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Studies on the application of dynamic surface tensiometry of serum and cerebrospinal liquid for diagnostics and monitoring of treatment in patients who have rheumatic, neurological or oncological diseases

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Abstract

Human biological liquids comprise various surfactants, which adsorb at liquid interfaces and lead to a variation in surface tension. The adsorption processes involving low molecular weight surfactants, proteins and phospholipids play a vital role in the physiological functions of the human organism, especially if large surfaces are involved (e.g., gas exchange in lungs, metabolism of kidneys, liver and brain). Dynamic surface tensiometric studies of biological liquids like serum and cerebrospinal fluid provide surrogate parameters that reflect surface tension phenomena. We provide dynamic surface tension data of serum and cerebrospinal fluid that were collected from healthy volunteers and patients with rheumatic, neurological or oncological diseases. Our studies indicate that dynamic surface tension data are helpful

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for diagnostic purposes and for monitoring of therapeutic interventions.

Keywords: Dynamic surface tension; Maximum bubble pressure tensiometry; Biological liquids; Diagnostic measures in medicine; Serum; Cerebrospinal liquid; Human study

Contents

1. Introduction

Human biological liquids comprise various surfactants which adsorb at liquid interfaces and lead to a variation in surface tension. The adsorption processes involving low molecular weight surfactants, proteins and phospholipids play a significant role in vital functions of the human organism, such as in respiratory processes and haematogenesis. In a recent review by Kazakov et al. [1] the results of dynamic surface tension studies of serum and urine obtained from healthy and ill persons were presented. It was shown that the parameters of dynamic surface tensiograms of biological liquids for healthy persons exhibit certain dependencies on the person's age and sex. In addition, referring to nephrologic diseases as an example, the dynamic surface tensiometry of serum and urine was demonstrated to be applicable as a diagnostic tool and for monitoring drug or surgical treatments.

The work of Polányi in 1911 [2] is probably some of the earliest research on surface tension of human biological liquids (cerebrospinal liquid). Künzel in 1941 [3] has published the first systematic research of surface tension of serum and cerebrospinal liquid. Recently, publications concerning studies of surface tension of blood, cerebrospinal and amniotic liquid, gastric juice, saliva, expired air condensate and other human biologic liquids become more frequent [4–11]. However, such studies are still incomprehensive, and the methods used are often not reliable enough. For the time being, only one branch of medical science, namely the studies of lung surfactant systems, can be regarded to as one where interfacial tensiometry is widely applied. In this particular area, significant clinical

progress was already achieved in the treatment of pathologies related to disorders in the lung surfactant system [12,13].

It should be stressed that all studies of dynamic surface tensions in the range from 1 ms to 50 s surface lifetime were performed using the maximum bubble pressure tensiometer MPT2 (LAUDA, Germany). The measuring cell and the capillary were developed especially for medico-biological experiments. The basic elements of the experimental methods and the theoretical explanation of the choice of dynamic surface parameters used in the analysis have been summarized recently [1]. A description of the theory and the experimental techniques related to the maximum bubble pressure method were presented in detail by Fainerman and Miller [14]. The rational choice of the geometry and material for the capillaries is essential for the stability and reliability of the data obtained from the maximum bubble pressure method and will be discussed below in more detail.

The main surface active compound of serum is albumin (HSA) with a concentration in human blood of 35–50 g/l. The great practical significance of the adsorption of proteins at liquid interfaces stimulated the development of various theoretical models to describe the equilibrium and dynamic behaviour of protein adsorption layers. A summary of the thermodynamic and adsorption kinetics models of protein adsorption at liquid interfaces was published recently by Fainerman and Miller [15]. An important specific feature of the adsorption behaviour of proteins is the capability of configuration variation, i.e. the large number of molecular states protein molecules can establish. Proteins also possess a significant unbound electric charge, which contributes to the surface tension of a solution [16]. These properties are responsible for some unusual features of protein solutions: steep decrease of equilibrium surface tension with small increase in the concentration; existence of the so-called induction period, when the adsorbed amount is already significant, while the surface pressure remains almost zero; anomalous increase of dynamic surface tension at low surface layer lifetimes, observed under some specific conditions; non-diffusive mechanism of adsorption for medium and high surface pressures. Yet more unusual, however, is the behaviour of interfacial layers of proteins mixed with other surfactants. Examples of such unusual behaviour were presented in Fainerman and Miller [15]. In this overview, we summarize recent results obtained in adsorption using HSA and the non-ionic surfactant decyl dimethyl phosphine oxide (C_{10}DMPO) [17].

The isotherms of equilibrium surface tension for pure and mixed C_{10}DMDO solutions are shown in Fig. 1. It is seen that for C_{10}DMDO concentrations higher than 10^{-4} mol/l, the isotherms are almost identical and the adsorption of HSA at these C_{10}DMDO concentrations is negligible.

The composition of the surface layer changes within a narrow range of C_{10}DMDO concentration. This view is supported by the analysis of the shear viscosity of the same mixed adsorption layers [17]. It was shown that for medium C_{10}DMPO concentrations 10^{-6} to 10^{-4} mol/l, the surface tension of mixtures exceeds that of the pure HSA solution. For C_{10}DMPO concentrations lower than 5×10^{-7} mol/l, the surface tension of mixtures was equal to that of the pure HSA solution. For concentrations between 4×10^{-5} mol/l and 10^{-4} mol/l this surface

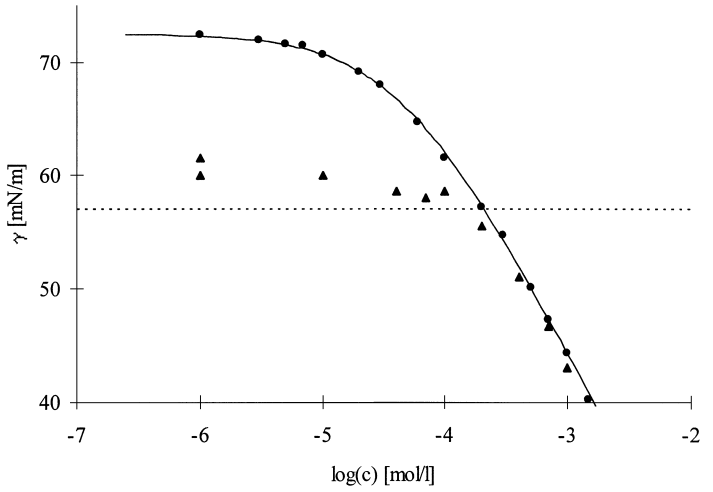

Fig. 1. Equilibrium surface tension isotherms for pure C_{10}DMPO solutions (● and solid line) and mixed C_{10}DMPO/HSA solutions for a HSA concentration of 10^{-7} mol/l (▲). The dotted line shows the equilibrium surface tension for pure HSA solutions at $c = 10^{-7}$ mol/l averaged from six measurements ($\sigma = 57 \pm 1$) mN/m.

tension exceeds only by 1–1.5 mN/m. However, for concentrations less than 4×10^{-5} mol/l, the surface tension of mixed solutions exceeds that of the respective HSA solution by 3–4 mN/m. Ordinary theoretical models are unable to explain such an intermediate surface tension increase. The hydrophobic interaction of the C_{10}DMPO hydrocarbon tails with HSA polypeptide chains can, in principle, lead to a hydrophilization of the protein molecule; this effect, however, is not the only possible explanation as only 10 molecules of C_{10}DMPO per HSA molecule (for $c = 10^{-6}$ mol/l) exist in a mixed solution, and HSA possesses 585 amino acid groups.

This anomalous adsorption behaviour of protein/surfactant mixture can also be explained in the framework of a theory developed recently [15–17]. An approximate expression was derived for the difference between the equilibrium surface tension (σ) of the pure HSA solution and that mixed with C_{10}DMPO

$$\Delta\sigma = \sigma_{\mathrm{HSA}} - \sigma_{\mathrm{HSA}+\mathrm{C_{10}DMPO}} = \frac{RT}{\omega}\ln\left(\frac{1}{1-b_2c_2}\right) - \frac{RTa}{\omega}\left(\frac{b_1c_1}{1+b_1c_1}\right)^2\left[2b_2c_2 - (b_2c_2)^2\right] \qquad (1)$$

where R is the gas constant, T is the temperature, ω is the partial molar area of HSA, b is the adsorption equilibrium constant, c is the bulk concentration, a is the inter-ion interaction constant for protein. Here the subscript '1' refers to HSA and

subscript '2' to C_{10}DMPO. It can be seen that the first term in this equation can be neglected as compared with the second term (it is known that for a protein solution, the main contribution to the surface tension is due to inter-ion interaction [15,16]). It follows from Eq. (1) that $\Delta\sigma$ is negative for low concentrations of C_{10}DMPO. Moreover, calculating the extremum of the second term of Eq. (1) one sees that a surface tension maximum of the mixture occurs at $b_2 c_2 = 1$. Therefore, the anomalous surface tension behaviour for HSA mixture with C_{10}DMPO results mainly from the large free charge in the protein molecule. This phenomenon can be explained rather simply from a physical point of view. The addition of C_{10}DMPO results in an increase of the total surface layer coverage, which leads to an increase in the first term of Eq. (1). However, the addition of C_{10}DMPO decreases the adsorption of HSA which results in a decrease of the second term of Eq. (1), which depends quadratically on the HSA adsorption. Therefore, an increase in surface tension of the mixture can occur.

This result is extremely important for dynamic surface tensiometry of human biological liquids. Any biological liquid is essentially a mixture of hundreds of inorganic and organic substances, including tens of surfactants (proteins, fatty acids, phospholipids, etc.). For such mixtures, unlike mixtures of surfactant homologues, the total decrease of surface tension cannot be regarded as merely the cumulative from each component. Thus, dynamic surface tensiograms cannot simply be correlated to the total amount of all surfactants contained in the biological liquid, and are therefore a complement to results of biochemical, spectroscopic and other quantitative and qualitative analyses. As the surface tension of protein solutions is extremely sensitive to variations of the pH of the medium, to additions of inorganic and organic electrolytes, carbohydrates and other surfactants, dynamic surface tensiometry allows study of some specific features of multi-component adsorption layers which cannot be accessed by other methods. Interfaces in the human organism are extremely extended; therefore one can expect that the results of dynamic surface tensiometry reflects the actual composition of human interfacial layers and chemical and exchange processes which take place at these interfaces. Various diseases affect the composition of interfacial layers and the physico-chemical processes within these layers. Therefore, dynamic surface tensiograms can be regarded as a comprehensive indicator of some pathologic disturbances.

The present review discusses problems concerning the parameters of dynamic surface tension of some biological liquids, mainly serum and cerebrospinal liquid, with special reference to rheumatic, neurological and oncological diseases. The choice of parameters was discussed in detail by Kazakov et al. [1]. Some of these parameters are related to asymptotic equations of the adsorption kinetics theory [18]. We selected six dynamic surface tensions: σ_0 is the surface tension extrapolated to $t \to 0$; σ_1 is the surface tension at $t = 0.01$ s; σ_2 is the surface tension at $t = 1$ s; σ_3 is obtained by extrapolation to $t \to \infty$; $\lambda_0 = -(\mathrm{d}\sigma/\mathrm{d}t^{1/2})_{t \to 0}$ and $\lambda = (\mathrm{d}\sigma/\mathrm{d}t^{-1/2})_{t \to \infty}$. The last two parameters describe the slope of the tensiograms plotted in specific co-ordinates of the surface lifetime t. According to the

theory developed by Fainerman et al. [18], λ_0 correlates with the total concentration of surfactants, while λ is a measure for the total equilibrium adsorption.

2. Hydrodynamics of the maximum bubble pressure method

Among all known methods, which can be used for the measurement of dynamic surface tensions in the very short lifetime range, the maximum bubble pressure method (MBPM) is most appropriate to study biological liquids. The main advantages of MBPM are the small sample volume (1 ml or less), and the wide range of surface lifetime (0.001–50 s) [1,14]. The method is based on the measurement of the maximum capillary pressure which is attained during the growth of a gas bubble at the tip of a capillary immersed in the studied liquid at the moment of time when the radius of curvature becomes minimum (and equal to the internal radius of the capillary.) The rate of air supply (or of another gas) through the capillary can be varied in a rather wide range and hence the time interval between the moment of formation of a fresh surface after the separation of the previous bubble, and the moment when the maximum pressure is attained. This yields a dependence of maximum pressure (which is a direct measure of the surface tension) as a function of surface lifetime.

