Fractionation of the Black Snake Venom (*Walterinnesia aegyptea*) by Sephadex Gel Filtration

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By the use of a column of Sephadex G-200, it was possible to separate from venom 7 protein components which showed different patterns in electrophoretic mobility. Only 2 of the separated fractions have toxic effects on albino rats.

SNAKE VENOMS are known to contain a number of enzymes. Attempts have been made to correlate the toxicity of snake venoms with their enzymatic activity (1). Most of these studies have been carried out on whole venoms (2) or after elimination of some components by heat treatment (3). A number of methods, such as electrophoresis, use of ion exchange resins such as Amberlite, and cellulose ion exchangers have been used for fractionation. All the methods used were not quite efficient for the complete separation of the different factors in the venom. Yang *et al.* (4) used electrophoresis on potato starch for the separation of the components of Hyopoda venom, and they found that the proteases and phosphatases occurred in the same fractions as the toxic components. Master *et al.* (5) stated that the use of paper and agar gel electrophoresis did not give a sufficiently good separation of the components of Indian cobra and Russel's viper venom.

Mohammed and Zaki (6) studied the properties of the black snake venom, and their chemical studies proved that the venom is a protein mixture. Sephadex gels act as molecular sieves allowing separation of protein mixtures (7). These gels are composed of small granules prepared by the crosslinking of dextran. Sephadex G-200 retains materials of molecular weight up to 200,000. Molecules larger than the pore size pass directly through the gel-packed column, whereas those small enough to enter the grains are retained until replaced by the eluant.

The present study was undertaken to see if any useful separation of the various protein factors in the black snake venom could be obtained by gel filtration.

METHODS

For protein, Sephadex G-200 is the most suitable type since the available capacity is high even for relatively large molecules. Gel filtration was performed by the method of Porath and Flodin (8). A column 7.5 × 41 cm. (volume of 1800 ml.) with Sephadex G-200 was used. Before being packed into the column, Sephadex G-200 was allowed to swell until equilibrium was attained. Forty-eight hours was allowed for swelling. The swelling of Sephadex was in an excess of water containing the buffer. The column was filled to about one-third of its height with the buffer. This consisted of 0.163 *M* NaCl in 0.0004 *M* trihydroxyaminoethane (Tris) previously adjusted to pH 7.4 with HCl. A ball of glass wool was placed at the bottom of the column, and a layer of small glass beads, sufficient to cover the glass wool to a depth of about 1 cm., was added. The suspension of Sephadex G-200 was poured into the column, filling an extension tube of about 20 cm. joined to the top of the column. The gel particles were allowed to sediment until a layer a few centimeters thick had formed. The outlet of the column was then gradually opened, and if the effluent was clear, packing was continued. When packing was finished, the extension tube was removed and the buffer reservoir connected.

The bed, stabilized by washing overnight, was ready for use. In each experiment the column was washed by NaCl–buffer solution (100:20). The venom (10 mg. in 2 ml. of buffered solution) was added to the column by a tip pipet. Before introduction of the sample, all the liquid above the surface of the bed was removed with care. After the sample had been layered on the bed, the bottom outlet was opened. A small volume of the buffer was added in the same manner and was allowed to enter the bed. The column was then filled with the buffer and the buffer reservoir was connected. In each experiment 32 fractions of 2 ml. each were collected. The rate was that each fraction was collected in 15 min. Then 0.4 ml. of each fraction was diluted by 3.6 ml. of buffered solution. The proportion concentration of each fraction was estimated from measure-

