10. K. H. Sanders, E. E. Klein, T. E. Mayer, et al., Brain Res., 186, 83 (1980).
11. C. Takeshige, M. Murai, C. P. Luo, et al., Neurosci. Lett., 13, 90 (1979).

ACTION OF MENINGOCOCCAL LIPOPOLYSACCHARIDE ON PLATELET FUNCTION

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In the generalized form of meningococcal infection toxemia is one of the factors which disturbs hemostasis and promotes thrombohemorrhagic complications. Definite views on the origin of these complications have now been formed. Disturbance of the integrity of the vessel wall [4, 5, 14] and injury to the blood cells [6, 13] under the influence of menigococcal endotoxin lead to the release of thromboplastic substances activating the blood clotting system into the blood stream. Developing intravascular blood clotting involves the consumption of procoagulants (especially fibrinogen), thrombocytopenia, and protective activation of the anticlotting component of the blood clotting system, which in conjunction with injury to the vessel wall, leads to hemorrhages [5].

In our opinion, in patients with generalized meningococcal infection (meningitis with meningococcemia) the aggregating properties of the platelets (in the first 2 days after admission to hospital) are depressed, especially in seriously ill patients. By the time of recovery (the 17th-20th day of treatment) this function of the platelets is back to normal. The ability of platelets to carry out reversible endocytosis likewise is modified. Uptake of the fluorescent label is increased, the liberation reaction is depressed during the first days of the disease, but starting with the 2nd day of treatment it is distinctly intensified and is not yet back to normal when the patient leaves hospital. We thus found that in patients with a generalized form of meningococcal infection not only is thrombocytopenia observed, but the functional properties of the membranes and subcellular apparatus of the platelets also are disturbed. The mechanism of action of meningococcal endotoxin on platelet function has virtually not been studied.

The object of the present investigation was to study the direct action of meningococcal endotoxin on the state of the plasma membranes and the subcellular apparatus of the platelets.

EXPERIMENTAL METHOD

Experiments were carried out *in vitro* on plasma from blood donors enriched with platelets. Platelet-enriched plasma was obtained by centrifugation (at 2000 rpm for 10 min) of blood stabilized with citrate (9:1). Samples of plasma were incubated with meningococcal endotoxin (in a concentration of between 0.5 and 75 μg ml plasma for 5 to 60 min). According to data in the literature [15], the toxic principle of the meningococcus is a lipopolysaccharide which, besides its toxic activity, also has pyrogenic and antigenic properties.

The complex antigen, consisting of cell wall lysate from group A *Neisseria meningitidis*, was obtained by treatment of this strain with a 0.5% solution of Triton X-100 in an alkaline medium.

The lipopolysaccharide was isolated by extraction with aqueous phenol and subsequently purified with Cetavlon.

The antigen complex in the culture fluid was obtained by chromatography of a supernatant of a liquid culture on a column with Acrylex P-6. The action of all three preparations on the state of platelet function was tested in the experiments.

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0007-4888/82/9303-0231$07.50

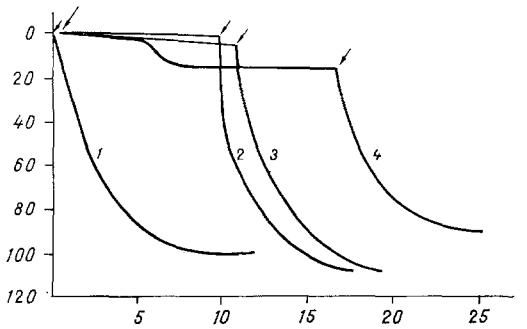

Fig. 1. Induction of platelet aggregation by meningococcal lipopolysaccharide and its effect on ADP-induced aggregation. 1) Aggregation of donor's platelets; 2-4) aggregation of platelets by different doses of endotoxin (1, 5, and 10 μg respectively). Short arrows indicate time of addition of toxin to specimen, long arrow denotes addition of ADP. Abscissa, time (in min); ordinate, degree of aggregation (in mm).

Platelet aggregation, induced by a 0.1% solution of ADP, was recorded by the method in [7], and the ability of the platelets to undergo reversible endocytosis was tested by the method in [2]. With this last method the quantity of fluorescent dye acridine orange taken up (initial concentration 3.7×10^{-6} mole/ml) and the degree of its liberation during recalcification of the plasma with 1.29% $CaCl_2$ solution could be recorded. Continuous recording of aggregation and endoexocytosis was carried out, first endotoxin, and then (after 5, 30, or 60 min) a solution of ADP or $CaCl_2$ being added to the sample of platelet-rich plasma placed in the instrument. By doing the experiments in this way it was possible to determine whether any of the preparations studied is an inducer of aggregation or of exocytosis. The investigation of the state of the surface and shape of the platelets was carried out on a scanning electron microscope (Iism-35, from Jeol, Japan). The sample was fixed with 2.5% glutaraldehyde at 37°C for 1.5 h and applied to nucleopores (diameter 0.2 μ). The samples were then dehydrated with alcohols. The specimens were sprayed with gold and palladium in a layer 15-20 nm thick. They were examined under an accelerating voltage of 25 kV, at an angle of 45°, under secondary emission conditions.

