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Comparative studies on Egyptian elapid venoms[1])

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With 3 figures and 2 tables

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Among a great number of venomous snakes, the members of the family Elapidae are distributed all over the world except in Europe. Its main genus Naja is also widely distributed, it ranges from Japan, Formosa, China, South East Asia, to India, Central Asia, Siberia and Iran. In Africa, it is found in the North, Central and East parts of the continent. It has been shown by a number of investigators that the chemical properties of venoms can be used as an index for taxonomy and phylogenetic relationship (1–3).

It is therefore of interest to investigate and compare the lethal, biochemical and immunological properties of morphologically similar snakes (Egyptian elapids) yet geographically separated. Also degree of neutralization of anti Naja haje of the Delta type (Nh) against all types of Egyptian elapids, Naja haje of the Western Desert (Nm), Naja haje of the Nile Delta (Nh), Naja nigricollis of Upper Egypt (Ng) and Walterinnesia aegyptia (W) from Sinai Desert.

Material and methods

Snakes were collected from their native localities and captured in our Agouza Serpentarium. Snakes of the same species were milked and venoms separately dried at 25 °C under reduced pressure. Univalent anti Naja haje (ACS) is raised in horses as a routine in our Institute.

Lethality of venoms was determined by intravenous injection of albino Swiss mice (16–18 mg) using 5–6 serial doses each injected into six animals. Results are recorded after 24 hrs and the LD_{50} expressed as μg/mouse were calculated according to methods of *Dragstedt* and *Lang* (6) and *Behrens* (1).

Neutralization tests were performed by intravenous injection of mixing different doses of venom and 0.25 ml of antivenin. Control tests with pure venom were carried out. Four albino Swiss mice (weighing 16–18 g) were used at each level dose, and number of survivors at 24 hrs was observed.

A 5 % venom solution in saline was used in the following tests:

Cellulose acetate electrophoresis was carried out by a *Beckman* microzone model R-101 at a current of 3.5–5.8 A and a voltage of 250 for 20 min. Results were registered by a *Beckman* analytrol Model R-110.

Antigen-antibody studies were performed by *Ouchterlony* (16) immunodiffusion technique. Immunoelectrophoresis was carried out as modified by *Schiedegger* (17) at 130 V for 2.5 hrs.

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Results and discussion

Table 1 shows the LD_{50} of the 4 venoms under investigation. It is clear that the most potent venom is the Delta Naja haje. Also its antiserum is most effective towards its homologous venom (1 ml neutralizes 631 LD_{50}) and least effective towards Naja nigricollis venom.

Table 1. Effectiveness of a univalent anti-naja-haje serum (Delta Nh) in neutralization of Egyptian elapid venoms.

Venom	LD_{50} µg/mouse	Neutralization capacity of 1 ml of univalent antiserum	
		mg venom	LD_{50}
Naja haje (Delta) (Nh)	1.9–2.00	1.2	600
Naja haje (Western desert) juvenile (Nmj)	40.0	—	—
Naja haje (Western desert) Mature (Nm)	8.0	0.50	62
Naja nigricollis (Ng)	6.0	0.25	40
Walterinnesia aegyptia (W)	13.0	1.10	85

Cellulose acetate electrophoresis results (table 2, figs. 1 and 2) showed the different localized protein components in the tested venoms. Naja haje (Nh) showed 7–8 protein fractions, 6 migrating to the cathode and 1–2 fractions towards the anode. The most concentrated fraction was the most cationic component, and it amounted to 38.2% of the whole venom.

The Western Desert Naja haje (Nm) showed similar migrating fractions, but with more protein concentration in cationic fractions 2 and 3 (59.2 %), while in contrast to (Nh) the most cationic fraction is the least concentrated (14.07 %).

Table 2. Cellulose acetate fractions in elapid venoms.

