LITERATURE CITED

1. F. Z. Meerson, Pathogenesis and Prevention of Stress-Induced and Ischemic Heart Injuries [in Russian], Moscow (1984).
2. F. Z. Meerson, Yu. V. Arkhipenko, I. I. Rozhitskaya, and V. E. Kagan, Byull. Éksp. Biol. Med., No. 8, 405 (1982).
3. F. Z. Meerson, L. Yu. Golubeva, V. E. Kagan, et al., Metabolism of the Myocardium [in Russian], Moscow (1981), pp. 237-250.
4. F. Z. Meerson, V. V. Malyshev, V. E. Kagan, et al., Arkh. Patol., No. 2, 9 (1980).
5. F. Z. Meerson, L. N. Medvedev, L. Yu. Golubeva, and E. E. Ustinova, Byull. Éksp. Biol. Med., No. 8, 61 (1982).
6. F. Z. Meerson, T. G. Sazontova, V. E. Kagan, et al., Byull. Éksp. Biol. Med., No. 12, 42 (1983).
7. F. Z. Meerson, N. V. Samosudova, E. V. Glagoleva, et al., Arkh. Anat., No. 2, 43 (1983).
8. O. Desiderato, J. McKinnon, and H. Hisson, J. Comp. Physiol. Psychol., 87, 208 (1974).
9. H. G. Glitsch, Am. J. Physiol., 236, 189 (1979).
10. H. G. Glitsch, Basic Res. Cardiol., 79, 611 (1984).
11. A. Kohama, W. Boyd, C. Ballinger, et al., J. Surg. Res., 11, 297 (1971).
12. B. Lown, R. L. Verrier, and R. Corbalan, Science, 132, 834 (1973).
13. B. Lown, R. A. De Silva, P. Reich, and B. J. Murawsky, Am. J. Psychiat., 137, 1325 (1980).
14. T. Tamai and S. Kagaijama, Circulat. Res., 22, 423 (1968).
15. M. Vassale, Circulat. Res., 27, 361 (1970).

ANTIAGGREGATING ACTION OF SODIUM HYPOCHLORITE ON PLATELETS

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Sodium hypochlorite (NaClO), obtained by electrolysis of sodium chloride solutions, possesses bactericidal properties and is therefore used in medicine as an external disinfectant [1, 2]. To study the possibility of further application of NaClO in medicine and, in particular, the possibility of its percutaneous absorption, its action on blood components must be studied.

EXPERIMENTAL METHOD

Platelet-enriched plasma (PEP) from rabbit blood was used [7]. Platelet aggregation induced by addition of 0.1 ml of ADP solution (pH 7.4, 10 μM) to 1 ml of PEP, was recorded by a turbidimetric method [3] on an aggregometer made in the writers' laboratory [5]. The main quantitative parameter of platelet aggregation was the maximal change of light transmission by PEP (degree of aggregation), recorded 7-8 min after addition of ADP.

Absorption spectra of ADP and mixtures of it with NaClO were recorded on the DU-7 spectrophotometer (Beckman, USA) in a cuvette 1 cm thick.

EXPERIMENTAL RESULTS

NaClO, added to PEP in a final concentration of 2-4 mM approximately 1 min before addition of ADP, had a strong antiaggregating action, leading to a decrease in the initial rate of platelet aggregation (the tangent of the angle of slope of the initial segment of the curve showing an increase in transmittance) and the degree of aggregation (Fig. 1; Table 1). A sim-

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ilar picture was observed on induction of aggregation with calcium chloride (Fig. 1). The degree of platelet aggregation (by ADP in a concentration of 10 μM) in PEP obtained from blood samples incubated for 2-3 h with NaClO (4.7 mM) was 41 ± 3% of the control (blood without NaClO). NaClO can weaken latent aggregation by modifying the aggregation inducer ADP, and (or) PEP itself. In the first place the action of NaClO was studied on ADP: Changes in the absorption spectrum of ADP and its ability to induce platelet aggregation were studied.

