An Electrophoretic Examination of Cobra Venoms

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The complex chemical nature of Indian cobra (*Naja naja*) venom was indicated by Ganguly & Malkana (1936) and Ganguly (1937), who reported its percentage composition as follows: total protein 87.56, globulin 20.31, albumin 39.69, primary protease 11.31, secondary protease 16.81. In addition to proteins, the venom was found to contain cholesterol, phospholipins, inorganic substances and colouring matter. The toxic principle was found to be associated with the secondary protease. Ganguly (1936) also reported the presence of proteolytic, lecithin-splitting and rennin-like enzymes in the venom.

The work recorded in this article is the result of an investigation of several cobra venoms by the method of electrophoresis.

MATERIALS AND TECHNIQUE

Venoms of the following snakes were investigated: (1) *Naja nigricollis* (M'fesi or spitting cobra), (2) *N. haje* (Egyptian cobra), (3) *N. naja* (Indian cobra), (4) *N. flava* (Cape cobra), (5) *Sepedon haemachates* (black ringhals).

Desiccated venoms were obtained from the South African Institute for Medical Research, Johannesburg, and several samples of venom in fluid form were obtained from Egyptian cobras caught in the grounds of the Institute at Onderstepoort. For electrophoresis the venoms were made up as 1% solutions in buffer at pH 6.2 containing 0.0224M- KH_2PO_4 , 0.0036M- Na_2HPO_4 and 0.044M- NaCl .

Technique used for the electrophoresis. This has been described in detail by Polson (1945); a brief description of the method follows.

The electrophoresis apparatus and technique used in the present work are slight modifications of those developed by Tiselius (1937). The U-shaped electrophoresis cell used by Tiselius can be cut into segments to facilitate the separation of the components from a protein mixture. This type of cell requires a special arrangement of pistons to shift the segments. For this reason, a less complicated electrophoresis cell was designed (see Figs. 1, 2) which was found suitable for analytical as well as for preparative work. It consists of a U-tube with an inlet tap in the bend and has two side arms of the same diameter fused to the limbs. The two side tubes are attached to two electrode vessels. The ends of the U-tube are open so that samples

may be withdrawn from the protein column in either limb.

A fine pipette with its point bent at right angles is used for taking samples. The movement of the pipette in and out of the U-tube is effected by a microscope rack and pinion. The rack and pinion are fixed to the cell holder just above the open ends of the U-tube. The cell holder is attached to an X-ray tube stand with which it can be lowered into and lifted out of the thermostat without disturbing the fluid in the electrophoresis cell.

A typical electrophoresis run on a venom is made as follows: The temperature of the thermostat is lowered to 2° and the cell filled with buffer solution. The silver chloride electrodes are lowered into their respective electrode vessels. These are then covered with saturated KCl solution and the protein inlet tube is fixed on to the capillary tap in the bend of the U-tube. The cell is then lowered into the thermostat, and when temperature equilibrium is attained the protein solution is run in to a level where the column can be photographed. To make an initial sharp boundary, which is essential in electrophoresis, the boundary is rectified by lowering the fine pipette into the U-tube to touch the protein level. A small quantity of fluid is then drawn off and a very sharp interface formed. The current is then switched on and passed through the apparatus until the required separation of components is effected. Using the Lamm (1928, 1937) scale method for observing the migration of the components, a photograph of the scale is taken. Samples are then drawn off at a fixed position in one of the limbs of the U-tube. After each sample is removed a photograph of the scale is taken. Each position where a sample was taken is indicated by a blurred region on the photograph of the scale. In this way the whole of the protein column in a limb is cut up into small samples. The electrophoresis current is now reversed and passed through the cell for the same period as before and the whole process of sampling and photographing repeated.

Determination of toxicity of venom fractions. The toxicity of each sample was tested by the intravenous injection of serial two-fold dilutions into mice, the volume of dose being 0.2 ml. The haemolysin content (Ganguly, 1937) was also determined by tests using 2% suspensions of washed guinea-pig red cells. In these tests it was found that complete haemolysis of all mixtures occurred on standing overnight in the water-bath; readings were therefore taken at the end of 20 min.

The activities of the samples were noted on the electrophoresis diagrams at the positions where they were taken; the position of the toxic component was taken as the peak in the region where the activities of the samples increase most rapidly. A component is considered the active one only if the activity in the ascending as well as the descending column is allocated to the same component.

Fig. 1. Electrophoresis apparatus with the cell lowered into the thermostat. *a*, source of illumination (mercury arc); *b*, water filter; *c*, light filter; *d*, vertical transparent scale; *e*, calcium chloride chamber; *f*, thermostat; *g*, tube holder; *h*, microscope rack and pinion; *i*, contact thermometer; *j*, bracket attached to cell holder; *k*, water-stirrer in thermostat; *l*, protein container; *m*, X-ray tube stand; *n*, camera.

