Venom of the lionfish Pterois volitans

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Intravenous injection into mice produced death in from less than a minute up to about one-half hour. The primary action in rabbits was a fall in blood pressure, accompanied by increase in respiratory rate; with larger doses there was evidence of myocardial ischemia or injury. After injection of fatal doses a variety of electrocardiographic changes occurred and the blood pressure fell to zero; respiratory arrest occurred terminally, but artificial respiration did not prolong the life of the animal. The active material was nondialyzable and the extracts contained considerable amounts of protein. Extracts retained substantial activity after lyophilization or addition of glycerol when stored for over a year at -20°C. The mean LD₅₀ following intravenous injection into mice was about 1 mg of protein/kg.

Fishes of the genus Pterois (family Scorpaenidae) have been known for many years to be capable of inflicting extremely painful and sometimes serious wounds through stings by the venomous spines (1-5). Species of Pterois are distributed chiefly in shallow water over wide areas of the tropical Indo-Pacific, and are often found in underwater caves or swimming about slowly in regions of rock or coral formations. These fishes present a spectacular appearance, with brilliant distinctive markings and long delicate fins, and are known by a variety of common names such as 'lionfish,' 'turkeyfish,' and 'zebrafish.'

Inflammation soon occur at the site of the wound and may spread and involve the entire leg. Systemic poisoning may also occur, with severe fall in blood pressure (unpublished observation).

The venom apparatus of the dorsal, anal and pelvic spines of Pterois volitans (Linnaeus) has been described by Halstead et al. (2). The venom-producing tissue is located beneath the integumentary sheath of the spines; the sheath is ruptured when the spine penetrates the flesh of the victim, and the venom diffuses into the puncture wound.

A number of specimens of Pterois volitans (Linnaeus) have been collected, and some physiological and other properties of extracts prepared from the spines are described for the first time in this report.

Materials and Methods

Collection of specimens. Specimens were collected at depths of 25 feet or less on the lagoon side of Parry Island at Eniwetok Atoll, Marshall Islands. They were captured alive by divers using hand nets made of chicken-wire; damage to the venomous spines by such nets was minimal, in contrast to our experience with nets made of twine. The fish were maintained alive in the aquarium until used. A total of 15 specimens (mean standard length 22 cm) were used in the work reported here.

Preparation of venom extracts. The fishes were killed by decapitation, and the 13 dorsal spines were cut free at the base. All subsequent manipulations were conducted at 1-5°C. The distal three-fourths of the integumentary sheath of each spine, together with the underlying glandular tissue within the anterolateral-glandular grooves (2) was scraped off into distilled water or 0.9% NaCl solution. The tissue was minced briefly with scissors, and then ground in an agate mortar in the presence of a small amount of clean sand. This initial extraction was carried out in 1.6 ml of water or saline per gm of spines. Grinding was continued for a few minutes until a homogeneous suspension was obtained; the suspension was then centrifuged at about 500 X g for 5 minutes, and the insoluble fraction was re-extracted for several minutes with an

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additional 0.4 ml of water or saline per gm of spines. The latter suspension was centrifuged and the supernatant fraction combined with that from the first centrifugation.

The crude extracts thus obtained as somewhat turbid, reddish-orange solutions with a pH of approximately 7 were stored at 3°C prior to preservation of activity by other procedures (see results).

Bioassay in mice. Extracts prepared as described above were diluted with 0.9% NaCl solution (usually about 1:10) immediately prior to bioassay and injected intravenously into male albino mice (Webster Swiss strain, mean weight 22 gm) via a tail vein. The solution was injected over a period of about 5 seconds; the volumes injected did not exceed 0.2 ml. All animals were observed for a minimum of 48 hours after injection.

Recording of blood pressure, respiration and electrocardiogram in anesthetized rabbits. Albino rabbits (mean weight 2.2 kg) were anesthetized with 1-1.5 gm/kg of urethan administered intraperitoneally. Blood pressure was recorded from a carotid artery with a mercury manometer and respiration recorded by a lever attached to a thread sewn to the skin above the diaphragm. The trachea was cannulated in all experiments; artificial respiration by means of a pulmotor valve attached to a compressed air source was initiated after the onset of respiratory arrest in some experiments. Venom solutions were injected intravenously through a catheter into a jugular vein. Electrocardiograms were taken at a chart speed of 25 mm/sec. (1 mv equal to 10 mm deflection); changes were most definite in lead I, although lead III was also used in some experiments.

Chemical methods. Nitrogen was determined by the micro-Kjeldahl method (6). Protein was determined with the biuret reagent of Gornall et al. (7); a calibration curve was prepared with crystalline bovine albumin (Armour) over the range 0.5-2.0 mg of protein, and the samples were read at 540 rnp in a Bausch and Lomb Spectronic 20 colorimeter.

