An Inhibitor of Complement in the Venom of the Brown Recluse Spider, *Loxosceles reclusa** (34124)

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(Introduced by W. O. Weigle)

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For 57 years cobra venom has been known to inhibit serum complement (C′) activity (1). Recently, a factor has been isolated from cobra venom that specifically inactivates the third component of complement (C′3) *in vitro* (2–4) and *in vivo* (3, 5–7). This factor is nontoxic, having been separated from the toxic substances in cobra venom (8). By depleting blood levels of C′3 the parenterally administered venom factor inhibits a variety of immunologically induced inflammatory lesions. Since studies to characterize the venom of the spider, *Loxosceles reclusa*, were underway in the laboratory of P. N. M., we decided to examine the spider venom for the presence of anticomplementary activity. That a potent inhibitor of C′ hemolytic activity is present in the spider venom is the subject of this report.

After humans are bitten by the brown recluse spider, *Loxosceles reclusa*, there may be a necrotic skin lesion at the site of puncture and a variable systemic reaction (9–13). Upon intradermal injection of venom into animals, there is the progressive appearance of erythema, edema, hemorrhage, and necrosis; ulceration and sloughing of the area may follow (9, 13–15). A remarkable spreading factor permits gravitational spread of the inflammation into contiguous dependent areas of skin. After intravenous administration in dogs, the venom causes marked thrombocytopenia, moderate intravascular hemolysis, and hemolytic anemia (16). In serially propagated cell cultures, there is marked cytotoxity for human epithelial cells (strain HeLa) and L-929 mouse fibroblasts (17).

Materials and Methods. To evaluate the effect of venom on C′, a standard assay for C′ hemolytic activity was devised by modifying a method recently described (18). Each tube contained 5×10^8 sensitized sheep erythrocytes (EA) suspended in 0.5 ml of veronal buffer (VB). To three tubes at 1°C were added 0.1 ml, 0.5 ml, and 1.0 ml of a particular serum previously diluted in VB. VB had been added to two of the tubes to make a final working volume of 1.5 ml in all tubes. After incubation for 1 hr at 37°C, 3.5 ml of saline was added to bring the total volume in all tubes to 5.0 ml. After centrifugation, supernatant fractions were analyzed spectrophotometrically at 541 mμ. Appropriate buffer and 100% lytic controls were employed to permit expression of C′ activity in C'_{H50} units/ml.

Venom from living adult female brown recluse spiders was obtained by electrical stimulation (19). It was collected in microcapillary tubes, quantitated by gravimetric and volumetric methods, and diluted in sterile physiologic saline to concentrations of 30–200 μg protein per ml. From one spider, an average of 0.36 ml venom could be obtained, containing 70 μg protein (15), as determined by the method of Waddell (20).

To study the effect of venom on C′ activity in a particular serum the following test system was used. To a series of acid-cleaned tubes, 0.5 ml of EA was added. Two to fivefold serial dilutions of venom solution in

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VB were added in amounts of 0.5 ml to successive tubes. Finally, 0.5 ml of diluted serum, containing 0.5–2.0 C'_{H50} units of hemolytic activity was added to each tube. Incubation, adjustment of final volume to 5.0 ml, centrifugation, and spectrophotometric readings were performed as described previously. In some cases, a duplicate set of tubes were prepared, employing nonsensitized sheep cells (E) at the concentration and volume employed for EA. In addition, there were buffer controls (0.5 ml of EA or E with 4.5 ml VB), 100% lytic controls (0.5 ml of EA or E with 4.5 ml of 0.1% Na_2CO_3), and C′ controls (0.5 ml EA or E, 0.5 ml of VB, and 0.5 ml of diluted serum).

Results and Discussion. Typical results are illustrated in Fig. 1. All tested concentrations of venom inhibited the lysis of EA when compared to control samples (100% lysis) containing the same reactants, but no venom. Maximal inhibition (95.2%) of one C'_{H50} unit of guinea pig C′ per ml occurred at a venom concentration of 0.033 μg protein per ml. With increasing concentrations of venom there was an increase of nonimmunologic hemolysis (see E curve). If this hemolytic effect were removed, the data suggest that C′ lytic inhibition at higher venom concentrations might be greater. In agreement with Denny *et al.* (16), we found that hemolysis of erythrocytes by concentrated venom is greatly enhanced by the presence of serum. This effect remains after heating serum at

