A NOTE ON THE TOXICITY OF THE HORSESHOE CRAB IN THE GULF OF THAILAND[1]

by

A.H. BANNER and BETTY JANE STEPHENS[2]

Two species of horseshoe or king crabs (order *Xiphosura*) are known to occur in the Gulf of Thailand, *Carcinoscorpinus rotundicaudata* (LATREILLE) and *Tachypleus gigas* (MÜLLER) (SUVATTI, 1937). To our knowledge no reports on the habitats of the crabs in Thai waters have been published, but CHUANG (1961) reports the following about the two species on the coasts of adjacent Malaya:

"*Carcinoscorpinus rotundicaudata* . . . 35 cm. in total length in the adult stage . . . smooth cylindrical caudal spine which exceeds half the body length . . . found in the littoral zone of sandy shores and estuarine mud flats . . . .

"*Tachypleus gigas* . . . depths to 40 meters . . . exceed a half meter in total length . . . caudal spine, which is serrated dorsally, equals half the total length of the body. The eggs of this species are a delicacy."

The two species in Thai waters are distinguished by the names *mangda*, the species with the smooth caudal spine or *C. rotundicaudata*, and *hela* or *hera*, the species with the dorsally serrate spine or *T. gigas*, according to Dr. Twesukdi PIYAKARNCHANA (Chulalongkorn University, Department of Zoology; personal communication). *Mangda* is used for food—its egg mass is eaten cooked, often served as a curry. *Hela*, on the other hand, is considered to be toxic and never eaten, yet it is the same species that CHUANG reports as a delicacy.

In Thailand cases of intoxication and death have been reported that were ascribed to the eating of one of these Xiphosuran crabs. In these cases the intoxication was attributed to the fact that the individuals confused the two species and inadvertently ate *hela*.

1 Contribution Number 252, Hawaii Institute of Marine Biology, University of Hawaii, Honolulu, Hawaii 96822. Report published at the request of Dean SUVATTI.

2 Department of Zoology, University of Hawaii.

HALSTEAD (1965) states, "Asiatic horseshoe crab intoxications reportedly result from eating the unlaid green eggs, flesh, or viscera during the reproductive season of the year." He reviews the symptoms reported by earlier workers and states there is no data available on the toxin. HALSTEAD does not distinguish between the toxicity of *mangda* and *hela* (but he lists the Thai names for *C. rotundicaudata* as "Mangda fai, mada tuey, hera", and *T. gigas* as"mangda tale").

While the senior author was in Thailand in 1960-61, he endeavored to obtain samples of *hela* to study its toxicity; he even advertised in the Cholburi newspaper a reward of 100 baht (U.S. $5.00) for any specimens of *hela*. His efforts were to no avail. In July, 1961 he approached Nai Boon INTARAMPHAN, then Dean of College of Fisheries of Kaesetsart University, for samples. Nai Boon left the job shortly thereafter, but he transmitted the request to Dean Chote SUVATTI of the same school.

Sometime in 1962 a shipment of two 5-gallon tins of these horseshoe crabs was received at the Hawaii Marine Laboratory of the University of Hawaii. The data on the specimens was sent by letter and received in time to be burned in the fire that destroyed the laboratory in December, 1961. However, the procurer of the specimens, Miss Kanitha SILTHORNVISUDH of Kaesetsart University, remembers purchasing the specimens in the central fish market of Bangkok, where they were being sold for human consumption. They probably were gathered near the mouth of the Chao Phya.

In the following tests, made three years after receiving the crabs, the initial extractive procedure developed for the study of fish toxins was used (BANNER, *et. al.*, 1960, 1964). We believe that it would be effective in the extraction of most toxins of small molecular size, but it probably would not be able to remove toxic proteins.

The authors wish to thank Dean SUVATTI for the specimens and Dr. PIYAKARNCHANA for supplying some of the information cited above.

