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Biochemical properties of Egyptian Cerastes venoms in relation to geographic distribution[1])

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With 4 figures and 2 tables

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A recent trend in the field of venomology is the use of venom properties as tools for taxonomic and phylogenetic studies. Besides their taxonomic implication, geographic differences might lead to medicobiochemical importance.

Many workers emphasized the remarkable effect of environmental changes due to geographical distribution on some species of venoms. The WHO committee on standardization (13) has pointed out the importance of reporting the specificity of antivenins produced against their appropriate zoogeographic venoms. Thus antisera for rattle snakes of Northern Brazil were found to be ineffective towards those of Southern areas (4, 5). Also Asian cobras from different parts of Asia were studied against specific antiserum of Thailand cobras, and it was found to be incompatible (11).

It is well established that most of the venom constituents responsible for toxic and enzymatic activity are proteinic in nature. It has also been demonstrated that proteins constitute 90–95% of dry elapid venoms (3) and 80–85% of viper venoms (6), respectively. Hence one should expect that variations in protein content or its distribution would contribute to a great deal to venoms' biological specificity, involving both their enzymatic activity and toxic power.

The present investigation deals with a study of protein changes and its consequences related to toxicity and its phospholipase activity due to geographical distribution in five members of Cerastes species living along the Nile Valley.

Material and methods

5 Cerastes vipers are dealt with in this study, namely:

- Cerastes cerastes (Cc_1[2]) captured near Cairo (Pyramid area)
- Cerastes cerastes (Cc_2[2]) captured at the New Valley (800 km south-west Cairo and located in the West. desert)
- Cerastes cerastes (Cc_3[2]) captured at Abu Simbil (1000 km south Cairo)

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[2]) These abbreviations are used through the whole text.

- Cerastes vipera (Cv_1)[2]) captured near Cairo (Pyramid area).
- Cerastes vipera (Cv_3)[2]) captured at Abu Simbil (1000 km south Cairo).

Vipers from different localities were captured and kept in the snake farm at Agouza Institute, Cairo. They were milked as soon as they were introduced to the farm. Venoms are desiccated under vacuum at room temperature, and 5% saline solutions were prepared for experimental investigations. Assay of lethal toxicity:

0.5 ml of crude venom of different dilutions are intravenously injected into 4 albino Swiss mice (16–18 g) at each level dose. LD_{50} was calculated according to *Dragsted* and *Lang's* formula (2).

Protein content

Protein content was estimated according to *Lowry* et al. method (8), and data were verified using micro-Kjeldahl method as described by *Wootton* (14).

Electrophoresis

Cellulose acetate

(A Beckman microzone cell Mod. R.–101 was used). Electrophoresis was run using veronal buffer of pH 8.6 and ionic strength 0.05 and applying 250 volts for 20 minutes. Acrylamide gel electrophoresis:

Cathodic and anodic migrations of the different proteinic fractions of the 5 Cerastes venoms were run on 15% acrylamide gel using pH 8.3 and 4.5 buffers respectively according to the method described by *Maurer* (10).

The gel systems used were

15% small pore gel at pH 8.3 (Buffer Tris/glycine pH 8.3)
15% small pore gel at pH 4.5 (Buffer B-alanine acetic acid pH 4.5)
100 and 200 µg of each venom were used, and electrophoresis was run for 3 to 5 hours in acidic or alkaline media, respectively.

The location and pattern of separated venom fractions were compared with those of human serum.

Phospholipase determination

Phospholipase activity was carried out on the concerned venoms according to the method described by *Marinetti* (9). It is a rapid assay of the enzyme that depends on the clearing of a suspension of egg yolk. This clearing is due to phospholipase A_2 of the venom acting on the lipoproteins to produce lysolecithin, which further is capable of solubilizing the egg-yolk suspension under controlled pH, temperature, and ionic strength. Different concentration of venoms ranging between 2 and 20 µg were used.

Stock egg yolk solution: One egg yolk is completed to 100 ml with physiological saline, kept at + 4 °C as concentrated substrate.

Working egg-yolk solution: 0.25 ml of the concentrated suspension is completed to about 6–10 ml with physiological saline so that the absorbance of the solution would read 0.6 at 925 µm.

Procedure: 0.2 ml containing 2–20 µg venom was added to equal aliquots (5.8 ml) of working egg-yolk solution. Temperature adjusted to 37 °C; and its absorbance at 925 µm is read at once.

The decrease in absorbance of the suspension was followed at 5 minutes-intervals for half an hour. Δ absorbance will be proportional to the activity of the enzyme.