Fig. 2. Electrophoresis cell fixed to holder. *g*, cell holder; *h*, microscope rack and pinion; *j*, bracket attached to X-ray tube holder and cell holder; *l*, protein container attached to U-tube; *o*, sampling pipette.

RESULTS

In Fig. 3 are shown the diagrams of the ascending (A) and descending (B) columns respectively after electrophoresis of *Naja nigricollis* venom at pH 6.2. The diagrams 1-9 in the ascending column (Fig. 3A)

and 1-7 in the descending column (Fig. 3B) show the relation of the samples taken to the other components.

From Table 1 it is seen that the toxic and haemolytic component in the ascending column is absent in sample 1 but is present in sample 2. In

the descending column the toxic and haemolytic activity begins with sample 5. When these results are correlated with those of Fig. 3, the activity of the venom can be ascribed to component *T*.

column, and 6.2×10^{-5} cm.²/sec./V. for the descending column.
In Figs. 4-8 are given the electrophoretic diagrams of other venoms examined. The haemolytic

Fig. 3. Electrophoresis diagrams of *Naja nigricollis* venom. A, ascending column; B, descending column; C, T, D, E, G, (H), I, various components in original diagram; 1, 3, 5, etc., diagrams of protein column after removal of samples.

Table 1. *Naja nigricollis*. Mouse toxicity and haemolytic activity of electrophoretic samples

Ascending column		Dilution							
Position of component	No. of sample	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256
C	1	-o-	-o-	-o-	-o-	-o-	-o-	-o-	--
T	2	H+H	H+H	H+H	H ^o -	-o-	-o-	-o-	--
D	3	H+H	H+H	H+H	H+H	H ^o H	o	o	--
	4	H+H	H+H	H+H	H+H	H+H	H ^o H	H ^o H	--
E	5	H+H	H+H	H+H	H+H	H+H	H ^o H	H ^o H	--
	6	H+H	H+H	H+H	H+H	+	H ^o H	H H	--
G	7	H+H	H+H	H+H	H+H	+	H ^o H	H H	--
	8	H+H	H+H	H+H	H+H	H+H	H ^o H	H H	--
I	9	H+H	H+H	H+H	H+H	H+H	H ^o H	H H	--
Descending column									
Position of component	No. of sample								
I	1	-o-	-o-	-o-	--	--	--	--	
	2	-o-	-o-	-o-	--	--	--	--	
	3	-o-	-o-	-o-	--	--	--	--	
G	4	-o-	-o-	-o-	--	--	--	--	
D	5	H+H	H+H	H ^o H	H ^o H	-o-	--	--	
T	6	H+H	H+H	H+H	H ^o H	H ^o H	--	--	
	7	H+H	H+H	H+H	H+H	H ^o H	H H	--	
C	8	H+H	H+H	H+H	H+H	H ^o H	H H	H -	
	9	H+H	H+H	H+H	H+H	H ^o H	H H	H -	
H H complete haemolysis		H - partial haemolysis				- - no haemolysis			
+ lethal for mice		o mice survived.							

Applying the formula of Tiselius (1930), the mobility of component *T* is calculated as 6.21×10^{-5} cm.²/sec./V. at pH 6.2 and 2° for the ascending and toxic properties of the various components of *N. naja* venom were not examined, but in both the ascending and descending columns of *N. flava* and

Fig. 4. *Naja flava* venom. Ascending column. Fig. 5. *Naja haja* venom. Ascending column. Fig. 6. *Naja haja* (var. *angulosa*) venom. Ascending column. Fig. 7. *Sepedon haemachates* venom. Ascending column. Fig. 8. *Naja naja* venom. Ascending column.

Figs. 4-8. Letters indicate various components in original diagram; numbers indicate diagrams of protein column after removal of samples.

N. haja and in the ascending column of *Sepedon haemachates* these activities were associated, as in the case of *Naja nigricollis*, with component *T*. By analogy, the *T* component of *N. naja* venom was accepted as being the active component.

Electrophoretic characteristics of cobra venoms. In Table 2 are shown the calculated mobilities of the chief components of the various venoms, the calculations being made from the diagrams depicted.

DISCUSSION

At the outset it must be pointed out that the conditions under which the electrophoresis experiments were carried out were identical for each venom, so that all the diagrams are strictly comparable. Consideration of Table 2 shows that the components of the different venoms may be tabulated into groups possessing the same mobility. Two components are common to all the venoms, the non-toxic component *I* and component *T* with which is associated the neurotoxins and haemolysins. This is in accordance with the observations of Grasset (1936) based on *in vitro* and *in vivo* neutralization tests, namely, that there is a common antigenicity between venoms of different species and genera of colubrid reptiles.

