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8

Biology of the Sea Snakes and Biochemistry of Their Venoms

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I. INTRODUCTION

With few exceptions, the venomous sea snakes are the only truly marine members of the broad range of modern caenophidian snakes. They are widely distributed throughout the warmer waters of the Indian and Pacific oceans and are conspicuously absent from the Atlantic Ocean. Sea snakes show several adaptations peculiar to marine life, including a skin which is much less permeable to water than that of land snakes and a specialized salt gland for the secretion of excess salt. Sea snakes prey largely on fish and, perhaps as a consequence of foraging on active prey in a three-dimensional forum, selection has favored the evolution of venoms among sea snakes which rapidly immobilize prey. Their venoms tend to be biochemically simple, with far fewer components than those of typical terrestrial venomous snakes; however, sea snake venoms are among the most toxic venoms known.

Potent neurotoxins are the main functional components of most sea snake venoms; they bind almost irreversibly to acetylcholine receptors. Toxin binding prevents nerve impulse transmission across the neuromuscular junction and death of prey (and occasionally snakebite

victims) occurs via muscular paralysis and subsequent respiratory failure. Prey are paralyzed almost instantaneously and neurotoxic symptoms in humans may occur quite suddenly.

Sea snake venoms have long attracted the attention of biochemists and pharmacologists because of their high toxicity. Isolated components have proved extremely useful as specific probes for the study of ion channels (neurotoxin: acetylcholine receptor) and for the study of cell membrane dynamics/composition and second messenger systems (phospholipase A_2). The relatively small size of these proteins has facilitated structure/function studies and X-ray diffraction determinations of crystal structure. Further, the neurotoxin gene has recently been cloned. Numerous scientific studies on sea snake venoms have made short-chain neurotoxins one of the best-defined ion channel probes; hence the acetylcholine receptor is one of the best understood receptors of vertebrates.

This chapter will review aspects of the biology of the sea snakes and the biochemistry of their venoms. Active research involving sea snakes encompasses many areas within pharmacology/toxicology, biochemistry, physical chemistry, ecology, behavior, morphology and natural history. The rich literature concerning these interesting creatures and their venoms thus makes a comprehensive review of the subject difficult. The authors hope that this chapter will further stimulate interested readers to pursue studies of these animals and their venoms.

II. BIOLOGY OF SEA SNAKES

A. Taxonomy and Classification

The taxonomic status of many species and, therefore, the higher-order classification of sea snakes has been in a state of flux for sometime. The truly venomous sea snakes have been assigned to various taxa by various workers. Smith (1926) considered the sea snakes a separate family, Hydrophiidae, while Harding and Welch (1980) assigned most of the sea snake species to the family Hydrophiidae, together with the Australian terrestrial venomous snakes; concomitantly, the latter workers transferred the species of *Laticauda* to the family Elapidae. More recently, Heatwole (1987) recognized two separate families, Hydrophiidae and Laticaudidae. Other authors had earlier placed the sea snakes in separate subfamilies of the Elapidae (McDowell, 1967, 1986; Underwood, 1978). McDowell (1967, 1986) provided convincing morphological evidence that the sea snakes have close affinities with certain terrestrial Australian elapids and represent a marine branch of ancestral forms. Current sea snake distribution and the high number of endemic Australian species of sea snakes, together with the

high degree of sequence homology of neurotoxins and of phospholipases from sea snakes and terrestrial elapids (Tu, 1990; Tamiya and Yagi, 1985), support the inclusion of these snakes in the single family Elapidae. However, numerous specializations, most related to marine life, suggest a close relation and/or a common origin for the various species of sea snakes, and the above-mentioned sequence homologies show greater similarities within the sea snakes than between the sea snakes and Australian elapids. For the purposes of this review, the sea snakes will be considered members of exclusive subfamilies of the Elapidae: the more generalized Laticaudinae (sea kraits) and the more specialized Hydrophiinae (true sea snakes). Species, subgenera and subfamilies of sea snakes are given in Table 1.

Presently there are approximately 52 species of venomous marine snakes known. The Laticaudinae, with the single genus *Laticauda* (includes *Pseudolaticauda* of Kharin, 1984), contains five described species. Species of *Laticauda* differ from hydrophiine sea snakes by having retained oviparity, larger ventral scales, greater mobility on land and more laterally placed nostrils. The Hydrophiinae, with 12–14 genera (depending on which synonymies and classifications are followed), includes several monotypic genera, several genera containing 2–7 species, and the large "collective" genus *Hydrophis* which contains ~22 species. Characteristic features of the subfamily include nostrils located on the top of the snout, a loosely attached premaxilla which allows flexion of the snout tip and a highly laterally compressed, paddlelike tail (McDowell, 1972).

B. Distribution

Sea snakes occur in most tropical marine waters with the notable exception of the Atlantic Ocean (Fig. 1). The most important physical parameters limiting the present-day distribution of sea snakes are surface water temperatures and water depth (Mao and Chen, 1980; Hecht *et al.*, 1974; Shuntov, 1971). Sea snake densities are often greatest in shallower coastal waters and even the broadly distributed, pelagic species, *Pelamis platurus*, is primarily an inhabitant of coastal regions. Contiguous land masses and the cold water currents along the southwestern coasts of South America and Africa effectively bar migration of sea snakes into Atlantic waters. The proposal to construct a continuous sea level canal through Panama created considerable concern in the lay press that *Pelamis platurus* would invade the Caribbean and threaten beachside resorts (Dunson, 1975a). These fears were probably justifiable, since it is likely that no biological barriers to sea snakes exist in the Atlantic. The subsequent cancellation of a continuous canal

TABLE 1
Species of sea snakes and their distribution

Species		Distribution
	Subfamily Laticaudinae	
Laticauda	*colubrina*	A, B, C, I, (IB), P, S, T
	crockeri	Solomon Ids.
	laticauda	A, B, C, I, (IB), P, S, T
	schistorhynchus	S
	semifasciata	C, I, P
	Subfamily Hydrophiinae	
Acalyptophis	*peronii*	A, C, T
Aipysurus	*apraefrontalis*	A
	duboisii	A, S
	eydouxii	A, C, I, P, T
	foliosquama	A
	fuscus	A
	laevis	A
	tenuis	A
Astrotia	*stokesii*	A, B, C, I, IA, IB, T
Disteira	*kingii*	A
	major	A
	nigrocinctus	B, IB
Emydocephalus	*annulatus*	A, S
	ijimae	C
Enhydrina	*schistosa*	A, B, C, (G), I, IA, IB, T
Ephalophis	*mertoni*	A
	greyi	A
Hydrelaps	*darwiniensis*	A
Hydrophis:	subgenus *Aturia*	
	belcheri	A, I, P, S
	bituberculatus	Sri Lanka
	caerulescens	B, C, I, IA, IB, P, T
	inornatus	A, I, P
	lapemoides	B, C, G, I, IA, (IB), T
	mammilaris	IA, IB
	ornatus	A, (G), (IA), IB, S
	stricticollis	B, IB
	torquatus	B, C, I, T
Hydrophis:	subgenus *Hydrophis*	
	brookii	A, B, C, I, T
	cantoris	B, IA, IB, T
	fasciatus	A, B, C, I, (IA), IB, T
	gracilis	A, B, C, G, I, IA, IB, T

Contd...

Table 1: Contd.

	klossi	B, T
	melanosoma	A, C, I
	obscurus	B, IB
	parviceps	S. Vietnam
Hydrophis:	subgenus *Leioselasma*	
	cyanocinctus	B, C, G, I, IA, (IB), T
	elegans	A
	melanocephalus	A, C
	semperi	Lake Taal, Philippines
	spiralis	B,C, G, I, IA, IB, (P)
Kerilia	*jerdoni*	B, C, IB, T
Lapemis	*annandalei*	C, (I), T
	curtis	IA, (IB)
	hardwickii	A, B, C, I, P, T
	viperina	B, C, G, I, IA, IB, T
Pelamis	*platurus*	A, B, C, (G), I, IA, IB, P, PA, S, T
Thalassophis	*anomalous*	I, C, T

Classification derived largely from Smith, 1926; McDowell, 1972; Cogger, 1975. Distributions largely from Minton, 1975; Tu, 1985.

Abbreviations: A = Australia/New Guinea; B = Burma/Malaysia (Andaman Sea); C = Southeast China; G = Persian Gulf; I = Indonesia; IA = West India (Arabian Sea); IB = East India (Bay of Bengal); P = Philippines; PA = Central/South America (W. Pacific Ocean); S = South Pacific (Polynesia, Micronesia, etc.); T = Thailand (Gulf of Thailand). () indicates rare or anomalous occurrence.

Figure 1: Distribution of sea snakes. Tropical and subtropical coastal regions of the Indian and Pacific oceans (shaded areas) provide suitable habitat for over fifty species of sea snakes. Sea snakes do not occur in the Atlantic ocean.

returned the question of potential survival of sea snakes in the Atlantic to academic circles.

Sea snakes are primarily marine-dwelling snakes; however, several species have been reported in fresh waters and one species, *Hydrophis semperi*, is found only in the freshwater Lake Taal on Luzon in the Philippines (Minton, 1975). *Hydrophis cyanocinctus* has also been reported from this lake (Taylor, 1922), but its occurrence in fresh water is likely sporadic and dependent on access to the lake via a narrow river. Another species of lake-dwelling sea snake is found on Rennell Island of the Solomons (Dunson, 1975b). Other species of sea snakes are occasionally reported in fresh waters (e.g., in Grand Lac, Cambodia, via the Mekong River; Bourret, 1934), typically in coastal reaches of rivers contacting the Pacific Ocean. An interesting feature of these usually marine snakes is their tolerance of low salinity; *Pelamis* has been kept in fresh water for six months without apparent ill effects (Dunson, 1957c).

The west coast of the Americas has been colonized by only one species, *Pelamis platurus* (Fig. 2A), which also has the widest distribution of all sea snakes. It is not clear why the Far Eastern Pacific should have such a depauperate sea snake fauna, but it may be that most species cannot make the long journey across open ocean required to reach the eastern Pacific coasts. Although many species (including *Pelamis*) are occasionally found far out in the open ocean, sea snakes are primarily occupants of coastal regions. Australia, Malaysia/Asia, India and eastern Africa are linked either by continuous land masses or via moderate to large islands which provide coastal habitats with only relatively short disjunctions across open waters. The present-day distribution of sea snakes may therefore represent a current or ancestral inability to disperse across large expanses of open water (such as the mid-eastern Pacific). Open water lacks refuges from marine predators and fish predation may have limited dispersion of most species (some sharks are known to prey on sea snakes: Heatwole, 1975). Foraging strategies may have also limited sea snake distribution. Most species feed in shallow (< 50 m) waters (Minton, 1975; Voris and Voris, 1983) and lack of suitable prey or forage areas may limit extended forays into open water. Regardless, coastal waters of the eastern Pacific and Indian Oceans are the centers for present-day diversity. The distribution of sea snake species is summarized in Table 1.

The tropical waters of Australia support the most diverse sea snake fauna and at least 32 species have been recorded there (Cogger, 1975). Of these, perhaps 15 species are endemic to Australian waters and representatives of all genera except *Kerilia* are found here. This high rate of endemism together with the evidence that Australian

