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AGGREGATION AND DISAGGREGATION OF ERYTHROCYTES IN WHOLE BLOOD: STUDY BY BACKSCATTERING TECHNIQUE

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ABSTRACT

The aggregation phenomenon is of great importance for the evaluation of performance of the microcirculation system because of its influence on the blood viscosity at low shear stresses. Some important features and consequences of this phenomenon *in vivo* can be predicted in the *in vitro* experiments using optical methods. These methods are considered to be the most informative and applicable not only for the basic study of the aggregation phenomenon, but also for the diagnosis of a number of diseases and for the monitoring of therapeutic treatment in clinics. Results presented in this paper prove that the backscattering technique allows one to detect different changes of aggregational ability and deformability of erythrocytes and to get reliable and reproducible results distinguishing normal blood and blood with different pathologies. © *1999 Society of Photo-Optical Instrumentation Engineers.* [S1083-3668(99)01001-1]

Keywords erythrocyte aggregation; disaggregation; whole blood; Couette viscometer; backscattering.

1 INTRODUCTION

When suspended in solution of macromolecules red blood cells (RBCs) have a tendency toward reversible aggregation. Primary aggregates comprising only two adjacent cells develop further into linear aggregates (rouleaux) of different lengths. End-to-side connections of the latter turn them into a three-dimensional (3-D) network that is tightened by cohesive forces into large dense aggregates separated by plasma layers.

It is commonly accepted[1] that such aggregation is mostly due to the nonspecific adsorption of the aggregating plasma proteins on the surfaces of adjacent RBCs. The experiments with different kinds of aggregating proteins showed that there is linear dependence between the velocity of aggregation and the concentration of proteins, temperature, and the deformability of RBCs.[2] In addition, different kinds of plasma proteins induce the formation of different types of aggregates: ''clumps'' or ''networks.''[1] Nonetheless there is evidence that protein adsorption is not the only mechanism of aggregation.[3,4]

The morphology of RBC aggregation in whole blood and in RBC suspensions at rest and in shear flow is successfully studied by optical methods. The photometric studies of blood aggregation characteristics were started by the works of Dognon et al.[5,6] who registered the deviation of the intensity of light scattered from a layer of blood under mixing and after its halt. They have shown the dependence of the signal (scattering intensity) on orientational aggregation of the erythrocytes.

In accord with the primary experiments two approaches have been developed in the aggregometry. The first is based on the registration of light transmission through the studied blood layer in a cone-plate type viscometer.[7,8] It is generally known that aggregation starts with the formation of pairs from which the rouleaux of different lengths are then formed.[2,7,9] These rouleaux are bound ''end-to-end'' and ''end-to side'' and form a 3-D network or clumps. Such aggregates containing thousands of erythrocytes are separated one from another by thick layers of plasma.[10] This stage of aggregation is impossible in blood layers as thin as those of the order of 25 μm which is typical of those sites of ''cone-plate'' aggregometers where the photometry is usually carried out.

In the cone-plate aggregometer the aggregation process reaches the stage of a 2-D network of rouleaux. This is manifested by the characteristic times of the aggregation process calculated from the exponentially decreasing scattering intensity and photocurrent. For example, after the halt of the flow the half-period of the single-exponential aggregation process in normal blood is around 3.5 ± 1.0 s (Ref. 8) while the majority of studies[10,11] have shown that

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the aggregation process in a stationary suspension preceding the sedimentation continues for 2–3 min. The 50% decrease in amplitude of the signal with respect to the full aggregation amplitude does not describe the morphological changes of the aggregates during the aggregation process.

The second approach is based on the registration of the backscattered light from a blood layer in a Couette type viscometer.[12–14] The commercial apparatuses are the erythroaggregometer Sefam[12,14] and the laser-assisted optical rotational cell analyzer (LORCA).[13] The latter instrument allows interpreting the aggregation phenomenon in terms of kinetics, structural, and rheological parameters. These parameters were found to be sensitive to some pathologies as well as to variations in temperature, hematocrit, and other factors, but they are determined phenomenologically and are not related to the real processes of aggregation and disaggregation. In the LORCA device measurements of the aggregation kinetics are performed on a 0.3 mm wide layer. This condition may be incorrect in some cases such as the hyperaggregation syndrome that is typical of a number of severe hemorheological disorders. The authors also suggest using a parameter such as the half-time of the aggregation kinetics as a quantitative parameter for the diagnosis of oxygenation, anticoagulant, and incubation time effects on the erythrocyte aggregation process.