The capillaries employed in MBPM are of small radius (less than 0.2 mm), thus the deformation of the bubble due to the buoyancy force can be neglected [14,19,20] and one can calculate the surface tension $\sigma(t_1)$ from the measured maximum bubble pressure $P_{\sigma\max}(t_1)$ at any given surface lifetime t_1 using the simplest form of the Laplace equation

$$P_{\sigma\max}(t_1) = \frac{2\sigma(t_1)}{r_0} \tag{2}$$

where r_0 is the capillary radius. The surface tension is determined from the maximum pressure at the time moment when the radius of curvature attains its minimum value, $r(t_1) = r_0$. The method does not require a simultaneous measurement of the radius with time $r(t)$, which makes the whole procedure easy to perform. Strictly speaking, if the surface pressure does not remain constant, the maximum pressure does not correspond exactly to the hemispherical bubble [21,22]. It is known from experiments, however, that this effect is rather small [21].

The MBPM does not involve the explicit measurement of the capillary pressure within the bubble $P_\sigma(t) = 2\sigma(t)/r(t)$ and the time t_1 when the maximum pressure is attained. Usually (for example in the MPT2 tensiometer manufactured by LAUDA, Germany) the parameters measured directly are the pressure P_S within the reservoir from which the air is supplied to the capillary, the time interval t_b between successive bubbles, and the air supply rate L. From these values, the capillary pressure and surface lifetime can be calculated. The capillary pressure is

related to the pressure within the reservoir via the expression

$$P_\sigma = P_S - P_H - \Delta P_{cap} - \Delta P_{liq} \quad (3)$$

Here P_H is the hydrostatic pressure at the capillary immersion depth, ΔP_{cap} is the pressure difference between the ends of the capillary, ΔP_{liq} is the excess pressure in the liquid at the surface of the bubble. In Eq. (3) the last two terms correspond to viscous resistance and inertia of the gas and liquid phase, respectively. When the pressure within the bubble approaches its maximum value, the velocity of the gas flux becomes significantly lower, and the contribution introduced by these two terms becomes small. It is assumed usually that in the moment of the maximum pressure, $P_\sigma \approx P_S - P_H$, and the surface tension is calculated via Eq. (2) from the measured pressure within the reservoir. It follows from a theoretical analysis and experimental data, however, that the corrections which account for aerodynamic and hydrodynamic effects are only negligible for rather long surface lifetimes, and the viscosity of the studied liquid is sufficiently small [23,24]. For studies of biological liquids, the surface tension in the entire lifetime range, including short times (less than 0.01 s) are of practical interest. Also the viscosity of these liquids is often high. Therefore, the corrections with respect to aerodynamic and hydrodynamic effects can be significant.

The surface lifetime t_l is calculated usually as the difference between the total bubble time t_b and the so-called dead time t_d, that is, the time interval between the moment of maximum pressure and the separation of the bubble

$$t_l = t_b - t_d \quad (4)$$

The dead time can be approximated from the pressure in the air reservoir P_S and the volume of a separating bubble V_b [25] which in turn is given by the air supply rate L and the bubble time, $V_b = t_b L$. The calculation of dead time becomes much simpler if the volume of separating bubbles is constant and independent of the bubble time. This can be achieved, for example, if the bubble growth is restricted by a pin (or another solid body) located opposite to the capillary tip. Such configuration is used in the MPT2 tensiometer [14,26] and the dead time can be approximated by

$$t_d = t_b \frac{LP_c}{L_c P_s} \quad (5)$$

where P_c and L_c are the pressure and air supply rate corresponding to the critical point in the dependence of pressure on air flow rate. This point corresponds to the transition from a regime of separate bubbles to a gas jet regime.

It should be mentioned that this calculation procedure of the dead time accounts for the viscous resistance of the capillary, but disregards the viscous friction in the liquid, inertial effects in the liquid, and the compressibility of the gas. These factors are quite insignificant for large and medium lifetimes (greater than 0.01 s), but can become noticeable at very short lifetimes [23,27].

Therefore, to substantiate the application of MBPM for surface tension studies at very short lifetimes and for highly viscous solutions, an additional analysis of the aerodynamic and hydrodynamic effects should be performed.

Another hydrodynamic problem, which has to be analysed, is the penetration of the studied liquid into the capillary after bubble separation. This penetration results in an increased bubble lifetime [28], and requires some extra pressure in the air reservoir [29]. This leads to an additional expansion and compression of the surface, with unpredictable effects on the measured surface tension of the surfactant solution [30]. If biological liquids containing dissolved components (macromolecules, colloid particles) are studied, these components can adsorb at the capillary surface. This affects the characteristics of the capillary, which finally results in an essential irreproducibility of the experimental data. To prevent the penetration of liquid into the capillary, a hydrophobization of the internal surface is often used [14,19,20,30]. This procedure is indeed helpful for aqueous surfactants solutions; for biological liquids, however, an inverse effect has been observed. The adsorption of the components of biological liquids at the capillary walls becomes more pronounced, which results in a non-stable formation of bubbles and, finally, to irreproducible results. This is possibly caused by the adsorption of lipids (phospholipids) at the boundary between the internal hydrophobic capillary surface and the external hydrophilic surface of the capillary tip. Whether penetration occurs can be best controlled by the geometric properties of the capillaries [28,29].

In wide short capillaries the inertia of the gas is more significant than the viscous forces; therefore the restoration of pressure in the entire capillary after bubble separation is very rapid, which can lead to pressure oscillations in the gas. For long narrow capillaries the viscous dissipation is significant, the inertial effects do not play any significant role, and the gas flow through the capillary is rather slow and aperiodic. The parameter to distinguish between the dynamic gas regimes is r_0^2/l [28,31]. Inertial forces are small, if

$$\frac{r_0^2}{l} < \frac{8\nu}{\pi c_0} \tag{6}$$

where ν is the kinematic viscosity of gas, and c_0 is the sound velocity. For air at 20°C $r^2/\mathrm{l} < 0.1$ μm.

The penetration depth of the liquid into the capillary after bubble separation, and the time of direct and reverse motion of liquid in the capillary were estimated in [28,31] using the Washburn equation with account for inertia of the liquid within the capillary and in the vicinity of the capillary orifice. In long narrow capillaries the restoration of gas pressure near the meniscus is slow, which allows a deep penetration of liquid into the capillary. If the condition in Eq. (6) is valid, the maximum meniscus rise can be estimated by [31]

$$h_{\max} \approx \sqrt{\frac{128\eta_g \sigma \cos\theta l^2}{\pi^4 \eta_l \gamma P_{\mathrm{atm}} r_0}} \tag{7}$$

where η_g and η_l are the dynamic viscosities of gas and liquid, respectively, γ is the adiabatic exponent, σ is the surface tension, θ is the contact angle of wetting and P_{atm} is the atmospheric pressure. For the air–water system, $\eta_g = 0.018 \times 10^{-3}$ Pa s, $\eta_l = 10^{-3}$ Pa s, $\sigma = 73$ mN/m, $\gamma = 1.4$. Assuming $\cos\theta = 1$, one obtains

$$h_{max} \approx l\sqrt{\frac{0.0124}{r_0}} \tag{8}$$

where r_0 is given in μm. The height of meniscus rise in the capillary after the bubble separation increases with increase of the capillary length and decrease of the capillary radius. For capillaries with a radius of 0.001–0.01 cm this height is 1–3% of the capillary length. For example, for a capillary of 3 cm length with a radius $r_0 < 0.005$ cm, the height of the meniscus rise exceeds 0.04 cm, which exceeds the capillary radius several times. Thus, long narrow capillaries are unsuitable for studies of biological liquids because of this strong penetration of liquid into the capillary after bubble separation.

For wide short capillaries, the pressure within the capillary near the meniscus is rapidly restored, and almost no liquid penetrates into the capillary. Only slight oscillations of the meniscus occur near the capillary entry. Numerical estimates for wide short capillaries ($r_0^2/l \gg 0.1$ μm) show that the penetration of liquid into

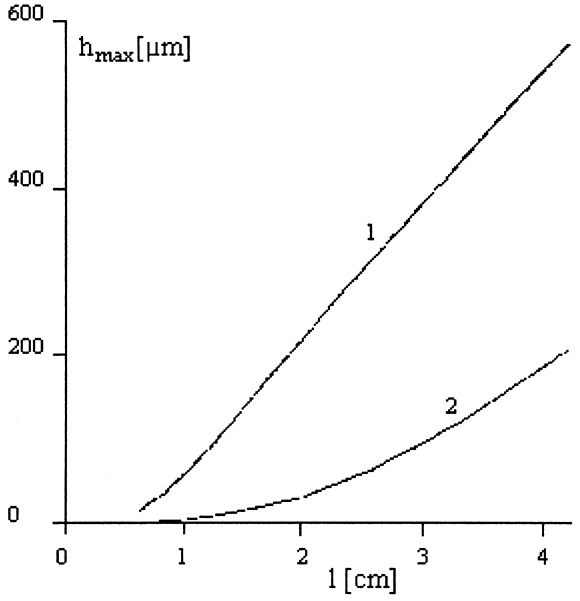

Fig. 2. Dependence of the depth of liquid penetration h_{max} into the capillary on the capillary length l for radius $r_0 = 0.005$ cm (curve 1) , and $r_0 = 0.01$ cm (curve 2) and pressure in the reservoir $P_s = 1.05$ $P_{\sigma max}$ for pure water; according to [31].

these capillaries is far less significant than that calculated from Eq. (7), see Fig. 2 [31].

Experimental data also confirm the conclusion that the meniscus rise decreases rapidly with the increase in capillary radius and decrease in capillary length [30]. It follows from the analysis of gas flow in the capillary [27] that for $r_0^2/l > 1$ μm the gas behaves like an incompressible medium. Our experiments have shown that wide ($r_0 = 0.1$–0.12 mm) short ($l = 5$–10 mm) capillaries are most suitable with respect to studies of biological liquids, because the liquid does not penetrate into the capillary irrespectively of the properties of the internal surface.

The proper account of aerodynamic and hydrodynamic effects mentioned earlier also suggests that wide short capillaries should be preferred. These effects are strongly dependent on the geometric characteristics of the capillaries. Here also the pressure restoration time plays a crucial role. In long narrow capillaries the process of pressure smoothening along the capillary is aperiodic and rather slow, and if the surface lifetime is small, at the time moment of maximum capillary pressure overcome, a significant pressure difference still exists between the two ends of the capillary [32]. Therefore the pressure in the gas reservoir exceeds appreciably than in the bubble. This leads to a significant overestimation of the surface tensions calculated from the measured pressure in the reservoir, see Fig. 3, capillary no. 1 [29]. In wide short capillaries the pressure smoothening process is rapid, therefore the aerodynamic resistance of the capillary does not introduce a significant error even for very short surface lifetimes, see Fig. 3, capillary no. 3.

With the decrease of the capillary length and the increase of its radius, the inertia of gas and liquid becomes more significant [23,27]. It should be mentioned that at the time moment when the maximum capillary pressure is achieved, the

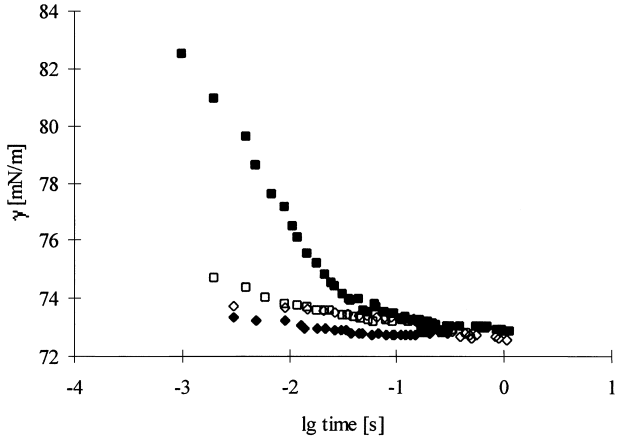

Fig. 3. Apparent dynamic surface tension of pure water at 18°C ($\sigma = 73$ mN/m) for various capillaries: No. 1 ($l = 7.3$ cm, $r_0 = 0.0117$ cm) (■); No. 2 ($l = 3$ cm, $r_0 = 0.0117$ cm) (□); No. 3 ($l = 1.1$ cm, $r_0 = 0.0117$ cm) (◆); No. 4 ($l = 1$ cm, $r_0 = 0.0075$ cm) (◇); according to [29].

motion of the meniscus becomes slower. In this case the direction of inertial forces coincides with the direction of the meniscus motion. The inertia of gas and liquid contributes to the overcoming of the maximum bubble pressure; therefore for wide short capillaries a large excess pressure in the gas reservoir cannot exist [33], and aerodynamic corrections can be neglected.

