METHODS

Analysis of Tensiograms of Cerebrospinal Fluid with a Kinetic Model

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We develop a model for estimation of the parameters of dynamic tensiometry of biological fluids based on equations of formal chemical kinetics. The model considers diffusion of low- and high-molecular compounds and conformational restructuring of these compounds in the surface layer. This model was tested on cerebrospinal fluid from patients with meningitis. Some correlations between the parameters of tensiometric curves and protein, chloride, neutrophil, and lymphocyte content in the liquor are detected.

Key Words: *surface tension; dynamic tensiometry; kinetic equation; cerebrospinal fluid; simulation*

Dynamic surface tension of biological fluids (blood, urine, cerebrospinal fluid, *etc.*) is used in medicine for integral evaluation of the disease severity, treatment efficiency, *etc.* [2,9]. However, there are no objective parameters of tensiometric curves (TC), which could make this evaluation more accurate; but kinetic nature of TC creates theoretical prerequisites for estimation of these parameters. The mathematical system for estimation of kinetic parameters in formal chemical kinetics is well developed (*e. g.*, [3]). In order to verify the expected advantages of TC formalization for the analysis of biological fluids it is logical to use one of these fluids as the first step. It is desirable that this liquid had not very complex composition and little changed under the effect of poorly controlled factors (ration, functional strain, *etc.*) that can modulate the content of surface-active compounds.

The cerebrospinal fluid (CSF) is an ideal model from this viewpoint, because of low protein content and the absence of many ingredients typical of other fluid (*e.g.* blood) [4]. Physicochemical characteristics of CSF normally little very (unlike, for example, urinary pH). In some pathological states protein concentration in CSF depends greatly on the disease type and severity, and thus modifies the saline composition.

Using the kinetic equations, we detected the relationships between TC parameters and concentrations of some substances in CSF from patients with meningitis.

MATERIALS AND METHODS

CSF was collected in accordance with medical indications from 86 patients with meningitis (60 with purulent and 26 with serous inflammation of the meninges); the disease was severe in 70 patients (19 of these with serous meningitis).

Neutrophil and lymphocyte counts, concentrations of protein [1], glucose, and anion chlorine (Cl^-) [6] in CSF were measured. Dynamic surface tension (σ) was measured in the 0.01-50 sec interval by the method of maximum pressure in a bubble (MPT2 tensiometer, Lauda) and in the 10-1500 sec interval by the

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droplet shape method (ADSA tensiometer) [2]. For additional information, a drop of the studied fluid was subjected to deformation (sharp extension of the surface by 5-7%) and the relaxation curve was plotted (Fig. 1). The data were statistically processed [2].

Tensiograms were processed using a model analogous to the previously used [7], based on the kinetic equations of σ changes in the first-order physicochemical reactions at the expense of 1) diffusion of low-molecular compounds from the surface layer (σ_1); 2) diffusion of high-molecular compounds into the surface layer (σ_2); 3) conformation changes in these compounds (σ_3):

$$\sigma=\Delta\sigma_1\exp(-k_1\times t)+\Delta\sigma_2\exp(-k_2\times t)+\Delta\sigma_3\exp(-k_3\times t)+\sigma_\infty, \quad (\mathbf{1})$$

where $\Delta\sigma_1$, $\Delta\sigma_2$, and $\Delta\sigma_3$ are deviation from the equilibrium resulting from processes 1, 2, and 3, respectively, at t=0, $\Delta\sigma_\infty$ is the steady-state σ value at $t\to\infty$, and k_1, k_2, and k_3 are the respective constants of the reaction rates. Equation (**1**) means that according to our model, the processes of direct and reverse diffusion of high- and low-molecular compounds (processes 1 and 2) take place at $t<t_{cr}$ and reach the equilibrium at t_{cr}. Conformation changes (process 3) take place in parallel with this and during the entire process. The region of t_{cr} is easily found on TC and corresponds to the decrease in the rate s changes, after which the rateof the parameter changing somewhat increases (Fig. 1). After t_{cr} changes in the parameter are determined only by conformation changes in high-molecular compounds in the surface layer. The t_{cr} time is determined from the condition $d^2\sigma/dt^2=0$ by numerical differentiation of experimental data. Since $k_3<<k_1$ and $k_3<<k_2$, at $t>t_{cr}$ equation (**1**) will be as follows:

