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Separation of proteins of "Elapidae" snake venoms on polyacrylamide gel

The disc electrophoresis technique, first developed for acid proteins by ORNSTEIN AND DAVIS[1] was modified later for basic proteins by REISFELD *et al.*[2]. Slight modifications of the original method have been proposed[3–9]; the further extension and numerous applications of the method are summarized by Canalco[10] and more recently by HANSL[11] and REICH *et al.*[12].

A few publications on the separation of acid proteins from snake venoms have been presented by RUSSELL *et al.*[13,14] but none have been published up to now on the electrophoretic behaviour of "Elapidae" snake venoms in disc electrophoresis.

Materials and methods

Our study was made on eight different Asiatic and African "Elapidae" snake venoms: *Naja naja atra* (kindly supplied by Prof. C. C. YANG, Kaoshiung Medical College, Formosa), *Naja Tripudians*, *Bungarus Caeruleus* (kindly supplied by Prof. P. BOQUET, Garches, France), *Naja Melanoleuca*, *Dendraspis Augustiseps*, *Naja naja* (kindly supplied by the Thaïland Red Cross), *Naja nivea* and *Naja naja Haje*.

All these venoms were obtained in a freeze dried state and were dissolved at concentrations of 5–10 % in an appropriate buffer solution.

All chemicals and reagents were of pure analytical grade and were used without further purification. Acrylamide and N,N'-methylenebisacrylamide were purchased from Fluka A. G. β-Alanine, glycine and TEMED* were supplied by Serva (Heidelberg).

A first run was made according to the method of ORNSTEIN AND DAVIS[1] in order to encapture the fractions moving toward the positive pole.

Operating conditions. Acrylamide gel: 7.5 % (ratio acrylamide:Bis, 1:37); 20 V/cm of tube; time of migration: 150 min.

A second run following the method of REISFELD[2] was made for the basic proteins.

Operating conditions. Acrylamide gel: 15% (ratio acrylamide:Bis, 1:150); 20 V/cm of tube; time of migration: 150 min.

Both experiments followed the original methods with some slight modifications: small pore gel and spacer gel were prepared as usual; large pore gel preparation was omitted and the sample was placed at the top of the spacer gel in a 5% sucrose solution.

Results and discussion

Table I shows under A the number of fractions obtained by the above mentioned method and under B the number of fractions obtained by agar gel electrophoresis using a phosphate buffer pH 7.0 (ionic strength = 0.1).

Fig. 1 represents the separations obtained from the eight snake venoms. Proteins were colored with Amidoblack 10 B. Runs for basic and acid proteins are presented on the same drawing. The numbering of the tubes corresponds to that of Table I.

Fig. 2 shows the densitometric scanning of the fractions of *Naja naja atra* venom (see Fig. 1 tube No. 1). Scanning was made on a Vitatron densitometer using a slot especially adapted for polyacrylamide gel scanning purposes.

* TEMED = N,N,N',N'-Tetramethylethylenediamine.