The diagrams of He–Ne laser light intensities backscattered and transmitted by a concentrated suspension of RBCs in the Couette flow have shapes closely related to the state of the suspension (at rest or subjected to a simple shear flow). An asymmetry in diagrams of backscattered light, absent in the diagrams of transmitted light, is observed when the suspension is in shear flow. This asymmetry is related to the deformation and orientation of the erythrocytes.[15–17]

The backscattered intensity from large structural aggregates is highly sensitive to the changes in their sizes and concentration. Moreover, the use of the backscattering regime is expedient because no moving parts of the device remain in the way of the light beams going into or out of the blood layer.

The goal of our study is to substantiate both experimentally and clinically the efficiency and the advantages of the method of determination of the aggregational properties of whole blood *in vitro* based on registration of the kinetics of backscattering intensity from a thick (1 mm) layer of blood. The aim of this paper is to summarize our experience in this field and to present some basic results, part of which has already been published.[18–24]

2 MATERIALS AND METHOD

2.1 ERYTHRONEPHELOMETER

The optical scheme of our experimental setup is shown in Figure 1. The sample of blood is placed into a Couette chamber—a 1 mm gap between two

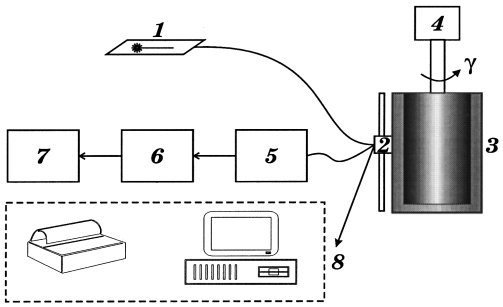

Fig. 1 Experimental setup of the aggregometer: 1—He–Ne laser, 2—the probing unit, 3—Couette chamber, 4—the motor drive with reductor, 5—photodiode, 6—amplifier, 7—plotting device, 8—computer processing unit.

coaxial cylinders. The outer cylinder is transparent and stationary, whereas the inner one is blackened and can be rotated. The shear rate, which is constant all through the gap except maybe close to the vicinity of the cylinders and the bottom of the couvette, can be varied and increased stepwisely. The radiation from a He–Ne or a diode laser in the red light spectrum range (power density 3 mW/mm^2) is used for probing. The probing unit, basically a light guide 2 mm in diameter, is coupled with four detecting fibers of similar diameter, placed so that the backscattered light can be registered from two pairs of sites located either along or across the direction of the flow. In this respect our setup is considerably different from other systems described, e.g., see Ref. 25. All detected light is fed to a photodiode. The alteration of the intensity of the backscattered light, and consequently, of the photocurrent with time related to the aggregation kinetics, is registered by a plotter or digitized and processed by a computer.

The registration time for one aggregation kinetics from the state of full disaggregation to the state of full aggregation takes about 100–120 s. Complete testing of one blood sample including the registration of one aggregation kinetics and one stepwise disaggregation kinetics takes about 10–15 min.

The process of spontaneous aggregation of RBCs in stasis and aggregates destruction under shear stress are usually represented as dependencies of the backscattered light intensity (remission) on time (in the case of aggregation) or on shear rate (in the case of disaggregation). A typical example of the aggregation kinetics is presented in Figure 2.

2.2 BLOOD SAMPLE PREPARATION

Blood drawn from an elbow vein of a donor was put into a glass test tube and stabilized with EDTA solution (0.3 ml of 7% EDTA solution for 10 ml of fresh blood) to prevent clotting. Measurements were usually performed within 6 h after drawing, although the RBCs, when kept in thermostabilized conditions (4–6 °C) in EDTA stabilized autologous plasma, keep their properties for about 30 h with-

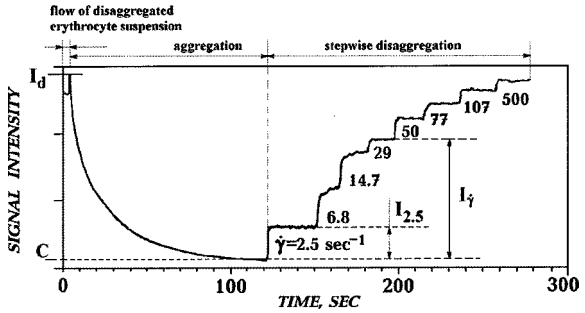

Fig. 2 The kinetics of spontaneous aggregation and disaggregation of erythrocytes in a sample of whole blood drawn from a healthy donor and measured parameters derived from the aggregation and disaggregation kinetics. Here I_d is the intensity of backscattered light at the state of total disaggregation, $I_{\dot\gamma}$ is that at shear rate $\dot\gamma$, $I_{2.5}$ is that at the shear rate $\dot\gamma$=2.5 s^{-1}. Numbers designate the values of shear rate fixed during the stepwise disaggregation process.

