DETERMINATION OF THE COMPOSITION OF Vipera lebetina VENOM BY COLUMN CHROMATOGRAPHY

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Fractionation of Vipera lebetina venom on Sephadex G-200 columns yielded fractions with lethal, coagulase, hemolytic, and hyaluronidase activity of this venom. Besides the principal molecular forms, additional fractions with lethal, hyaluronidase, and hemolytic activity also were detected. Fractionation of the venom on DEAE-cellulose columns led to the isolation of the hemolysin of V. lebetina venom, uncontaminated by lethal factor.

Therapeutic antivenom sera are the main method of treatment for poisonous snake bite at the present time. However, the standardization of antivenins is a complicated task. Along with many other factors, the complex antigenic structure of the venom is also responsible for these difficulties. It has been stated [1, 2, 4, 6] that snake venom possesses various types of activity. As yet there is no general agreement about the factor actually associated with the toxic action of the venom, or whether antivenin should be titrated with respect to only one of these factors or to all of them.

In the investigation described below an attempt was made to fractionate the venom of Vipera lebetina by column chromatography and to determine the toxicity of its components.

EXPERIMENTAL METHOD

The dried venom of V. lebetina was used in the experiments. The characteristics of the venom were as follows: LD_{50} 0.018 mg; 1 MCD (minimal coagulating dose of the venom) = 0.01 mg; 1 MHyD (minimal hyaluronidase dose of the venom) = 0.004 mg; 1 MHD (minimal hemolytic dose of the venom) = 0.015 mg.

Gel filtration was carried out on Sephadex G-100 columns measuring 500 × 22 mm and Sephadex G-200 columns measuring 620 × 22 mm. Borate buffer, pH 7.3 (borax 1.28 g, boric acid 7.6 g, sodium chloride 6.18 g, distilled water 1 liter) was used. The material was applied to the column in a dose of 15-30 mg venom in a volume of 5 ml. Samples of 5-ml of filtrate were collected, and the rate of filtration was 18-20 ml/h. Ion-exchange chromatography was carried out on DEAE-cellulose (Serva) columns with a working size of 100 × 22 mm. Proteins adsorbed on the columns were eluted stepwise, first with phosphate buffer (pH 7.3; 0.005 M), and later with the same solution containing sodium chloride in an increasing molar concentration (0.1, 0.2, 0.3, 0.5, and 2 M). The columns were regenerated with 1% NaOH solution. The content of nitrogenous substances in the samples was determined by the Lowry–Folin method. The lethal activity was determined by intravenous injection into albino mice weighing 16-18 g. The hyaluronidase activity of the samples was determined by the MCP (muciba clotting prevention) test. Hyaluronic acid was prepared from umbilical cords. The coagulase activity of the samples was determined with citrated rabbit plasma, and their hemolytic activity with a 5% suspension of horse erythrocytes [3].

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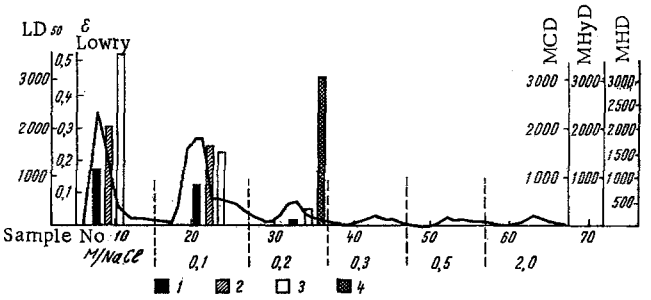

Fig. 1. Fractionation of V. lebetina venom on DEAE-cellulose column measuring 100 × 22 mm. Curve shows extinction with Lowry–Folin reagent. 1) LD_{50} for albino mice in whole fraction (volume 25 ml); 2) number of MCD in whole fraction; 3) number of MHyD in whole fraction; 4) number of MHD in whole fraction. Quantity of venom applied 50 mg (LD_{50} – 2800, MCD – 5500, MHD – 3300, MHyD – 8300). Eluate collected in 5-ml samples.

