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ACTION OF TOXIC SUBSTANCES OF *Staphylococcus aureus* ON PLATELET FUNCTION

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The toxemia which accompanies infectious diseases is one factor leading to activation, and sometimes to injury of platelets. Under these circumstances biologically active substances (serotonin, ADP, platelet factors 3 and 4, etc.) which play an essential role in hemostasis and in the microcirculation are secreted from the platelets. It has been shown that endotoxins of Gram-negative bacteria have a direct action on the morphologic and functional state of the blood cells [2, 4, 5, 8, 11, 12]. The action of toxin of Gram-positive bacteria on platelet function has been studied extremely inadequately.

This paper gives data on the direct action of a toxic substance of *Staphylococcus aureus* and of an antigen isolated from staphylococcal cell membranes, namely teichoic acid (TChA).

EXPERIMENTAL METHOD

Standard staphylococcal toxin of batch 271.82, produced by the N. F. Gamaleya Research Institute of Epidemiology and Microbiology was used diluted with physiological saline (1:20, 1:10) and undiluted, and TChA in concentrations of $1 \cdot 10^{-3}$, $1 \cdot 10^{-2}$, $1 \cdot 10^{-1}$, 1, and 10 μg/ml. Experiments were carried out on plasma obtained from healthy blood donors, enriched with platelets. The platelet-rich plasma (PRP) was obtained by centrifugation (at 2000 rpm for 10 min) of citrate-stabilized blood (9:1). Platelet aggregation induced by 0.1% ADP solution (control) was recorded by the method in [7], and ability of the platelets to produce reversible endocytosis was tested by the method in [3], by means of which the quantity of absorbed fluorescent marker (acridine orange — AO, initial concentration $3.7 \cdot 10^{-6}$ mole/ml) and the degree of its release during plasma recalcification with 1.29% $CaCl_2$ solution could be recorded. Aggregation and endo-exocytosis were recorded continuously; initially the toxic substance, later ADP or $CaCl_2$, were added to the sample of PRP in the instrument. By conducting the experiments in this order it was possible to discover whether any one of the preparations studied was an inducer of aggregation or exocytosis. The quantity of the fluorescent marker AO absorbed by the platelets was determined quantitatively with the LYUMAM IUF-1 luminescence microscope, with photometric attachment. Preparations of living platelets were obtained from PRP, 0.1 ml of which was diluted with an equal volume of physiological saline and incubated for 30 min with 0.1 ml AO in a final concentration of $5 \cdot 10^{-5}$ M. A drop of the fluorochromed sample was applied to a defatted slide and covered with a coverslip. Those areas of the preparation where the platelets were spread out in a monolayer were examined under the microscope. The intensity of luminescence of the background and platelets was measured. The difference between these values corresponded to the quantity of AO absorbed by the platelets. Fluorescence of at least 25 platelets was measured in each preparation [11]. The surface and shape of the platelets were studied with the scanning electron microscope (JSIM-35, from Jeol, Japan). The sample was fixed with 2.5% glutaraldehyde solution at 37°C for 1.5 h and transferred to filters (Nucleopore, USA, diameter 0.2 μ). The samples were then dehydrated with alcohol. The specimens were sprayed with gold together with palladium in a layer 15-20 nm

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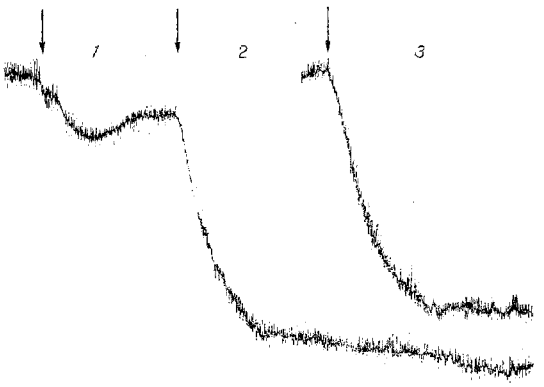