However, a severe experimental problem arises when wide capillaries are used. The inertia of liquid and gas can lead to a regime where instead of a regular creation of separate bubbles, a series of bubbles occur. Such a series consists of several bubbles, with a large time interval between two series [32]. This effect hampers the measurements and has to be generally avoided. This is achieved by using narrow capillaries with large resistance [19,20], or by introducing an additional resistance into the gas flow path [34]. It is essential that the effect of series formation occurs only in pure liquids and dilute solutions of surfactants [32], and does not occur in concentrated solutions like biological liquids.

It should also be noted that the use of wide short capillaries eliminates the influence of the liquid viscosity on surface tension measurements. This is also very important for biological liquids, which are often highly viscous. It can thus be concluded that with respect to a number of properties, short wide capillaries are most suitable for studies of dynamic surface tension of biological liquids using the maximum bubble pressure method.

Table 1
Surface tension parameters of serum and urine for healthy persons with respect to sex

Biological liquid	Parameter[a]	Sex[b]	
		Male	Female
Serum	σ_1, mN/m	69.2 ± 0.50	70.8 ± 0.59
	σ_2, mN/m	67.1 ± 0.39	68.3 ± 0.54
	σ_3, mN/m	59.3 ± 0.18	61.3 ± 0.65[c]
	λ_0, mN m^{-1} s$^{-1/2}$	4.9 ± 0.90	3.6 ± 0.70
	λ, mN/m^{-1} s$^{1/2}$	15.3 ± 0.61	8.2 ± 0.60[c]
Urine	σ_1, mN/m	71.6 ± 0.24	71.5 ± 0.21
	σ_2, mN/m	69.2 ± 0.27	69.3 ± 0.32
	σ_3, mN/m	56.6 ± 1.81	61.1 ± 0.36[c]
	λ_0, mN m^{-1} s$^{-1/2}$	5.5 ± 0.70	5.2 ± 0.65
	λ, mN/m^{-1} s$^{1/2}$	11.7 ± 0.43	15.2 ± 0.54[c]

[a] σ_1, surface tension at $t = 0.01$ s; σ_2, surface tension at $t = 1$ s; σ_3, σ_∞ derived obtained by extrapolation for $t \to \infty$; $\lambda = (d\sigma/dt^{-1/2})_{t\to\infty}$; $\lambda_0 = -(d\sigma/dt^{1/2})_{t\to 0}$.

[b] Data are given as interval $M \pm m$, where M is the average value of a parameter and m^2 the distribution of this measured value ($m^2 = \varepsilon^2/n$) , where ε is the standard deviation, and n is the number of volunteers. The interval $M \pm m$ corresponds to a probability of 0.6827 that the measured value occurs within the interval $[M - m, M + m]$. The interval $M \pm 3m$ corresponds to a probability of 0.9973 that the measured value occurs within the interval $[M - 3m, M + 3m]$ and may serve as a normal value.

[c] Significant difference between males and females.

3. Dynamic surface tensiometry of serum and cerebrospinal liquid obtained from healthy subjects

The results of tensiometric studies of serum and urine taken from healthy subjects [1,7,35–38] show that there are certain specific features in dynamic surface tensiograms, which are determined by the gender and age of the volunteers. Table 1 summarizes the averaged tensiographic parameters of these biological liquids with respect to gender.

Equilibrium surface tensions of serum and urine obtained from females are higher than those from males. The slope of the tensiograms λ are also different when comparing gender: it is higher in serum but lower in urine obtained from males. The relatively high σ_3 values for female serum can in part be attributed to lower contents of some proteins, lipids and hydrocarbons in their blood. In

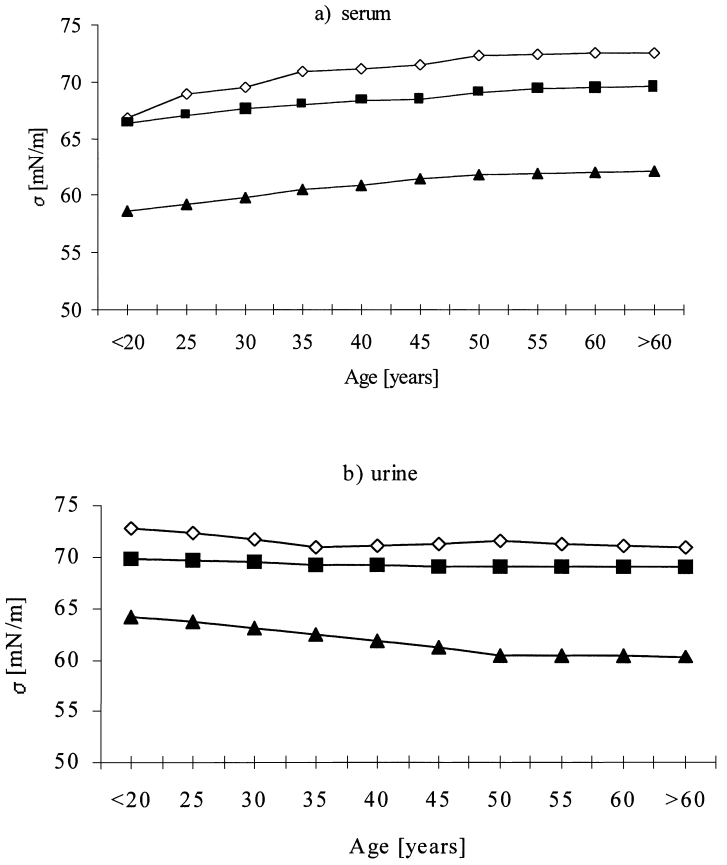

Fig. 4. Surface tension parameters measured in serum and urine obtained from healthy persons as a function of age; (◇), σ_1; (■), σ_2; (▲), σ_3 parameters.

particular, female serum has a lower physiological level of low density and very low density lipoproteins, and of a number of ferments (creatine kinase, α-glutamyl transpeptidase, γ-glutamate dehydrogenase, etc.). In addition, sex-related differences exist regarding variations of fractions of phospholipids, cholesterol, triglycerides, free fatty acids, polysaccharides (galactose, galactose amine, hexose, fucose) and uric acid. Furthermore, sex-related differences exist in the occurrence of protein molecules that contain amino acids possessing hydrophilic radicals [39,40]. It should be stressed that the content of fibronectin in serum obtained from healthy males is much higher than for females [41,42].

After describing gender-related dependencies on dynamic tensiograms, age-related dependencies will now be described. With increasing age, a gradual growth of surface tension of serum, and a gradual decrease of surface tension of urine take place [1], with most pronounced changes occurring in the very short time range, see Fig. 4.

The increase of σ_1 values for serum during ageing may be in part due to changes of the biosynthesis and metabolism of proteins and lipids, that leads to changes in the level of surfactants in biological liquids. Examples are insulin and steroid and thyroid hormones, the production and secretion of which gradually decrease during ageing. In addition, the response of receptors with respect to arginine-vasopressin, adrenaline and thyroxin of target cells in kidney, liver, and the hypothalamo–hypophysiary system deteriorates during ageing [35,37].

Some differences were observed in the behaviour of dynamic tensiograms for children. Sixty-five healthy children (boys and girls) from the age of 2 months to 15 years have been screened. The σ_1 value of serum for children is 1.2–2 mN/m higher than for adults, while σ_2 and σ_3 of serum and urine differ from corresponding values for adults by < 1 mN/m. It should be noted that the average tensiometric value of λ of serum for the two sexes comparing adults is virtually equal (12 mN m^{-1} $s^{1/2}$). In contrast, this value measured in samples from girls exceeds that of

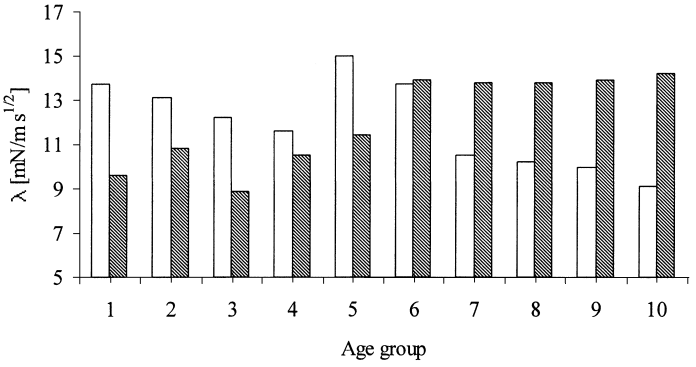

Fig. 5. Values of λ for serum (white) and urine (hatched) for children and adults of various age; age: 1, less than 1 year; 2, 1–5 years; 3, 6–10 years; 4, 11–15 years; 5, 16–20 years; 6, 21–30 years; 7, 31–40 years; 8, 41–50 years; 9, 51–60 years; 10, 60 years and more.

adult females by 50%, while for boys this value is by 20% lower than for adult males. Therefore, this parameter increases with males' age, and decreases with female's age. Fig. 5 summarizes the dependence of this parameter for children and adults as a function of age.

The values for λ in serum are rather high for children younger than 1 year, decrease towards the puberty period, and then sharply increase, attaining its maximum in the age of 16–20. Subsequently, a gradual decrease of this parameter takes place. The λ-values of urine for juveniles are rather stable and low (10–12 mN m^{-1} $s^{-1/2}$), and increase with age to 14 mN m^{-1} $s^{-1/2}$. While the value of λ for urine tensiograms increases over the first 40 years of life and then remains virtually constant, the λ value of serum gradually decreases with age.

Dynamic tensiograms of serum, urine and cerebrospinal liquid obtained during various pathologies have been compared with the tensiograms of liquid obtained from healthy persons of corresponding sex and age. The tensiograms of cerebrospinal liquid for patients suffering from different neurological diseases are compared with the averaged tensiogram of healthy persons (control group). The control group consisted of patients with diseases involving no damage of the nervous system (vascular dystonia, Harris facial sympathalgy, porencephalic cyst, residual encephalopathy with liquor-vascular decirculation). Averaged values of dynamic tensiographic parameters of cerebrospinal fluid for the control group (16 patients) were: $\sigma_1 = 71.7$ mN/m, $\sigma_2 = 66.6$ mN/m, $\sigma_3 = 60.4$ mN/m and $\lambda = 7.6$ mN m^{-1} $s^{1/2}$.

For some pathologies, a significant increase of the dynamic surface tension for serum and cerebrospinal liquid was found in the short time range. Initially, no explanation can be given for values of $\sigma_1 = 73$–75 mN/m, which exceed the surface tension of pure water by 1–3 mN/m. However, similar anomalies in the short surface lifetime range were found for some proteins (β-casein, β-lactoglobulin) in a number of studies [43–45]. To give a qualitative explanation for this

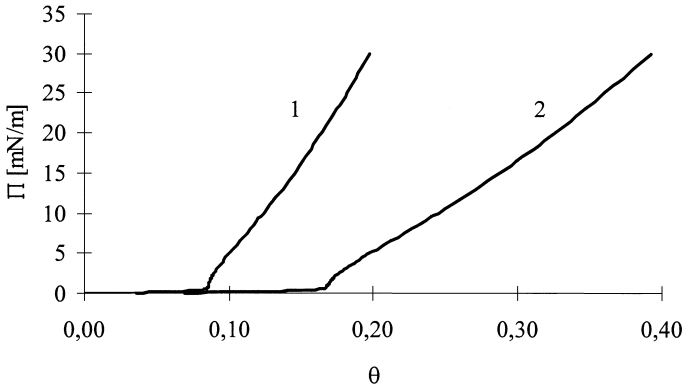

Fig. 6. Dependence of surface pressure on the adsorption layer coverage for a protein solution ($M = 24\,000$, $\omega_{min} = 2$ nm^2, $\omega_{max} = 60$ nm^2) , $a = 400$ (curve 1) and $a = 100$ (curve 2).

apparent contradiction, the protein adsorption theory summarized by Fainerman and Miller [15] can be used. It is seen from Fig. 6 [15] that no decrease in the surface tension of protein solutions takes place for monolayer coverage less than 10%.

