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COLLECTION, TOXICITY, AND PRELIMINARY PHARMACOLOGY OF VENOM FROM THE SEA SNAKE, 'PELAMIS PLATURUS'

James A. Vick, et al

Edgewood Arsenal
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August 1973

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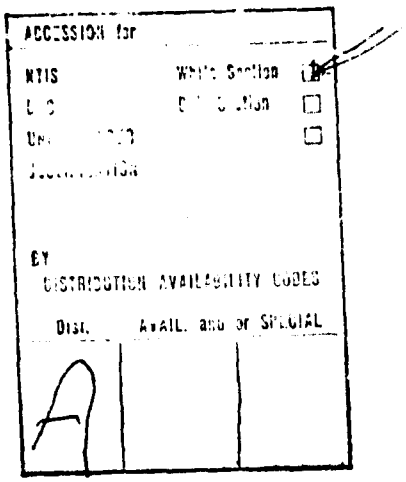

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13. ABSTRACT

A series of scientific studies was carried out to determine the feeding habits, distribution, and toxicity of three representatives of the Sea Snake *family Hydrophidae*. Mating and migratory habits were charted during specific times of each year in the South China Sea and in the South Pacific. The amazing diving and breath-holding capabilities of the sea snakes were also explored using deep diving gear and underwater photography. One hundred of each of three species of sea snakes (*Laticauda semifasciata, L. Laticaudita, Pelamis platurus*) were captured and their venom collected by a series of "milkings." The venoms were freeze dried, weighed, and reconstituted with saline to determine their lethality. This allowed for a comparison of the sea snake venoms, on a milligram per kilogram basis, with venoms obtained from land snakes (cobra, krait, rattlesnake, etc.). Results indicate that sea snake venom is one of the most potent venoms known to man, and it is capable of producing respiratory paralysis within 12 to 15 minutes following envenomation. The actual lethal dose of sea snake venom in mice is 0.05 mg/kg as compared with 0.5 mg/kg for the cobra, and 5.0 mg/kg for the rattlesnake. In addition, sea snake venom appears to be effective against both warm-blooded and cold-blooded prey even though the sea snake feeds mainly on cold-blooded fishes. The possible importance of the migratory habits of the sea snake in maintaining or disturbing the balance of nature between the Atlantic and the Pacific Oceans has also been explored. The ecological significance of these findings are discussed, as well as the potential problem that these reptiles pose to professional and amateur divers.

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Task 1W662710AD2502

DEPARTMENT OF THE ARMY
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FOREWORD

Most of the work described in this report was sponsored by the US Navy. The one member of the team from Edgewood Arsenal was invited to take part in the exercise because of his past work with toxins and venomous snakes. The laboratory work that was conducted at Edgewood Arsenal was authorized under Task 1W662710AD2502, Medical Defense Against Chemical Agents, Prophylaxis and Therapy for Lethal Agents, as venoms cause the same kind of shock seen in anticholinesterase poisoning. This work was started in March 1971 and completed in January 1973.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences–National Research Council.

Acknowledgments

We wish to thank the Mexican government for permitting us to collect and study sea snakes in Mexican territorial waters. Conversations with Dr. Soto and his assistants at Puerto Vallarta, and with Captain Mario A. Mucharraz F. have been very rewarding.

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A. J. Hutchinson and J. M. Nixon who provided valuable technical assistance during pharmacological tests.

DIGEST

A series of scientific studies was carried out to investigate the feeding habits, distribution, and toxicity of three representatives of the sea snake *family Hydrophidae.* Mating and migratory habits were charted during specific times of each year in the South China Sea and in the South Pacific. The possible importance of the migratory habits of the sea snake in maintaining or disturbing the balance of nature between the Atlantic and the Pacific Oceans has also been explored. The ecological significance of these findings are discussed in this report, as well as the potential problem that these reptiles pose to professional and amateur divers.

The amazing diving and breath-holding capabilities of the sea snake were also explored by the use of deep diving gear and underwater photography.

