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The Protein Neurotoxins in Scorpion and Elapid Snake Venoms

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A concise review of the chemistry and biological properties of the protein neurotoxins in scorpion and elapid snake venoms is given. Although there is considerable homology in amino acid sequences within the group of snake toxins and within the scorpion toxins, respectively, there is very little homology between the two groups of toxins. The snake toxins cause an irreversible antidepolarizing block of the endplate, whereas the scorpion toxins produce an irreversible effect (depolarization) of several different target cells (*e.g.*, acetylcholine and catecholamines are released from their respective tissues and ion distributions in certain cells are altered). We propose the hypothesis that both groups of toxins combine with the membrane through disulfide interchange between the toxin and the membrane. Data, recently obtained by us, demonstrate that the lethal effects of both scorpion and cobra venoms are alleviated by administering, at the site of the envenomation, agents that reduce disulfide bonds.

Venoms from several species of scorpions are lethal because they contain polypeptides that depolarize certain target cells. Consequently, toxic venoms cause release of acetylcholine and catecholamines from the corresponding nerve endings. Conversely, the principal lethal factors in venoms from many snakes of the family *Elapidae* produce a nondepolarizing block of skeletal muscle. In the past few years intensive studies have been made of the snake venom toxins. Consequently several review articles, symposia, and books have been published recently (Bücherl and Buckley, 1971; Bücherl *et al.*, 1968; Lee, 1972; Russell and Saunders, 1967; Simpson, 1971; de Vries and Kochva, 1973). Somewhat less is known about the scorpion toxins, however.

This presentation is concerned primarily with scorpion venoms and the neurotoxins present in them. Reference will be made, however, to the neurotoxic polypeptides in the elapid snake venoms for purposes of comparing the two types of toxins. The term "neurotoxin" as we will use it refers to those polypeptides in venoms that block transmission at the neuromuscular junction.

PROPERTIES OF VENOMS AND THEIR TOXINS

The family *Elapidae* comprises a large group of different species of snakes that includes, among others, cobras, coral snakes, kraits, and death adders. Venoms from the elapid snakes contain neurotoxins which characteristically cause death in the untreated subject within 24 to 48 hr. Among the lethal scorpions, the most dangerous to man are species belonging to the genera *Centruroides* (North America), *Tityus* (Brazil), and *Leiurus, Buthus,* and *Androctonus* (Africa and Asia).

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The neurotoxins in snake and scorpion venoms are single-chain, basic polypeptides with molecular weights between 6000–10,000 daltons. These peptides are tightly folded and stabilized with four or five disulfide bridges per toxin molecule. The toxins are thermostable, resistant to enzymatic hydrolysis when in their native forms, and pass slowly through cellulose acetate dialyzing tubing. Although antisera are available for many neurotoxic venoms, the actual neutralizing powers are relatively low when compared with antitoxins for some of the bacterial antigens, for example (Reid, 1968).

The elapid snake venom toxins are divided into two groups based upon the numbers of amino acid residues per molecule of toxin. One group, the "61" residue toxins, contains 60–62 amino acid residues per toxin molecule whereas the other group, designated "71" residue toxins, contains 70–74 amino acids per molecule. The scorpion venom toxins contain approximately 65 amino acids per molecule. Unique features of the amino acid compositions of these toxins are as follows. (1) Methionine is almost completely absent in both the snake and scorpion toxins (4 of 32 toxins have one methionine residue and 1 of 32 has two residues). (2) Alanine is missing in all of the "61" residue toxins except toxin α from *Dendroaspis polylepis,* whereas the "71" residue toxins and many scorpion toxins contain alanine. (3) Phenylalanine is frequently missing in the snake toxins. (4) The high content of lysine and arginine contributes to the basic character of these molecules (pH_I above 9.0). (5) The dicarboxylic amino acids and their amides are present in relatively high proportions (Table I).

Neurotoxins in Scorpion Venoms. Venom from the North American scorpion, *Centruroides sculpturatus* (range in the Southwestern U. S. A.) is a mixture of at least 12 different proteins plus other components, *e.g.*,

Table I. Characterizing Amino Acid Residues in Snake and Scorpion Venom Neurotoxins

Amino acid	Variations in amino acid residues		
	Snake venom toxins		Scorpion toxins
	"61" residue[a]	"71" residue[a]	
Methionine	0–1[b]	0–2[b]	0–1
Arginine	3–6	3–6	0–4
Lysine	3–7	4–9	4–8
Arginine + lysine	9–13	9–13	6–11
Glutamic + glutamine	5–8	1–5	0–5
Aspartic + asparagine	5–8	4–10	7–10
Dicarboxylic amino acids	10–15	9–13	9–14
Alanine	0–1[c]	2–4	0–5
Phenylalanine	0	1–3	0–2
Half cystine	8	10	8

[a] "61" and "71" designations refer to those toxins with 60–62 and 71–74 amino acids residues. [b] Three toxins have one methionine residue. One toxin has two methionine residues (32 toxins are represented in the table). [c] One toxin has one alanine.

Table II. Responses of Rodents and Chicks to Scorpion Venom

Time scale, min	
0	Rodents
2	1. Immediate severe local pain and rapid swelling.
5	2. Restlessness, anxiety, hypersensitivity to touch and sound.
	3. Salivation blocked by atropine.
10	4. Convulsions, especially in response to touch or sharp noises.
	5. Semi-comatous yet respond to noise or touch.
20	6. Labored breathing, muscle twitchings and weakness.
	7. Death in 15 min to 24 hr. Respiratory failure.
	Chicks
	1. Symptoms as above excepting restlessness.
	2. Salivation is intense, the crop becoming engorged with viscous fluid. Blocked by atropine.
	3. Muscle weakness and irreversible spastic paralysis begins within 10 min from envenomation.

mucous and small molecules (McIntosh and Watt, 1973). The venom is secreted by secretory epithelial cells including mucous cells (Keegan and Lockwood, 1971). Thus far we have isolated at least eight different polypeptide toxins, which are toxic in varying degrees to vertebrates and invertebrates, from the venom of *C. sculpturatus*. Characterization of four of these toxins has already been reported (McIntosh and Watt, 1973).

