On the mechanism of stomatocyte – echinocyte transformations of red blood cells: Experiment and theoretical model

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Aim of the Study

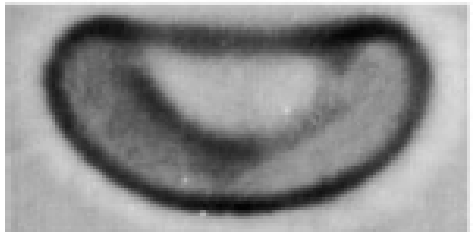
To understand why a transition stomatocyte–discocyte–echinocyte occurs in the shape of red blood cells, when the ionic strength in the outer medium is increased.

This transition is accompanied by a variation of the transmembrane electric potential (Glaser, 1993):

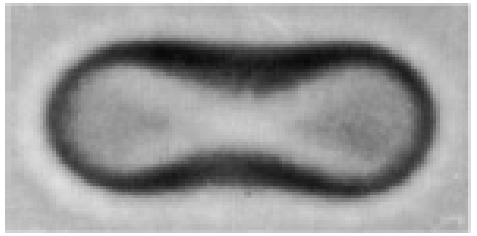
$\Delta\psi = \psi_{in} - \psi_{out} > 0$ for stomatocyte

$\Delta\psi = \psi_{in} - \psi_{out} \approx 0$ for discocyte

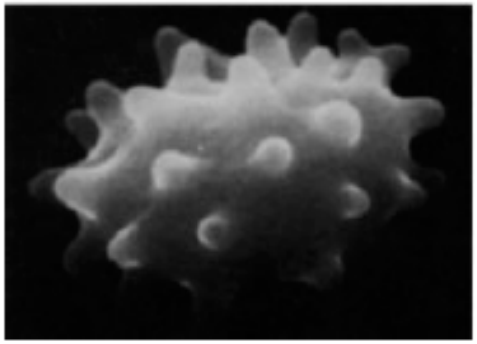
$\Delta\psi = \psi_{in} - \psi_{out} < 0$ for echinocyte



stomatocyte

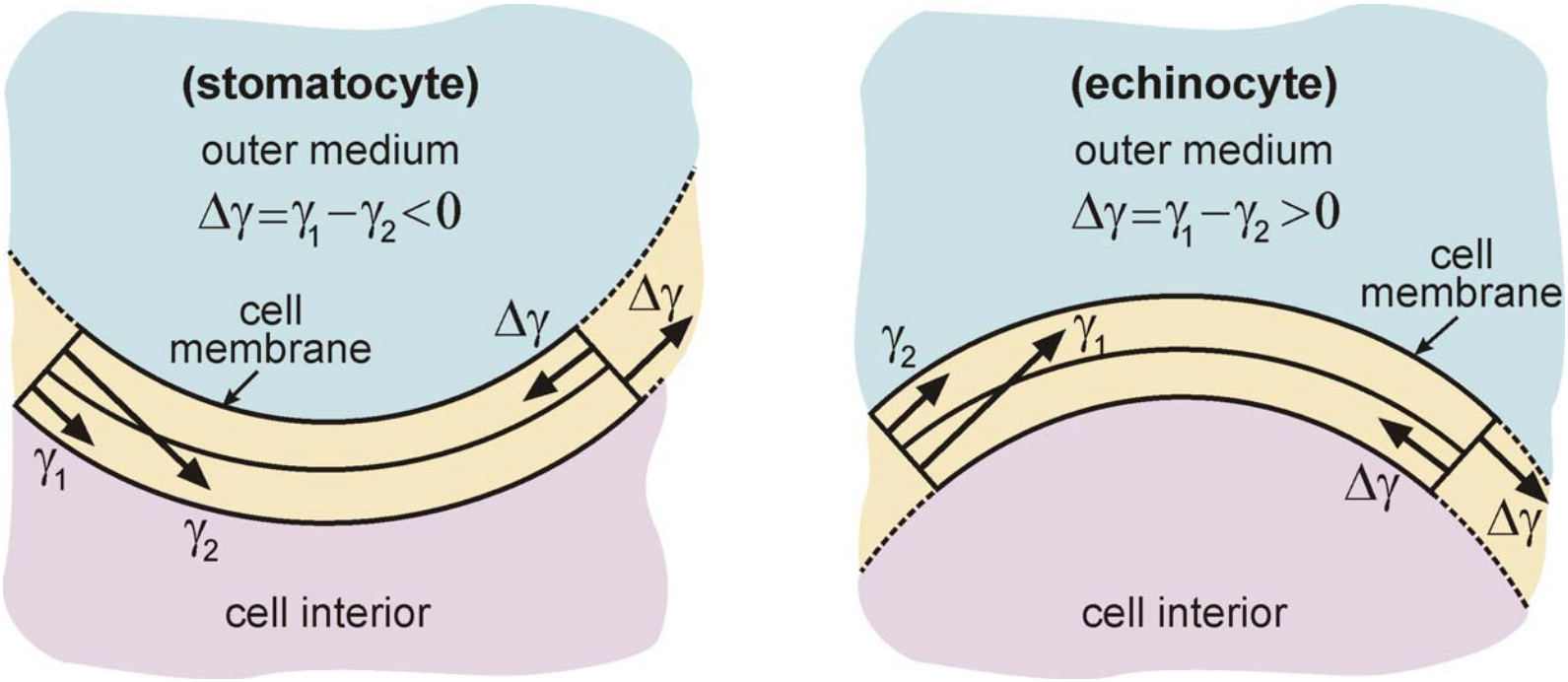


discocyte



echinocyte

The bilayer-couple hypothesis



(Greater tension, γ , $\Rightarrow$ Greater contraction; Sheetz & Singer, 1974)

Any contraction of the outer leaflet, relative to the inner one, favors the formation of cavities (stomatocytosis) to accommodate the extra area.

