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Sea Snake Venoms and Neurotoxins

Anthony T. Tu

Sea snakes are more numerous than terrestrial venomous snakes and are common in tropical and subtropical regions bordering the Indian and Pacific Oceans. The venoms of sea snakes contain potent neurotoxins which are more toxic than those of terrestrial snakes. Like neurotoxins of cobras, sea snake neurotoxins bind to the acetylcholine receptor of the neuromuscular junction. Toxins have been isolated from six varieties of sea snakes by a number of investigators. Among them, three were investigated in our laboratory. Molecular weights of sea snake neurotoxins are about 6800 and belong to neurotoxin Type I. They contain eight half-cystines. Unlike cobra or krait toxin, some sea snake toxins contain methionine. All sea snake toxins contain 1 mol of tryptophan which is important for toxicity but not for antigen-antibody binding activity. Current progress on sea snake toxin research by a number of investigators is summarized. Similarity of toxins in cobra, krait, and sea snake venoms is discussed.

The venom of sea snakes (family: Hydrophiidae) contains potent neurotoxins which are some of the most toxic substances in the world. They are more toxic than venoms of terrestrial snakes, including rattlesnakes, copperheads, and cobras. The sea snake venom neurotoxins act at the neuromuscular junctions and paralyze the victims.

Sea snakes are more numerous than terrestrial venomous snakes and are common in tropical and subtropical regions bordering the Indian and Pacific Oceans. They are found in the coastal waters of Baja California, Mexico, Central and South Americas, Southeast Asia, the Far East, Australia, Indonesia, Burma, India, Iran, the Arabian Peninsula (excluding the Red Sea coast), and Eastern Africa.

There have been many conflicting reports on the aggressiveness and danger of sea snakes. Apparently, some sea snakes are more aggressive, while some are quite tame. A further complexity is that the aggressiveness of sea snakes also depends on many factors, such as season and breeding cycles. However, sea snake bites are quite common and death is not unusual among the fishermen in Southeast Asia. Reid (1963) called sea snake poisoning one type of occupational hazard, as it occurs mainly in fishermen who have daily contact with the sea. Because of the highly toxic nature of the venoms, sea snake poisoning is a potential public health hazard and should not be considered lightly (Halstead, 1970; Pickwell and Evans, 1972).

YIELD OF VENOMS

In contrast to land snakes, sea snakes and their venoms have not been investigated extensively. Many of these snakes spend their entire lives in the ocean, which makes capturing enough specimens for scientific study of their venoms and neurotoxins very difficult. Moreover, the amount of crude venom that can be obtained from sea snakes is very small, ranging from 0.2 to 19 mg per snake, depending on the size of snakes (Tu and Tu, 1970). A comparison of the yield of venom from sea snakes and land snakes is made in Table I (see Figures 1-3).

TOXICITY

Even crude sea snake venoms are extremely toxic. In order to compare the toxicity of sea snake venoms to those of land snakes, the LD_{50} values are listed in Table II. The lower the LD_{50} values, the higher is the toxicity. Therefore, it is evident that most of the sea snake venoms are as toxic as cobra venoms or even slightly more toxic. Sea snake venoms are ten times more toxic than venoms of rattlesnakes (genus: *Crotalus*).

When the nontoxic components were removed by fractionation, pure toxins are even more toxic than crude venoms. Toxicities of a number of pure toxins isolated from sea snake venoms are shown in Table III.

NEUROTOXIC ACTION

The paralyzing action of *Enhydrina schistosa* venom on the isolated rat phrenic nerve diaphragm preparation clearly demonstrates a peripheral action of the venom on the neuromuscular junction (Carey and Wright, 1961). Venom of *Laticauda semifasciata* has a curare-like action as well as a direct paralytic action on the isolated frog gastrocnemius muscle (Tu, 1961). Both *Laticauda semifasciata* (Tu, 1961) and *Laticauda laticaudata* (Tu, 1967) produce marked inhibition of respiration.

Cheymol *et al.* (1967) studied the neuromuscular blocking action of venoms of *Enhydrina schistosa, Hydrophis cyanocinctus,* and *Lapemis hardwickii* from Vietnam. They concluded that all three venoms behaved similarly and specific receptors of the postsynaptic membrane were blocked almost irreversibly. On the other hand, muscle fibers and nerve fibers were not affected directly.

Erabutoxin a from *Laticauda semifasciata* venom inhibits the contraction of the rectus abdominis muscle of a frog caused by acetylcholine (Tamiya *et al.*, 1967). Crude

Department of Biochemistry, Colorado State University, Fort Collins, Colorado 80521.