The scorpion venom neurotoxins are isolated by a series of column chromatographic separations, the initial separation being on carboxymethylcellulose (CMC), followed by rechromatography on Amberlite CG-50. The toxins already characterized elute from CMC in the region 230–280 (Figure 1). In addition to these toxins, another family of at least four toxic proteins has been isolated recently from *C. sculpturatus* venom. We are currently characterizing these proteins. Isolation procedures are the same as described above, with an additional final purification stage on DEAE-Sephadex. In Figure 1 this latter group of toxins elutes in the region of fractions 90–180. The zone 190–220 contains mixtures of toxic materials. These latter four toxins are similar in many respects to those toxins isolated previously (McIntosh and Watt, 1973). They all contain four disulfide bridges, eight lysine residues, and six tyrosine residues and have a total of 65 amino acids.

The Edman phenylthiohydantoin degradation of three of the latter native toxins reveals that they have the amino terminal sequence Lys-Glu-Gly-Tyr. The fourth protein has the sequence Lys-Lys-Asp-Gly-Tyr. Two of these proteins, which were obtained in good yields from *C. sculpturatus* venom, were reduced and alkylated, and the alkylated products were then hydrolyzed with trypsin. The tryptic peptides were isolated by chromatography on a cation exchange resin, using a gradient of volatile buffers. Peptides with the sequences Lys-Glu-Gly-Tyr-Leu-Val-Lys and Lys-Glu-Gly-Tyr-Leu-Val-Asn-Lys were obtained. It is evident that these peptides are derived from the amino terminus of their respective neurotoxin molecule. The determination of the complete amino acid sequence of these proteins is now in progress. These limited data show that the amino terminal sequences of these toxic proteins from venom of *C. sculpturatus* correspond well with those sequences published for the insect and mice toxins from the scorpion, *Androctonus australis*, Figure 2 (Rochat *et al.*, 1970a, 1972; Zlotkin *et al.*, 1971, 1972).

Although complete sequences of three neurotoxins, toxins I, II and I′, from *A. australis* have been determined (Figure 2), only two sequences are shown because toxin I′ differs from toxin I only by substitution of an isoleucine residue for valine at position 17. Partial sequences of several toxins from other species of scorpions have been determined and presumably complete sequences will be forthcoming (Rochat *et al.*, 1970b). Three of these partial sequences, toxin III from *Buthus occitanus tunetanus*, toxin III from *Leiurus quinquestriatus*, and the insect toxin from *A. australis*, are also included in Figure 2. Therefore, another family of homologous proteins, much like the snake venom toxins, appears to be present in venoms from scorpions throughout the world.

Cobratoxin in position 1 (Figure 2) is included for purposes of comparison. There is considerable difference in primary structures between the snake toxins and those from scorpion venoms. The cysteine residues in scorpion toxins are located in different positions, which means the disulfide bridges will be located differently. The locations of the disulfide bridges in scorpion toxins have not yet been determined, however. There are more hydrophobic residues in the scorpion toxins than in the snake toxins and the distribution of these residues lies throughout the molecules. The extensive hydrophilic region of the "61" residue snake toxins (residues 3–24) is missing in the scorpion toxins and in this respect they resemble the "71" snake toxins.

Primary Structures of Snake Venom Neurotoxins. Primary structures of more than 20 toxins from venoms of several different species of snakes have been determined (Botes, 1971, 1972; Botes and Strydom, 1969; Botes *et al.*, 1971; Eaker and Porath, 1967; Karlsson *et al.*, 1972; Nakai *et al.*, 1971; Strydom, 1972; Strydom and Botes, 1971; Yang *et al.*, 1969). A prominent fact emerges from comparison of these structures, *viz.*, the snake venom toxins comprise a family of structurally homologous polypeptides with great similarity in amino acid sequence.

In the seven "61" residue toxins of Figure 3, 21 of the 62 possible amino acid residues show identical homologies when alignment is started at the carboxyl terminus. Six additional alignments are obtained at the N terminus when there is a deletion at position 18. Toxins 1 to 5 (Figure 3) have homologies in 4 of the 61 amino acids, with replacements of hydrophobic residues in 3 instances and in 10 instances of hydrophilic residues (ionizable and nonionizable). These seven toxins were selected bethey are from seven different species of snakes and because they show the greatest variation in primary struc-

Figure 1. Elution profile of whole scorpion venom from carboxymethylcellulose (CMC) chromatographic columns. Discontinuous gradient elution with ammonium acetate buffers as follows (5.0-ml fractions; 14.0 ml/hr): Tubes 1–31, 0.01 *M*, pH 4.6. Tubes 32–61, 0.05 *M*, pH 5.1. Tubes 62–92, 0.10 *M*, pH 5.6 Tubes 93–161, 0.10 *M*, pH 6.0. Tubes 162–206, 0.25 *M*, pH 6.15. Tubes 207–279, 0.50 *M*, pH 6.5.

Line	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	LEU	GLU	CYS	HIS	ASN	GLN	GLN	SER	SER	GLN	THR	PRO	THR	THR	THR	GLY	CYS	SER	GLY	GLY
2	LYS	ARG	ASP	GLY	TYR	ILE	VAL	TYR	PRO	ASN	ASN	CYS	VAL	TYR	HIS	CYS	VAL	PRO	PRO	
3	VAL	LYS	ASP	GLY	TYR	ILE	VAL	ASP	ASP	VAL	ASN	CYS	THR	TYR	PHE	CYS	GLY	ARG	ASN	ALA
4	VAL	LYS	ASP	GLY	TYR	ILE	VAL	ASP	ASP	ARG	ASN	CYS	THR	TYR	PHE	CYS	GLY	ARG	ASN	ALA
5	VAL	ARG	ASP	ALA	TYR	ILE	ALA	LYS	ASN	TYR	ASN	CYS	VAL	TYR	GLU	CYS	PHE	ARG	ASP	SER
6	LYS	LYS	ASN	GLY	TYR	ALA	VAL	ASP	SER	SER	LYS	LYS	ALA	PRO	GLU					
7		LYS	GLU	GLY	TYR	LEU	VAL	LYS												
	LYS	LYS	ASP	GLY	TYR															
		LYS	GLU	GLY	TYR	LEU	VAL	LYS												