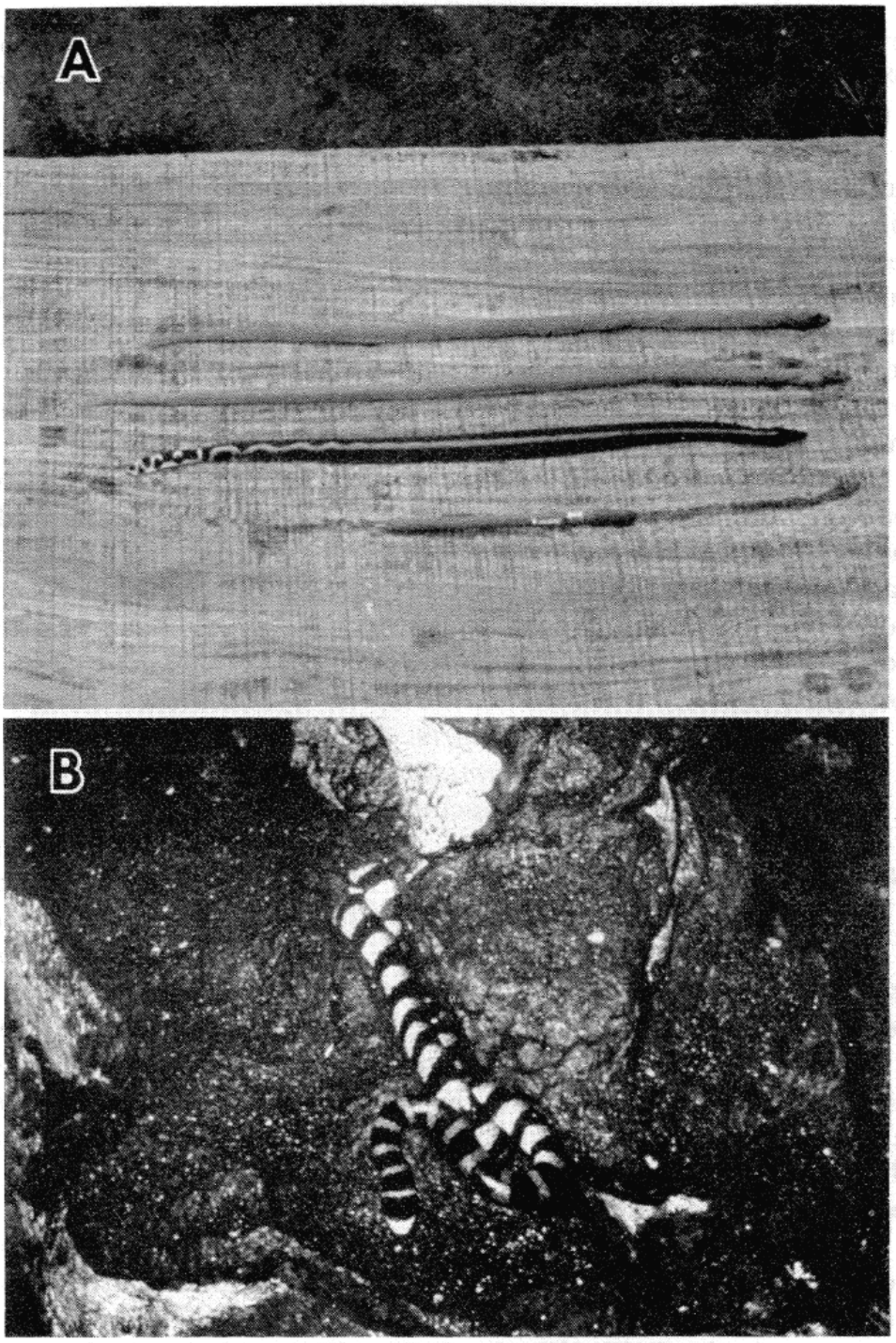

Figure 2: Representatives of the two subfamilies of sea snakes. A—The yellow-bellied sea snake (*Pelamis platurus*) is a hydrophiine sea snake and is the most widely distributed species. The specimens pictured here are a typical color morphs seen occasionally in coastal Costa Rica; the two (top) specimens are xanthic and the third is melanistic. B—A yellow-lipped sea snake (*Laticauda colubrina*) is seen among rocks in a cave on Gato Island; this species is a member of the subfamily Laticaudinae.

terrestrial elapids and sea snakes have a common ancestor (McDowell, 1986; Tamiya and Yagi, 1985) suggest that Australia was the center of the original sea snake offshoot from terrestrial ancestors.

Approximately 21 species of sea snakes are found in the coastal waters of India and Sri Lanka. However, there is little recent detailed information concerning sea snake distribution in these areas (see Walls, 1909; Deraniyagala, 1955; Deoras, 1965). Minton (1975) provided a more recent summary of sea snake distribution in Indian waters. Additional species with more recent distribution information include *Hydrophis lapemoides* (Toriba and Sawai, 1981), *Lapemis curtis* (Gawada and Bhide, 1977a) and *Enhydrina schistosa* (Gawade and Bhide, 1977b).

To the west, Indian coastal waters are part of the Arabian Sea, and to the east the Bay of Bengal borders India and Sri Lanka. For convenience, sea snake distribution off the Indian coast is considered either the Arabian Sea (IA of Table 1) or the Bay of Bengal (IB). The following species have been recorded in the Arabian Sea: *Astrotia stokesii, Enhydrina schistosa* (Fig. 4B), *Hydrophis caerulescens, H. cantoris, H. cyanocinctus H. gracilis, H. lapemoides, H. mammaliaris, H. spiralis, Lapemis curtis, L. viperina* (Fig. 3D), and *Pelamis platurus* (Fig. 2A). Species known to occur in the Bay of Bengal include: *Laticauda colubrina* (Fig. 2B), *L. laticauda, Astrotia stokesii**, *Disteira nigrocinctus, Enhydrina schistosa**, *Hydrophis bituberculatus, H. caerulescens**, *H. cantoris**, *H. fasciatus, H. gracilis**, *H. mammalaris**, *H. obscurus, H. ornatus, H. spiralis**, *H. stricticollis, Kerilia jerdoni* (Fig. 3C), *Lapemis viperina**, and *Pelamis platurus** (* denotes species which occur in both seas). Several species are found in both the Arabian Sea and the Bay of Bengal but species diversity is greatest in the Bay of Bengal. Only one species is endemic to Indian waters: *Hydrophis bituberculatus* has only been found off the coast of Sri Lanka. The distribution of the many other species of sea snakes was recently summarized (Tu, 1988b) and need not be repeated here.

Sea snake species diversity is greatest in Australian waters (approximately 32 species; Cogger, 1975) and the Malacca Straits (approx. 24 species; Minton, 1975). However, sea snake *abundance* may be greatest in the Gulf of Thailand (Tu, 1974b and unpub. data) and in the vicinity of Borneo (Sabah) (Voris, 1964; Stuebing and Voris, 1990). In both of these areas, sea snake collections, obtained primarily from fish trawlers, consisted mostly of *Lapemis hardwickii* (Fig. 3B). This species is the most commonly encountered sea snake, outnumbering all other species by at least a factor of 10. In 1989, approximately 10,000 *L. hardwickii* were obtained from Thai fishermen in a one-month pe-

riod (A.T.T.). Sea snakes are frequently netted by trawlers (Figs. 3A and B) and sea snake hides are now a profitable business venture for fishermen. In the Philippines, more than 100,000 *Laticauda semifasciata* are killed annually for their skins (Tu and Fulde, 1987). It is unclear how long sea snake populations can sustain such massive levels of harvesting.

Occasionally, sea snakes may be highly concentrated in slicks or drift lines of still waters formed by the convergence of surface water

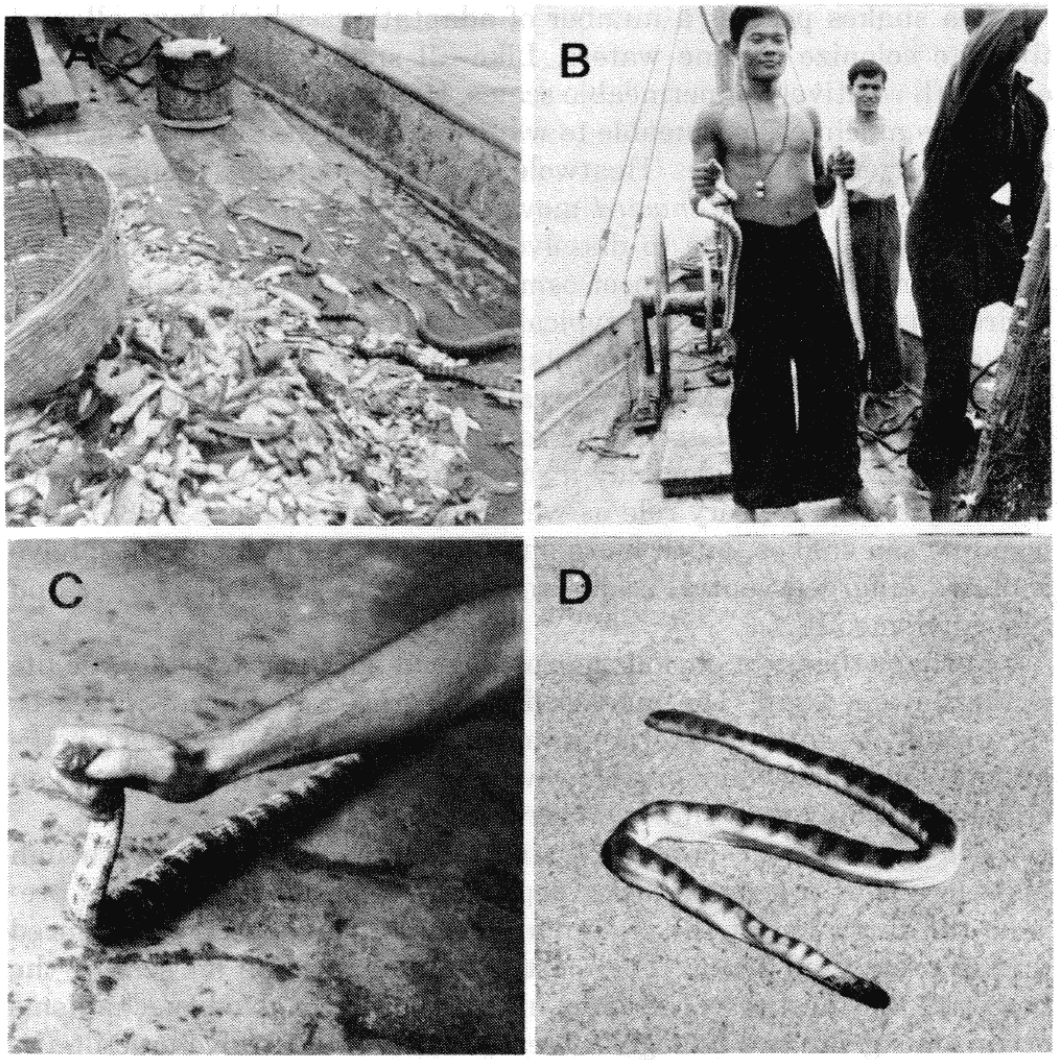

Figure 3: A—Typical net contents on a Philippine fishing boat. *Enhydrina schistosa* (far right) and two *Hydrophis* sp. (banded) are among the fish caught. B—A Philippine fisherman holds two specimens of *Lapemis hardwickii*. C—*Kerilia jerdoni*. D—*Lapemis* (formerly *Praescutata*) *viperina*.

currents. Aggregations of several hundred snakes off the coast of Central America have been reported for *Pelamis platurus* (Dunson and Ehlert, 1971; Kropach, 1971), and these aggregations appear to be the passive result of local currents. The most impressive observation of sea snake aggregations is that of W.P. Lowe (as quoted in Dunson, 1975c), who estimated that millions of *Astrotia stokesii* were concentrated in a slick ten feet wide and sixty miles long.

C. Adaptations to Marine Life

1. External Morphology

Sea snakes possess a number of adaptations which have allowed them to colonize marine waters. Like all snakes, their skin is covered with relatively impermeable scales. However, the skin of most sea snakes is much less permeable to water than are the skins of freshwater and terrestrial snakes (Heatwole, 1987). In addition, though somewhat permeable to the *inward* movement of water, sea snake skin is essentially impermeable to dissolved salts, providing the snakes an initial barrier to disruption of osmotic balance. Dorsal scales of the hydrophiine sea snakes are typically small and often exhibit tubercles and other surface adornments, and ventral scales, usually quite broad in terrestrial snakes, are greatly reduced in size. A recent study of scale structure of *Lapemis hardwickii* described surface features and suggested that scales play a role in maintaining osmotic balance and may have a sensory role as well (Gopalakrishnakone, 1985). Laticaudine sea snakes, much more amphibious than hydrophiines, have retained enlarged ventral scales and the dorsal scales are larger and typically smooth.

Perhaps the most obvious morphological adaptation to aquatic life is the flattened tail. The increased surface area of the tail increases the propulsive force of lateral undulations of the body and sea snakes are quite capable swimmers. However, on land, most hydrophiines can crawl about only very slowly (Heatwole, 1978).

Sea snakes are capable of diving to depths of 50–100 m (though most forage at much shallower depths) and specialized features prevent the intrusion of salt water at the great pressures experienced during protracted diving. The most unique adaptation for sealing the body are the nostril valves and independently evolved mechanisms occur among the two lineages of sea snakes. Both involve erectile tissues which block the nares when engorged with blood. In hydrophiines, a flap-like valve closes the opening, while in laticaudines swelling of the adjacent tissues closes the nostril.

2. ANATOMY

a. Lung and respiration: Sea snakes are highly adapted to marine life and many species can remain submerged for 30 minutes or more. This ability is partially explained by the low basal metabolic rate of reptiles in general. In addition, reptiles can function anaerobically for sometime but an oxygen debt is incurred, which must be repaid via aerobic metabolism. Like most terrestrial snakes, sea snakes have only one functional lung (the left lung is extremely reduced) but in sea snakes this lung is much larger than the lung of terrestrial species. There are three structurally distinct lung regions and only the anterior two (the trachial and bronchial regions respectively) are involved in gas exchange. The saccular lung, elongated posteriorly, serves as an air storage device (Heatwole, 1987).

Cutaneous respiration is an important means of gas exchange for most amphibians and some reptiles (e.g., Porter, 1977). For most terrestrial snakes, it is of minor importance, but for hydrophiine sea snakes cutaneous respiration is an important mode of oxygen uptake. Sea snakes such as *Lapemis, Aipysurus* (Fig. 4A), and *Acalyptophis* have a rate of cutaneous oxygen uptake approximately 10 times that of terrestrial snakes (Heatwole, 1987), and the semipelagic species *Pelamis platurus* has a capacity 35 times that of land snakes. Conversely, two tested species of *Laticauda* showed cutaneous respiration rates not significantly different from terrestrial snakes. Again, features of laticaudines differ from hydrophiine sea snakes, supporting the arguments that these subfamilies represent different derivations from terrestrial ancestors.

b. Salt gland: Vertebrates which have limited access to fresh water (e.g., desert dwellers) or which live in marine environments frequently have a specialized organ known as a salt gland (Schmidt-Knielsen and Fange, 1958; Schmidt-Knielsen, 1979). This gland secretes "excess" salt obtained in foods or salt water which the kidneys could not otherwise process. The salt gland of sea snakes is uniquely located beneath the tongue (Dunson *et al.*, 1971) and secreted salts are carried out by tongue extension. Aspects of the ultrastructure of the gland cells and gland function are further discussed by Dunson (1975b). Surprisingly, there is great variation in the size and capacity of the salt gland from various species, but even in a freshwater form (*Hydrophis semperi*) it remains functional. This variation may result from dietary differences or differential abilities of various species to regulate skin permeability to salts (primarily NaCl); a direct positive relation of secretory capacity to degree of cutaneous respiration has also been suggested.