Any factor leading to contraction of the inner leaflet relative to the outer one, produces convex structures, as the echinocytic spicules.

Electric Double Layer (EDL) Paradox

Contribution of EDL to the tension of the outer leaflet, γ_2 :

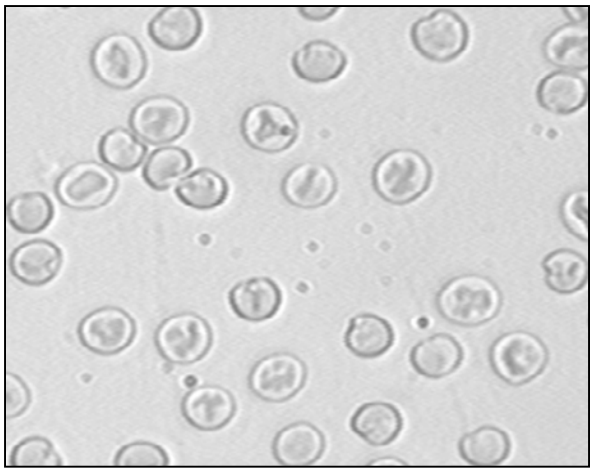
$$\gamma_2 = \gamma_0 - 8kT\frac{I_2}{\kappa_2}\left[\cosh(\Phi_{2s}/2)-1\right]; \qquad \Phi_{2s} = \frac{e\psi_{2s}}{kT}$$

Φ_{2s} – dimensionless potential of the outer membrane surface

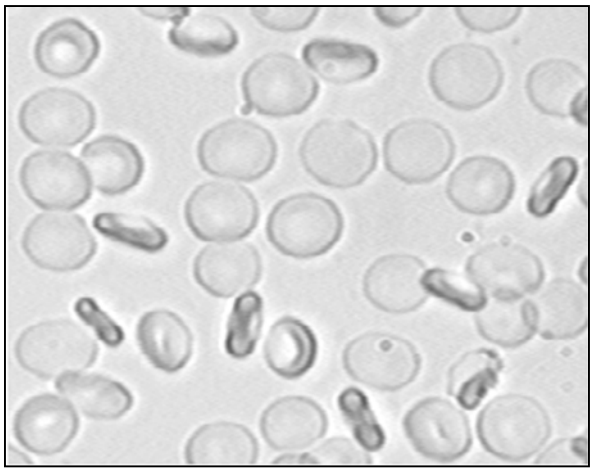
Paradox: With the increase of the ionic strength, I_2, in the outer medium, $\cosh(\Phi_{2s}/2)$ decreases, and the tension γ_2 increases. This tendency is exactly the opposite to the experimental observations! (Rasia, Bollini, 1998).

Key to solve the paradox: The increase of I_2 leads not only to suppression of the diffuse EDL, but also to an increasing binging of counterions at the outer membrane surface.