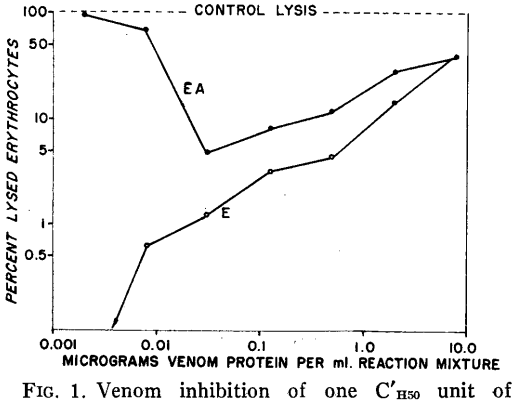

FIG. 1. Venom inhibition of one C'_{H50} unit of guinea pig C′ per milliliter of reaction mixture. EA = sensitized sheep erythrocytes. E = nonsensitized sheep erythrocytes.

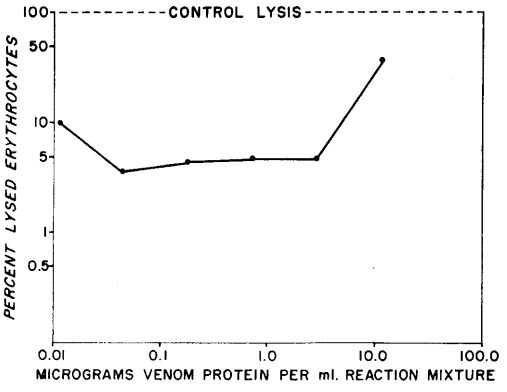

FIG. 2. Venom inhibition of one C'_{H50} unit of human C′ per milliliter of reaction mixture.

56° for 30 min, but is lost upon the addition of EDTA. Enhancement of venom hemolytic activity by serum therefore appears to be dependent upon available free cations, but not upon an intact C′ system.

Marked inhibition of human C′ activity is demonstrated in Fig. 2. Maximal inhibition (over 95%) of hemolysis occurred at venom concentrations of 0.045–0.290 μg venom protein/ml. The sharp increase of hemolysis at the highest venom concentration probably reflects nonimmunologic destruction of erythrocytes.

The results of our studies to date are summarized in Table I. Recluse venom consistently was found to inhibit the hemolytic C′ activity of sera from three species to an equivalent degree. The venom effect was not due to alterations of sheep erythrocytes or amboceptor; EA incubated with venom and subsequently washed were lysed normally in the presence of C′. Venom inhibition of C′ was equivalent in cation-fortified VB and in Tris–HCl and NaCl buffer systems. Additions of large amounts of Ca^{2+} and Mg^{2+} to reaction mixtures did not diminish inhibition of lysis by venom. These latter findings indicate that the venom reaction with C′ is not dependent upon buffer quality or upon chelation of important cations.

The C′ inhibiting activity is nondialyzable and is in a fraction that is excluded from Sephadex G75. It is soluble in distilled water and precipitates in trichloracetic acid. The venom activity is stable in a pH range of

TABLE I. Inhibition of Hemolytic Complement Activity by *L. reclusa* Venom.

C′ source	Number of sera tested	Percentage of maximal inhibition of C′ Range (mean)	Minimal concentration of venom protein for maximal inhibition of C′ (μg/ml)
Human	18	74–99 (90)	0.016–0.261
Rabbit	14	86–97 (92)	0.022–0.144
Guinea pig	8	79–95 (89)	0.033–0.300

4–10 and upon storage at room temperature for at least 2 months. Heating of venom at 56° for 80 min diminished the C′ inhibiting activity only slightly.

Studies are in progress to isolate and characterize further the C′ inhibiting material in spider venom. Preliminary observations, recently presented, suggested that the venom blocked the 3b component of guinea pig C′ and the equivalent fifth component of the human system (21). Investigations are underway to determine whether the venom factor acts directly or requires interaction with serum constituents to exert its antagonistic effect. Because parenteral administration of crude venom causes depression of C′ hemolytic activity *in vivo* in rabbits, it is possible that C′ inhibiting factor, once isolated, may be useful in the modification of various immunopathologic lesions.

Summary. Venom of the brown recluse spider, *Loxosceles reclusa*, contains a potent inhibitor of hemolytic complement activity *in vitro*. Up to 2 C_{H50} units of C′ activity in human, rabbit, and guinea pig sera could be maximally inhibited by 0.016–0.300 μg venom protein per ml. The venom factor is nondialyzable, excluded from Sephadex G75, and is relatively stable over a wide range of pH, heat, and storage conditions.

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