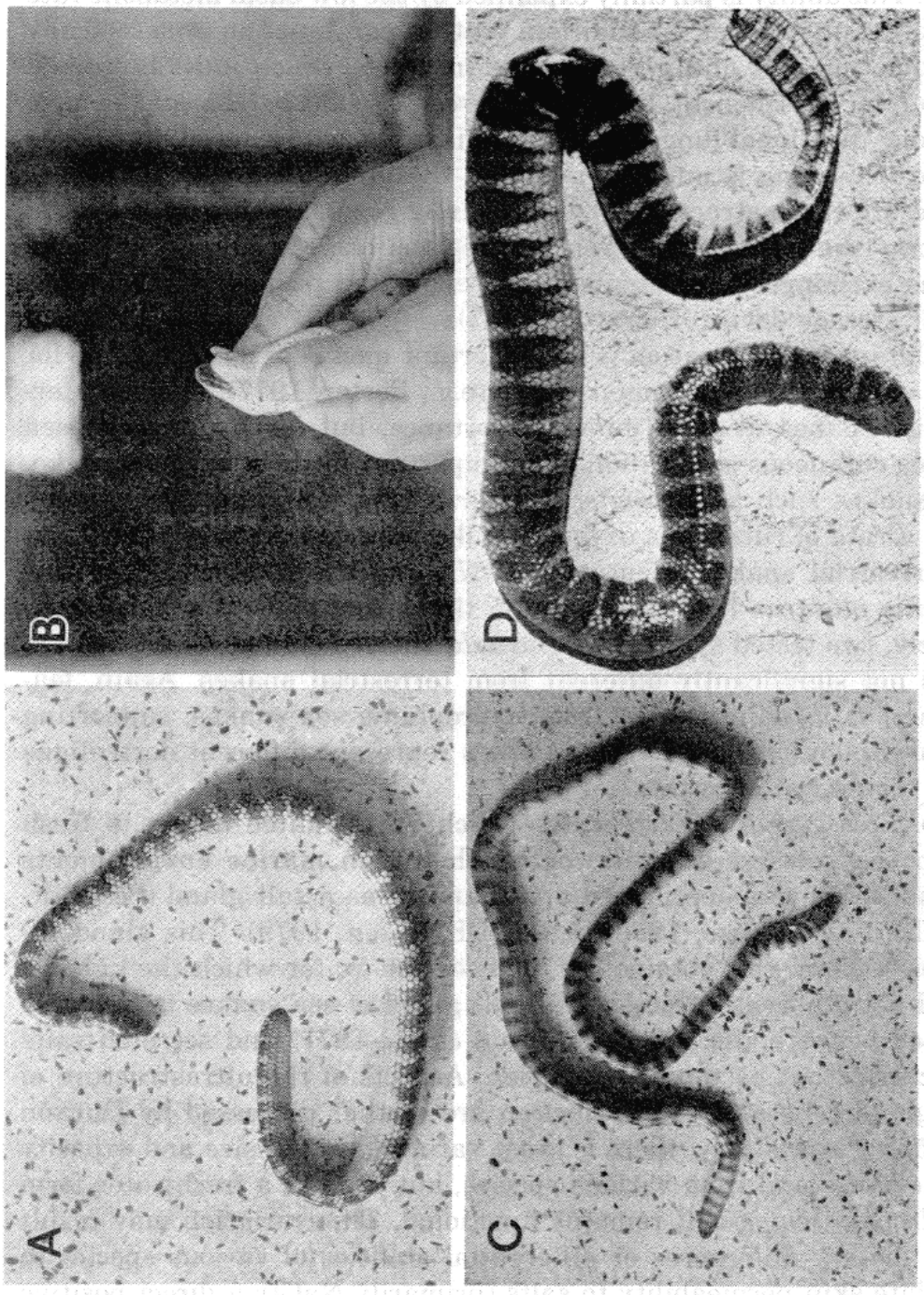

Figure 4: Representatives of several genera of sea snakes. A—*Aipysurus eydouxii*. B—*Enhydrina schistosa* (note characteristic "split lip"). C—*Hydrophis cyanocinctus*. D—*Laticauda semifasciata*.

D. Diet Specializations

Not surprisingly, the diets of most sea snakes consist largely of fish (Voris *et al.*, 1978; Glodek and Voris, 1982; Voris and Voris, 1983; Zimmerman *et al.*, 1990). The most extensive report on the feeding behavior of sea snakes is that of Voris and Voris (1983) and involved analysis of >1060 stomach contents from 39 species. Several generalizations followed from the Voris' work. Although nearly 1/3 of the families of fish from shallow marine waters of the Indo-Australian region are prey of sea snakes, many species of sea snakes show low prey diversity, with a single fish species accounting for ~50 percent of the total diet (Glodek and Voris, 1982). Fish belonging to the eel and goby families are taken by sea snakes in the greatest numbers; most of these fish are bottom-dwelling, relatively sedentary species. Specialization on particular prey is characteristic of some species, but no clear patterns of foraging strategy relation to phylogeny appeared from analysis. Eel specialists include *Laticauda colubrina* (Pernetta, 1977), *L. laticauda* (Moriguchi, 1988), *Hydrophis brooki, H. fasciatus, H. gracilis, H. melanocephalus* and *H. melanosoma* (Voris and Voris, 1983). Some of these eel specialists, such as *H. fasciatus*, have extremely small heads but are capable of swallowing prey ~ 1.5 times the neck diameter. *Emydocephalus annulatus* (Voris, 1966) and *Aipysurus eydouxi* (McCarthy, 1987) appear to specialize on fish eggs; short fang length (0.6 and 1.0 mm respectively) and diminutive venom glands (Gopalakrishnakone and Kochva, 1990) may be correlated with this unusual diet.

Contrasting sharply with the eel and fish egg specialists are the generalists such as *Lapemis hardwickii*. Dietary analyses revealed that this species includes 21 families of fish, cuttlefish and squid as prey (Glodek and Voris, 1982). *Aipysurus laevis* and *Pelamis platurus* also exhibit a generalist feeding strategy, though the latter appears to feed primarily on juvenile and larval fish of many species (Voris and Voris, 1983; Kropach, 1971; Paulson, 1967).

Dietary overlap of any two species of sea snakes is greatest when they are not sympatric. When two or more species occur at one locality, diet divergence may lessen potential competition. For further discussion on sea snake feeding strategies and diet, the reader is referred to Voris and Voris (1983). For a general discussion of sea snake feeding, see Heatwole (1987).

E. Reproduction

Laticaudine and hydrophiine sea snakes represent divergent lineages of the venomous marine snakes, and their reproductive biology

differs as well. Species of *Laticauda* are oviparous (M.C. Tu *et al.*, 1990; Nakamoto and Toriba, 1986; Toriba and Nakamoto, 1987) and possibly ovoviviparous; all hydrophiine species are viviparous (Lemen and Voris, 1981; Smith, 1926). *Laticauda* thus must come ashore to lay eggs, and these snakes often form "breeding colonies" on small isolated islands such as Gato Island in the Philippines (Tu, 1988b) and Orchid Island near SE Taiwan (M.C. Tu *et al.*, 1990).

The incubation period for *L. semifasciata* (Fig. 4D) eggs is relatively long and both lab (Nakamoto and Toriba, 1986) and field (Tu *et al.*, 1990) incubations required 4–5 months. On Orchid Island, eggs were laid in muddy fresh waters, protected from sea water, and the average clutch was 4 eggs. *Laticauda* eggs may be less permeable to water than the eggs of terrestrial snakes since the latter will usually rot if even partially submerged in water (pers. obs.)

The reproductive strategies of 13 species of hydrophiine sea snakes have been summarized by Lemen and Voris (1981). Like *Laticauda*, most clutches were small (averaging 3–7 young) for these viviparous species, with the notable exception of *Enhydrina schistosa* (Voris and Jayne, 1979). The clutch for this large species averaged 17 young, with a maximum number of 34. The average size for neonate *Enhydrina* is ~220 mm, approximately 1/2 that of newly hatched *L. semifasciata*. Female *Enhydrina* first give birth in their second year and few individuals survive past four years. Clutch size correlated positively with female body weight for most of the species studied.

F. Venom Apparatus: Gland and Fangs

1. Morphology

Compared to terrestrial snake species, the morphology of sea snake venom apparatus has received very little attention (Kochva, 1978). Halstead *et al.* (1978) described the venom apparatus of *Lapemis hardwickii* and Limpus (1978a) described the venom apparatus of several Australian species. Recently, however, Gopalakrishnakone and Kochva (1990) have described the morphology of venom glands of 16 species of sea snakes. These works allow some generalizations to be made about the venom apparatus of sea snakes.

Like most terrestrial elapids, the short hollow fangs of sea snakes are positioned at the anterior end of the maxilla, which is capable of only limited anterior/posterior movement. Sea snake fangs are usually quite small and in adult snakes range in length from 0.6 mm (*Emydocephalus*, a fish egg specialist) to 4.2 mm (*Aipysurus laevis*, a generalist) (Voris and Voris, 1983). Contraction of the well-developed compressor glandulae muscle forces venom through the fangs and into

the prey and muscular paralysis via the action of venom neurotoxins rapidly immobilizes the prey.

The venom gland structure of sea snakes is similar in many respects to that of terrestrial elapids but the gland is notably reduced in size. The accessory gland, an anterior specialized region of the venom gland, is quite reduced and in this respect resembles the gland structure of the Australian elapid *Notechis* (Gopalakrishnakone and Kochva, 1990). The accessory gland shows extreme reduction in some species, and is present as a single row of mucous tubules in the glands of *Lapemis hardwickii* and *Acalyptophis peronii*. The venom glands of those species specializing on fish eggs are further reduced, and it appears that selective pressures favoring the elaboration of the venom gland as a trophic adaptation have been relaxed for these specialized forms.

Laticauda colubrina has a somewhat larger venom gland than found in hydrophiine sea snakes, with features similar to a seperate group of terrestrial elapids (Gopalakrisnakone and Kochava, 1990). As with the hydrophiines, the accessory gland is also narrow and quite reduced. Sea snake gland morphology is structurally quite conservative, with much greater similarity to typical elapid patterns than to a typical crotalid, such as *Crotalus viridis oreganus* (Mackessy, 1991).

2. Adaptive Significance of Sea Snake Venoms

Sea snake venoms lack the biochemical complexity (in terms of number of components) found in venoms of other venomous caenophidian snakes, but they are among the most toxic snake venoms known (Tu, 1977). Snake venoms are primarily trophic adaptations, assisting the snake in prey manipulation and/or conditioning (Gans, 1961; Savitsky, 1980). Two main general trends in venom composition are seen among the front-fanged venomous snakes. It should be noted, however, that many exceptions to these trends exist; in general, the following points apply to the majority of species within the respective groups.

One trend, typified by viperid and crotalid snakes, is to produce a venom rich in lytic enzymes and toxins which initiate prey digestion and immobilize prey rapidly. These snakes tend to feed on larger mammalian prey and the predigestive function of these venoms has allowed crotalids and viperids to exist at higher latitudes and altitudes than similarly proportioned (but non-venomous) advanced snakes (Thomas and Pough, 1979). Venoms with high proteolytic activity towards structural proteins also appear to have allowed crotalids and viperids to

colonize thermally variable environments, such as north temperate deserts (Mackessy, 1988).

A second general trend in venom composition is seen among the elapids (here including the sea snakes). Elapid venoms have fewer lytic enzyme components but typically include numerous neurotoxic components. Many elapids feed on elongate, narrow prey, such as eels, other snakes and caecilians, and the large relative surface area of these prey (available to degradation by snake gut digestive enzymes) precludes the adaptive advantage of a highly lytic venom. Further, most elapids occur in subtemperate to tropical regions, where daily and seasonal temperature fluctuations are not as extreme as in temperate latitudes. Elapids are therefore largely freed from ecological constraints incurred by bulky prey and temperature instability, and their venoms have become specialized as agents for killing prey rapidly. Elapid venoms are typically 10–20 times more toxic than cortalid/viperid venoms and the primary selective pressure favoring these highly toxic venoms is rapid paralysis/immobilization of dangerous or elusive prey.