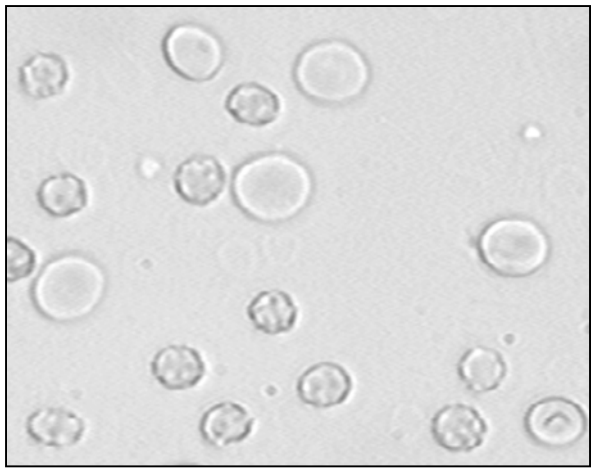
Experiment: Shapes of Erythrocytes



I_2 = 46 mM, stomatocytes



I_2 = 96 mM, discocytes

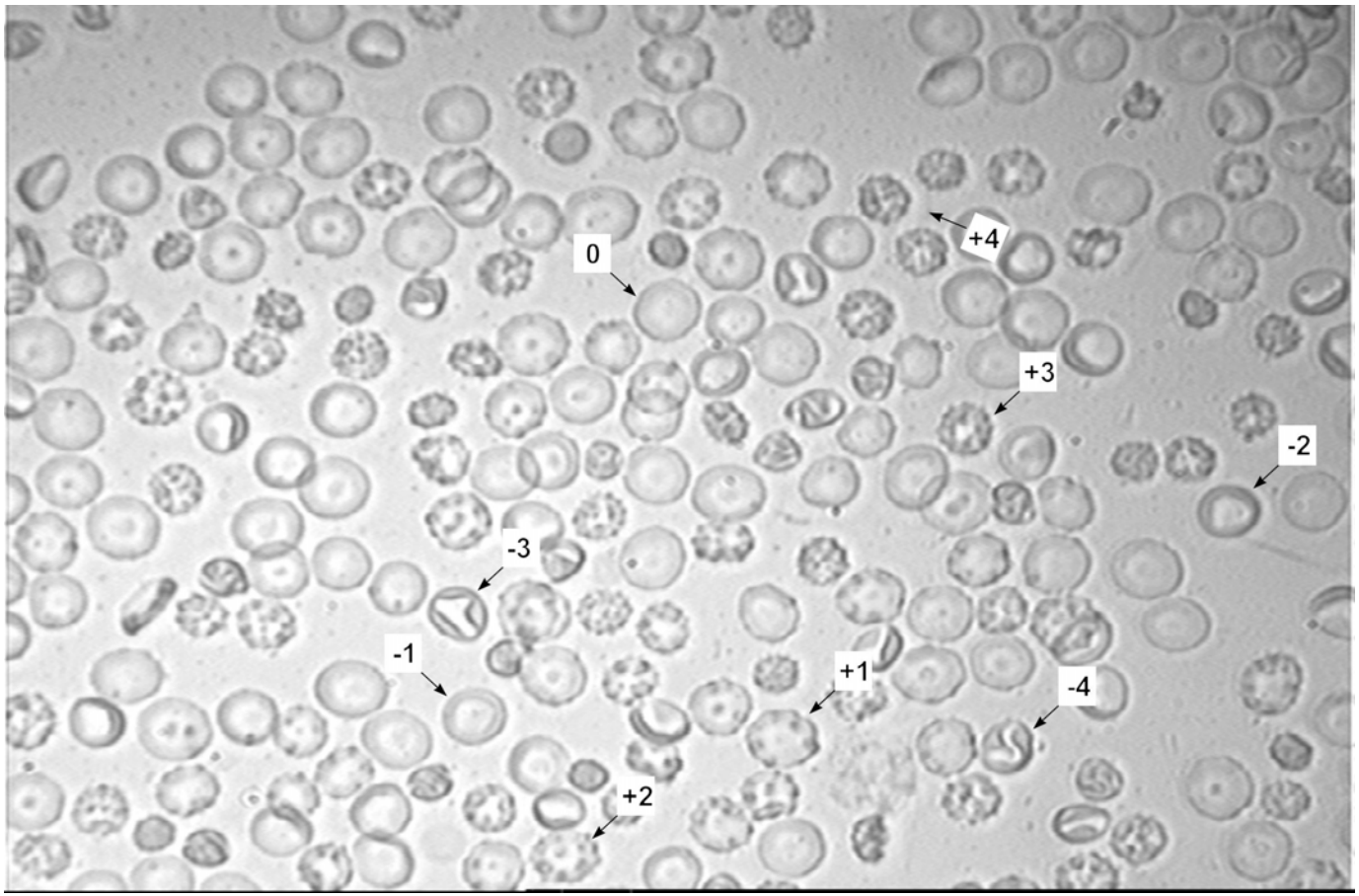


I_2 = 300 mM, echinocytes

The osmolarity is adjusted 290 mOsm by addition of sucrose and Tris-buffer.

In some experiments, the RBC were treated by the enzyme trypcin, which hydrolyses a part of the cell glycocalyx thus decreasing the negative electric charge at the outer membrane surface.

I_2 is varied, while I_1 is calculated – model by Lew & Bookchin (1986)