Results and discussion

Data for venom lethality (table 1) show that the venom of Cerastes snakes living in Abu Simbil (Upper Egypt), where day temperature around the year varies between 35 and 52 °C, are the most toxic if com-

Table 1. Lethal Toxicity of Different Species of Cerastes Venoms and their Neutralization Power Against Specific Bivalent Antiserum of Cerastes cerastes and Cerastes vipera of Cairo.

Venom species	Locality	LD_{50} µg/mouse	Mg of venom neutralized by 1 ml of antiserum	No. of LD_{50} neutralized by 1 ml antiserum
C. cerastes Cc_1	Pyramid desert	8-9	5.2	611
C. cerastes Cc_2	New Valley	6-7	4.8	738
C. cerastes Cc_3	Abu Simbil	5-6	4.6	836
C. vipera Cv_1	Pyramid	12-13	4.0	320
C. vipera Cv_3	Abu Simbil	9-10	3.6	379

pared of their corresponding species of Cairo. Also the toxicity of Cerastes snakes of the New Valley, where the temperature during the day varies between 35 and 48 °C all over the year, is still more than that of Cairo. The lethality of the studied venoms could be given in the following sequence $Cc_3 > Cc_2 > Cc_1 >$ for the 3 Cerastes-cerastes snakes and $Cv_3 > Cv_1$ for the two Cerastes-vipera snakes.

The protein content (table 2) of the dry venoms of the 5 studied snakes showed the sequence $Cc_3 > Cc_2 > Cc_1 >$ and $Cv_3 > Cv_1$. It seems that the environmental high temperature exerts an influence on the moisture content of the venoms; a view that receives support from the finding that the moisture content of the fresh venoms showed a sequence opposite to that of toxicity.

Cellulose-acetate electrophoresis of the 3 Cerastes-cerastes venoms revealed great qualitative similarity in the protein pattern regarding the number of the fractions and their mobility. On the contrary, the venoms of the two Cerastes vipers showed different protein-electrophoretic patterns; that of Abu Simbil (Cv_3) revealed more negatively-charged fractions moving towards the anode than that of Cairo (Cv_1) (fig. 1).

Table 2. Total protein and soluble nitrogen in g/100 ml in all cerastes species.

Viper	Tot. N_2	prot. N_2	Sol. N_2	Total protein		% Moisture content
				micro-Kjeldahl	Folin	
Cerastes cerastes (dry) Cc_1	16.50	13.90	2.6	87.50	86.00	
Cerastes cerastes (fresh) Cc_1	4.00	3.30	0.70	20.60	20.47	76
Cerastes cerastes (dry) Cc_2	17.23	14.43	2.80	90.20	91.20	
Cerastes cerastes (fresh) Cc_2	4.43	3.70	0.73	23.10	23.52	73.5
Cerastes cerastes (dry) Cc_3	17.36	14.62	0.74	91.40	92.60	
Cerastes cerastes (fresh) Cc_3	4.52	3.83	0.69	23.93	24.44	73
Cerastes vipera (dry) Cv_1	15.10	13.10	2.00	82.00	84.00	
Cerastes vipera (fresh) Cv_1	3.80	3.30	0.50	20.60	21.30	75
Cerastes vipera (dry) Cv_3	17.40	14.81	2.59	92.60	94.00	
Cerastes vipera (fresh) Cv_3	4.63	3.95	0.68	24.68	25.29	72.3

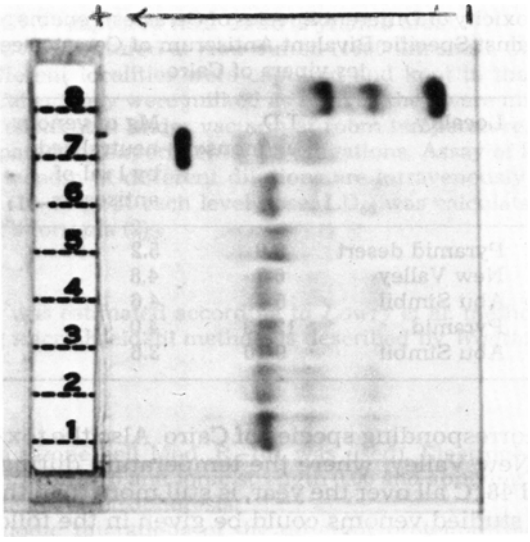

Fig. 1 shows cellulose acetate electrophoresis of (1) Cv_3, (2) Cc_3, (3) Cc_1, (4) Cc_2, (5) Cv_1, (6) Cv_3 (7) normal serum, and (8) cobra venom.

