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TOXICITY AND TREATMENT OF SEA SNAKE ENVENOMATION,(U)
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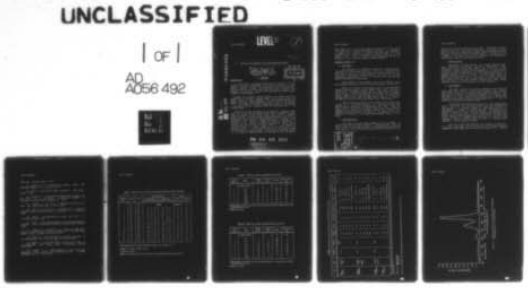

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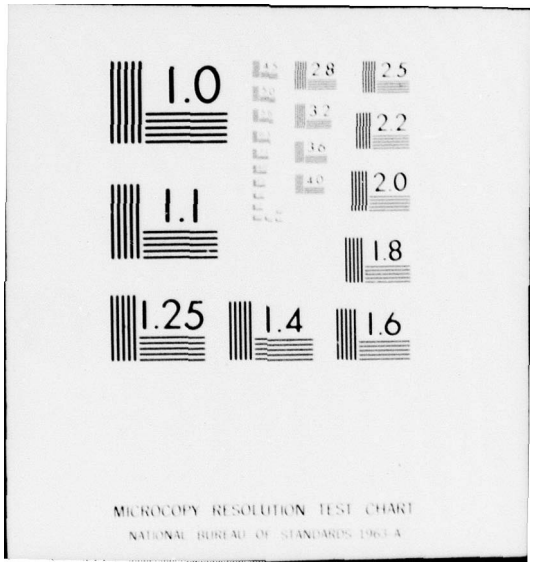
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(6) TOXICITY AND TREATMENT OF SEA SNAKE ENVENOMATION (U)

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INTRODUCTION.

In recent years, increasing interest has been directed to the sea snakes of the family Hydrophidae with special attention to their behavior general biology, and the chemical nature of their venoms. Several television features concerning sea snakes, together with newspaper and magazine articles, have generated substantial public interest in these animals (1-3).

Perhaps the greatest current interest in sea snakes lies in the probability of one species, Pelamis platurus (the yellow bellied sea snake), being an early immigrant into the Caribbean Sea from the Pacific Ocean when a Middle American sea-level canal is constructed (4-6). At present no resident, reproducing population of sea snakes of any species has been described from the Caribbean Sea or the Atlantic Ocean.

Concern on the part of members of the scientific community stems from the lack of adequate knowledge with which to judge the probable effect that Pelamis a fish-eating snake, would have on Caribbean pelagic ecology. A second and more immediate problem is the threat to human safety, particularly in regions frequented by tourists. Some scientists have predicted disastrous economic consequences to Caribbean tourist centers (presumed reduction in tourist trade) arising from the presence of this sea snake (6,7). The problem in this latter point of concern seemed to us to center about the following points (1) the actual potency of this sea snake s venom; (2) the quantity of venom available for delivery by individual snakes; (3) the degree of aggressiveness or willingness to bite displayed by

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this snake, as well as the total number of snakes; and (4) the degree of fear expressed by people who encounter this snake. In attempting to answer these questions, we hope to shed some light on the true seriousness of the threat to man posed by marine snakes in a new habitat. This paper is a report on our findings, and includes an evaluation of the currently fielded sea snake antivenin

MATERIALS AND METHODS.

A. Sea Snakes.

Our specimens of Pelamis platurus were all collected within the confines of Bahia Banderas, Mexico. This is a deep water bay indenting the Pacific coast some 30 miles and having a mouth 10 to 12 miles across The town of Puerto Vallarta at the foot of the bay (population approximately 25,000) is a tourist resort popular among Mexican and American citizens alike.

The distribution of sea snakes in this area is similar to that which might be expected in the area around the proposed sea way canal.

While our research vessels were hove-to or at anchor during the Naval Undersea Center cruises "MINOXI (July 1970) PELACAN I (March 1971), and MINOXII (March 1972)", we collected the sea snakes by dip-netting. Captured specimens were held aboard ship in gimbaled aquaria supplied with air bubblers and filters. The snakes were usually milked of their venom and tagged within moments of capture, but some were held for several days before their first milking

When our holding capacity for live snakes aboard ship were reached (30 to 40 adult specimens), we continued milking newly captured snakes, immediately returning them to the water. Upon the ship s return to San Diego, the captured snakes were placed in aquaria supplied with seawater and filters; aquaria were located in a temperature-controlled room at 80 degrees F. The snakes were fed live goldfish at intervals of 7 to 10 days and were milked every 3 to 4 weeks.

