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Parameters characterizing acid–base equilibria between cell membrane and solution and their application to monitoring the effect of various factors on the membrane

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Abstract

The cell membrane is an extremely complicated object. It participates in a large number of equilibria. For this reason, it is impossible to determine the parameters of all of them. It is the purpose of this work to define a limited number of averaged parameters in order to describe the equilibria between cell membrane components and environmental components. These parameters are the total acidic functional group concentration as well as the basic group concentration and their association constants with hydrogen or hydroxyl ions. The parameters were determined using the pH dependence of the electric surface charge density.

The usefulness of these parameters was checked by studying the effect of green tea on liver cells in ethanol poisoning. Ethanol provokes an increase in concentration of functional groups, positively and negatively charged, as well as an increase in the basic groups association constant and a decrease in acidic groups association constant. Administering green tea partly compensates the changes provoked by ethanol poisoning. The parameters proposed in this work, C_{TA}, C_{TB}, K_{AH} and K_{BOH}, are suited for monitoring the changes caused by various factors.
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Keywords: Surface charge density; Liver cell; Ethanol; Green tea

1. Introduction

The liver cell membrane is negatively charged in physiological pH [1]. Its charge value is determined by the number of negative and positive charge carriers, i.e. phospholipids and amino groups of proteins and amino phospholipids, respectively. Electric properties of the membrane are determined by acid–base and complex formation equilibria in which membrane and surrounding medium components are involved [2,3]. The majority of the membrane components are involved in those equilibria but phospholipids and proteins play a major role.

Any perturbation in the action of the cell is manifested by variations in the action of the cell membrane, i.e. in its electric layer [4–6]. An essential property of the electric double layer is its electric charge which can be altered by various xenobiotics or by metabolic transformations [7–9]. For this reason, studies of the electric charge can furnish much information on equilibrium existing within the membrane and between the membrane and its environment, both in physiological and in non-physiological conditions [2,3].

The structure of a natural membrane is very complex implying numerous equilibria between the cell components and between the cell components and the cell environment. It is impossible to determine the parameters of so numerous equilibria numerically. Therefore, a small number of parameters should be adopted to characterize the equilibria between membrane components and the environment that would contain the values averaged for all equilibria. Both concentration sum of functional acidic and as well as of sum basic groups on the membrane surface and their averaged association constants with

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hydrogen or hydroxyl ions and sodium or chlorine ions can be used as such parameters.

It is the purpose of the present work to propose the above magnitudes as parameters characterizing the cell membrane, to check their utility for following changes in the membrane surface caused by toxic agents, and to elaborate the method to determine the parameters from electrophoretic mobility of cells. This work is also aimed at evaluation of the effect of green tea on the proposed parameters in the case of rat liver cells membrane in chronic ethanol intoxication.

Green tea is an example of such a natural antioxidant preparation. It contains relatively large amounts of polyphenols [10]. Tea polyphenols comprise mainly catechins and their derivatives, which are reported to have antioxidant properties. Under in vitro conditions they can inhibit free radicals generation, scavenge hydroxyl and superoxide radicals, reduce lipid peroxyl radicals and inhibit lipid peroxidation [11]. Catechins incorporation into cell membranes was shown to prevent or reduce the morphological and biochemical alterations of hepatocytes induced by hepatotoxic agents [12,13]. It has been shown that catechins and their derivatives can be absorbed and metabolized, and the catabolites of tea polyphenols have been found in rat and human urine and plasma [14,15]. These results indicate that ingested catechins are absorbed and can have biological effects.

2. Theory

Dependence of surface charge density of cell membrane on pH of electrolyte solution can be described with the help of four equilibria. There are two equilibria of negative groups, with the sodium and hydrogen ions and two equilibria of the positive groups, with the hydroxide and chloride ions. The existing equilibrium can be written in the form:

$$\mathrm{M} + \mathrm{N} \Leftrightarrow \mathrm{MN} \tag{1}$$

M can represent either A^- or a basic B^+, whereas N can represent H^+ Na^+, OH^-, and Cl^- ions which can associate either with A^- or with B^+ group.

The association constant is expressed by Eq. (2):

$$K_{\mathrm{MN}} = \frac{a_{\mathrm{MN}}}{a_{\mathrm{M}} \cdot a_{\mathrm{N}}} \tag{2}$$

with K_{MN} representing the association constant.