Line	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
1	GLU	THR	ASN	CYS	TYR	LYS	LYS	ARG	TRP	ARG	ASP	HIS	ARG	GLY	TYR	ARG	THR	GLU	ARG
2		CYS	ASP	GLY	LEU	CYS	LYS	LYS	ASN	GLY	GLY	SER	SER	GLY	SER	SER	CYS	PHE	LEU
3	TYR	CYS	ASN	GLU	GLU	CYS	THR	LYS	LEU	LYS	GLY	GLU	SER	GLY	TYR		CYS	GLN	TRP
4	TYR	CYS	ASN	GLU	GLU	CYS													
5	TYR	CYS	ASN	ASP	LEU	CYS													

Line	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
1	GLY	CYS	GLY	CYS	PRO	SER	VAL	LYS	ASN	GLY	ILE	GLU	ILE	ASN	CYS
2	VAL		PRO	SER	GLY	LEU	ALA	CYS	TRP	CYS		LYS	ASP	LEU	PRO
3	ALA	SER	PRO	TYR	GLY	ASN	ALA	CYS	TYR	CYS	TYR	LYS		LEU	PRO

Line	55	56	57	58	59	60	61	62	63	64	65	66	67
1	CYS	THR	THR	ASP	ARG	CYS	ASN	ASN					
2	ASP	ASN	VAL	PRO	ILE	LYS	ASP	THR	SER	ARG	LYS	CYS	THR
3	ASP	HIS	VAL	ARG	THR	LYS	GLY	PRO	GLY	ARG		CYS	HIS

1. N. naja atra
2. A. australis I
3. A. a. II
4. B. o. tunetanus III
5. L. q. quinquestriatus III
6. A. a. Insect T.
7. C. sculpturatus

Figure 2. Amino acid sequences of neurotoxins from scorpion and snake venoms. Line 1 is the complete sequence of cobratoxin from *Naja naja atra;* lines 2 and 3 complete sequences of mammalian toxins I and II from *Androctonus australis;* lines 4 and 5 partial sequences of toxin III from *Buthus occitanus tunetanus* and *Leiurus quinquestriatus,* respectively; line 6 partial sequence of the insect toxin from *A. australis;* line 7 and below, beginning sequences of several toxins from *Centruroides sculpturatus.* Gaps are spaces left intentionally for purposes of alignment of homologous amino acids.

ture. The toxins all have approximately the same degree of lethality.

Other interesting observations are: (1) there is a very high incidence of homodipeptides (identical adjacent amino acids); (2) the only tryptophan is aligned uniformly throughout; (3) the segment Glu-Arg-Gly-Cys-Gly-Cys-Pro (residues 38 to 44) is common to all; and (4) the high concentration of basic amino acids in the region 25 to 35 is apparent. This region forms a loop in the two-dimensional representation of the toxin structures and is believed to be associated with biological activity.

The "71" residue toxins (Figure 4) likewise show considerable homology in their primary structures. Note particularly the identical alignments between positions 40 and 49. Three deletions are made in the first four toxins in order to accommodate the extra amino acids in toxins α and γ of *B. multicinctus* and *D. polylepis*, respectively.

A common primary structure exists between the "71" and "61" residue toxins (Figure 5). With deletions to accommodate the longer interval between Cys 3 and Cys 17 in the "61" residue toxins and the insertions of additional amino acids in the case of the "71" residue toxins, the

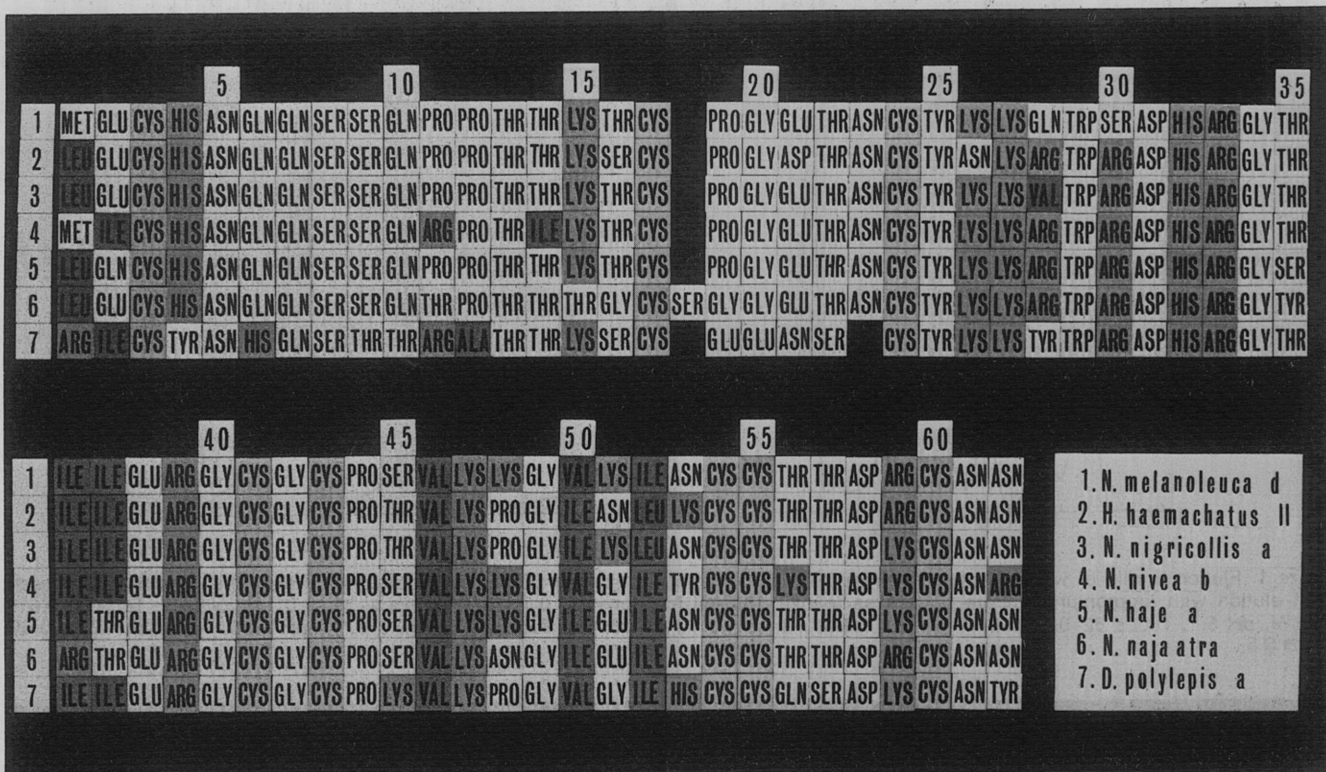

Figure 3. Amino acid sequences of selected "61" residue toxins from venoms of elapid snakes. Abbreviations: Line 1, *Naja malanoleuca*, toxin δ. Line 2, *Haemachatus haemachatus*, toxin II. Line 3, *Naja nigricollis*, toxin α. Line 4, *Naja nivea*, toxin β. Line 5, *Naja haje*, toxin α. Line 6, *Naja naja atra*, cobratoxin. Line 7, *Dendroaspis polylepis*, toxin α.