One hundred of each of three species of sea snakes (*Laticauda semifasciata, L. Laticaudita, Pelamis platurus*) were captured and their venoms collected by a series of "milkings." The venoms were freeze dried, weighed, and reconstituted with saline to determine their lethality. This allowed for a comparison of the sea snake venoms, on a milligram per kilogram basis, with venoms obtained from land snakes (cobra, krait, rattlesnake, etc.). Results indicate that sea snake venom is one of the most potent venoms known to man, and it is capable of producing respiratory paralysis within 12 to 15 minutes following envenomation. The actual lethal dose of sea snake venom in mice is 0.05 mg/kg as compared with 0.5 mg/kg for the cobra, and 5.0 mg/kg for the rattlesnake. In addition, sea snake venom appears to be effective against both warm-blooded and cold-blooded prey even though the sea snake feeds mainly on cold-blooded fishes.

CONTENTS

COLLECTION, TOXICITY, AND PRELIMINARY PHARMACOLOGY OF VENOM FROM THE SEA SNAKE, *PELAMIS PLATURUS*

I. 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) (figure A-1),* 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 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 first report on our findings.

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*Figure A-1 through A-7 are in appendix A.

[1] Cropp, B. Sea Snakes. Oceans Magazine *3*(2), 48-54 (1970).

[2] Dunson, W. A. The Sea Snakes are Coming. Natural History Magazine *80*(9), 52-61 (1971).

[3] MacLeish, K. Diving with Sea Snakes. National Geographic *141*(4), 565-578 (1972).

[4] Weathersbee, C. Linking the Oceans. Science News *94*(23), 578-581 (1968).

[5] Rubinoff, I., and Kropach, C. Differential Reactions of Atlantic and Pacific Predators to Sea Snakes. Nature, London *228*, 1288-1290 (1970).

[6] Gilluly, R. H. Consequences of a Sea-level Canal. Science News *99*(3), 52-53 (1971).

[7] Bailey, D. A Thermal Barrier for Snakes. Biomed. News 12-70. December 1970.

II. 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 [MINOX I (July 1970), *PELACAN* I (March 1971), and MINOX II (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 was 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°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 this sea snake has fangs only 2 mm in length (figure A-2). 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 (figure A-3). 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 B-I).* The product from a number of specimens was pooled for various tests and analyses. Fresh *Pelamis* venom was invariably water-clear (figure A-4).

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 × 300-cm glass column for maximum single pass resolution[9] or a 2 × 150-cm glass column for

*Tables B-I through B-VI are in appendix B.

[8] Barme, M. Venomous Sea Snakes (Hydrophiidae) in Venomous Animals and Their Venoms. Vol. I. Venomous Vertebrates. W. Bucherl, E. E. Buckley, and V. Deulofeu, eds., pp 285-308, Academic Press, New York, New York. 1968.

[9] Shipman, W. H., and Cole, L. J. A Surfactant Bee Venom Fraction: Separation on a Newly Devised Constant Flow Rate Chromatographic Column and Detection by Changes in Effluent Drop Volume. Anal. Biochem. 29 490-497 (1969).

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.

Additional pharmacological tests were performed on adult beagle dogs (Army test stock) weighing 10 ± 2 kg. In these animals, the venom was injected into an 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]

III. RESULTS.

A. Venom Production.

Bahia Banderas appears to be the northernmost 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 B-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 B-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.

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*Shipman, W. H., and Pickwell, G. V. Venom of the Yellow-bellied Sea Snake (*Pelamis platurus*): Some Physical and Chemical Properties. (In preparation.)

[10] Miller, L. C., and Tainter, M. L. Estimation of the ED50 and Its Error by Means of Logarithmic-probit Graph Paper. Proc. Soc. Exp. Biol. Med. *57*, 261-264 (1944).

[11] Finney, D. J. Probit Analysis. 3d Ed. pp 333. Cambridge University Press. 1971.

[12] Vick, J. A., and Herman, E. H. An Isolated Dog or Monkey Heart Preparation for Studying Cardioactive Compounds. Pharmacology *6*, 290-299 (1971).