Surface charge density (δ) is expressed as follows:

$$\delta = (a_{\mathrm{B}^+} - a_{\mathrm{A}^-}) \cdot F \tag{3}$$

Here: $a_{\mathrm{A}-}$, $a_{\mathrm{B}+}$ are surface concentrations of corresponding groups on the membrane surface and $F = 96487 \left[\frac{\mathrm{C}}{\mathrm{mol}}\right]$ is the Faraday constant.

Functional group concentration balances are expressed as follows:

$$C_{\mathrm{TA}} = a_{\mathrm{A}^-} + a_{\mathrm{AH}} + a_{\mathrm{ANa}} \tag{4}$$

$$C_{\mathrm{TB}} = a_{\mathrm{B}^+} + a_{\mathrm{BOH}} + a_{\mathrm{BCl}} \tag{5}$$

Here: C_{TA}, C_{TB} $\left[\frac{\mathrm{mol}}{\mathrm{m}^2}\right]$ are total surface concentrations of functional groups, and a_{AH}, a_{ANa}, a_{BOH} and a_{BCl} are surface concentrations of corresponding groups on the membrane surface. The $a_{\mathrm{A}\,-}$, a_{AH}, $a_{\mathrm{B}\,+}$, and a_{BOH} values can be eliminated from the equation system obtained by Eq. (2) for each equilibrium and from Eqs. (4) and (5). The following equation results:

$$\frac{\delta}{F} = \frac{C_{\mathrm{TB}} \cdot a_{\mathrm{H}^+}}{a_{\mathrm{H}^+}(1 + K_{\mathrm{BCl}} \cdot a_{\mathrm{Cl}^-}) + K_{\mathrm{BOH}} \cdot K_{\mathrm{W}}} - \frac{C_{\mathrm{TA}}}{K_{\mathrm{AH}} \cdot a_{\mathrm{H}^+} + K_{\mathrm{ANa}} \cdot a_{\mathrm{Na}^+} + 1} \tag{6}$$

Here: K_{AH}, K_{ANa}, K_{BOH}, K_{BCl} are association constants and $a_{\mathrm{H}\,+}$, $a_{\mathrm{Na}\,+}$, $a_{\mathrm{OH}\,-}$ and $a_{\mathrm{Cl}\,-}$ are corresponding concentrations in solution.

It is difficult to carry out the regression of Eq. (6) to determine the C_{TA}, C_{TB}, K_{A}, and K_{B} constants. In order to carry out calculations based on Eq. (6) it will be reduced to linear equations for low and high H^+ ion concentrations. Assuming $a_{\mathrm{H}^+} \rightarrow \infty$ and $a_{\mathrm{OH}^-} \rightarrow 0$ results in simplification of the above equation to $\delta = \frac{C_{\mathrm{TB}} \cdot F}{1 + K_{\mathrm{BCl}} \cdot a_{\mathrm{Cl}^-}}$.

The second term of Eq. (6) can be neglected because of the high a_{H^+} value in the denominator. On the other hand, it can be accepted that $K_{\mathrm{BOH}} \cdot K_{\mathrm{w}}$ is considerably smaller than a_{H^+} $(1 + K_{\mathrm{BCl}} \cdot a_{\mathrm{Cl}^-})$ in the denominator of the first term of Eq. (6). Analogous to assuming $a_{\mathrm{H}^+} \rightarrow 0$ and $a_{\mathrm{OH}^-} \rightarrow \infty$ yields $\delta = \frac{C_{\mathrm{TA}} \cdot F}{1 + K_{\mathrm{ANa}} \cdot a_{\mathrm{Na}^+}}$.

