keeping, are given in Fig. 2. It will be clear from Fig. 2B that with an increase in the duration of ultrasound treatment the mean diameter of the liposomes fell from 0.17 to 0.06 μ (determined from Fig. 1B), whereas during keeping (Fig. 2D) $\bar{d}_\lambda$ increased from 0.2 μ to approximately 2 μ, for on the 10th day of keeping at room temperature the relative refractive index of these liposomes was 1.06 ± 0.01. By separating the suspension into fractions, by gel-filtration for example, the distribution of liposomes by size can be obtained. The turbidity spectrum method can also be used to analyze fractions of liposomes after ultracentrifugation, to monitor the process of preparation of liposomes, and to verify their state after exposure to various factors.

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DETERMINATION OF THE PROBABILITY OF AGGREGATION TO ASSESS THE FUNCTIONAL STATE OF PLATELETS

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Investigations of platelet aggregation induced by addition of ADP, serotonin (or other factors) in platelet-enriched blood plasma provide information on the functional state of the platelets and are widely used at the present time. The turbidimetric method, developed by Born [2], is used for this purpose.

However, there is as yet no satisfactory approach to the interpretation of results obtained by Born's method which would allow quantitative evaluation of the state of platelets on the basis of analysis of a series of aggregatograms; moreover, it is difficult to compare aggregatograms obtained for different samples of blood plasma.

A method of determining the probability of platelet aggregation due to collision at the beginning of the aggregation process, based on analysis of the initial portion of the aggretogram, is suggested in this paper. The parameters usually obtained by analysis of aggregatograms (a change in light transmittance of the cell suspension at the maximum of aggregation, the rate of change of light transmittance during aggregation and disaggregation) depend es-

KEY WORDS: method of determination of probability of platelet aggregation.

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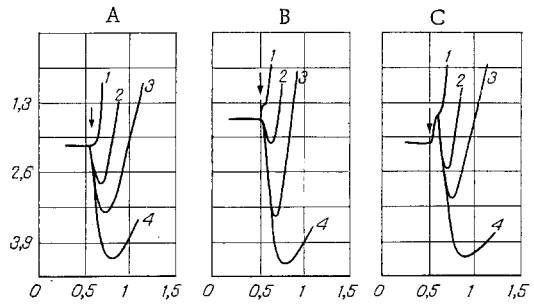

Fig. 1. Initial portions of aggregatograms for different shear velocities. A) Theoretical curves calculated for values of (h:/b)0.1 (1), 0.05 (2), 0.035 (3), 0.01 (4), B, C) experimental curves with inducers of aggregation: B) ADP (0.5 μM); C) serotonin (1 μM). Frequency of rotation of mixer: 60 Hz (1), 32 Hz (2), 16 Hz (3), and 8 Hz (4). Abscissa, time (in min); ordinate, extinction $\times 10^{-2}$

sentially on the hydrodynamic conditions in the aggregating suspension and on its light-scattering properties. Unlike these parameters, the probability of aggregation due to collision is determined entirely by the functional state of the platelets at the moment of collision and it can thus be suggested for quantitative evaluation of the state of the platelets.

EXPERIMENTAL METHOD

To describe the process of aggregation a kinetic equation of coagulation in dispersed media in Smolukhovskii's approximation [1] was used:

$$\frac{dN_\infty}{dt} + \frac{4G\alpha\Phi}{\pi} N_\infty = 0,$$

where N_∞ is the total concentration of particles (aggregates, single platelets); Φ the relative volume of platelets; α the probability of aggregation due to collision; G the shear velocity in the flow, which has the solution:

$$N_\infty(t) = N_\infty(0)\, e^{\frac{-4G\alpha\Phi}{\pi} t},$$

where $N_\infty(0)$ is the initial concentration of particles. The solution obtained is valid at initial moments of time when the aggregates are not large enough and when the destructive action of shear stresses can be disregarded.

During aggregatometry the intensity of light scattered by the platelets is measured, and in the case of a single scatter it is described by the equation:

$$I = I_0 \exp(-\varkappa N_\infty \chi),$$

where I_0 is the intensity of incident light; χ the length of the optical path; $\varkappa$ a coefficient determined by the physical parameters of the particles and, in particular, by their shape.

Since the signal in many aggregatometers is given in logarithmic form, the extinction of the platelet suspension can be taken as the parameter for measurement: $E = \varkappa N_\infty \chi$.

To allow for the effect of a change in shape of the platelets on light transmittance, the following relationship was obtained by analysis of the experimental data for the change in the parameter with time:

$$\varkappa(t) = (\varkappa_0 - \Delta\varkappa\, e^{-bt}),$$

where $\varkappa_0$ is the value of $\varkappa$, corresponding to the spherical state of the platelet; $\Delta\varkappa$ is the change in the value of $\varkappa$ accompanying the change of shape; b is the time constant of the process in change in $\varkappa$.