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 B-I; lambda = microliter). The venom was always waterclear and colorless (figure A-4) 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°C for no more than 4 days from time of milking to time of testing) was found to have an LD50 of 0.092 mg/kg in adult male mice (table B-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 B-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 (table B-IV) 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* (figures A-5 and A-6).

In the first of two series of tests on lyophilized *Pelamis* venom fractions (fraction series 1), the cuts were obtained and labeled as indicated in figure A-5. 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 (table B-IV).

*Shipman, W. H., and Pickwell, G. V. Venom of the Yellow-bellied Sea Snake (*Pelamis platurus*): Some Physical and Chemical Properties. (In preparation.)

**Grenan, M. M. Observations on the Toxicity of *A rhodostoma* Venom in Male and Female Mice. (In preparation.)

[13]Vick, J. A. Venomous Sea Snakes. pp 974-975 in Poisonous and Venous Marine Animals of the World. Vol. 3 – Vertebrates continued. B. W. Halstead. US Government Printing Office, Washington, DC. 1970.

In a second series of tests (fraction series 2), cuts were made on the fractionated venom as indicated in figure A-6. 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 (table B-V). 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 occurred approximately 15 minutes postinjection, accompanied by mydriasis. 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) parallel 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 (table B-IV, Nos. 1 and 2). 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.

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[14] Vick, J. A., and Grenan, M. M. Evaluation of Antivenoms for Common Cobra (*Naja naja*) and Blue Krait (*Bungarus caeruleus*) Venoms. In Handbook of Dangerous Animals for Field Personnel, G. V. Pickwell and W. E. Evans, eds., Naval Undersea Center, San Diego, California. Technical Report. 1972.

A series of graded doses of *Pelamis* whole venom (batch 1) was tested on the isolated, perfused dog heart.[12] 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.

2. Venom Fractions.

Although there were differences 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 (table B-IV, No. 6), the sequence of events was nearly identical to the sequence seen with high doses of whole venom (especially table B-IV, No. 2). In the animals receiving fraction 2 (table B-IV, No. 7), no symptoms directly attributable to the effects of envenomation were observed throughout the 3-hour experiment.

IV. 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.[17] 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 on sea snake venom published to date have been done with optimal numbers of laboratory animals, particularly mice, from suitably homogeneous

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[15] Rogers, L. On the Physiological Action of the Poison of the Hydrophidae. Part II. Action on the Circulatory, Respiratory, and Nervous Systems. Royal Society, London. Proceedings *72*, 305-319 (1903).

[16] Reid, H. A. Sea-Snake Bite Research. Trans. Roy. Soc. Trop. Med. Hyg. *50*(6), 517-542 (1956).

[17] Reid, H. A. Snake Bite in the Tropics. British Med. J. *3*, 359-362 (1968).

[18] Carey, J. E., and Wright, E. A. The Toxicity and Immunological Properties of Some Sea-Snake Venoms with Particular Reference to That of *Enhydrina schistosa*. Trans. Roy. Soc. Trop. Med. Hyg. *54*(1), 50-67 (1960).

[19] Barme, M. Venomous Sea Snakes of Viet Nam and Their Venoms. pp 373-378 in Venomous and Poisonous Animals and Noxious Plants of the Pacific Region. H. L. Keegan and W. V. Macfarlane, eds., Pergamon (Macmillan), New York, New York. 1963.

[20] Tu, A. T., and Ganthavorn, S. Immunological Properties and Neutralization of Sea-Snake Venoms from Southeast Asia. Am. J. Trop. Med. Hyg *18*(1), 151-154 (1969).

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 (table B-VI).

Pelamis produces much less venom than does other species of sea snakes. Even in large specimens (table B-I) 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[13] 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 (cf. table B-I and table B-*II* Methods Section). 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 with 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. This may be largely because of its life habits and habitat[24] 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.

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*Grenan, M. M. Observations on the Toxicity of *A. rhodostoma* Venom in Male and Female Mice. (In preparation.)