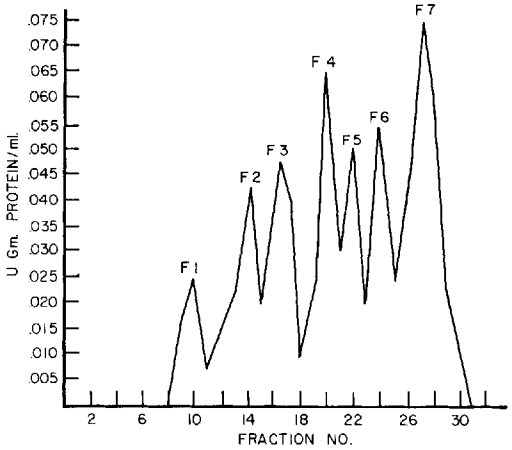

Fig. 1.—The position of the different factors in the black snake venom according to their protein extinction when the venom is fractionated with Sephadex G-200. The extinction of each fraction, of 32 fractions, is plotted on the curve. The summit of each fluctuation in the curve is taken as a definite fraction and these fractions are represented on the curve as F_1, F_2, F_3, F_4, F_5, F_6, and F_7. Eluant, 0.163 *M* NaCl in 0.004 *M* Tris buffer. Fraction size, 2 ml.

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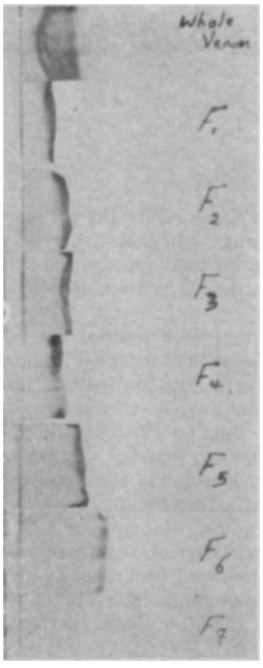

Fig. 2.—The electrophoretic pattern of the Egyptian black snake venom and that of the 7 fractions (F_1, F_2, F_3, F_4, F_5, F_6, and F_7) obtained by fractionation of the venom using a column of Sephadex G-200.

ments of the extinction 280 mμ using a Unicam S.P. 500 spectrophotometer.

Paper strips electrophoresis was carried out on the whole venom as on each of the fractions. A horizontal Shandon tank was used, with constant current and constant voltage. Oxoid 2.5 × 12 cm. strips were used. The volume of venom added to each strip was 0.005 ml. of 1:1000 venom solution. The buffer used in the electrophoresis had pH 8.6 and was of the following composition: sodium acetate, 6.5 Gm.; sodium barbital, 8.87 Gm.; barbital, 1.13 Gm.; in 1 L. of distilled water.

The electrophoretic run was continued for 2 hr. using a current slightly below 0.4 ma./cm. width of the strip. After electrophoresis, the strips were removed with forceps and were dried by suspending them in a hot air over for 20 min. at 100°. The dry strips were left overnight in the staining solution (0.001% nigrosine in 2% aqueous acetic acid). After staining, the strips were washed by running tap water and then dried.

The protein fractions collected from the column were tested for their toxicity. This was done by the subcutaneous injection of 0.5 ml. of the diluted fraction into albino rats weighing about 100 Gm. The fraction which caused the death of the rats within 24 hr. was assumed to contain the toxin. This experiment was repeated several times to confirm the position of the toxic components.

RESULTS AND DISCUSSION

Considerable separation of the protein venom factors was achieved by Sephadex G-200 filtration. Seven fractions showed definite protein extinctions (Fig. 1). By studying the electrophoretic pattern of the whole venom, 6 separate components could be recognized (Fig. 2), indicating that 2 of the gel filtration fractions had the same electrophoretic mobility. The paper strips electrophoresis carried out on each fraction showed that factors 2 and 4 (F_2 and F_4) had the same mobility.

When the toxicity of the fractions was tested, it was found that the fraction corresponding to F_4 and F_6 killed the rats. The death in case of F_6 injection had the same typical symptoms of paralysis as that which occurred when the whole venom was injected (6). This suggested that presence of a neurotoxin in this fraction.

When the animals were injected by F_4, they showed severe itching, excessive salivation, difficult breathing, and then respiratory failure followed by circulatory failure. There were no paralytic symptoms.

The results showed that Sephadex gel filtration may be of considerable value in the separation of the components of the snake venoms.

Probably the most useful application will be in the preparation, physiological study, and the study of enzymes in each factor of the venom separately. Such experiments are being conducted in these laboratories.

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