NUCLEIC ACIDS IN ORGANS AND TISSUES IN *Vipera lebetina* VENOM INTOXICATION

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The mechanism of action of *Vipera lebetina* L. venom in animal organisms has not been completely elucidated until the present time. The variety of mechanisms of pathogenesis has been said to be caused by the complexity of substances which make up the venom.

The venom contains a spreading factor and a component which is identical with proinvasine I of Haas [7]. The venom is characterized by its proteolytic activity [8] which is the reason for its cytotoxic effect. This is related to the presence in the venom of amino acid oxidase-1, which hastens the action of proteolytic enzymes in the cells of the intoxicated organism, which subsequently results in cytolysis [9].

Among the components of venoms of snakes belonging to the family Viperidae there are substances causing coagulation and having a marked proteolytic activity; these substances may be blocked by heparin [1]. The action of the venom of *V. lebetina* may be blocked by a number of non-specific agents which retard the free radical reactions [4, 5]. These two publications [4, 5] have indicated that either the direct or indirect action of the venom is directed towards nucleic acids in the cells and tissues of the intoxicated animal.

In order to confirm this hypothesis we have studied the nucleic acid contents in organs and tissues of animals subjected to the action of the venom of *V. lebetina*.

TABLE 1. Changes in Nucleic Acid Content in Organs and Tissues of Mice Intoxicated with *V. Lebetina* Venom. (2 MLD)

Organ of tissue	Stat. parameter	Units of estimation of nucleic acids	Nucleic acid content					
			in control	after injection of venom				
				After 1 h	After 2 h	After 3 h	After 4 h	After 5 h
Blood	M	μ g/0.0005 ml of whole blood	6.25	3.33	3.38	3.56	4.85	3.82
	m		0.269	0.291	0.340	0.329	0.429	0.471
	t			7.12	6.57	6.34	2.96	4.48
	P			<0.001	<0.001	<0.001	<0.01	<0.001
Spleen	M	μ g/1 mg of fresh tissue	31.73	27.67	26.81	27.74	28.52	30.36
	m		0.373	1.399	0.979	0.736	1.108	0.391
	t			2.80	4.69	4.84	2.66	2.54
	P			<0.02	<0.001	<0.001	<0.02	<0.05
Liver	M	μ g/1 mg of fresh tissue	17.28	16.23	17.98	16.40	17.97	19.19
	m		0.394	0.933	0.642	0.531	0.590	0.390
	t			1.04	0.83	1.34	1.37	3.44
	P			<0.5	<0.5	<0.2	<0.2	<0.01

MATERIALS AND METHODS

Experiments were conducted on white mice weighing 20-25 g. The dessicated venom, dissolved in neutral saline was injected subcutaneously into mice in one or two minimum lethal doses (MLD). Mice were killed 1-8 h after injection. Nucleic acid concentrations were determined spectrophotometrically according to Spirin [3] in fresh liver and spleen tissues. Two samples were taken from each animal; these were 10 mg fragments of the liver tissue and the splenic pulp. Tissues were homogenized in 10 ml of 0.5 N chloric acid solution, following which the homogenate was hydrolyzed for 20 min in water bath at 100°C. After chilling on ice the homogenate was centrifuged for 15 min at 2500 rpm. The supernatant fluid was examined in a spectrophotometer at wave lengths of 260, 270, and 290 mμ. The total concentration of all the nucleic acids and of free nucleotides was calculated from the optical density.

TABLE 2. Changes in Nucleic Acid Content in Organs and Tissues of Mice Intoxicated with V. Lebetina Venom (1 MLD)

Organ or tissue	Stat. parameter	Units of estimation of nucleic acids	Nucleic acid content after injection of venom							
			After 1 h	After 2 h	After 3 h	After 4 h	After 5 h	After 6 h	After 7 h	After 8 h
Blood	*M*	μ g/0.0005 ml of whole blood	6,25	6,49	3,65	3,24	3,58	4,92	4,03	4,87
	m		0,269	0,912	0,625	0,378	0,334	0,348	0,334	0,310
	t			0,25	3,82	6,49	5,69	3,03	5,16	3,40
	P			>0,5	<0,01	<0,001	<0,001	<0,01	<0,001	<0,01
Spleen	*M*	μ g/1 mg of fresh tissue	31,73	31,09	29,76	28,01	31,36	29,94	25,20	25,59
	m		0,373	1,239	1,241	0,545	1,056	1,821	0,890	0,443
	t			0,50	1,11	5,63	0,33	1,28	6,78	10,62
	P			>0,5	<0,5	<0,001	>0,5	<0,5	<0,001	<0,001
Liver	*M*	μ g/1 mg of fresh tissue	17,28	17,51	17,09	16,57	16,54	17,83	15,81	16,71
	m		0,394	0,450	0,877	0,694	0,534	0,707	0,433	0,925
	t			0,38	0,19	0,89	1,11	0,68	2,52	0,57
	P			>0,5	>0,5	<0,5	<0,5	=0,5	<0,05	>0,5

Nucleic acid concentration in the blood was determined by the same method modified by Simakov [2]. For this, 0.1 ml of whole blood obtained by cardiac puncture, was hemolysed in 1.4 ml of distilled water and added to 13.5 ml of 0.6 N chloric acid solution. Subsequently the method used was that described above. The quantities determined by this method represented the concentration of all nucleic acids and free nucleotides in 0.1 ml of whole blood diluted 150 times.