EXPERIMENTAL RESULTS

Effect of Meningococcal Endotoxin on Platelet Aggregation. The experiments showed that meningococcal lipopolysaccharide can induce platelet aggregation. The intensity of the effect of the endotoxin depended on its concentration, within certain limits. In one experiment (Fig. 1), for instance, 1.0 μg of lipopolysaccharide caused weak aggregation of platelets (degree of aggregation H = 2 mm): after the addition of ADP the degree of aggregation reached the control level (100 mm). A dose of 5.0 μg caused more marked aggregation (H = 4 mm), but after the addition of ADP it increased to 102 mm. A dose of 10.0 μg caused considerable platelet aggregation (H = 10 mm), but after the addition of ADP it did not reach the control level (H = 84 mm).

A decrease in the total degree of platelet aggregation was observed in four of 19 experiments. It can be postulated that in these cases a certain number of platelets changed into a refractory state under the influence of the toxin and did not react to addition of ADP. The possibility that such a state may arise after addition of the next two doses of ADP was demonstrated previously [1].

An increase in the total degree of platelet aggregation after successive addition of meningococcal lipopolysaccharide and ADP was observed in 15 of 19 experiments, whereas the inducing action of the lipopolysaccharide itself was observed in all experiments without exception. The two other preparations had a similar action.

It can be concluded from these observations that meningococcal endotoxin induces platelet aggregation. As we know, the aggregation power of platelets is determined by the state