Sea snakes have taken the reduction of the number of venom components to an extreme. Venom from a single species may contain very few components and the major functional components of these venoms are the short-chain postsynaptic neurotoxins (Mori and Tu, 1988 and others). A single neurotoxin (and isoforms) is the major component of venom expressed from the fangs and such toxins are extremely potent (typical LD_{50} values are < 100 ng/g mouse; Tu, 1988a, 1990). Feeding on rapidly moving prey in a marine environment has favored the evolution of these potent neurotoxins. Prey is typically held until quiescent, as struggling prey could damage the snake. Conversely, a strike-and-release strategy (typical of crotalids feeding on adult rodents) may be ineffective in water, since prey could then retreat in any of three dimensions. Sea snakes do not have high visual acuity and scent cues are probably absent or unstable due to local currents, so prey which is released is most likely lost unless paralyzed or killed more or less instantaneously. In an evolutionary sense, sea snakes seem to have opted for a venom largely dependent on a single component, the postsynaptic neurotoxins. The few enzymatic activities detected in sea snake venoms (see Tu, 1990) may therefore represent evolutionary remnants of their elapid ancestry, and the enzyme components are more typical of elaped venom enzyme components than those of crotalid/viperid venoms. Selection has favored the production of biochemically complex venoms among crotalids and viperids, while among sea snakes, secondary loss of complexity with concomitant increase in toxicity has been favored. In this context, it may be noteworthy that the *least* toxic sea snake venoms ($LD_{50} \sim 2.5$–12 μg/g mouse, 10–100 times less toxic

than most species) are produced by species feeding on fish eggs (Limpus, 1978b; Minton, 1983). A rapid acting, highly toxic venom has little function against immobile, defenseless prey; it would be interesting to determine whether these venoms contain a greater repertoire of digestive enzyme components or if enzymes account for a greater proportion of venom components.

III. TOXICOLOGY, PHARMACOLOGY AND PATHOLOGY OF SEA SNAKE VENOMS

A. LD_{50} and Toxicity Data

1. Crude Venoms

Sea snake venoms are among the most potent snake venoms known. Only among the Australian elapids are venoms of comparable toxicity found and, in fact, several protein components share a high degree of amino acid sequence homology. The primary reason for this high toxicity is the preponderance of the main functional component, a postsynaptic neurotoxin. Sea snake venoms lack enzymatic complexity and therefore the neurotoxin(s) make up a sizeable fraction of the crude venom protein. Most enzymatic components are much less toxic and their relative absence from sea snake venoms results in a venom with a very high percentage of neurotoxin content.

A list of representative sea snake venom toxicities is given in Table 2. LD_{50} values are typically derived from experiments with lab mice (often female Swiss/Webster strain, 20–25 g) and these data provide a comparative index of toxicity which is easily replicated. However, it is probable that these venoms evolved as adaptations to immobilize fish prey and mammalian test animals may overlook the biological relevance of specific venom fractions; only a few workers have investigated the effect of these venoms of fish prey (Berman, 1983); Zimmerman *et al.*, 1990).

Sea snake venoms typically have IV LD_{50} values below 0.5 μg/g mouse (Table 2). Toxicity is apparently correlated with diet: those species which are fish egg specialists (such as *Aipysurus eydouxii)* have much less toxic venoms, while related forms which feed on fish (such as *Aipysurus duboisii*) produce highly toxic venoms. Venom yields from sea snakes are low compared to most venomous land snakes but high toxicity compensates for low venom availability. Low yields are likely responsible for the low incidence of fatality following human envenomation by sea snakes.

TABLE 2

Toxicities of sea snake venoms

Table 2: Contd.

Species		Route	LD_{50} (μg/g)	Reference
		Subfamily Laticaudinae		
Laticauda	*colubrina*	SC	0.42	Tu *et al.* (1963)
		SC	0.45	Levey (1969)
		IV	0.40	Vick *et al.* (1975)
	crockeri	—	—	
	laticauda	IV	0.17	Sato *et al.* (1969)
		IV	0.16	Tu & Salafranca (1974)
	schistorhynchus	—	—	
	semifasciata	SC	0.34	Tu (1961)
		SC	0.37	Baxter and Gallichio (1976)
		IV	0.21	Tu (1961)
		IV	0.23	Tu *et al.* (1971)
		IV	0.39	Tu and Salafranca (1974)
		IV	0.30	Vick *et al.* (1975)
		IV	0.33	Baxter and Gallichio (1976)
		Subfamily Hydrophiinae		
Acalyptophis	*peronii*	SC	0.08	Minton (1983)
Aipysurus	*apraefrontalis*	—	—	
	duboisii	SC	0.04	Minton (1983)
	eydouxii	IV	> 4.00	Tu (1974a, b)
		IV	> 11.70	Limpus (1978a, b, c)
	foliosquama	—	—	
	fuscus	—	—	
	laevis	SC	0.08	Barber *et al.* (1974)
		SC	0.30	Baxter and Gallichio (1976)
		IM	0.50	Barber *et al.* (1974)
		IV	0.18	Maeda and Tamiya (1978)
	tenuis	—	—	
Astrotia	*stokesii*	SC	0.30	Baxter and Gallichio (1976)
		IM	3.50	Barber *et al.* (1974)
		IV	0.19	Baxter and Gallichio (1976)
		IV	0.32	Limpus (1978a, b, c)
Disteira	*kingii*	—	—	
	major	IV	0.21	Limpus (1978a, b, c)
	nigrocinctus	SC	0.34	Baxter and Gallichio (1976)
Emydocephalus	*annulatus*	SC	<2.50	Minton (1983)
	ijimae	—	—	
Enhydrina	*schistosa*	SC	0.13	Baxter and Gallichio (1976)
		SC	0.16	Broad (1979)
		IP	0.11	Carey and Wright (1960)
		IV	0.35	Cheymol *et al.* (1967)
		IV	0.09	Tu and Ganthavorn (1969)
		IV	0.14–0.21	Tu (1974a, b)
		IV	0.10–0.34	Baxter and Gallichio (1976)
		IV	0.07–0.21	Gawade *et al.* (1981)

Table 2: Contd.

Ephalophis	*mertoni*	—	—	
	greyi	—	—	
Hydrelaps	*darwiniensis*	—	—	
Hydrophis:	subgenus *Aturia*			
	belcheri	IM	0.07	Barber *et al.* (1974)
		IM	0.24	Tamiya and Puffer (1974)
	bituberculatus	—	—	
	caerulescens	—	—	
	inornatus	—	—	
	lapemoides	—	—	
	mammilaris	—	—	
	ornatus	IV	2.20	Tu (1974)
		IM	0.12	Baxter and Gallichio (1976)
	stricticollis	SC	0.16	Baxter and Gallichio (1976)
	torquatus	—	—	
Hydrophis:	subgenus *Hydrohis*			
	brookii	—	—	
	cantoris	—	—	
	fasciatus	IV	0.18	Barme (1963)
	gracilis	SC	0.55	Madsen and Lundstrom (1979
	klossi	IP	0.20–0.53	Carey and Wright (1960)
	melanosoma	IP	0.40	Carey and Wright (1960)
	obscurus	—	—	
	parviceps	—	—	
Hydrophis:	subgenus *Leioselasma*			
	cyanocinctus	SC	0.53	Romer (1965)
		IP	0.24	Carey and Wright (1960)
		IP	0.18	Tu and Toom (1971)
		IP	0.20	Yang and Lee (1976)
		IV	0.35	Tu and Ganthavorn (1969)
		IV	0.31	Baxter and Gallichio (1976)
		IV	0.57	Bhise and Bhide (1978)
		IV	0.67	Madsen and Lundstrom (1979)
	elegans	SC	0.30	Baxter and Gallichio (1976)
		IM	0.30	Cheymol *et al.* 1967)
		IV	0.28	Baxter and Gallichio (1976)
		IV	0.12–0.27	Limpus (1978a, b, c)
	melanocephalus	SC	0.11	Minton (1983)
		IM	0.08	Tamiya and Puffer (1974)
	semperi	—	—	
	spiralis	IP	0.25–0.38	Carey and Wright (1960)
Kerilia	*jerdoni*	IP	0.53	Carey and Wright (1960)
Lapemis	*annandalei*	—	—	
	curtis	—	—	
	hardwickii	IP	0.26	Carey and Wright (1960)
		IV	0.44	Cheymol *et al.* (1967)
		IV	0.71	Tu and Ganthavorn (1969)
		IV	0.70	Tu and Toom (1971)
		IV	0.40–1.37	Tu (1974a)

Contd...

Table 2: Contd.

	viperina	IV	4.50	Tu and Salafranca (1974)
Pelamis	*platurus*	SC	0.07	Minton (1983)
		IV	0.18	Tu and Ganthavorn (1969)
		IV	0.44	Bolanos (1972)
		IV	0.09–0.11	Pickwell and Evans (1972)
		IV	0.29	Maeda and Tamiya (1978)
Thalassophis anomalous		—	—	

Route refers to method of administration of venom: SC = subcutaneous; IP = intraperitoneal; IM = intramuscular; IV = intravenous (tail vein). Venom toxicities are expressed in μg venom/g mouse body weight. Females of Swiss/Webster strains are typically used. — = LD_{50} values not available.

2. Purified Venom Components

High toxicity of venoms and paralysis after envenomation strongly indicate a neurotoxic component in sea snake venoms and a number of postsynaptically active short-chain neurotoxins have now been isolated from venoms of several species of sea snakes. They show very high toxicity (Table 3) and most have LD_{50} values in mice well below 0.1 μg/g. These toxins all bind very tightly to the acetylcholine receptor derived from the neuromuscular junction or *Torpedo* electroplax organ tissue (Tu *et al.*, 1976; Allen and Tu, 1985).

A second toxic component of sea snake venoms is phospholipase A_2. This enzyme has several biological activities, which include membrane disruption and myonecrosis, and some exhibit neurotoxic actions as well. Phospholipase A_2 is also a major component of sea snake venom. Many of these enzymes are quite toxic, although a "nontoxic", nonenzymatic homologue of phospholipase A_2 (from *Laticauda colubrina* venom) has recently been isolated and sequenced (Takasaki *et al.*, 1988.

B. Neurotoxins—Interaction with the Acetylcholine Receptor

Snake venoms contain several types of neurotoxins which act pre- or postsynaptically at the neuromuscular junction; presynaptic neurotoxicity is apparently due to phospholipase A_2. Most sea snake toxins act postsynaptically, binding to the acetylcholine receptor. These toxins are small basic proteins of approximately 6,000–8,000 daltons and consist of 60–70 amino acids. These toxins produce a flaccid paralysis by blocking the acetylcholine binding site on the receptor, thereby making it nonreceptive to acetylcholine. Sea snake neurotoxins bind very tightly and specifically to the acetylcholine receptor and its primary effects are mitigated at the neuromuscular junction. However, neurotoxin affects different muscle tissues differently, suggesting that

TABLE 3
Toxicities of purified sea snake venom neurotoxins

Species and Toxin	Route	LD_{50} (μg/g)	Reference
	Subfamily Laticaudinae		
Laticauda semifasciata			
Erabutoxin a	IM	0.15	Tamiya and Arai (1966)
Erabutoxin b	IM	0.15	Tamiya and Arai (1966)
Toxin a	IV	0.07	Tu *et al.* (1971)
Toxin b	IV	0.05	Tu *et al.* (1971)
	Subfamily Hydrophiinae		
Aipysurus laevis	IM	0.8	Maeda and Tamiya (1978)
Astrotis stokesii			
Toxin a	IM	0.13	Maeda and Tamiya (1978)
Toxin b	IM	0.10	Maeda and Tamiya (1978)
Toxin c	IM	0.10	Maeda and Tamiya (1978)
Enhydrina schistosa			
Cm-IV-Sa	IM	0.05	Gawade and Bhide (1978)
Major toxin	IV	0.04	Tu and Toom (1971)
Enhydrotoxin a	IV	0.04	Gawade and Gaitonde (1982a)
Enhydrotoxin b	IV	0.05	and Gaitonde (1982b)
Enhydrotoxin c	IV	0.05	Gawade and Gaitonde (1982b)
Hydrophis cyanocinctus			
Toxin	IV	0.05	Su *et al.* (1984)
Lapemis hardwickii			
Lapemis toxin	IV	0.06	Tu and Hong (1971)
Pelamis platurus			
Pelamis toxin a	IV	0.4	Tu *et al.* (1971)
Pelamis toxin b	IV	0.15	Tu *et al.* (1971)
Pelamis toxin c	IV	0.31	Tu *et al.* (1971)

Route refers to method of administration of toxin. SC = subcutaneous; IP = intraperitoneal; IM = intramuscular; IV = intravenous (tail vein). Venom toxicities are expressed in μg venom/g mouse body weight. Females of Swiss/Webster strains are typically used.

differences exist in the affinity of neurotoxin for various subtypes of acetylcholine receptors (Walker and Yeoh, 1974; Ishikawa and Shimada, 1983). Mammals typically succumb from respiratory paralysis (Tu *et al.*, 1976; Yang and Lee, 1976), suggesting that the neurotoxins may bind preferentially to the acetycholine receptors of the phrenic nerve-diaphragm endplate.

C. Phospholipase A_2

Phospholipase A_2 is hydrolytic enzyme common to most animal venoms (Rosenberg, 1990) and is a component of sea snake venoms as well. Sea snake phospholipases have numerous potential biologi-

cal activities, including neurotoxic, myotoxic and hemolytic activities. Considerable discussion has been generated concerning the relation of enzymatic and toxic activities of various phospholipases A_2 (see Rosenberg, 1990) and the role of enzyme activity to toxicity is not yet settled. Unlike other neurotoxic snake venom phospholipases, which possess presynaptic neurotoxicity, phospholipase A_2 from *Laticauda semifasciata* venom appears to exhibit postsynaptic neurotoxicity (Harvey *et al.*, 1978; Harvey and Tamiya, 1980). The mechanism of this action has not been elucidated.