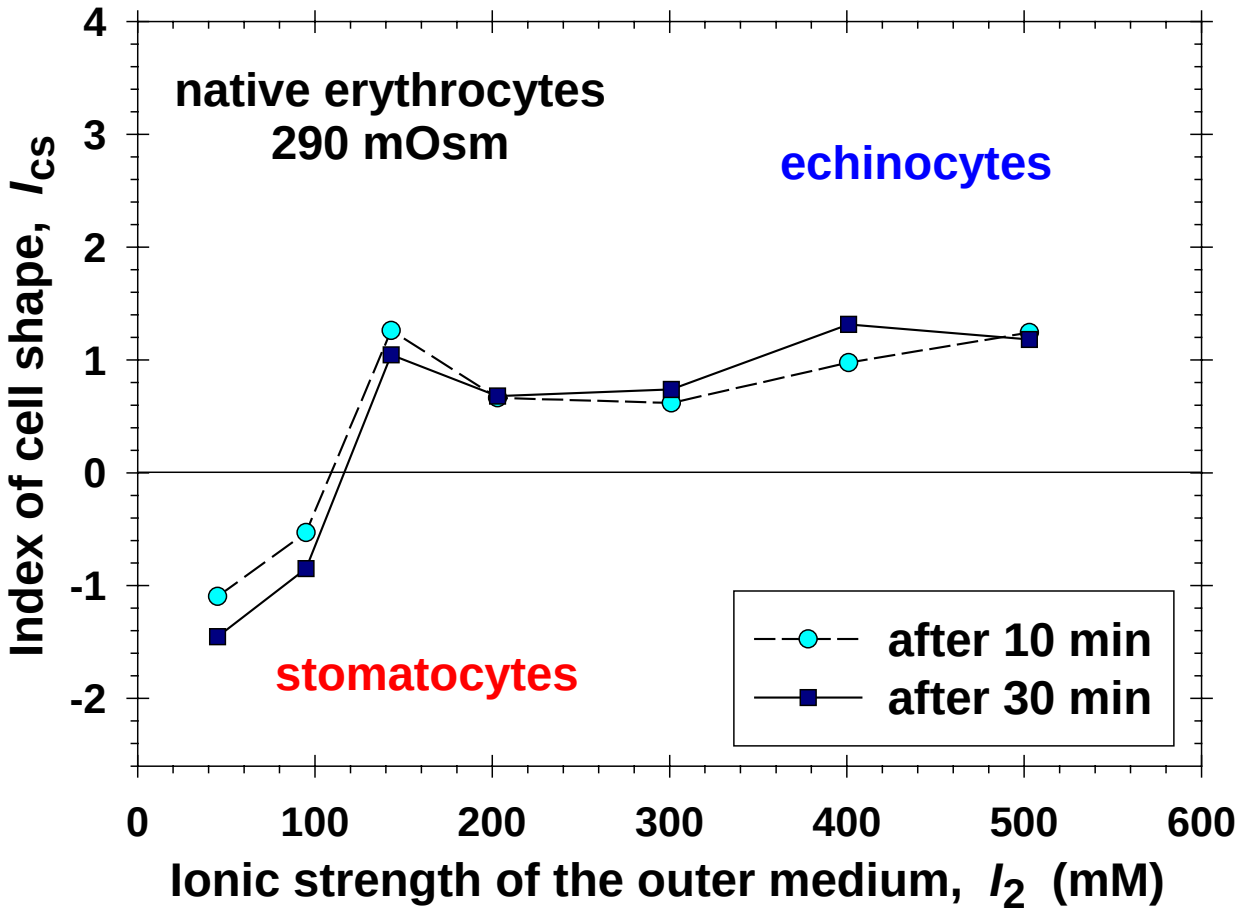


Index of Cell Shape: **Biconcave disc (shape index 0);**

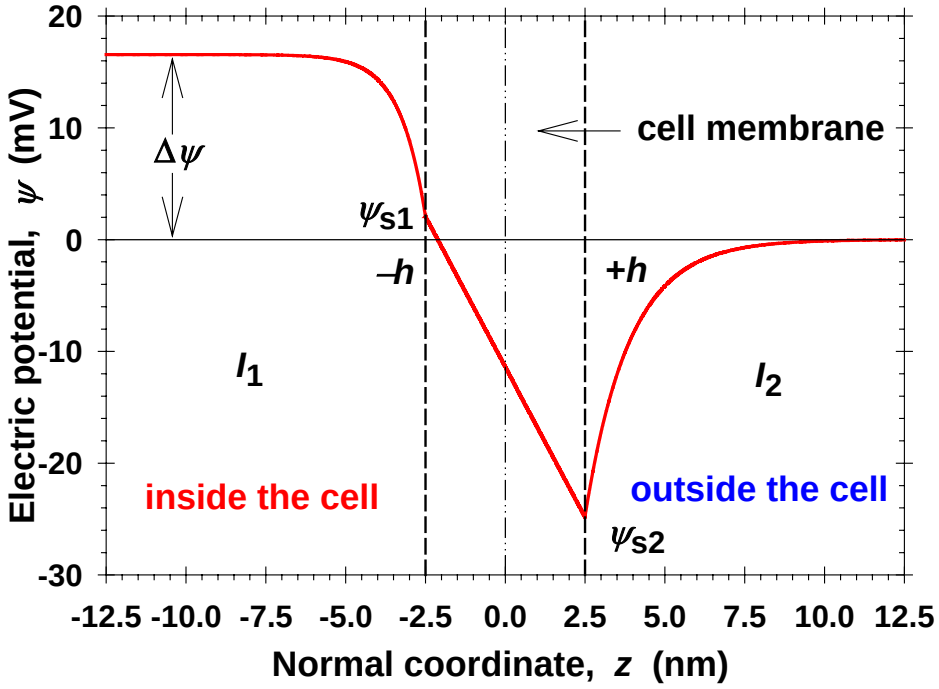
Three types of stomatocytes (indexes –1, –2 and –3) to spherostomatocyte (index –4);

Three degrees of echinocytes (indexes +1, +2 and +3) to spheroechinocyte (index +4).

Dependence of the Shape Index on the Ionic Strength



Plot of the experimental index of cell shape, I_{cs}, vs. the ionic strength of the outer medium: values measured 10 and 30 min after the immersion of the cells in the respective solutions.