[21] Trevan, J. W. The Error of Determination of Toxicity. Royal Society, London. Proceedings. B. *101*(712), 483-514 (1927).

[22] Russell, F. E. To be or Not to be . . . . . the LD50. Editorial. Toxicon. *4*, 81-83 (1966).

[23] Pickwell, G. V. Sea Snakes of Viet Nam and Southeast Asia. In Handbook of Dangerous Animals for Field Personnel, G. V. Pickwell and W. F. Evans, eds., Naval Undersea Center, San Diego, California. Technical Report. 1972.

[24] Pickwell, G. V. The Venomous Sea Snakes. Fauna. The Zoological Magazine. No. 4, pp 17-32. 1972.

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, that *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 Becke'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 occurred 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 these areas (figure A-7).[24,30-32] Once on the beach, the sea snake is comparatively helpless since it lacks the broad ventral scutes possessed by land

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[25] Becke, L. *Neath Austral Skies*. pp 305-310. John Milne, London. 1909.

[26] Halstead, B. W. Poisonous and Venomous Marine Animals of the World. Vol. 3. pp 627-681 -- Vertebrates continued. US Government Printing Office, Washington, DC. 1970.

[27] Swaroop, S., and Grab, B. Snakebite Mortality in the World. Bull. World Health Org. *10*, 35-76 (1954).

[28] Reid, H. A., and Lim, K. J. Sea-Snake Bite. A Survey of Fishing Villages in Northwest Malaya. British Med. J. *ii*, 1266-1272 (1957).

[29] Fayrer, J. The Thanatophidia of India. pp 131,133. J. & A. Churchill, London, 1872.

[30] Viquez, C. Distribucion geografica de nuestras serpientes venenosas. Inst. Defensa Cafe Costa Rica. Revista. *11*(87), 608-611 (1942).

[31] Dunson, W. A., and Ehlert, G. W. Effects of Temperature, Salinity, and Surface Water Flow on Distribution of the Sea Snake *Pelamis*. Limnol. Oceanog. *16*, 845-853 (1971).

[32] Kropach, C. *Pelamis platurus* as a Potential Colonizer of the Caribbean Sea. Bull. Biol. Soc. Wash. 2, 267-269 (1972).

snakes. We have reports of large numbers of *Pelamis* stranded along the beaches of Panama and northern 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, this 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.

V. CONCLUSION.

The general toxicity and physiological action of *Pelamis* venom resembles that of other sea snake species, but the quantity of venom delivered by *Pelamis* is substantially less than that obtained from some of the very dangerous Southeast Asian sea snakes.

The venom of *Pelamis* is highly toxic, however, and is easily capable of causing human death if available in large enough quantities (on a milligram per kilogram basis). Casual handling or molesting of living *Pelamis* should always be avoided. Because of the frequency that numbers of *Pelamis* are stranded along certain heavily populated beach areas, the possibility of serious envenomation in children or in small, infirm or elderly adults should be borne in mind and guarded against. At present, we have no evidence for other than mild avoidance reactions on the part of this sea snake toward human swimmers.

On the basis of the foregoing evidence, we believe that concern over possible adverse public health aspects occasioned by introduction of the *Pelamis* into the Caribbean has been exaggerated. Kropach[32] has come to much the same conclusion as a result of his studies of *Pelamis* in Panamanian waters. While we agree with others in a general concern over introduction of species into new environments, we believe that the present evidence does not support claims that this species constitutes a serious threat to human life. The small quantity of available venom in *Pelamis* and its apparently nonaggressive behavior have led us to the conclusion that it will be no more dangerous to people on the Caribbean coast than to the people on the Pacific coast. On the other hand, it might develop into a serious ecological problem as other newly introduced species have in the past. One needs only cite the imported fire ant and the Japanese beetle in support of this possibility.