B. Obtaining Venom.

The adult of the sea snake has fangs only 2 mm in length. We therefore followed the method of Barme [8], using capillary tubes of known volume for obtaining the venom. The milking operation involved three operators one restraining and manipulating the snake while the

other two each fitted a capillary over a fang . Frequently, the snake voluntarily expelled venom into the capillaries. Subsequent pressure applied along the venom glands and ducts (maxillary margin) usually extruded an additional quantity of venom (table I). The product from a number of specimens was polled for various tests and analyses. Fresh Pelamis venom was invariably water-clear.

C. Venom Treatment.

Depending on the tests planned, the freshly obtained whole venom was either refrigerated, frozen, or lyophilized. Fresh, unrefrigerated venom was, in some cases, placed on one of our gel filtration columns, submitted to pH determination, or percent solids and specific gravity measurements For whole venom fractionation, we employed Sephadex G75 40 in either a 2 X 300 cm glass column for maximum single pass resolution [9] or a 2 X 150 cm glass column for more rapid separations and use aboard ship. Gel filtration columns were calibrated for molecular weight determinations using primary standards supplied for this purpose in kit form (Pharmacia).

D Test Animals.

All basic toxicity tests were performed on adult male and female albino mice (age 8 to 10 weeks) from the Walter Reed randomly bred ICR/FG strain. Final toxicity assays employed either male mice weighing 30 to 35 grams, or female mice weighing 25 to 30 grams. Male and female mice were never mixed in toxicity tests. The LD50 was estimated using the graphic method of Miller and Tainter [10] and the statistical procedures described by Finney [11].

Stock venom test solutions were made up in cold physiological saline. Dilutions from stock were made so that venom concentration was contained in a standard volume for injection equivalent to approximately 1 percent of mouse body weight. Graded doses of the venom were injected into a tail vein of the mice For each venom dose tested, 10 mice were used. The LD50 evaluation was determined from several pooled assays performed over a 24-hour period. The mice were closely observed for symptomatology and death for 4 hours following injection. Deaths occurring within 48 hours of injection were included in the evaluation of the LD50.

Five lots of sea snake antivenin, batch numbers 549-009(1964) 011 1(1967), 020-1(1969) 23-1(1970) and 25-1(1972), obtained from the Commonwealth Serum Laboratories, Australia were tested for efficacy

against sea snake venom in Walter Reed ICR strain mice (Table IV)

Additional pharmacological tests were performed on adult beagle dogs (Army test stock) weighing 10 ± 2kg. In these animals, the venom was injected into a indwelling catheter in the femoral vein. A single primate test was run on a 6-kg male Rhesus monkey. In all these tests, the dogs and the monkey were anesthesized with sodium pentobarbital (30 mg/kg). Tests for vasoconstrictive and cardiotoxic effects of the venom were performed on the isolated perfused dog heart following the method of Vick and Herman [12].

RESULTS

A. Venom Production.

Bahia Banderas appears to be the northermost site of a year-round population of the Pelamis in the eastern Pacific and in addition, it functions as a nursery area where gravid females congregate to give birth to their free-swimming, precocious young. Consequently, the majority of the specimens listed in table-I were gravid and, therefore, probably heavier than non-gravid specimens of comparable length.

Even when there was no evidence for a broken or unregenerated fang, the venom production from right and left venom glands tended to be unequal by as much as a factor of two or more (table I). Frequently, a varying amount of venom would be voluntarily injected into the capillaries by the snake accompanied by visible contractions of the marginal maxillary musculature. Following this venom expulsion an additional amount was usually available by milking. Venom production from apparently healthy, vigorous adult snakes ranged from a few lambda to a maximum of 33 lambda, but averaged 15 to 20 lambda per snake (table I; lambda = microliter). The venom was always waterclear and colorless except for the final few lambda expelled by milking which were occasionally cloudy with cellular debris.