Materials and methods

The crabs in the two lots had the following characteristics:

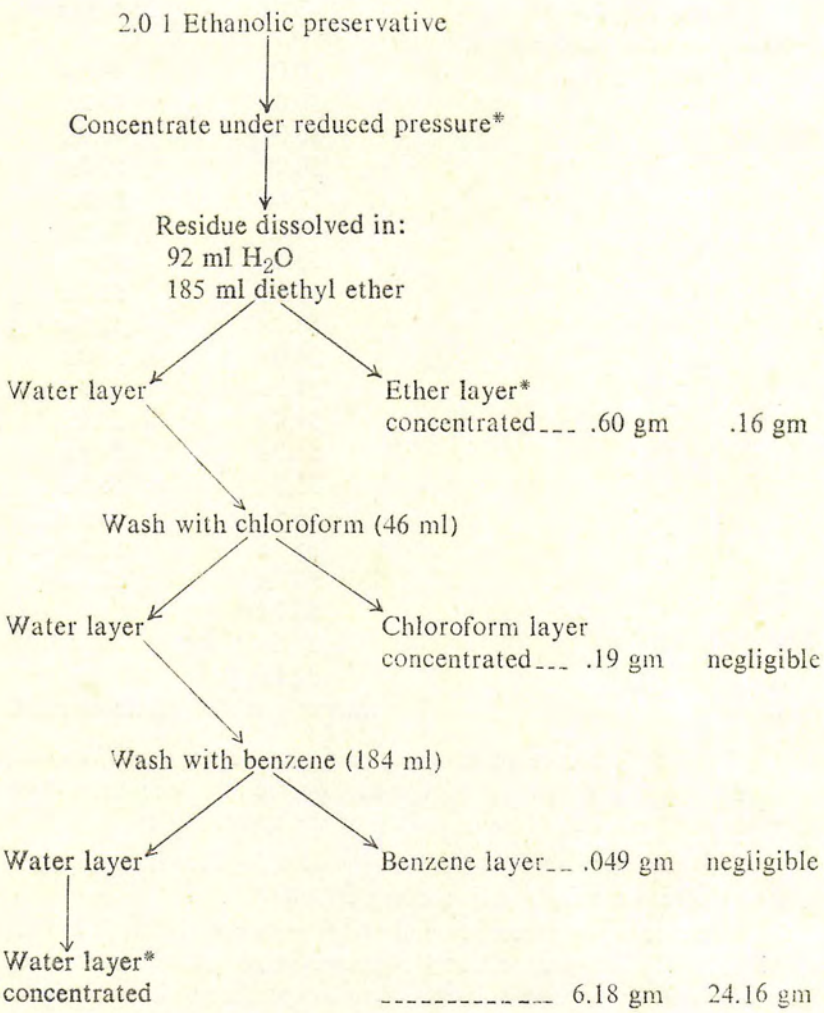
	Can I	Can II
Number of specimens	21	19
Number with broken caudal spines	1	2
Size range of intact specimens (total length)	25-31 cm	25.5-34 cm
Ratio of length of caudal spine to total length	50.0%	44.4%
	50.0%	48.2%
	50.0%	49.0%
	50.0%	50.0%
	52.8%	50.8%
	53.2%	51.6%
	53.4%	51.6%
	53.6%	51.6%
	53.6%	51.7%
	54.0%	53.0%
	54.3%	53.6%
	54.4%	53.6%
	55.2%	54.4%
	55.2%	55.2%
	55.5%	55.8%
	55.9%	57.2%
	56.7%	57.6%
	56.7%	
	58.6%	
	57.1%	
Nature of caudal spine	Smooth in all	Smooth in all

We have interpreted all specimens to be *C. rotundicaudata*, believing that the shorter caudal spines on the few specimens to be the results of accidental loss of the tip of the spine.

The specimens were preserved in sealed 5-gallon tins (approximately 40 liter capacity) in 70 percent ethanol.

The extractive procedure and yields are shown in the following diagram. The original ethanolic preservative was decanted and filtered; it and subsequent solvents were evaporated to dryness under reduced pressure with a "Swissco" rotary evaporator at temperatures below 50°C. Solvent-solvent extraction, as indicated, was done with separatory funnels after vigorous shaking by hand.

Extraction Procedure



* Samples tested

Those extracts which were obtained in sufficient quantity were homogenated in 0.9% normal saline solution with Tween 60 and injected intraperitoneally in Swiss Hirac white mice from the Pacific Biomedical Research Center, University of Hawaii. The mice ranged from 17 to 20 grams in weight, averaging 18.4 grams. They were watched for 3 hours; if no results appeared within that time the test was considered to be negative. The results are presented in Table I.