If ADP is mixed with NaClO (60 μM and 2.15 mM, respectively) it is destroyed, as is shown by transformation of the absorption band in the spectral region 230-290 nm (Fig. 2). The character of this transformation can be interpreted as follows. Through the action of NaClO, ADP is converted into a product with lower absorbance in the 240-270 nm region and with an absorption maximum in the longer waveband. As a result of accumulation of this product and of destruction of a considerable quantity of ADP the absorption spectrum of the mixture in the 240-290 nm region shifts within a few minutes into the region of longer wavelengths. The product described above is unstable: Due to its disintegration and total destruction of ADP the absorption band at 240-290 nm disappears after 30 min. Destruction of ADP, evidently, ought itself to weaken platelet aggregation in the presence of NaClO. Under these circumstances, however, the end result of interaction betwen ADP and NaClO may be different, for ADP modification products may possess proaggregation properties. In the next experiments ADP and NaClO were incubated together in concentrations of 60 μM and 2.15 mM, respectively, after which the mixture was added, in a volume of 0.1 ml, to 1 ml of PEP. In the samples thus obtained NaClO could not have had any effect on platelet aggregation because of its low concentration. This is shown by the result of the control experiment: If NaClO was added to PEP in a concentration of 0.215 mM before the addition of ADP, platelet aggregation was virtually unchanged. The main effect of NaClO on ADP was to reduce the ability of the latter to induce platelet aggregation: The degree of aggregation after preliminary incubation of this mixture for 1, 4, 20, and 30 min was 75 ± 5, 74 ± 6, 73 ± 6, and 53 ± 4% of the control respectively. Meanwhile, the degree of platelet aggregation was reduced by the same amount during incubation of ADP with NaClO for 1-20 min, although the ADP concentration fell steadily. This may easily be explained on the grounds that the intermediate product of ADP modification, with peak absorption at about 270 nm (Fig. 2) has the ability to potentiate, or to induce de novo, platelet aggregation. Its formation compensates functionally for destruction of ADP in the course of 1-20 min, so that during this period the ADP + NaClO mixture induces the same degree of platelet aggregation. Quite strong platelet aggregation was observed on the addition of a mixture of ADP and NaClO, preincubated for 30 min, during which the ADP and the intermediate were virtually completely destroyed, to PEP (Fig. 2). Hence it follows that when ADP is destroyed by NaClO, besides the intermediate product mentioned above, a secondary product capable of inducing platelet aggregation also is formed.

The velocity constant of bimolecular destruction of ADP in a concentration of 60 μM with NaClO in a concentration of 2.15 mM was found to be not more than 0.7 liter/(mole•sec). Let us assume that the NaClO concentration in PEP is virtually unchanged with time and that ADP destruction thus proceeds in accordance with the law of first-order reactions. In that case, with NaClO in concentrations of 2.15 and 4.3 mM, not less than 90 and 80% of ADP respectively would remain 1 min after its addition to PEP, and about 50 and 25% would remain 8 min after its addition. If ADP destruction is a second-order reaction, these values will be too low. Since platelet aggregation reaches a maximum with ADP in a concentration of about 8 μM [4], on its addition in a concentration of 10 μM this process ought not to be significantly changed due to destruction of ADP in the initial period, let us say for 1 min. Meanwhile during this period the rate of aggregation obtained in the presence of NaClO was much lower than in the control (Fig. 1). By the time that the degree of aggregation was recorded (7-8 min after addition of the inducer) much of the ADP may have been destroyed and in that case the antiaggregating effect of NaClO would be reduced when the ADP concentration was raised. In reality platelet aggregation with ADP in concentrations of 30 and 50 μM was reduced by virtually the same degree in the presence of NaClO (Table 1). It can thus be concluded that NaClO causes changes in PEP and that this modification is responsible for the weakening of platelet aggregation when induced by ADP in a concentration of 10-50 μM, although this degree of platelet aggregation is in any event reduced if ADP is used in a concentration of 10 μM rather more strongly than in a concentration of 50 μM (Table 1). This indicates that platelet aggregation with reduced ADP concentrations is weakened partly as a result of direct destruction of the inducer also. Changes in platelet aggregation may be linked with changes both in the cells and in the components of the plasma. To determine what part of PEP is modified by the action of NaClO, experiments were carried out with concentrated platelet suspensions. NaClO was added