Note that in the calculation of surface pressure, only the contributions from the entropy of mixing and Coulomb interaction were taken into account. At the same time, 10% coverage of the monolayer by protein corresponds to a protein adsorption of approximately 0.3–0.5 mg/m^2, or $(3–5) \times 10^{-6}$ mol/m^2 (per mole of amino acid groups). For an adsorption layer of 1 nm thickness, this results in a concentration of 3–5 mol/l for ions within the surface layer. It is well known that in aqueous solutions of inorganic electrolytes of a similar concentration the surface tension increases by some mN/m.

4. Surface tensiometry in rheumatology

Degradation of connective tissue which occurs during rheumatic diseases is caused by the disturbance in the biosynthesis of macromolecules, in particular, various types of collagens, proteoglycans, elastin and structural glycoproteins. Collagen and elastin undergo a degradation, which results from the decreased activity of lysonoxydase and production of tropoelastin, and enhanced elastolytic processes. The turnover of connective tissue metabolism takes place from the ordinary exocrine to endocrine route. Elastase is responsible for the control of metabolic processes in the connective tissue. One important class of components in connective tissue are the glycosaminoglycanes, which largely determine the flexibility, strength and elasticity of articular (arthroidal) cartilage, the course of ossification, calcification and fibrillogenesis processes. Disintegration of collagen and its destruction is manifested by the increase of the amount of oxyproline, which is virtually contained in collagen fibres only. For rheumatoid arthritis and other rheumatological diseases, significant increases in the elastolytic activity and the contents of glycosaminoglycanes and oxyproline in blood were observed [46–48].

During the development of rheumatoid arthritis the proteolytic ferments play a significant role (due to their universal ability to impact the cartilage and other articular structures). Proteolysis leads to the formation of biologically-active substances which influence the course of inflammatory processes, participate in the production of the rheumatoid factor and cause degradation of the connective tissue. The level of proteolytic ferments in blood increases with the concentration of α_2-globulins, and does not depend on the level of seromucoids, C-reactive protein and γ-globulins [49].

One of the factors of the pathogenesis of inflammatory rheumatic diseases is the change of the rheological properties of blood, related to the immune imbalance (mainly with the enhanced synthesis of immunoglobulin and circulating immune complexes). The increased viscosity of blood is determined by the molecular composition and configuration of proteins [50–54]. A phenomenon common to rheumatic diseases is the increase in the contents of degradation products of fibrin

Table 2
Surface tension variation of biological liquids during various rheumatic diseases may serve as differential diagnostic indicators[a]

Diseases	n[b]	Surface tension parameter							
		Serum				Urine			
		σ_1	σ_2	σ_3	λ	σ_1	σ_2	σ_3	λ
Rheumatism	33		+	+					−
Systemic lupus erythematosus	45	+			+		−		−
Sclerodermia systematica	20	+	+	+	+	+	−		
Haemorrhagic vasculitis	22	+	+			+	+		
Rheumatoid arthritis	43	+	+	+					−
Bechterew's disease	12				−			+	
Reiter's disease	29	+			−	+		+	
Psoriatic arthropathy	23						+	+	−
Gout	46				−	+	+	+	
Osteoarthrosis	49					+	+	+	−

[a] +, statistically significant increase of parameter compared with normal values; −, statistically significant decrease of parameter compared with normal values.
[b] n, number of patients studied.

and fibrinogen in the blood. These products, can exist as high molecular compounds or molecular fragments of monomeric fibrin or fibrinogen.

The quantitative and qualitative variations of proteins in blood, quite naturally, produce their effect on the state of surface tension in rheumatic diseases. The total number of patients studied with rheumatic diseases was 322 of which 33 had

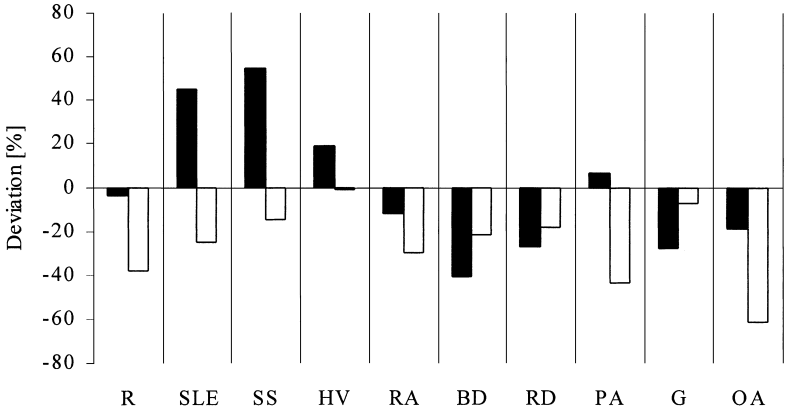

Fig. 7. Variation of λ-values in serum (black) and urine (white) obtained from patients with rheumatic diseases. The changes are given in % compared with corresponding healthy controls. R, rheumatism; SLE, systemic lupus erythematosus; SS, sclerodermia systemica; HV, haemorrhagic vasculites; RA, rheumatoid arthritis; BD, Bechterew's disease; RD, Reiter's disease; PA, psoriatic artropathy; G, gout; OA, osteoarthrosis; black, serum; white, urine.

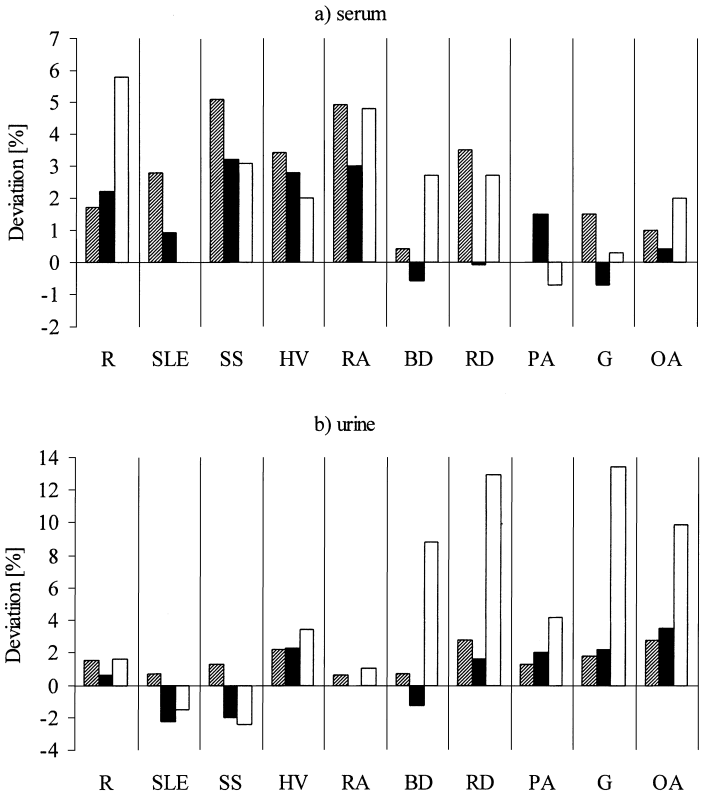

Fig. 8. Changes of surface tension parameters measured for biological liquids obtained from patients with rheumatic diseases. Changes are given in % compared with corresponding healthy controls. R, rheumatism; SLE, systemic lupus erythematosus; SS, sclerodermia systematica; HV, haemorrhagic vasculitis; RA, rheumatoid arthritis; BD, Bechterew's disease; RD, Reiter's disease; PA, psoriatic arthropathy; G, gout; OA, osteoarthrosis; hatched, σ_1; black, σ_2; white, σ_3.

rheumatism, 45 systemic lupus erythematosus, 20 sclerodermia systemica, 22 haemorrhagic vasculites, 43 rheumatoid arthritis, 12 Bechterew's disease, 29 Reiter's disease, 23 psoriatic artropathica, 46 gout, 49 osteoarthrosis.

It is seen from Table 2 and Figs. 7 and 8 that peculiarities in the dynamic surface tensions and the slope of the curves are observed for all rheumatologic diseases, with particular features for each disease. This fact is very important from a differential diagnosis point of view.

We studied 45 patients with systemic lupus erythematosus and compared dynamic tensiograms of serum and urine with data obtained for normal probands. Systemic lupus erythematosus is characterized by increased surface tensions of serum in the short time range, and by a decrease of σ_2 and λ for urine. These deviations which exceed the range $M \pm 3m$ as determined for healthy persons, were detected in 62.7%, 57.3% and 71.6% of all cases, respectively. It should be

noted that differences in the variations of dynamic surface tensiograms exist with respect to sex. We believe that these ambiguous inconsistencies in surface tension parameters of biological liquids with respect to patients' sex can be explained by a number of specific features characteristic to the clinical progress of a disease.

It should be noted that if no kidney manifestation occurs, then an increase of equilibrium surface tension of serum and a corresponding decrease of λ is usually observed, while the development of glomerulonephritis generally results in quite opposite changes of surface tensiograms. Obviously, this fact is especially important, because the prognosis of systemic lupus erythematosus is usually determined by kidney manifestation, the presence of lupus glomerulonephritis. Urine sampled from patients with lupus glomerulonephritis had a decreased σ_2 value and significantly decreased λ values. In fact, the value of urine tensiographic λ indicates the development of kidney manifestation of systemic lupus erythematosus, because the absence of nephritis is characterized by a very high value for λ in urine.

When the nephritic syndrome occurs, we found the following characteristics for serum and urine tensiograms. Tensiograms of serum had decreased σ_2 and σ_3 values. Tensiograms of urine had decreased σ_1, σ_2 and λ values. If kidney function deteriorates we found decreased values of σ_1, σ_2 and σ_3 in serum and decreased values of σ_2, σ_3 and λ in urine, see Fig. 9.

The closest correlation links between surface tension parameters of serum were shown to exist with respect to the concentration of immunoglobulin. It should be noted that a similar correlation was observed between surface tensiographic parameters and the level of binding ability of immunoglobulins with amino acids (proline, oxyproline, arginine, glutaminic acid, lysine). Some comments should be made in this regard. As no protein other than collagen contains proline and oxyproline (these substances are specific labels of collagen and its degradation products), the increased binding capability of immunoglobulin to these amino acids and increased concentrations of these amino acids in blood specifically reflect the extent and intensity of destructive processes in the connective tissue.

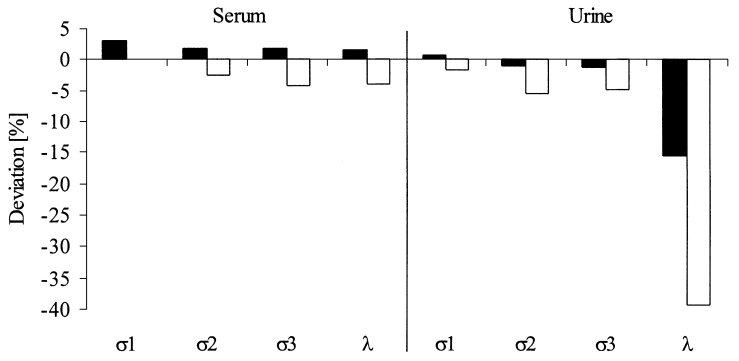

Fig. 9. Variations of dynamic surface tension parameters of serum and urine for patients suffering from lupus glomerulonephritis with non-deteriorated (black) and deteriorated (white) kidney function ($\pm$% deviations from parameters for healthy persons).

Comparing the parameters which reflect an increase in the concentration of serum immunoglobulins G, A and M with increased binding ability to amino acids we have shown that in patients with grave manifestation of systemic lupus erythematosus, the amount of these immunoglobulins had increased approximately 2.3, 2.1 and 1.3 times, respectively, as compared with normal concentrations. The corresponding increase in their binding ability with amino acids was increased 3.4–5.7, 2.7–4.7 and 2.4–2.6 times [50]. In patients with moderately severe systemic lupus erythematosus, no changes in the concentrations of these immunoglobulins were found as compared with mild systemic lupus erythematosus. The binding ability of immunoglobulin with amino acids increases with severity of the disease. The most significant increase was found for the binding ability of immunoglobulin-G with arginine (13.5 times), glutaminic acid (7.8 times) and lysine (5.3 times) [53]. The data presented above provide an argument that the dynamic surface tensions of serum are necessary integral parameters for the estimation of the activity of the pathologic processes of systemic lupus erythematosus.