TABLE I

Results of Intraperitoneal Injection of Various Extracts of *Carcinoscorpinus rotundicaudata* in Mice

Extract	Amount mg/gm.	number of mice	LD/50	Symptoms
Concentrated Ethanolic Decantate				
Can I	4	8	1-2 hrs.	General sluggishness, later ataxia, profuse sweating, paralysis of hind lims.
Can II	4	6	No deaths	No apparent symptoms.
Diethyl ether EXtract				
Can I	4	6	2 hrs.	Slight sweating, general sluggishness, paralysis of all limbs.
Can II	4	2	LD/100 2 hrs.	Dyspnea, muscular spasms, paralysis of all limbs.
Final Water Layer (Concentrated)				
Can I	4	6	1 hr.	Initial symptoms of abdominal distress, muscular spasms, dyspnea, and paralysis of all limbs.
Can II	4	6	No deaths	No apparent symptoms.

Discussion

The results of these preliminary experiments are baffling. The following differences between the two lots are not understood:

1. Why was the yield per liter of the preserving ethanol in Can I less than a third that of Can II?

2. Why were the ethanolic concentrate and water extract of Can I toxic and those of Can II nontoxic?

3. Why were the ethanolic concentrate and water extracts of Can II nontoxic, but the diethyl ether extract of the same material toxic?

4. Why did the diethyl ether extracts of the two cans produce different symptoms?

Two suggestions may account for these observed facts. The first explanation is that among 19-21 horseshoe crabs in each cans, only a very few were toxic. Either all or most of the toxic crabs were in Can I, while in Can II there was at most a single or a few toxic crabs. This variation in toxicity of individuals from the same area is well known in the fish causing ciguatera (BANNER *et al.*, 1964), and variation in toxicity with area has been noted in the invertebrates *Tridacna maxima* (Mollusca, Pelecypoda) and *Palythoa* sp. (Coelenterata, Anthozoa) (data from studies at the Hawaii Institute of Marine Biology, as yet unpublished).

The other explanation suggests that the crabs in both containers were toxic, but varying amounts of other substances were extracted with the toxin. If the amounts of toxin extracted were uniform, and in small quantity, then in Can I the toxin would have been mixed with 7.6 g of extract, in Can II, with 25.8 g of extract. This 1 : 3.4 dilution may have dropped the concentration of the toxin below a threshold value in Can II and the toxin did not appear until it was concentrated in the diethyl ether layer.

In any case, it would appear that at least some individuals of *C. rotundicaudata* contain one or more substances that are toxic when injected intraperitoneally into mice, and that this toxin is soluble in polar organic solvents.

It is regretted that the samples available were inadequate to carry on more exhaustive and precise tests. This inconclusive report is presented merely to indicate that there may be confusion about the species of Xiphosurans in Thailand that may be toxic, that some specimens of the commonly marketed species of horseshoe crab may be toxic, and that further work is demanded upon fresh specimens studied individually. We further suggest that whole crabs be bioassayed both by feeding tests and by intraperitoneal injection of extracts.

BIBLOGRAPHY

BANNER, A.H., P. SCHEUER, S. SASAKI, P. HELFRICH, and C. ALENDER. 1960. Observations on ciguatera-type toxins in fish. Annals N.Y. Acad. Sci. 90(3): 770-787.

BANNER, A.H., P. HELFRICH, P. SCHEUER, and T. YOSHIDA. 1964. Research on ciguatera in the tropical Pacific. Proc. Gulf and Carib. Fish. Inst. 16th Annual, 1963 : 84-98.

CHUANG, S.H. 1961. On Malayan Shores. Pp. xvi + 225, 112 plates. Muwu Shosa, Singapore.

HALSTEAD, B.W. 1965. Poisonous and Venomous Marine Animals of the World. Vol. I, Invertebrates. Pp. xxxv + 994, many plates. Washington, U.S. Government Printing Office.

SUVATTI, C. 1937. A Checklist of the Aquatic Fauna in Siam (excluding Fishes). Pp. 1-116.