Fig. 1

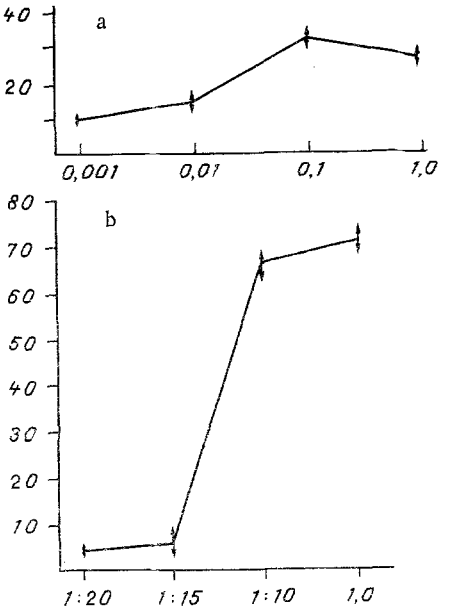

Fig. 2

Fig. 1. Platelet aggregation curves: 1) TChA (1 μg/ml, latent period of aggregation 30 sec, degree of aggregation 28 mm, duration of aggregation 10 min, duration of deaggregation 180 sec); 2) ADP after action of toxin (degree of aggregation 120 mm, duration of aggregation 810 sec); 3) ADP-induced aggregation (control, degree of aggregation 112 mm, duration of aggregation 450 sec).

Fig. 2. Dependence of degree of platelet aggregation on concentration of staphylococcal toxic substances: a) TChA; b) native toxin. Abscissa, concentration of toxic substance (in μg); ordinate, degree of aggregation (in mm).

TABLE 1. Intensity of Fluorescence of Platelets before and after Incubation with Antigen (staphylococcal TChA)

Platelets	Intensity of fluorescence, conventional units			
	control	incubation with TChA, min		
		5	30	60
A cells	17,2±0,5	13,2±0,5 $P<0,005$	11,8±0,6 $P<0,005$	11,0±0,6 $P<0,05$
B cells	57,6±3,0	46,4±2,4 $P<0,05$	0	0
B cells, in % of total number	10,5±2,0	1,1±0,5 $P<0,001$	0	0

thick. The specimens were examined under an accelerating voltage of 25 kV, at an angle of 45°, under secondary emission conditions [5, 6].

EXPERIMENTAL RESULTS

The results showed that the toxic substances from *Staph. aureus* are inducers of platelet aggregation. As an illustration, aggregation curves obtained in one experiment are shown (Fig. 1). Clearly the addition of TChA (final concentration $1 \cdot 10^{-2}$ μg/ml) to the sample of PRP caused unstable platelet aggregation. Subsequent addition of ADP caused a new wave of aggregation. The combined degree of aggregation (138 mm) was higher than the control (112 mm). With an increase in the concentration of toxic substances the degree of platelet aggregation also increased (Fig. 2). Characteristic features of the action of the standard toxin were the presence of a latent period of the reaction (from 15 to 300 sec) and instability of the aggregates. After only 30-180 sec, in 13 of 21 experiments a process of deaggregation had be-

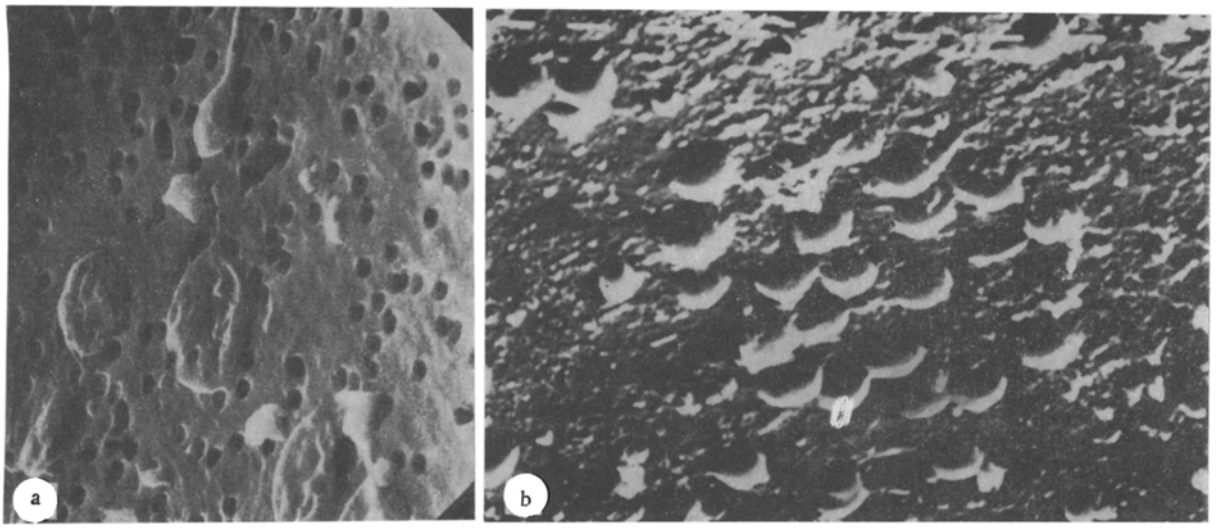

Fig. 3. Photomicrographs of platelets: a) normal, b) after action of toxie substance from *Staph. aureus* ($1 \cdot 10^{-1}$ mg/ml).

