VENOM OF THE MAMUSHI *Agkistrodon halys halys*.

III. FRACTIONATION OF DEAE-SEPHADEX A-50

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The composition and properties of the venoms of Central Asian snakes are being widely studied, but the venom of the mamushi has scarcely been investigated. Nevertheless, in the Central Asian fauna the numerous family of crotalid snakes is represented only by the genus *Agkistrodon*. We have continued a systematic investigation of mamushi venom undertaken in connection with its influence on the blood-clotting reaction [1-3]. In the present paper the composition of the protein-peptide fraction of this venom is characterized from the results of its chromatography on DEAE-Sephadex A-50.

When it was adsorbed on the anion-exchange resin equilibrated with 0.05 M ammonium acetate buffer (pH 8.2), part of the proteins of the venom was not adsorbed but issued from the column in the form of two protein peaks. After the column had been washed with the equilibrating buffer, elution was begun with the same buffer solutions the concentration and pH of which were varied from 0.05 M (pH 8.2) to 0.25 M (pH 5.7). It was possible to desorb a considerable part of the protein components of the venom, which issued from the column in the form of 13 protein peaks. In the last stage of chromatography, the concentration and pH of the buffer solution were changed from 0.25 M (pH 5.7) to 1.0 M (pH 5.2), and this gave another two protein peaks. Thus, we succeeded in separating the whole mamushi venom into 17 protein fractions. The results of their biological testing, which are given in Table 1, indicate that with respect to their influence on the clotting of blood they can all be

TABLE 1. Biological Effects and Enzymatic Activities of the Mamushi Venom and Its Fractions

Venom or fraction (100 μg)	Clot-forming time, sec			Activity, units/mg		Phospholipase A_2 (coagulation time, min)	Fibrinolytic activity (area of lysis, sq. mm) of fibrin platelets	
	directly on the addition of the venom or the fraction	on recalcification	in the presence of thromboplastin	thrombin-like	caseinolytic		heated at 85°C	unheated
Control	—	130 0	36,0	—	—	2,0	—	—
Venom		30 0	15 0	—	92,0	>30,0	110,0	256,0
Fraction 1	10,0	10 0	10 0	13,3	52,6	2,0	—	132,0
2	Inst. clot	Inst. clot	Inst. clot	200,0	—	2,0		—
3	6,0	5 0	5,0	45,4	—	2,0	—	—
4	4,0	3,0	3 0	51,0	—	2 0	—	—
5	4,0	3,0	3 0	52,0	—	2 0	—	—
6	2,0	Inst. clot	Inst. clot	91.0	—	2,0	—	—
7	22 0	20,0	20,0	9,5	—	2,0	—	—
8	30 0	22,0	26.0	5,0	—	2,0	—	—
9	—	110,0	86.0	—	—	2,0	—	360.0
10	—	—	—	—	—	2 0	—	441.0
11	—	—	—	—	—	13 0	—	48,0
12	—	—	—	—	—	>30,0	—	42,0
13	—	—	—	—	—	>30.0	—	34,0
14	—	—	—	—	—	>30 0	—	35.0
15	—	—	—	—	—	11 0	—	35 0
16	—	210,0	78,0	—	65,0	7.0	—	63,0
17	—	135,0	39,0	—	76,4	5 0	156.0	153,0

Note. A minus sign indicates absence of effect (or activity); inst. clot — instantaneous formation of a fibrin clot.

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differentiated as fractions of coagulant and anticoagulant components. The coagulant fractions 1-8 were found to contain caseinolytic and thrombin-like proteases, probably causing a stimulation of clotting, while in the anticoagulant fractions 10-17 and claiming the role of active principle were found "direct" and "indirect" fibrinolysins, phospholipases A_2, and components blocking the effects of thrombin and thrombin-like proteases on fibrinogen (fibrogenin treated with them lost its capacity for forming clots under the influence of thrombin or thrombin-like proteases from the mamushi venom).

The results of electrophoresis and of isoelectric focussing revealed a predominant content of basic components in the fraction of the coagulant group, while the anticoagulant fractions were represented mainly by acidic proteins.

The same results were obtained when the mamushi venom was chromatographed on DEAE-cellulose, but in this case it was possible to detect and collect preparatively a smaller number (13) of protein fractions likewise differentiated with respect to their influence on the clotting of blood and the pI values of the proteins that they contained.

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PURIFICATION AND CHARACTERIZATION OF THE PROTEIN HEMOPOIESIS STIMULATOR FROM THE SPLEEN OF HORSFIELD'S TERRAPIN *Testudo horsfieldi*

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The literature contains information indicating the participation of humoral factors from the spleens of many animals in the process of postradiation recovery [1, 2]. However, there is no single opinion in the nature, properties, and mechanism of the action of this substance, known by its effective influence on hemopoiesis.

In the present work we consider the isolation and physicochemical characterization of the component (or components) of terrapin spleen affecting hemopoiesis and promoting postradiation recovery.

In the first stage of purification, an extract of terrapin spleen was fractionated by gel filtration on Ultragel AcA-34. The gel chromatograms of the spleen extract showed up to seven protein peaks, the best separation of the fraction being achieved by the use as eluent of 0.01 M Tris hydrochloride buffer with pH 7.5. However, for preparative purposes only three fractions were collected. It has been shown previously [3] that up to 26% of the whole weight of the terrapin spleen extract consists of proteins, 1.46% of lipids, and 11.5% of sugars. On gel filtration, the bulk of the proteins (47.3%) in the extract issued in the second fraction which, in accordance with the theory of gel filtration [4], contained components with molecular weights of 60-80 daltons. The same fraction was characterized by the most powerful stimulating activity. The protein hemopoiesis stimulator (PHS) contained in it was subjected to further purification with the aid of ion-exchange chromatography on CM-cellulose. As a result, five protein fractions were obtained of which a powerful biological efficacy was possessed by the last two, 4 and 5, containing the most basic components of the material

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