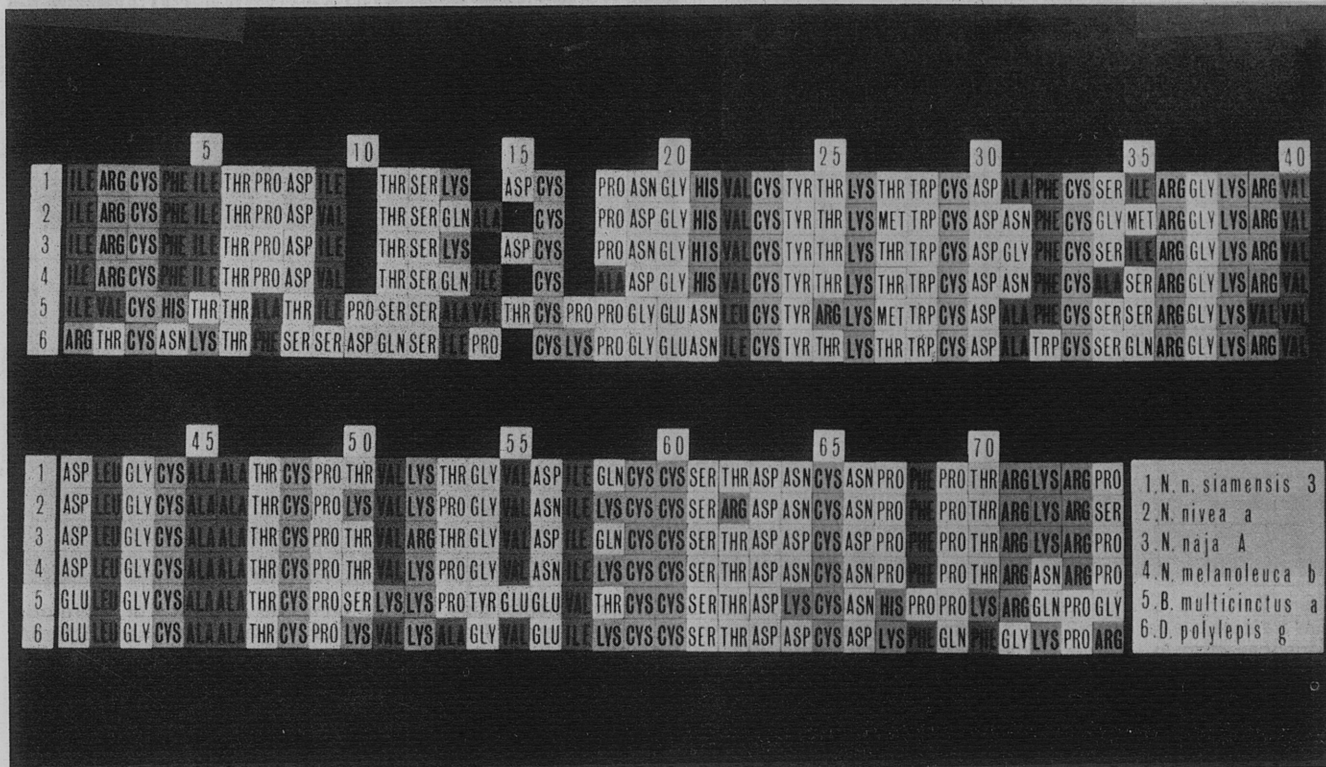

Figure 4. Amino acid sequences of "71" residue toxins from elapid snakes. Abbreviations: Line 1, *Naja naja siamensis*, toxin 3. Line 2, *Naja nivea*, toxin α. Line 3, *Naja naja*, toxin A. Line 4, *Naja melanoleuca*, toxin β. Line 5, *Bungarus multicinctus*, toxin α. Line 6, *Dendroaspis polylepis*, toxin γ.

structural homology common to both classes of toxins becomes apparent. It is interesting to note that the three methionine residues of the "71" toxins lie within the chain, whereas the two methionine residues of the "61" toxins are on the amino terminus.

The positions of the disulfide bridges have been located for cobratoxin (62 residues) and for toxin α (71 residues) from *Naja nivea* (Figure 6). The locations are 3–24, 17–41, 43–54, and 55–60 for cobratoxin (Yang *et al.*, 1970) and 3–20, 14–41, 26–30, 45–56, and 57–62 for toxin α (Botes,