Venom 5% solution	Dry venom total protein mean± S. D. gm %	% of protein in electrophoretic fractions								
		A_2	A_1	O	C_1	C_2	C_3	C_4	C_5	C_6
N.mj	80.2 + 3.6	5.5	8.00		10.0	20.0	46.0	3.5		7.00
N.m	88.5 + 5.9	2.5	6.00		12.8	17.06	42.26	5.31		14.07
N.h	92.0 + 4.42	3.6	5.7		11.4	20.5	4.9	12.3	3.27	38.20
N.g	96.0 + 2.14	6.6	8.8	7.0	5.6	9.5	13.5	46.5	2.50	
W	90.0 + 2.75	33.57	23.92	13.07		12.86		6.72	9.86	

A: anodic
O: non-movable
C: cathodic
Nmj: Naja haje Western desert (juvenile)
Nm: Naja haje Western desert (mature)
Nh: Naja haje (Delta)
Ng: Naja nigricollis
W: Walterinnesia aegyptia

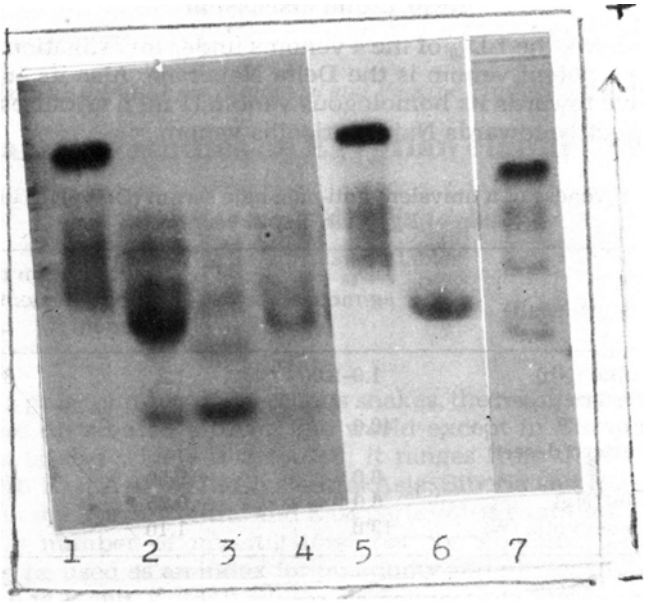

Fig. 1. Cellulose-acetate electrophoresis of Normal serum (Ns), (2) Naja haje Western Desert juvenile (Nmj), (3) Naja haje (Nh), (4) Naja haje Western Desert (Nm), (5) Normal serum (Ns), (6) Naja nigricollis (Ng), (7) Walternesia aegyptia (W).

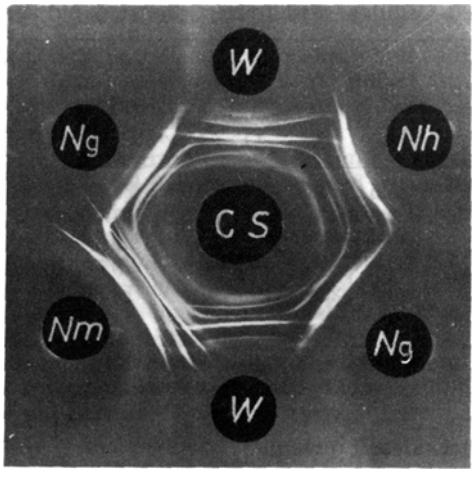

Fig. 2. Immunodiffusion of venoms; Naja haje Delta (Nh), Naja nigricollis (Ng), Walternnesia aegyptia (W), and Naja haje of Western Desert (Nm), against anti Naja haje serum (ACS) in central well.

Of interest in the juvenile Western Desert cobra (Nmj), the concentration of fractions 3 and 4 are maximal, and they grow less on maturation of the snake. On the other hand, W. aegyptia showed 7 protein fractions, 2 towards the anode, the fastest of which is the most concentrated fraction in the venom (33.57 %), the other 5 fractions were all cathodic. The N. nigricollis showed less migrating cathodic fractions, 3–4 only; in addition to 2–3 more concentrated anodic fractions. It lacked the fast cathodic fraction of the Delta cobra.

Immunodiffusion results of different elapid venoms against the antiserum Naja haje showed 4–5 and 6–7 precipitin bands being shared between Naja haje (Nh) and the venoms of W. aegyptia and N. nigricollis, respectively.

The two mature Naja haje venoms of Delta and Western Desert (Nm) showed almost similar, but not identical patterns. They differed again in the concentration of some of the shared precipitin lines (fig. 2).

