Protein Components of Loxosceles reclusa Venom

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The availability of certain spider venom preparations through biological supply houses, such as Sigma Chemical Co. (1966), and the recognition that spiders other than *Lathrodectus mactans* create a medical problem indicate that more information is needed concerning spider venom components. A recent report on arachnidism in Texas by Micks (1963) revealed that *Loxosceles reclusa,* the brown or fiddleback spider, is a growing problem for this region. Studies on the characteristics of venom from this spider by Denny and co-workers (1964) showed that it was stable when freeze-dried or in suspension, was inactivated by heating at 56 C for 10 min, and was active as a red blood cell hemolytic agent. These workers also report the appearance of antibodies "to some portion of the crude venom extract . . . by immunodiffusion studies."

From the venom characteristics reported by Denny (1964) and the nature of the cytotoxic reaction, enzymatic activity for the active component of this venom was suspected. Separation of the protein components of *L. reclusa* venom was accomplished by an electrophoretic technique which utilized the polyacrylamide gel disc apparatus. Protein components obtained by this method were checked for biological activity and further studies on the enzymatic activity of these components are in progress.

EXPERIMENTAL PROCEDURES

The development of a technique for recovery of pure spider venom (Grothaus and Howell, 1967) has provided material for examination by electrophoretic separation methods. Since the spider *L. reclusa* yields approximately 10 μg of venom per "milking," 10 spiders were used to provide venom for one electrophoretic separation. Duplicate samples were required as it is necessary to stain one separated sample to mark the position of protein components in the second sample. Twenty spiders were thus required for each experiment. The Conalco polyacrylamide gel disc electrophoresis technique was used with a glycine-tris buffer, pH 8.5. The paper pad used to collect the venom was inserted in the upper end of the electrophoresis tube and gel suspended in buffer was poured around the pad. The samples were subjected to electrophoresis for approximately 35 min at 5 ma per tube. Voltage was from 100 to 250 v depending on the number of tubes used in the apparatus. After electrophoresis, one tube was stored at 4 C while the other was stained and destained to show the position of protein components. The unstained gel was sectioned to separate individual protein components by using the stained gel as a pattern. This system has the sensitivity to separate and detect the protein components in 0.1 mg of pure venom.

Resuspension of the protein was effected with 0.2 ml of normal saline. The gel and saline were mixed in the tip of a conical 15-ml centrifuge tube. This mixture was held at 4 C overnight; then low speed centrifugation gave about 0.1 ml of clear supernatant for bio-assay. Biological activity of the resuspended protein fraction was determined by injection of this

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resuspended protein into an adult male American cockroach. A 5-hour observation time was used as the most representative of toxic effects. The control (c) would represent extraction of a section of the sample gel strip which shows no protein bands.

RESULTS

The number and relative intensity of protein components in *L. reclusa* venom for each sampling date are shown in Figure 1. The fraction number in this figure is used in Table I to designate protein component.

DISCUSSION OF RESULTS

The disc electrophoretic system described under methods has been used to separate the major protein components of pure *L. reclusa* venom. Five to seven bands or protein components were observed using this system with 0.1 mg of venom. Two to three of these protein components were found to have biological activity when saline suspensions were injected into cockroaches. Protein fractions designated 3, 4 and 5 show the greatest biological activity based on the roach assay. Further work in the characterization of these biologically active components as hemolytic agents and as specific enzymes is in progress. Variation in the number of bands and position is being studied with reference to electrophoretic technique and date of venom collection.

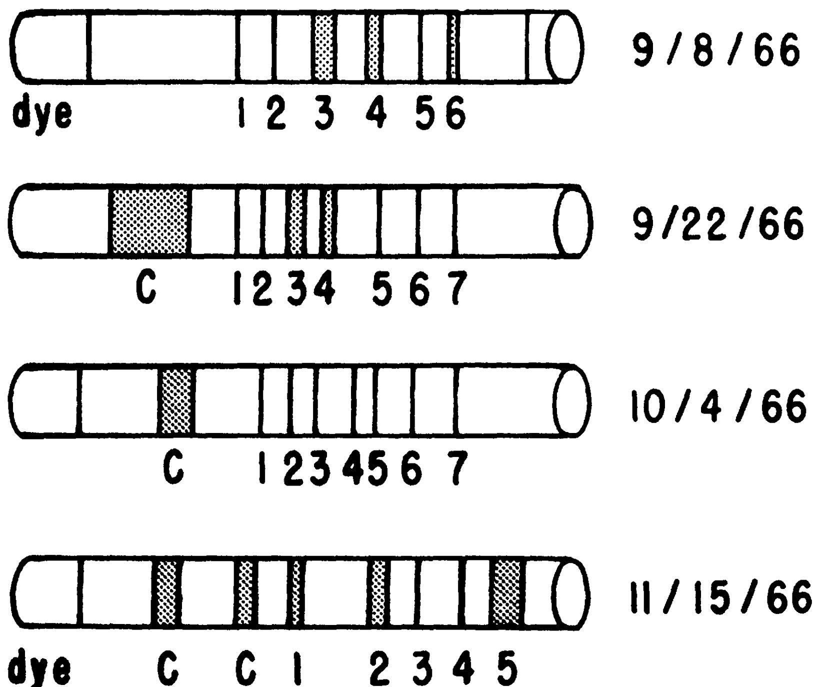

Figure 1. Polyacrylamide gel electrophoretic separation of *L. reclusa* venom (0.10 mg)

TABLE I. TOXICITY* OF PROTEIN COMPONENTS OF *L. reculsa* VENOM

Fraction	Condition of Roach 5 Hours After Administration of Venom — Protein Component 9/8/66	9/22/66	10/4/66	11/15/66
Control	....	No change	No change†	No change
Control	....	No change	No change	No change
1	No change	No change	No change	No change
2	No change	No change	No change	No change
3	Dead	No change	No change	No change
4	Dead	On back	No change	Dead
5	No change	On back	No change	On back
6	No change	No change	No change	....
7	....	No change	No change	....

*Resuspended protein band equivalent to 0.05 mg venom injected into American cockroaches.

†Venom protein concentration very low.

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