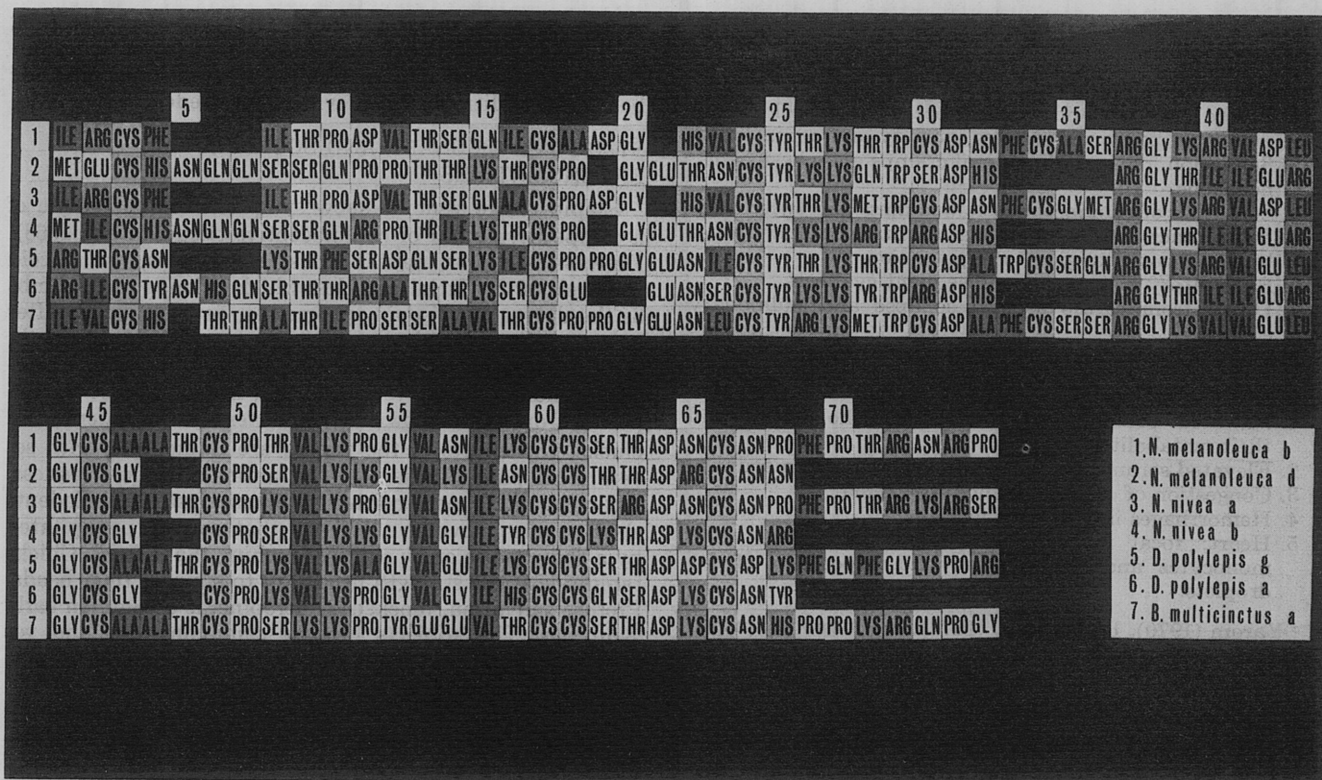

Figure 5. Comparison of "61" and "71" residue toxins from elapid snake venoms. Abbreviations are as before.

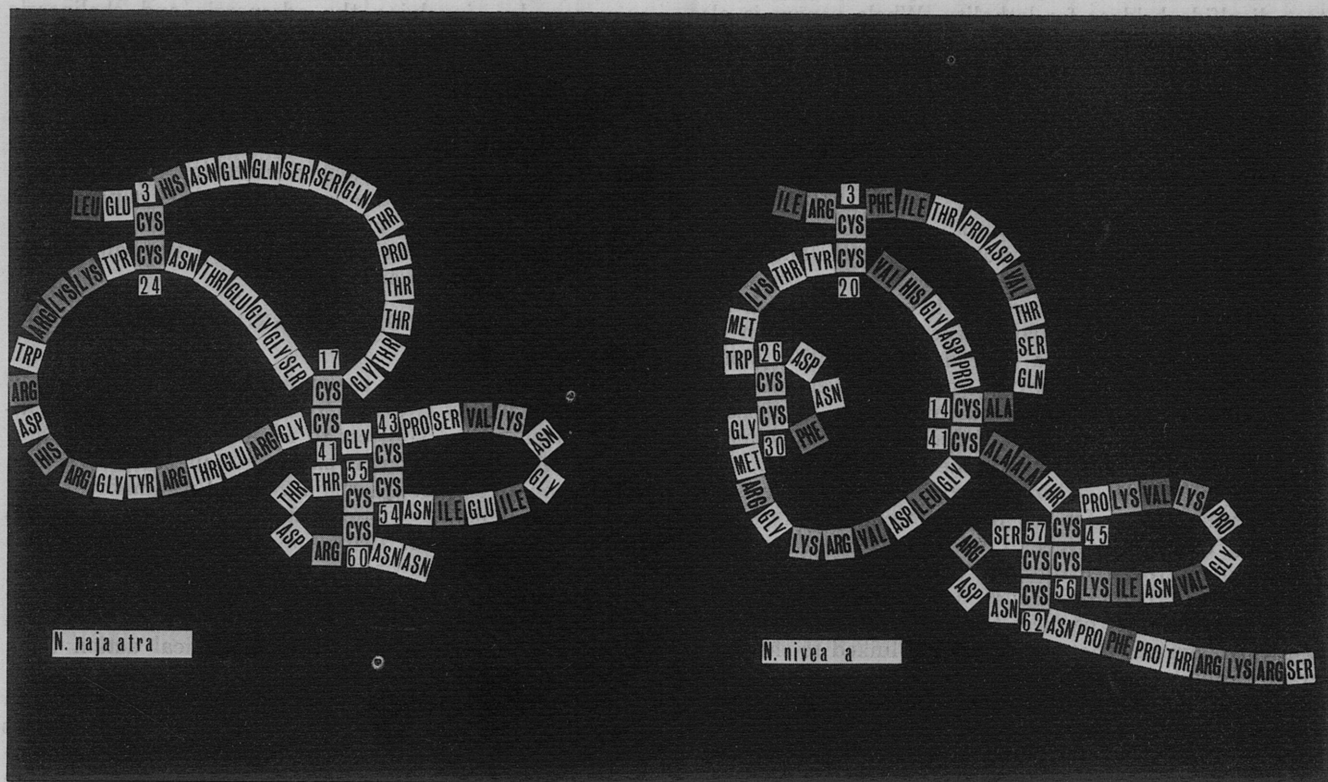

Figure 6. Positions of disulfide bonds in cobratoxin (*N. n. atra*) and toxin α (*N. nivea*).

1971). Cobratoxin has a loop of hydrophilic amino acids between positions 3–24 followed by a loop of basic, positively charged residues between positions 24–41. This latter loop is believed to have special significance in attachment of the toxin molecule to the acetylcholine receptor. Cobratoxin contains two additional small loops between positions 43–54 and 55–60. The "71" residue toxin has a very tight loop at positions 26–30 and the hydrophobic amino acids are more abundant and more evenly distributed throughout the molecule. Both toxins produce a nondepolarizing block at the neuromuscular junction, however.