In the cases of high or low hydrogen ion concentrations, Eq. (6) can be simplified to a corresponding linear equation. In the high H^+ concentration range, the numerator of each term can then be divided by the denominator leaving two initial terms only.

$$\frac{\delta}{F} a_{\mathrm{H}^+} = \frac{C_{\mathrm{TB}}}{1 + K_{\mathrm{BCl}} \cdot a_{\mathrm{Cl}^-}} \cdot a_{\mathrm{H}^+} - \frac{K_{\mathrm{BOH}} \cdot K_{\mathrm{w}} \cdot C_{\mathrm{TB}}}{(1 + K_{\mathrm{BCl}} \cdot a_{\mathrm{Cl}^-})^2} + f_1 \cdot a_{\mathrm{H}^+}^{-1} - f_2 \cdot a_{\mathrm{H}^+}^{-2} + \ldots - \frac{C_{\mathrm{TA}}}{K_{\mathrm{AH}} \cdot a_{\mathrm{H}^+}} + f_1' \cdot a_{\mathrm{H}^+}^{-1} - f_2' \cdot a_{\mathrm{H}^+}^{-2} + \ldots \tag{7}$$

f_1, f_2 …, are functions of K_{BOH}, K_{BCl}, whereas f_1', f_2'…are functions of K_{AH}, K_{ANa},

The terms with the negative a_{H^+} powers can be neglected for high a_{H^+} concentrations. These operations yield a straight line equation in the a_{H^+} and $\frac{\delta}{F} a_{\mathrm{H}^+}$ coordinate system; it is correct in a high hydrogen ion concentration only:

$$\frac{\delta}{F} a_{\mathrm{H}^+} = \frac{C_{\mathrm{TB}}}{1 + K_{\mathrm{BCl}} \cdot a_{\mathrm{Cl}^-}} \cdot a_{\mathrm{H}^+} - \left(\frac{K_{\mathrm{BOH}} \cdot K_{\mathrm{w}} \cdot C_{\mathrm{TB}}}{(1 + K_{\mathrm{BCl}} \cdot a_{\mathrm{Cl}^-})^2} + \frac{C_{\mathrm{TA}}}{K_{\mathrm{AH}}} \right) \tag{8}$$

Here: $\frac{C_{\mathrm{TB}}}{1 + K_{\mathrm{BCl}} \cdot a_{\mathrm{Cl}^-}}$ is the line slope and $-\left(\frac{K_{\mathrm{BOH}} \cdot K_{\mathrm{w}} \cdot C_{\mathrm{TB}}}{(1 + K_{\mathrm{BCl}} \cdot a_{\mathrm{Cl}^-})^2} + \frac{C_{\mathrm{TA}}}{K_{\mathrm{AH}}} \right)$ is its intersection point with the ordinate axis.

For the simplification Eq. (6) in a low H^+ ion concentration it should be written in the form:

$$\frac{\delta}{F} = \frac{C_{\mathrm{TB}} \cdot a_{\mathrm{H}^+}}{K_{\mathrm{BOH}} \cdot K_{\mathrm{w}} + a_{\mathrm{H}^+}(1 + K_{\mathrm{BCl}} \cdot a_{\mathrm{Cl}^-})} - \frac{C_{\mathrm{TA}}}{K_{\mathrm{ANa}} \cdot a_{\mathrm{Na}^+} + 1 + K_{\mathrm{AH}} \cdot a_{\mathrm{H}^+}},$$

Analogously to high H^+ ion concentration, the numerator of each term should be divided by the denominator leaving two initial terms only. These operations yield a straight line equation which is correct in a high hydroxyl ion concentration only.

$$\frac{\delta}{F} a_{H^+}^{-1} = \frac{-C_{TA} \cdot a_{H^+}^{-1}}{1 + K_{ANa} \cdot a_{Na^+}} + \left(\frac{C_{TB}}{K_{BOH} \cdot K_w} + \frac{K_{AH} \cdot C_{TA}}{(1 + K_{ANa} \cdot a_{Na^+})^2} \right) \tag{9}$$

Here $\frac{-C_{TA}}{1 + K_{ANa} \cdot a_{Na^+}}$ is the slope of the straight line and $\left(\frac{C_{TB}}{K_{BOH} \cdot K_w} + \frac{K_{AH} \cdot C_{TA}}{(1+K_{ANa} \cdot a_{Na^+})^2}\right)$ is its intersection point with the ordinate axis. The association constants have been assumed to be the same as those determined for phosphatidylcholine liposomes [16]: K_{ANa}= 0.051 [m^3/mol] and K_{BCl}=0.218 [m^3/mol].