⇐ **Distribution of the electric potential across the membrane at the lower ionic strengths (stomatocyte).**

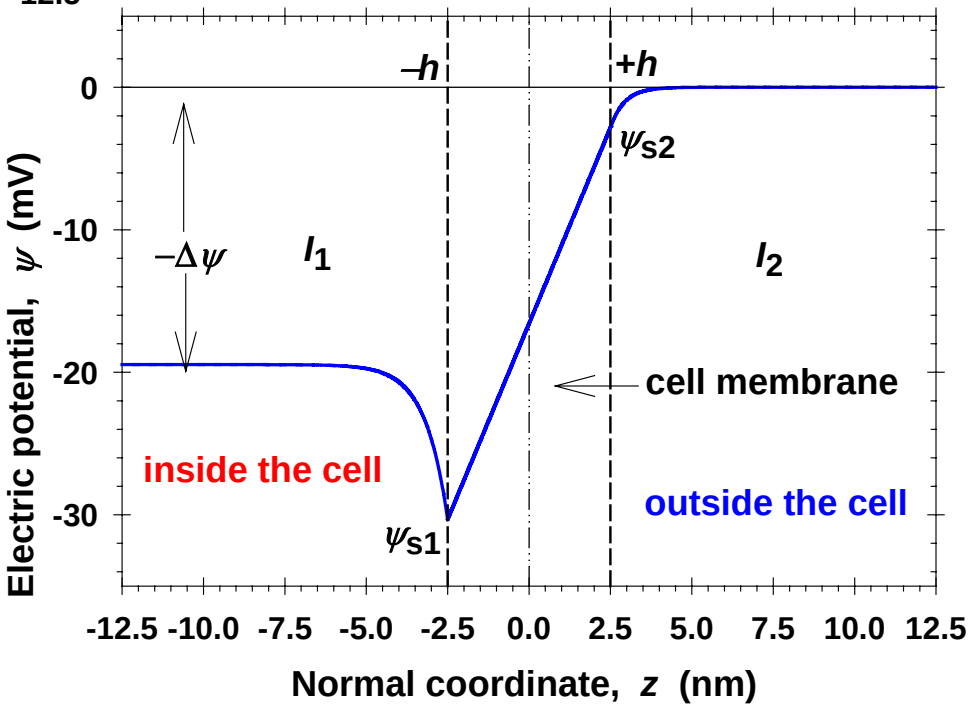
Distribution of the electric potential across the membrane at the higher ionic strengths (echinocyte). ⇓

$$\Delta\Phi = \frac{e\Delta\psi}{kT}, \quad \Phi_i = \frac{e\psi_i}{kT},$$

$$\Phi_{si} = \frac{e\psi_{si}}{kT}, \quad (i = 1, 2)$$

$$\tanh\left(\frac{\Phi_2}{4}\right) = \tanh\left(\frac{\Phi_{s2}}{4}\right)\exp[-\kappa_2(z-h)]$$

$$\tanh\left(\frac{\Phi_1 - \Delta\Phi}{4}\right) = \tanh\left(\frac{\Phi_{s1} - \Delta\Phi}{4}\right)\exp[\kappa_1(z+h)]$$



Theoretical Model: Distribution of the Electric Potential

Standard EDL theory + Stern isotherm of counterion adsorption:

$$\frac{\Gamma_2^{(1)}}{\Gamma_1^{(1)}} = \frac{K_1 I_1 \exp(\Delta\Phi - \Phi_{s1})}{1 + K_1 I_1 \exp(\Delta\Phi - \Phi_{s1})} \qquad \frac{\Gamma_2^{(2)}}{\Gamma_1^{(2)}} = \frac{K_2 I_2 \exp(-\Phi_{s2})}{1 + K_2 I_2 \exp(-\Phi_{s2})}$$

$\Gamma_1^{(1)}$ – surface density of ionizable groups at the inner membrane surface due to phosphatidylserine (4 nm^2 per charge).

$\Gamma_1^{(2)}$ – surface density of ionizable groups at the outer membrane surface due to sialic acid in glycocalyx (8 nm^2 per charge).

$\Gamma_2^{(1)}$ and $\Gamma_2^{(2)}$ – adsorptions of counterions at the inner and outer surfaces; K_1 and K_2 – adsorption constants.

Full set of equations is obtained to determine all parameters of the model, see:

Tachev, K., et al., *Colloids Surf. B, Biointerfaces* 34 (2004) 123-140.

Effect of Ionic Strength on the Membrane Tension

Tension differences between the two leaflets:

$$\Delta\gamma = \Delta\gamma_{ad} + \Delta\gamma_{el}, \qquad \Delta\gamma_{ad} = \gamma_{1,ad} - \gamma_{2,ad}, \qquad \Delta\gamma_{el} = \gamma_{1,el} - \gamma_{2,el}$$

Adsorption components of the tension (Langmuir adsorption isotherm):

$$\gamma_{1,ad} = kT\,\Gamma_1^{(1)} \ln[\frac{1 + K_1 I_1 \exp(\Delta\Phi - \Phi_{s1})}{1 + K_1 I_{1,st} \exp(\Delta\Phi^{(st)} - \Phi_{s1}^{(st)})}], \qquad \gamma_{2,ad} = kT\,\Gamma_1^{(2)} \ln[\frac{1 + K_2 I_2 \exp(-\Phi_{s2})}{1 + K_2 I_2^{(st)} \exp(-\Phi_{s2}^{(st)})}]$$

Electric components of the tension (from the theory of the diffuse EDL):

$$\gamma_{1,el} = 8kT\{\frac{I_1^{(st)}}{\kappa_1^{(st)}}[\cosh(\frac{\Delta\Phi^{(st)} - \Phi_{s1}^{(st)}}{2}) - 1] - \frac{I_1}{\kappa_1}[\cosh(\frac{\Delta\Phi - \Phi_{s1}}{2}) - 1]\}$$

$$\gamma_{2,el} = 8kT\{\frac{I_2^{(st)}}{\kappa_2^{(st)}}[\cosh(\frac{\Phi_{s2}^{(st)}}{2}) - 1] - \frac{I_2}{\kappa_2}[\cosh(\frac{\Phi_{s2}}{2}) - 1]\}$$

All tensions are defined relative to the state of a RBC at standard physiological conditions (discocyte) – assumed to be tension free state.