Results

Acute toxicity in mice. Intravenous injection of venom killed animals in about 30 seconds to 30 minutes, depending upon the dose; animals which did not die within a half-hour usually survived indefinitely with no apparent ill-effects. Amounts of venom fatal in 2-10 minutes produced any or all of the following symptoms initially: paresthesia, circling movements and partial or complete paralysis of legs. A period of inactivity usually followed, with evidence of muscular weakness (e.g. the head often rested on the floor of the cage unless the animal was prodded, in which case the mouse usually moved about slowly). This apparent skeletal muscular weakness was more pronounced with the venom of Pterois volitans than with the venoms of the stonefishes Synanceja verrucosa, Bloch and Schnieder (8), and Synanceja horrida (Linnacus) (unpublished observations). The effects in mice of all three of these venoms were similar in most other respects.

Rolling or peddling movements (less violent in general than after the injection of stonefish venom) usually occurred terminally, and were followed immediately by respiratory arrest. The auricles and ventricles were usually still contracting when the chest was opened within a minute after respiratory arrest; the auricles usually were still beating with a regular rhythm. Approximately 2500 LD50 doses for mice were present in extracts prepared from the combined dorsal spines of each individual fish.

Venom extracts injected intraperitoneally were lethal at 5-10 times the intravenous LD50 dose. The mice became very inactive within a few minutes after the injection and when prodded to move displayed a slow ataxic gait which persisted until death, which occurred in 1-6 hours at the dosage levels used. Collapse, respiratory arrest and death were preceded by marked slowing of the respiration and intermittent twitching of the legs for a few minutes.

Extracts prepared in 0.9% NaCl from the skin adjacent to the dorsal spines (using the same ratio of volume of saline to weight of tissue as was used with the spines) were inactive in mice at doses 10 times as large as were lethal with extracts prepared from the spines (dose calculated on basis of weight of tissue used for extraction).

It was not possible to carry out protein analyses on freshly prepared extracts in the field. Subsequent analyses on stored glycerol-treated samples (after centrifuging and discarding the precipitate as described later) and on reconstituted lyophilized samples indicated a mean LD50 for four separate extracts of 1.1 mg of protein/kg. By the time at which the protein analyses were performed, these stored extracts had lost an average of 30% of their activity, by comparison with the freshly prepared extracts which had been bioassayed immediately after preparation.

Stability of venom under various conditions of storage. Freshly prepared extracts were treated as described below in

![FIG. 1: Effect of small dose of venom of Pterois volitans on blood pressure, respiration and electrocardiogram of a rabbit.](http://ajplegacy.physiology.org/DownloadedFrom)
order to maintain the potency of the venom during transport to the United States and subsequent storage.

Extracts were bioassayed in mice within one hour after preparation, the pH was adjusted to 7.5, and the extracts were then preserved either a) by lyophilization or b) by addition of glycerol (analytical reagent) to a final concentration of 40%. The lyophilized samples were stored subsequently in sealed glass vials in vacuo and the glycerol-treated samples in sealed glass vials in the presence of air. Samples were maintained for as long as 2 weeks at -20°C, then for 6 days packed in dry ice while being transported from Eniwetok to the United States. Subsequent storage has been at -20°C. A flocculent reddish-brown precipitate appeared in the glycerol-treated samples over a period of several weeks at -20°C; centrifugation yielded a clear, almost colorless supernatant solution and a precipitate (which was discarded). Little or no further precipitation occurred on further storage. Lyophilized samples were reconstituted with water to the original volume and centrifuged to give a clear supernatant solution and a small amount of reddish precipitate which was discarded. Such reconstituted samples usually lost about one-fourth of their activity on storage at 3°C for 24 hours.

Bioassays of representative lyophilized and glycerol-treated samples after about 1 year of storage has indicated that from 40 to 90% of the original activity was retained. Precise determination of the stability was not possible because of the limited number of mice (about 15) available in the field for bioassay of each freshly-prepared extract. It is clear, however, that substantial activity was retained on storage; furthermore, the symptoms produced in mice by stored extracts appeared identical to those evoked by extracts prepared from fresh spines.

Stability of the venom on storage of the spines themselves was also investigated. Spines from several fish, removed as described under MATERIALS AND METHODS, were scaled in polyethylene bags, which were then packed in dry ice for 6 days and stored subsequently at -20°C. Extracts prepared from these spines in the usual manner after storage for periods of up to 3 months were of comparable potency and produced in mice symptoms apparently identical to those evoked by extracts prepared from fresh spines.

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Extracts prepared in deionized water were of comparable potency and produced in mice effects which appeared to be identical with those produced by extracts prepared in 0.9% NaCl solution.

