

ETHANOL EFFECTS ON THE IN VITRO STABILITY OF HUMAN ERYTHROCYTES

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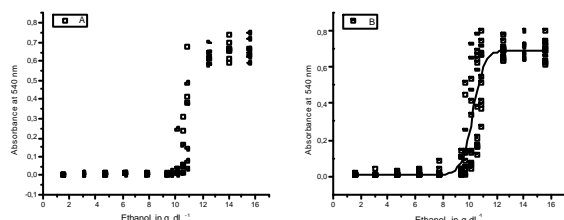
Introdução

In vitro, ethanol presents chaotropic effects on membrane¹ and cytoskeleton proteins². It is a known denaturant agent that can promote the exposure of apolar groups in the protein unfolding process³. For the same reason, incorporation of ethanol to the membrane environment will be associated to promotion of exposure of apolar groups in a membrane denaturation. The direct study of ethanol on membrane *in vitro* can show the basic principles about important effects of this drug in cells' metabolism. This work subject is investigate and discuss the direct effects of ethanol on stability of erythrocyte membrane.

Resultados e Discussão

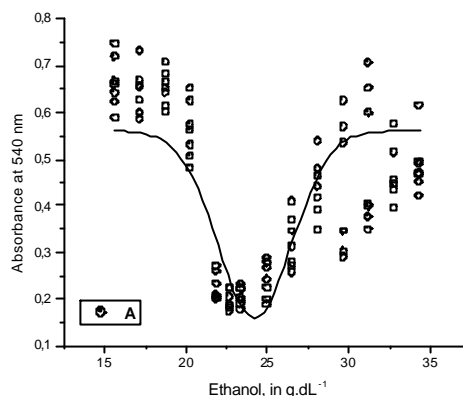
The stability of erythrocytes against ethanol presented a sigmoidal shape, with the half transition points (D_{50}) at 11.05 ± 0.25 and 10.35 ± 0.25 g.dL⁻¹ ethanol in 0.9 and 0.6% NaCl, respectively (Figure 1A and B).

Figura 1. Effect of the ethanol concentration on the stability of human erythrocytes in 0.9 (A) and 0.6% (B) NaCl.



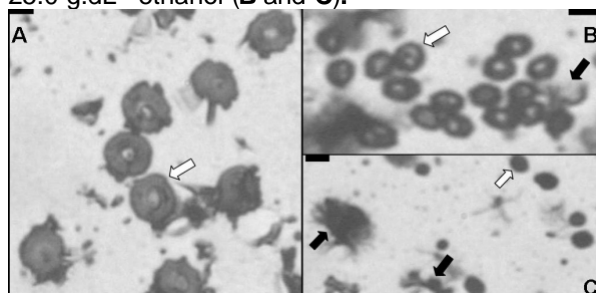
Unexpected, after values of 20,0 g.dL⁻¹ (figure 2), begins a type of protection on erythrocyte membrane, with maximum protection in 24,0 g.dL⁻¹ and end in 30,0 g.dL⁻¹.

Figure 2. Ethanol's protective effects on membrane.



The light microscopy demonstrated the shape of erythrocytes in the various curves described above (figure 3). In maximum protection post-translational curve (figure 2), little erythrocytes intact morphologically are visible associated with destructed erythrocytes (figure 3C). These facts implies that the effect of NaCl on the preservation of membranes (Figure 2) isn't solely an osmolarly based phenomenon and depends on electrical effects of the solute to keep integer the erythrocyte membrane.

Figure 3. Light microscopy images of human erythrocytes in 0.9% NaCl solutions with 1.56 (A) and 25.0 g.dL⁻¹ ethanol (B and C).



Elevated concentrations of ethanol can interfere in the Nernst potential of Na⁺ and Cl⁻, changing the physical behavior of the membrane. The dielectric constant is highly perturbed by the presence of solutes⁴. Incorporation of ethanol to the saline solution will decrease the dielectric constant and increase the attractive forces between reciprocal charges. The polar forces between ethanol and ions and ethanol and water are bigger than the ethanol apolar force between tails of lipids of membrane. Close to the transition region, the ethanol concentration in the solution is high enough to compete with the hydrophobic regions of the membrane lipids for the hydrophobic contacts.

Conclusões

Ethanol presents a denaturant effect on the erythrocyte membrane with a half transition point that can be significantly decreased from 11.05 ± 0.25 to 10.35 ± 0.25 g.dL⁻¹ with a decrease in the NaCl concentration from 0.9 to 0.6%. It also presents a protective effect on the erythrocyte membrane with maximal protective points in 25.20 ± 1.71 and 26.26 ± 2.05 g.dL⁻¹ in the presence of 0.9 and 0.6% NaCl, respectively.

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