Relation between Cell Shape and Membrane Tension

Hooke's law for the area difference between the two leaflets, ΔA_0 :

$$\frac{\Delta A_0}{A} = \frac{1}{K_s}\Delta\gamma$$

***A* – total area of the membrane; K_s – dilatational elastic modulus for one membrane leaflet.**

The cell shape index is expected to depend on the area difference ΔA_0 :

$$I_{cs} = I_{cs}(\Delta A_0) = a_0 + a_1\Delta A_0 + \ldots$$

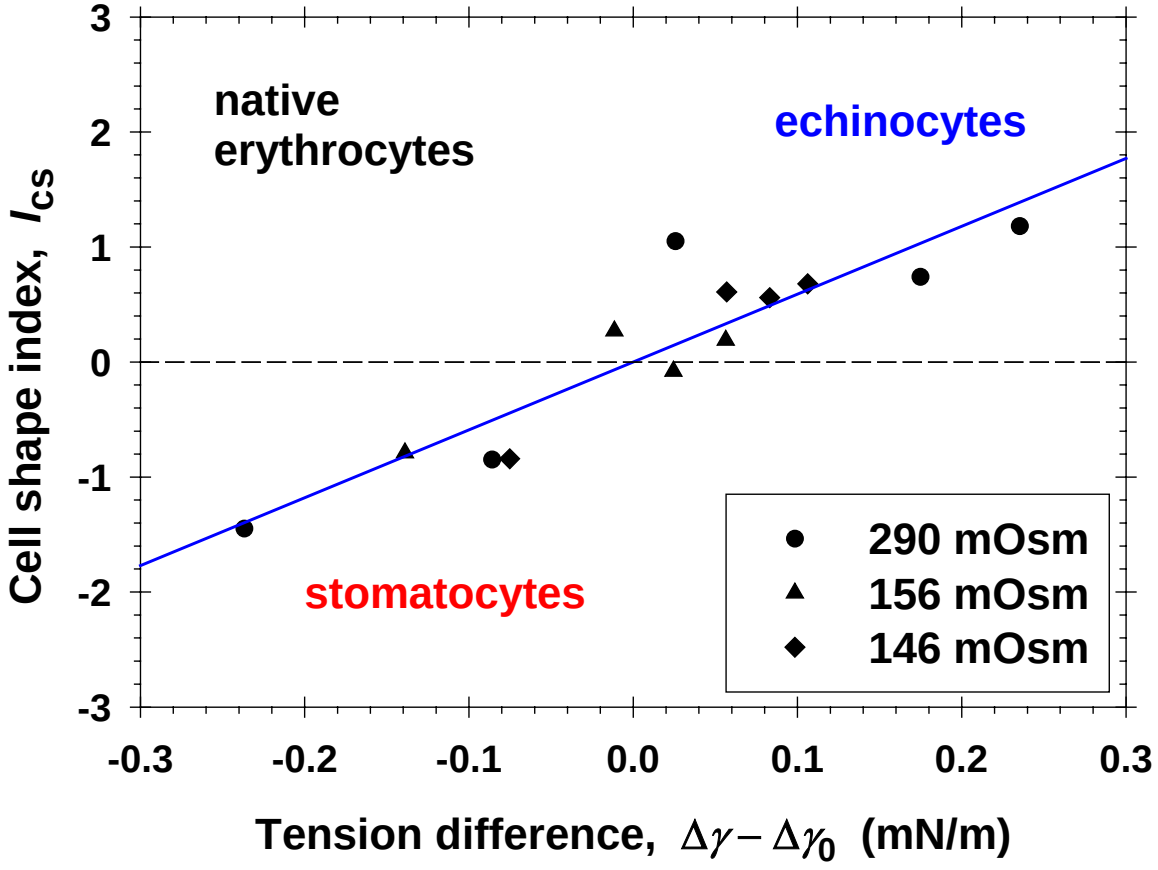
(series expansion for small ΔA_0)

$$\Rightarrow \qquad I_{cs} = a_0 + a_1\frac{A}{K_s}\Delta\gamma + \ldots \approx C(\Delta\gamma - \Delta\gamma_0)$$

$\Delta\gamma_0$ is the tension difference for a discocyte, for which I_{cs} = 0.

***C* = 5.9 m/mN is a coefficient, which is determined from the experiment.**

Experimental index I_{cs} vs. the theoretical $\Delta\gamma - \Delta\gamma_0$



$\Delta\gamma - \Delta\gamma_0$ varies because of the variation of the outer ionic strength, I_2, and the osmolarity.