Table I. Yield of Sea Snake Venoms

Snakes	Yield, mg/snake	Reference
Sea snakes		
Acalyptophis peronii	1.5	Tu (1973b)
Aipysurus eydouxii	0.6	Tu (1973b)
Enhydrina schistosa	6.9–14.0	Tu (1973b)
	4.6	Reid (1956)
Hydrophis cyanocinctus	18.0	Tu (1973b)
	4.3	Reid (1956)
Hydrophis ornatus	19.0	Tu (1973b)
Lapemis hardwickii	2.4–5.2	Tu (1973b)
	1.2	Reid (1956)
Pelamis platurus	2.0	Tu and Tu (1970)
	2.8	Pickwell *et al.* (1973)
Praescutata viperina	0.2	Tu (1973b)
Land snakes		
Naja naja kaouthia	263	Ganthavorn (1969)
Ophiophagus hannah	420	Ganthavorn (1969)
Bungarus fasciatus	114	Ganthavorn (1969)
Agkistrodon rhodostoma	59	Ganthavorn (1969)
Vipera russellii siamensis	133	Ganthavorn (1969)

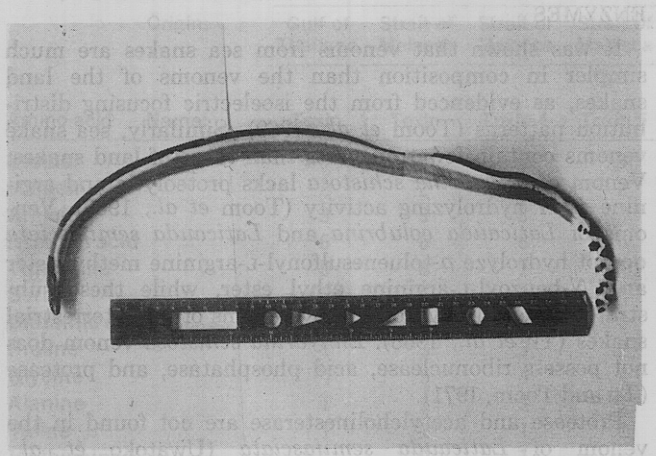

Figure 1. *Pelamis platurus* (yellow-bellied sea snake). The scale of ruler is 30 cm.

venom of *Laticauda semifasciata* stimulates the peristaltic movement of the isolated rabbit's small intestine, while pure toxins do not (Uwatoko-Setoguchi, 1970). Crude venom as well as erabutoxins a and b block the neuromuscular junction of the isolated sciatic nerve-gastrocnemius muscle or sciatic nerve-sartorius muscle preparation of frog (Uwatoko-Setoguchi, 1970). Autoradiographic studies of radioactive erabutoxin b indicate that the toxin specifically binds to the endplates of the mouse diaphragm (Sato *et al.*, 1970). Both erabutoxins a and b block the end-plate receptors without affecting the muscle fibers or acetylcholine output at the nerve ending (Cheymol *et al.*, 1972).

From the above evidence, it can be concluded that sea snake venoms and neurotoxins act on the peripheral nerves specifically by binding to the acetylcholine receptors. Thus, the action of sea snake neurotoxins is very similar to that of various cobra toxins and α-bungarotoxin. It is remarkable that the venoms of two types of different snakes, Hydrophiidae (sea snakes) and Elapidae (elapids), possess strikingly similar neurotoxic action although the former spend their entire lives in the sea while the latter live on land.

IMMUNOLOGY

Venoms of various species of sea snakes seem to contain a common antigen. By using the double diffusion method, Carey and Wright (1960) showed that venoms of *Enhydrina schistosa, Kerilia jerdonii, Microcephalus gracilis, Lapemis hardwickii, Hydrophis cyanocinctus,* and *Hydrophis spiralis* formed precipitin lines with antisera of *Enhydrina schistosa* venom. Tu and Ganthavorn (1969) demonstrated that venoms of *Hydrophis cyanocinctus, Pelamis platurus,* and *Lapemis hardwickii* cross-reacted immunologically with anti-*Enhydrina schistosa* sera. Similarly, venoms of *Praescutata viperina* show a precipitation line in immunoelectrophoresis with anti-*Enhydrina schistosa* sera, but *Laticauda semifasciata* and *Laticauda laticaudata* venoms do not form precipitins with antisera (Tu and Salafranca, 1974). Tu and Ganthavorn (1969) also showed that antivenin for *Enhydrina schistosa* is effective for neutralizing the venoms of *Hydrophis cyanocinctus, Pelamis platurus,* and *Lapemis hardwickii.* Antivenin for *Laticauda semifasciata* is effective for the neutralization of venom of *Hydrophis cyanocinctus* (Okonogi *et al.*, 1967). Barme (1963) reported that antivenin for *Lapemis hardwickii* manufactured in Vietnam effectively neutralized the venoms of *Enhydrina schistosa* and *Hydrophis cyanocinctus.* Our recent work (Tu and Salafranca, 1974) indicates that the commercial antivenin for *Enhydrina schistosa* manufactured at the Commonwealth Serum Laboratory, Melbourne, Australia, is effective for neutralizing the venoms of sea snakes *Pelamis platurus* from Central America, *Praescutata viperina* from the Gulf of Thailand, *Laticauda semifasciata* from the Philippines, and *Laticauda laticaudata* from Japan. Similarity in the toxins of cobras and sea snakes is also reflected in their immunological properties, since different cobra venoms are neutralized by the sea snake antivenin for *Enhydrina schistosa* (Minton, 1967).