B. Whole Venom Toxicity.

Fresh, liquid Pelamis venom (batch 1, refrigerated at 5 degrees C for no more than 4 days from time of milking to time of testing) was found to have an LD50 or 0.092 mg/kg in adult male mice (table II) based on an estimated 15.3 percent solids.* A second pooling of Pelamis whole venom that had been lyophilized (batch 2) showed an LD50 of 0.111 mg/kg in adult female mice (table III). Until

further testing we cannot say whether the apparent difference in toxicity of the two batches may be ascribed to a loss of activity in the freeze dried venom, a possible greater resistance on the part of female mice as has been shown for female over male mice in the Malayan pit viper venom or some other factor.

A limited series of toxicity tests on dogs in conjunction with pharmacological evaluation suggested a somewhat lower LD50 for whole Pelamis venom (fresh, liquid) in dogs than in mice, but insufficient data are available for valid statistical treatment. Using dogs, Vick [13] found the lowest LD50 (0.05 mg/kg) yet reported for any sea snake venom.

C. Toxicity of Major Venom Fractions.

Separation of Pelamis whole venom into its main components on our gel columns produced two major fractions (one a composite) together constituting about 90 percent of the total venom, and a series of minor components* (figure 1).

In the first of two series of tests on lyophilized Pelamis venom fractions, the cuts were obtained and labeled as indicated in figure 1. Although there was subsequent evidence of possible degradation in some of the fractions due to an error in handling during transport, both fractions 2 and 3 were found to be toxic for male albino mice (LD50 of 7.5 and 0.89 mg/kg, respectively) Neither fraction was as toxic as the fresh whole venom obviously. Fraction 1 was found to be totally nontoxic for mice at concentrations from 50 to 500 mg/kg. Whereas fraction 3 from this batch proved quickly fatal for a single dog at the concentration tested, the same dose of fraction 2 in a second dog of equivalent size was not fatal.

In a second series of tests, cuts were made on the fractionated venom. In this case, some of the minor fraction residues were also tested. Again, only fractions 2 and 3 demonstrated toxic activity, whereas the remaining components were nontoxic at the maximum concentrations tested. Careful assessment of the LD50's for fractions 2 (0.201 mg/kg) and 3 (0.086 mg/kg) in female albino mice indicated that fraction 3 was more toxic than whole Pelamis venom. Fraction 2 is about as toxic as whole venom from the common cobra (Naja naja) [14].

D. Pharmacology.

1. Whole Venom.

The symptoms of envenomation in male and female mice were essentially identical. The dose-response relationship was very steep, typical of highly toxic substances. As the venom dose decreased survival times increased. Major symptoms observed included deep and rapid respiration, always accompanied by severe mydriasis. At doses greater than the LD50 level, the mice were lethargic or semi-comatose and died almost instantaneously. At doses near the LD50, the symptoms of labored and rapid respiration occured approximately 15 minutes postinjection, accompanied by mydrdriasis. The heart beat seemed to increase. The mice at this stage appeared severely depressed and lethargic, showing impaired reflexes and occasional convulsions. As the effects progressed, respiration decreased and became more shallow until death occurred. If the mice remained in this state for more than 2 hours following injection, they usually recovered.

Physiological data obtained from dogs receiving varying doses of Pelamis whole venom (batch 1) paralled results obtained by Vick[13] with dogs receiving venom of the sea snakes Laticauda laticaudata and L. semifasciata. This was particularly apparent for the arterial blood pressure, heart rate, and EKG, all of which remained unchanged for periods of minutes even at the highest venom doses. In common with envenomated mice, the respiration rate in some test dogs was unaffected at first, only gradually increasing in the final preterminal minutes as inspiratory volume declined. In the dogs receiving the highest doses, however, respiration rate remained almost unchanged until time of death.

In animals nearing death, arterial pressure rose simultaneously with a decrease in inspiratory volume while heart rate gradually declined. The abruptness of onset of these latter events and the speed with which they developed seemed directly related to venom dosage. The EKG wave form remained almost unchanged until time of termination.

Physiological events in poisoned monkeys were practically the same as in dogs except that bradycardia developed very abruptly and only in the terminal phase.

A series of graded doses of Pelamis whole venom (batch 1) was tested on the isolated, perfused dog heart. At dose levels equivalent to 0.5, 1.0, 1.5, 2.0, and 2.5 mg/kg of intact animal, no change in rate or force of contraction was shown by the perfused, beating

hearts.