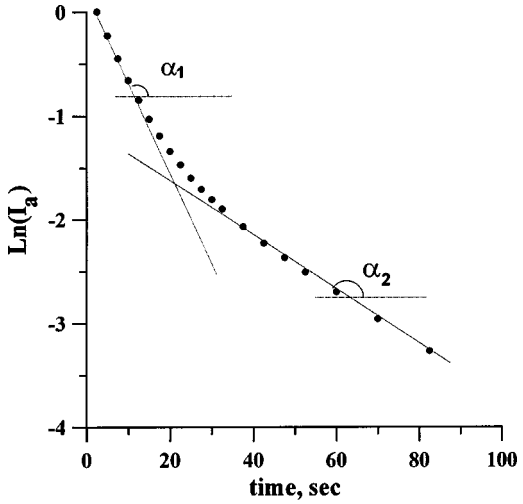

Fig. 3 The aggregation kinetics in the semilogarithmic scale. α_1 and α_2 are the slope angles of different lines distinguished.

out an alteration of their discoid form and aggregation properties. Before the experiment the volume concentration of erythrocytes (hematocrit) in the sample was measured by means of centrifugation in a capillary tube. The hematocrit values in all studied blood samples ranged from 0.4 to 0.44. The RBC's shape was visually controlled by microscopic observations.

2.3 EXPERIMENTAL PROCEDURE

We shall describe the regular experimental procedure with the help of a diagram presented in Figure 2. A 2.4 ml sample of stabilized blood at room temperature is placed into the gap of the aggregometer with a syringe. Then the sample is subjected to high shear rate (about 840 s^{-1}) to destroy all aggregates to single cells. The corresponding shear stress at typical blood viscosity ν=0.04 Ps is around 33.6 dyn/cm^2. At this shear stress normal RBCs start to deform into stretched discoids and to orient relative to the flow direction.

Then the flow is halted. At this moment we observe an instantaneous increase of the backscattered light intensity that is attributed to the transformation of RBCs to their normal shape. Immediately after that the aggregation process starts, lasting for 100–120 s up to complete aggregation. Photometrically this is manifested by the exponential decrease of the backscattered light intensity until saturation.

After that the disaggregation process induced by the stepwise increase of shear rate is detected. At every shear rate the registration of the signal lasts about 10–60 s to avoid the transitional process. In the experiments with normal blood the corresponding stepwise increase of the backscattered light is observed. It is worth noting that in some cases of pathological blood the scattering signal may become lower at low shear rates than at rest because of the shear-induced aggregation.[18] As for this part of the measurement procedure, the backscattered light intensity during the disaggregation process may be subsequently registered at the stepwise increase and decrease of shear rate.

2.4 SIGNAL PROCESSING AND DATA EVALUATION PROCEDURE

Now there are some algorithms that are widely used in the aggregometry. The method of exponential expansion of the kinetic curve[13,21] allows us to determine temporal parameters (see below) which are reliably sensitive to different aggregational states of blood and to the effect of physical and chemical factors on blood.[19,20] The method of evaluation of the aggregation rate at a 50% decrease in amplitude of the signal with respect to the full aggregation amplitude[8,13] is also used for the diagnosis of the aggregation properties of erythrocytes.

The representation of the original curve in the semilogarithmic scale (Figure 3) allows us to distinguish two (or in some cases three) lines with different slopes by means of the method of linear regression. Characteristic parameters of these lines are cotangent of the slope angles designated as T_1 and T_2, corresponding to the subsequent stages of the linear and 3-D aggregates formation. Therefore, phenomenologically the time dependence of the scattering signal intensity I_a can be described as follows:

$$I_a(t) = Ae^{-(t_1/T_1)} + Be^{-(t-t_1/T_2)} + C.$$

Here C is the signal intensity at the end of the aggregation process. The digital processing of the signal allows compensating for the C level, so in Figure 2 the scattering signal designated as 0 really corresponds to the C level.