Several studies using pure sea snake toxins have been made. When the tryptophan residue of two toxins isolated from *Laticauda semifasciata* (Philippine origin) venom was chemically modified with *N*-bromosuccinimide, the

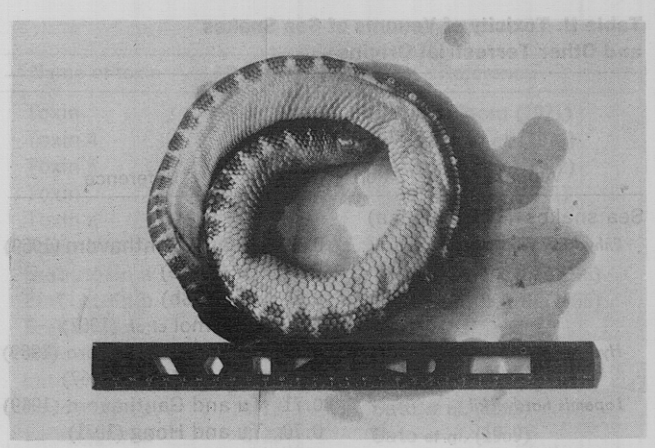

Figure 2. *Lapemis hardwickii* (Hardwick's sea snake). The length of ruler is 30 cm.

Figure 3. *Enhydrina schistosa* (common sea snake).

Table II. Toxicity of Venoms of Sea Snakes and Other Terrestrial Origins

Venoms	Route of administration	LD_{50} (mg/kg) in mice	Reference
Sea snakes (Hydrophiidae)			
Enhydrina schistosa	iv	0.09	Tu and Ganthavorn (1969)
	iv	0.14	Tu (1973b)
	iv	0.21	Tu (1973b)
	iv	0.35	Cheymol *et al.* (1967)
Hydrophis cyanocinctus	iv	0.35	Tu and Ganthavorn (1969)
	iv	0.67	Cheymol *et al.* (1967)
Lapemis hardwickii	iv	0.71	Tu and Ganthavorn (1969)
	iv	0.70	Tu and Hong (1971)
	iv	1.37	Tu (1973b)
	iv	1.40	Tu (1973b)
	iv	0.44	Cheymol *et al.* (1967)
Laticauda semifasciata	sc	0.34	Tu (1961)
	iv	0.28	Tu *et al.* (1971)
	iv	0.39	Tu and Salafranca (1974)
	iv	0.21	Tu (1961)
Laticauda laticaudata	iv	0.17	Sato *et al.* (1969)
	iv	0.16	Tu and Salafranca (1974)
Laticauda colubrina	sc	0.42	Tu *et al.* (1963)
	iv	0.4	Sato *et al.* (1969)
Pelamis platurus	iv	0.18	Tu and Ganthavorn (1969)
	iv	0.09	Pickwell *et al.* (1973)
	iv	0.11	Pickwell *et al.* (1973)
	iv	0.44	Tu and Salafranca (1974)
Praescutata viperina	iv	4.5	Tu and Salafranca (1974)
Land snakes[a]			
Elapidae (Elapids)			
Naja naja	iv	0.13	Friederich and Tu (1971)
Naja naja atra	iv	0.29	Friederich and Tu (1971)
Viperidae (Vipers)			
Bitis arietans	im	2.0	Tu *et al.* (1969)
Bitis gabonica	im	5.2	Tu *et al.* (1969)
Bitis nasicornis	im	8.6	Tu *et al.* (1969)
Vipera aspis	im	4.1	Tu *et al.* (1969)
Vipera russellii siamensis	im	2.1	Tu *et al.* (1969)
Crotalidae			
Agkistrodon acutus	iv	0.38	Friederich and Tu (1971)
Bothrops atrox	iv	1.4	Tu and Homma (1970)
Bothrops nasuta	iv	4.6	Tu and Homma (1970)
Bothrops nummifer	iv	2.4	Tu and Homma (1970)
Bothrops pieadoi	iv	1.6	Tu and Homma (1970)
Bothrops schlegelii	iv	1.6	Tu and Homma (1970)
Crotalus atrox	iv	3.6	Friederich and Tu (1971)
Crotalus adamanteus	iv	2.4	Friederich and Tu (1971)
Crotalus harridus	iv	2.6	Friederich and Tu (1971)

[a] No attempt is made to cover the toxicity of all land snake venoms. For those who wish to investigate the toxicity of various venoms of land snakes, please refer to other references such as Vick (1971) and Russell and Puffer (1971).

toxicity was completely lost (Hong and Tu, 1970). However, detoxified neurotoxins formed a precipitation line with antibody made to original venom (Tu *et al.*, 1971). Thus, the tryptophan residue is important for toxic action but not necessarily important for immunological reaction. Although erabutoxins a and b of *Laticauda semifasciata* (Japan origin) differ slightly, they formed a fused precipitation line with antierabutoxin b (Sato and Tamiya, 1970). Moreover, erabutoxin b with iodinated histidine also binds to antierabutoxin b. When erabutoxin a was modified chemically, the toxicity was lost. However, modified erabutoxin a formed a precipitation line with antierabutoxin a (Seto *et al.*, 1970).