Moreover, patterns of antigen-antibody reaction were further resolved by immunoelectrophoresis, thus confirming both cellulose acetate and immunodiffusion results in adult and juvenile cobras (fig. 3, a & b).

Electrophoretic studies revealed the individual protein levels in Egyptian elapids. Almost all venom components migrated to the cathode as expected for true elapids (*Grassman* and *Hanning* (7)). However, remarkable differences in migration or concentration of some protein fractions were observed even within the same species (Naja) as reported by *Tu* and *Ganthavorn* (19), *Bertke* et al. (2), working on Asian cobras. Also the electrophoretic analysis helped in identifying the most cationic neuro-

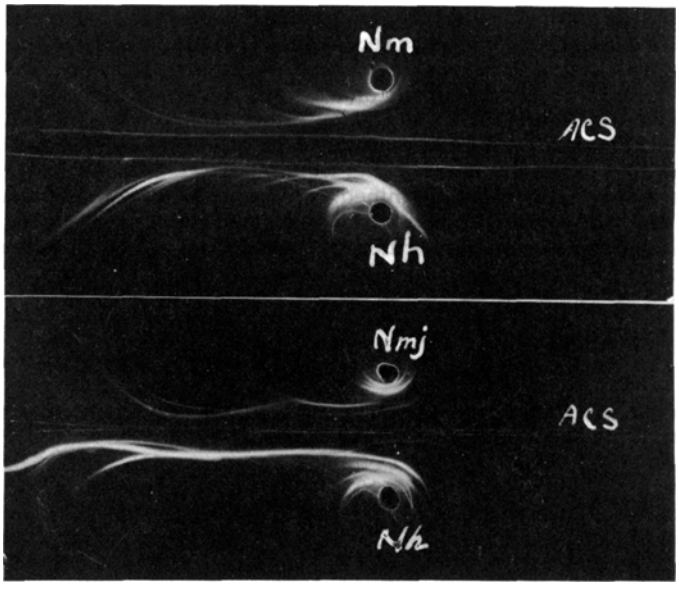

Fig. 3. Immunoelectrophoresis of adult Naja haje Delta (Nh), Naja haje Western Desert and Naja haje juvenile of Western Desert (Nmj) against specific serum of anti N.h.

toxic fraction (C_6) in the Egyptian Delta cobra (Nh), reported by lots of workers (3, 5, 11–14). In addition, the electrophoretic results confirmed roughly the test of magnitude of lethality of the Naja haje venoms as the latter could be proportional to the concentration of (C_6) fraction. This was noticed in the juvenile samples of the Western Desert cobra where (C_6) fraction was negligible and accordingly its toxicity was minimal.

The neutralization capacity of the anti Naja haje (ACS) serum showed its effectiveness towards its homologous venom as well as the venom of W. aegyptia and its moderate effect towards Western Desert Naja haje (Nm) venom. It was least effective towards Naja nigricollis venom. This is parallel to a similar finding reported by *Mohamed* et al. (15) and may be due to different lethal mechanism included in Naja nigricollis venom as suggested by *Bouquet* et al. (4).

Although immunodiffusion and immunoelectrophoresis techniques showed great similarities between the antigenic structure of the Egyptian elapid venoms, yet neutralization test could not always correlate with such data as reported by *Keegan* et al. (10), *Hassan* and *El Hawary* (8). This study may lead to the necessity of preparing regional types of antisera as reported by *Jimenez Porras* (9). However, these results support the idea that biochemical differences due to geographical distribution might change the potency or mechanism of action of some venoms of the same species.

Summary

The immunological properties of *Naja haje* from Western Desert, *Naja haje* of the Nile Delta, *Naja nigricollis* from Upper Egypt and Walternnesia aegyptia from Sinai Desert were compared using horse serum antivenin prepared from the Delta *Naja haje venom*. All elapid venoms showed very similar precipitin lines with immunodiffusion or immunoelectrophoresis on agar gel. Results of cellulose-acetate electrophoresis showed either different concentration of certain similar protein components or the absence of some major protein fractions. However, different migration and localization of protein components were observed. LD_{50} of the 4 elapids and their degree of lethality was determined. Naja haje (Delta) antivenin had different degree of neutralization capacity on the investigated elapid venoms. No correlation between immunodiffusion similarities and the degree of neutralization could be deducted.

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