Similar fit is obtained for the trypsin-treated cells.

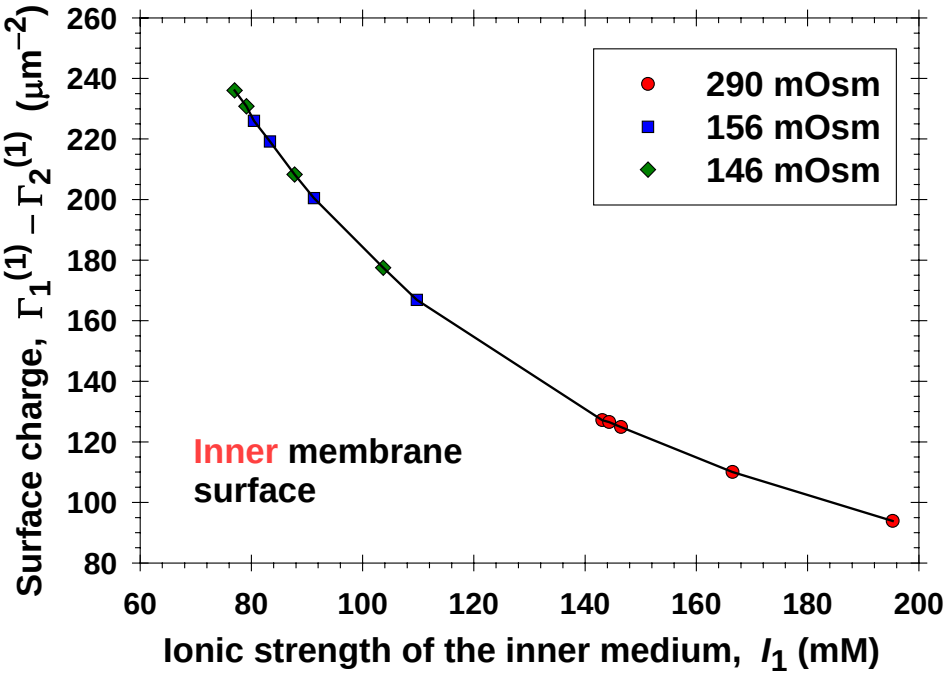
Stern adsorption constants:

$$K_i = \frac{\delta_i}{\Gamma_1^{(i)}} \exp\left(\frac{\Delta\mu^{(i)}}{kT}\right)$$

The counterion adsorption energies are obtained as adjustable parameters:

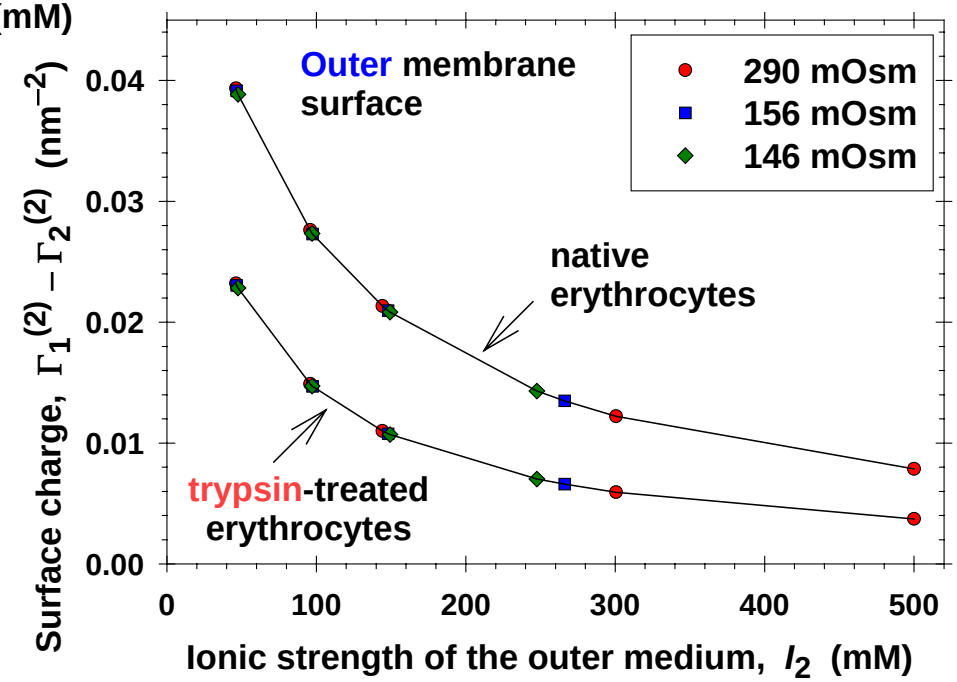
$\Delta\mu^{(1)} = 9\,kT$ for the inner interface (at the phosphatidylserine headgroups)