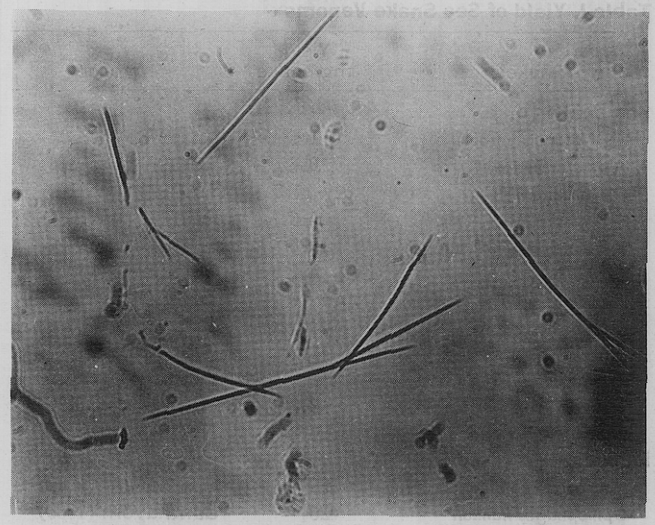

Figure 4. Crystal of purified neurotoxin isolated from the venom of *Lapemis hardwickii*.

ENZYMES

It was shown that venoms from sea snakes are much simpler in composition than the venoms of the land snakes, as evidenced from the isoelectric focusing distribution patterns (Toom *et al.*, 1969). Similarly, sea snake venoms contain fewer enzymes than those of land snakes. Venom of *Enhydrina schistosa* lacks proteolytic and arginine ester hydrolyzing activity (Toom *et al.*, 1969). Venoms of *Laticauda colubrina* and *Laticauda semifasciata* do not hydrolyze *p*-toluenesulfonyl-L-arginine methyl ester and *N*-benzoyl-L-arginine ethyl ester, while these substrates are easily hydrolyzed by venoms of some terrestrial snakes (Tu *et al.*, 1966). *Enhydrina schistosa* venom does not possess ribonuclease, acid phosphatase, and protease (Tu and Toom, 1971).

Protease and acetylcholinesterase are not found in the venom of *Laticauda semifasciata* (Uwatoko *et al.*, 1966a,b).

The presence of phospholipase A in the venom of *Enhydrina schistosa* was recognized by Carey and Wright (1960) and Ibrahim (1970). The enzyme was isolated from the venom of *Laticauda semifasciata* (Tu and Passey, 1971; Tu *et al.*, 1970; Uwatoko-Setoguchi and Obo, 1969; Uwatoko-Setoguchi 1968).

The venom of *Laticauda semifasciata* also contains phosphomonoesterase and phosphodiesterase (Uwatoko-Setoguchi, 1970). *Enhydrina schistosa* venom shows the following enzyme activities: fibrinogen clotting activity, hyaluronidase, alkaline phosphatase, phosphodiesterase, deoxyribonuclease, acetylcholinesterase, and leucine aminopeptidase (Tu and Toom, 1971). Leucine aminopeptidase activity can also be detected in the venoms of *Laticauda laticaudata* and *Hydrophis cyanocinctus* (Tu and Toom, 1967).

Although several enzymes can be found in sea snake venoms, they are not directly involved in neurotoxic action. Carey and Wright (1960b) observed that 90% of the phospholipase A activity of *Enhydrina schistosa* venom was retained inside the dialysis tube, while more than 90% of the initial toxicity passes through. Acid phosphomonoesterase and phospholipase A were seperated from the toxic components of *Laticauda semifasciata* venom (Uwatoko-Setoguchi, 1970). Several enzymes were found in the venom of *Enhydrina schistosa*, but they were separated from the toxic component (Tu and Toom, 1971).

Phospholipase A isolated from *Laticauda semifasciata* venom exhibited hemolytic activity which was greatly intensified by the addition of lecithin. The purified enzyme was nonlethal, nonhemorrhagic, and exhibited only

Table III. Toxicity of Pure Toxin

Snakes	Origin	Name of toxin	LD_{50}, mg/kg	Reference
Enhydrina schistosa	Strait of Malacca	Toxin	0.044	Tu and Toom (1971)
	Strait of Malacca	Toxin 4	0.085	Karlsson *et al.* (1972)
		Toxin 5	0.085	Karlsson *et al.* (1972)
Lapemis hardwickii	Gulf of Thailand	Toxin	0.06	Tu and Hong (1971)
Laticauda semifasciata	South China Sea, Philippines	Toxin a	0.07	Tu *et al.* (1971)
		Toxin b	0.05	Tu *et al.* (1971)
	East China Sea, Okinawa	Erabutoxin a	0.15	Tamiya and Arai (1966)
		Erabutoxin b	0.15	Tamiya and Arai (1966)
		Erabutoxin c		
	Japan	Laticatoxin III	0.015	Uwatoko *et al.* (1966a)
		Laticatoxin IV	0.021	Uwatoko *et al.* (1966a)
Laticauda laticaudata	Japan	Laticotoxin a	0.13	Sato *et al.* (1969)
Laticauda colubrina	Japan	Laticotoxin a	0.13	Sato *et al.* (1969)

Table IV. Neurotoxins from the Venoms of Sea Snakes (Family: Hydrophiidae): Amino Acid Compositions and Total Residues

