ACTION OF THE VENOM OF THE CENTRAL ASIAN COBRA (Naja oxiana EICH.) ON THE BLOOD CLOTTING SYSTEM

L. Sh. Mitel'man

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As regards their action on the process of blood clotting, snake venoms are divided into two groups: coagulating and anticoagulating. The first group includes the venoms of most vipers and rattlesnakes, the second the venoms of the cobras [1,2,7,10,12]. These properties of snake venoms have long been used both in laboratory practice, where the toxins of snakes have been used to investigate the blood clotting process, and for the treatment of patients with various forms of hemorrhagic syndrome [3-6,8,9,12,15].

During recent years, attention has been concentrated on cobra venoms, possessing marked analgesic, antispastic, vasodilator, and anticoagulating activity. Meanwhile the mechanism of action of these venoms on the blood clotting process has been insufficiently studied.

Nearly all experiments to study the effect of cobra venoms on the blood clotting mechanism have been performed with the toxin of the Indian cobra (Naja naja). Arthüs [7] was one of the first investigators to show that the addition of this venom to the blood causes a complete loss of its ability to form clots.

O'Brien [13] concluded that cobra venom inactivates the plasma component of thromboplastin (coagulation factor IX). It has also been reported that the venom of the Indian cobra possesses an antithromboplastin action and irreversibly inactivates the tissue thromboplastin, and this effect, moreover, is not abolished by specific immune serum [16].

The object of the present investigation was to study the action of the Central Asian cobra on the blood clotting process.

EXPERIMENTAL METHOD

Experiments were carried out with freshly diluted dried toxin in dilutions of 1:100, 1:800, 1:1000, and 1:50,000. The effect of the venom was studied on the recalcification time of citrated plasma, the prothombin time, the AC-globulin and proconvertin activity, the thromboplastin activity of the plasma, and the activity of the plasma factors of thromboplastin. In addition, the action of the cobra venom on the process of formation of plasma thromboplastin was determined by means of the Biggs–Douglas test. Experiments were carried out with plasma from the blood of healthy persons and patients with hemophilia B.

EXPERIMENTAL RESULTS

The venom of the Central Asian cobra in dilutions of 1:100-1:800 completely arrested the process of blood clotting, and this disturbance was not prevented by the addition of tissue thromboplastin to the blood. As the dilution of the venom increased, its action gradually became weaker, and it was least perceptible in a dilution of 1:50,000 (Table 1).

In the thromboplastin–generation test of Biggs–Douglas, the venom of the Central Asian Cobra inhibited the formation of plasma thromboplastin (Table 2).

The venom tested had no inactivating action on thrombin.

Because of O'Brien's statement that the effect of the venom of the Indian cobra is mainly on coagulation factor IX, it was decided to repeat his experiments using the toxin of the Central Asian Cobra. In experiments with the blood serum of patients with hemophilia B (factor IX deficiency), it was found that the ability of the cobra venom

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TABLE 1. Effect of Various Concentrations of Cobra Venom on Blood Clotting Revealed by Determination of Various Plasma Factors (mean data)

Concentration of venom	Clotting time (in sec) shown by determination of				
	Prothrombin time	AC-globulin	Proconvertin	Thromboplastin activity	Plasma factors of thromboplastin
Control	20.0	83.5	71.0	54.9	58.3
1:100	103.7	0	0	0	0
1:800	55.2	0	0	0	0
1:1,000	30.6	328.3	0	262.0	0
1:50,000	23.0	121.8	159.1	127.5	145.8

Note: In all experiments with the venom when clotting was observed, instead of a clot only tiny separate freely floating fibrin floccules were observed. 0 indicates that no clotting took place.

TABLE 2. Effect of Venom of the Central Asian Cobra on Formation of Plasma Thromboplastin in Biggs–Douglas Test (mean data)

Concentration of venom	Time of incubation of mixture (in min)				
	2	4	6	8	10
	Clotting time (in sec)				
Control	15.1	10.2	12.8	16.0	17.9
1:100	90.3	78.1	81.4	78.3	85.5

TABLE 3. Effect of Venom of the Central Asian Cobra in Dilution of 1:1000 on Thrombin Generation Test and Recalcification Time

Ingredients added	Recalcification time (in sec)	Time of thrombin generation test (in sec)				
		Time of incubation of mixture (in min)				
		1	2	3	4	5
Normal	105	50	30	22	27	34
Venom	205	No clotting detected				
Venom + normal serum	115	60	45	31	50	55
Hemophilia B serum + venom	300	No clotting detected				

to disturb the formation of plasma thromboplastin is mainly dependent on its inactivating action on the plasma component of thromboplastin (Table 3). The dynamics of this process is revealed by the results of the thrombin generation test [11].

The anticoagulating principle of the venom, unlike its main toxin [14], is thermolabile and is almost totally inactivated by heating for 10 min at 80-100°. Hence, the main anticoagulating fraction of the venom may be separated from its toxic fraction, and this suggests that it may have potential value as an anticoagulant.

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