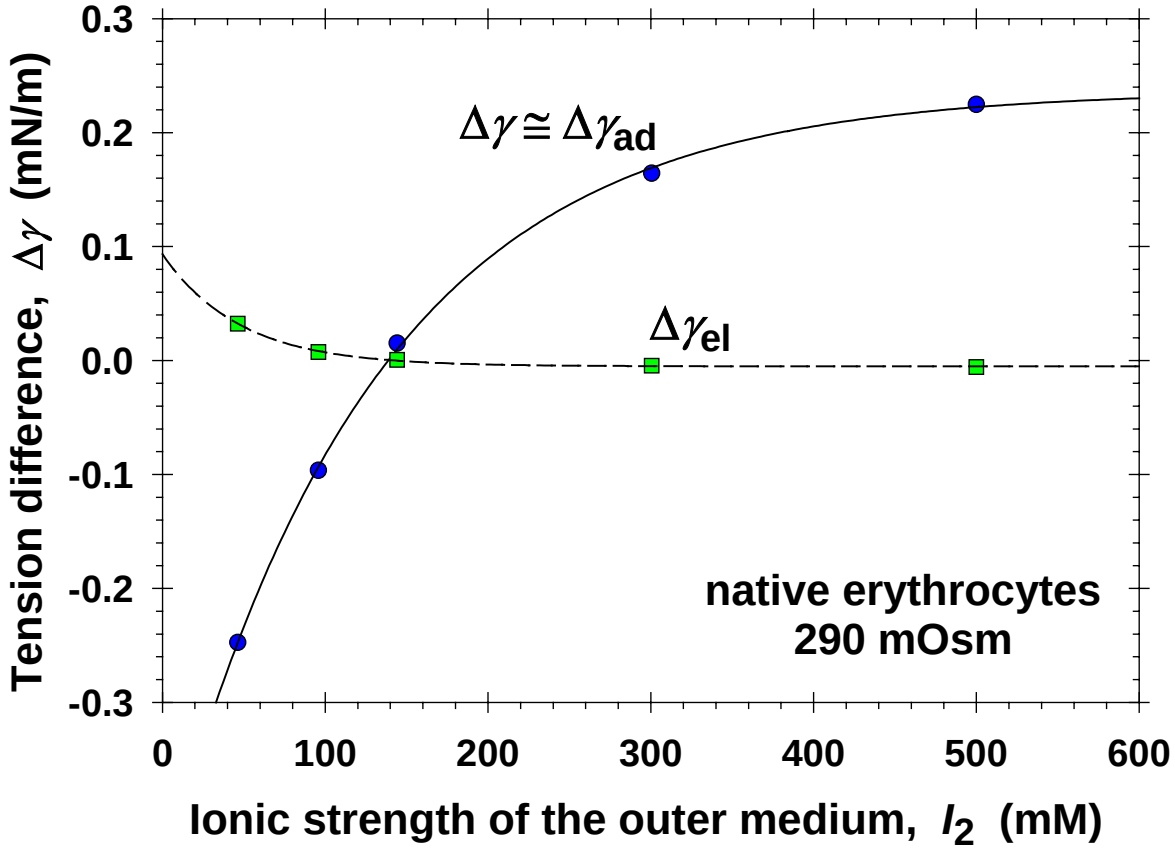
$\Delta\mu^{(1)} = 2.1\,kT$ for the outer interface (at sialic acid headgroups / glycocalyx)



The net charge density at the inner surface is about 100 times lower than on the outer surface. This considerable difference is due to the greater adsorption energy of the counterions at the inner surface: $\Delta\mu^{(1)} \approx 9\ kT$ versus $\Delta\mu^{(2)} = 2.1\ kT$ at the outer surface.

The charge density at the outer surface is pronouncedly smaller for the treated cells. The trypsin is known to remove a part of the glycocalyx and to reduce the density of negative ionizable groups at the outer membrane surface.





Plot of the tension differences due to the counterion adsorption and electric double layer, $\Delta\gamma_{ad}$ and $\Delta\gamma_{el}$ vs. the ionic strength of the outer medium, I_2.

$\Delta\gamma_{el}$ decreases, whereas $\Delta\gamma_{ad}$ increases with the ionic strength. The behavior of $\Delta\gamma_{el}$ could be considered as a contradiction with the bilayer-couple hypothesis .

It turns out that $|\Delta\gamma_{el}| << |\Delta\gamma_{ad}|$, and that $\Delta\gamma$ is completely dominated by $\Delta\gamma_{ad}$, which varies in the opposite to $\Delta\gamma_{el}$ direction. Hence, $\Delta\gamma_{ad}$ governs the changes in the cell shape, and there is no contradiction between the bilayer-couple hypothesis and the electric double layer theory.

Summary and Conclusions

1. The index of cell shape is experimentally determined at various ionic strengths and osmolarities for native and trypsin-treated erythrocytes.

2. We described theoretically the electric double layers formed on both sides of the cell membrane, and derived expressions for the tensions of the two membrane leaflets.

3. Taking into account that the cell-shape index depends on the tension difference between the two leaflets, $\Delta\gamma$, we fitted the experimental data with the constructed physicochemical model.

4. The model agrees well with the experiment and indicates that $\Delta\gamma$ is governed by the different adsorptions of counterions at the two membrane surfaces, rather than by the contribution of the diffuse electric double layers.

5. With the rise of the ionic strength, the counterion adsorption increases stronger at the outer leaflet, whose stretching surface pressure becomes greater, and whose area expands relative to that of the inner leaflet.

6. The developed quantitative model can be applied to predict the shape index of cells upon a stomatocyte-discocyte-echinocyte transformation at varying composition of the outer medium.

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