The hyperbolic approximation of the original scattering intensity curve allows us to represent $1/I_a$ as a function of time t (Figure 4). In this case we

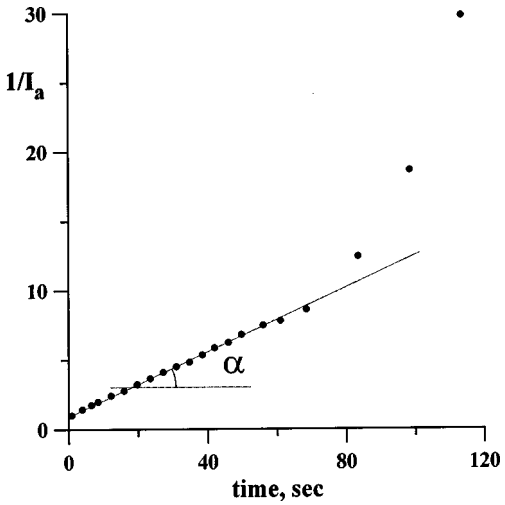

Fig. 4 The aggregation kinetics presented as a time function of the value $1/I_a$. tan α determines the rate of the aggregation process in whole.

distinguish only one line, and some points at the end of the aggregation process are placed randomly. This means that the relation

$$I_a \cdot t = \text{const}$$

characterizes the aggregation process as a whole. This parameter is calculated as the tangent of the slope angle (tan α) as shown in Figure 4.[22]

The process of shear-induced disaggregation should be regarded as a relaxational one with approximation of the kinetics by a sum of exponents representing different disaggregation stages. Each of these stages seems to start after the previous one is completed. The shear rate dependence of backscattering intensity is defined as follows:

$$I_d - I_{\dot{\gamma}} = \text{Fe}^{-(\dot{\gamma}/\beta)},$$

where I_d is the intensity of backscattered light for complete disaggregation, $I_{\dot{\gamma}}$ is the intensity at shear rate $\dot{\gamma}$, and β is the hydrodynamic strength of aggregates. This technique enables reliable determination of a single value of β in the case of normal blood and two different values β_1 and β_2 in cases of different pathologies.

Thus the basic parameters which we use for the description of the microrheological state of blood are: T_1 is the time of quick aggregation of RBCs and of the linear aggregates formation; T_2 is the time of slow aggregation of RBCs and of the 3D aggregates formation; β_1 is the hydrodynamic strength of large aggregates; β_2 is the hydrodynamic strength of aggregates; $I_{2.5}$ is the alteration of backscattered light intensity relative to the state of total aggregation level; and tan α is the rate of the aggregation process as a whole. The measured parameters characterize not only the morphology and dimensions of aggregates but also the kinetics of aggregation. The latter is not restricted to 1-D or 2-D but is extended to 3-D structures.

Measured in different blood preparations (whole blood, whole blood with additives of different polymeric molecules, and washed erythrocytes with additives of different polymeric molecules), these parameters serve much better for the characterization of blood as a complex fluid than mere photographs of the aggregates, and can form a basis for the development of more advanced mathematical models of aggregation. We also measure the deformability of RBCs under shear stress but do not discuss this issue here (see, e.g., Ref. 16).

3 RESULTS

During the last few years we have tested more than 1000 samples of blood with the described technique. The experiments with blood samples from healthy donors ($n=40$) showed that the temporal parameters of the aggregation process are: $T_1 = 12.5 \pm 3.8$ s, $T_2 = 41.4 \pm 5.1$ s. We found some peculiarities of the aggregation in cases of different pathologies, that are discussed below.

The aggregation process was significantly altered compared to healthy controls both regarding T_1 and T_2 in all diseases studied except for *psoriasis without arthritis*. An increase of T_2 accompanied by the decrease of T_1 compared to healthy controls was found in the blood of patients with *chronic glomerulonephritis* with and without *nephritic syndrome*, *systemic lupus erythematosus*, and *psoriatic arthritis*. In contrast, these parameters for *psoriasis* patients were not different from those of the healthy controls. An increase of T_2 with normal values of T_1 was found in blood from patients with *pulmonary hypertension* and in preoperative patients with *intestinal tumors* aged above 60 years, while *hereditary hypercholesteremia* is characterized by a decrease of T_2 and a normal value of T_1 parameter. Decreased T_1 complemented with a decrease of T_2 was observed in patients with *coronary disease* with and without *diabetes*. A considerable increase of the spontaneous aggregation rate was observed in the blood of patients with these diseases. The number of patients, values of parameters with their standard deviations and comparison with normal ones for all studied pathologies are presented and discussed in more detail elsewhere.[23]

Figure 5 shows that by combining T_1 and T_2, the aggregation characteristics of pathological blood can be divided into three diagnostic groups:[23] decreased T_1 and increased T_2 (autoimmune diseases); decreased T_1 and decreased T_2 (ischemic heart diseases); and increased T_1 and increased T_2 (other diseases). The combination of increased T_1 and decreased T_2 was not found. The above stated existence of three diagnostic groups defined according to the values of T_1 and T_2 relative to the norm