By assuming a short platelet activation time and considering the process of change of shape to take place parallel with aggregation, the change in extinction of the platelet suspension can thus be approximated by the following equation:

$$E(t) = N_\infty(0)\, \varkappa_0 \cdot e^{\frac{-4G\alpha\Phi}{\pi} t} \left(1 - \frac{\Delta\varkappa}{\varkappa_0} e^{-bt}\right). \quad (1)$$

To confirm the validity of this approximation experimentally a series of experiments was carried out to study the effect of shear velocity on aggregation processes and on changes in shape of the platelets. Experiments with incubation of platelet-enriched plasma with EGTA, after which ADP- and serotonin-induced changes in shape took place, but aggregation was in-

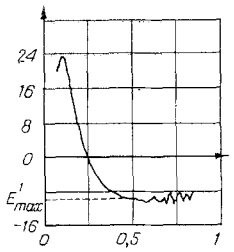

Fig. 2

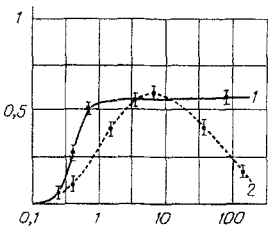

Fig. 3

Fig. 2. Rate of change (derivative) of extinction as a function of time after addition of ADP. Final concentration of ADP 1 μM. Abscissa, time (in min); ordinate, rate of change of extinction $\times 10^4$.

Fig. 3. Change in probability of platelet aggregation under the influence of various doses of ADP and serotonin. 1) ADP; 2) serotonin. Abscissa, concentration of inducer (in μM); ordinate, change in probability of extinction.

hibited (the probability of aggregation was zero), showed that shear velocity does not affect the process of change of shape, as also follows from Eq. (1). Theoretical aggregation curves and curves obtained in experiments with ADP- and serotonin-induced aggregation at different velocities of rotation of the mixer, are given in Fig. 1. Good agreement between the theoretical and experimental data is evidence in support of the view that the platelet activation time is short compared with the spherulation time and that aggregation processes run parallel with the change in shape. Moreover, by analysis of Eq. (1) a method of determining the probability of platelet aggregation based on measurement of the maximal negative value of the derivative of the change in light transmittance can be suggested. This value is determined by the following equation:

$$E'_{max} = -N_\infty(0)\cdot\varkappa_0 k\cdot\frac{b}{k+b}\cdot\left[\frac{\varkappa_0}{\Delta\varkappa}\cdot\frac{k^2}{(k+b)^2}\right]^{\frac{k}{b}},$$

$$\text{where } k = \frac{4G\alpha\Phi}{\pi}.$$

The value of $\varkappa_0 N_\infty(0)$ is the greatest possible amplitude of aggregation, which can be taken to be the maximal amplitude of aggregation, for example, after addition of a large dose of ADP (in a final concentration of over 10 μM).

The value of $\frac{b}{k+b}\cdot\left[\frac{\varkappa_0}{\Delta\varkappa}\cdot\frac{k^2}{(k+b)^2}\right]^{\frac{k}{b}}$ varies within limits of 1 to 0.69; during a change in k/b by a factor of 2 it changes by not more than 1.1 times, and for that reason it can be classed as a technical error of measurement and the probability of aggregation can be determined as the ratio of the maximal value of the derivatives to the maximal amplitude of aggregation:

$$\alpha = \frac{\pi}{4G\Phi}\cdot\frac{E'_{max}}{N_\infty(0)\,\varkappa_0}.$$

The coefficient $\pi/4G\Phi$ is determined entirely by the shear velocity G and the relative volume of the platelets Φ. If the shear velocity cannot be calculated, for example because of the complex pattern of distribution of velocities in the aggregatometer cuvette during mixing of the plasma with an ordinary cylindrical mixer, the value $k = (4G\Phi/\pi)\alpha$, measured at a fixed shear velocity (constant rate of rotation of the mixer) can be used. Henceforward the value of k will be taken to mean a value corresponding to a frequency of mixing of 1000 rpm, which was used in the present situation.

EXPERIMENTAL RESULTS

As an illustration of the use of this method results obtained during a study of ADP- and serotonin-induced aggregation of rabbit platelets will be given. Blood was obtained by direct puncture from a rabbit's heart and stabilized with 3.8% Na citrate in the ratio of 9:1.

Platelet-enriched plasma was obtained from the blood by centrifugation. To determine the maximal value of the derivative of the change in light transmittance of the sample, the channel recording the derivative of the aggregatometer signal was used. A typical recording from this channel is given in Fig. 2.

The maximal value of the derivative E'_{max} was determined as the greatest negative value of the curve, as shown in Fig. 2.

As an example of the use of the suggested method, graphs of the change in probability of platelet aggregation under the influence of various doses of ADP and serotonin are given in Fig. 3. With an increase in the ADP concentration the probability of aggregation also increased, until a constant value was reached in concentrations of over 1 μM. The relationship between the probability of aggregation and serotonin concentration differed in form. After addition of serotonin in a final concentration of 10 μM the probability of aggregation reached its maximal value, about equal to the maximal probability of aggregation induced by ATP, but later it declined progressively. Incidentally, probability of aggregation did not correlate with amplitude of aggregation. For instance, in the case of aggregation induced by large doses of ADP maximal probability of aggregation could be significantly increased by the addition of Ca^{++}, but the amplitude of aggregation remained unchanged in this case, and equal to its former maximum.

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