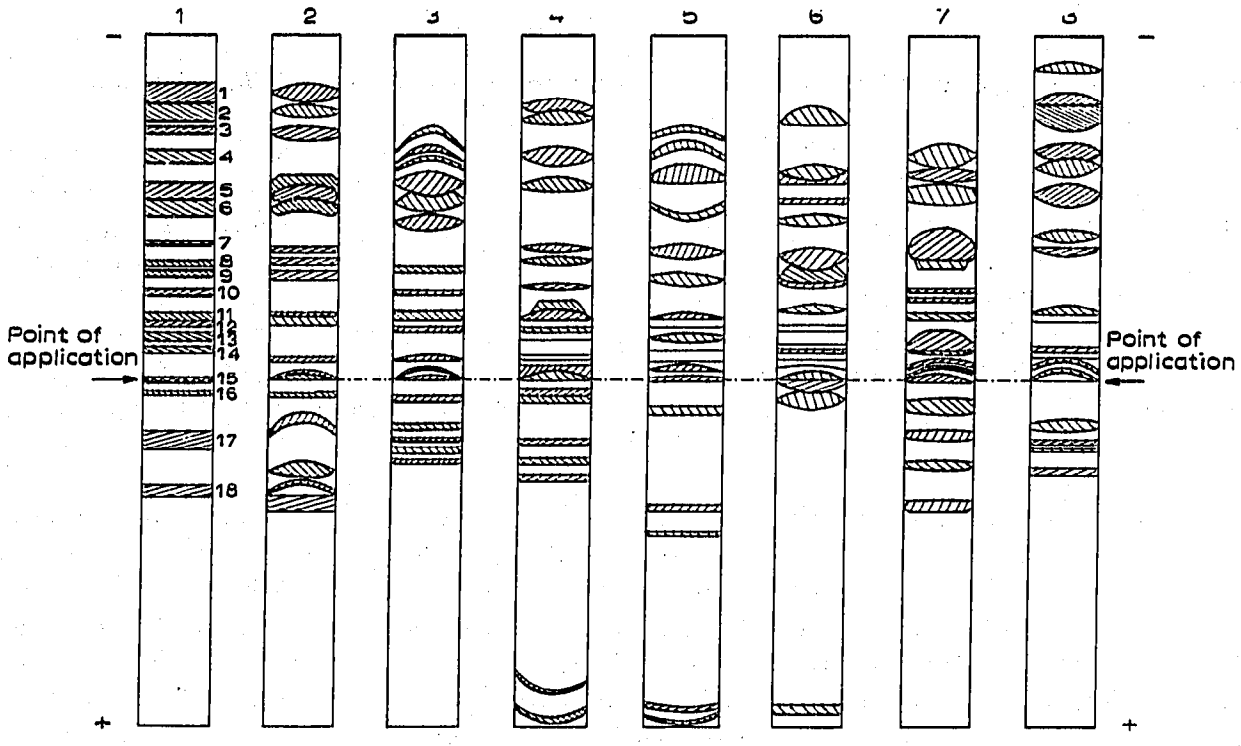

Fig. 1. Electrophoretic separations of eight "Elapidae" venoms.

TABLE I

No.	*Kind of venom*	*Number of fraction*	
		A	*B*
1	*Naja naja atra*	18	7
2	*Naja naja* (Thaïland)	19	4
3	*Naja nivea*	18	5
4	*Naja melanoleuca*	23	6
5	*Naja naja haje*	19	3
6	*Bungarus Caeruleus*	19	6
7	*Naja Tripudians*	17	5
8	*Dendraspis Augustiseps*	18	4

Disc electrophoresis is a very adequate separation tool for proteins in general and has many advantages over the other techniques commonly used in zone electrophoresis. As can be seen from Table I, the number of fractions obtained is great; moreover the reproducibility of the method is fairly good, resolution is excellent and the separation is accomplished in quite a short time.

Refrigeration during the separation was not necessary, the diffusion of the different fractions being small. The interpretation of the identity of the different fractions obtained and the measure of their enzymic activity are now being studied in our laboratory.

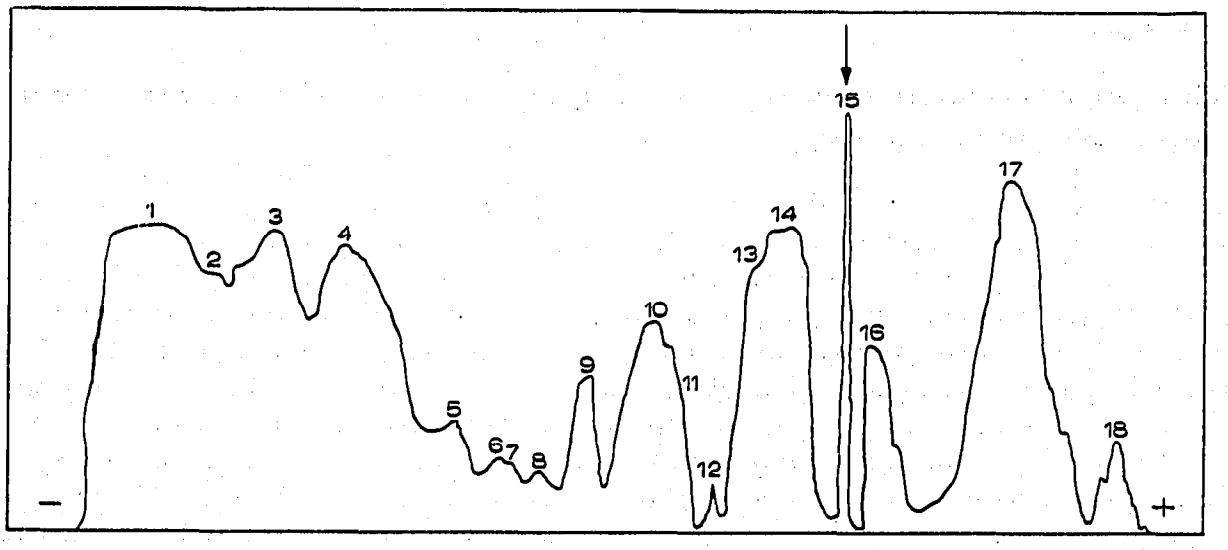

Fig. 2. Densitometric scanning of *Naja naja atra* venom (Vitatron densitometer). The point of application is indicated by the arrow.

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