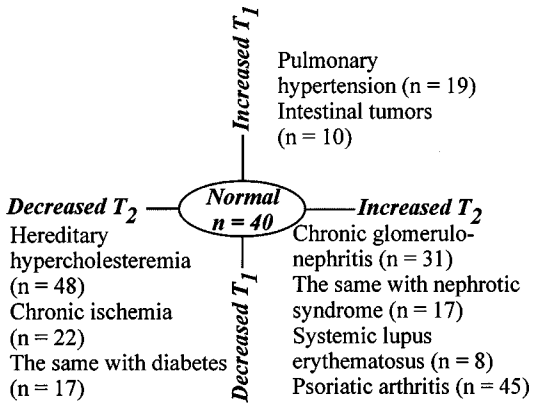

Fig. 5 Diagram of combination of T_1 and T_2 alterations related to different groups of diseases.

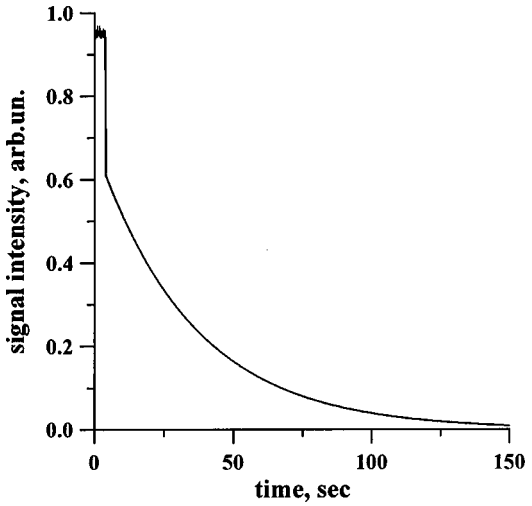

Fig. 6 The abnormal aggregation kinetics with the transitional process in the shape of a break.

is so far qualitative and is based on the fact that there exists no linear correlation between the above temporal parameters of the aggregation process measured in each sample. Quantitative substantiation of these diagnostic groups needs additional studies with more and various diseases.

The representation of registered aggregation curve in semilogarithmic scale permits us to usually observe two consequential processes of aggregation and a transitional process. The last is related to that state of blood when both linear and primary 3-D aggregates occur. In some cases this part of the whole process comprised 9%–15% of the whole aggregation amplitude and can be also characterized by an additional exponential parameter T_1'. This parameter is inessential from the viewpoint of diagnostics, but it is supposed to reflect the homogeneity of cohesive properties of erythrocytes. When applying the therapy aimed at the destruction of erythrocyte aggregates by means of dextran DX-40, the transitional process disappears and two characteristic times, T_1 and T_2, are reliably determined.

The single-exponential process was observed only in the case of *inherited hemoglobinopathy*. This is characterized by a single time parameter with magnitude close to T_2 value. In the case of *inherited hyperlipemia* the spontaneous aggregation process differs from the normal one. The primary aggregation process was found to be of relatively low amplitude but very rapid with characteristic time on the order of 0.5 s (instant aggregation).

We observed the appearance of abnormal kinetics of spontaneous aggregation after plasmapheresis (plasma exchange up to 2 ℓ) performed with patients suffering from *hypercholesterinemia*. The parameter T_1 disappeared and the amplitude of instant process strongly increased up to 50% of the whole aggregation amplitude. Then slow one-exponential aggregation process with characteristic time on the order of T_2 was registered. The transitional process was observed in the shape of a break (Figure 6), a cupola-shaped peak (Figure 7) or a delay of aggregation. Such a delay of the aggregation process can be as long as 19 s (Figure 8) and was reliably reproduced in experiments on the same blood sample.

In the experiments focused on the study of the aggregation kinetics in blood samples of normal subjects at different hematocrit (H) values we observed the slowing down of the aggregation process (T_1) along with the decrease of hematocrit. However no regular dependence of T_2 on H has been found so far.[24] The study of the disaggregation kinetics showed that the hydrodynamic strength of aggregates determined by the monoexponential algorithm without distinguishing two different parameters of aggregate strength, is inversely proportional to the hematocrit. Variation of hematocrit in the range from 0.2 to 0.8 was performed by diluting the erythrocyte suspension in the autologous blood plasma.

