1. Development of the humoral immune response in inflammatory diseases of the female genitalia is characterized by successive phases of immunoglobulin production and CIC formation, and this must be taken into account when the clinical-immunological diagnosis is made.

2. By carrying out repeated investigations the sequence of phases of the immune response was confirmed.

3. A phase of the humoral immune response characterized by a below normal level of immunoglobulin(s) and by a high CIC concentration, distinguishing it from an ID state in which the CIC level is within normal limits, is distinguished for the first time.

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# STATE OF COAGULATION HEMOSTASIS AND FIBRINOLYSIS IN THE RAPIDLY PROGRESSIVE FORM OF BOTULISM


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The pathogenic action of botulinus toxin is known not to be restricted to blockade of acetylcholine release in myoneural synapses and synapses of the autonomic and central nervous system, but it is also accompanied by marked changes in functional activity of the system regulating the aggregation state of the blood (RASB). In particular, marked disturbances of vascularization of different parts of the brain and spinal cord and of the internal organs, manifested as a combination of signs of ischemia and congestion, by the presence of petechial and larger hemorrhages, thrombosis, and stasis in the capillaries against the background of destructive changes affecting the endothelium and walls of the blood vessels, have been described [1-4, 6, 7]. As yet, however, the character and mechanisms of disturbances of the pro- and anticoagulant components of the hemostasis system, and also the state of fibrinolysis in the course of botulinus poisoning, have not been studied. In was accordingly decided to investigate this problem.

## EXPERIMENTAL METHOD

Type C botulism was induced by intraperitoneal injections of botulinus toxin in a dose of 0.025 mg/kg body weight (1 MLD for mice is 0.0005 mg of the dry toxin). Noninbred rats weighing 250 g were used. The experiments were carried out 6 h after injection of the toxin, in the absence of any marked clinical signs of poisoning, and also 24 and 48 h later, against a background of the development of generalized pareses and paralyses of the skeletal muscles. The following parameters of the state of the coagulation mechanism of hemostasis were studied at intervals: the blood clotting time in ordinary and siliconized

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TABLE 1. Parameters of Coagulation Hemostasis and Fibrinolysis during Course of Type C Botulism ($M \pm m$)

| Parameter | Control | Preclinical period of botulism | | Paralyses of skeletal muscles | | Paralyses of skeletal muscles | |
|---|---|---|---|---|---|---|---|
| Blood clotting time, min | 2,77±0,2 | 2,2±0,2 | $p<0,05$ | 2,75±0,1 | $p>0,5$ | 3,6±0,1 | $p<0,001$ |
| Silicone blood clotting time, min | 4,9±0,3 | 2,3±0,2 | <0,001 | 3,7±0,3 | <0,02 | 5,8±0,3 | 0,05 |
| Index of range of contact activation, per cent | 41,2±3,1 | 9,1±4,4 | <0,001 | 20±2,5 | <0,001 | 30,6±2,9 | <0,05 |
| Antithrombin III, sec | 21,1±0,8 | 43,6±3,1 | <0,001 | 31,5±0,2 | <0,001 | 24±1,0 | <0,01 |
| Thrombin time, sec | 14,3±0,3 | 20,6±0,9 | <0,001 | 18,3±0,9 | <0,01 | 21±1,8 | <0,01 |
| Fibrinogen, g/liter | 2,5±0,03 | 3,1±0,21 | <0,05 | 3,5±0,1 | <0,001 | 3,6±0,18 | <0,001 |
| Total fibrinolytic activity, mm | 110,3±1,1 | 225,4±2,7 | <0,001 | 191,2±4,1 | <0,001 | 168,5±2,9 | <0,001 |
| Plasminogen concentratin, $mm^2$ | 61,2±2,1 | 109,4±1,4 | $p<0,001$ | 112,3±3,9 | $p<0,001$ | 99,5±2,7 | $p<0,001$ |
| Plasminogen activators, $mm^2$ | 52,4±2,3 | 116,0±1,3 | <0,001 | 78,9±2,6 | <0,001 | 68,9±1,5 | <0,001 |
| Fibrinogen B, per cent of positive tests | 0 | 91,7 | | 100 | | 100 | |
| Ethanol test, per cent of positive tests | 0 | 91,7 | | 100 | | 100 | |
| Protamine sulfate test, per cent of positive tests | 0 | 75 | | 50 | | 66,7 | |

**Legend.** In each series of experiments 15-20 animals were used.

tubes, the index of the range of contact activation, total antithrombin activity of the blood, antithrombin III activity, the blood heparin level, the fibrinogen concentration in the blood and fibrin-monomer complexes, and total fibrinolytic activity of the blood — traditional methods of investigation [5].

## EXPERIMENTAL RESULTS

In the preclinical period of botulism the blood clotting time in ordinary and siliconized tubes was reduced and the index of the range of contact activation depressed (Table 1). Meanwhile the thrombin time, antithrombin III activity, total fibrinolytic activity, blood plasminogen level, and activity of plasminogen activators were all increased. Thus a characteristic feature of the early period of the rapidly progressive form of botulism is simultaneous marked activation of the pro- and anticoagulant components of the hemostasis system, and also of the fibrinolytic system.

In the period of development of pareses of the skeletal muscles due to botulism, shortening of the blood clotting time was observed only in siliconized tubes, whereas in nonsiliconized tubes it remained within normal limits. The index of the range of contact activation remained low, just as in the previous period. The blood fibrinogen concentration at this period of botulism rose, but total antithrombin activity of the blood and antithrombin III activity were a little lower than in the preclinical period of botulism, although higher than the corresponding parameters for the group of control animals.

Meanwhile fibrinolytic activity of the blood was increased due to an increase in the plasminogen concentration and in activity of plasminogen activators. Just as in the previous period of botulism, the appearance of fibrin-monomer complexes and of other fibrin degradation products was found.

The results are evidence that the rapidly progressive form of botulism is characterized by intravascular activation of blood clotting with a simultaneous increase in activity of anticoagulant mechanisms, and increased fibrinolytic properties during the period of development of generalized pareses of the skeletal muscles.

With an increase in the severity of botulism in the period of development of paralyses of the skeletal muscles the blood clotting time was lengthened in both siliconized and nonsiliconized tubes, and the index of the range of contact activation decreased, evidence of the onset of hypocoagulation changes. The fibrinogen concentration in the late stage of botulism was appreciably higher than normal. Meanwhile total antithrombin activity, activity of antithrombin III, the total fibrinolytic activity of the blood, and its plasminogen level and activity of plasminogen activators all remained high. Thus the terminal stage of botulism is characterized by the development of failure of the mechanisms of formation of the prothrombinase activity of the blood, accompanied by simultaneous activation of the anticoagulant mechanisms and the fibrinolysis system.

To sum up the results it may be concluded that a characteristic feature of the rapidly progressive form of botulism is a marked disturbance of function of the RASB system, resembling a thrombohemorrhagic syndrome in type, and appearing appreciably before the onset of the neuroparalytic syndrome and the increasing severity of the course of the specific manifestations of this neurotoxicosis.

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