1. Myonecrosis

General tissue necrosis is not a typical manifestation of sea snake envenomation, but the occurrence of myonecrosis is apparent from the frequent observation of myoglobinuria in humans bitten by sea snakes (Reid, 1974, 1975a, b). Muscle damage results from the action of phospholipase A_2 in the venom of *Laticauda semifasciata*; however, this enzyme has low lethal toxicity (Tu and Passey, 1972). The enzyme from *Enhydrina schistosa* venom is both toxic (Fohlman and Eaker, 1977) and myonecrotic (Sutherland *et al.*, 1981) and has several other biological activities as well (Tu, 1988b). LD_{50} values for phospholipases isolated from sea snake venoms are given in Table 4.

TABLE 4

Toxicities of purified sea snake phospholipases A_2

Species and Toxic	Route	LD_{50} (μg/g)	Reference
	Subfamily Laticaudinae		
Laticauda colubrina			
Lc PLH-I	IV	> 4.50	Takasaki *et al.* (1988)
Lc PLA-II	IV	0.05	Takasaki *et al.* (1988)
Laticauda semifasciata			
Ls PLA-I	IV	9.00	Takasaki *et al.* (1988)
Ls PLA-II	IV	> 6.00	Takasaki *et al.* (1988)
Ls PLA-IV	IV	> 6.00	Takasaki *et al.* (1988)
	Subfamily Hydrophiinae		
Enhydrina schistosa			
Toxin VI:5	IV	0.11	Fohlman and Eaker (1977)

Route refers to method of administration of toxin. IV = intravenous (tail vein). Venom toxicities are expressed in μg venom/g mouse body weight. Females of Swiss/Webster strains are typically used.

2. Membrane Disruption

Phospholipase A_2 enzymes in general promote the hydrolysis of diverse phospholipids at the SN-2 position. Substrate prefer-

ences/specificities vary depending on the source of the enzyme, but most common cell membrane phospholipids can serve as substrates. The specificity of their catalysis has made these enzymes useful probes of cell membrane and model membrane organization (Davidson and Dennis, 1991).

The catalytic action of phospholipase A_2 can lead to cell membrane damage and eventual cell lysis (Harris, 1985) via direct hydrolytic activity and via the autopharmacological action of the lysophospholipid products (Dennis *et al.*, 1991). In addition, the release of fatty acids (such as arachidonic acid) may have a wide variety of secondary effects, such as the generation of lipid second messengers (Dennis *et al.*, 1991). Differences in substrate specificity (and hence in products formed) may account in part for the large differences in toxicity among the single-chain phospholipases A_2. The mechanism of action of the toxic phospholipase A_2 from *Laticauda colubrina* venom was not defined (Takasaki *et al.*, 1988). However, a much less toxic phospholipase A_2 from *L. semifasciata* venom apparently acts in a fashion similar to the postsynaptic neurotoxins, binding to the acetylcholine receptor and preventing acetylcholine binding (Harvey and Tamiya, 1980). A myotoxic phospholipase A_2 from *Enhydrina schistosa* venom was shown to possess presynaptic neurotoxicity (Fohlman and Eaker, 1977), apparently the only known example of a presynaptic neurotoxin in sea snake venom.

D. Other Venom Components

Sea snake venom have been shown to contain several different enzyme activities in addition to phospholipase A_2 (Table 5; see also Tu, 1988b). However, very little is known about the contribution of these components to venom toxicity and pharmacology. Only one, a phosphomonoesterase from *L. semifasciata* venom, has been isolated (Uwatoko-Setoguchi, 1970). Most are hydrolytic enzymes and some, such as hyaluronidase, may facilitate the distribution of phospholipase A_2 and neurotoxin(s) within prey.

IV. BIOCHEMISTRY OF SEA SNAKE VENOMS

A. Neurotoxins

Short-chain postsynaptic neurotoxins (type I, α-neurotoxin) are the dominant component of sea snake venoms, both functionally and biochemically. For venom obtained from the fang, neurotoxins may account for 75 percent of the total protein in the venom (e.g. *Aipysurus laevis*: Maeda and Tamiya, 1976). Sea snake venom

TABLE 5

Enzyme activities present in sea snake venoms

Activity	Species	Reference
Acetylcholinesterase	*Enhydrina schistosa*	Tu and Toom (1971)
	Hydrophis cyanocinctus	Su *et al.* (1984
Hyaluronidase	*Enhydrina schistosa*	Tu and Toom (1971)
Leucine aminopeptidase	*Enhydrina schistosa*	Tu and Toom (1971)
5'-nucleotidase	*Laticauda semifasciata*	Setoguchi *et al.* (1968)
	Enhydrina schistosa	Gawade and Bhide (1977)
	Hydrophis cyanocinctus	Su *et al.* (1984)
Phosphodiesterase	*Enhydrina schistosa*	Tu and Toom (1971)
	Hydrophis cyanocinctus	Su *et al.* (1984)
Phosphomonoesterase	*Laticauda semifasciata*	Setoguchi *et al.* (1968)
	Enhydrina schistosa	Su *et al.* (1984)
	Hydrophis cyanocinctus	Su *et al.* (1984)
Phospholipase A_2	*Laticauda colubina*	
	Laticauda semifasciata	Uwatoko-Setoguchi *et al.* (1968)
	Enhydrina schistosa	Carey and Wright (1960)
	Hydrophis cyanocinctus	Durkin *et al.* (1981)
	Pelamis platurus	Durkin *et al.* (1981)

toxicity is therefore largely dependent on neurotoxin content. Type I neurotoxins bind very tightly to the α-subunits of the acetylcholine receptors found in neuromuscular junction of the skeletal muscle and the electroplax organs of electric fish. These toxins have proved extremely useful as probes of the acetylcholine receptor structure and function, and many toxins have been purified from sea snake venoms. A comprehensive review of structure/function studies is given in Endo and Tamiya (1987).

1. Toxin Structure

a. Amino acid sequence: Most sea snake venoms contain one major toxin, accounting for the majority of neurotoxins and one to several minor toxins; all show considerable sequence homology. Approximately 20 of these α-neurotoxins from sea snake venoms have been sequenced (Table 6). The primary sequence and location of disulfide bonds of *Lapemis* toxin is shown Fig. 5; most short-chain α-neurotoxins are quite similar. They consist of 60–62 amino acids (molecular weights 6,800 daltons) with eight half-cystines which define three functional loops (A, B & C) of the molecule (Fig. 6). A single free cysteine is present in these toxins and has been demonstrated by the characteristic S-H stretching vibrations at 2578 cm^{-1} in Raman spectra (Fox and Tu, 1977; Ishizaki *et al.*, 1984; Tu, 1985).

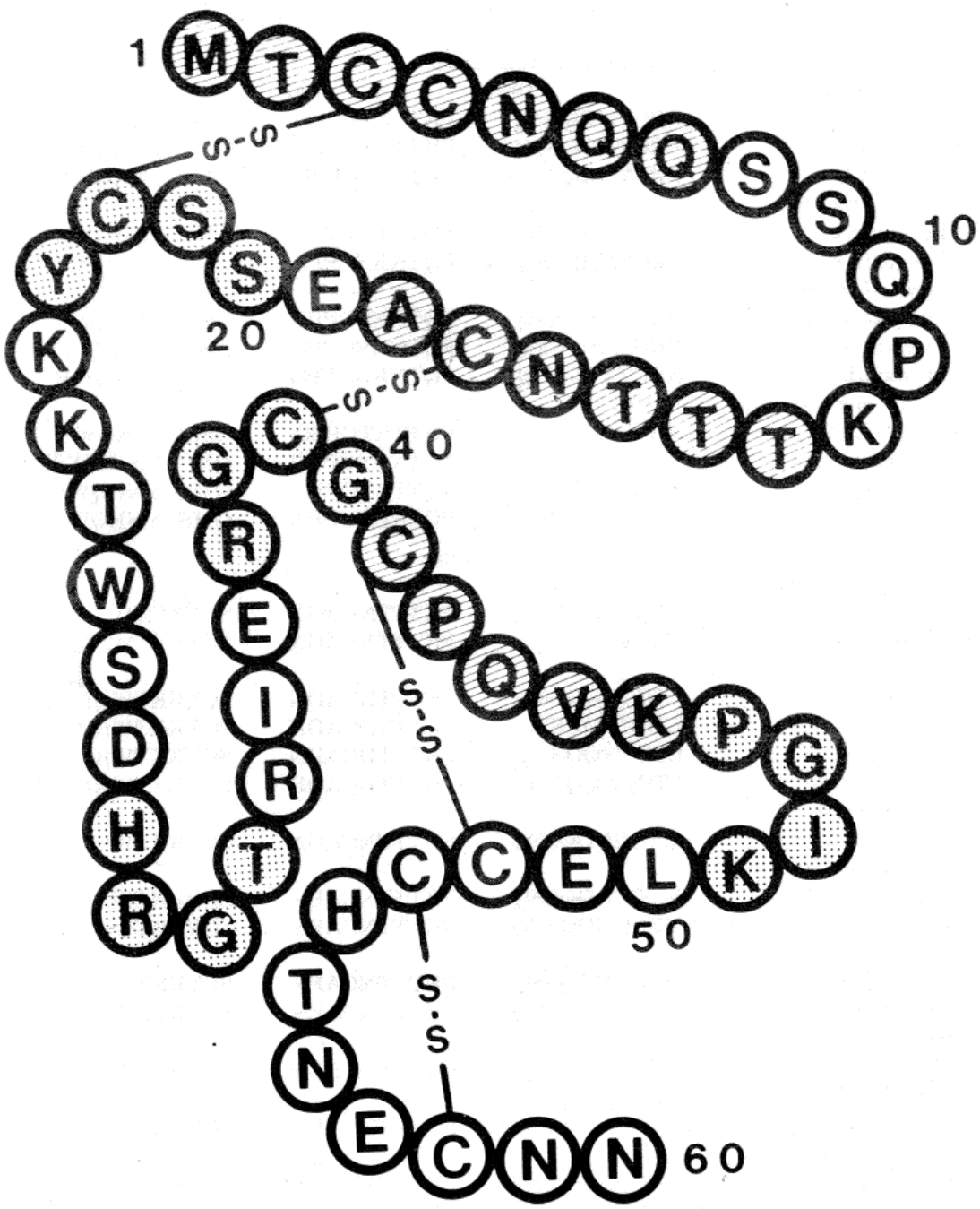

Figure 5: Primary structure of *Lapemis* toxin, a representative postsynaptic neurotoxin isolated from the venom of *Lapemis hardwickii*. The four disulfides produce a tightly compacted structure and add to the stability of the toxin.

TABLE 6

Amino acid sequences of short-chain neurotoxins

Species and Toxin			
	Subfamily Laticaudinae		
Laticauda colubrina	10	20	30
1. Lc toxin c	RRCYNQQSSQ	PKTTKSCPPG	ENSCYNKQWR
2. Lc toxin d	RRCYNQQSSQ	PKTTKSCPPG	ENSCYNKQWR
3. Lc toxin II	RRCYNQQSSQ	PKTTKSCPPG	ENSCYNKQWR
Laticauda crockeri			
4. Lcr toxin a	RRCFNHPSSQ	PQTNKSCPPG	ENSCYNKQWR
5. Lcr toxin b	RRCFNHPSSQ	PQTNKSCPPG	ENSCYNKQWR
Laticauda laticauda			
6. Laticotoxin a	RRCFNHPSSQ	PQTNKSCPPG	ENSCYNKQWR
7. Ll toxin a	RRCFNHPSSQ	PQTNKSCPPG	ENSCYNKQWR
8. Ll toxin b	RRCFNHPSSQ	PQTNKSCPPG	ENSCYNKQWR
Laticauda semifasciata			
9. Erabutoxin a	RICFNQHSSQ	PQTTKTCPSG	QSSCYNKQWS
10. Erabutoxin a	RICFNQHSSQ	PQTNKSCPPG	QMSCYNKQWS
11. Erabutoxin c	RICFNQHSSQ	PQTTKTCPSG	QMSCYNKQWS
12. Toxin b	RICFNQHSSQ	PQTNKSCPPG	QMSCYNKQWS
	Subfamily Hydrophiinae		
Acalyptophis peronii			
13. Major toxin	MTCCNQQSSQ	PKTTTNCAGN	SCYKKTWSDH
14. Major toxin	MTCCNQQSSQ	PKTTTNCAGN	SCYKKTWSDH
Aipysurus laevis			
15. Toxin a	LTCCNQQSSQ	PKTTTDCADN	SCYKKTWQDH
16. Toxin b	LTCCNQQSSQ	PKTTTDCADN	SCYKKTWRDH
17. Toxin c	LTCCNQQSSQ	PKTTTDCADN	SCYKKTWKDH
18. Toxin d	LTCCNQQSSQ	PKTTTDCADD	SCYKKTWKDH
Astrotia stokesii			
19. Toxin a	MTCCNQQSSQ	PKTTTNCAGN	SCYKKTWSDH
Enhydrina schistosa			
20. Toxin 4	MTCCNQQSSQ	PKTTTNCAES	SCYKKTWSDH
21. Toxin 5	MTCCNQQSSQ	PKTTTNCAES	SCYKKTWSDH
Hydrophis cyanocinctus			
22. Hydrophitoxin a	MTCCNQQSSQ	PKTTTNCAES	SCYKKTWSDH
23. Hydrophitoxin b	MTCCNQQSSQ	PKTTTNCAES	SCYKKTWSDH
Hydrophis lapemoides			
24. Hl toxin a	MTCCNQQSSQ	PKTTTNCAES	SCYKKTWSDH
Lapemis hardwickii			
25. Lapemis toxin	MTCCNQQSSQ	PKTTTNCAES	SCYKKTWSDH
Pelamis platurus			
26. Pelamitoxin a	MTCCNQQSSQ	PKTTTNCAES	SCYKKTWSDH
27. Pelamis toxin b	MTCCNQQSSE	PKTTTNCAES	SCYKKTWSDH

* Toxin sequence was deduced from the nucleotide sequence of cDNA.