$$\sigma=\Delta\sigma_3\exp(-k_3\times t)+\sigma_\infty. \quad (\mathbf{2})$$

After estimation of t_{cr} and parameters of equation (**2**) the estimation of other constants in equation (**1**) is a simple task.

The relaxation characteristics were estimated using an equation similar to the kinetic equation for the first-order reaction:

$$\Delta\sigma=\Delta\sigma_4\exp[-(t-t_0)/\tau], \quad (\mathbf{3})$$

where $\Delta\sigma_4$ is the initial rise of σ at $t=t_0$ (t_0=1200 sec), $\tau=1/k_4$ is the time of relaxation.

The equilibrium σ value after relaxation was found to be more than σ_∞, while $\Delta\sigma_4<<\sigma_0$, this indicating transfer of the system after excitation into a new thermodynamically more beneficial state, presumably due to conformational changes in the protein molecules in the surface layer.

Analyses of regressions and correlations were carried out.

RESULTS

The relative content of neutrophils and lymphocytes in CSF was 1-99%, concentration of protein 0.17-10 g/liter, glucose 2.9±0.9 mmol/liter, and chlorides 110±13 g-ion/liter. The value of σ varied from 63 to 54 range (σ_∞) mN/m, the $\Delta\sigma_1$, $\Delta\sigma_2$, $\Delta\sigma_3$, and $\Delta\sigma_4$ values were within 3-10 mN/m, t_{cr} was about 700-800 sec, and τ was 43-57 sec.

Since only lymphocytes and/or neutrophils can migrate into CSF, their percentage was in negative correlation (Table 1). The count of neutrophils in CSF was proportional to protein concentration, which mediates the correlations between tensiometry values and neutrophil (or lymphocyte) count, which are not discussed further and are not presented in Table 1. It was found that glucose concentration in CSF correlates with none of tensiometry parameters (at the specified level of significance). The effects of sugars on σ values remain unclear [9].

Protein content determines the parameters of tensiometry, which depend mainly on conformational changes and were obtained at $t\to\infty$ (longer life span of the surface) or during the relaxation process (Table 1). On the one hand, Cl^- concentration is essential for the tensiometric parameters obtained by superposition of elementary constants of the kinetic equation system (**1**) found at $t\to\infty$ and at t=0, as well as t_{cr} (longer, shorter, and medium life spans of the surface). This effect is quite expected, because diffusion of low-molecular compounds (chloride) is more rapid than diffusion of high-molecular compounds (protein) in the opposite direction. Addition of NaCl to albumin solution leads to a decrease in σ in the intervals of longer and medium times [8]. For these reasons chlo-

TABLE 1. Correlation ($p<0.05$) between Protein Concentration and Cl^- in CSF and Parameters of Tensiometric Curve

Parameter	Parameter of tensiometric curve	
	protein	Cl^-
$\Delta\sigma_1$	—	0.47
$(\Delta\sigma_3+\sigma_\infty)$	—	-0.45
$\Delta\sigma_3$	0.63	—
t_{cr}	—	-0.50
σ_∞	-0.47	—
$(\Delta\sigma_4+\sigma_\infty)$	0.73	—
$\Delta\sigma_4$	-0.65	—

Note. Dash means the absence of correlations with the specified level of significance.