The acid–base equilibria are more important for determination of parameters characterizing the membrane. However, taking into consideration the association constants with the sodium and chloride ions results in the C_{TA}, C_{TB}, K_{AH}, and K_{BOH} values which are closer to the real ones, even if the K_{ANa} and K_{BCl} assumed by us are burdened by error. The coefficients could be determined by linear regression and they could be used to determine the C_{TA}, C_{TB}, K_{AH}, and K_{BOH} values. In each case, it should be determined which points should be considered in the regression, both in high and low H^+ concentration range.

3. Materials and methods

3.1. Animals

The experiment was carried out with 12 months old Wistar race rats (520–550 g b.w.) which had been receiving an isocaloric liquid diet according to Lieber and deCarla commonly used in chronic ethanol poisoning. The animals which the blood originated from were classed into four groups (6 animals in each group): the control group, the group to which green tea was administered, the group receiving ethanol and the group receiving ethanol together with green tea. The methodology was described in detail in the following paper [17].

3.2. Electrochemical method

In order to determine surface charge density of cell membrane, liver tissue from rats was exposed to trypsin action. Thus, the cells removed from the tissue to be investigated were put into the measuring vessel and then the electrophoretic mobility was measured by using DTS5300 ZETASIZER 3000 apparatus (MALVERN INSTRUMENTS). The surface charge density has been determined using equation: $\sigma = \eta u / d$; here u — electrophoretic mobility, η — viscosity of solution, d — diffuse layer thickness [18]. The diffuse layer thickness was determined from the formula, $d = \sqrt{\frac{\varepsilon \cdot \varepsilon_0 \cdot R \cdot T}{2 \cdot F^2 \cdot I}}$, where R is the gas constant, T is the temperature, F is the Faraday number, I is the ionic strength of 0.9% NaCl, and $\varepsilon\varepsilon_o$ is the permeability of the electric medium [19].

3.3. Statistical analysis

The data obtained in this study are expressed as mean ± SD. The data was analyzed by the use of standard statistical analyses: one way ANOVA with Scheffe's F test for multiple comparisons to determine significance between different groups. The values for $p < 0.05$ were considered significant.

4. Results and discussion

It can be seen in the presented figures that surface charge density dependences of liver cells on pH are similarly shaped

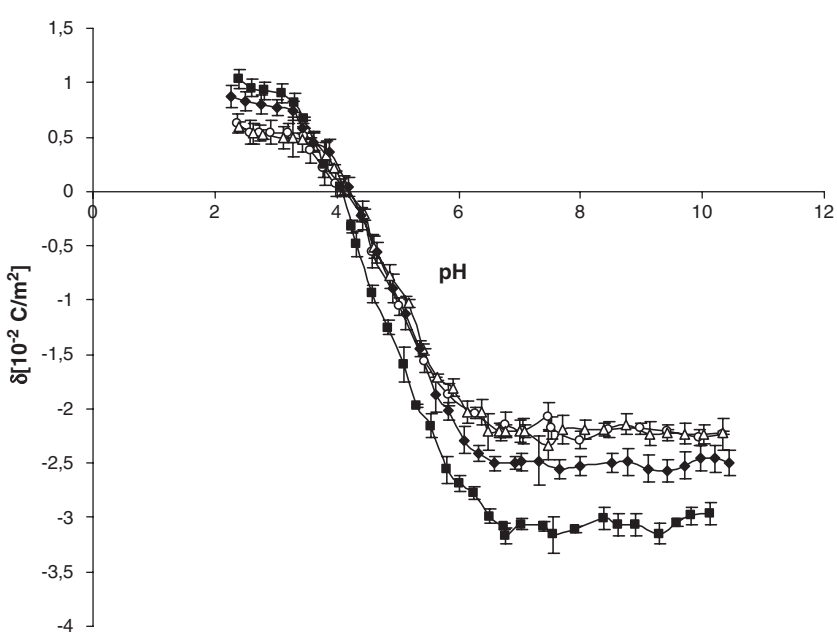

Fig. 1. Effect of green tea on changes in electric properties of liver cell membrane of the rats treated with green tea, or ethanol, and together ethanol and green tea as function pH (○ — control group, △ — green tea group, ■ — ethanol group, ♦ — ethanol + green tea group).