The mean protein content (biuret) of extracts which had been stored for 6-12 months in 40% glycerol was determined after centrifuging and discarding the reddish-brown precipitate described above. The mean protein content (calculated for the original volume of extract prior to the addition of glycerol) was 0.29%. Lyophilized samples reconstituted to the original volume of the extract possessed a mean protein content of 0.55% and a mean nitrogen content of 0.095%.

**Effect on blood pressure, respiratory rate and electrocardiogram of anesthetized rabbits.** Both glycerol-treated and lyophilized extracts were used in these experiments, and identical results were obtained; the extracts used had been stored from 6 to 12 months after preparation from fresh material. There was no evidence of a cumulative effect or tachyphylaxis following repeated injection of small to moderate doses at intervals of about 30 minutes. Seventeen rabbits were used in the experiments reported here.

Minimal doses produced a slight fall in blood pressure accompanied by an increase in respiratory rate, with no effect upon the heart rate or electrocardiogram (fig. 1); these effects disappeared within about 20-30 minutes. Larger doses caused a more marked fall in blood pressure and increase in respiratory rate, with evidence of myocardial ischemia or injury (flattening or inversion of T wave, or displacement of the S-T segment), or conduction defects such as bundle branch block. Little or no change in cardiac rate occurred, and the effects disappeared within about 30 minutes (fig. 2). The blood pressure usually returned, after the first small or moderate dose administered to a given animal, to a value which was about 20% above the preinjection pressure, and this elevated pressure usually persisted for a half-hour or
longer. Injection of fatal doses produced effects initially which were similar to those described above; a variety of additional electrocardiographic changes soon appeared (e.g. extrasystoles, bundle branch block, ventricular tachycardia, ventricular fibrillation). The respiration slowed and finally ceased, and the blood pressure continued to decrease (fig. 3). The auricles (and sometimes the ventricles) were usually still beating when the chest was opened within a minute or two after respiratory arrest; some irregular electrical activity in the heart usually persisted for an additional 5 minutes or more. Initiation of artificial respiration in eight animals within about one-half minute after respiratory arrest was ineffective in prolonging the life of the animal (fig. 3).

The mean lethal dose in the above experiments was approximately 200 µg protein/kg.

DISCUSSION

The authors are not aware of any well documented account of fatal poisoning from a wound by any species of *Pterois*. A near-fatal case occurred several years ago in the Marshall Islands following a sting by a specimen of *Pterois solitans* (unpublished observation), in which the victim's blood pressure fell to a very low value, and death appeared imminent. However, epinephrine was administered intravenously, and the patient's heart rate was opened within a minute or two after respiratory arrest, and blood pressure soon began to return to normal; no permanent after-effects of the sting were noted.

The work reported here indicates that the primary action of the venom on the cardiovascular system is the production of a marked hypotension (together with an increased respiratory rate), with essentially no change in the electrocardiogram following small doses. Amounts of venom sufficient to lower the blood pressure to about one-half to two-thirds the preinjection value produced also evidence of myocardial ischemia or injury which was reversible if the animal recovered; cardiac rate changes were again minimal, but this of course may have been due to depression of cardiac reflexes by anesthesia. We have no evidence at present to indicate whether these effects on the electrocardiogram were due to a direct action on the myocardium or coronary vessels, or were secondary to the hypotension.

REFERENCES


Respiratory arrest occurred as a terminal event; artificial respiration was ineffective in prolonging the life of the animal. Therapeutic measures directed toward the support of the circulation would appear to be indicated in the treatment of seriously poisoned victims, together with artificial respiration if needed.

The actions of the venom of *Pterois solitans* upon mice and upon the cardiovascular and respiratory systems of anesthetized rabbits appear very similar to those of the venoms of the stonefishes *Synanceja verrucosa* (8) and *Synanceja horrida* (unpublished observations). Mice appeared to suffer more from skeletal muscular weakness prior to death from lionfish venom, and in addition, the duration of action upon the cardiovascular system of rabbits was somewhat greater than with stonefish venoms. In other respects, however, the effects were essentially indistinguishable. Certain other properties of the venoms were also similar (e.g. their stability on storage under various conditions, and the nondialyzability of the active components). The common local effects (i.e. pain and swelling) seen in victims stung by *Pterois* and *Synanceja* also suggest the similar nature of the venoms. The apparently less dangerous consequences of most lionfish as compared to stonefish stings may reflect mainly a difference in the amount of venom entering the wound, rather than important differences in the nature of the venoms.

The active substance or substances in *Pterois* venom are nondialyzable; this fact, together with the presence of substantial amounts of proteins in the extracts, indicates that the active material is identical with or closely associated with protein.

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