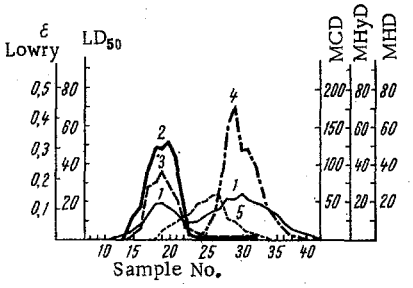

Fig. 2

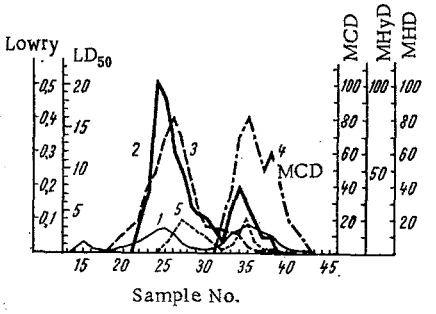

Fig. 3

Fig. 2. Fractionation of V. lebetina venom by filtration through Sephadex G-100 on column measuring 500 × 22 mm: 1) results of analysis of samples by Lowry–Folin method; 2) LD_{50} for albino mice per ml; 3) number of MCD per ml; 4) number of MHD per ml; 5) number of MHyD per ml. Quantity applied 30 mg. Eluate collected in 5-ml samples.

Fig. 3. Fractionation of V. lebetina venom by filtration through Sephadex G-200 gel on column measuring 620 × 22 mm: 1) results of analysis of samples by Lowry–Folin method; 2) LD_{50} for albino mice per ml; 3) number of MCD per ml; 4) number of MHD per ml; 5) number of MHyD per ml.

EXPERIMENTAL RESULTS

By ion-exchange chromatography on DEAE-cellulose it was impossible to separate the lethal factor, the coagulase, and the hyaluronidase (Fig. 1). They had a closely similar electrical charge, they were loosely adsorbed on the anion-exchange resin, and they were extracted either by phosphate buffer without sodium chloride or with 0.1 M sodium chloride. The hemolysin of the viper venom was adsorbed more firmly and extracted only by sodium chloride in a concentration of 0.2 M. After intravenous injection of hemolysin alone into albino mice in a dose of 15-20 MCD (corresponding to the quantity of whole venom containing 20 LD_{50} for albino mice), no visible pathological changes or death of the mice were observed.

More complete fractionation of the toxic factors was obtained by molecular gel-filtration on Sephadex columns, separating substances in accordance with their molecular weight. When V. lebetina venom was fractionated on Sephadex G-100 (Fig. 2), as the graph of the results of Lowry's test show, two protein peaks were found. The first peak was connected with lethal factor and coagulase, the second with hemolysin. Hyaluronidase was connected with both the first and second protein peaks. Fractionation on Sephadex G-200, with greater resolving power, enabled all four factors to be separated (Fig. 3).

Besides the principal molecular forms, additional forms possessing lethal, hyaluronidase, and hemolytic activity were also found. Calculations by Squire's formula [5] gave the following values for their molecular weights: lethal factor in 1st fraction 104,000; lethal factor in 2nd fraction 16,580; coagulase 76,800; hemolysin in 1st fraction 12,800; hemolysin in 2nd fraction 5600; hyaluronidase in 2nd fraction 12,800. The discovery of these two molecular forms of lethal activity, hyaluronidase, and hemolysin, the molecular weights of which are multiples of each other, suggests the possibility of dissociation of these factors into subunits possessing specific activity. The probability that two types of lethal activity, hemolysin, and hyaluronidase may also exist likewise cannot be completely ruled out.

The results indicate the V. lebetina venom contains several components, each of which has its own molecular characteristics, and can be isolated in a relatively pure form.

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