Intact disulfide bridges, tryptophan at position 29, tyrosine at 25, and glutamic acid at 21, are essential for biological activity of cobratoxin (Chang and Hayashi, 1969; Chang *et al.*, 1971a,b). Alteration of the free amino groups

Table III. Responses in Man to Scorpion Envenomations[a]

Constitutional symptoms
1. Severe local pain and swelling; occasional discoloration.
2. Sweating, palor, restlessness, anxiety and confusion.
3. Salivation, nausea, abdominal cramps and chest pains, headache.
4. Sensation of choking, muscle weakness and twitching.
5. Initial tachycardia changing to bradycardia. Initial hypertension changing to hypotension.
6. Respiratory distress and subsequent cyanosis.
7. Death from cardiovascular collapse and pulmonary edema.
8. Time to death less than 1 hr to several days.

Pathological findings
1. Elevated urinary excretion of catecholamines and their metabolites.
2. Elevated serum potassium and lowered serum sodium.
3. Congestion of organs; pulmonary edema.
4. Hemorrhages in various organs.
5. Heart; focal myocardial necrosis, infiltration with monocytes and lymphocytes and deposition of fat droplets.

[a] Yarom (1970). Reddy *et al.* (1972). Watt and McIntosh (1967).

of cobratoxin also destroys activity (Chang *et al.*, 1970). Data from X-ray crystallography of cobratoxin and erabutoxin b (from the sea snake, *Laticauda semifasciata*) suggest that these toxins are flat, disk-shaped molecules (Low *et al.*, 1971; Wong *et al.*, 1972).

Unfractionated venom from *C. sculpturatus* requires intact disulfide bridges for lethality. Whole venom is also inactivated by *O*-methylisourea, a reagent specific for ϵ-amino groups of lysine and by diazobenzene-sulfonic acid, which suggests that tyrosine and/or histidine may be associated with lethality (Watt and McIntosh, 1972). Additional studies with isolated toxins are currently being carried out to establish which specific amino acids are essential for lethality.

Biological Activities of Elapid Snake and Scorpion Toxins. The polypeptide neurotoxins in snake and scorpion venoms affect impulse transmission at the neuromuscular junction. The snake venom toxins produce an irreversible nondepolarizing block, whereas the scorpion toxins irreversibly depolarize (Parnas *et al.*, 1970; Zlotkin and Shulov, 1969). The toxins from snake venoms either combine with the postjunctional membrane to cause an irreversible curare-type block or decrease acetylcholine release at the presynaptic membrane (Karlsson *et al.*, 1972; Mebs *et al.*, 1972). α-Bungarotoxin, because of an especially great affinity for the postjunctional membrane, is currently used extensively to isolate and characterize the acetylcholine receptor protein (Changeux *et al.*, 1970; Miledi *et al.*, 1971; O'Brien *et al.*, 1972, a review).

The great affinity of these toxins for membranes of certain cells suggests that the toxins are linked to the membrane through covalent bonds. Two likely possibilities are the formation of a Schiff's base between the ϵ amino groups of lysine in the toxin and a carbonyl group in the membrane and/or interchange between disulfide bridges of the toxin and either disulfide bonds or sulfhydryl groups of the membrane. Both free amino groups and intact disulfide bridges are necessary for toxicity of snake and scorpion venoms. It is known that agents which react with sulfhydryl groups and disulfide bridges in membranes alter the response by conductile tissues to acetylcholine (Karlin, 1969). Studies with reducing agents and their effects upon the toxin-receptor combinations support the concept of a covalent linkage through disulfide groups (Berg *et al.*, 1972; Clark *et al.*, 1972). Additional evidence is found in enzyme inhibition studies in which enzymes that require intact sulfhydryl groups are inhibited by snake and scorpion venoms (Babu *et al.*, 1971; Yang and Tung, 1954). These studies, however, do not offer unequivocal proof that the toxins act through their disulfide bonds and additional direct evidence is needed.

SYMPTOMS OF SNAKE AND SCORPION ENVENOMATIONS

Elapid snake venoms show symptoms characteristic of a competitive block of cholinoceptive tissues (Reid, 1968). Drowsiness, thirst, nausea, muscle weakness, and flaccid paralysis of the facial muscles, which extends to the limbs as the degree of poisoning progresses, develop shortly after envenomation. Respiratory distress increases until death, in man, occurs usually within 48 hr. Symptoms of scorpion envenomation are very different, in fact almost the opposite, from those shown by snake venoms. In rodents and chicks, the effects of the venom are immediate, with severe local pain (Table II). The animals become restless, anxious, and hypersensitive to external stimuli. Rodents show severe convulsive activity that can be precipitated by a sharp noise or by simply touching the animal. Chicks rapidly lose the strength necessary to support their heads. Profuse salivation, blocked by atropine, is a common early symptom. The subjects become semi-comatous and yet respond to external stimuli. Physostigmine-like muscle twitches, spastic paralysis in the chick, and tail undulations in the rodent are apparent. Labored breathing ensues and the animal dies of respiratory failure and cardiovascular collapse. Symptoms in man (Table III) resemble those in rodents. Death in man probably results from cardiovascular collapse and pulmonary edema.

The physiological effects of toxic scorpion venoms are very complex, involving the adrenergic and cholinergic nervous systems, cellular electrolyte imbalances, and tissue degeneration (Bertke, 1964; Reddy *et al.*, 1972; Yarom, 1970). The general effect of scorpion venom appears to result from responses of several different target cells and tissues. Excitable membranes are most certainly target tissues of the venom neurotoxins. The complex physiological effects of the venom, therefore, appear to result from direct action upon susceptible target cells or indirectly by release of biologically active substances such as catecholamines and acetylcholine.

IN VIVO DETOXICATION OF SNAKE AND SCORPION VENOMS

Paralysis produced by snake and scorpion venoms is not necessarily totally irreversible, as evidenced by the ability of envenomated subjects to recover. Chicks, for example, showing serious spastic paralysis from scorpion venom may recover completely within 24 hr. Presumably, therefore, a mechanism for detoxifying these toxic polypeptides exists in the animal. Reduction of disulfide bonds is a common biochemical process in cellular metabolism. Detoxication by disulfide bond reduction is a reasonable postulate because the toxins are absolutely dependent upon intact disulfide bridges for their biological action (Lee, 1972; Watt and McIntosh, 1972).