The effectiveness of sea snake antivenin against the venom of 3 sea snake species is shown in Table IV. Results indicate that the antivenin is highly effective against the venoms of Laticauda laticaudata, Laticauda semifasciata, and Pelamis platurus and in addition retains its potency for at least 8 years.

2. Venom Fractions.

Although there were difference in toxicity between fractions 2 and 3 of Pelamis venom, and between these fractions and whole venom, the symptoms produced in mice by each fraction were the same as those elicited by whole venom. That is, the mice envenomated with either fraction in the toxic range showed dyspnea, mydriasis, and impairment of reflexes, particularly the righting reflex. Death, with whole venom, appeared to occur from respiratory failure.

In the dog poisoned with fraction 3, the sequence of events was nearly identical to the sequence seen with high doses of whole venom. In the animals receiving fraction 2, no symptoms directly attributable to the effects of envenomation were observed throughout the 3-hour experiment.

DISCUSSION.

A. Cause of Death from Pelamis Venom.

From the foregoing, it appears that Pelamis venom acts to produce respiratory arrest in animals critically poisoned. No evidence for cardio toxicity was found in whole venom or its toxic fractions. This is essentially in agreement with earlier work indicating respiratory arrest to be a major cause of death in experimental animals [15,16] and in human beings who are victims of sea snake bite. Respiratory failure arising from myoneural junction blockade at the level of the diaphragm was the cause of death in experimental animals injected with whole venom from the sea snakes Laticauda laticaudata and L. semifasciata [13].

B. Hazard to Man from Bites of Pelamis.

The venom of Pelamis is somewhat less toxic than that of other sea snakes studied in recent years [13,16]. In some cases, its

potency is equal to or greater than that of other sea snake species [8,16,18,20]. However, few toxicity studies of sea snake venom published to date have been done with optimal numbers of laboratory animals, particularly mice, from suitably homogeneous populations; or perhaps this important facet has been left undisclosed by the respective authors (exceptions -Vick [13] and Tu and Ganthavorn) [20]. Data obtained from test groups of fewer than 30 are frequently not statistically reliable, [21,22] whereas populations of widely divergent size or age, or mixed gender* can all contribute to substantial deviations in LD50 values. Nonetheless, keeping in mind these difficulties, it appears that Pelamis venom toxicities are equal to or greater than those determined by others for this species.

Pelamis produces much less venom than does other species of sea snakes. Even in large specimens (table 1) the quantity available for injection will not be fatal to an adult man if the mouse toxicity data can be extrapolated to mammals, including man. Tests on monkeys suggest that primates may be less susceptible to sea snake venom than are mice. This becomes particularly true considering the work of Reid [16] who was of the opinion that sea snakes infrequently inject venom when biting in defense, and also considering the further probability that a sea snake is unlikely to deliver its total venom supply in a single bite. Under certain circumstances, however, it is possible to visualize how a bite from a large Pelamis might prove serious for a small adult, especially an infirm or elderly person or a child.

C. Aggressive Behavior of Pelamis Toward Man.

Reid [16] and Barme [19] have documented the incidence of sea snake bites among native fishermen of Southeast Asia. The bites occurred most frequently around the fishermen's hands and arms while they were handling nets and sorting fish, or on their feet and ankles while they were wading in their nets, stepping on or otherwise disturbing unseen snakes in the muddy water. Pelamis was not implicated as the source of envenomation in these reports.

Pickwell [23] has summarized the available information concerning the behavior of sea snakes toward swimmers and divers. The evidence indicates that swimmers on the surface or divers in transit are unmolested by the sea snakes they encounter although a number of instances have been reported of swimmers being followed some distance by sea snakes. Divers working in static situations such as salvage operations have, however, been bitten by sea snakes. There are no verified reports of loss of life under these circumstances, and it is

not clear what attracts the snakes to such relatively large animals as the stationary human divers.

