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Studies on the nerve growth factor (NGF) from snake venom

Gel filtration patterns of crude venoms

A specific nerve growth factor (NGF) was found by LEVI-MONTALCINI AND COHEN[1,2] in snake venom. The remarkable biological effects elicited by the NGF have been thoroughly reviewed in recent articles[3,4]. COHEN[5] devised a procedure for the isolation and purification of the NGF activity from venom, which was identified as a protein molecule with a molecular weight of approximately 20,000. As for many other proteins, the relationships between the biological activity and the molecular properties of NGF have not been clarified. A pre-requisite to a better understanding of the structural–functional relationships of the nerve growth factor is a clear knowledge of its molecular properties. In the original purification procedure[5], 6*M* urea was used in the first step in order to avoid loss of biological activity. Because this treatment might not allow the recognition of NGF at various levels of complexity, milder fractionation procedures were tested. The results of gel filtration experiments are reported in this paper.

Samples of dried venoms from various species of the three families of poisonous snakes (Elapidae, Viperidae and Crotalidae) were used. The Butantan Institute of Brazil kindly supplied the venoms of *Bothrops jararaca* and *Crotalus terrificus*. The following venoms were purchased from Sigma Chemical Co. (St. Louis): *Ancistrodon contortrix lactocinctus*, *Crotalus adamanteus*, *Crotalus atrox*, *Naja Naja*, and *Vipera russelli*.

From 20 to 30 mg of each venom were weighed and dissolved in 0.5 ml of 0.050 *M* Tris–HCl buffer, pH 7.4, containing 0.100 *N* NaCl. After centrifugation for 20 min at 10,000 r.p.m. in a Sorvall centrifuge, the supernatant was applied on a column of Sephadex G-100 medium grade (Pharmacia, Uppsala), 1.0 cm × 115.4 cm column size, and equilibrated with the solvent buffer. The column was calibrated with Blue Dextran (Pharmacia, Uppsala) and purified cytochrome c, chymotrypsin, and bovine serum albumin form Sigma Chemical Co. (St. Louis). All operations were performed in a cold room. Fractions of 1 ml were collected in all experiments, and the optical density at 280 mμ was recorded for each with a Zeiss spectrophotometer. Nerve-growth promoting activity was assayed in each fraction by the tissue-culture method devised by LEVI-MONTALCINI *et al.*[6,7], using sensory ganglia from 8-day chick embryos. Series of five-fold dilutions were tested for each fraction. One Biological Unit was defined as the amount of each fraction necessary to produce a 3+ response *in vitro* (nerve fiber outgrowth).

Fig. 1 shows the chromatographic patterns obtained by gel filtration of the various venoms. As can be seen, the protein profiles were distinct for each venom. Despite the variations of the protein patterns among the venoms, particularly regarding the proportions of the various peaks and the positions of the intermediate peaks, the NGF activity was generally concentrated in an area corresponding to molecular weights from 40,000 to 20,000. NGF activity was, however, found throughout all fractions when tested *in vitro* at very low dilutions. The total recovery of NGF activity was approximately 100% in each experiment. In several venoms, such

