

Observations on the Toxic Sea Anemone, *Rhodactis howesii* (Coelenterata)

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THE SEA ANEMONE *Rhodactis howesii* W. S. Kent 1893 belongs to the phylum Coelenterata, order Corallimorpharia, family Actinodiscidae. This animal occurs on reefs in tropical areas of the Pacific Ocean (Cutress, 1957). The present study was done with specimens from the reefs of the Samoa Islands. American Samoans call *R. howesii* "matamalu" and attribute a form of fatal poisoning to either suicidal or inadvertent ingestion of the raw sea anemone. Such cases have repeatedly been mentioned by local medical authorities. However, cooking in water destroys the poison and cooked "matamalu" is commonly eaten by the natives.

While on a tour of duty at the Hospital of American Samoa, the author observed three cases of this poisoning. Shortly after the alleged ingestion of the sea anemone the patients went into stupor which lasted from 8 to 36 hours, depending on the case. During this period, knee jerk and pupillary light reflexes were absent but blood pressure and pulse rate were normal. All patients finally went into prolonged shock. They died with pulmonary edema. The clinical history and course of the poisonings recalled paralytic shellfish poisoning (Meyer, 1928). The phase of stupor suggested that the poison had either a curare-like action or affected primarily the central nervous system. The long duration of this phase suggested that the poison was different from known paralytic marine poisons and that it would be worthwhile to investigate it.

The paucity of research facilities on the islands restricted us to a study of general properties of the poison. We hoped that information so obtained would be adequate for both comparing this poison with other "marine poisons" and studying the conditions of preserva-

tion under which the highly perishable sea anemone could be shipped overseas to research laboratories with its poison intact. A bio-assay had to be worked out, using what was available. Various snails and fishes and the toad *Bufo marinus* L. (Oliver, 1953)² were tried. All showed some response to the poison, but that of *B. marinus* was the most suitable for a bio-assay. When the toads were injected with homogenates of sea anemone their survival time showed a definite relation to the injected dose.

METHODS

The toads were captured the evening before the experiments. From the time of their capture till the end of the observations they were given no food and were kept moist in darkness at an environmental temperature of 25–30° C. Fourteen to 16 hours after capture the toads were weighed and injected intraperitoneally with homogenates, five toads being used at each dose level. After injection each toad was turned over on its back at intervals, and its alertness and ability to return to normal posture were noted. The toads showed no change in behavior and reactions for several hours after injection. Then, suddenly, their responsiveness to change in posture and their mean frequency of respiration decreased, and within the following half-hour to 2 hours they died. In most of the toads the parotoid glands turned white immediately before or as death occurred.

For the bio-assay whole sea anemones were homogenized in an Osterizer with 4 times their volume of distilled water and then were further diluted with 0.9 per cent NaCl solution for easier handling. In assays with "fresh" sea anemones the time elapsed from the harvesting of a batch on the reef until the last injection of its homogenate into the toads was 4–6 hours.

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² *Bufo marinus* was introduced on Tutuila, American Samoa, by D. H. Butchart in 1951 (Butchart, 1957), and presently abounds on that island.

During this period the environmental temperature was 25–30° C. The tolerance of toad peritoneal cavity to distension by the volume of injected homogenate was estimated at about 8 ml. of homogenate for 100 g. of toad. This set the upper limit of the doses at 8 μ l. of sea anemone per gram of toad.

Survival time was measured as the elapsed time, in hours, from the moment of injection until death. In recording this, actual survival time was rounded out from the half-hour mark to the nearest full hour. Observations were not extended beyond 48 hours after injection. The lowest dose which caused death in a high proportion of toads within this period of time was found to be 1 μ l/g.

EXPERIMENTS AND RESULTS

1. Basic experiments showed the following results: (1) Toads of different weights, ranging from 30 to 100 g., were equally susceptible to the poison. (2) Various amounts of diluent added to the homogenate did not affect the dose effect curve. (3) Toads did not die after injection of any of the following: (a) a clear fluid which spurts from the sea anemone upon touch and slight pressure; (b) a jelly-like substance which the sea anemone secretes upon exposure to air; (c) sea water. (4) Homogenates which were dialyzed with running chlorinated rain water of pH 6 for 12 hours at 22° C. yielded the same dose effect curves as the fresh material of the batches from which they had been taken.³ It was noticed that the pungent smell of the original material disappeared during the dialysis. (5) Homogenates which had been heated in a boiling water bath for 15 minutes caused no mortality among injected toads.