		Subfamily: Hydrophiinae					Subfamily: Laticaudinae						
	Genus, species, subspecies:	*Lapemis hardwickii*	*Enhydrina schistosa*	*E. schistosa*	*E. schistosa*	*Hydrophis cyanocinctus*	*Laticauda semifasciata* (Philippines)		*Laticauda semifasciata* (Japan)			*Laticauda laticaudata*	*Laticauda colubrina*
	Origin:	Gulf of Thailand	Strait of Malacca	Strait of Malacca	Strait of Malacca	Formosa	Sulu Sea		Okinawa			Okinawa	Okinawa
									Erabutoxin				
Amino acid	Name:	Toxin	Toxin	Toxin 4	Toxin 5	Hydrophitoxin a	a	b	a	b	c	Laticotoxin	Laticotoxin
Lysine		5	5	5	5	6	4	5	4	4	3	4	4
Histidine		2	2	2	2	2	1	1	1	2	2	2	2
Arginine		3	3	3	3	3	3	2	3	3	3	5	5
Aspartic acid		6	6	6	6	6	5	4	5	4	5	9	9
Threonine		8	8	7	7	7	6	5	5	5	5	4	4
Serine		6	6	5	6	5	7	6	8	8	8	6	6
Glutamic acid		8	8	8	8	8	8	8	8	8	8	7	7
Proline		3	3	3	2	2	4	4	4	4	4	5	5
Glycine		4	5	4	4	4	5	6	5	5	5	5	5
Alanine		1	1	1	1	1	0	0	0	0	0	0	0
Valine		1	1	1	1	1	2	3	2	2	2	1	1
Methionine		1	0	1	1	1	0	0	0	0	0	0	0
Isoleucine		2	2	2	2	2	4	4	4	4	4	2	2
Leucine		1	1	1	1	1	1	1	1	1	1	1	1
Tyrosine		1	1	1	1	1	1	1	1	1	1	1	1
Phenylalanine		0	0	0	0	0	2	2	2	2	2	1	1
Half-cystine		8	9	9	9	8	8	8	8	8	8	8	8
Tryptophan		1	1	1	1	1	1	1	1	1	1	1	1
Total residue		61	62	60	60	59	62	61	62	62	62	62	62
References		Hong (1970) Tu and Hong (1971)	Tu and Toom (1971)	Karlsson *et al.* (1972)		Liu *et al.* (1973)	Hong (1970) Tu *et al.* (1971)		Tamiya and Arai (1966)		Tamiya and Abe (1972)	Sato *et al.* (1969)	

slight myolytic activity (Tu and Passey, 1971; Tu *et al.*, 1970).

CHEMISTRY OF NEUROTOXINS

Toxins have been isolated from six varieties of sea snakes (three Laticaudinae and three Hydrophiinae) by many investigators from different laboratories. Toxins were isolated from the venom of *Laticauda semifasciata* in Japan and designated as crystalline laticatoxins III and IV by Uwatoko *et al.* (1966b). Two toxins isolated from the same venom were called erabutoxins a and b by Tamiya and Arai (1966), and a third component, erabutoxin c, was also isolated (Tamiya and Abe, 1972; Uwatoko-Setoguchi, 1970). Laticotoxin was isolated from the venoms of *Laticauda laticaudata* and *Laticauda colubrina* (Sato *et al.*, 1969). Toxins a and b were isolated from the venom of *Laticauda semifasciata* from the Philippines and contain 62 and 61 amino acids, respectively (Hong, 1970; Tu *et al.*, 1971).

Neurotoxins isolated from the subfamily Hydrophiinae are from the venom of *Enhydrina schistosa* (Karlsson *et al.*, 1972; Tu and Toom, 1971), *Lapemis hardwickii* (Hong, 1970; Tu and Hong, 1971), and *Hydrophis cyanocinctus* (Liu *et al.*, 1973). Chemical properties of neurotoxins obtained from the venoms of Hydrophiinae and Laticaudinae subfamilies are not only very similar but are also similar to those of Elapidae. But the toxins isolated from Hydrophiidae and Elapidae venoms are different from those of Viperidae and Crotalidae (Tu, 1973a). Amino acid composition (Table IV) and molecular weight (Table V) of neurotoxins are very similar regardless of geographic origins and different species.

Table V. Molecular Weight of Toxins from the Venoms of Sea Snakes (Hydrophiidae)

Snake	Origin	Toxin	Methods: Amino acid composition	Methods: Sedimentation equilibrium	Methods: Gel	Methods: S and D	Reference
Hydrophiinae							
Enhydrina schistosa	Strait of Malacca	Toxin	6878			7300	Tu and Toom (1971)
		Toxin 4	6689				Karlsson *et al.* (1972)
		Toxin 5	6679				Karlsson *et al.* (1972)
Lapemis hardwickii	Gulf of Thailand	Toxin	6774	6800		6800	Hong (1970)
							Tu and Hong (1971)
Hydrophis cyanocinctus	Formosa	Hydrophitoxin a	6635				Liu *et al.* (1973)
Laticaudinae							
Laticauda colubrina	Okinawa	Laticotoxin a	6970				Sato *et al.* (1969)
Laticauda laticaudata	Okinawa	Laticotoxin a	6970	6520			Sato *et al.* (1969)
Laticauda semifasciata	Okinawa	Erabutoxin a	6850	7430			Sato *et al.* (1969)
					7000		Uwatoko-Setoguchi (1970)
		Erabutoxin b	6870	7430			Sato *et al.* (1969)
					7000		Uwatoko-Setoguchi (1970)
		Erabutoxin c			7000		Uwatoko-Setoghchi (1970)
			6847		7000		Tamiya and Abe (1972)
	South China Sea	Toxin a	6840	6800	6600		Hong (1970), Tu *et al.* (1971)
		Toxin b	6677	6500	6400		Hong (1970), Tu *et al.* (1971)