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APPENDIXES

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APPENDIX A

FIGURES

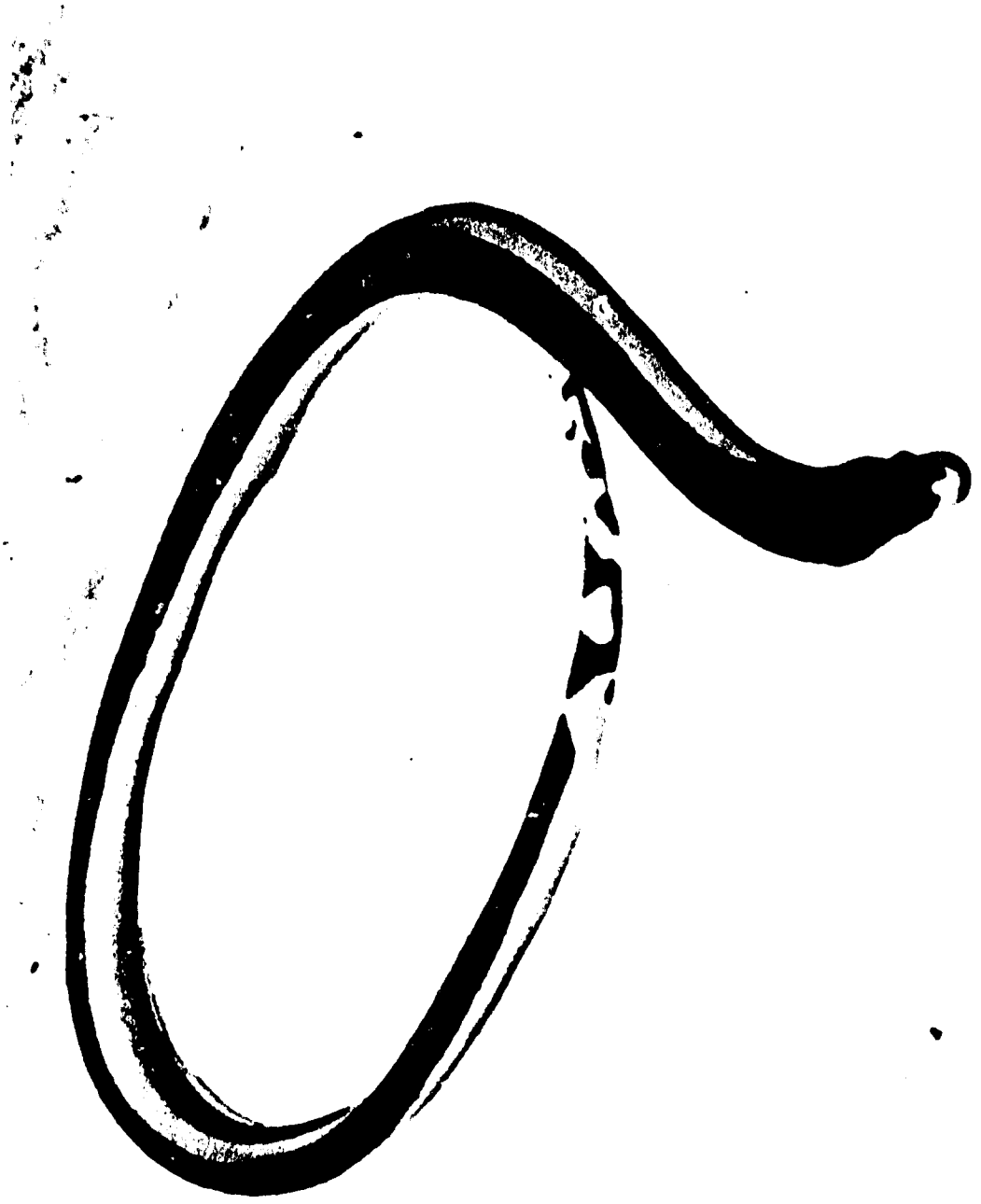

Figure A-1. The Yellow-Bellied Sea Snake, *Pelamis platurus*, (L.)

A probable early colonizer of the Caribbean via the proposed sea-level canal (photo by J. Sneed).

