

SHORT COMMUNICATIONS

LOXOSCELES LAETA VENOM—PARTIAL PURIFICATION*

GERARDO SUAREZ,¹ HUGO SCHENONE² and TERESA SOCÍAS

¹Department of Biochemistry and Chemistry and

²Department of Microbiology and Parasitology, University of Chile, Santiago, Chile

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THE BITE of the Chilean spider *Loxosceles laeta* causes lesions in man and higher animals, which may have cutaneous or visceral locations (PIZZI *et al.*, 1957; SCHENONE and PRATS, 1961). To help understand the mechanism of action of the venom we have attempted to isolate the active materials.

For this purpose 100 venom glands, physically removed from female individuals were homogenized and suspended in 1 ml saline. Alternatively, the venom was extruded from the spider over a glass slide under microscopic observation. No appreciable difference in results was seen using either method. The homogenate was clarified by centrifugation at 30,000 g and dialysed at 0–4°C overnight against a large volume of 0.9% (w/v) NaCl. The dialysate thus obtained was active when assayed in the skin of the abdomen of the rabbit where it caused the typical necrotic plaque. The activity of this material was lost upon treatment with pepsin and after precipitation by 5 per cent trichloroacetic acid.

The fractionation of the dialysed material was carried out in a 2.2 × 30 cm G-100 Sephadex column previously equilibrated with 0.9 per cent NaCl at 0–4°C. Effluent fractions (0.5–1.0 ml) were monitored by absorbance at 230 nanometers (nm) and some by their necrotizing activity on the rabbit. Two main areas of elution were obtained, only the second possessing necrotizing activity. To assay this activity a constant amount of O.D. units (e.g. 0.03) was injected i.d. in the rabbit. The homogeneity of the preparation was investigated by its electrophoretic behaviour in polyacrylamide gels. Electrophoresis was performed at pH 7.3 using Tris buffer and 4 mA per tube. The unfractionated extract gave about six bands, whereas the active fraction gave only one distinct band and two faint ones. We conclude that the active materials are proteins which can be obtained in a high degree of purity. Work is in progress attempting to correlate effects of venom to its enzyme activities. We have not detected in the unfractionated venom extract any of the following enzyme activities: phospholipase C, collagenase, protease, phosphodiesterase.

REFERENCES

- PIZZI, T., ZACARÍAS, J. y SCHENONE, F. (1957) Estudio histopatológico experimental en el envenenamiento por *Loxosceles laeta*. *Biológica* 23, 33.
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