(Type I) from sea snake venoms

			References
40	50	60	
DHRGSITERG	CGCPKVKPGI	KLRCCESEDC MN	Tamiya *et al.* (1983b)
DHRGSITERG	CGCPKVKPGI	KLRCCESEDC NN	Tamiya *et al.* (1983b)
DHRGSITERG	CGCPKVKPGI	KLRCCESEDC NN	Tamiya *et al.* (1983b)
DHRGTITERG	CGCPTVKPGI	KLTCCQSDDC NN	Tamiya *et al.* (1983b)
DHRGTIIERG	CGCPQVKSGI	KLTCCQSDDC NN	Tamiya *et al.* (1983b)
DHRGTITERG	CGCPTVKPGI	KLTCCQSEDC NN	Maeda and Tamiya (1974)
DHRGTITERG	CGCPTVKPGI	KLTCCQSEDC NN	Tamiya *et al.* (1983b)
DHRGTITERG	CGCPTVKPGI	KLTCCQSEDC NN	Tamiya *et al.* (1983b)
DFRGTIIERG	CGCPTVKPGI	KLSCCMSESC NN	Maeda and Tamiya (1977)
DFRGTIIERG	CGCPTVKPGI	KLSCCESEVC MN	Maeda and Tamiya (1977)
DFRGTIIERG	CGCPTVKPGI	KLSCCESEVC NN	Maeda and Tamiya (1977)
DFRGTIIERG	CGCPTVKPGI	KLSCCQSEDC NN	Tsernoglou *et al.* (1977)
RGTIIERGCG	CPQVKSGIKL	ECCHTNECNN	Morei and Tu (1988)
RGTIIERGCG	CPQVKSGIKL	ECCHTNECNN	Morei and Tu (1988)
RGTRIERGCG	CPQVKPGIKL	ECCKTNECNN	Maeda and Tamiya (1976)
RGTRIERGCG	CPQVKPGIKL	ECCKTNECNN	Maeda and Tamiya (1976)
RGTRIERGCG	CPQVKPGIKL	ECCKTNECNN	Maeda and Tamiya (1976)
RGTRIERGCG	CPQVKPGIKL	ECCKTNECNN*	Ducancel *et al.* (1989)
RGTIIERGCG	CPQVKSGIKL	ECCHTNECNN	Maeda and Tamiya (1978)
RGTRIERGCG	CPQVKPGIKL	ECCHTNECNN	Frykland *et al.* (1972)
RGTRIERGCG	CPQVKSGIKL	ECCHTNECNN	Frykland *et al.* (1972)
RGTRIERGCG	CPQVKKGIKL	ECCHTNECNN	Liu and Blackwell (1974)
RGTRIERGCG	CPQVKSGIKL	ECCHTNECNN	Liu and Blackwell (1974)
RGTRIERGCG	CPQVKPGIKL	ECCHTNECNN	Tamiya *et al.* (1983)
RGTRIERGCG	CPQVKPGIKL	ECCHTNECNN	Fox *et al.* (1977)
RGTRIERGCG	CPQVKSGIKL	ECCHTNECNN	Wang *et al.* (1976)
RGTRIERGCG	CPQVKSGIKL	ECCHTNECNN	Mori *et al.* (1989)

TABLE 7

Amino acid sequences of long-chain neuro

Species and Toxin				
		Subfamily Laticaudinae		
Laticauda colubrina	10	20	30	40
1. Lc toxin a	RICYLAPRDT	QICAPGQEIC	YLKSWDDGTOG	FLKGNRLEFG
2. Lc toxin b	RICYLAPRDT	QICAPGQEIC	YLKSWDDCTG	SIRGNRLEFG
Laticauda semifasciata				
3. Ls toxin III	RECYLNPHDT	QTCPSGQEIC	YVKSWCNAWC	SSRGKVLEFG
		Subfamily Hydrophiinae		
Astrotia stokesii				
4. As toxin b	LSCYLGYKHS	QTCPPGENVC	FVKTWCDAFC	NTRGERIIMG
5. As toxin c	LSCYLGYKHS	QTCPPGENVC	FVKTWCDAFC	STRGERIVGM

Some sea snake venoms also contain long-chain (type II) postsynaptic neurotoxins (Table 7). These toxins contain 66–72 amino acids, five disulfides and no free cysteines. Type II toxins have some structural similarities to type I toxins but there is much less sequence homology between them. Toxins which are structural "hybrids" between type I and II toxins have also been sequenced (Kim and Tamiya, 1982; Tu, 1990).

b. Conformation: Sea snake neurotoxins are single-chain polypeptides which are extensively disulfide linked, producing a compact structure which is highly stable. When *Pelamis* toxin b was heated at 94°C for 15 minutes, very little change occurred in the Raman spectra, indicating thermal conformational stability (Mori *et al.*, 1989). Short-chain neurotoxins are also considerably resistant to cystine reduction and alkylation.

It is assumed that the β-sheet structure of neurotoxins is essential to acetylcholine receptor binding, and Raman spectroscopy identified β-sheet structure in *Enhydrina schistosa* toxin (Yu *et al.*, 1975), *Laticauda semifasciata* toxin (b. Tu, 1990) and *Pelamis* toxin (Mori *et al.*, 1989). Antiparallel β-sheet structure accounts for ~ 38 percent of the secondary structure of these toxins, while the remainder is random coil; α-helical structure is absent from these toxins.

c. X-ray crystallography determination of sea snake neurotoxin structure: Crystals of pure sea snake neurotoxin can be grown, and the tertiary structure of several toxins has been analyzed. The three-dimensional structure of toxin b (= erabutoxin b) from *L. semifasciata* venom has been studied most extensively (Low *et al.*, 1976; Tsernoglou and Petsko, 1976; Low and Corfield, 1986). The

toxins (Type II) from sea snake venoms

			Reference
50	60	69	
CAATCPTVKP	GIDIKCCSTD	KCNPHPKLA	Kim and Tamiya (1982)
CAATCPTVKR	GIHIKCCSTD	KCNPHPKLA	Kim and Tamiya (1982)
CAATCPSVNT	GTEIKCCSAD	KCNTYP	Maeda and Tamiya (1974)
CAATCPTAKS	GVHIACCSTD	NCNIYAKWGS	Maeda and Tamiya (1978)
CAATCPTAKS	GVHIACCSTD	NCNIYTKWGSGR	Maeda and Tamiya (1978)

presence of $\sim$ 40 percent twisted antiparallel β-sheet structure was confirmed by X-ray crystallography, and the three functional domains of the toxin (as defined by disulfide-bridged loops) were assigned spatial distributions (see Fig. 6). Loop B was believed to be important to acetylcholine receptor binding since it contains appropriately charged side chains which could interact with the cationic pocket of the receptor (Tsernoglou and Petsko, 1976). The reverse structural association, whereby residues on the receptor fit into an "active site" on the toxin molecule, has also been suggested (Low *et al.*, 1976). Currently, it is believed that a tryptophan residue on the acetylcholine receptor α-subunit (trp184 or 187) fits into the toxin tryptophan cleft upon binding (Low and Corfield, 1986). The crystal structure of toxin *a* from *L. semifasciata* venom has recently been determined (Corfield *et al.*, 1989). This isotoxin, which differs from toxin *b* in amino acid sequence only at residue 26, has the same overall structure as toxin *b* but differs in several fine structural details.

The long chain neurotoxin from cobra venom (α-cobratoxin: *Naja naja siamensis*) has been shown to have a crystal structure similar to toxin *b* (Walkinshaw *et al.*, 1980), and it is likely that sea snake type II neurotoxins are also structurally similar.

2. Toxin Interaction with the Acetylcholine Receptor

The acetylcholine receptor of vertebrate skeletal muscle is a five-subunit protein/carbohydrate complex ($\alpha_2\beta\gamma\delta$) which, upon binding acetylcholine released from nerve terminals, undergoes a shift in conformation to allow passage of Na^+ and K^+ ions. Sea snake neurotoxins (as well as many other postsynaptic neurotoxins) bind at the acetyl-

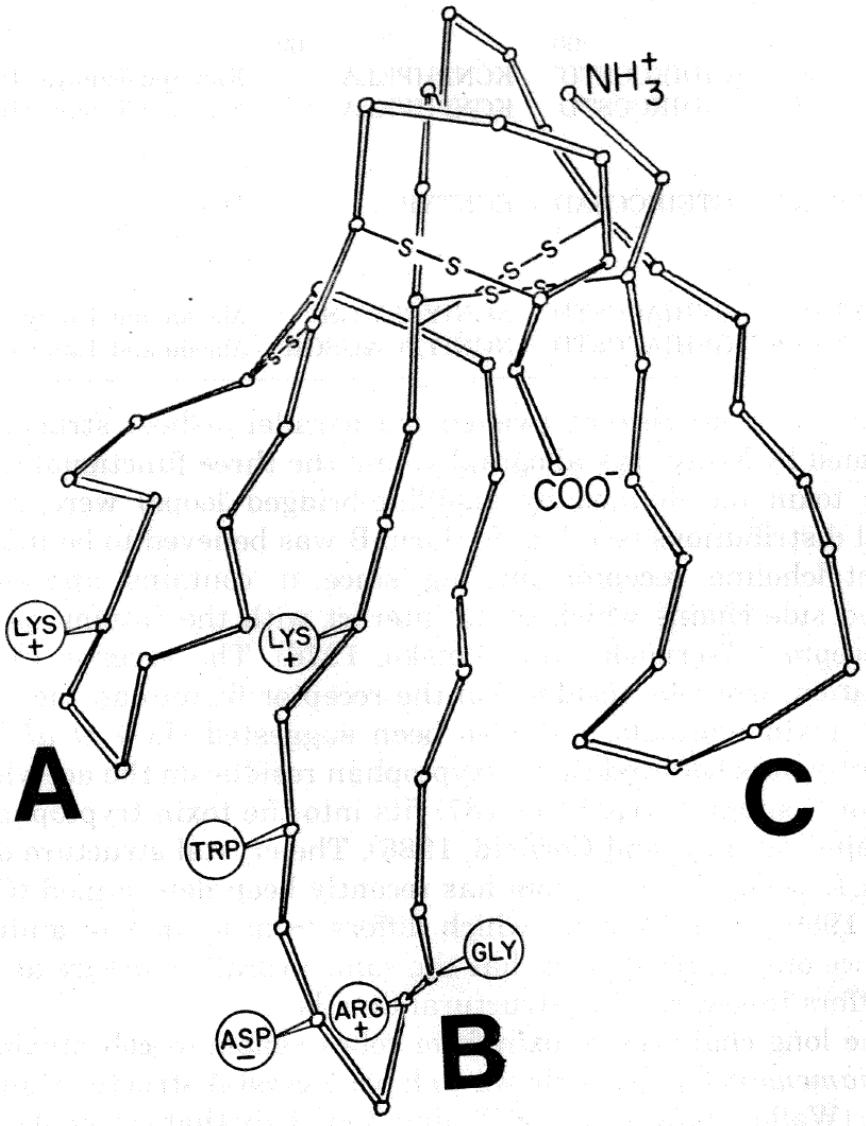

Figure 6: Tertiary structure of *Lapemis* toxin. The disulfides define three main structural domains (A, B, C). This structure is based on that of the homologous toxin b from *Laticauda semifasciata* venom. The structure of most short-chain postsynaptic neurotoxins from sea snake venom is assumed to be quite similar.

choline binding site, blocking neurotransmitter binding and resulting in disruption of nerve impulse transmission to the muscle endplate.

Snake venom toxins have long been used as probes to study the structure of the acetylcholine receptor and other ion channels (reviewed in Mebs and Hucho, 1990) and, in fact, most isolation procedures for the purification of the acetylcholine receptor (e.g., Lindstrom *et al.*, 1980) utilize affinity columns of α-cobratoxin (first isolated by Yang, 1965). Type I (α) neurotoxins have also been used to demonstrate the reduction in acetylcholine receptor number in individuals afflicted with the autoimmune disease myasthenia gravis (Drachman, 1983).

Sea snake α-neurotoxins, like those from other venomous snakes, bind nearly irreversibly to the acetylcholine receptor and dissociation constants for toxin/receptor binding are in the nanomolar to subnanomolar range (Low and Corfield, 1986). Such tight binding confers high specificity on these toxins, which in turn helps explain their high toxicity. Sea snake toxins and other elapid toxins compete for the same site on the α-subunits of the receptor, a fact which has been demonstrated by displacement binding studies (Ishizaki *et al.*, 1984).

a. Peptide models of toxin: acetylcholine receptor binding: Synthetic peptides corresponding to portions of the primary structure of type I neurotoxins will bind to the acetylcholine receptor (Atassi, 1991; McCormick and Atassi, 1984). Reciprocal experiments, utilizing synthetic peptides corresponding to the primary structure of the acetylcholine receptor (derived from gene sequences) *and* that of neurotoxins, have shown that even these peptide fragments will interact, though dissociation constants are considerably higher (Mulac-Jericevic and Atassi, 1986, 1987). These studies have been used to map the spatial orientation of the acetylcholine receptor with bound toxin (Ruan *et al.*, 1990; reviewed in Atassi, 1991). α-bungarotoxin, a type I toxin purified from *Bungarus* venom, has been used for most peptide model studies.

