for the sample in CsCl is a somewhat higher than that observed for the other ionic species.

This anomalous behaviour of Cs^+ ions is also reflected in the corresponding value of σ_s, as can be seen in Fig. 6.

Whereas the effect of different ions is moderate for the dielectric constant ε_s, a more pronounced differentiation is present for the conductivity σ_s, demonstrating that the permeation of the membrane is strongly dependent on the nature of the surface layers in contact with the saline solution.

The monovalent cations studied line-up in the following series $Na^+ > Cs^+ > Li^+ > K^+$ for normal membrane and $Cs^+ \gg Na^+ \cong Li^+ \cong K^+$ for pathological membranes.

These series differ both from that corresponding to the conductivity of the bulk solution and that of the structure-breaking properties of these cations as $Cs^+ > K^+ > Na^+ > Li^+$.

It is noteworth the fact that the difference in the σ_s values between the normal and pathological membrane could be due to the presence of proteins which increase the dielectric constant of the normal membrane. In fact, it has been pointed out by Dilger *et al.* [20] that the permeability of a phospholipid bilayer membrane to ions depends on the dielectric constant of the membrane. These indications seem to be consistent with the results shown in Fig. 6 where low values of the dielectric constant correspond low values of the conductivity σ_s, that, although in an indirect manner, must be related to the membrane permeabilities.

As pointed out by Rachmilewitz [21] the cation permeability of thalassemic membrane, particularly for K^+ ions, should be altered in comparison with normal cells. Fig. 6 shows, on the contrary, that Li^+, Na^+, K^+ ions produce approximately the same influence on the σ_s values, even if these lie on a lower level. This may result from the fact that σ_s describes the conductivity behaviour of the hydrophylic region of the membrane, whereas the efflux of different ions across the membrane is governed by the properties of the membrane as a whole.

The qualitative features of the results lead to the conclusion that striking differences in the electrical parameters exist between the normal and β-thalassemic membrane of erythrocyte cells.

The present work provides some of the experimental data useful to suggest consequences for the different types of hydrophobic layer-ion interactions which can be of interest in membrane models or which occur in the pathological membrane, since ionic interactions are critically important for the structure and function of many biomembranes.

Acknowledgements

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