2. For evaluation of "fresh" homogenates, twelve batches of *R. howesii*, harvested on nine irregularly spaced days, were assayed. Figure 1 summarizes the dose effect curves of these assays.

A total of 225 toads was injected with doses of 2, 4, and 8 μ l/g. Eight of them, with the three doses distributed at random, survived for

more than 48 hours. A total of 75 toads was injected with 1 μ l/g, and 20 of these survived for more than 48 hours. Only the data from toads which died within less than 48 hours were used for Figure 1.

The data of Figure 1 are derived from dose effect curves from batches which were harvested during all seasons of the year over a period of 14 months. Included were batches from a yellow-brown and a dark blue variety of *R. howesii*, both of which occur on the reefs of American Samoa; from each of two different colonies of sea anemones which grew a mile apart from each other; and from a colony which had been transplanted from its normal habitat on the reef to the shore line of the lagoon and kept there for 6 months. The narrow range of variability of the dose effect curves indicates that the poison content of *R. howesii* is not subject to seasonal variations and that it is the same in varieties of two different colors, in colonies of different locations of the area, and in colonies transplanted under the experimental conditions.

3. Evaluation of effects of preserving procedures: Two portions of a given batch of sea anemone were assayed to establish dose effect curves of the fresh material (controls). Two other portions of the same batch were preserved under a given condition and assayed after 8–14 days of preservation (experimental series). Dose effect curves of the experimental series were compared with those of the controls. The significance of differences of mean survival time at any one dose (Burn *et al.*, 1950) was estimated by the *t* test. The 2 per cent level of *P* was taken as the limit of significance.

4. Experiments with preserved homogenates: (1) Sea anemones were mixed with their weight of sodium chloride. A 5 per cent sodium carbonate solution was added until the mixture showed a pH of about 8. The mixture was kept at 25–30° C. for 8 days, after which it was dialyzed and assayed. It was found that the survival times, as compared with the controls, were significantly prolonged at doses of 2–8 μ l/g and that there was no mortality at the dose of 1 μ l/g. (2) The same experiment was done with a 2 per cent HCl solution added until the mixture showed a pH of about 5. It gave the same results as the experiment at alkaline

³ pH was estimated with nitrazine paper.

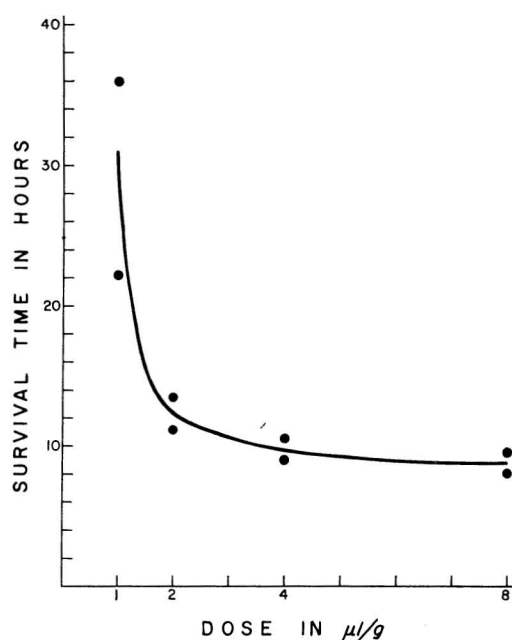


FIG. 1. Relation of dose to survival time of *Bufo marinus* injected with "fresh" *Rhodactis howesii*. At each dose level, two dots show the highest and lowest mean obtained in 15 assays. In each assay, five toads were used at each dose level.

reaction. (3) Sea anemones were mixed with 3 times their volume of ethyl alcohol and kept at 25–30° C. for 14 days, after which the mixture was dialyzed and assayed. It was found that mean survival times at doses of 4 and 8 $\mu\text{l/g}$ were the same as in the controls, but at doses of 1 and 2 $\mu\text{l/g}$ they were significantly prolonged. (4) Sea anemones, without addition of any substance, were kept at 3° C. for 14 days, after which they were assayed. The dose effect curves were found to be the same as in the controls.