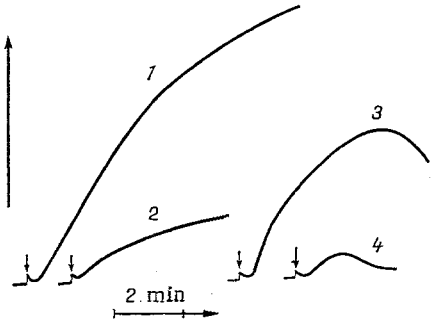

Fig. 1

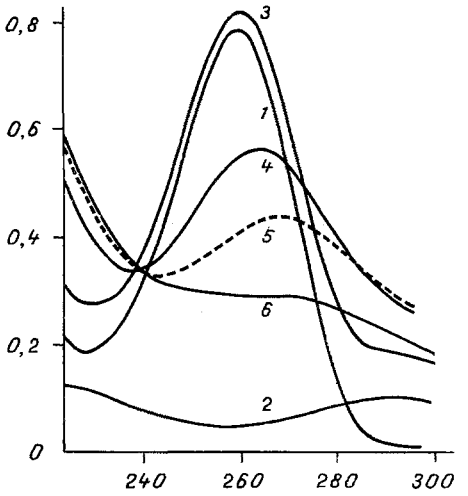

Fig. 2

Fig. 1. Typical kinetic curves of platelet aggregation in control samples of PEP (1, 3) and in the presence of NaClO (4.3 mM), added 1 min before the inducers (2, 4). Vertical axis — transmittance (in relative units). Arrows pointing downward — times of addition of inducers. 1, 2) Aggregation was induced by ADP (10 μM); 3, 4) aggregation was induced by calcium chloride (60 mM).

Fig. 2. Absorption spectra of mixture of ADP and NaClO. Abscissa, wavelength (in nm); ordinate, optical density. 1, 2) Pure solutions of ADP (60 μM) and NaCl (2.15 mM), respectively; 3-6) mixture after incubation for 0.18, 4, 20, and 30 min, respectively at room temperature.

TABLE 1. Antiaggregating Action of NaClO on PEP When Added 1 min before ADP in Different Concentrations

NaClO concentration, mM	Degree of aggregation, %		
	ADP concentration, μM		
	10	30	50
0	100	100	100
2,15	42±4	53±8	60±12
4,30	20±3	26±4	31±5

(physiological saline in the control) to the suspension in a final concentration of 4.3 mM, and after the necessary incubation period if was diluted 1:10 with native plasma and platelet aggregation induced by ADP (10 μM) was recorded. Weakening of aggregation was found in these experiments, and on incubation with HClO for 1 and 30 min its value was 51 ± 7 and 51 ± 10% of the control respectively. Platelets in the concentrated suspension were directly modified by NaClO and lost their ability to aggregate, since addition of NaClO in a concentration of 0.43 mM to the native platelet suspension after its dilution with plasma caused a decrease in the degree of aggregation by not more than 2-3%. Correspondingly, the antiaggregating action of NaClO on the original PEP (Fig. 1; Table 1) was presumably due mainly to modification of the platelets.

The results are evidence that NaClO in concentrations of over 1 mM inhibits platelet aggregation in PEP. This takes place mainly as a result of direct modification of the cells. Platelet aggregation may be weakened partly as a result of ADP destruction. Products of destruction of ADP by NaClO can induce platelet aggregation.

LITERATURE CITED

1. I. A. Malevskaya and L. A. Yaroslavskaya, Modern Methods and Agents for Disinfection and Sterilization [in Russian], Moscow (1979), pp. 43-46.
2. V. A. Nikulin and B. L. Shura-Bura, Vrach. Delo, No. 2, 135 (1977).

3. G. V. R. Born, Nature, 194, 275 (1962).
4. G. A. Marguerie, E. F. Plow, and T. S. Edgington, J. Biol. Chem., 254, 5357 (1979).
5. D. I. Roshchupkin, A. K. Anosov, and A. Ya. Potapenko, Photobiochem. Photobiophys., 5, 307 (1983).

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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