Neither has Pelamis been implicated in any of the diving and swimming encounters reported to us. In contrast to the behavior of other species, Pelamis has not been reported to approach or to bite man.[24] This may be largely because of its life habits and habitat which include spending much of its life at the sea surface where it alone among the 50 species of sea snakes is able to feed on small pelagic fishes. Pelamis thus does not dive for feeding or hunting purposes and is not tied to inshore areas where wading fishermen or divers would most frequently be encountered. Nevertheless, Pelamis does occur in such areas as well as on the open sea, and we have had opportunity to test its possible aggressiveness or curiosity for human divers and swimmers.

In Bahia Banderas, Mexico we have swum with Pelamis where they occurred in some numbers several hundred yards offshore. None of us were ever approached by the sea snakes even when we had been treading water in a stationary position for some time. While in the water, one of our group once attempted to photograph specimens of this species. In two separate instances the snakes swam away from the diver just rapidly enough to maintain their distance, regardless of how fast the diver swam in pursuit. It seems, on the basis of these experiences, the Pelamis is not among the sea snake species likely to be aggressive or, for that matter, annoyingly curious about people.

There are very few accounts of human deaths from Pelamis envenomation. Such accounts as do exist are impossible to verify and tend to be anecdotal. An example of these is Beck's[25] account of a young diver (working in the Torres Straits, Cape York Peninsula Australia) who was bitten on the finger by a yellow-bellied sea snake (presumably Pelamis). The man became convulsive and died 48 hours later despite the fact the finger was amputated within an hour of receiving the bite.

Halstead[26] in his monumental treatise on venomous and poisonous marine animals cited a total of four deaths from the bite of Pelamis. He also included more generalized statements from the literature regarding the mortal fear in which this snake is held in such areas as Taiwan. Swaroop and Grab[27] in their summary on worldwide snake bite mortality stated that deaths from Pelamis bite have occured near Mozambique and along the coast of Central America. No data or references were given, however, and we do not know the

source of these statements. Reid and Lim [28] in the course of a survey of Malay fishing villages, learned of the "ular belerang," a mythical sea snake feared more than any other by the natives. The description of this snake (claimed to have been seen by a few of the native people) suggests a young Pelamis (belerang is Malay for sulfur). Fayrer [29] quoted a Mr. Stewart as saying that Indian natives in the region of Puri, in the state of Orissa, on the east coast, believe Pelamis to be the most deadly of all sea snakes. As "kullunder samp," this species figures prominently in local folklore.

d. Pelamis on the Beach.

Although it is able to feed and survive in the open sea and is not committed to shallow water, Pelamis not only occurs near shore in such places as Bahia Banderas, Mexico, and the Bay of Panama but frequently is washed ashore in thes areas [24,30-32]. Once on the beach, the sea snake is comparatively helpless since it lacks the broad ventral scutes possessed by land snakes. We have reports of large numbers of Pelamis stranded along the beaches of Panama and northrn Peru, and we have observed and counted stranded Pelamis along the beaches at Puerto Vallarta, Bahia Banderas [24]. Many of these snakes were still vigorous in the early morning when we made our counts, and they readily swam away when returned to the water. The possibility of receiving a bite from accidentally treading upon a beached Pelamis seemed real enough since the beaches in this resort area are heavily patronized by Mexican and American tourists alike. Yet our conversations with the town mayor, a local physician, and the port captain for Puerto Vallarta failed to disclose any incidents of sea snake bite within their memory. Evidently, the sea snake fails to intimidate the tourists frequenting the beaches of this popular resort and, at least in this area, has caused no fatalities or serious cases of envenomation.

e. Sea Snake Antivenin.

The currently available antivenin is effective against at least 3 species of sea snake and most probably many more. In addition it retains its potency for at least 8 years which makes it quite useful in actual field situations. Early results in our laboratory further indicate that short term artificial respiration, in addition to antivenin therapy, may increase survivor populations.

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Table I Venom Production by Newly Captured Adult, Female Sea Snakes (*Pelamis platurus*)[a]