For patients with rheumatoid arthritis, antibodies for the hydrolysis of hyaluronic acid are produced. It is known that, after binding to antigen, the antibodies change their conformation and form immune complexes. These complexes are capable of stimulating the superoxide dismutase activity. The presence of superoxide dismutase enhance inflammation and introduce an imbalance in the surfactant composition of serum and in the interstitium.

For rheumatoid arthritis, the presence of various antibodies in serum is characteristic, along with an increase of circulating immune complexes that activates the complement system leading to concentration increases of C_3, C_{3a}, C_{3bi}, C_{3dg} components of the system [55]. The proteins of the complement system, during its activation, acquire new surface active properties, which eventually leads to variations in surface tensions of serum. The 100–1000 times increase in the contents of

Table 3
Differences between values for various surface tension parameters measured in serum and urine from rheumatoid arthritis patients and corresponding controls[a]

Biological liquid	Surface tension parameter	Parameter deviation (%)	
		$> M + 3m$	$< M - 3m$
Serum	σ_1	76.7	18.6
	σ_2	62.8	14.0
	σ_3	67.4	9.3
	λ	7.0	53.5
Urine	σ_1	41.9	16.3
	σ_2	51.6	27.9
	σ_3	53.5	37.2
	λ	9.3	74.4

[a] Differences are given as the frequency (in %) of values for patients that lie above $M + 3m$ or below $M - 3m$ for controls.

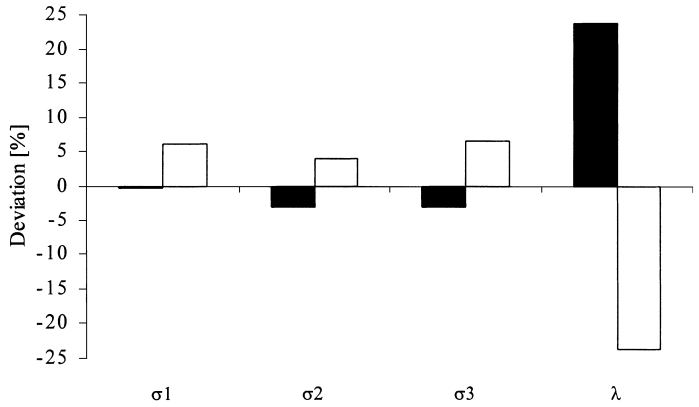

Fig. 10. Changes in surface tension parameters measured in serum obtained from patients with rheumatoid arthritis for various serologic activities of the disease: black, seronegative; white, seropositive. Changes are given in % compared with corresponding healthy controls.

amyloid acute phase SAA-protein is similar to C-reactive protein. Note that the concentration of other acute phase proteins (α_1-antitrypsin, α_1-antichymotrypsin, fibrinogen, haptoglobin, α_1-acidic glycoprotein) increase only by a factor of 2–4 [56].

The tensiographic parameters for serum in the short and medium time range for rheumatoid arthritis exceed significantly those characteristic of healthy persons. This behaviour of σ_1 ($> M + 3m$) was found for more than three-quarters of patients, and for σ_2 for almost two-thirds of the patients (cf. Table 3).

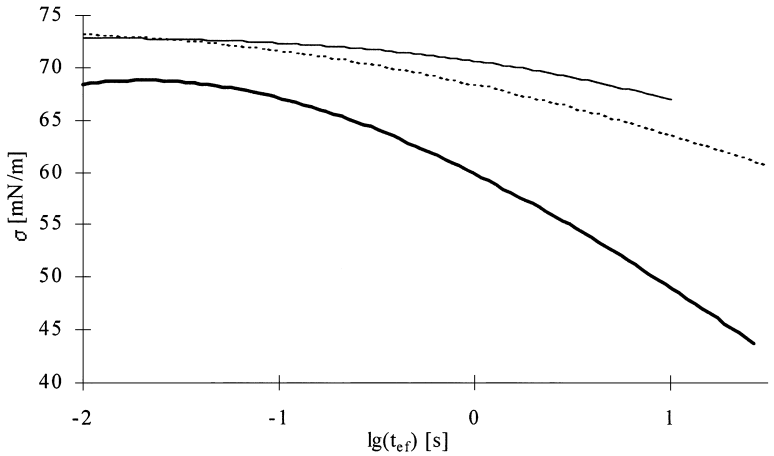

Fig. 11. Examples for serum tensiograms obtained from patients with rheumatoid arthritis, one with seropositive version (female, age 69, thin line); one with seronegative version (female, age 52, thick line); dotted curves correspond to average values for healthy females of corresponding age.

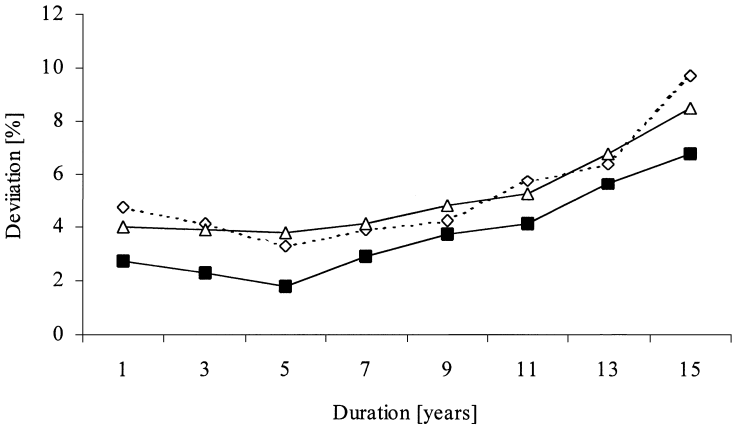

Fig. 12. Changes of surface tension parameters measured in serum obtained from patients with rheumatoid arthritis as a function of the disease duration. Changes are given in % compared with corresponding healthy controls. (◇), σ_1; (■), σ_2; (△), σ_3.

Of some interest are the data concerning the variations in the tensiograms of serum for patients with the seropositive version of the disease, when the rheumatoid factor in the serum leads to a sharp increase of the surface tension. One can presume therefore that the concentration of immunoglobulins, which contributes to the formation of the rheumatoid factor, can affect the surface tension of serum for rheumatoid arthritis (Figs. 10 and 11).

It should be noted that the dynamic surface tension of serum increases with the duration of rheumatoid arthritis (cf. Fig. 12), becoming apparent only after 7 years with a two-fold increase of the parameters after some 15 years. We believe this phenomenon can be explained either by the formation of some new substances in blood, or by the fact that substances already existing can acquire unusual surface active or inactive properties (e.g. due to medical applications).

The synovial fluid is the most available indicative medium regarding the character of the articulatory lesion. It should be kept in mind that σ_1 and λ for synovial fluid are lower than those for serum. At the same time, synovial fluid of patients with rheumatoid arthritis possess large concentrations of immunoglobulins-G, immunoglobulins-M and immune complexes which contain large quantities of immunoglobulins G, M and A [57]. The activity of acidic phosphatase exceeds eight times that characteristic for patients suffering from post-traumatic arthritis, while the acetyl-β-D-glucosaminepeptidase activity is three times as high as that for post-traumatic arthritis, and two times higher than that for patients suffering from Reiter's disease. The level of β_2-microglobulin is twice as high as the contents of this protein in blood, and correlates with the total amount of protein in synovial fluid. Synovial fluid of patients with rheumatoid arthritis contains large amounts of immunoglobulin-G complexes, which can react with the immunoglobulin-M-rheumatoid factor forming very large, stable and insoluble intercross-reacting

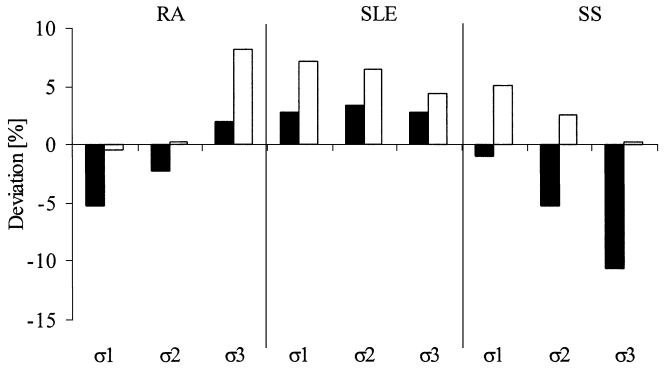

Fig. 13. Changes in surface tension parameters measured in serum obtained from patients with rheumatic diseases before (black columns) and after glucocorticoid therapy (white columns). Changes are given in % compared with corresponding healthy controls. RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SS, sclerodermia systematica.

compounds. Rheumatoid arthritis is accompanied by a decreased activity of the fibrinolytic system in synovial fluid. Eventually, the contents of fibrinogen and fibrin in synovial fluid increases. For rheumatoid arthritis an increase of the fibronectin concentration is observed [58,59]. It should be stressed that for arthritis with another origin, the contents of fibronectin in synovial fluid virtually does not change.

Application of glucocorticoids is a basic therapy of rheumatic diseases. During glucocorticoid therapy a trend towards normalization of dynamic tensiographic parameters occurs. Fig. 13 shows variations of surface tension parameters of serum before and after administration of glucocorticoids to patients with rheumatoid arthritis, systemic lupus erythematosus and sclerodermia systematica. It can be concluded that glucocorticoid therapy increases surface tensions of serum, especially for sclerodermia systematica.

For cases with rheumatoid arthritis and sclerodermia systematica, this is true mostly for σ_3, while for systemic lupus erythematosus the important parameter is σ_1. For patients with rheumatoid arthritis the glucocorticoids treatment leads to a normalization of σ_1 and σ_2, while for sclerodermia systematica the normalized parameters are σ_2 and σ_3. For systemic lupus erythematosus (patients who do not suffer from lupus glomerulonephritis were also incorporated in the screened group) increased surface tensions of serum were observed.

The application of plasmapheresis to patients suffering from rheumatoid arthritis leads to higher surface tensions of serum. This effect is caused by the excretion of some proteinic surfactants from the organism, while the contents of macroproteins is decreased along with an increase of the parameters indicative of relative albuminaemia. During a selective plasmapheresis, fibronectin, complement components, cryoglobulins, circulating immune complexes and cryofibrinogen are removed. As there are no significant changes in the level of albumin, the total

protein content at the end of a series of plasmapheretic procedures is similar to its initial value. The comparison of surface tensiometric parameters measured before and after plasmapheresis once again shows that pathologic proteins affect the surface tension of serum for rheumatoid arthritis. The elimination of pathological proteins from blood during plasmapheresis is clearly accompanied by the return of surface tensiometric parameters to normal values.

5. Surface tensiometry in neurology

In modern neurology, cerebrospinal fluid and blood are widely used as diagnostic tools. Cerebrospinal fluid protects the brain from physical injuries, maintains a stable intracranial and osmotic pressure in brain tissue, participates in metabolic processes, neurohumoral and neuroendocrinal regulation, and reacts with compensatory-protective mechanisms during central nervous system diseases. Any pathological processes in the central nervous system are accompanied by changes of cerebrospinal fluid, i.e. changes in composition and properties of surfactants contained therein.

Table 4 summarizes the results obtained in comprehensive studies of dynamic surface tension of cerebrospinal fluid and serum obtained from patients suffering from various diseases of the nervous system. The control group consisted of patients without diseases of the nervous system [41].

All patients were subdivided into the following groups:

1st group: 49 patients with neurological infection diseases (encephalitis, meningoencephalitis, arachnoiditis, myelitis, polyneuritis and slow infections-encephalomyelitis and multiple sclerosis);
2nd group: 27 patients suffering from vascular brain diseases (decirculatory encephalopathy and acute brain circulatory disturbances);

Table 4
Surface tension variation of biological liquids during various types of nervous system diseases may serve as differential diagnostic indicators[a]

Nervous system disease type	n[b]	Serum				Liquor			
		σ_1	σ_2	σ_3	λ	σ_1	σ_2	σ_3	λ
Infection	49		−	−			+	+	+
Vascular	27					+	+	+	+
Spondylogenic	46			−	+	+	+		+
Neoplasm	36		−	−					+
Trauma	38		−	−			+	+	+

[a] +, statistically significant increase of parameter compared with normal values; −, statistically significant decrease of parameter compared with normal values.
[b] n, number of patients studied.