RESULTS

Forty-nine white mice were used in the experiment which involved two MLD. Six mice were killed after 1 h, nine after 2 and 3 h, six after 4 h and seven after 5 h; 12 control mice were also killed.

In this experiment 98 spleen samples, 91 blood samples and 93 liver samples were studied. The results were treated statistically by means of Student's t test (Table 1).

Sixty-three mice were used in the experiment involving 1 MLD of the venom. Five mice were killed after 2 h, 6 after 3 h, 11 after 4 h, 9 after 5 h, 7 after 6 h, 9 after 7 h and 4 after 8 h; 12 controls were used. 122 spleen samples, 118 blood samples and 123 liver samples were studied. The results are presented in Table 2.

Thus, a total of 645 samples were examined for DNA content in two experiments.

The results obtained illustrate a sharp decrease in nucleic acid content in blood and spleen after the injection of 2 MLD of the venom. This decrease was statistically significant as early as 1 h after injection of the venom and was retained during the second and the third hours of observation. At the fourth and fifth hours the amounts of nucleic

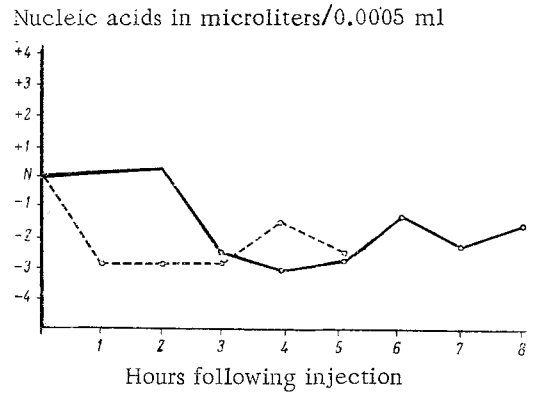

Fig. 1. Changes in nucleic acid content in blood following the intoxication of mice with different doses of V. lebetina venom (deviation from the norm).

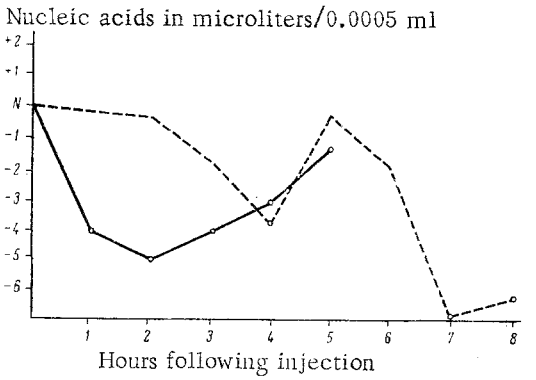

Fig. 2. Changes in nucleic acid content in spleen following the intoxication of mice with different doses of V. lebetina venom (deviation from the norm).

acids became increased to a certain degree (with maxima on the fourth hour for blood and on the fifth hour for spleen). This increase in the nucleic acid level, however, did not reach normal values.

In the liver the nucleic acid content during the first 4 h of observation was not statistically different from that of the controls. However, at the fifth hour there was a statistically significant increase in the nucleic acid level in the liver parenchyma.

When 1 MLD of the venom was injected, the nucleic acid content in the organs and tissues examined was also altered, but in a way different to that in the preceding experiment (Table 2).

In animals which received 1 MLD, the nucleic acid content in blood and spleen began to fall only after 3-4 h following injection.

The statistically significant increase in the acid concentration in spleen at the fifth hour and in blood at the sixth hour was most characteristic of this experiment. This was followed by a second sharp decrease in the nucleic acid level culminating in death. It must be noted that throughout the entire period of observation the nucleic acid content of liver remained within normal limits and only after 7 h there occurred a statistically significant decrease in its content; this was usually followed by death of the animal.

A comparison of the changes in the amounts of nucleic acids in blood and spleen depending on the dose of the venom, as seen in Figs. 1 and 2 which were constructed from data in Tables 1 and 2 had led to a conclusion on the existence of general patterns in the development of these changes. Thus, intoxication with the venom is followed by a decrease in the amount of nucleic acids, followed by a certain increase in their level, followed by a sharp decrease and death of the mice.

It must be added that injection with 2 MLD's led to an intense poisoning and the rapid death of the experimental animals. As a result of this, in the experimental series involving 2 MLD's, we were not able to note the sharply defined second fall in the nucleic acid level in the spleen tissue, such as was seen in experiments with 1 MLD which produced a lower degree of intoxication.

The increase in the nucleic acid content in tissues of mice injected with the venom may be regarded as a defense reaction which ultimately fails because of the large amount of the venom injected. The defense mechanisms fail as a result of this, and the animals die.

The data obtained indicate also that one of the main reasons for the death of animals is the breakdown in nucleic acid metabolism, especially so in the reticulo-endothelial system. This deprives the animal of its ability to protect itself from the venom.

SUMMARY

Spirin's method was used in the study of nucleic acid content in the organs and tissues of mice, intoxicated with the Vipera lebitina venom. A reduction of nucleic acids in the blood and spleen was revealed; the intensity of this drop depended on the dose of the venom administered. The evidence obtained led to a supposition that one of

the chief causes of the animals' death is the disturbance of nucleic acid metabolism, especially in the reticulo-endothelial system.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
