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ractionation of cobra venom by electrofocusing

Electrofocusing has been well known for several years[1–3]. The development of his technique has recently been made possible by the availability of ampholine. In ecent months, several substances, which were previously difficult to obtain by clas- ical means of fractionation, have been purified by this new technique. A general eview and several very interesting applications of this technique were recently escribed at the *XVIIth Annual Colloquium on the Protides of the Biological fluids Bruges 1969)* which was partially devoted to this problem[4].

As part of our investigations on Cobra venom components[5,6], it seemed that t would be of interest to study the application of this method to our material. Because of its high resolving power, electrofocusing is usually applied as one of the ast steps in a fractionation process. However, its direct application to a mixture nay provide interesting indications regarding the isoelectric points of the constituents ınd sometimes lead to the isolation of one of these in an almost pure state. This new ractionation method was therefore applied to the venom of a Formosan cobra *(Naja ıaja atra)*. The experiments were carried out in an LKB analytical column.

A density gradient was formed with sucrose solutions. The ampholine carriers consisted of a mixture of low molecular weight aliphatic polyamino- polycarboxylic ıcids (available from LKB Produktor, Stockholm). A 1% concentration in a total volume of 110 ml was used.

Focusing was carried out for 72 h at a temperature of 4°, the voltage being initially adjusted to maintain a maximum input power of 2 W. After 24 h running, the voltage was fixed at 750 V for the next 48 h; the current finally dropping to less than

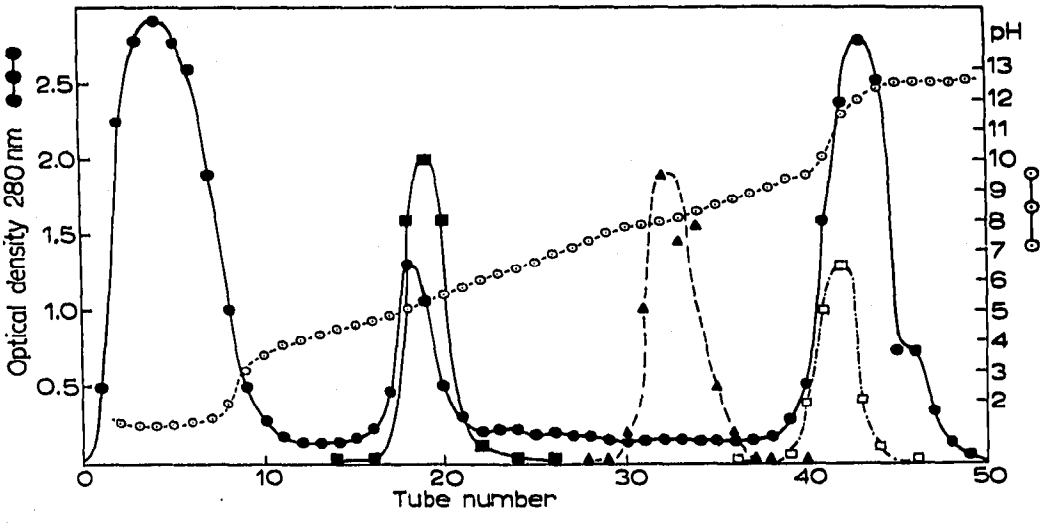

Fig. 1. Electrofocusing of *Naja naja atra* venom; pH range 3–10. ●—●, absorbance at 280 nm; ⊙—⊙, pH; □—□, toxicity[7]. Arbitrary enzymatic activities are plotted as follows: ■—■, phospholipase A (E.C. 3.1.1.4.) detected by estimation of the lecithin degradation products by thin-layer chromatography; ▲—▲, L-amino acid oxidase (E.C. 1.4.3.2.) estimated by the ZELLER[8] manometric method.

1 mA. At the end of the run, the column was emptied and 2 ml fractions were automatically collected. Absorbancies at 280 nm, pH and enzymatic activities were determined on each tube without removing the ampholines and sucrose.

As shown in Figs. 1 and 2, electrofocusing is a very useful technique for the separation of cobra venom components. All the enzymatic activity seemed well preserved after fractionation except that of the hyaluronidase and phosphodiesterase. Nearly all the enzymes were focused at sufficiently different pH's, which permitted their separation by a second run in a narrower pH range.