3rd group: 41 patients with spondylogenic diseases (spinal osteochondrosis, radiculitis, vertebrogeneous myelopathy, spondylolisthesis);

4th group: 36 patients with neoplasms of the nervous system (accusticus neurinoma, meningioma, tumours of cerebellum, fourth ventricle, posterior cranial fossa, trunk and spinal cord); and

5th group: 38 patients with traumatic brain damages (contusions of various degree of severity).

The control group consisted of patients with diseases involving no damage of the nervous system (vascular dystonia, Harris facial sympathalgy, porencephalic cyst, residual encephalopathy with liquor-vascular decirculation).

Our results that infection led to decreased surface tension parameter are supported by studies of other physico-chemical and biochemical parameters of serum obtained from such patients. For example, plasma viscosity increased during infection. Infection led to increased levels of amino acid and circulating immune complexes in blood, while the concentration of serotonin decreased [60]. One of the most important findings in patients with slow damage of the nervous system is the decrease in the contents of unsaturated fatty acids in blood. Some of the most significant changes were observed in multiple sclerosis: a reduction of the ratio of linolic to arachidonic acid in the high density lipoprotein fraction and complex ethers fraction of cholesterol [61]. For vascular pathology, the level of serum orosomucoid was increased [62]. After cerebral damage, the level of total cholesterol and the low density lipoprotein fraction in blood increase, while the concentrations of the high density and very low density lipoprotein fractions remain virtually constant. At the initial stage of the disease, a tendency to hypotriglyceridaemia was observed [63]. It is quite reasonable that these (and other) substances can affect the values of surface tensiographic parameters of serum.

For infection, tumour and traumatic diseases, the general feature is a decrease in σ_2 and σ_3 of serum. A decrease of σ_3 of serum is also observed for spondylogeneous diseases, while σ_2 tends to increase (Fig. 14).

Some increase of all surface tension characteristics of serum was found for patients suffering from vascular brain diseases. It is to be noted that only a pathological process in the spinal column results in a significant increase of the λ value for serum (Fig. 15).

An example of a dynamic serum tensiogram for a patient with slow encephalomyelitis is shown in Fig. 16.

For all types of neurological diseases except trauma some of the surface tension parameters of liquor increase: σ_2 increased in the 1st, 2nd and 3rd group, σ_2 in the 2nd and 3rd group, and σ_3 in the 1st and 2nd group. The increase of λ values for liquor was characteristic for all studied groups; however, the most significant increase was observed for patients with vascular brain pathologies. Low surface tension values at $t = 0.01$ s and $t \to \infty$ might be significant for intracranial neoplasm.

An example of cerebrospinal fluid tensiogram obtained from patient with acute

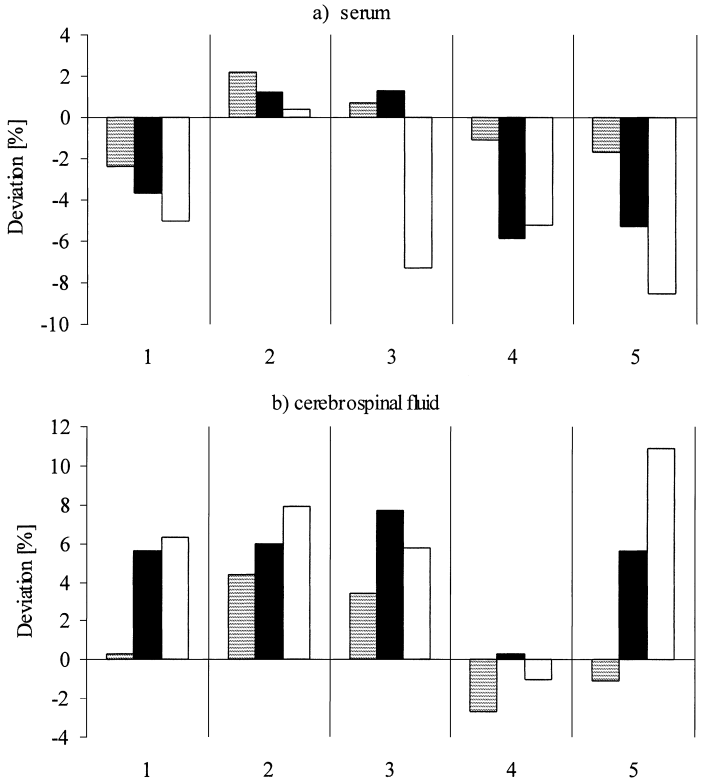

Fig. 14. Changes in surface tension parameters measured in biological liquids obtained from patients with nervous system diseases. Changes are given in % compared with corresponding healthy controls. 1, infection; 2, vascular diseases; 3, spondylogenic diseases; 4, neoplasm; 5, trauma; hatched, σ_1; black, σ_2; white, σ_3.

meningoencephalitis is shown in Fig. 17. The duration of disease, and age of patient also affect the dynamic surface tensiograms.

Cerebrospinal fluid is composed of water (98–99%), organic (proteins, amino acids, carbohydrates, urea, glycoproteins and lipoproteins), and inorganic (electrolytes and microelements) substances. The amount of proteins in cerebrospinal fluid is usually 200–400 times lower than in serum. In addition, two other fractions, prealbumin and θ-fraction (intermediate between β- and γ-globulins) are present. The proportion of prealbumin in liquor sampled from different sites is different. The fraction of prealbumin in ventricular liquor is 13–20% with respect to total protein, while this amount is 7–13% in liquor sampled from major cistern, and 4–7% in the lumbar liquor. In some cases this fraction cannot be detected in the cerebrospinal liquid, because it can be masked by albumins, and appears to be entirely absent when the amount of protein is large.

The protein composition of normal cerebrospinal fluid is essentially constant.

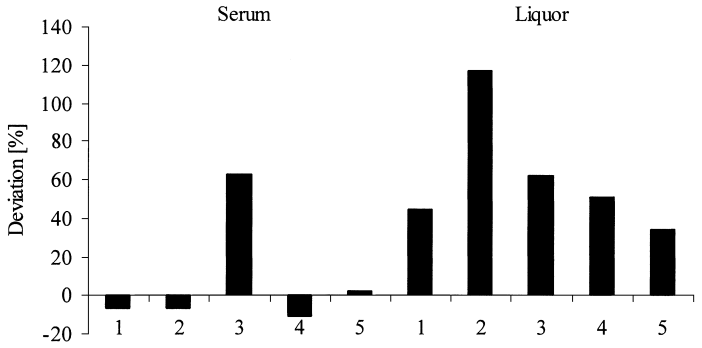

Fig. 15. Changes in λ values of serum and liquor tensiograms obtained from patients with various nervous system diseases. Changes are given in % compared with healthy controls. 1, infection; 2, vascular diseases; 3, spondylogenic diseases; 4, neoplasm; 5, trauma.

For pathologies which result in a malfunction of liquor circulation and hampering of venous deflux (e.g. intracranial tumours), pronounced typical variations in both qualitative and quantitative composition of proteins in cerebrospinal liquid are noticed. It should be stressed that dysproteinrachia was observed also in cases where the total concentration of proteins in the cerebrospinal fluid did not exceed the normal values. The increase in the total concentration of proteins in cerebrospinal fluid is accompanied by a simultaneous increase in the amount of transferrin, cholesterol, some enzymes, zinc, cuprum, etc. [64].

The possible correlations between surface tension parameters of cerebrospinal liquid and the amount of various proteins therein were studied. No interrelation between the values can be detected for patients with craniocerebral trauma. The level of total protein shows a very close correlation with σ_1 for vascular and spondylogenic diseases of the nervous system; here the correlation in the 2nd

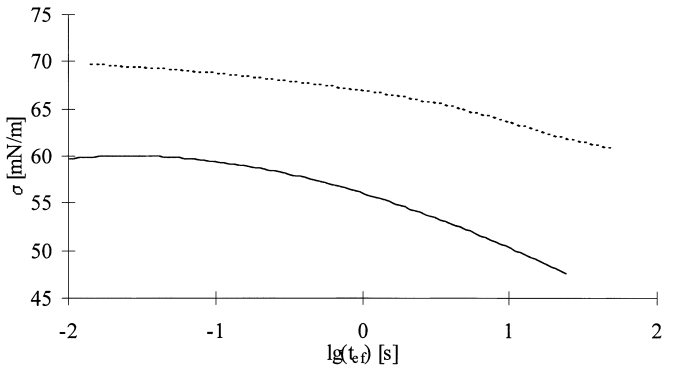

Fig. 16. Example for serum tensiogram obtained from a patient (male, age 27) with slow encephalomyelitis; dotted line corresponds to average values for healthy males of corresponding age.

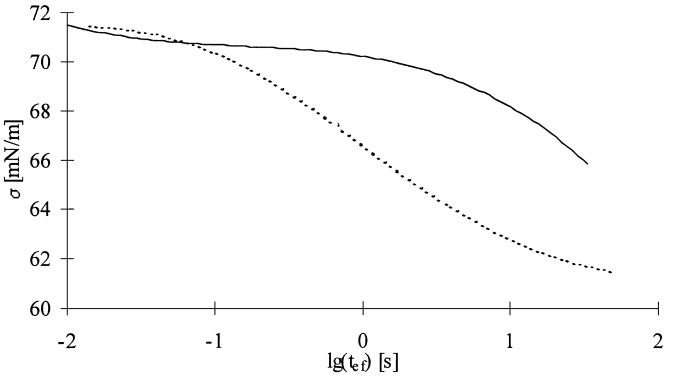

Fig. 17. Example for cerebrospinal fluid tensiogram obtained from a male patient, age 46 with acute meningoencephalitis; dotted line corresponds to average values for the control group.

group is negative, while in the 3rd group a positive correlation exists. The equilibrium surface tensions inversely depend on the total concentration of proteins in the cerebrospinal fluid for patients with spondylogenic diseases of the nervous system and brain neoplasms. Surface tensions of liquor in the medium time range show moderate correlations with parameters of total protein and albumin: for patients with vascular encephalopathy and acute derangements of blood circulation in the brain a positive correlation was observed, while for other groups of screened patients this correlation was negative.

The amount of proteins in the albumin and pre-albumin fraction in cerebrospinal fluid shows a negative correlation with σ_2 and σ_3 values for infection, and σ_1 for vascular diseases of the nervous system. For tumours and trauma, the dependence of surface tension parameters on the extent of protein level in cerebrospinal fluid is somewhat less pronounced. Therefore, the same substances are characterized by opposite signs of the correlation coefficients either for different pathologies of the nervous system, or for different surface lifetimes. In addition, in a number of cases, some surface active substances (e.g. albumin) show no correlation with dynamic surface tension parameters of the liquor. It has to be stressed once more that cerebrospinal liquor is an extremely complicated biological liquid comprising a great variety of proteins, lipids and other compounds. The composition of liquor undergoes quantitative and qualitative variations during any pathology of the nervous system, and therefore the results obtained in dynamic surface tension studies of liquor in some cases can disagree with the results of in vitro studies performed with model solutions.