Treatment of snake and scorpion venoms, *in vitro*, with reduced glutathione (GSH) and dihydrolipoate inactivates the neurotoxins (Kurihara and Shibata, 1971; Watt, unpublished results). Dihydrolipoate has been used in the treatment of experimental envenomation with a nonneurotoxic venom from the snake *Trimeresurus flavoviridis* (Sawai *et al.*, 1963). Unfortunately these investigators did not use sufficient numbers of test animals to permit statistical evaluation of the data.

We wish to report, in this symposium, results which show that lethality from scorpion and cobra venoms is significantly reduced by injecting reducing agents, *e.g.*, GSH and dithiothreitol (DTT) at the site of the envenomation. Approximately two LD_{100} dosages of venom were injected into the back of the neck of chicks and mice. The test re-

Table IV. ***In Vivo*** **Protection by Reduced Glutathione (GSH) and Dithiothreitol (DTT) against Lethality from Scorpion Venom** *(C. sculpturatus)*

Exp	Treatment	No. of deaths[a] / No. tested	p values χ^2	p values Order of death
	Chicks as Test Animals			
1.	GSH dose response (GSH administered 5 min prevenom)			
	Control: saline[b] + venom 0.4 mg/kg	20/20		
	GSH 400 mg/kg + venom 0.4 mg/kg	14/20	<0.05	>0.1
	GSH 600 mg/kg + venom 0.4 mg/kg	12/20	<0.01	<0.01
	GSH 800 mg/kg + venom 0.4 mg/kg	9/20	<0.001	<0.001
2.	DTT dose response (DTT administered 5 min prevenom)			
	Control: saline + venom 0.4 mg/kg	15/15		
	DTT 30 mg/kg + venom 0.4 mg/kg	12/15	>0.2	<0.05
	DTT 90 mg/kg + venom 0.4 mg/kg	5/15	<0.001	<0.001
	DTT 150 mg/kg + venom 0.4 mg/kg	11/15	>0.1	>0.6
3.	GSH–DTT (pooled data from several experiments)			
	Control: saline + venom 0.4 mg/kg	141/160		
	GSH–DTT 200–15[c] mg/kg + venom 0.4 mg/kg	48/162	<0.001	
	Mice as Test Animals			
4.	GSH–DTT			
	Control: saline + venom 0.4 mg/kg	28/30		
	GSH–DTT 1200–90 mg/kg + venom 0.4 mg/kg			
	5 min prevenom	7/30	<0.001	<0.001
	5 min postvenom	5/30	<0.001	<0.001

[a] Number of deaths per total number of animals tested. [b] Volume of saline equivalent to maximum volume of reducing agent used.[c] GSH–DTT 200–15 means GSH 200 mg/kg and DTT 15 mg/kg, respectively. All animals were fasted before use. Reducing agents themselves showed no symptoms in dosages used. [d] χ^2 was calculated from a 2 × 2 contingency table. Order of death, refer to Heath and Irwin (1962).

agent, adjusted to pH 8.0–8.3, was likewise injected into the same general area as the venom, either before or after administration of the venom. Data recorded are survival times after envenomation and the numbers of animals dying in a 24-hr period. Appropriate statistical methods were used to evaluate the significance of the results, the cutoff point being $p = 0.05$. Control animals received venom plus an amount of physiological saline equivalent to the volume of test solution used. If the animal responded to the test reagent alone, then the dosage used with the venom was 50% or less of the minimum dosage of reagent that produced overt symptoms by itself. Solutions of GSH were maintained in a reducing atmosphere by adding 1% sodium borohydride (1% of the weight of GSH).

Results (Table IV) with GSH, DTT, and a combination of the two reagents show that statistically significant protection is afforded chicks and mice by these reagents. GSH gives an increased degree of protection as the dosage is increased. DTT has a very narrow effective dosage range because of an apparent synergistic action between the venom and this reagent. If DTT is administered before the venom, then the synergistic effect is reduced somewhat. Combinations of GSH–DTT are effective at dosages considerably less than the dosage of either agent when used alone. The results in experiment 3 (Table IV) are pooled data (tested for homogeneity). The protective effect is exhibited in chicks and mice although the chick is approximately ten times more sensitive to scorpion venom than is the mouse.

Protection in chicks and mice against lethality from cobra venom is shown by GSH–DTT mixtures. In the chick, GSH–DTT can be injected up to 20 min after cobra venom with significant protection against lethality and survival times are increased significantly if injections are as late as 40 min after the envenomations (Table V).

Cysteamine (Table VI), a common radiation protective agent, is ineffective both *in vitro* and *in vivo* in preventing lethality, although this agent significantly increases survival times from scorpion envenomations. Iodine–KI mixtures are effective if administered to chicks before the scorpion venom. Atropine, which offers some degree of protection by itself, does not significantly alter the protection shown by GSH–DTT (Table VI).

Three possible mechanisms are proposed to explain the *in vivo* effects of the reagents we have used. The most apparent mechanism is reduction of the toxins by sulfhydryl reagents. In this connection, oxidized glutathione has no effect upon lethality of the toxins. A second possible mechanism is reaction of the reagents with susceptible tissues at the site of the injection, thereby reducing the rate of absorption of the toxin. This effect is supported by results with iodine and the reduction of lethality of curare by GSH–DTT (Table VI). A third possible mechanism is alteration of the postjunctional receptor membrane by which the membrane then becomes less susceptible to the toxins.

Whether the approach we are using to inactivate the neurotoxic components in venoms *in vivo* has any therapeutic value cannot be determined from our data. We do believe, however, that reagents of the type we are using may be valuable tools in studying the biological action of this very interesting and important group of polypeptide neurotoxins.

