basic properties (pI 9.5) and contained valine at the N-end.

The possibility of the existence of multiple forms of TP in mamushi venom is shown by the results of the chromatography of the venom and its fraction on anion-exchange resins, revealing a large number of individual fractions with thrombin-like activity, and by the isolation of two individual TPs differing in the values of their isoelectric points. At the same time, in connection with our experiments performed in TP fractions homogeneous with respect to protein, the presence of sugars (8-10%) may show that the heterogeneity of the population of TPs in mamushi venom is explained by the "certain degree of natural polydispersity" that is characteristic of glycoproteins in general [2].

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VENOM OF THE MAMUSHI Agkistrodon halys halys.

V. COMPONENTS WITH AN ANTICOAGULANT ACTION

É. S. Sadykov, N. A. Barabanshchikova,
I. A. Shuvalova, and L. Ya. Yukel'son

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By chromatography on DEAE-Sephadex A-50, the venom of the mamushi Agkistrodon halys halys can be separated into two groups of fractions, of which one contains mainly the basic and the other the acidic protein components of the whole venom. The first group of fractions of the basic components is characterized by a capacity for stimulating the clotting of blood and is defined as the "coagulant fraction" of the venom. Conversely, in the presence of the components of the second group of fractions the clotting time is extended.

In these fractions of the acid components of the venom forming its "anticoagulant fraction," we have detected a phospholipase A_2 and substances lysing heated and unheated fibrin plaques. We have also shown that in the presence of the components of the anticoagulant fraction of the venom thrombin and the thrombin-like proteases isolated from the coagulant fraction from the mamushi venom lose their capacity for forming a fibrin clot in solutions of purified fibrinogen or in plasma.

In the subsequent experiments we attempted to identify and make a preliminary characterization of the active principle (or principles) of the anticoagulant fraction of the mamushi venom. Figure 1 gives the results of the gel filtration and ion-exchange chromatography of the fraction of acid components of the mamushi venom. When various molecular sieves were used (Acrilex, Bio-Gel, Sephadex) it was possible to separate a phospholipase A_2 from a protein anticoagulant with an antithrombin action. The phospholipase A_2 itself, which, with some modification of the purification procedure, can be obtained in the individual state, had a molecular weight of about 20 kD and a pI of 4.7, i.e., it was an acid phospholipase A_2. Like other acid phospholipases A_2 of snake venoms [1], it possessed no anticoagulant action. The same separation of the protein with antithrombin activity and the phospholipase A_2 was achieved with the aid of ion-exchange chromatography on CM-cellulose.

Thus, the phospholipase A_2 that we isolated in the pure form must be eliminated from among the probable anticoagulants of the mamushi venom while, as the active principle ensuring the anticoagulant properties of the venom, attention is attracted by a protein with the antithrombin properties and by proteins present in all the fractions with a direct and

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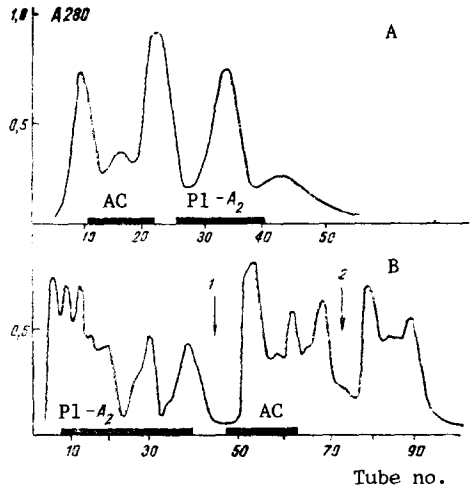

Fig. 1. Graph of the gel filtration (A) and of the chromaography on CM-cellulose (B) of the fraction of the acid components of the mamushi venom. The zones containing activity are hatched: AC – anticoagulant with the properties of an antithrombin; P1-A_2 – phospholipase A_2. A) Column (2.5 × 90 cm) equilibrated with 0.05 M ammonium acetate buffer, pH 6.7; elution with the same solution. B) Column (2 × 40 cm) equilibrated and eluted with 0.05 M amonium acetate buffer, pH 4.7. Elution program: 1) linear gradient, 0.05 M, pH 4.7 → 0.3 M pH 6.8; 2) 0.5 M, pH 8.1.

an indirect fibrinolytic action. Attempts at the further purification of the antithrombin anticoagulant with the aid of chromatography on SP-Sephadex and gel filtration on Sephadex have permitted the separation of a considerable part of the ballast but have not yet been crowned with the isolation of the desired substance in the individual state.

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