Table VI. Physicochemical Data of Sea Snake Neurotoxins

	Isoelectric point	Sedimentation coefficient, $S_{20}°$, w	Diffusion coefficient	Partial specific volume	Reference
Hydrophiinae					
Enhydrina schistosa	9.2	1.40	15.5×10^{-7}	0.70	Tu and Toom (1971)
Lapemis hardwickii	9.9	1.13	13.7×10^{-7}	0.70	Hong (1970)
					Tu and Hong (1971)
Laticaudinae					
Laticauda semifasciata					
Toxin a	9.2	1.52		0.71	Hong (1970)
Toxin b	9.3	1.43		0.71	Tu and Hong (1971)
Erabutoxin a	9.4				Uwatoko-Setoguchi (1970)
Erabutoxin b	9.4				Uwatoko-Setoguchi (1970)
Erabutoxin c	7.5				Uwatoko-Setoguchi (1970)
	9.2				Tamiya and Abe (1972)

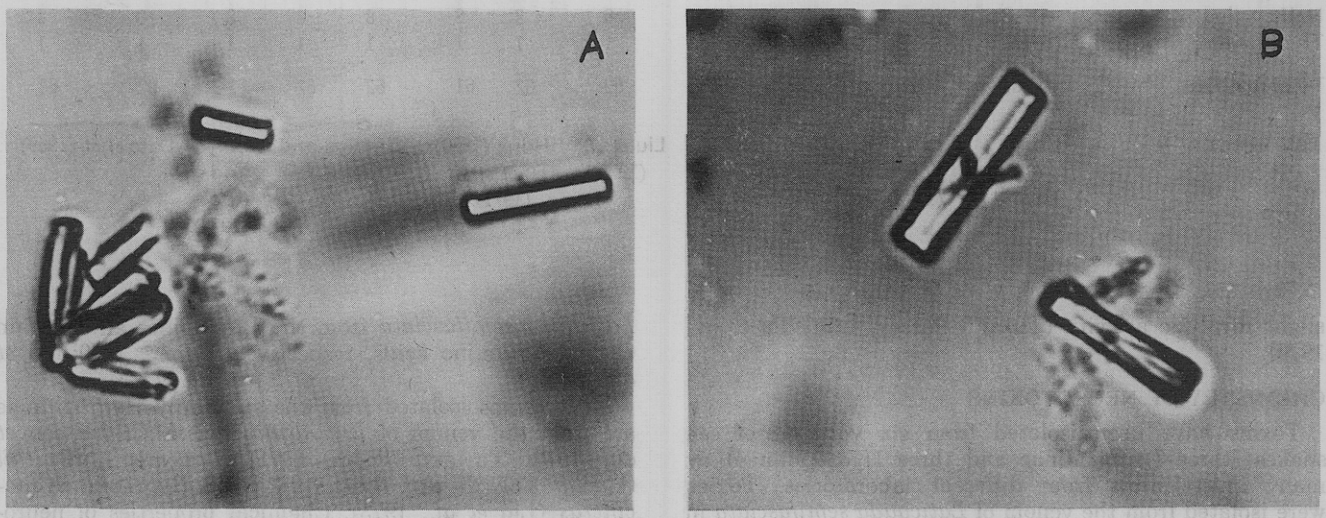

Figure 5. Crystal of purified neurotoxin a (A) and b (B) isolated from *Laticauda semifasciata* from the Philippines.

A number of sea snake neurotoxins were isolated in crystalline form (Hong, 1970; Tamiya and Arai, 1966; Tu and Hong, 1971; Tu *et al.*, 1971; Uwatoko *et al.*, 1966b). Three examples of crystals obtained from sea snake neurotoxins are illustrated in Figures 4 and 5.

A preliminary X-ray diffraction study has been made on sea snake neurotoxins (Low *et al.*, 1971; Tsernoglou *et al.*, 1972; Tu *et al.*, 1973).

Other physicochemical data of sea snake neurotoxins are shown in Table VI. Isoelectric points of all neurotoxins