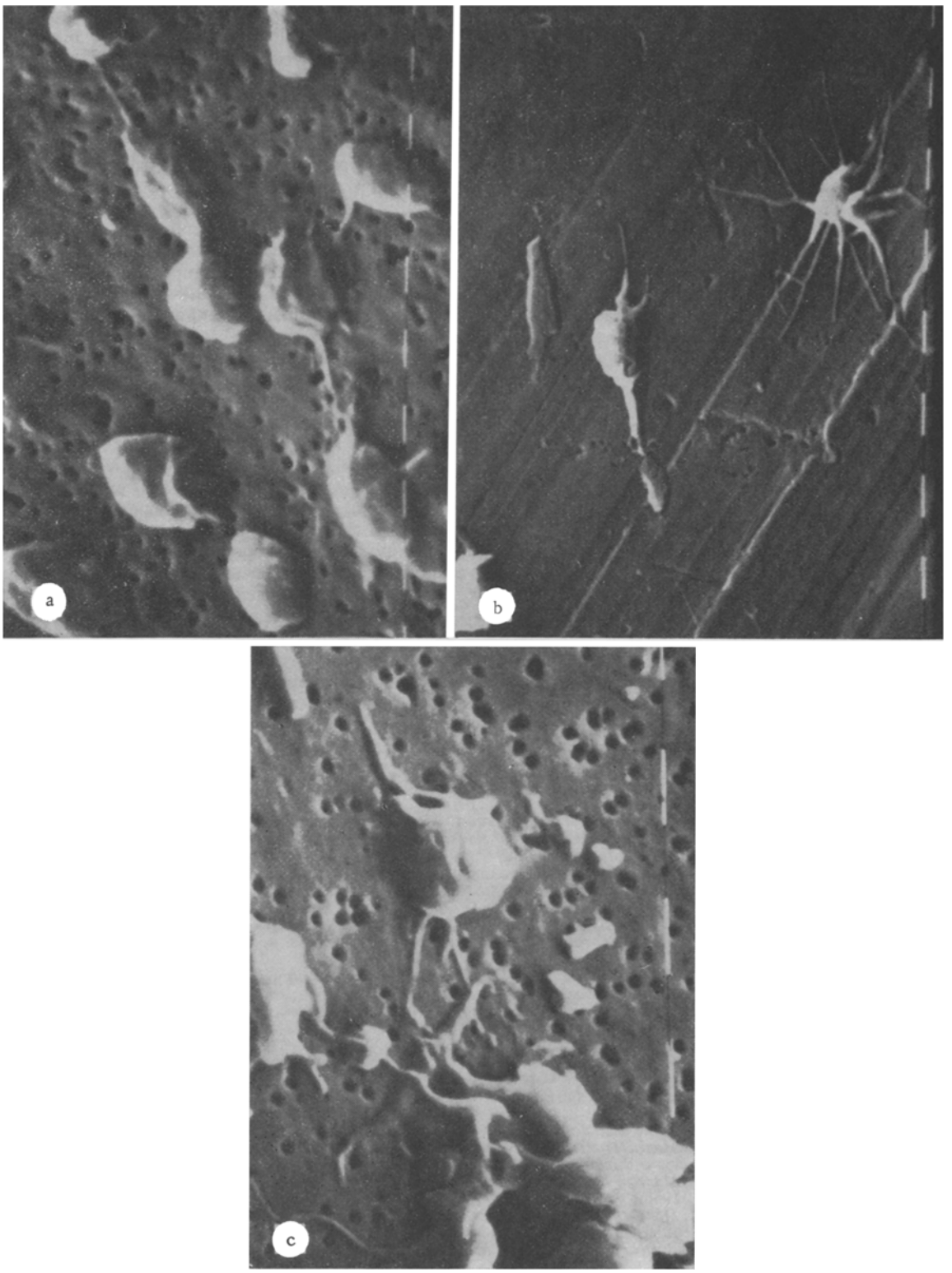

Fig. 2. Changes in platelets under the influence of meningococcal lipopolysaccharide: a) donor's platelets, b) after incubation for 10 min with 10 μg meningococcal lipopolysaccharide (spherulated forms of platelets with pseudopodia can be seen); c) platelet aggregate. 10, 000 ×.

of their membranes [8, 9]. Consequently, meningococcal endotoxin acts directly on the platelet plasma membranes.

This conclusion was confirmed by the results of an investigation of the surface and shape of the platelets before and after incubation with meningococcal lipopolysaccharide. The results showed that the lipopolysaccharide, in a dose of 10.0 μg/ml and after incubation for 10 min, caused change in shape of the platelets from disk-like (Fig. 2a) to spherical, with many pseudopodia (Fig. 2b). Many aggregates can be seen in the test specimen (Fig. 2c).

Effect of Meningococcal Endotoxin on Exocytosis (Liberation Reaction) of Platelets. The ability of platelets to take up fluorescent dyes (mepacrine, acridine orange) and to accumulate them in their subcellular organelles, especially in 5-HT granules [3, 10, 11, 14], and later to liberate them during viscous metamorphosis, provided an approach to the study of the function of the subcellular apparatus under normal and various pathological conditions [9]. Liberation of biologically active substances such as serotonin, adrenalin, noradrenalin, and ADP from 5-HT granules plays an essential role in intravascular blood clotting processes and in the subsequent hemorrhages, by affecting the adhesion and aggregation of platelets and the permeability of blood vessel walls [9, 12].

The effect of meningococcal endotoxin on the function of the subcellar apparatus of platelets has not hitherto been studied. Our observations showed that meninogococcal lipopolysaccharide, like the other two preparations, does not directly induce the liberation reaction, for it is evidently not an inducer of exocytosis. However, after incubation of a specimen of platelet-rich plasma with endotoxin (for 5, 30, or 60 min), addition of $CaCl_2$ solution caused a greater degree of liberation of the fluorescent label than in the control.

Mackay [12], who studied the effect of meningococcal endotoxin on plasma coagulability, concluded that it does not act through thrombin formation. The present experiments showed that the action of meningococcal endotoxin on the intracellular apparatus of platelets likewise does not involve thrombin formation. Meanwhile the increase in exocytosis found under the influence of meningococcal endotoxin is evidence that the state of the intracellular apparatus has changed.

LITERATURE CITED

1. R. I. Volkova, "Regulation of ADP-induced platelet aggregation at different stages of individual development," Candidate's Dissertation, Moscow (1978).
2. V. K. Kozlov, Tsitologiya, No. 7, 762 (1975).
3. V. K. Kozlov, R. A. Markosyan, and L. I. Buryachkovskaya, Byull. Eksp. Biol. Med., No. 7, 5 (1979).
4. B. I. Kuznik and V. I. Skipetrov, Blood Cells, Vessel Wall, Hemostasis, and Thrombosis [in Russian], Moscow (1974).
5. V. I. Pokrovskii, L. A. Favorova, and N. N. Kostyukova, Meningococcal Infection [in Russian], Moscow (1976).
6. A. M. Chernukh, P. N. Aleksandrov, and O. V. Alekseev, The Microcirculation [in Russian], Moscow (1975).
7. G. Born, Nature, 194, 927 (1962).
8. J. Gordon and J. Milner, in: Platelets in Biology and Pathology, Amsterdam (1976), p. 3.
9. H. Lorez, M. da Prada, and A. Pletcher, Experientia, 31, 593 (1975).
10. H. Lorez and M. da Prada, Experientia, 34, 663 (1978).
11. D. MacIntary, in: Platelets in Biology and Pathology, Amsterdam (1976), p. 61.
12. D. Mackay, S. Shapiro, and J. Shanberge, J. Exp. Med., 107, 369 (1958).
13. D. Mackay, Thromb. Diath. Haemorh. (Stuttgart), 29, 11 (1973).
14. G. Stewart and M. Anderson, Br. J. Exp. Path., 52, 75 (1971).
15. O. Westphal, Int. Arch. Allergy, 49, 1 (1975).