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30. SEPARATION OF TOXIC COMPONENTS FROM THE BRAZILIAN SCORPION — *TITYUS SERRULATUS* — VENOM

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Separation of protein components from *T. serrulatus* venom has already been made using mainly electrophoresis techniques (1). In the present paper results will be reported on the separation of toxic components from the venom of *T. serrulatus* by a combination of extraction and chromatographic techniques using dextran gels and the resin carboxymethylcellulose. The procedure used allows the separation of a toxic component in highly purified form. Columns of 29×2.5 cm were packed with the fine grade suspension of Sephadex G-25 in distilled water, following the instructions of Gelotte (2). CM-cellulose was used in 0.6×22 cm columns prepared according to Peterson and Sober (3). Buffers were prepared from analytical grade reagents dissolved in glass distilled water. Scorpion venom obtained by electrical stimulation was supplied by Instituto Butantan and was used in all the experiments. Cellulose acetate paper electrophoresis was run according to Kohn (4). Determinations of the LD_{50} (5) were made by the intraperitoneal route in mice.

Results

Venom obtained by electrical excitation contained insoluble substances that were separated by successive cold water extractions. The water soluble extract was chromatographed on Sephadex G-25 columns using water and 0.1 TRIS buffer or 0.1 M ammonium acetate as eluents. Two main protein peaks P_1 and T_1 (Fig. 1) appeared in the water eluates. Component T_1 contained approximately 30% of the toxic activity evaluated by injection in mice. TRIS buffer eluted two additional components P_2 and T_2 (Fig. 1), the bulk of toxicity being found in peak T_2. Elution with ammonium acetate following water elution (Fig. 2), showed a small inactive protein peak and a large peak where the toxic activity was found. Electrophoresis on cellulose acetate paper revealed that peak T_2 obtained either with TRIS or acetate buffers still contained several components. For further purification elution with ammonium acetate was chosen and

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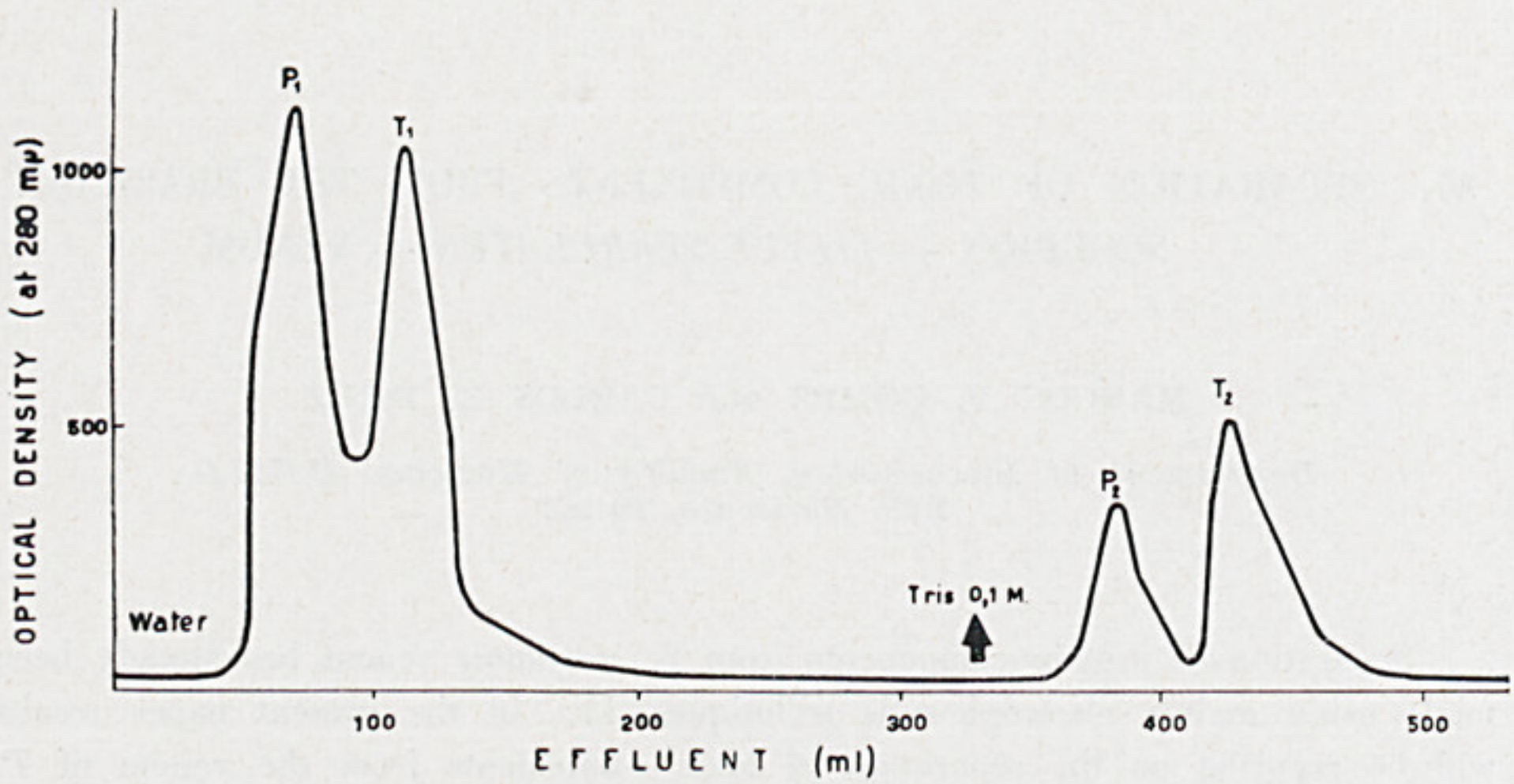