SUMMARY

Comparison of the neurotoxins from snake and scorpion venoms permits the following conclusions: 1. Two separate families of homologous proteins constitute the toxic principles in snake and scorpion venoms. The neurotoxins from snake venoms exert their action primarily upon the postjunctional acetylcholine receptor at the neuromuscular junction to produce a nondepolarizing block. The scorpion venoms produce a general depolarizing effect on certain target cells and consequently cause complex symptomatology in the intact animal and various actions on isolated preparations. 2. The toxic proteins from elapid snake venoms are homologous in primary structure and primarily in mechanism of action. The toxins from scorpion venom are likewise homologous in mechanism of action and, as additional sequences are known, probably in primary structure also. 3. There are no great similarities between the primary structures of the snake toxins and the scorpion toxins although they do resemble each other in

Table V. *In Vivo* Protection by Reduced Glutathione (GSH) and Dithiothreitol (DTT) Against Lethality from Cobra Venom (*Naja naja atra*)

Exp	Treatment	No. of deaths/ no. tested	*p* values	
			χ^2	Order of death
	Chicks as Test Animals			
1.	Control: saline + venom 0.5 mg/kg	20/20		
	GSH–DTT 400–30 mg/kg + venom 0.5 mg/kg[a]	6/20	<0.001	<0.001
	GSH–DTT 600–45 mg/kg + venom 0.5 mg/kg	2/20	<0.001	<0.001
	GSH–DTT 800–60 mg/kg + venom 0.5 mg/kg	2/20	<0.001	<0.001
2.	Control: saline + venom 0.5 mg/kg	20/20		
	GSH–DTT 800–60 mg/kg + venom 0.5 mg/kg			
	Given 5 min postvenom	3/20	<0.001	<0.001
	Given 20 min postvenom	10/20	<0.001	<0.001
	Given 40 min postvenom	16/20	>0.2	<0.01
	Given 60 min postvenom	18/20	>0.3	>0.3
	Mice as Test Animals			
3.	Control: saline + venom 0.5 mg/kg	15/15		
	GSH–DTT 400–30 mg/kg + venom 0.5 mg/kg[a]	9/15	<0.05	>0.2
	GSH–DTT 800–60 mg/kg + venom 0.5 mg/kg	5/15	<0.001	<0.01
	GSH–DTT 1600–120 mg/kg + venom 0.5 mg/kg	9/15	<0.05	>0.05
4.	Control: saline + venom 0.5 mg/kg	15/15		
	GSH–DTT 800–60 mg/kg + venom 0.5 mg/kg			
	Administered 5 min postvenom	5/15	<0.001	<0.01
	Administered 10 min postvenom	12/15	>0.2	

[a] Administered GSH–DTT 5 min after venom. See Table IV for meaning of GSH–DTT dosages.

Table VI. Effects of Various *In Vivo* Treatments upon Lethality of Scorpion Venom and Tubocurarine in the Chick

Treatment	No. of deaths / No. treated	*p* values	
		χ^2	Order of death
Chicks as Test Animals			
Cysteamine (2-aminoethanethiol)			
200 mg/kg + venom 0.4 mg/kg 5 min prevenom	13/15	>0.3	<0.02
200 mg/kg + venom 0.4 mg/kg 5 min postvenom	15/15	1.0	<0.02
Control: venom 0.4 mg/kg + saline	15/15		
Iodine in KI (12.5 mg of I_2/ml and 25 mg of KI/ml)			
I_2 100 mg/kg + venom 0.4 mg/kg 5 min prevenom	11/34	<0.001	
Control: venom 0.4 mg/kg + saline 5 min prevenom	34/34		
I_2 100 mg/kg + venom 0.4 mg/kg 5 min postvenom	19/20	1.0	<0.001
Control: venom 0.4 mg/kg + saline 5 min postvenom	20/20		
Atropine (ATR)–GSH–DTT (ATR 15 min prevenom)			
Control: venom 0.4 mg/kg + saline	32/35		
ATR 30 mg/kg + venom 0.4 mg/kg	30/40	<0.05	
GSH–DTT 200–15 mg/kg, ATR 30 mg/kg, venom 0.4 mg/kg[a]	9/35	<0.001	
GSH–DTT 200–15 mg/kg, venom 0.4 mg/kg GSH–DTT 5 min postvenom	6/35	<0.001	
GSH–DTT detoxication of tubocurarine			
Control: saline + curare 4.5 mg/kg	24/30; 51/57		
GSH–DTT 200–15 mg/kg + curare 4.5 mg/kg			
GSH–DTT 10 min precurare	10/30	<0.001	
GSH–DTT 5 min precurare	27/57	<0.001	
GSH–DTT 5 min postcurare	23/30	1.0	

[a] See Table IV for meaning of GSH–DTT dosages.

amino acid composition. 4. It is important to note that snake venoms contain other toxins with different actions than the ones we have discussed. These toxins include cardiotoxins, cytotoxins, and many enzymes with varied activities. 5. Structure and/or amino acid residues associated with lethality are the disulfide bridges, ϵ amino groups of lysine, glutamic acid (in cobratoxin), and tryptophan. Of these side-chain substituents, the disulfide bridges are most vulnerable and hence constitute a point of attack on the molecule that results in ready inactivation and destruction of lethality *in vitro* and *in vivo* after the venom has been given.

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Procamine and Other Basic Peptides in the Venom of the Honeybee (*Apis mellifera*)

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Honeybee venom is a complex mixture of substances, among them a number of basic peptides. Several of these demonstrate potent biological activity. Most recently the venom has been shown to contain a histamine-terminal peptide, procamine, which was the first such peptide isolated from a natural source. The synthetic preparation of procamine offers the opportunity of studying the biological properties of this previously unknown component of the venom.

The venom of the honeybee has been of interest to scientists for many years. A variety of medical uses for the venom have been suggested, the most widely known being in the treatment of certain arthritic conditions (Beck, 1935; Broadman, 1962), apparently through action of the venom in stimulating the pituitary–adrenal corticol system (Alfano *et al.*, 1973; Couch and Benton, 1972; Vick *et al.*, 1972). The effectiveness of bee venom in affording protection against radiation damage in mice (Ginsberg *et al.*, 1968; Shipman and Cole, 1967) has further stimulated investigation of the activities of major components of the venom.

Many persons evince severe reactions to bee sting, and death from a single sting, generally attributed to anaphylactic shock, is not uncommon (O'Connor *et al.*, 1964). The usual pattern in such cases is one of increasingly severe reactions to bee sting, even though these may be spaced over several years. Hyposensitization treatments have proved reasonably effective and injection of a pressor amine, such as epinephrine, within a few minutes of the onset of the anaphylactic reaction is recommended as emergency treatment (O'Connor *et al.*, 1964). The venom does contain toxic compounds, but the small amount injected in a single sting is of no real consequence in this respect. The allergic reaction is a serious problem and the wives of bee keepers are particularly susceptible, possibly by development of a hypersensitive condition from inhalation of the dust from clothes worn by their husbands while working with the bees.

The composition of bee venom is now reasonably well known (Table I) and a number of its components have

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