Specimen number	Length[b]	Weight[b]	Venom produced[c]				Total	
			Left fang		Right fang		Liquid venom	Equivalent dry venom[d]
			Voluntary	Milked	Voluntary	Milked		
	cm	gm	lambda				lambda	mg
1	70	160	0	5.8	4.2	1.5	12	1.8
2	75	180	9.6	1.5	5.8	0	17	2.6
3	83	310	8.1	7.7	15.8	1.5	33	5.0
4	75	200	12.7	3.8	13.1	0	30	4.6
5	68	150	1.1	3.8	0	11.1	16	2.4
6	71	160	1.9	1.9	1.9	0.4	6	0.9
7	72	170	4.2	0.8	0.8	2.3	8	1.2
8	80	200	10.0	2.3	4.6	2.7	20	3.1
9	70	160	3.8	2.7	5.8	0	12	1.8
10	77	210	14.6	0.8	6.9	0	22	3.4
11	75	210	5.8	0.8	0.4	3.1	10	1.5
12	73	190	13.1	1.1	8.8	5.0	28	4.3
13	62	110	4.2	2.7	3.8	5.8	17	2.6

[a] Data obtained aboard R/V CAPE on Bahia Banderas, Mexico, 21 to 24 March 1971.

[b] Lengths, ±1 cm, approx; weights, ±10 gm.

[c] 1 lambda = 1 microliter.

[d] Based on 15.3 average percent solids in liquid *Pelamis* venom (Shipman and Pickwell, in preparation).

Table II LD50* Assay – *Pelamis* Liquid Whole Venom in Mice**

Dose group	Dosage	Number of mice	Number dead	Mortality	Mean survival time
	mg dry venom/kg			%	min
1	0.20	5	5	100	27
2	0.12	10	7	70	40
3	0.10	10	6	70	68
4	0.095	10	6	60	68
5	0.086	10	3	30	51
6	0.078	10	2	20	63

*This was fresh, liquid whole venom, batch 1 (see text). Mice were all males, 8 weeks old, 30 to 35 gm of the Walter Reed ICR strain.

**LD50 = 0.092 mg/kg.

Table III LD50* Assay – *Pelamis* Lyophilized Whole Venom in Mice**

Dose group	Dosage	Number of mice	Number dead	Mortality	Mean survival time
	mg dry venom/kg			%	min
1	0.5	5	5	100	5
2	0.2	5	5	100	23
3	0.14	10	10	100	33
4	0.13	30	25	83	40
5	0.12	30	19	63	48
6	0.11	20	11	55	64
7	1.10	20	5	25	64
8	0.075	10	0	–	–

*This was *Pelamis* venom batch 2 (see text). Mice were all females, 9 to 10 weeks old, 25 to 30 gm, of the Walter Reed ICR strain.

**LD50 = 0.111 mg/kg.

Table IV Effectiveness of Sea Snake Antivenin* Against the Venom of Three Heterologous Sea Snake Species

Species	Date of tests	Venom LD50 for mice	Venom LD99 for mice	Antivenin and venom LD50	Potency factor**	Venom (mg) 1 ml antivenin	Neutralized by 1 unit antivenin	Antivenin		
								Batch No	Date of batch	Strength
		mg/kg								units†/ml
Pelamis platurus	September 1972	0.11	0.15	1.06	9.6	0.1	0.004	25-1	February 1972	26.6
				1.20	10.8	0.1	0.004	23-1	June 1970	27.5
				1.12	10.1	0.1	0.004	020-1	March 1969	23.1
				1.48	13.3	0.1	0.002	011-1	26 June 1967	44.8
				1.17	10.5	0.1	0.003	549-009	24 November 1964	34.4
Laticauda laticaudata	December 1972	0.16	0.26	1.31	8.2	0.3	0.011	25-1	February 1972	26.6
	January 1973			1.03	6.4	0.2	0.007	23-1	June 1970	27.5
				1.10	6.9	0.3	0.013	020-1	March 1969	23.1
				0.90	5.6	0.2	0.004	011-1	26 June 1967	44.8
				0.65	4.1	0.2	0.006	549-009	24 November 1964	34.4
Laticauda semifasciata	April 1973	0.30,	0.48	1.90	6.3	0.4	0.015	25-1	February 1972	26.6
	May 1973			1.70	5.7	0.4	0.014	23-1	June 1970	27.5
Laticauda semifasciata				1.65	5.5	0.4	0.017	020-1	March 1969	23.1
				1.30	4.3	0.3	0.007	011-1	26 June 1967	44.8
				1.20	4.0	0.3	0.009	549-009	24 November 1964	34.4

* Commonwealth Serum Laboratories, Australia – equine, liquid, sea snake antivenin (AV) produced from the venom of the common sea snake, *Enhydrina schistosa*, five batches of varying age, all injections IV in mice.