Among the preserving procedures tested, refrigeration proved to be the only one suitable for our purpose.

DISCUSSION

The following discussion is based on the assumption that the poison which caused death of the toads is the same as that which caused death of the humans. Its nature is unknown.

According to one classification, "marine poisons" may be of infectious, allergic, or paralytic

type (Muller, 1935). Neither the symptoms of our patients nor prevailing environmental conditions suggested banal infectious agents as the cause of the poisonings. Furthermore, persons who repeatedly manipulated both the sea anemone on the reefs and its homogenates in the laboratory with their bare fingers did not experience the sensation of being stung nor did they manifest any skin lesions at later dates. Thus it is uncertain whether the discharge of the nematocysts of *R. howesii* is capable of injuring the human tegument and whether the poison is contained in the nematocysts or in the tissues (Phillips, 1956). But it is certain that for human skin the allergenic potentialities of *R. howesii* are negligible as compared with those of some other sea anemones and of squids (Sonderhoff, 1936; Halstead, 1957).

The poison of *R. howesii* seems to be of the paralytic type and, as is the case with the paralytic poisons of mussels and clams, it can be assumed that it is composed of more than one toxic principle (Sommer and Meyer, 1937). However, it differs from these in several respects. In poisonings with *R. howesii* the stage of stupor is long and cannot escape any observer, while mussel and clam poisonings seem more rapidly fatal, and a stage of stupor—if it exists—seems not to be impressive (Meyer, 1928; Medcof *et al.*, 1947; Tennant *et al.*, 1955). The degree of toxicity of mussels and clams changes with the seasonal variations of the density of poisonous dinoflagellates in the plankton (Sommer *et al.*, 1937), but *R. howesii* showed no seasonal change in toxicity.

Some toxic marine invertebrates have been found to derive their poison from one or the other of the following: their own tissue metabolism (Erspamer and Benati, 1953), the metabolism of a symbiont which lives in the tissue of the invertebrate (Zahl and McLaughlin, 1957), and the ingestion of poisonous plankton (McFarren *et al.*, 1957). By analogy it can be speculated that one or the other of these modalities may apply to *R. howesii*.

Various sea anemones have been found to contain homologues of amines and ammonium bases of varying degrees of toxicity (Ackermann *et al.*, 1923; Ackermann and Janka, 1953; Welsh, 1955) and high proportions of fatty acids and

sterols (Bergmann *et al.*, 1956); and for the sea anemone *Metridium senile* it was suggested that a mucoprotein participates in the poison of its nematocysts (Phillips, 1956). Substances of these classes probably occur in *R. howesii*, but it cannot be said whether they contribute to its toxicity. Since *R. howesii* loses its toxicity when heated, but not when dialyzed, it is suggested that proteins may play an important role in the composition of its poison.

From the clinical symptoms observed in our patients we could not determine whether the poison had a curare-like action or affected primarily the central nervous system. The symptoms of poisoning in *B. marinus* could not be interpreted, since we had no basis of comparing them with the effects of pure drugs on the toad.

The present preliminary study was terminated when we had established that refrigeration was suitable to preserve the poison of *R. howesii* during shipment.

CONCLUSIONS

1. *R. howesii* contains a paralytic poison which differs from other known "marine poisons" of this category. The duration of the phase of stupor observed in three cases of poisoning in humans was comparatively very long. The human skin is not affected by contact with *R. howesii*.

2. The poison does not dialyze at pH 6 and is inactivated within 15 minutes in the boiling water bath. There is no detectable loss in toxicity when the raw sea anemone is kept at 3° C. for 2 weeks.

3. The poison content of *R. howesii* does not depend on the color of the sea anemones and shows no seasonal variations. The toad *B. marinus* L. is suitable for bio-assaying *R. howesii*.

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