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Figure A-2. Head and Venom Fang of an Adult *Pelamis*

Note that fang is little more than 1 mm in length. Shortest interval on scale equals 1 mm (photo by W. H. Shipman).

Figure A-3. Procedure for Obtaining Venom from the Yellow-Bellied Sea Snake Using Capillary Tubes of Known Volume

(Photo by E. G. Barham)

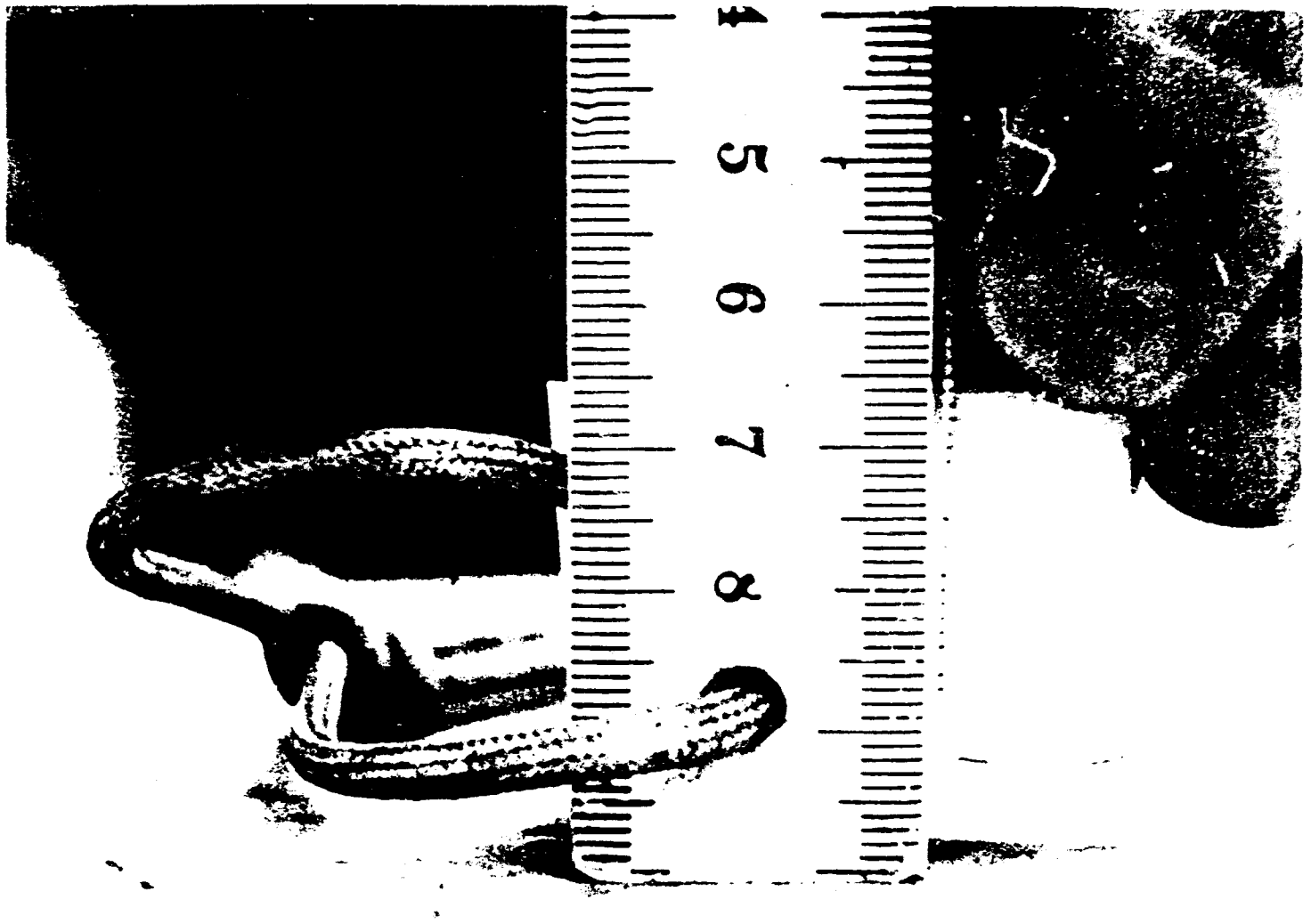

Figure A-4. The Venom Obtained from Milking 24 Yellow-Bellied Sea Snakes (*Pelamis platurus*)

Volume of liquid venom shown is 308 microliters, equivalent to approximately 47 mg of dry venom. Note water-like clarity of this colorless venom (photo by E. G. Barham).