Acrylamide electrophoresis demonstrated significant pronounced differences between the pure venoms. At acidic buffer of pH 4.5 (fig. 2), proteins migrating towards the cathode showed almost similar patterns in the 5 Cerastes venoms; except for a dilution or concentration of some of the fast-migrating components. At alkaline pH 8.3 (fig. 3) remarkable

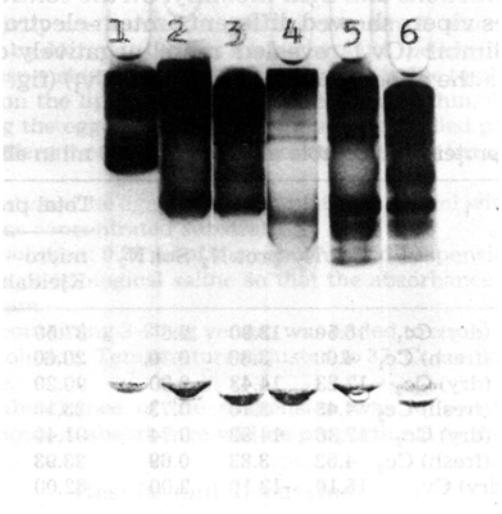

Fig. 2 shows acrylamide gel electrophoresis run at pH 4.5 for (1) human serum, (2) Cc_1, (3) Cv_1, (4) Cc_2, (5) Cc_3, and (6) Cv_3.

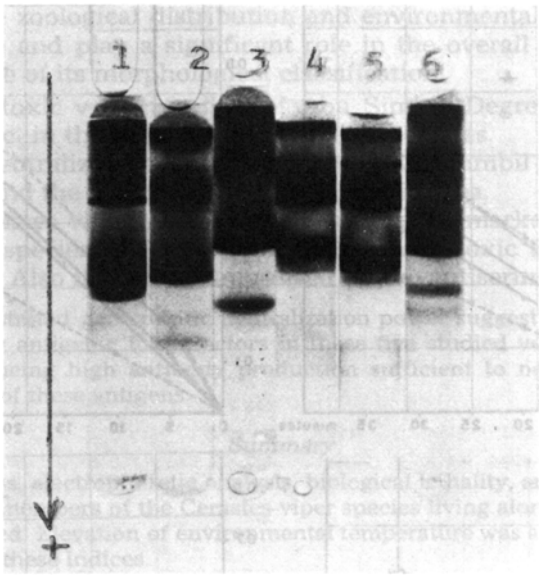

Fig. 3 shows acrylamide gel electrophoresis run at pH 8.3 for (1) human serum, (2) Cc_1, (3) Cc_2, (4) Cc_3, (5) Cv_3, and (6) Cv_1.

differences were observed. Variations in the mobility of the faster bands and presence of new bands in the area of slow migration were demonstrated in Cv_3 venom when compared to that of Cv_1. In Cerastes-cerastes groups, similar patterns could be observed; with the exception of two heavy bands in the middle area of Cc_2 and Cc_3 venoms as compared to a single band in the same area for Cc_1 venom. Such differences in the protein patterns could help in differentiating between the five studied venoms, which in turn would facilitate the identification of the locality of the snake.

All the 5 venoms tested showed remarkable phospholipase A_2 activity; in harmony with high activity reported by *Klibansky* et al. (7) working on Vipera palaestiniae and *Bradlow* and *Marcus* (1) working on Vipera russelli. On the other hand, *Marinetti* (9) reported lower activity for elapid venoms.

The sequence of phospholipase A_2 activity (fig. 4) in the studied venoms was $Cv_3 > Cc_3 > Cc_2 > Cc_1 > Cv_1$. At venom concentrations of 2–5 μg per reaction mixture, the enzyme activity showed a linear relationship with time over the 30-minutes period of the experiment. At venoms of higher concentrations (10–20 μg) except for venoms of low phospholipase activity this linear relationship was only limited to the initial 5–10 minutes of the reaction, followed by an early plateau. Apparently one can draw a correlation between venom-phospholipase activity and environmental conditions, viz., venoms of snakes living in hot areas, e.g. Abu Simbil and the New Valley, are characterized by very high phospholipase activity. This is in concordance with the fact that the enzyme is extremely heat-stable (12).