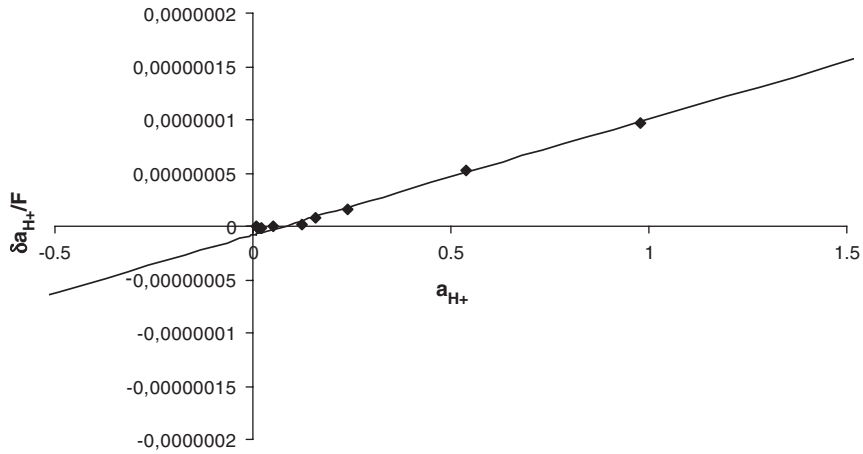

Fig. 2. Determination of the parameters on the base of Eq. (8) (the experimental values are marked by points and the theoretical ones by line).

for all studied animal groups: in the case the control rats, of the rats to which green tea only was administered, of the ethanol-intoxicated rats, and of the ethanol-intoxicated rats to which green tea was simultaneously administered. There is an increase in positive surface charge density of the cells at low pH values until a plateau is reached. At high pH values, the negative charge of the cells also increases reaching a plateau.

Ethanol intoxication of the rats caused an increase in negative charge by 36% at low pH values as well as in positive charge by 70% at high ones compared with the control rats (Fig. 1). No significant differences could be observed in the surface liver cells' charge density of rats watered with green tea compared with the control group. On the other hand, administering green tea with alcohol to the rats provoked a considerable decrease in negative charge of liver cell membrane at high pH values (22%) and in positive charge at low ones (19%) compared with the animals to which alcohol only was administered.

The C_{TA}, C_{TB}, K_{AH}, and K_{BOH} parameters of the cell membrane were determined from Eqs. (8) and (9). Eq. (8) was used to calculate the straight-line slope corresponding to the $\frac{C_{TB}}{1+K_{BCl}\cdot a_{Cl^-}}$ value and the intersection point with the ordinate axis equal to $-\left(\frac{K_{BOH}\cdot K_w\cdot C_{TB}}{(1+K_{BCl}\cdot a_{Cl^-})^2}+\frac{C_{TA}}{K_{AH}}\right)$. A determination of $\frac{C_{TB}}{1+K_{BCl}\cdot a_{Cl^-}}$ and of $-\left(\frac{K_{BOH}\cdot K_w\cdot C_{TB}}{(1+K_{BCl}\cdot a_{Cl^-})^2}+\frac{C_{TA}}{K_{AH}}\right)$ values of cell membrane is shown in Fig. 2. The experimental results presented in the plot are those of liver cell membrane cells of rats belonging to the group to which ethanol and green tea was administered.

On the other hand, Eq. (9) used the slope of straight line corresponding to $\frac{-C_{TA}}{1+K_{ANa}\cdot a_{Na^+}}$, and the intersection point with the ordinate axis is equal to $\left(\frac{C_{TB}}{K_{BOH}\cdot K_w}+\frac{K_{AH}\cdot C_{TA}}{(1+K_{ANa}\cdot a_{Na^+})}\right)^2$. A typical determination in the case of liver cell membrane of rats belonging to the group to which ethanol and green tea was administered is presented in Fig. 3.

The C_{TA}, C_{TB}, K_{AH}, and K_{BOH} constants resulting from these determined results were substituted to Eq. (6) yielding a theoretical curve. Typical experimental points and the theoretical curve are presented forthwith in Fig. 4. It can be observed that the theoretical and experimental surface charge density values agree.

Administering ethanol to the rats provokes an increase in the C_{TA} value of the liver cell membrane and in C_{TB} compared with the control group (Table 1). A decrease was observed in concentration of acidic groups and of basic groups in the liver cell membrane of the animals to which ethanol was fed and which were at the same time watered with green tea compared with the animals to which ethanol only, was fed.