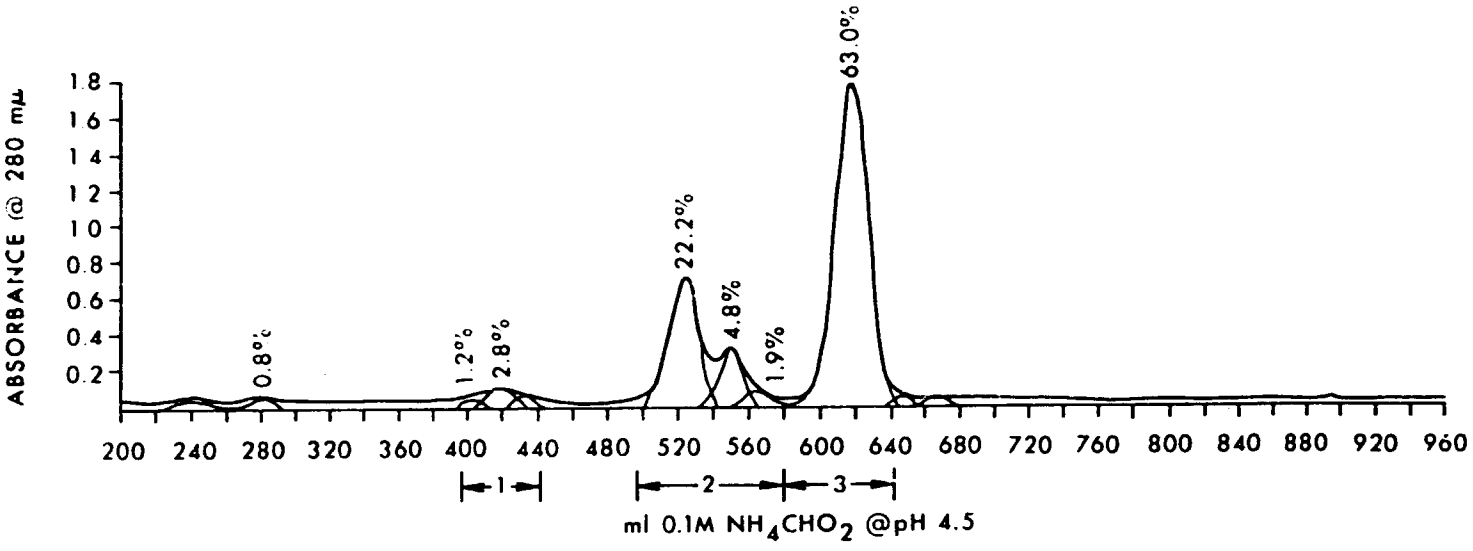

Figure A-5. Separation of the Major Components in *Pelamis platurus* Venom by Single Pass Gel Filtration on a 2 X 300-cm Column

Position of the cuts for fractions 1, 2, and 3 (fraction series 1) are indicated by arrows and bars. Estimated molecular weight for fraction 1, 24,500; fraction 2 main peak, 11,700; and fraction 3, 6,000.

Figure A-6. Separation of *Pelamis* Venom Components by Single Pass Gel Filtration on a 2 × 150-cm Column

Position of fraction cuts (fraction series 2) are indicated by arrows and bars; fractions 1, 2, and 3 same as in figure A-5. For toxicity tests on these fractions, see table B-V.