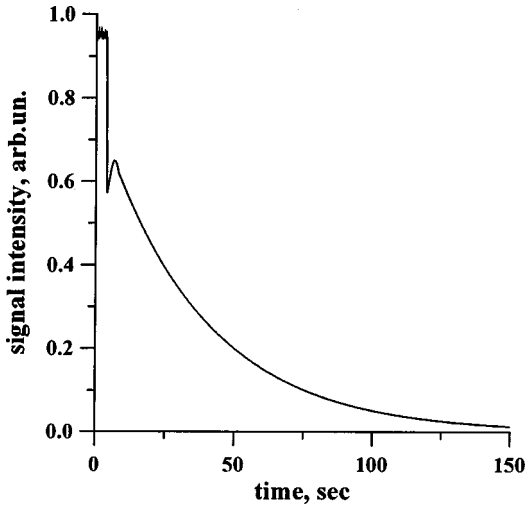

Fig. 7 The abnormal aggregation kinetics with transitional process in the shape of a cupola-shaped peak.

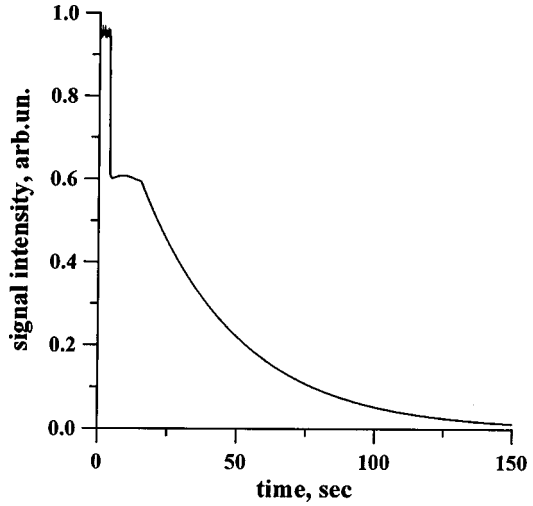

Fig. 8 The abnormal aggregation kinetics with a delay of aggregation that can be as large as 19 s.

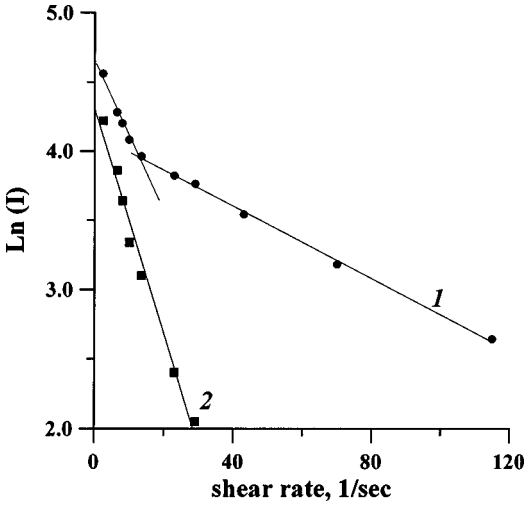

Fig. 9 Disaggregation kinetics in the case of *pulmonary hypertension* blood (1) and normal blood (2). The corresponding values of aggregates strength are: $\beta_1 = 21.2\ s^{-1}$ and $\beta_2 = 73.1\ s^{-1}$ (1); $\beta = 24.7\ s^{-1}$ (2).

The influence of aging of the blood sample *in vitro* on the aggregation kinetics was also studied.[24] Keeping the blood sample for a long period of time even under thermostabilized conditions at t=4–6 °C induces changes in the erythrocyte shape. Normal discocytes are transformed into echinocytes in 2 days and into spherocytes in 4 days. When the form of RBCs is changed the advantage of the side-by-side aggregation disappears and the aggregation kinetics reduces to one exponent with characteristic time close to T_2 value. The analysis of the T_1 parameter in aged blood samples showed that the increase of the H value results in the decrease of T_1, which asymptotically approaches a certain value T_{1as}=4.0±1.5 s.[23]

The analysis of the hydrodynamic strength of aggregates showed that there are several types of disaggregation kinetics:

(i) normal disaggregation kinetics with one index of aggregates strength ($\beta \sim 30\ s^{-1}$);

(ii) two-component process of disaggregation with the strength of large aggregates β_1 close to the normal index and the strength of small aggregates β_2 increased by 2–4 times (for example in the case of *hereditary glomerulonephritis* $\beta_1 = 30 \pm 2\ s^{-1}$, and $\beta_2 = 99 \pm 3\ s^{-1}$, and in the case of *Sjogren disease* $\beta_1 = 30 \pm 2\ s^{-1}$, and $\beta_2 = 132 \pm 4\ s^{-1}$);

(iii) abnormal two-component disaggregation process with significantly increased strength of large aggregates ($\beta_1 = 140 \pm 5\ s^{-1}$ and $\beta_2 = 81 \pm 3\ s^{-1}$) that we observe only for some samples of blood in the case of *rheumatoid arthritis.*

The case of a two-component disaggregation process in blood of an individual with *pulmonary hypertension,* which is characterized by the low strength of large aggregates β_1 and the high strength of small aggregates β_2, as well as a one-component process in the case of normal blood are presented in Figure 9.