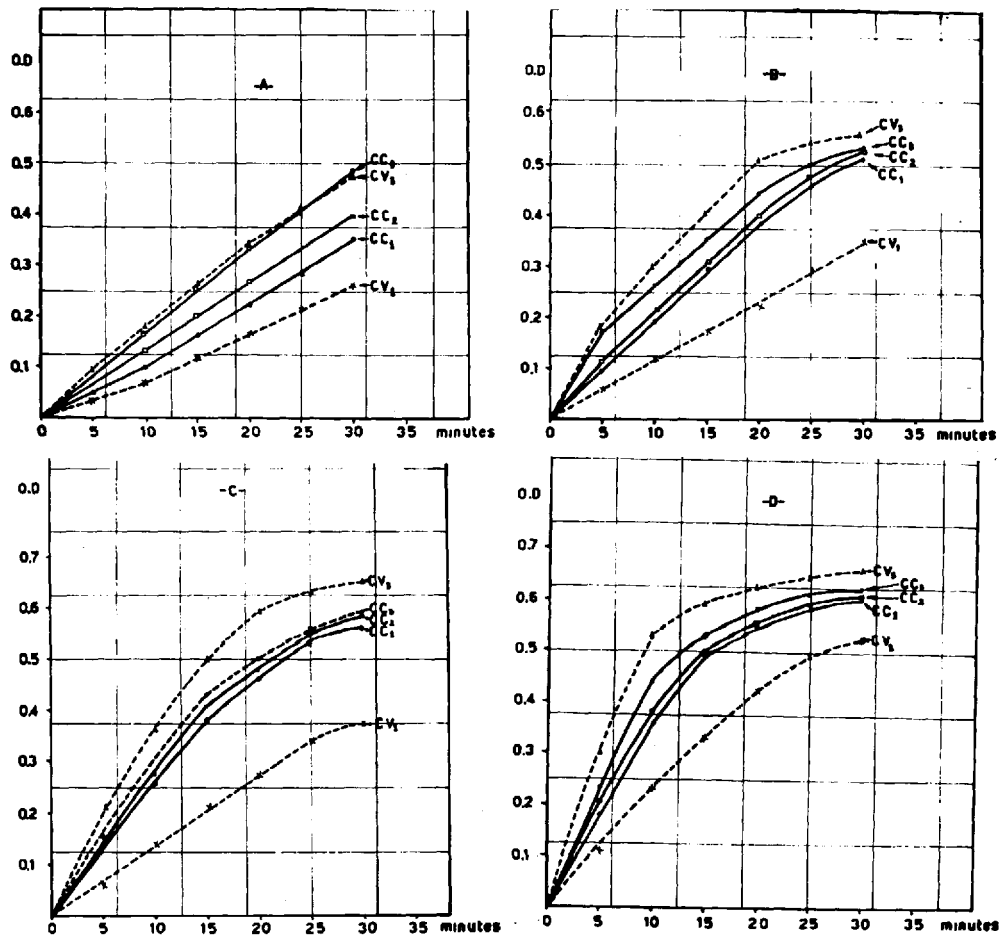

Fig. 4 shows curves of phospholipase A_2 activity in Cc_1, Cc_2, Cc_3, Cv_1, and Cv_3 using (A) 2 µg, (B) 5 µg, (C) 10 µg and (D) 20 µg of venom per reaction mixture.

Cellulose-acetate and acrylamide-gel electrophoresis showed certain similarities between the protein patterns of snakes living under similar environmental conditions. Thus the patterns of venom proteins of Cv_3 and Cc_3 inhabitants of hot areas are quite matching. Also those of Cc_1 and Cv_1 inhabitants of mild climatic areas were similar, but greatly different from those of Cv_3 and Cc_3.

Of value to add is that there is apparent possible correlation between the venom protein content, its toxicity and its enzymatic phospholipase activity; and all three indices seem to increase with elevation of temperature according to geographical distribution. Also one may add that the toxic symptoms that are caused by one or more than one protein entity of a venom species that would be distributed in different amounts and patterns due to climatic changes would cause overall new symptomatic

results. Hence zoological distribution and environmental conditions are most effective and play a significant role in the overall reaction of the venoms inspite of its morphological classification.

The most toxic venom is that of Abu Simbil. Degree of toxicity is $Cc_3 > Cc_2 > Cc_1$ in the horned viper Cerastes cerastes.

The best-neutralized venom is also that of Abu Simbil followed by the New Valley, and the least is that of the Pyramid area.

In the Cerastes vipera, our two species show a marked difference in toxicity. The species of Abu Simbil is still more toxic than that of the Pyramid area. Also it is more neutralized by the antiserum.

This demonstrated paraspecific neutralization power suggests the presence of common highly antigenic toxic factors in these five studied venoms, which are capable of inducing high antibody production sufficient to neutralize elevated concentrations of these antigens.

Summary

Total proteins, electrophoretic analysis, biological lethality, and phospholipase activity of five members of the Cerastes-viper species living along the Nile Valley were investigated. Elevation of environmental temperature was accompanied with high values for these indices.

It is concluded that zoological distribution and environmental conditions could influence the overall biological behaviour of snake venoms of the same species.

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