At this point, some general conclusions could be made, which are interesting not only from a theoretical point of view, but have practical consequences (for the estimation of the amount of particular proteins, the determination of the activity of pathological processes and the stage of development of a disease, the control of treatments, etc.). In particular, it can be concluded that for infection of the nervous system, surface tension parameters of the cerebrospinal fluid correlate primarily

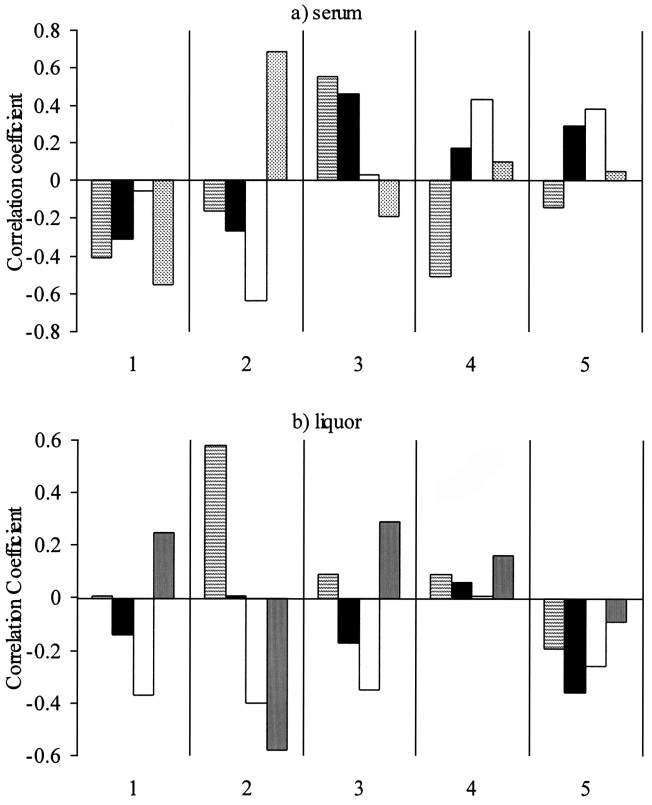

Fig. 18. Correlations between surface tension characteristics of serum and liquor obtained from patients with various type and severity of nervous system diseases. Surface tension parameters are: hatched, σ_1; black, σ_2; white, σ_3; grey, λ. Nervous system diseases are: 1, infection; 2, vascular diseases; 3, spondylogenic diseases; 4, neoplasm; 5, trauma.

with the amount of albumins and immunoglobulin-G in the liquor, for vascular diseases a correlation with albumin and fibrinogen exists; for spondylogenic diseases there is a correlation with immunoglobulin-M and transferrin; for brain tumours a correlation was found with the extent of β_2-microglobulinemia, while the λ values of liquor exhibit the most pronounced correlation with the protein level for spondylogenic diseases.

It seems interesting to estimate the possibility of prognosis of the severity of a disease using the data obtained from the dynamic surface tensiometry of biological liquids. Corresponding correlations are illustrated in Fig. 18.

The factors that predict a bad prognosis are:

- for infection → low serum values of σ_1 and λ;
- for vascular diseases → high serum values of λ for serum and high σ_1 values

for cerebrospinal fluid accompanied by small λ values for liquor and low equilibrium surface tensions for both liquids;
- for spondylogenic diseases → increase σ_1 and σ_2 values for serum;
- for neoplasm → decrease of σ_1 and increase of σ_3 values for serum;
- for trauma → increase of σ_2 and σ_3 for serum with a decrease of σ_2 values for liquor.

These data are important from a practical point of view, because the application of tensiometric analysis of biological liquids might be helpful for monitoring the course of treatment of neurological disorders.

In summary, dynamic surface tensiometry of serum and cerebrospinal fluid is useful for diagnostic and for prognostic purposes. We believe that further studies of surface phenomena in biological liquids taken from patients with neurological diseases should be extended into the following three areas:

1. the determination of unambiguous surface tensiometric parameters of biological liquids with respect to specific infection, vascular, spondylogenic, neoplasm and trauma-related diseases should include patients sex and age;
2. the detection of surface active and surface inactive compounds which affect the state of surface tension of biological liquids, should include experimental in vitro studies employing the modelling of the composition of cerebrospinal liquor; and
3. estimation of the dynamic properties of surface tensiograms of biological liquids for neurological diseases with respect to treatment and prognosis.

6. Surface tensiometry in oncology

Neoplasms are associated with compositional changes of blood. These variations change dynamic surface tension parameters tremendously. Therefore, dynamic interfacial tensiometry has a potential role concerning diagnostics of certain tumours and monitoring of its treatment.

For oncological diseases of various localization, a pronounced structural change of serum albumin along with a decrease in its concentration was observed. Large amounts of lipids, products of peroxide oxidation of lipids and polyunsaturated fatty acids of the ω_6 family in albumin is indicative of a disturbance of metabolic processes in the patient's organism, and also of large capabilities of albumin to transport lipid-related ligands. For patients suffering from cancer of various organs, increased concentrations in γ-globulin, circulating immune complexes, β_2-microglobulin, α_1-antitrypsin, haptoglobin, ferritin, orosomucoid, α_2-glycoprotein, T-globulin, C-reactive protein and polyamines in blood were observed [65–68]. An intensive synthesis of surface active fibronectin is performed by epithelial tumour cells, which leads to a hyperfibronectinaemia [66]. Amyloid P-component-glycoprotein is produced by hepatocytes. This glycoprotein is increased in serum obtained from patients with malignant tumours [56]. During oncological diseases,

significant decreases in the level of vitamin-K-dependent glycoprotein C (molecular mass 62 kDa) in serum was observed [69]. It was shown by Baskies et al. [70] that direct correlations exist between the extent of tumoural processes and the concentration of haptoglobin, orosomucoid and α-antitrypsin in blood, while there are inverse correlations with the concentration of albumin, pre-albumin and α_2NS-glycoprotein.

The ability of hepatomas to synthesize the hepatic embryonic protein, α-fetoprotein, was discovered 30 years ago. This fact strongly promoted the searches for new proteins, which arise during the development of neoplasms. The detection of oncofetal antigens can be regarded in some cases as an indication of tumoural development at its early stage, because the presence of these antigens depends on the degree of differentiation of tumour cells and the damage of intercellular links, and does not depend on the extent of the tumour.

The characteristic feature of epithelial tumours is the increase of carbohydrate antigen 19.9 and carcinoembryonic antigens α_1-, α_2-, α_2H-, β-, γ_1-, γ_2-fetoprotein and sulfo-glycoprotein in blood. The characteristic feature of tumours of the ovary is increased amounts of carbohydrate antigen 125, sialyl-SSEA, tissue polypeptide antigen, acid glycoprotein IAP and ferritin. The comedocarcinoma is accompanied by high levels of mucin-like glycoprotein and carbohydrate antigens 15.3 and 549. In serum obtained from patients with lung cancer, the concentrations of neurone-specific enolase and mucin-like glycoprotein are increased.

Malignant tumours that arise from tissue that normally do not produce any hormones, often start hormone production. This secretion of hormones is often called ectopic secretion. It should be kept in mind in this regard that, under normal conditions the production of hormones happens not only in the endocrine glands, but also in the cells of so-called amino precursor uptake and decarboxylation (APUD) system. This (diffuse neuroendocrinal) APUD system consists of a complex of hormone-synthesizing cells, specialized in the secretion of more than 35 various hormones and amines. It consists of neurosecretory cells of the brain, lungs, gastrointestinal tract, anterior lobe of the hypophysis, epiphysis, substantia medullaris of adrenal gland, C-cells of thyroid gland and D-cells of pancreatic gland. Therefore, the ectopic secretion is performed by cells which are neither endocrine cells, nor APUD system cells, and for which the production of hormones is not inherent.

Usually a decreased glucose level in blood is observed for extended tumoural processes. Possible sources of hypoglycaemia are the ectopic production of somatostatin, somatomedin, proinsulin and insulin, the formation of insulinase inhibitors, retardation of glycogenesis in the liver, an increase in the glucose consumption by the tumour, intensification of glycolysis due to the suppression of lipolysis and the production of tryptophan. Changes in the glucose concentration of a biological liquid can affect its surface tension. In fact, it was shown that increasing glucose levels in blood of patients with cancer is accompanied by increased surface tensions of serum in the short and medium time range. Interfacial tensiometric parameters for $t \to \infty$ correlate negatively (however, less pronouncedly) with the glycemic level.

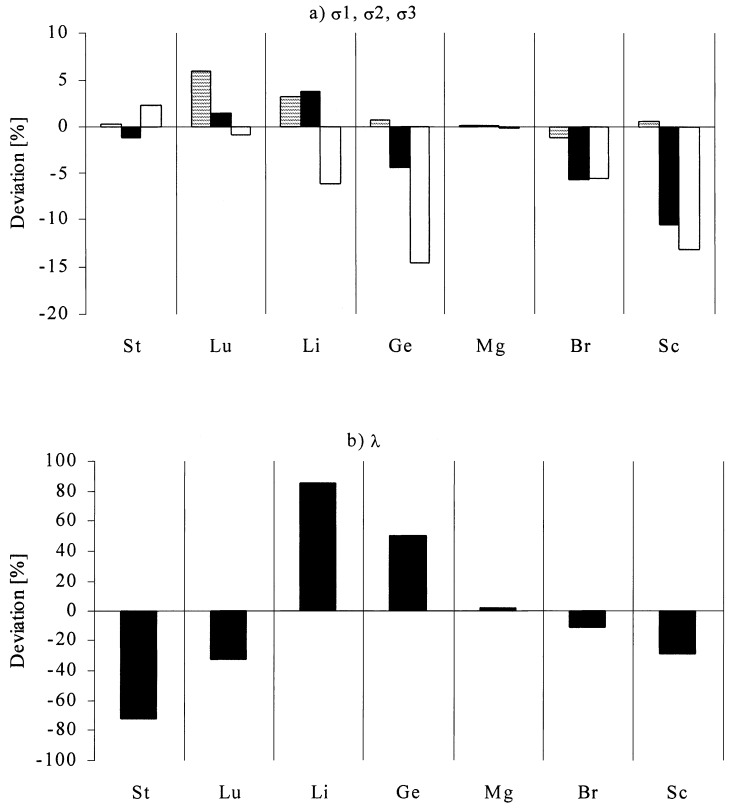

Fig. 19. Changes of surface tension parameters in serum obtained from patients with tumours of different location. Changes are given in % compared with healthy controls. St, stomach tumours; Lu, lung tumours; Li, liver tumours; Ge, genitals tumours; Mg, mammary gland tumours; Br, brain tumours; Sc, spinal cord tumours. The upper graph gives changes for σ_1, hatched; σ_2, black; σ_3, white. The lower graph gives changes for λ.

The compositional changes of blood due to neoplasm depend on the localization, size and histologic structure of the tumour. The total number of patients with malignant tumours studied was 165. Most changes in blood composition were observed for carcinoma of the stomach, lung and liver. At the same time, surface tension parameters of serum obtained from patients with carcinoma of the stomach (26 patients) show almost no differences in values of the reference group (healthy persons). Lung malignant neoplasm (17 patients) was characterized by increased surface tensions in the short time range, while for liver malignant neoplasm (23 patients) equilibrium surface tension decreased, as shown in Fig. 19.

Some ambiguity in the changes in surface tension parameters can be ascribed to various hormonal and fermental disturbances, variations in the eicosanoid and peroxide oxidation of lipid systems, peculiar features of the electrolyte and immune imbalance which finally results in complex variations of the composition of surfac-

tants. Previous pharmacotherapies, duration of a disease, patient's sex and age are also important.

It should be mentioned that metastases in the liver following carcinoma of the stomach or lungs lead to a decrease in the equilibrium surface tension and an increase of λ values of serum. In such cases, the interfacial tensiometric parameters approach those characteristic for primary hepatoma. These data permit the conclusion that decreases in equilibrium surface tensions of serum for patients with carcinoma of the stomach or lungs indicates metastatic spreading of the tissue into the liver, and represents evidence of the involvement of the liver in the formation of additional surfactants which can affect the dynamic surface tensions of biological liquids.

For tumours of the female reproductive organs (42 patients) the lowest equilibrium surface tensions of serum was detected, and these changes do not depend on metastatic spreading into the liver [71]. For neoplasm of genitals, the λ values are increased up to values higher than those characteristic of patients suffering from primary carcinoma of the liver. It should be noted that the parameters of interfacial tensiograms virtually do not depend on the particular localization of a tumour. No significant deviations from the normal amounts of the total protein in blood, its fractions of cholesterol, triglycerides and lipoproteins of various density were detected; however, an increase in the level of some ectopically secreted hormones (in particular, chorionic gonadotropin and somatotrophic hormone), which can probably determine the serum surface tensions (either directly or indirectly via other surfactants) takes place. For malignant neoplasms of female reproductive organs, a significant increase of the β_2-microglobulin in serum was observed. However, its concentration does not exceed a value characteristic for stomach or lung tumours, i.e. for cases when either no change in the λ values takes place, or these values become lower. In addition, no correlations exist between surface tension parameters and β_2-microglobulinemia.

For tumours of the mammary gland (21 patients) virtually no changes in averaged surface tension parameters of serum were detected. In some observations the dependence of equilibrium surface tension on the stage of pathological process was found, and correlations with some parameters of peroxide oxidation of lipids were detected which partly determine the composition of serum surfactant.