In some cases no disaggregation was found at low shear rates. Instead a decreased intensity of the backscattered light as a sign of the intensified aggregation process was observed at low shear rates (Figure 10). This effect was characterized quantitatively as the percentage of the complete amplitude of the aggregation and called $I_{2.5}$. We have given this parameter a negative sign when the backscattered light intensity increased and a positive sign when the light intensity decreased at the introduction of shear. In the blood of healthy donors the

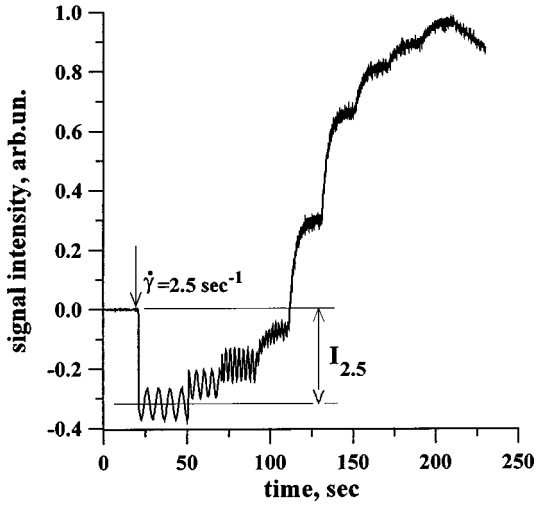

Fig. 10 The shear-induced aggregation process at the shear rate of 2.5 s^{-1} under *Sjogren disease.* In this case the parameter $I_{2.5}$ is positive, as different from Figure 2, and is about 30% lower than the level of total amplitude of aggregation.

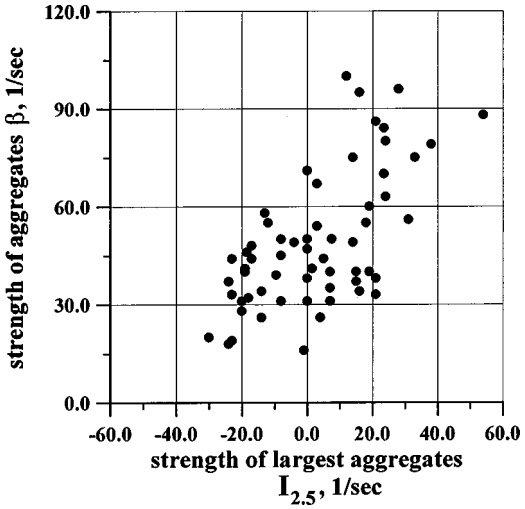

Fig. 11 The correlation between $I_{2.5}$ and β in patients with *psoriatic arthritis* (r=0.68, $p<0.05$) indicates that shearinduced aggregation is the result of the increased strength of the erythrocyte aggregates. The overall number of points includes the results of multiple measurements performed with blood samples drawn from the same donor on different days.

mean of $I_{2.5}$ was $-25.0\pm0.8\%$ ($n=40$). Strong and significant correlation ($r=0.68$; $p<0.05$) between $I_{2.5}$ and β in the case of *psoriatic arthritis* was obtained (Figure 11).[23]

4 DISCUSSION

The values of characteristic times obtained by the aggregometer described above do not coincide with those obtained by other authors with an aggregometer of a different type.[8] The characteristic time of the aggregation process obtained by means of the cone-plate aggregometer was 3.5 ± 1.0 s for normal blood. The big difference results from different construction of the device, namely a different thickness of blood layer ($\sim$50 μm in the cone-plate and 1 mm in a Couette type aggregometer) and different orientation of the sedimentation force relative to the gap thickness. In the case of cone-plate design it is directed across the gap and in the Couette flow along it. The sedimentation process, shear forces, and diffusion of the RBCs strongly influence the aggregation kinetics *in vitro*.[26,27] The aggregation of red blood cells combined with the sedimentation due to the gravitational force induces the formation of gradients of concentration that can influence the stability of the blood flow.

Owing to the inhomogeneity of the cohesion properties of erythrocytes the 3-D aggregates, linear ones, and single red blood cells can be simultaneously present in the measured volume. We suppose that at every moment of the aggregation process there is a certain distribution of the sizes of erythrocyte aggregates. The aggregates of the most probable size mostly contribute to the scattering signal registered by the photometer. At the present time the theory of multiple light scattering in suspensions of aggregating particles is still under development, therefore it is difficult to bring the experimentally registered aggregation kinetics in accordance with the sizes of formed aggregates.