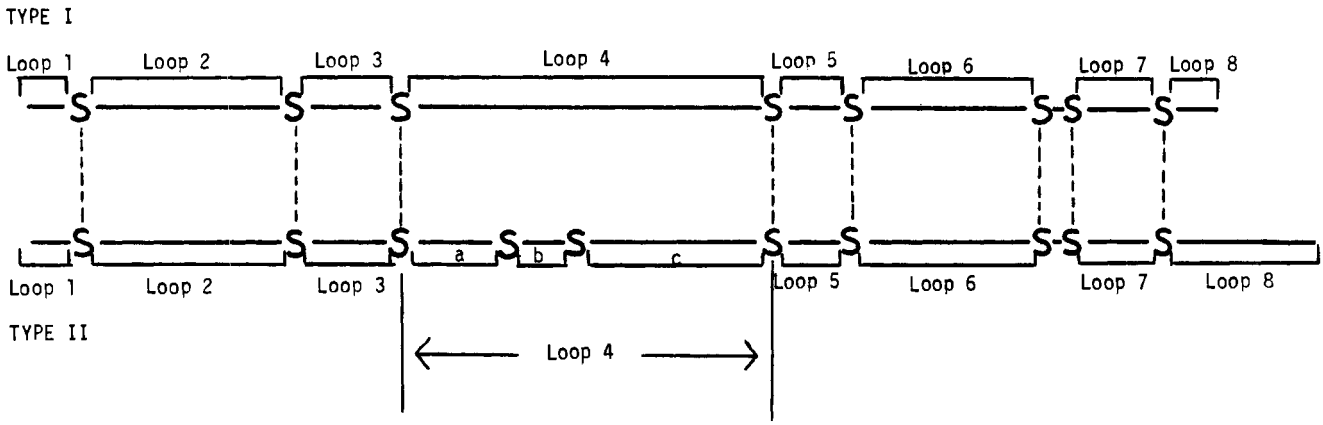

Figure 6. Schematic diagram of two types of neurotoxins. Note the similarities in the relative positions of the half-cystine residue. The figure is reprinted from *Annu. Rev. Biochem.*, **42**, (1973) by permission of the copyright owners, the Annual Review, Inc.

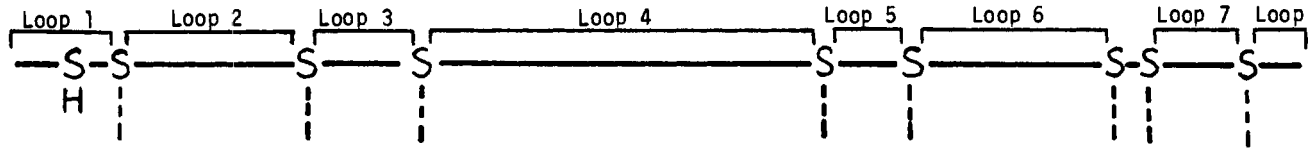

Figure 7. Schematic diagram of structure of neurotoxin isolated from *Enhydrina schistosa* venom. The complete amino acid sequence was identified by Fryklund *et al.* (1972). In this diagram, eight half-cystines used for disulfide bonds are expressed by the dotted line while the free half-cystine is expressed by –SH.

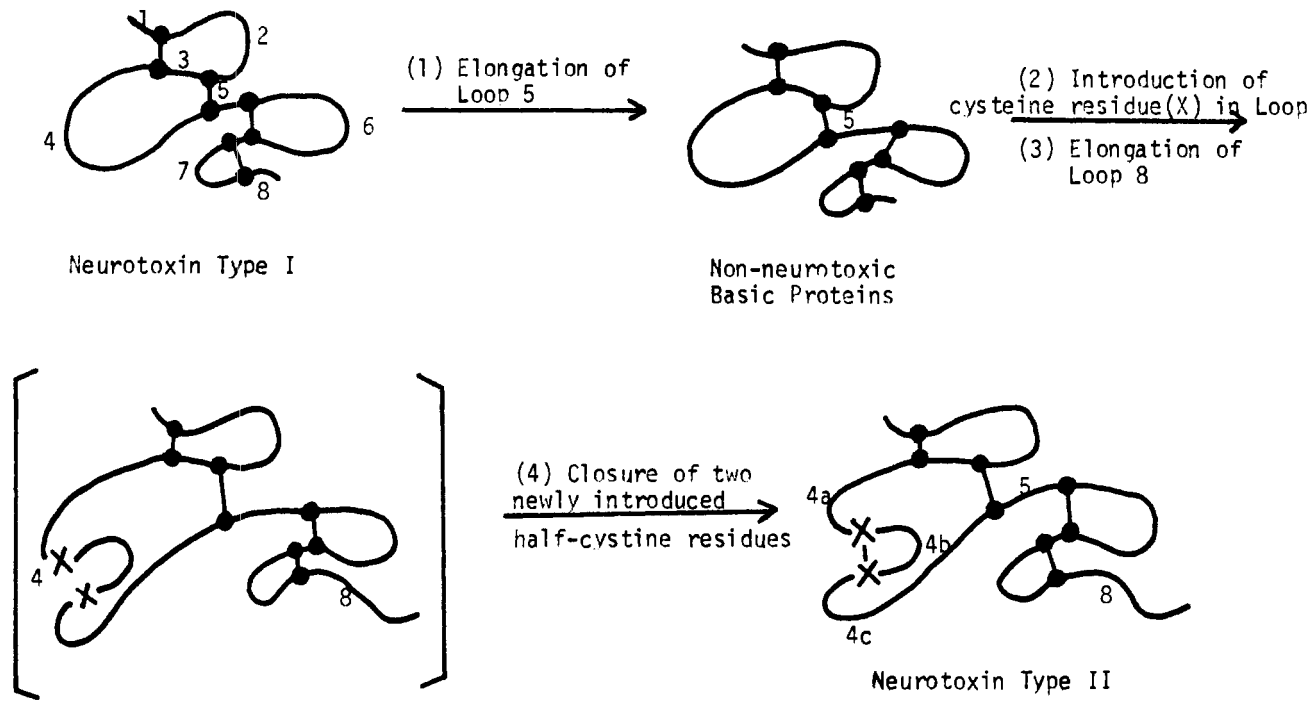

Figure 8. Two-dimensional structure of Type I and Type II neurotoxins, and nonneurotoxic basic proteins. The figure also shows the possible molecular evolution of Type I neurotoxin to nonneurotoxic basic proteins and to Type II neurotoxins. Reprinted from *Annu. Rev. Biochem.* **42**, (1973), by permission of the copyright owners, the Annual Review, Inc.

are very high, indicating that the toxins are highly basic proteins.