Recently, a synthetic peptide fragment of *Lapemis* toxin (corresponding to loop B; see Fig. 6), was shown to bind to isolated acetylcholine receptor (Miller and Tu, 1991). Loop B, a domain defined by disulfides, is believed to be the primary functional domain of postsynaptic neurotoxin binding to the acetylocholine receptor. Four peptides corresponding to portions of the primary sequence of *Lapemis* toxin were synthesized and one of them (B1) bound to the acetylcholine receptor. In addition, peptide B1 was found to be relatively nontoxic (LD_{50} at least 100-fold less than the native toxin); the authors suggest

that this fragment may potentially serve as an antagonist to the *in vivo* effects of the native toxin.

3. Chemical Modifications of Toxins and Receptor

Specific amino acid residues of sea snake type I neurotoxins have been chemically modified in an attempt to determine their importance to toxicity and to acetylcholine receptor binding. When tryptophan 27 of *Lapemis* toxin was modified with either 2-hydroxy-5-nitrobenzylbromide or N-bromosuccinimide, toxicity was abolished (Tu and Hong, 1971) and binding to the acetylcholine receptor was essentially eliminated (Allen and Tu, 1985). Tryptophan, an invariant residue in sea snake neurotoxins (see Table 6), is likely involved in receptor binding and toxicity. Reduction of disulfides also abolishes *Pelamis* toxin neurotoxicity, probably due to loss of secondary structure. Modification of one or more residues of arginine, free cysteine, histidine or lysine had little effect on toxicity of several sea snake neurotoxins, but modification of tryptophan or tyrosine residues resulted in loss of toxicity (summarized in Tu, 1988).

The acetylcholine receptor from *Torpedo californica* electroplax organ has also been chemically modified, and the effects on toxin binding were studied. When the receptor subunits were crosslinked with dimethylsuberimidate (through primary amines; Fig. 7), ^{125}I-*Lapemis* toxin binding was not affected (Mori and Tu, 1988). However, when the receptor subunits were crosslinked with N, N'-1, 4-phenylenedimaleimide (sulfhydryl groups), toxin binding to the receptor was greatly decreased (Lin and Tu, 1989). It was concluded that although tight association of the acetylcholine receptor subunits (via crosslinking) does not in itself inhibit toxin binding, the sulfhydryl groups which were crosslinked are required for neurotoxin binding.

4. Homology of Sea Snake Neurotoxins

As can be seen from Table 6, sea snake α-neurotoxins (type I) show a very high degree of homology. This similarity is seen among toxins from both subfamilies of sea snakes, suggesting that selection has strongly favored the retention of this gene product in spite of the diversification of sea snake species. Sea snake type I toxins are also homologous with the short-chain neurotoxins isolated and sequenced from numerous terrestrial elapid venoms (Tamiya, 1985). Surprisingly, type II (long chain; Table 7) neurotoxin sequences differ considerably from type I toxins (Table 6), though some homology is obvious. The invariant tryptophan residue is present in all sea snake neurotoxins, though it occupies residue position 25 in the type II and "hybrid" neurotoxins.

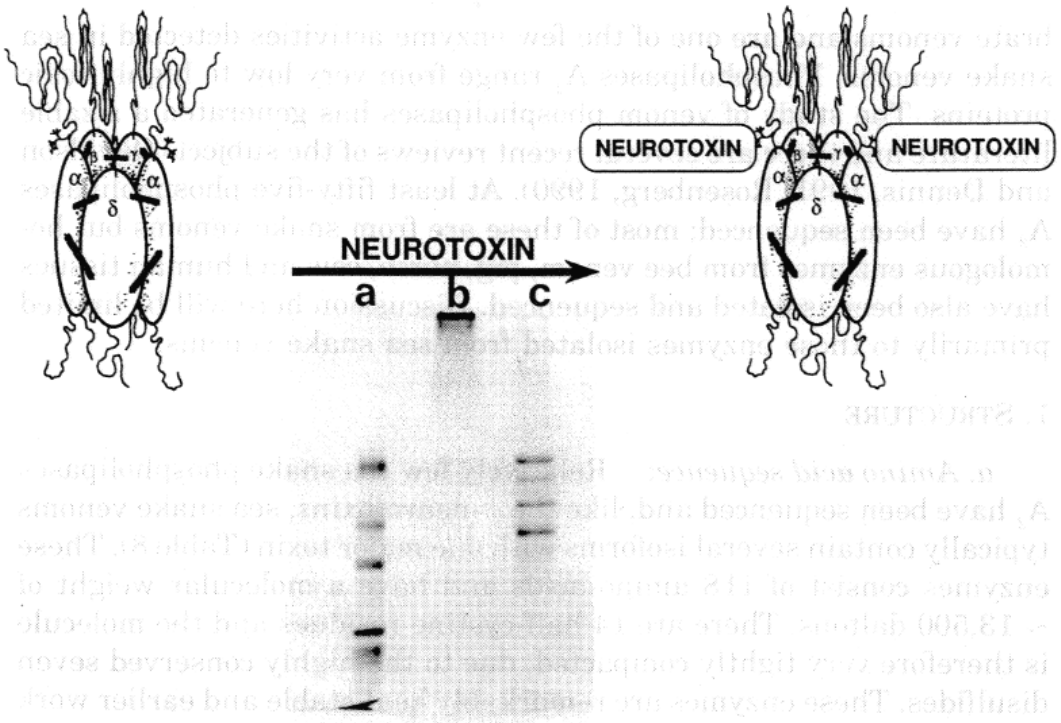

Figure 7: Effect of crosslinking the acetylcholine receptor subunits on binding of neurotoxin. Crosslinked receptor subunits can be demonstrated by SDS-PAGE (lane b); noncrosslinked receptor (lane c). However, crosslinking through primary amine groups does not affect neurotoxin binding. Lane a—molecular weight standard markers.

Structural homology of short-chain neurotoxins also extends to a family of α-neurotoxins from a quite different source. Marine snails of the genus *Conus* produce a variety of potent α-neurotoxins (α-conotoxins) which are 13–15 amino acids in length, have two disulfide bridges which tightly constrain structure, and bind to the acetylcholine receptor (Gray *et al.*, 1988; Cruz, 1989; Olivera *et al.*, 1990). Conotoxins show structural similarity to specific regions of sea snake α-neurotoxins (Dufton *et al.*, 1989) and may represent the minimum structure necessary for receptor blockage. The region of similarity in the snake toxins is peripheral to that region usually considered important for receptor binding. It would be interesting to see whether these natural peptide "conformational fragments" can elucidate structure/function relation of α-neurotoxins and the acetylcholine receptor.

B. Phospholipase A_2

Phospholipase A_2 enzymes are found in virtually all types of verte-

brate venoms and are one of the few enzyme activities detected in sea snake venoms. Phospholipases A_2 range from very low to highly toxic proteins. The study of venom phospholipases has generated a sizable literature and there are several recent reviews of the subject (Davidson and Dennis, 1991; Rosenberg, 1990). At least fifty-five phospholipases A_2 have been sequenced; most of these are from snake venoms but homologous enzymes from bee venom, pig, horse, cow and human tissues have also been isolated and sequenced. Discussion here will be limited primarily to those enzymes isolated from sea snake venoms.

1. Structure

a. Amino acid sequence: Relatively few sea snake phospholipases A_2 have been sequenced and, like the α-neurotoxins, sea snake venoms typically contain several isoforms with one major toxin (Table 8). These enzymes consist of 118 amino acids and have a molecular weight of $\sim$ 13,500 daltons. There are 14 half-cystine residues and the molecule is therefore very tightly compacted, due to the highly conserved seven disulfides. These enzymes are remarkably heat stable and earlier work with venoms employed heating at >65°C to inactivate other components (though neurotoxins were also not affected). One mole of calcium is bound per mole of enzyme and activity is dependent on this cation (Teshima *et al.*, 1989). The calcium binding site for homologous phospholipases A_2 has been shown to involve residues 28, 30, 32 and 49 (White *et al.*, 1990); these are invariant residues in all snake venom phospholipases A_2, including an inactive homologue from *Laticauda colubrina* venom (Takasaki *et al.*, 1988).

b. X-ray crystallography data: No sea snake phospholipases A_2 have as yet been subjected to X-ray diffraction studies. However, two homologous enzymes from other snake venoms have recently been crystallized and studied (Tomoo *et al.*, 1989; White *et al.*, 1990). The enzyme from *Naja naja atra* venom was crystallized in the presence of a transition state analog as a model of how the fatty acyl chains of the substrate are accommodated by the enzyme during catalysis (White *et al.*, 1990). A tertiary structural model for the bovine phospholipase A_2 was compared with a crotalid phospholipase A_2 and the backbone configurations found to be essentially identical (Renetseder *et al.*, 1985). Global structural features of these studies could most likely be extended to sea snake phospholipases as well.

2. Enzymatic Activity/Specificity

Phospholipase A_2 enzymes hydrolyze the fatty acyl chains of phospholipids at the SN-2 position. The amino acid side chains involved

in hydrolysis of fatty acids include residues 48, 52 and 92 (White *et al.*, 1990); these residues are also invariant for all active snake venom phospholipases A_2. An inactive phospholipase A_2 homologue from *L. colubrina* venom has asparagine 48 instead of histidine 48 (Takasaki *et al.*, 1988); this substitution at the catalytic center is likely responsible for the lack of enzymatic activity. When histidine 48 of an active phospholipase A_2 from the same venom or from the venom of *Notechis scutatus* (Volwerk *et al.*, 1974) was modified with p-bromophenacyl bromide, both enzymes lost activity and toxicity.

It has been demonstrated that phospholipases A_2 recognize polar head groups of several types of phospholipids rather than simply a hydrophobic environment, since Triton X-100 micelles lacking phospholipids will not bind phospholipase A_2 (Roberts *et al.*, 1977). A "dual phospholipid" model has been proposed to explain the activity of phospholipases A_2 on micelles and membrane vesicles (Hendrickson and Dennis, 1984a, b; Davidson and Dennis, 1991), which suggests that two phospholipid binding sites exist on the enzyme. One site is required for enzyme activation and the other includes the catalytic site. Phospholipase A_2 from *L. semifasciata* venom has also been shown to be activated by free fatty acids (Yoshida *et al.*, 1979).

In the past, rates of hydrolysis of various phospholipids led to the ranking of substrate specificities for phospholipases A_2 from a given source (e.g., Adamich and Dennis, 1978). Since some phospholipases are extremely toxic while others are essentially non-toxic, and since phospholipid distribution in cell membranes is known to be asymmetric, it was hoped that substrate ranking would lead to the identification of particular "target" phospholipids. However, as has been noted (Rosenberg, 1990; Davidson and Dennis, 1991), seemingly small differences in assay procedures gave rise to strikingly different apparent substrate specificities, making it difficult to compare experimental results from different investigators.

3. Interdependence of Toxicity and Enzymatic Activity

A lively debate has continued for some years now as to the relation between phospholipase A_2 presynaptic neurotoxicity and enzymatic activity. Various investigators have shown that in some cases the pharmacological and catalytic activities could be dissociated (Karlsson, 1979; Yang *et al.*, 1981; Rosenberg, 1986). However, phospholipases A_2 from different sources behave differently, and with some enzymes, catalytic and toxic activities appear to be linked. The exact mode of action of toxic phospholipases A_2 at the neuromuscular junction is not known. However, at least one phospholipase A_2 (from *L. semifasciata* venom) appears to act like an α-neurotoxin by binding to the acetylcholine

TABLE 8

Amino acid sequences of phospholipases

Species and Toxin	10	20	30	40
	Subfamily Laticaudinae			
Laticauda colubrina				
1. LcPLA-II	NLIQFSELIQ	CANKGKRATY	YYMDYGCYCG	KGGSGTPVDD
2. LcPLH-I	NLIQFSQLIQ	CANKGKRPTL	HYMDYGCYCG	PGGSGTPVDD
Laticauda laticauda				
3. LlPLA$_2$	NLAQFALVIK	CADKGKRPRW	HYMDYGCYCG	PGGSGTPVDE
Laticauda semifasciata				
4. LsPLA I	NLVQFSNLIQ	CNVKGSRASY	HYADYGCYCG	AGGSGTPVDE
5. LsPLA III	NLVQFTNLIQ	CANSGKRASY	HYADYGCYCG	AGGSGTPVDE
6. LsPLA IV	NLVQFSYLIQ	CANTGKRASY	HYADYGCYCG	AGGSGTPVDE
	Subfamily Hydrophiinae			
Aipysurus laevis				
7. Al PLA$_2$	NLYQFDNMIQ	CANKGKRATW	HYMDYGCYCG	SGGSGTPVDA
Enhydrina schistosa				
8. PL	NLVQFSYVIT	CANHNRRSSL	DYADYGCYCG	AGGSGTPVDE

*Toxin sequence was deduced from the nucleotide sequence of cDNA.

receptor and preventing acetylcholine uptake (Harvey and Tamiya, 1980). Other sea snake venom phospholipases A_2 do not appear to interact with the acetylcholine receptor.