For brain tumours (26 patients) the values of σ_1 and σ_2 decrease. The variations of these parameters for spinal cord neoplasm (ten patients) are still more pronounced, and the λ value also becomes lower. For tumoural processes in the brain the behaviour of surface tensions of serum depends on the tumour location (Fig. 20).

It is seen that each kind of tumour has its specific features, which is important from a practical point of view. The differences in the dynamic surface tensions are caused by different compositions of surfactant in tumours and, in addition, can be determined by the age of patients and the duration of the disease. For example, tumours of the cerebellum, ventricle IV and posterior cranial fossa were characteristic primarily for children, whose liquor contains, even for healthy children, levels of γ-globulins and β_2-microglobulin lower than for adults, while the concentrations

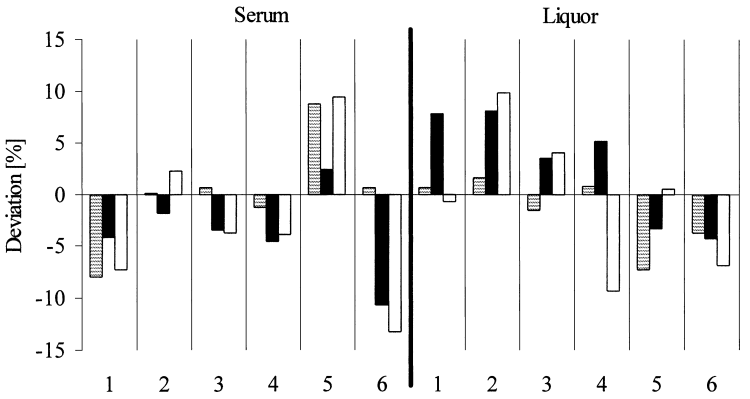

Fig. 20. Changes surface tension parameters measured in serum and liquor obtained from patients with nervous system neoplasms. Changes are given in % compared with corresponding controls. 1, accusticus neurinoma; 2, meningioma; 3, cerebellum tumour; 4, IV ventricle tumour; 5, posterior cranial fossa tumour; 6, spinal cord tumour; hatched, σ_1; black , σ_2; white, σ_3.

of amino acids are higher. In addition, the values of σ_2, σ_3 and λ inversely correlate with the duration of the disease, so that this factor should be considered in the analysis of interfacial tensiograms along with the patient's age. These data are of significant practical importance, enabling the prognosis of the morphologic type of tumoural processes before surgical treatment. Also, it was found that primary tumours of the spinal cord lead to a sharp increase of λ of liquor, while for metastatic spreading into the spinal cord a decrease of λ of serum occurs, as one can see in Fig. 21. These data are of certain practical importance for the differential diagnosis of pathologic processes in the spinal cord.

The application of radiotherapy results in a normalization of the state of the

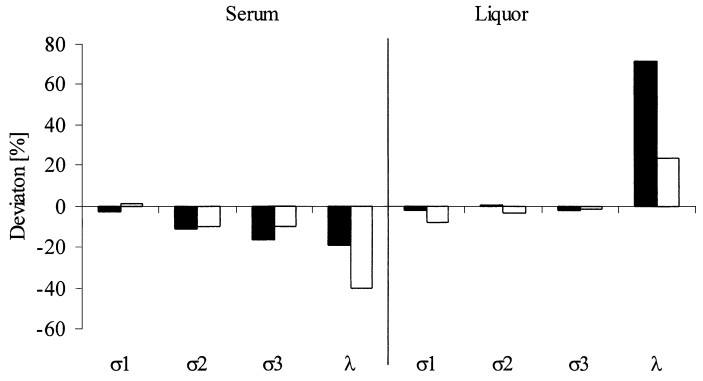

Fig. 21. Changes in surface tension parameters measured in serum and liquor obtained from patients with primary (black columns) and metastatic (white columns) various spinal cord neoplasms. Changes are given in % compared with corresponding controls.

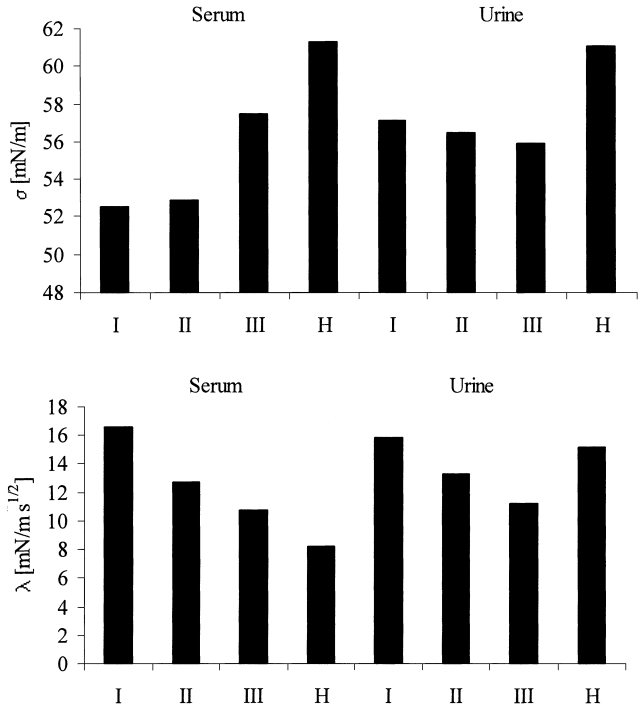

Fig. 22. Influence of radiation therapy on equilibrium surface tension and λ values in serum and urine obtained from patients with genital tumours and healthy controls. Upper graph gives the equilibrium surface tension for patients and healthy controls in mN/m. Lower graph gives the λ values in mN/m $s^{1/2}$ for patients and healthy controls. I, before the treatment; II, after remote radiation therapy (22–26 Gy) with intracavitary radiotherapy (20 Gy); III, after remote radiation therapy (46–48 Gy) with intracavitary radiotherapy (40–50 Gy); H, healthy females.

antioxidant system and of the phospholipid levels: these characteristics become closer to those common for patients at early stages of tumoural processes [72,73]. The irradiation leads to decreased concentrations of prostaglandins E_2 and $F_{2\alpha}$ in serum. These changes in the state of eicosanoids are accompanied by a reduction of peroxide oxidation of lipids and the activation of the antioxidant system [74,75]. For female patients with tumours of the reproductive organs, the application of a combined (remote and intracavitary) radiation therapy results in a decrease of the concentrations of malonic dialdehyde and diene conjugates in serum, while the amount of α-tocopherol becomes higher. Such variations in the peroxide oxidation system of lipids correlate with the equilibrium surface tension [71]. It is interesting that these variations are accompanied by a decrease in the interfacial tensiographic parameters of urine at $t \rightarrow \infty$, see Fig. 22.

Serum tensiograms for patients suffering from uterus body or neck carcinoma, treated by remote γ-therapy are presented in Fig. 23.

The results obtained from a screening of patients with tumours performed

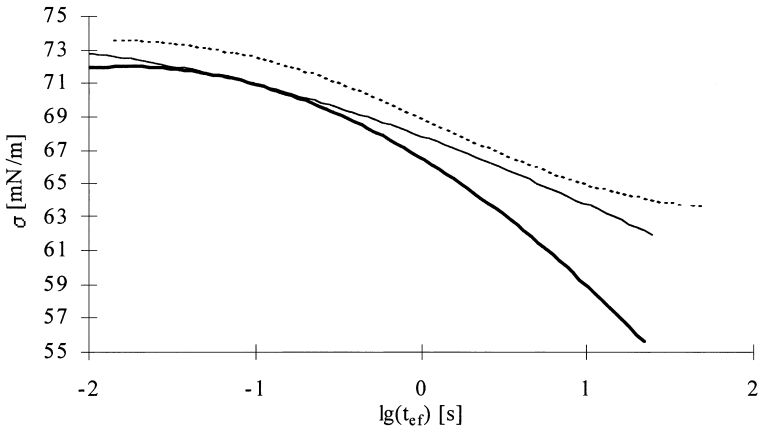

Fig. 23. Examples of serum tensiograms obtained from a patient with uterus carcinoma during the radiation therapy. Thick line, before treatment; thin line, after treatment; dotted line corresponds to average values for healthy controls.

before and after surgical treatment are rather interesting. Here the group of patients with nervous system neoplasm will be considered as an example. It is seen from Fig. 24 that the excision of brain tumour leads to a normalization of the surface tension of the cerebrospinal fluid.

Here the positive dynamics of the parameter σ_2 is especially demonstrative for patients with accusticus neurinoma and ventricle IV tumours, while for σ_3 the most representative patients in this sense are those with meningioma and cerebellum tumours. In these cases, decreased λ values were observed, and the average values of this parameter after an operation were virtually the same as those in the

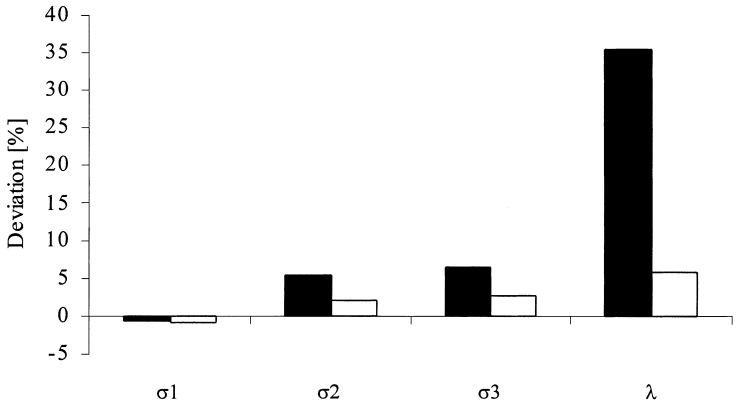

Fig. 24. Changes in cerebrospinal liquid surface tension parameters before (black) and after (white) surgical treatment for patients suffering from brain tumours. Changes are given in % compared with controls.

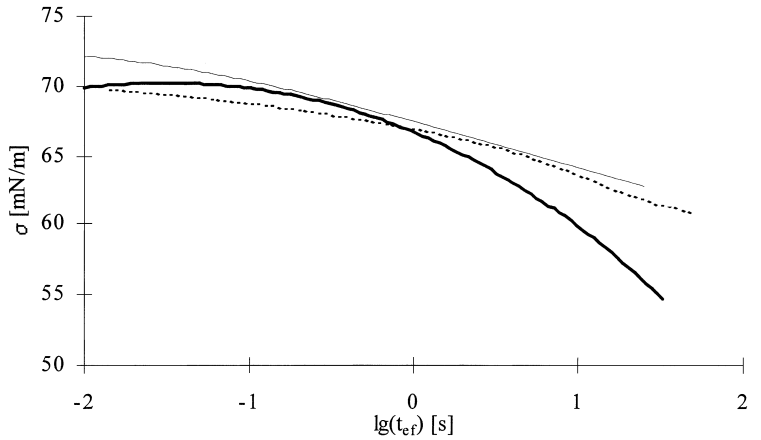

Fig. 25. Examples of serum tensiograms obtained from a male patient, age 31, with spinal cord tumour. Thick line, before surgical treatment; thin line, after surgical treatment; dotted line corresponds to average values for healthy males of corresponding age.

control group. Only for patients with tumours of the posterior cranial fossa the positive dynamics of interfacial tensiograms following any surgical treatment was not so pronounced; however, for these patients deviations of the initial parameters were also rather insignificant. Nevertheless, for this group of patients, the trend to a normalization of dynamic surface tensions was also observed, and the equilibrium surface tension had attained its initial values.The excision of spinal cord tumours is also characterized by a subsequent trend to normalization of the dynamic interfacial parameters for both serum and cerebrospinal fluid, as can be seen in Fig. 25.

In summary, we believe that studies of dynamic surface tensions of biological liquids are of significant practical interest, due to its capability of providing a differential diagnosis and monitoring of the efficiency of therapy. With regard to the above analysis of correlations between interface tensiographic parameters and the contents of surfactants it is now possible to indicate some surfactants which affect the surface tension state of serum, urine, synovial fluid and liquor. In turn, the dynamic surface tensiometry of biological liquids for different diseases is capable of providing rapid and rather accurate reflection of the total composition of surfactants, including pathological proteins and other compounds formed and accumulated during the development of the disease.

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