Thus in the case of blood aggregometry it is impossible to strictly distinguish the stages of formation of the aggregates of certain sizes only on the basis of the absolute amount of scattered light. The analysis of the kinetic coefficients is the only reasonable approach possible at the current stage of research.

The original scattering signal curves were processed according to both exponential and hyperbolic algorithms. The analysis of the obtained parameters T_1 and $\tan\alpha$ for 20 samples of blood shows[22] that the dependence between these indices is hyperbolic with the constant $(T_1\cdot \tan\alpha)=5.95 \pm 0.25$. This means the similarity of both algorithms but the exponential method gives more detailed information about the different stages of the aggregation process.

The instant aggregation process observed in the case of *inherited hyperlipemia* (Figures 6–8) corresponds, as we suppose, to the formation of primary red cell aggregates. Basically these are pairs of erythrocytes and their formation is vastly induced both by the remaining convection, and by that circumstance that pairs are formed due to the rotation of cells already having contact. Following this process a delay of aggregation is difficult to explain, but this effect can be interpreted in favor of theories of long-range interaction between erythrocytes.[28] In a suspension with a small amount of macromolecules after forming the pair aggregates (the instant component) a certain amount of time is necessary for creating a macromolecular structure in erythrocyte ''atmosphere,'' in order to overcome the formed plasma layer and to join erythrocytes in rouleaux. The appearance of the cupola-shaped peak on the aggregation kinetics can be explained by changes in the orientation of pair aggregates after their forming.

The study of disaggregation of erythrocyte aggregates in shear flow has shown that this process is different not only for different diseases but for different samples of blood under the same pathology and for different hematocrit values. Usually the value of β_1 was lower than β_2. This may be a result of the homogeneity of cohesion properties of the erythrocytes manifested by the successive destruction primarily of clumps or network and then by the destruction of the rouleaux oriented along the flow. The effect of shear stress is low on linear aggregates and complete disaggregation can only occur at high shear rates.

The effect of intensified erythrocyte aggregation induced by low shear rates was observed even in the blood of healthy donors. Copley et al.[29] found this effect in whole blood at a shear rate of $\dot{\gamma}$

$=0.1\ s^{-1}$. Chen et al.[30] registered an intensified aggregation process in whole blood at low hematocrit by adding 0.5% Dextran DX-500. The phenomenon of the shear-induced aggregation (hyperaggregation) was caused by the formation of pathological aggregates with high strength.

Strong and significant correlation (r=0.68; $p<0.05$) between $I_{2.5}$ and β in the case of *psoriatic arthritis* indicates that the effect of the shear-induced aggregation is the result of the increased strength of the erythrocyte aggregates. At normal hematocrit and positive $I_{2.5}$ the process of spontaneous aggregation has been completed when the 3-D network of the linear erythrocyte aggregates had been formed. The shear stress causes the destruction of this network and spheroid-like aggregates are then formed. This process can be observed when shear stress at the shear rate of $2.5\ s^{-1}$ is lower than the strength of the spheroid-like erythrocyte aggregates.

The analysis of H dependencies of T_1 for normal blood and *hereditary glomerulonephritis* allowed us to conclude that this parameter is proportional to H^{-2}, the coefficients requiring additional measurements.[24] The data obtained on the disaggregation kinetics showed that the strength of RBC aggregates is inversely proportional to the hematocrit. Such dependence is explained by the fact that the decrease in the concentration of erythrocytes results in the increase of the quantity of plasma proteins (fibrinogen, globulins, etc.) inducing the aggregation, per one RBC.[24] At low H values the shear-induced aggregation and sedimentation processes are manifested simultaneously. Thus the aggregation and disaggregation parameters measured by the backscattering technique are sensitive to the hematocrit changes. So it is important that the experiments be carried out with blood samples at one fixed value of hematocrit, e.g., $H=0.4$.

The system of aggregation parameters presented and discussed in this paper makes possible a quantitative estimation of processes of linear erythrocyte aggregates and 3-D aggregates (clumps) formation. In the case of hyperaggregation syndrome it is sometimes possible to evaluate separately the strength of the largest aggregates ($I_{2.5}$) as well as that of large (β_1) and linear (β_2) red blood cells aggregates. So we believe that the combination of aggregation and disaggregation parameters can be considered as a diagnostically meaningful pattern of hemorheological pathology.

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