fect the LPO intensity. The spleen was found to be the most sensitive tissue, especially at an early stage of poisoning. The long-term influence of NB and its chloro-substituted derivatives mobilizes the adaptive systems of the organism, which brings the LPO processes under control, although their capacity is practically exhausted, judging from the AOA decline. Administration of the test substances results in an intensive redistribution of vitamin E reserves in the organism, which implies its efficiency as a prophylactic.

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Ability of Low-Molecular Heparin (Fraxiparine) to Counter the Action of Exogenous Coagulases: a Fundamental Difference from Nonfractionated Heparin

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Snake venoms are known to contain proteases inducing blood coagulation by activating the hemocoagulation cascade on various levels [1-9,12,16,19, 21]. For example, in our previous studies, as well as in some reports from other laboratories, it was established that *Vipera lebetina turanica* venom contains a coagulase activating factor X, *Echis carinatus* and *Echis multisquamatus* venoms contain the enzyme directly activating prothrombin, factor II [5,7,9,10-13,17,20], while *Agkistrodon halys halys* venom includes a protease which con-

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TABLE 1. Effect of Heparin and Fraxiparine on Parameters of Coagulation Tests

Test	Coagulation time, sec ($M\pm m$)				
	without anticoagulant	with anticoagulant in final concentration of (IU/ml)			
		0.125	1.15	12.5	25.0
	Heparin				
PATT	45.0±2.1	48.0±2.3	77.8±2.3	350.0±5.9	no clotting
with *Echis carinatus* venom	32.2±1.9	34.8±2.4	35.4±2.6	35.8±2.1	35.8±2.4
with *Agkistrodon halys halys* venom	31.9±2.2	34.8±2.4	35.3±2.5	36.1±2.9	35.9±2.5
	Fraxiparine				
PATT	45.0±2.1	56.2±2.2	190.5±2.6	350.5±6.7	no clotting
with *Echis carinatus* venom	32.2±1.9	33.0±2.5	34.6±2.3	88.3±4.5	158.6±2.6
with *Agkistrodon halys halys* venom	31.9±2.2	32.8±2.4	37.4±2.6	44.2±2.6	78.2±3.5

verts fibrinogen to incomplete des-AA-fibrin [4,7-9,11]. At the same time, venom coagulases differ considerably from natural blood coagulation enzymes, and these distinctions are very useful for diagnostic purposes [2,7,9,12]. For example, *Echis carinatus* venom and its coagulase (ecarin) produce mesothrombin (or E_M-thrombin), whose coagulating activity cannot be blocked by ordinary (nonfractionated) heparin and the heparin-antithrombin III complex [3-5,7,10,12,13,18]. The same thrombinlike effect of *Agkistrodon halys halys* venom cannot be blocked by nonfractionated heparin either [7-9,11,14,19,21]. Heparinization of experimental animals does not prevent their death from the hemocoagulation shock occuring when these venoms are injected intravenously in lethal doses [2,4,6].

In the present study we compared the effects of partially depolarized low-molecular heparin SU 216 (Fraxiparine, Sanofi) and nonfractionated heparin (Serva, Germany) on the coagulation activity and mortality caused by prothrombin-activating (*Echis carinatus*) and fibrin-coagulating (*Agkistrodon halys halys*) venoms.

MATERIALS AND METHODS

The effects of Fraxiparine (FP) (Sanofi) and nonfractionated heparin (NH) on the hemocoagulatory properties of *E. carinatus* and *A. halys halys* venoms were studied *in vitro* using platelet-deficient citrate plasma samples (PDP) from 10 healthy subjects with a normal coagulogram. The plasma samples were routinely obtained as described previously [1]. Working solutions of venoms were prepared by diluting freeze-dried venoms (Biotekhnologiya diagnostic kits, Barnaul) in 0.3 ml distilled water. To this end the above-mentioned volume of distilled water was added to each well of a plate and stirred thoroughly for 1 min with a glass stick; thereafter the solutions obtained were considered to possess enough coagulating activity for the clotting of normal human PDP at 37°C for 25-30 sec (26.0±2.0 sec on average). In the control series 0.02 ml Michaelis buffer solution (pH 7.35) was added to 0.1 ml PDP at 37°C, 30 sec after 0.1 ml venom solution had been added, and the coagulation time was determined. In the experimental series the effect of FP and NH (introduced in PDP instead of Michaelis buffer solution) on venom-induced clotting was studied in the same plasma samples. Both NH and FP were introduced in PDP in a volume of 0.02 ml in the following concentrations: 1.25, 12.5, 125.0, and 250.0 IU/ml, and, correspondingly, the final concentrations in the experimental system were 10-fold lower. After the addition of the anticoagulants, 0.1 ml venom solution was introduced into the system and the clotting time was recorded. It should be borne in mind that the units for mea-

TABLE 2. Effect of Heparin and Fraxiparine on Acute Hemocoagulation Shock Induced by Injection of LD_{100} of *Echis carinatus* venom in Rats

Preparation	Dose, IU/kg	Total number of rats	Number of rats dead from shock	Time of death, min
Heparin	100	10	10	10–20
	200	20	20	5–20
	800	10	10	5–20
Fraxiparine	250	2	2	10
	500	5	1	5
	750	3	0	–

suring NH and FP activity do not correspond: the first is expressed in antithrombin units, while the second (similar to other low-molecular heparins) in anti-factor X units (anti-Xa). Hence, together with assessing the effect of these preparations on venom coagulating activity, we investigated their effect on the partial activated thromboplastin time (PATT) in the same plasma samples.

In vivo experiments were performed on 50 outbred albino rats weighing 150-230 g. Various doses of NH and FP (Table 2) were injected through the jugular vein under ether anesthesia, followed 10 min later by an injection of 3.5 mg/kg (LD_{100}) *E. carinatus* venom, which, as was previously found, causes the development of lethal hemocoagulation shock with disseminated intravascular coagulation and death within 5-25 min [4,6]. NH was used in doses of 100, 200, and 800 units/kg, FP in doses of 250, 500, and 750 anti-Xa units per kg.

RESULTS

As follows from Table 1, NH does not inhibit coagulation induced by *E. carinatus* and *A. halys halys* venoms, which confirms the results obtained by us [3-5,7-9] and by other investigators [10-14,19,21]. On the other hand, Fraxiparine was found to be capable of greatly inhibiting the coagulation induced by factor II (prothrombin)-activating venom and, to a lesser extent, by thrombinlike coagulase from *A. halys halys* venom, a reliable lengthening of the clotting time ($p<0.05$-0.001) being observed with concentrations of FP starting from 12.5 anti-Xa units.

In light of this it seemed important to clarify whether FP is able to prevent the death of experimental animals from hemocoagulation shock induced by intravenous injection of *E. carinatus* venom in an absolutely lethal dose. NH was previously shown have no such protective effect. The data in Table 2 show that preliminary intravenous injection of FP in a dose of more than 500 anti-Xa units per kg prevents the death of the majority of experimental animals from acute toxic hemocoagulation shock induced by *E. carinatus* venom.

Thus, a fundamental distinction between the effects of FP and NH has been discovered by us, namely, that FP is capable of weakening the effect of not only exogenous but also endogenous coagulases which occur in prothrombin-activating and thrombinlike snake venoms. This effect of low-molecular heparin (FP) manifests itself not only *in vivo* but also in *in vitro* experiments by preventing mortality among experimental animals from hemocoagulation shock induced by an absolutely lethal dose of *E. carinatus* venom.

The results obtained here support the published data on the mechanism of the anticoagulation effect of low-molecular heparins and open up new avenues for their preventive and therapeutic application.

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