Further observations by Grasset were that the anavenom (toxoid) prepared from the venom of *Sepedon haemachates* confers upon an animal a satisfactory degree of immunity to the homologous venom but a much less marked immunity to other venoms of the *Naja* and *Dendropsis* groups, while anti-*Naja flava* serum has approximately the same neutralizing effect upon the various colubrid venoms of its own group as upon those of *Sepedon* and *Dendropsis*. For these and other reasons, notably one of availability, *Naja flava* venom was chosen as the group anti-cobra antigen for the preparation of South African polyvalent serum at the South African Institute for Medical Research.

Consideration of Table 2 in conjunction with the respective electrophoretic diagrams brings out several additional points of interest. *Sepedon haemachates* venom is the least complex, since only four well-defined components were detectable. For this reason it might be anticipated that it would prove to be a good antigen owing to the relatively small number of non-specific protein components present. Actually it was found to be the least efficient of the antigens investigated (Grasset, 1936), a fact which could be ascribed to the exceedingly limited size of the *T* component in the diagram (i.e. to a relatively high proportion of non-specific protein). This conclusion is supported by the finding that in spite of its greater complexity (six well-defined components), *Naja flava* venom proved to be a superior antigen and in the diagram shows a larger *T* component. If the antigenicity of a venom is a function of the

Table 2. *Mobilities of the components of various cobra venoms*(Expressed as 10^{-5} cm.²/sec./V. at pH 6.2 and 2°.)

Cobra	Components									
	A	B	C	T	D	E	F	G	H	I
<i>Naja nigricollis</i>	—	—	8.02	6.21	5.24	4.12	—	—	2.11	0.63
<i>Naja haja</i> (var. <i>angulosa</i>)	—	—	8.5	6.40	5.4	4.0	3.6	—	2.1	0.3
<i>Naja haja</i>	—	—	8.6	6.6	5.6	4.4	3.6	—	2.07	0.65
<i>Naja naja</i>	11.7	9.3	8.1	6.52	5.3	4.3	—	3.01	—	0.74
<i>Naja flava</i>	—	9.00	8.0	6.44	5.6	—	3.6	—	—	1.1
<i>Sepedon haemachates</i>	—	9.3	—	6.46	—	—	—	2.94	—	1.4

mass of the *T* component shown in the electrophoretic diagram, then *N. nigricollis* venom should be only slightly inferior to *N. flava*. *N. naja* venom should be at best only slightly superior to *Sepedon haemachates* venom and markedly inferior to the others. It would be of interest to show whether *Naja naja* venom is superior as an antigen to *N. flava* or whether the greater mass of the *T* component is counterbalanced by the greater complexity as shown by the presence of an additional well-marked component.

SUMMARY

1. The electrophoresis of a number of cobra venoms is described, together with the method of identification of the toxic component in the electrophoresis diagram.

2. The venoms of *Naja nigricollis*, *N. haja*,

N. naja, *N. flava* and *Sepedon haemachates* were examined electrophoretically, and the respective diagrams obtained under identical conditions at pH 6.2 are shown.

3. The neurotoxic and haemolytic activity is found to be associated with the *T* component having a mobility of about 6.4×10^{-5} cm.²/sec./V.

4. The various components of the different venoms fall into groups having the same mobility.

5. *Sepedon haemachates* venom is the least complex and *Naja naja* venom the most complex.

6. The contention that the efficiency of an antigen may be gauged by the mass of the *T* component is discussed.

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REFERENCES

- Ganguly, S. N. (1936). *Indian J. Med. Res.* **24**, 287.
 Ganguly, S. N. (1937). *Indian J. Med. Res.* **24**, 1165.
 Ganguly, S. N. & Malkana, M. T. (1936). *Indian J. Med. Res.* **24**, 281.
 Grasset, E. (1936). *Quart. Bull. Hlth Org. L.o.N.* July 1936.
 Lamm, O. (1928). *Z. phys. Chem. A*, **138**, 313.
 Lamm, O. (1937). *Nova Acta Soc. Sci. upsal.* Ser. iv, **10**, no. 6.
 Polson, A. (1945). *Onderstepoort J. vet. Sci.* **20**, 159.
 Tiselius, A. (1930). *Nova Acta Soc. Sci. upsal.* Ser. iv, **7**, no. 4.
 Tiselius, A. (1937). *Trans. Faraday Soc.* **33**, 524.

The Metabolism and Functioning of Vitamin-like Compounds

2. A COMPARISON OF PANTOTHENATE METABOLISM BY PROLIFERATING AND BY NON-PROLIFERATING BACTERIA

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Suspensions of non-proliferating haemolytic streptococci were previously found (McIlwain & Hughes, 1944) to destroy the growth-promoting powers of pantothenate, for streptococci and for other organisms, during glycolysis. Such pantothenate inactivation was inhibited by pantoyltaurine, which

inhibited also the growth of streptococci; the activities of pantoyltaurine in these two respects towards a series of organisms were quantitatively correlated, as were also the effects of a series of compounds structurally similar to pantoyltaurine, upon a given organism (McIlwain & Hughes, 1945). Preformed