Fig. 1 — Chromatogram of 100 mg of total venom on a column of Sephadex G-25, 29 × 2.5 cm Flow rate 30 ml/h. Stepwise elution with water and Tris 0.1 M.

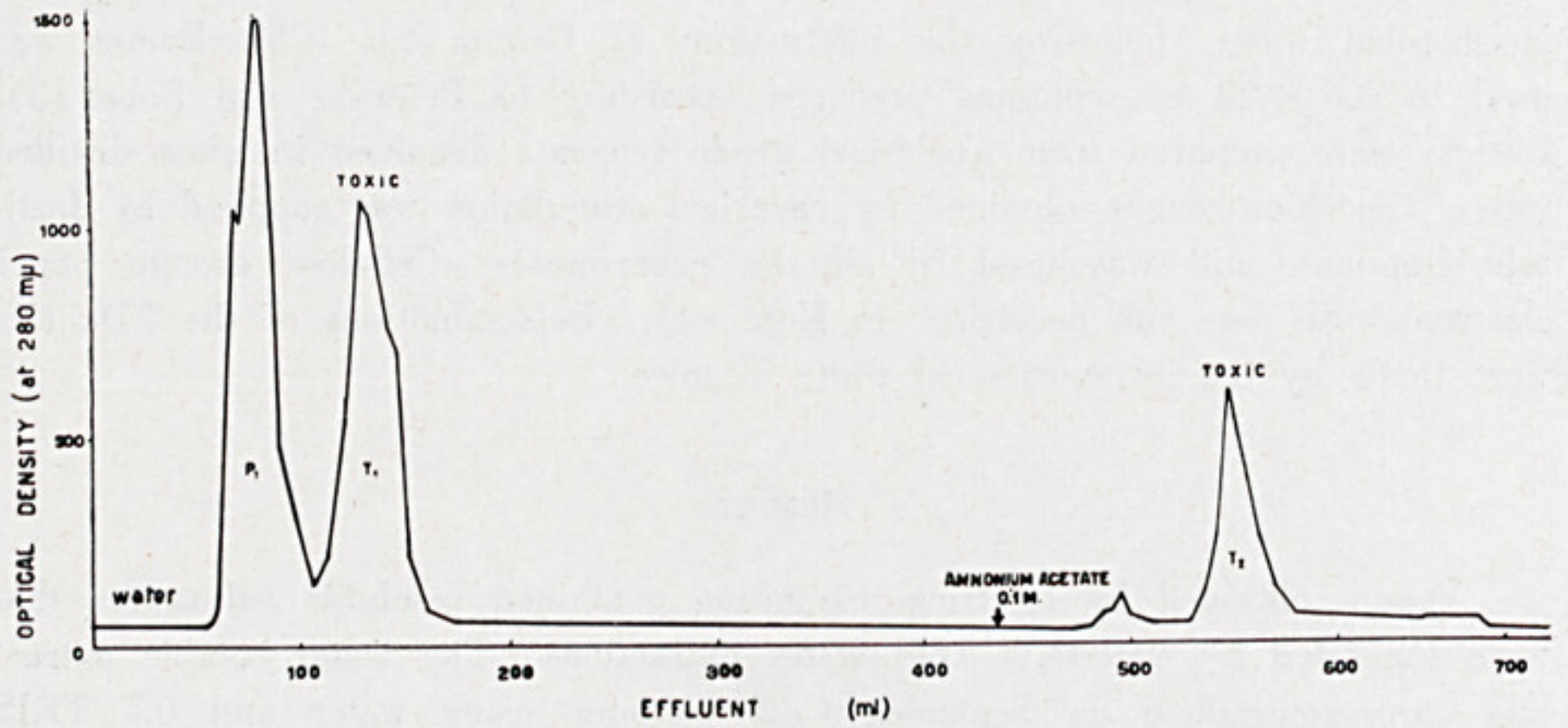

Fig. 2 — Chromatogram of 80 mg of total venom on a column of Sephadex G-25, 37 × 2.5 cm Flow rate 30 ml/h. Stepwise elution with water and ammonium acetate 0.1 M.

the T_2 component was lyophylized and suspended in 0.01 M ammonium acetate pH 7.7. An inactive precipitate formed and was removed by centrifugation. The soluble supernatant was transfered to the CM-cellulose column and eluted with 0.01 M ammonium acetate pH 7.7. Several inactive protein peaks appeared but the toxic material was retained. Following several attempts the elution of the toxic fraction could be achieved by 0.15 M ammonium acetate adjusted at pH 9.0 (Fig. 3).