V. IMMUNOLOGY AND MOLECULAR BIOLOGY OF SEA SNAKE VENOMS

A. Immunodiffusion Studies

Immunodiffusion studies provide a first approximation of the relatedness of biological compounds derived from different sources. Typically, related venoms show similar precipitation line patterns while

A_2 from sea snake venoms

50	60	70	80	90
LDRCCKTHDD	CYGQAEKKGC	FPFLTLYNFI	CFPGGPTCDR	GTTCQRFVCD
LDRCCKTNDD	CYAQAEKKGC	SPLSTNYNFD	CFPGGPQCGK	GTTCQRFVCD
LDRCCKTHDQ	CYGEAEKMGC	YPKLTMYSYY	CDDGDPYCNS	KTECQRFVCD
LDRCCKIHDN	CYGEAEKMGC	YPKWTLYTYD	CSTEEPNCST	KTGCQGFVCA
LDRCCKIHDN	CYGQAEKMGC	YPKLTMYNYY	CGTQSPTCDD	KTGCQRYVCA
LDRCCKIHDN	CYGVAEDNGC	YPKLTMYNYY	CGTQSPTCDN	KTGCQRYVCA
LDRCCKAHDD	CYGVAEDNGC	YPKWTLYSWQ	CTENVPTCNS	ESGCQKSVCA
LDRCCKIHDD	CYGEAEKQGC	YPKMLMYDYY	CGSNGPYCRN	VKKKCNRKVC

venoms of distantly related snakes (such as elapid venom: crotalid venom antibodies) do not (Tu *et al.*, 1980).

Commercial antivenin prepared from *Enhydrina schistosa* venom (Commonwealth Serum Laboratories, Melbourne, Australia) showed 1–4 precipitation lines when tested against several different sea snake venoms (Tu and Salafranca, 1974). Two of these precipitin lines resulted from phospholipase A_2 and short-chain neurotoxin, indicating that these toxins from various seas snake venoms are quite similar antigenically. Venoms which showed immunological cross-reactivity with *Enhydrina schistosa* antivenin included *Lapemis hardwickii, Hydrophis cyanocinctus*, and *Pelamis platurus*.

Table 8: Contd.

Species and Toxin				References
	Subfamily Laticaudinae			
Laticauda colubrine	100	110	118	
1. LcPLA-II	CDIQAAFCFA	RSPYNNKNYN	INISKRCK	Takasaki *et al.*(1988)
2. LcPLH-I	CDLKAALCFA	KSPYNNKNFN	IDTKKRCK	Takasaki *et al.* (1988)
Laticauda laticauda				
3. LlPLA$_2$	CDVRAADCFA	RYPYNNKNYN	INTSKRCK*	Guignery Frelat *et al.* (1987)
Laticauda semifasciata				
4. LsPLA I	CDLEAAKCFA	RSPYNNKNYN	IDTSKRCK	Nishida *et al.* (1982)
5. LsPLA III	CDLEAAKCFA	RSPYNNKNYN	IDTSKRCK	Nishida *et al.* (1982)
6. LsPLA IV	CDLEAAKCFA	RSPYNNKNYN	IDTSKRCK	Nishida *et al.* (1982)
	Subfamily Hydrophiinae			
Aipysurus laevis				
7. Al PLA$_2$	CDATAAKCFA	EAPYNNKNYN	INTSNCQ*	Ducancel *et al.* (1989)
Enhydrina schistosa				
8. PL	DCDVAAAECF	ARNAYNNANY	NIDTKKRCK	Lind and Eaker (1981)

B. Antibody/Antivenin Production

Antivenins are usually produced in horses by initial inoculation with venom(s) and then follow-up booster injections; goats, sheep and rabbits also produce antibodies to venom proteins which are useful as antivenins, but the size of horses makes them more suitable for commercial production (Latifi, 1978). When antibodies to venom proteins have reached a sufficient level, blood is removed and the cell-free serum containing antibodies is lyophilized. Currently, only the Australian manufacturer (Commonwealth Serum Laboratories) supplies sea snake antivenin commercially; it is produced from *E. schistosa* venom only. However, this antivenin also neutralizes venoms from *P. platurus, Hydrophis cyanocinctus, H. ornatus, H. spiralis, Lapemis hardwickii, L. viperina, Laticauda laticauda,*

and *L. semifasciata* (Kaire, 1964; Tu and Ganthavorn, 1969; Tu and Salafranca, 1974; Gawade *et al.*, 1980). Since lethality of sea snake venom is largely dependent on the action of short-chain neurotoxin(s), this broad-spectrum neutralization must result from antibody interaction with neurotoxin. Antivenin to *E. schistosa* venom is also effective against cobra venoms (*Naja naja* and *Ophiophagus hannah*), demonstrating the antigenic similarity between the long-chain neurotoxins of these venoms and the short-chain neurotoxins of sea snake venoms (Gawade and Gaitonde, 1980). Antivenins to venoms of several terrestrial Australian elapids effectively neutralized the venom of *E. schistosa* as well, indicating that there is antigenic similarity between the toxic components of these venoms. An unusual observation was that venom from *Vipera russelli* was also neutralized by sea snake antivenin (Gawade and Gaitonde, 1980). The antivenin to *L. hardwickii* venom (produced experimentally) was also shown to neutralize the lethal effects of several sea snake venoms (Okonogi *et al.*, 1972; Kawamura *et al.*, 1981).

One difficulty in producing antivenins is the inherent toxicity of the compound to be neutralized. This is particularly true of neurotoxins (see Table 3). Higher concentrations of initial and booster injections of toxins can be utilized if the toxins are first formaldehyde-denatured (Sato *et al.*, 1972). The relevant antigenic sites of the neurotoxin do not appear to be altered significantly and higher levels of nuerotoxin antibodies can be obtained. It may also be possible to achieve higher levels of antibody production if polylysine peptide (which stimulates a greater host immune response) is covalently attached to the toxin prior to injection.

C. Cloning of Sea Snake Toxins

A recent approach to the study of sea snake toxins involves the production of complementary DNA (cDNA) to toxin mRNA derived from gland tissue. The cDNA is then inserted into plasmids and cloned into bacteria (usually strains of *E. coli*). Cloned DNA can then be isolated and sequenced. This technique has the advantage of providing precursor sequence information (if present) as well as the sequence of the mature protein.

The first sea snake toxin to be cloned and sequenced was the short-chain neurotoxin (erabutoxin *a*) from *L. semifasciata* venom (Tamiya *et al.*, 1985). This toxin, and all sea snake toxins which have been sequenced from cDNA thus far, contains a signal peptide at the N terminus of the molecule. This signal peptide is rich in hydrophobic residues and consists of 21 amino acid residues (neurotoxins) or 27 residues (phospholipases A_2), with one free cysteine residue, which may be re-

quired for correct folding of the proteins (Ducancel *et al.*, 1989, 1990). The signal peptide sequences for neurotoxin (or phospholipase) show a high degree of homology with other neurotoxin (or phospholipase) signal peptides, but there is little homology between neurotoxin and phospholipase signal peptides. These proteins are therefore initially synthesized as precursors and must undergo proteolytic cleavage before or during export to the gland lumen.

DNA cloning techniques are likely to become quite prominent in sea snake venom research. cDNA sequencing is a more efficient method for sequencing many larger proteins and this method detects the precursor sequence as well. In addition, cloning techniques should allow determination of the functional roles of specific amino acid residues via the use of site-directed mutagenesis and subsequent expression and analysis of the modified proteins. Fusion proteins involving sea snake neurotoxins have also been produced (Ducancel *et al.*, 1989); such studies should further enhance our understanding of the structure/function aspects of neurotoxin actions.

VI. CLINICAL ASPECTS OF SEA SNAKE POISONING

Snakebite is a minor health problem in most temperate areas, but in tropical and semitropical regions treatment of bites by venomous snakes is an important clinical concern. In India alone, perhaps as many as two million people are annually bitten by snakes (venomous and nonvenomous) and of these, approximately 15,000 fatalities result (Murthy, 1990). The relatively high frequency of snakebite in these areas results from the combined factors of high diversity of venomous snake species, abundance of these animals, and the large number of people living in rural/agricultural areas. Administration of antivenin is still the only effective treatment for snakebite, but its availability in rural areas is limited. Specific details of snakebite in Asia are covered in a separate chapter in this book.

A. Occurrence of Sea Snakebites

Sea snakebites occur mainly among marine fishermen working in tropical waters of the Indian and Pacific oceans, often while snakes are being removed from nets. Also at risk are individuals engaged in recreational or professional diving in areas where sea snakes are known to occur. However, since sea snakes frequent shallow, warm waters, sunbathers, swimmers and others involved in activities in coastal tropical waters should be aware of the potential danger that sea snakes present. The occurrence of sea snakes is often seasonal, so that an area which is snake-free at one time of the year may not be at

another time. Particularly dense populations of sea snakes have been found in the coastal waters of the Philippines and of Thailand (Minton, 1975) and the western Pacific coasts of Central America (Tu, 1976). Sea snakes in general are not particularly aggressive, so bites are rare even in these circumstances. The venom reserve of most sea snakes is much less than that of most terrestrial snakes due to the small size of the head and glands, and venom injection does not always accompany bites. However, the high toxicity of their venoms make sea snakes a potentially quite dangerous group of animals and bites should be taken seriously.

B. Symptoms of Sea Snake Poisoning

Clinical symptoms following snakebite are quite variable and depend on the amount of venom injected, the site of injection, the physical state of the victim, and several other factors. Snakebite results can vary from very minor puncture wounds to death. Venom yields from sea snakes are quite low compared to most terrestrial venomous snakes, a factor which likely contributes to the low incidence of systemic effects following sea snakebites (Reid, 1981; Limpus, 1978a, b). Local reactions at the site of the bite are usually minor or asymptomatic and the puncture wounds are small and often not visible without careful inspection. Since many other marine organism stings produce intense local reaction or pain, the absence of local reaction is an important clinical clue implicating sea snake poisoning when signs of neuromuscular toxicity (such as muscle spasms, diplopia, facial weakness, general weakness, stiffness and trismus) are present.

The major manifestations of sea snake envenomation result from the action of the predominant neurotoxin(s) and can include muscle pain, paralysis (local and general), and respiratory arrest (Tu, 1987). Respiratory arrest is frequently the most immediate life-threatening symptom of sea snakebite; the diaphragm/phrenic nerve endplate is blocked by neurotoxin binding (Carey and Wright, 1961; Karunaratne and Pannabokke, 1972). In rare cases, death can occur rapidly from respiratory paralysis of the diaphragm at the phrenic nerve. Artificial respiration can delay fatality, but the neurotoxin(s) present in the venom must be neutralized with the appropriate antivenin to prevent death. One of us (S.P.M.) witnessed a middle-aged man in southwestern Mexico receive a bite in the chest by a small *Pelamis platurus*; he complained shortly thereafter of numbness of the hands, lips and throat, shortness of breath, blurred vision, and a metallic taste. Further observations were not possible, but it is likely he received a moderately severe bite. However, medical case reports for sea snake bites are rare and other symptoms may be present as well. Further clinical

observations of human victims of sea snakebites are given in Tu and Fulde (1987) and Reid (1975a, b).

Renal failure and myoglobinuria may result in cases of severe poisoning, presumably from the myotoxic action of phospholipase A_2. Whole venom from *Laticauda semifasciata* did not appear to affect the cells of the proximal tubule of the kidneys of experimentally envenomated mice (Schmidt *et al.*, 1976), though localized intracellular swelling of visceral epithelium was noted. Widespread hyaline myonecrosis of the skeletal muscles was noted postmortem in a patient bitten by a sea snake, and myoglobinuria is also a common observation in human victims (Karunaratne and Panabokke, 1972; Reid, 1973). Purified phospholipase A_2 was shown to induce myonecrosis in experimental animals (Tu and Passey, 1972; Lind and Eaker, 1981) and is probably the causative agent of myonecrosis in human sea snakebite victims.

Clinical management of sea snakebites can be complicated by patient uncertainty as to whether he or she has been bitten by a snake and inability to identify the offending species. Yet a case history can provide important cues for ruling out various types of biting and stinging animals. Since sea snakes are primarily aquatic, most bites occur in the water or when handling fishing trawling nets; however, snakes may wash ashore and be picked up or stepped on so bites are not limited to strictly aquatic activities. Sutherland (1983) has developed a flow chart for the treatment of snakebite in Australia, and most features of the chart are applicable to snakebite worldwide, particularly those resulting from elapid snakes. Physicians and others confronted with treatment of snakebite are referred to this chart (Sutherland, 1983; pp. 196–197). The basic first-aid features are outlined below:

1. Examine patient for fang wounds (often obscure in sea snakebites).
2. Proceed with first aid if positive signs of bite or envenomation are present. These include but are not limited to:
   a. pressure-bandage at site of bite
   b. immobilize affected limb
   c. keep limb and victim at rest
   d. transport victim to hospital immediately.

Antivenin treatment of sea snakebites remains the best method for preventing morbidity and mortality (Tu, 1987). Additional therapy may also be required, such as mechanical ventilation in cases involving respiratory failure and hemodialysis in cases of myoglobinuria and renal failure (Sitprija *et al.*, 1971). More complete summaries of sea snake envenomation symptoms and treatments can also be

found in Reid (1975a, b; 1978; 1981), Sutherland (1983), Tu (1987), Halstead (1988), Auerbach (1987) and Gopalakrishnakone and Chou (1990).

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