Figure A-7. Recently Stranded Yellow-Bellied Sea Snakes (*Pelamis platurus*) on Playa del Sol, Puerto Vallarta, Mexico

These snakes were very healthy and vigorous when encountered by the authors (photo by G. V. Pickwell).

APPENDIX B

TABLES

Table B-1. 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).

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Table B-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 B-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 B-IV. Toxicity of *Pelamis* Venom in Adult Beagle Dogs and a Monkey*

Test	Weight	Animal/gender	Venom dosage	Time to death
	kg ± 2		mg dry venom/kg	min
1	10	Dog – female	0.39	15
2	10	Dog – female	0.08	30
3	10	Dog – female	0.05	80
4	10	Dog – female	0.01	Survived
5	6	*Rhesus* – male	0.16	20
6	10	Dog – male	2.0 Fraction 3	12
7	10	Dog – female	2.0 Fraction 2	Survived

*Except where otherwise indicated, this was whole, liquid venom, batch 1 (see text), maintained at 5°C for 4 days prior to tests. Route of administration was in all cases intravenous; all animals were under sodium pentobarbital anesthesia. The venom fractions given in tests 6 and 7 were lyophilized prior to reconstitution in saline.

Table B-V. LD50 Assay – Lyophilized Fractions of *Pelamis* Venom in Mice[a]

Fraction group	Dosage	Number of mice	Number dead	Mortality	Mean survival time
	mg dry venom fraction/kg			%	min
1	3	5	0	–	–
2[b]	40	2	2	100	Immediate death
	4.0	2	2	100	Immediate death
	0.40	17	17	100	15
	0.30	10	10	100	30
	0.28	10	8	80	39
	0.25	19	15	79	43
	0.23	20	16	80	40
	0.20	39	20	51	63
	0.18	23	11	48	77
	0.17	22	3	14	40-98
3[c]	18	3	3	100	Immediate death
	1.8	3	3	100	Immediate death
	0.18	3	3	100	11-15
	0.15	10	10	100	31
	0.13	10	10	100	34
	0.11	9	6	67	58
	0.10	10	7	70	44
	0.09	10	7	70	52
	0.08	20	7	35	59
	0.07	10	1	10	105
4	37	5	0	–	–
5	45	3	0	–	–
6	10	5	0	–	–

[a]These were fractions from *Pelamis* venom, fraction series 2 (see text and figure A-6). Mice were all females, 9 to 10 weeks old, 25 to 30 gm, of the Walter Reed ICR strain.

[b]LD50 = 0.201 mg/kg.

[c]LD50 = 0.086 mg/kg.

Table B-VI. Toxicity Studies on *Pelamis* by Other Workers

Test animal	Number of animals used	Venom dosage	Route of administration	Mortality data	Reference/remarks
"A small fowl"	1	Unknown	Bite – probably on the thigh	Dead in 3 hours, 24 minutes	Fayrer.[29] Probably first experiment on effects of *Pelamis* venom.
Pigeon	Not given	0.075 mg/kg	Subcutaneous	"Minimal lethal dose" – no times given	Rogers[33]
Mud-fish	Not given	0.25 mg/kg	Subcutaneous	"Minimal lethal dose" – no times given	Rogers[33]
Guinea pig	1	1 mg/kg	Probably subcutaneous	Dead in 2 hours	Nauck.[34] Venom obtained from venom gland secretions expelled from dead snake
Mice, 20 gm	Not given	0.5 mg/kg	Intravenous	LD100	Barme[8,19]
Swiss white mice	30	Graded – six dilutions; LD50 equals 0.180 mg/kg	Intravenous	LD50 determined 24-hour observation period	Tu and Ganthavorn[20]

[33]Rogers, L. On the Physiological Action of the Poison of the Hydrophidae. Royal Society, London. Proceedings *71*, 481-496 (1902-1903).

[34]Nauck, E. G. Untersuchungen über das Gift *einer Seeschlange (Hydrus platurus)* des Pazifischen Ozeans. Deutsche Tropenmed. Zeit. *33*, 167-170 (1929).