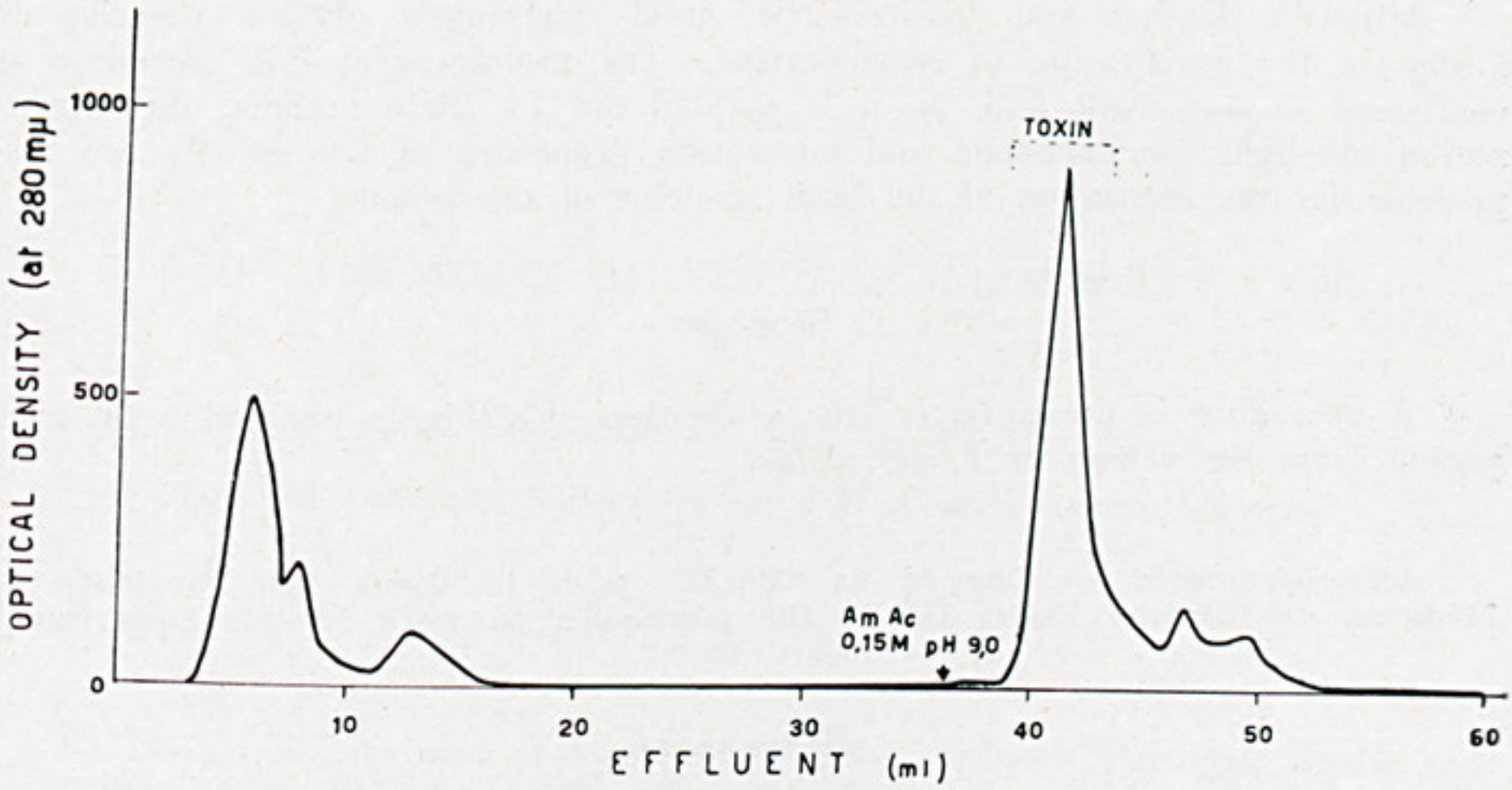

Fig. 3 — CM-cellulose column 0.6 × 22 cm. Chromatogram of 5.5 mg T_2 obtained by gel filtration. Stepwise elution. Starting buffer: Ammonium Acetate 0.01 M, pH 7.7. Flow rate 1.5 ml/h.

The main toxic peak behaved as a single component when submitted to paper electrophoresis or rechromatography. Specific activities and yields of the toxic component in the various steps are shown in Table I.

TABLE I — PURIFICATION OF A TOXIC COMPONENT FROM *T. SERRULATUS* VENOM

Fractions	Protein mg	LD_{50} μg	Toxic units μg	Specific activity	Yield %
1) Total venom	205,0	65	3.150	15,3	100
2) Aqueous extract	136,0	43,4	3.140	23,0	99,5
3) Sephadex G-25	26,9	20,6	1.300	48,5	41,5
4) C.M.-cellulose	7,5	8,6	870	116,2	27,5

Discussion

The procedure now reported to separate the protein components from the venom of *T. serrulatus* may be useful for preparative purposes. The toxic activity, which was assayed on mice by LD_{50} measurements and by observing intoxication symptoms, particularly salivation and lacrimation, was resolved in two components on Sephadex G-25. Further investigations are necessary to interpret this finding as meaning the presence of at least two toxins in the venom. Chromatography on CM-cellulose of the main toxic component from the Sephadex columns, disclosed non toxic components and led to the preparation of a highly purified toxin, which is homogeneous on paper electrophoresis at different pHs.

Miranda, Rochat and Lissitzky (6), used dextrangels of low porosity as a step in the purification of scorpiotoxins. The usefulness of this procedure is confirmed in our work and, as it is pointed out by these authors, the combination of slight ion exchange and adsorption properties of the gel (2) are responsible for the separation of the basic proteins of the venom.

Summary

A procedure is presented for the separation of a highly purified toxic component from the venom of *T. serrulatus*.

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References

1. DINIZ, C. R., and GONÇALVES, J. M., *Biochim. Biophys. Acta*, **41**, 470, 1960.

2. GELOTTE, B., *J. Chromatog.*, **3**, 330, 1960.

3. PETERSON, E. A., and SOBER, H. A., in S. P. COLOWICK, and N. O. KAPLAN (Editors), *Methods of Enzymology*, Vol. 5, Academic Press, Inc., New York, 1962, p. 3.

4. KOHN, J., in I. SMITH (Editor), *Chromatographic and Electrophoretic Techniques*, Vol. 2, William Heinemann Books Ltd., London, 1960, p. 56.

5. BURN, J. H., *Biological Standardization*, Oxford University Press, London, 1937.

6. MIRANDA, F., ROCHAT, H., and LISSITZKY, S., *Toxicon*, **2**, 51, 1964.