It has been shown by many investigators that neurotoxins from Elapine venoms consist of either 60–62 or 70–74 amino acid residues. Thus, they can be conveniently grouped into two groups on the basis of the number of amino acids. The first group is designated as Type I neurotoxins and the second as Type II (Figure 6). Detailed descriptions of these neurotoxins are discussed in a recent review article (Tu, 1973a).

All the neurotoxins so far isolated from sea snake venoms belong to Type I.

Normally, Type I neurotoxins contain four disulfide bonds and those of Type II possess five disulfide bonds (Botes, 1971; Endo *et al.*, 1971; Yang *et al.*, 1970). By comparing the two types, striking similarities in chemical structure appear (Figure 6). If we consider the fourth and fifth cysteine residues of Type II as insertion to the Loop 4 of Type I, the relative position of the rest of the toxin corresponds well for two different types of neurotoxins. Therefore, it is logical to make the assignment of Loops 4a, 4b, and 4c for the portion from the third to sixth cysteine residues in Type II neurotoxin.

In contrast to all neurotoxins from Elapidae and most

sea snake venoms, neurotoxin isolated from *Enhydrina schistosa* contains 9 mol of half-cystine instead of the usual number of 8 (Tu and Toom, 1971). However, the toxin is Type I basically, as it contains four disulfide bonds with one free cysteine residue (Fryklund *et al.*, 1972), as shown in Figure 7.

Basic proteins which are not neurotoxic have been isolated by a number of investigators from the venom of cobras (Larsen and Wolff, 1968; Narita and Lee, 1970). Although they are nonneurotoxic, the chemical structure of these basic proteins is closely related to both Type I and Type II neurotoxins. The neurotoxins and nonneurotoxic basic proteins must have evolved from a common ancestral molecule (Tu, 1973a). Investigations thus far have not shown nonneurotoxic basic proteins to be present in the venoms of the sea snakes. Based on the assumption that the more complex molecule evolved from a simpler structure, a possible chemical evolution of Type I neurotoxin to the basic proteins and then to Type II is illustrated in Figure 8.

Total amino acid sequences of neurotoxins were determined for two sea snakes. They are *Laticauda semifasciata* (Sato and Tamiya, 1971; Tamiya, 1973) and *Enhydrina schistosa* (Fryklund *et al.*, 1972).

STRUCTURE-FUNCTION RELATIONSHIP

Chemical modification of a specific functional group has been used extensively for the study of structure–function relationships in enzymes. Since the pure neurotoxins have been isolated, many investigators have applied the same techniques to these toxins. In this review article I would like to summarize the chemical modifications applied to sea snake neurotoxins.

Through iodination and nitration experiments, the single tyrosine residue contained in the neurotoxin isolated from the venom of *Lapemis hardwickii* has been shown to be essential for toxic action (Raymond, 1973; Raymond and Tu, 1972). Complete loss of lethality was achieved with up to 84% iodination of tyrosine. The concept of a somewhat buried tyrosine residue is supported by the incompleteness of nitration, even with a large excess of reagent.

Modification of the single tryptophan residue in the toxins isolated from the venom of *Lapemis hardwickii* (Hong, 1970; Tu and Hong, 1971), *Enhydrina schistosa* (Tu and Toom, 1971), and *Laticauda semifasciata* (Hong, 1970; Hong and Tu, 1970; Seto *et al.*, 1970; Tu *et al.*, 1971) caused complete loss of toxicity. Since there is only one tryptophan residue, it is clear that the tryptophan is an essential residue for toxicity.

Toxins a and b isolated from *Laticauda semifasciata* from the Philippines contain 3 and 2 mol of arginine, respectively. After modification with 1,2-cyclohexanedione, the numbers of arginine residue were reduced to 2 and 1, respectively. Thus, 1 mol of arginine per mole of toxin was modifed. No appreciable change in toxicity was observed (Hong, 1970; Tu *et al.*, 1971). When the toxins from *Laticauda semifasciata* from the Philippines were modified with *O*-methylisourea, 3 out of 4 mol in toxin a and 4 out of 5 mol in toxin b were converted into homoarginine without appreciable loss of toxicity (Hong, 1970; Tu *et al.*, 1971).

It was reported that iodination of histidine in the toxin from *Laticauda semifasciata* (from Japan) did not affect toxicity (Sato and Tamiya, 1970).

Results of chemical modification, molecular weight, amino acid composition, and other chemical studies indicate that sea snake neurotoxins are indeed similar to Elapine toxins such as cobras and kraits. Thus, toxins from Elapidae and sea snakes should be considered as "isotoxins."

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The Protein Neurotoxins in Scorpion and Elapid Snake Venoms

Dean D. Watt,* Donald R. Babin, and Ronald V. Mlejnek

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).

Department of Biochemistry, School of Medicine, Creighton University, Omaha, Nebraska 68178.

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.*,