TABLE 1. Aggregation of Platelets Induced by ADP in Suspension After Addition of Plasma Factor $XIII_a$ (200 units/ml)

Statistical index	Components added			
	0.85% NaCl solution	factor $XIII_a$	inactive factor XIII	thrombin in dose used to activate factor XIII
$M \pm m$	1,78±0,14	2,27±0,09	1,88±0,15	1,85±0,19
n	6	6	6	6
P		<0,01	>0,01	>0,01

Note. P calculated by comparison with control (addition of 0.85% NaCl solution).

The experiments described above thus yielded evidence to show that platelet factor XIII, like plasma factor XIII, increases the degree of platelet aggregation induced by ADP.

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USE OF A LASER PHOTOMETER TO STUDY PLATELET SHAPE AND AGGREGATION IN A CONTINUOUS-FLOW SYSTEM

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UDC 612.111.7-087.5

An original apparatus is described for photometric investigation of the shape of platelets during their aggregation and disaggregation in a continuous flow system. Experiments on a model of stenosed blood vessel showed that in the presence of ADP (1 μM) stenosis potentiates platelet aggregation in platelet-enriched rabbit plasma.

A change in the shape of platelets and in their aggregation and disaggregation may play an essential role in thrombus formation [1]. Methods of investigation of the functional properties of platelets used at the present time enable the behavior of a platelet suspension in plasma to be studied in a test tube, but not in a continuous-flow system, although hemodynamic forces can have a significant effect on the functional properties and behavior of platelets during thrombus formation [2]. The optical density of a suspension of platelets in plasma has been shown to depend on their shape and orientation [3], for the scatter diagram of an ellipsoid of rotation is determined by its position relative to the direction of incidence of light. The investigation described below

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(Presented by Academician of the Academy of Medical Sciences of the USSR E. I. Chazov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 87, No. 2, pp. 101-103, February, 1979. Original article submitted February 27, 1978.

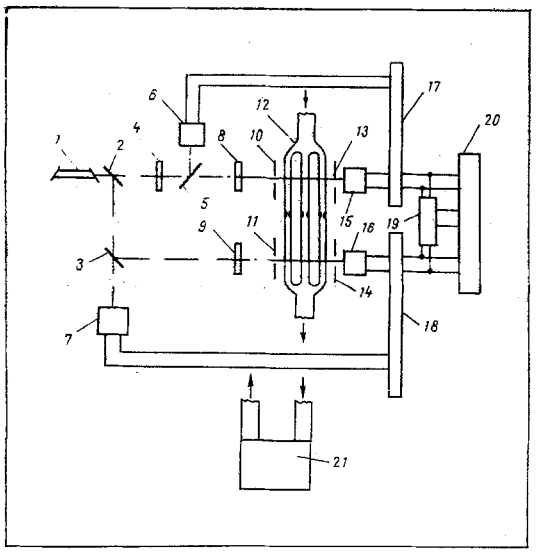

Fig. 1. Block diagram of apparatus for studying effect of various geometric parameters of the vascular system on functional properties of platelets.

showed that a photometric method can be used to study the properties of platelets in plasma while flowing through a system simulating a segment of the vascular system under normal conditions and in the presence of pathological factors manifested as a change in the geometric parameters of the segment of the vascular system, namely stenosis.

EXPERIMENTAL METHOD

To study the effect of different geometric conditions on functional properties of the platelets an apparatus (Fig. 1) was devised whereby the orientation of nonspherical blood cells in the blood flow, the shape of the platelets and its changes with time, aggregation and disaggregation of the platelets, and the effect of the vascular system and, in particular, of a stenosed region, on the functional properties of the platelets could be studied.

The apparatus consists (Fig. 1) of a helium-neon laser (1), dividing plates (2,3,5), filters (4,8,9), diaphragms (10,11,13,14), photodetectors (6,7,15,16), a model of the vascular system (12), ratio meters (17-19), a system for maintaining the flow (21), and a recorder (20). The apparatus works as follows. Light from the laser is divided by the dividing plate (2) into two beams of equal intensity. The first beam passes through the filter (4) and is divided by the dividing plate (5) so that some of the light falls on the comparison photodetector (6), whereas the rest passes through the filter (8), diaphragm (10), model of the vascular system (12), and diaphragm (13), and falls on the photodetector (15). Signals from the photodetectors (6 and 15) are led to the ratio meter (17) and the result is recorded by the recorder (20) and is also led to the ratio meter (19). The second beam from the dividing plate (2) falls on the dividing plate (3) so that some of the light falls on the comparison photodetector (7), whereas the rest passes through the filter (9), diaphragm (11), model of the vascular system (12), and diaphragm (14) and falls on the photodetector (16). Signals from the photodetectors (7 and 16) are led to the ratio meter (18), and the result is recorded by the recorder (20), and also led to the ratio meter (19), where it is compared with the signal arriving from the ratio meter (17); the result of comparison is also recorded by the recorder (20).

