nism [6]. This hypothesis is also confirmed by the results of the writers' previous investigations, which showed a marked fall in the Ca^{++} content in the mast cells and endothelium of the mesenteric microvessels in experimental hypoparathyroidism [2].

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INHIBITION OF PLATELET AGGREGATION BY IMMUNE COMPLEXES.

I. CLINICAL STUDIES

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There is as yet no general agreement regarding physiological interaction of immune complexes (IC) and platelets. Precipitates of IC are known to induce platelet aggregation (PA) [14]. This lay at the basis of a method of detecting IC in blood serum by their ability to induce PA *in vitro* [12]. At the same time it was noted that human platelets, unlike platelets of laboratory animals, are not aggregated by IC [4], and it was also suggested that the presence of F_c-receptors for IgG on the surface of the platelets determines their aggregation only under the influence of IC formed by class G antibodies [9].

The physiological mechanism determining interaction between platelets and circulating IC assume special significance in atherosclerosis and its clinical manifestations. In the pathogenesis of atherosclerosis, hypotheses postulating the primary nature of the lesion in the endothelial layer of blood vessels and/or proliferation of smooth-muscle cells of the arterial wall are being increasingly accepted [2, 13]. One of the possible mechanisms of damage to the endothelium may be the pathological action of antigen–antibody complexes, the level of which rises in ischemic heart disease (IHD) and myocardial infarction [5, 10, 16]. We also know that in atherosclerosis platelet dysfunction is observed, with an increased tendency toward aggregation [3]. Finally, it has been shown that smooth-muscle cells are powerful stimulators of PA [1]. Consequently, in the process of atherogenesis, injury to the

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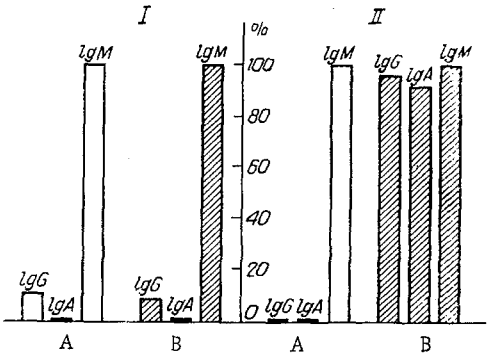

Fig. 1. Immunoglobulin spectrum in IC precipitated in 3.5% (I) and 7% (II) solution of PEG from blood serum of nine healthy blood donors (A) and 24 patients with IHD (B).

TABLE 1. IC Level and PA Parameters in Patients with IHD and Healthy Donors

Group of subjects	IC in 3.5% PEG solution, mg%	IC in 7% solution, units	Degree of aggregation, mm	Size of aggregates, mm	Time of aggregation, sec	Rate of aggregation, mV/min
Patients with IHD	261±7,8 (n=100)	1692±48,1 (n=51)	66±1,6 (n=100)	5,1±0,2 (n=100)	150±5,1 (n=100)	6,8±0,7 (n=100)
Blood donors	143±5,5 (n=120)	1059±53,1 (n=55)	62,5±0,5 (n=16)	4,3±0,05 (n=16)	107±1,1 (n=16)	5,2±0,08 (n=16)
P	<0,001	<0,001	<0,05	<0,01	<0,001	=0,02

Legend. n) Number of observations.

vascular wall by IC and proliferation of smooth-muscle cells may lead to sharp intensification of PA and of thrombus formation.

It must be pointed out that large and small IC (with different sedimentation constants) are distinguished, and they differ in their biological effects on organs and tissues [14].

The aim of this investigation was to study the effect of IC, circulating in the blood stream, on platelet function. For this purpose the level of large and small IC was compared with the parameters of PA in patients with IHD accompanied by coronary atherosclerosis, confirmed in most cases by angiography.

EXPERIMENTAL METHODS

Altogether 100 patients with IHD and with postinfarction cardiosclerosis (the presence of coronary atherosclerosis confirmed in two-thirds of cases by angiography) and 120 clinically healthy blood donors were investigated. The number of large and small soluble IC in the blood serum was determined by the method described previously [6]. The method of determination of large IC was based on precipitation of IC in a 3.5% solution of polyethylene glycol 6000 (PEG) followed by measurement of the scattering of light by the test samples on a laser nephelometer (Behringwerke, West Germany). To construct a standard curve, Kohn's fraction II, aggregated at 63°C for 50 min, was used. The quantity of IC was expressed in mg%, equivalent to the concentration of aggregated γ-globulin. The IC level in sera diluted in 7% PEG solution was determined in the same way, which enabled small IC, not detectable in low concentrations of PEG, to be precipitated as well as large IC [11, 15]. To precipitate IC the sera were diluted in 3.5% and 7% solutions of PEG and incubated for 1 h at 20°C. The precipitates were obtained by centrifugation at 18,000 rpm for 30 min at 4°C. Immunoglobulins of classes G, A, and M were determined in the residue of precipitated IC by means of antisera and the laser nephelometer (Behringwerke). PA was determined in citrated blood plasma with a platelet concentration of 250,000-300,000/mm^3 [7]. ADP-induced PA was analyzed in an aggregometer (Chronolog, England).