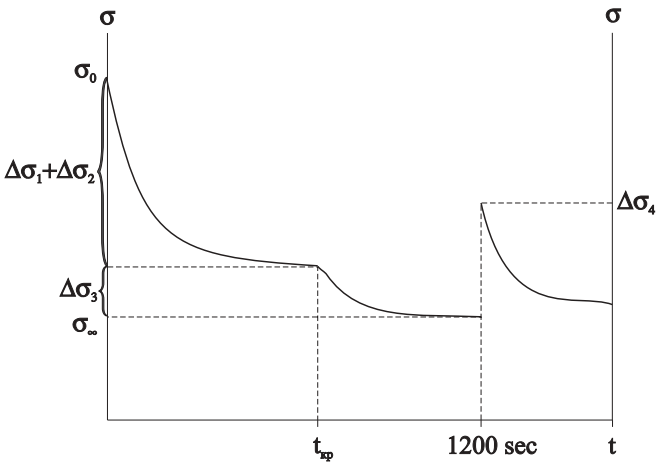

Fig. 1. Results of dynamic tensiometry of human cerebrospinal fluid in meningitis. $\Delta\sigma_1$, mN/m: σ deviation due to diffusion of low-molecular compounds from thesurface layer; $\Delta\sigma_2$, mN/m: σ deviation due to diffusion of high-molecular compounds into the surface layer; $\Delta\sigma_3$, mN/m: σ deviation due to conformational changes in proteins; $\Delta\sigma_4$, mN/m: σ deviation after excitation; σ, mN/m: steady-state σ value; σ_4, mN/m: $\sigma_\infty+\Delta\sigma_1+\Delta\sigma_2+\Delta\sigma_3+\Delta\sigma_4$; t_{cr}, sec: critical time.

ride content determines the tensiometric characteristics of CSF during the entire process of σ measurements, including the period when diffusion of Cl^- from the surface layer is over ($t>t_{cr}$). Proteins ensure an appreciable contribution to σ only at sufficiently long life span of the surface, primarily at the expense of conformational restructuring and mainly when the chloride contribution to σ value is rather low. Hence, the results of correlation analysis confirm the adequacy of model (**1**) to dynamic σ of actual biological fluids.

As proteins decrease σ_∞ [5], protein concentration in CSF is in negative correlation with σ_∞, the higher protein concentration, the greater the contribution of conformational changes to σ value, which leads to positive correlation between protein concentration and $\Delta\sigma_3$. Contrary to this, inorganic salts increase σ [5], which results in positive correlation between $\Delta\sigma_1$ and Cl^- concentration. These latter modulate the conformational structure of protein molecules by stabilizing or destabilizing certain structural conformations, which is reflected in negative correlation between Cl^- content and $(\Delta\sigma_3+\Delta\sigma_\infty)$. It seems that the correlation between Cl^- and t_{cr} is negative for this reason.

The fact that none of tensiometry parameters determined as described previously [2] with the level of significance used in our study does not correlate with CSF concentrations of protein, chlorides, and glucose is one more proof in favor of equation (**1**) for processing the biological fluids TC. It seems that, as after the excitation the changes in σ are determined predominantly by conformational restructuring of biopolymers, there are many strong correlations between protein concentration and relaxation parameters of TC, estimated using equation (**3**). Negative correlation between protein concentration and $\Delta\sigma_4$ also indicates that the lower protein content, the greater conformation changes molecules after extension of the surface. This fact is justified, because the decrease of macromolecule concentration due to reduction of the diffusion limitations opens new possibilities for conformational restructuring, leading to new higher value of σ and to a positive correlation between protein content and $(\Delta\sigma_4+\sigma)$.

The detected regularities are intrinsic of not only σ of CSF and reflect the common regularities in the surface layer of various biological fluids. Our hypothesis can be experimentally verified and is in line with the search for TC parameters most adequately reflecting the composition of biological fluids with more complex composition than CSF. The detection of these integral parameters will improve the accuracy of evaluation of the disease course and efficiency of treatment and rehabilitation measures.

Hence, analysis of dynamic tensiograms of CSF carried out using a kinetic model indicates a relationship between TC parameters and the content of cellular and biochemical components in CSF.

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