gun, and amounted in some experiments to 50-60%. This was probably connected with the presence of hemolysins, procoagulases, and staphylokinases, which activate fibrinolysis, in the staphylococcal toxic substance, leading to the appearance of fibrin dry products (FDP) and to inhibition of aggregation. During platelet activation, biosynthesis of intermediate products of the arachidonic acid cascade also took place, with subsequent accumulation of cAMP, which also facilitates deaggregation.

Investigation of samples of PRP with the scanning microscope after incubation for 5-10 min with native toxin (Final concentration $1 \cdot 10^{-1}$ µg/ml) revealed:

1) the presence of activated platelets, as shown by a change in their shape from discoid to spherical, the appearance of small pseudopodia, and swelling of the plasma membranes;

2) the presence of small aggregates of two to five platelets (Fig. 3).

Under the influence of TChA the latent period of $CaCl_2$-induced release in 10 of 12 experiments was shortened from 147.0 ± 20.7 sec to 70.4 ± 12.1 sec ($P < 0.01$). The degree of exocytosis, i.e., the quantity of fluorescent marker released, increased from 18.9 ± 3.8 to 24.7 ± 3.6%, i.e., by one third.

Native toxin, unlike TChA, is itself an inducer of release: in low concentrations (1:15, 1:10) in three of 11 experiments, and undiluted in all 15 experiments. The average release of fluorescent label was 14.4% of the total taken up. Additional release of AO under the influence of $CaCl_2$ in these cases was very small (on average 2.5%). The main mass of platelets, having reacted to the toxin, probably became refractory to the subsequent action of $CaCl_2$.

The staphylococcal toxic substance thus has a stimulating action on platelet release. This was confirmed by luminescence microscopy.

The platelet pool of healthy subjects is known to be heterogeneous with respect to many parameters and, in particular, to the intensity of their luminescence [9, 10, 13]. The present investigation showed that 80-90% of cells have an intensity of fluorescence of between 15 and 30 conventional units (group A platelets), whereas the remaining 10-20% give off brighter fluorescence (from 50 to 130 conventional units — group B). The intensity of fluorescence of the group A platelets from the 20 blood donors investigated averaged 17.2 ± 0.5 conventional unit, whereas for group B cells it was 57.6 ± 3.0 conventional unit. The number of bright cells in this case was 10.5 ± 2.0%.

After incubation with TChA the intensity of fluorescence fell in both groups of cells, as also did the number of bright cells (Table 1). The decrease in the intensity of fluorescence of the platelets increased and the duration of incubation of the cells with the toxin increased. After incubation for only 30 min the brightly fluorescent B cells disappeared and fluorescence of the A cells was considerably reduced.

The decrease in the intensity of fluorescence of platelets of groups A and B in these experiments was evidently the result of their degranulation under the influence of the staphylococcal toxic substance.

Degranulation of platelets, i.e., enhancement of their secretory function, leads to the release of biologically active substances (serotonin, ADP, calcium ions, platelet factor 3), contained in the 5-HT-organelles, into the blood stream, activating the blood clotting system, potentiating aggregating properties and, as a result, giving rise to thrombohemorrhagic manifestations and microcirculatory disturbances.

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PREVENTION OF STRESS-INDUCED DYSLIPIDEMIA BY ADAPTATION OF ANIMALS TO PERIODIC HYPOXIA

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Negative correlation exists [1, 12] between the blood level of high-density lipoproteins (HDL) and the degree of atherosclerosis of the coronary vessels in man, whereas correlation between low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL), on the contrary, is positive [2, 4]. These facts, confirmed in extensive epidemiologic [2] and experimental [4] investigations, lay at the basis of the view that HDL have an anti-athermogenic role, whereas LDL have an atherogenic role, and they have been used to determine the so-called "cholesterol coefficient"(ChC) of atherogenicity, or ratio between the concentration of cholesterol (Ch) in lipoproteins of different density

$$\frac{(Ch_{ldl} + Ch_{vldl})}{Ch_{hdl}}$$

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