Administering green tea to the animals provoked an increase in the association constant (K_{AH}) of the liver cell membrane of

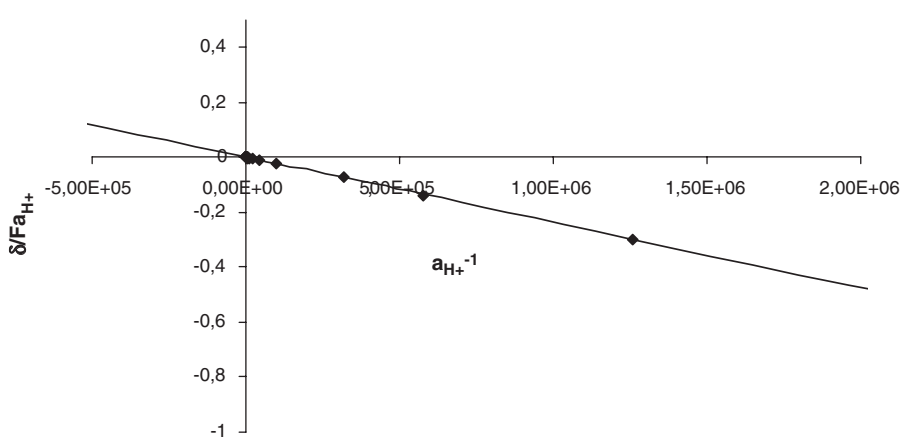

Fig. 3. Determination of the parameters on the base of Eq. (9) (the experimental values are marked by points and the theoretical ones by line).

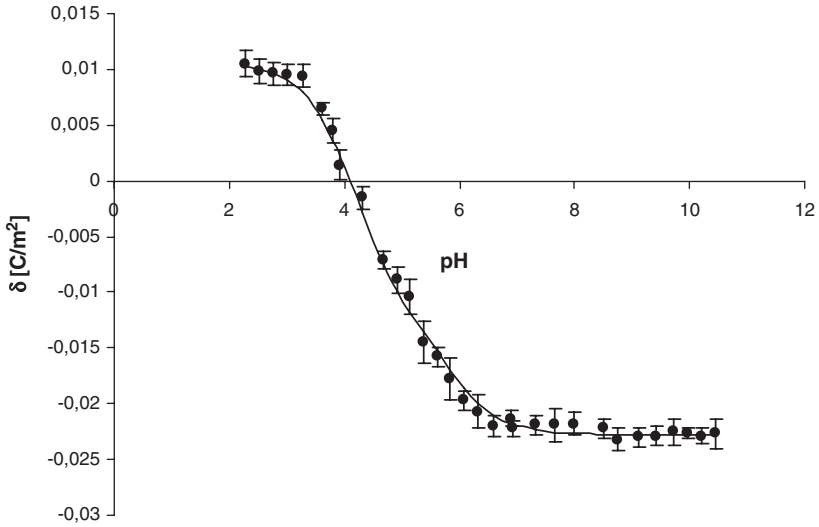

Fig. 4. The dependence of the surface charge density of liver cell membrane on the pH (the experimental values are marked by points and the theoretical ones by line).

the rats with respect to the control groups. In both groups, the K_{BOH} constant of the liver cell membrane of the rats decreased after administering green tea compared with the control group.

Administering ethanol provokes decrease in K_{AH}, the association constant of the acidic groups, and increase in K_{BOH}, the association constant of the basic groups, in the liver cell of rats compared with the control groups. Administering green tea with ethanol also provoked increase in the K_{AH} value and decrease in the K_{BOH} value compared with the ethanol groups.

The experimental results indicate that a chronic ethanol intoxication of rats provokes an increase in negative and positive charge numbers at the liver cell surface. It corresponds to increased surface concentration of acidic groups (C_{TA}) and basic groups (C_{TB}). Changes in functional groups composition on the membrane surface are due to appearance of new functional groups and/or to disappearance of the existing ones as the result of reaction of the alcohol or of its metabolism products with membrane components. Variations in the number and kind of functional groups bring about variations in C_{TA} and C_{TB} and, in turn, in their association constant values. Ethanol intoxication makes the association constant of negatively charged groups (K_{AH}) lower and the association constant of positively charged ones (K_{BOH}) higher.