Electrofocusing, in association with another fractionation procedure, could lead

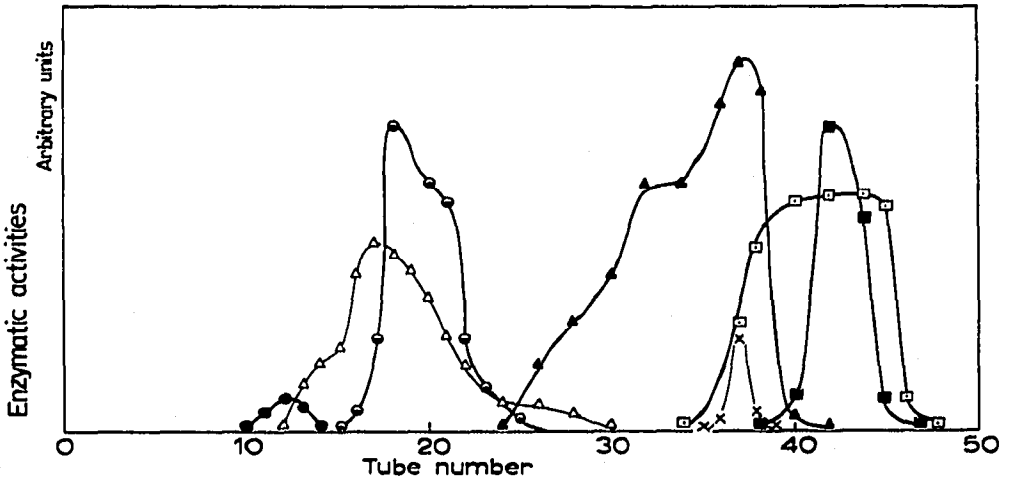

Fig. 2. Electrofocusing of *Naja naja atra* venom. Activities are represented as follows: ●—●, hyaluronidase (E.C. 4.2.99.1.) estimated by the TOLKSDORF turbidimetric method[9]; △—△, phosphomonoesterase, estimated by the LEVINTHAL spectrophotometric method[10]; ◒—◒, cholinesterase (E.C. 3.1.1.8.) determined by the KRAMER colorimetric method[11]; ▲—▲, 5′-nucleotidase (E.C. 3.1.3.5.) tested by the BABKINA method[12]; ×—×, phosphodiesterase (E.C. 3.1.4.1.) determined by the BOMAN spectrophotometric method[13]; ■—■, endonuclease estimated by the SHAPIRA method[14]; ⊡—⊡, inhibitor of anaerobic glycolysis tested manometrically on Ehrlich ascites cells[15].

to an easy means of purification of any one of these enzymes. This new technique is certainly an invaluable analytical method for p*I* determination. With regard to its application to preparative work, two points remain to be elucidated. Firstly, whether the enzymatic and physiological activities are wholly conserved, and secondly, the problem of the removal of sucrose and "ampholine" from small proteins and large peptides. Further investigations are being conducted in this field.

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Note added in proof

Since this manuscript was completed a paper has been published[16] dealing with the fractionation by isoelectric focusing of a crotalidae snake venom *(Agkistrodon rhodostoma)*.

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1 H. SVENSSON, *Acta Chem. Scand.*, 15 (1961) 325.
2 H. SVENSSON, *Acta Chem. Scand.*, 16 (1962) 456.
3 H. SVENSSON, *Arch. Biochem. Biophys., Suppl.*, 1 (1962) 132.
4 H. PEETERS (Editor), in *Protides of Biological Fluids, Proc. 17th Coll. Bruges, 1969*, Elsevier, in press.
5 L. GILLO, *Annales Soc. Roy. Sci. Med. Nat. Bruxelles*, 19 (1966) 121.
6 L. BRISBOIS, N. RABINOVITCH-MAHLER, P. DELORI AND L. GILLO, *J. Chromatog.*, 37 (1968) 463.
7 B. BEHRENS AND G. KARBER, *Arch. Exptl. Pathol. Pharmacol.*, 177 (1935) 378.
8 E. A. ZELLER AND A. MARITZ, *Helv. Chem. Acta*, 27 (1944) 1888.
9 S. TOLKSDORF, *Methods Biochem. Anal.*, 1 (1957) 439.
10 G. A. LEVINTHAL, *Biochim. Biophys. Acta*, 38 (1960) 470.
11 N. KRAMER AND B. M. GAMSON, *Anal. Chem.*, 30 (1958) 251.
12 G. T. BABKINA AND S. K. VASILENKO, *Biokhimiya*, 29 (1964) 230.
13 H. G. BOMAN AND V. KALLETA, *Biochim. Biophys. Acta*, 24 (1957) 619.
14 R. SHAPIRA, *Anal. Biochem.*, 3 (1962) 308.
15 O. WARBURG, in *Metabolism of Tumors*, Smith, New York, 1931.
16 P. M. TOOM, P. Q. SQUIRE AND A. T. TU, *Biochim. Biophys. Acta*, 181 (1969) 339.

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