** $\text{Potency factor} = \frac{\text{LD50 AV + venom}}{\text{LD50 venom only}}$

† One unit of Commonwealth Serum Laboratories' sea snake antivenin is defined as that quantity which will neutralize 0.01 mg of *Enhydrina schistosa* venom.

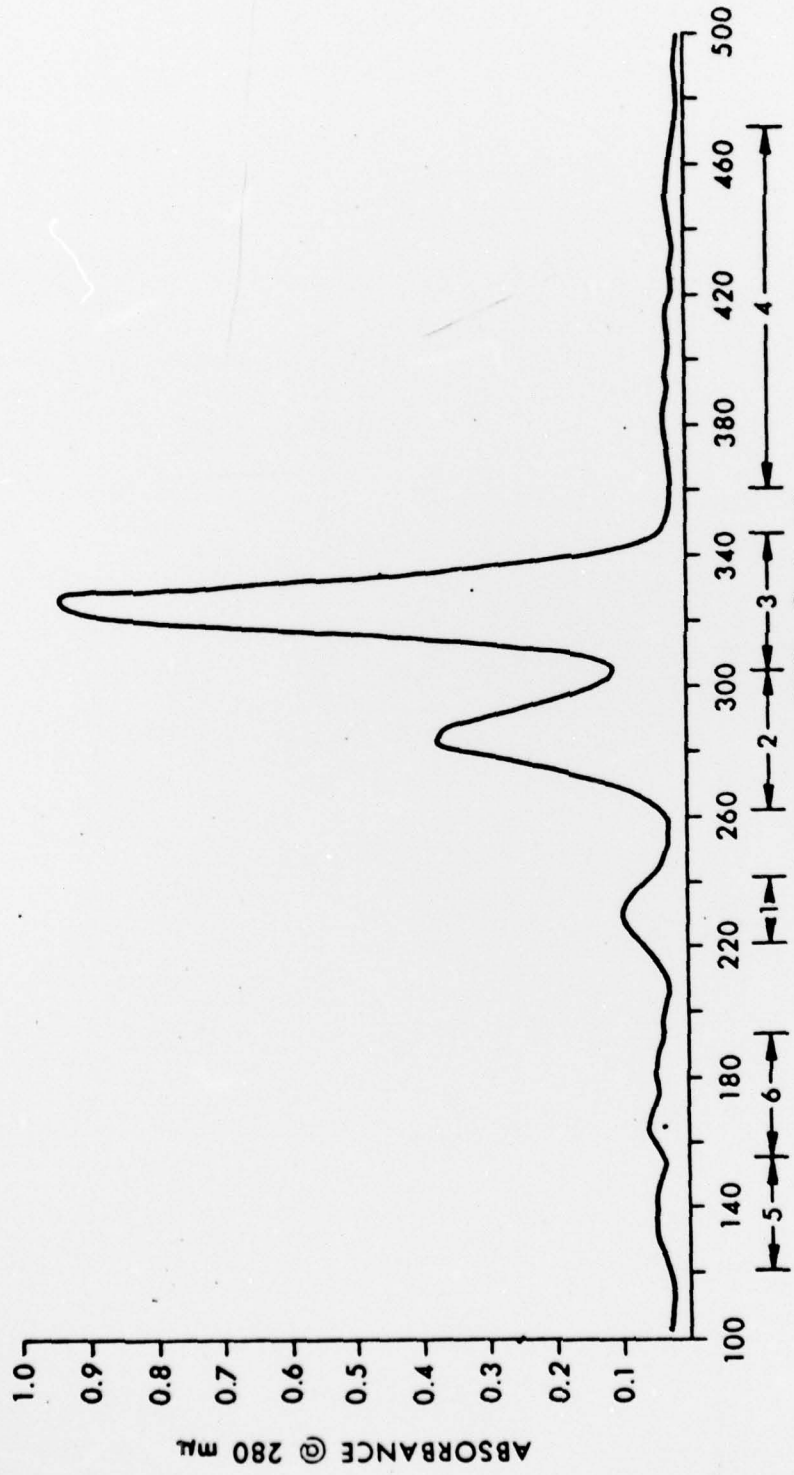

Figure 1 Separation of *Pelamis* Venom Components by Single Pass Gel Filtration on a 2 × 150-cm Column