Table 1
Effect of green tea on the value C_{TA}, C_{TB}, K_A, K_B of liver cells membrane intoxicated with ethanol

Group	Parameters			
	C_{TA} [10^{-7} mol/m^2]	C_{TB} [10^{-7} mol/m^2]	K_A [m^3/mol]	K_B [10^6 m^3/mol]
Control				
Green tea	2.31±0.11	0.61±0.04	36.31±2.19[a]	0.79±0.04[a]
Ethanol	3.12±0.11[a,b]	1.08±0.05[a,b]	23.43±1.22[a,b]	1.67±0.06[a,b]
Ethanol+ green tea	2.58±0.07[a,b,c]	0.91±0.06[a,b,c]	26.47±1.18[a,b,c]	1.21±0.05[a,b,c]

Statistically significant differences for $p<0.05$.
[a]In comparison with control 1; [b]in comparison with green tea group 1; [c]in comparison with ethanol group.

It has been demonstrated hitherto that ethanol poisoning provokes modifications in lipid bilayer membrane [20]. The alcohol molecules which penetrate into the lipid bilayer can destroy hydrogen bonds, they can assume the role of water molecules and hereby they can reduce membrane surface hydration [20]. The alcohol molecule can form one hydrogen bond only, either with a protein or with a lipid, hereby liberating a functional group; it can result in changed electric charge of the cell membrane due to increased surface concentration of positively and/or negatively charged groups. Change in the group number can bring about change in association constants of positively and negatively charged groups. These changes are accompanied by decrease in the sialic acid amount, both free and bonded in glycolipids and in glycoproteins, in the liver cell membrane [21,22]. A lower amount of this acid is supposed to expose positive and negative groups of lipids or proteins; it can result in changed association constants. The results obtained in this work are presented in Table 1; they suggest that the negatively charged groups appearing on the surface are more acidic and the positively charged ones have weaker basic properties then groups before intoxication.

In alcohol intoxication the cell membrane components are also subjected to the action of alcohol metabolites. The first ethanol metabolite–acetaldehyde being very reactive can modify amino acid residues of membrane proteins by reacting with free amino and sulfhydryl groups [23,24]. Consequently, it can reduce the positive charge of proteins and charge of lipids of the liver cell membrane.

Membrane lipids and proteins can also be modified by reactive oxygen species (ROS) appearing as the result of alcohol metabolism [25–27]. The ROS action results in lipid peroxidation and to lipid hydroperoxide formation. As the consequence, phosphatidylserine molecules, physiologically present on the inner side of the membrane become exposed on its outer side [28,29]. Exposing the phosphatidylserine molecule to the outer side of the membrane makes an additional negatively charged group appear there. It provokes an increase in the acid group surface concentration as it has been confirmed in this work

(Table 1). The presence of additional functional groups of phosphatidylserine can result in a decreased association constant of negatively charged groups of the membrane and in increased association constant of positively charged ones because their K_{AH} value is lower and the K_{BOH} value is higher than those of original cell membrane groups [30].

It is considered that most changes in the cell membrane occurring in alcohol intoxication are due to an increasing formation rate of reactive oxygen species and to reduced antioxidant abilities of the organism. For this reason, it was attempted to prevent these changes by administering green tea extract to the ethanol-intoxicated animals. Green tea is an example of such a natural antioxidant preparation. It contains a large amount of polyphenols, mainly catechin and its derivatives of strong antioxidant properties [31]. For this reason, the ingested catechins can exert biological effects. Consumption of green tea by ethanol-intoxicated rats provokes a decrease in surface concentration of acidic and basic groups. In addition, it effects the value of the association constant of positively charged groups as well as an increase in association constant of negatively charged groups of the liver cell membrane compared with the group of ethanol-intoxicated rats to which the green tea was not administered. Administration of green tea to animals does not induce statistically important changes in C_{TA} and C_{TB} compared with the control groups, it lowers the K_{BOH} value and it increases K_{AH}. In conclusion, the constants proposed in this work, C_{TA}, C_{TB}, K_{AH} and K_{BOH}, are suited for monitoring the changes caused by various compounds.

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