When the optical density of the sample is constant, signals at the output of the ratio meters (17-19) also are constant. Measurement of the optical density of the specimen throughout its volume simultaneously leads to mismatching of the signals from the photodetectors (6 and 15 and also 7 and 16). These signals are recorded, after the ratio meters (17 and 18), by an automatic writer (20). The restricting diaphragms (10,11,13,14) shape the optical channels proximally and distally to the model of stenosis.

As a model of the stenosed vessel a glass tube with a bore of 2.5 mm was used. The middle part of the tube was narrowed to a diameter of 0.6 mm and the length of the stenosis was 10 mm. Blood was obtained by direct puncture from the 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.

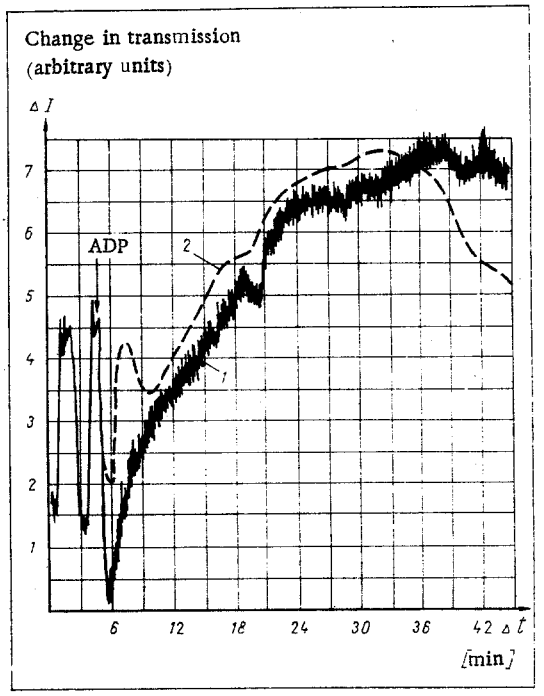

Fig. 2. Aggregation of platelets in optical channels proximally and distally to stenosed region of model blood vessel after addition of ADP (1 μM) to platelet-enriched plasma. Curve 1) aggregation in proximal channel; 2) aggregation in distal channel.

EXPERIMENTAL RESULTS

Stationary platelet-enriched plasma was set in motion by starting up a peristaltic pump, giving a plasma flow rate of 0.3 ml/sec. The optical density of the plasma under these circumstances fell sharply (the light transmission was increased), evidence of an orderly orientation of the system of disk-shaped platelets (Fig. 2). After the flow had stopped under the influence of brownian fluctuations the orientation of the platelets became irregular again and returned to its initial state with chaotic orientation of the cells. The light transmission correspondingly returned to its initial level. Starting and stopping the pump creating flow thus enabled the initial state of the platelet suspension to be determined and its changes assessed in the course of the experiment.

On the addition of ADP in a final concentration of 1 μM to the plasma an increase in optical density of the specimen was observed in both the proximal and the distal optical channel, due to spherulation of the platelets. However, immediately after addition of ADP the optical density in the channel distally to the stenosis increased by a lesser degree than in the proximal channel. The change in optical density was due to changes in the shape of the platelets from disk-shaped to spherical, on the one hand, and on the other hand, to the formation of aggregates, leading to an increase in transmission of the platelet suspension. Spherulation increases whereas aggregation reduces optical density. Competition between these two processes determined the shape of the curve of transmission versus time. Since aggregation in the distal channel reached a higher level than in the proximal, it can be concluded that in the presence of ADP stenosis facilitates aggregation. The effect of stenosis was manifested only in the presence of ADP. During breakdown of the ADP the course of the aggregation—disaggregation curves distally and proximally to the stenosis approximated, and later the curves virtually coincided and behaved in the same way.

The action of stenosis in potentiating aggregation under physiological conditions, it can tentatively be suggested, could lead to embolism of the small peripheral vessels distally to the stenosis. This possibility is also determined by the relaxation time of platelet aggregates arising under the influence of stenosis.

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