EXPERIMENTAL RESULTS

Analysis of IC precipitates obtained from blood sera of nine donors and 24 patients with IHD, in 3.5% and 7% solutions of PEG, revealed considerable differences in the composition of

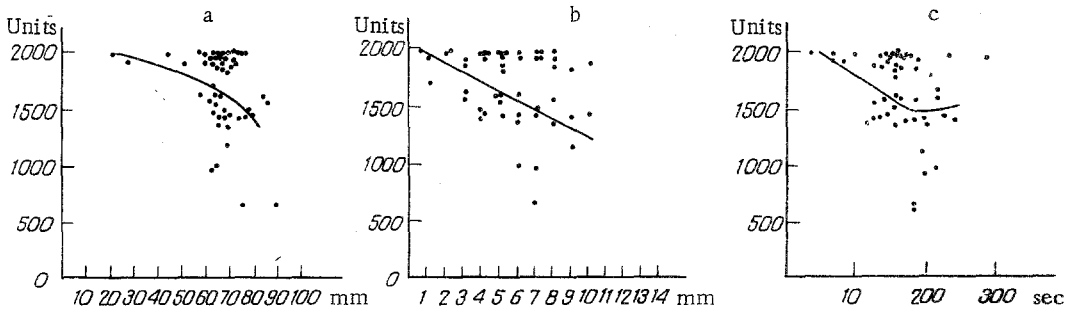

Fig. 2. Correlation between level of IC detectable in 7% PEG solution with degree of PA (a), with size of aggregates (b), and with time of PA (c) in patients with IHD (n = 51). Coefficient of correlation (r) for a, b, and c is −0.37, −0.40, and −0.39 respectively. In every case $P < 0.01$.

the immunoglobulins in IC precipitated in 7% PEG solution (Fig. 1). Unlike healthy subjects, in patients with IHD IgG and IgA were found as well as IgM. These data confirm that, besides large IC, it is also possible to detect so-called small IC, capable of circulating in the blood stream for a long time in certain pathological states and, in particular, in IHD, as well as large IC in 7% PEG solution.

In a 3.5% solution of PEG the content of IC in 120 healthy donors was 143 ± 5.5 mg%, compared with 216 ± 7.8 mg% for patients with IHD (Table 1). The difference is highly significant ($P < 0.001$). Samples of blood serum from 55 healthy donors and 51 patients with IHD were tested in a 7% solution of PEG. In the 7% solution of PEG the light scatter of the test sera was 4-5 times greater than in a 3.5% PEG solution. Since no similar effect was observed during analysis of aggregated γ-globulin (evidence that only large protein aggregates were present, equally detectable in high and low concentrations of PEG), the IC level in 7% PEG solution was determined from the scattering of light of the laser beam of the nephelometer. Its value was 1059 ± 53.1 units in donors and 1692 ± 48.1 units in patients with IHD, further evidence of a significant rise in the level ($P < 0.001$) of both large and small IC in patients with coronary atherosclerosis. A significant increase also was observed in all parameters of PA in patients with IHD, evidence of platelet dysfunction in atherosclerosis.

Parallel studies — PA tests and determination of the IC level in 3.5% PEG solution — were carried out on 97 patients with IHD and PA tests and determination of IC in 7% PEG solution on 51 patients. Analysis of correlation between PA and the IC level determined in 3.5% PEG solution showed absence of significant correlation between the degree of aggregation ($r = -0.12$), the size of the aggregates ($r = -0.05$), the aggregation time ($r = -0.04$), and the aggregation rate ($r = -0.20$) and the quantity of circulating IC. Comparison of the values for IC in 7% PEG solution with data for PA showed absence of significant correlation only with the aggregation rate ($r = -0.10$). With the other parameters of PA, significant negative correlation was found (Fig. 2), evidence of depression of the aggregation function of the platelets in the presence of a high level of small IC, detectable in 7% PEG solution.

Analysis of these experimental results suggests that the reduction in aggregating power of the platelets in patients with a high level of small IC, detectable in 7% PEG solution, may be due to blocking of the receptor apparatus of the platelets by these IC. This evidently largely determines the resistance of the intima of the vessels to thrombosis when there is the possibility of damage to the endothelial layer by circulating IC.

The study of the effect of preformed IC on platelets of animals of several species and of man [8] led to the conclusion that platelets active against immune adhesion (in rabbits, dogs, guinea pigs, rats, and mice) and inactive platelets (those of man and of the even-toed ungulates) exist.

However, the results of the present investigation preclude the absence of an active functional connection between human platelets and IC and they suggest the presence of a mechanism of inhibition of PA in man by small IC, which must be a subject for a special experimental study.

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