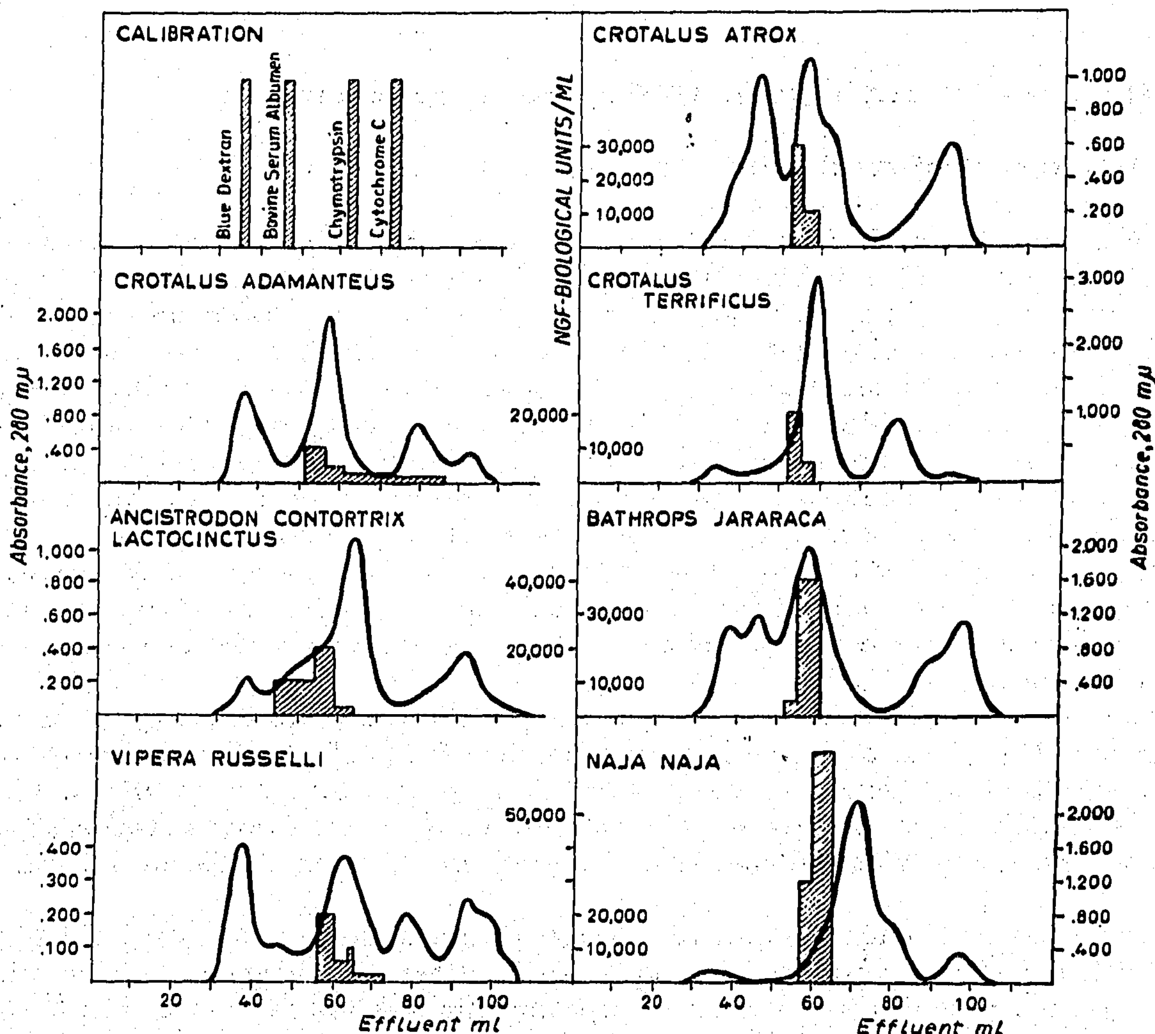

Fig. 1. Gel filtration patterns of various snake venoms on Sephadex G-100. Column size, 1.0 cm × 115.4 cm. The upper left chromatogram shows the elution volumes of some standard proteins. Solid line: Absorbance at 280 mμ. Hatched areas: NGF activity in biological units/ml.

as *Ancistrodon contortrix lactocinctus*, *Crotalus terrificus* and *Crotalus atrox*, the biological activity was found predominantly in the area corresponding to 50,000–40,000 molecular weight; whereas in other venom samples, for example *Naja naja*, the activity was found prevalently in the molecular weight range closer to 20,000–25,000.

It seems, therefore, that in crude venoms the NGF is present in aggregates of various molecular weights, ranging prevalently from 20,000 to 40,000. These forms may be in dynamic equilibrium with each other and may represent the most stable molecular forms of the active molecule. It remains to be seen whether this aggregation results from polymerization of identical subunits, aggregation of different subunits, or interaction of the NGF molecule with unrelated macromolecules present in the venom. The presence of NGF activity, although in small amounts, in other areas of the chromatogram may also be explained by one of these mechanisms. Experiments now in progress with highly purified preparations of NGF from *Crotalus adamanteus* and *Bothrops jararaca* favor the first possibility. Under appropriate conditions, it appears that a 40,000 molecular weight NGF can aggregate into forms of 80,000–

120,000 or higher molecular weight, or dissociate into smaller molecular weight forms. The smallest form so far obtained has a molecular weight of approximately 6,000 and retains full biological activity. Studies now in progress are aimed at the complete clarification of the chemical structure of this unit.

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Use of tetracyanoethylene as a thin-layer chromatographic spray reagent

Methods presently used for the detection of aromatic compounds in thin-layer chromatography (TLC) involve irradiation of the plate with ultraviolet (U.V.) light, the use of spray reagents, or transparency methods[1]. The use of U.V. light for the detection of aromatics is restricted to the availability of U.V. lamps, provided, of course, that the compound of interest is U.V. active. Transparency methods do not distinguish between aromatics and aliphatics. Most spray reagents are specific for functional groups, while the corrosive and universal spray reagents do not distinguish between aromatics and aliphatics.

Tetracyanoethylene (TCNE) has been found to undergo a variety of reactions, including addition, replacement and cyclization reactions[2]. Furthermore, TCNE forms 1:1 π-complexes with a variety of aromatic compounds, particularly hydrocarbons, oxy-substituted hydrocarbons, and other electropositively substituted aromatic hydrocarbons[3]. The formation of these characteristically colored complexes makes TCNE highly useful as a rapid means for the detection and identification of aromatic compounds.

We have investigated TCNE as a possible spray reagent for the detection of aromatics in TLC.