Ciguatera Fish Poison: A Cholinesterase Inhibitor

Abstract. *The substance responsible for the poisoning effect of ciguatera poison from fish is an anticholinesterase. In rats, mice, and rabbits ciguatera poison causes death by asphyxiation. Protopam chloride with atropine is an effective antidote.*

Ciguatera, a serious and sometimes fatal disease caused by the ingestion of a variety of tropical marine fishes, has long been known in the Pacific and Caribbean (*1–3*). The substance responsible for ciguatera poisoning was first isolated at the Hawaii Marine Laboratory from the red snapper, *Lutjanus bohar* (Forskål) (*1*). Although much has been reported about ciguatera poison, little is known of its physiological and pharmacological properties (*3, 4*). We have now confirmed, by both pharmacological and biochemical assays, that the poison inhibits the action of cholinesterase.

Because of a shortage of pure poison, we used a semipurified poison extracted from a single batch of the musculature of *L. bohar*. The extract was diluted with 4.5 percent Tween 60 (polyoxy ethylene sorbitan mono-stearate) in 0.9 percent saline solution as emulsifier; this mixture is referred to hereafter as ciguatera toxin (CT). The lethal dose (LD_{100}) of CT in the mouse, when injected intraperitoneally, was approximately 12 mg per kilogram of body weight; based on previous work (*1*), this was approximately equivalent to a 0.7-percent solution of the pure poison. Because of the dangers associated with the crude poison, it is essential that our results be confirmed by experiments with the pure poison when it is available.

Toxic extracts from the flesh of the moray eel *Gymnothorax javanicus* (Richardson) and the grouper *Epinephelus fuscoguttatus* (Forskål), and from the liver of the gray shark *Carcharhinus menisorrah* (Müller and Helne), were also tested and the effects of each were similar to the effects of the extract from *L. bohar*.

Male albino rats (47), each weighing from 250 to 300 g, were anesthetized with pentobarbital sodium, 50 mg/kg of body weight, given intraperitoneally. Recordings of systemic blood pressure from the common carotid artery and of respiration from the trachea were made by means of pressure transducers on a Grass polygraph (*5*). One external jugular vein was cannulated for intravenous administration of CT and drugs.

Intravenous injection of a nonlethal dose of CT (0.5 μg/g) caused, in an anesthetized rat, a prompt fall in blood pressure (about 40 percent) and a slight increase in respiration. After a few minutes both blood pressure and respiration gradually returned to normal. No changes were observed in electrocardiograms.

Intravenous injection of a sublethal or lethal dose of CT (2 to 3 μg/g) caused the blood pressure to decrease at first, then to increase slightly and remain below normal. Initially, respiration was stimulated both in rate and depth. Respiration then decreased, and irregularities developed following the pattern of Cheyne-Stokes respiration. Electrocardiograms showed brachycardia (350 to 210 heartbeats per minute after 1 hour) and frequent ventricular blocks. Occasionally there were arrhythmic heartbeats with displacement of the QRS–T wave interval and omission of the T wave, as shown in Fig. 1*B*. If the dose proved to be lethal, respiration stopped after several hours, the blood pressure having remained only slightly lower than normal until death.

Intravenous injection of a large lethal dose (4 to 6 μg/g) of CT caused a sudden increase in blood pressure (Fig. 1*A*), the effects being similar to those caused by the injection of a large amount of adrenaline. Brachycardia, ventricular block, and arrhythmic heartbeat always occurred (Fig. 1*B*). In a few rats, prolonged ventricular blocks occurred prior to cessation of respiration. Only a very short period of slightly stimulated respiration could be observed. The changes in respiration, associated with the fall in blood pressure, usually led to complete respiratory arrest within a few minutes.

The effects of intraperitoneal injections of sublethal or lethal doses of CT on intact mice or rats were similar to those caused by the administration of an anticholinesterase. Initial restlessness and increasing abdominal distress was followed by the appearance of muscular fasciculation, diarrhea, salivation, lacrimation, profuse sweat, and general weakness. With doses sufficient to cause death, respiration became stressed as tremor developed, followed by ataxia; dyspnea then usually led to respiratory arrest. The heartbeat slowed down but continued a short time after cessation of respiration.

When CT (2 drops of a 1-percent solution) was applied topically to the eyes of three rabbits, miosis (contraction of the pupils of the eyes) occurred. Subsequent application of a 1-percent solution of atropine immediately counteracted the cholinergic effect.

When a small piece of rabbit intestine was suspended in warm Tyrode's solution (37°C) to which a solution of blood and acetylcholine chloride (prepared so that the final concentration was 0.1 μg/ml, and allowed to stand 30 minutes before use) was added, contractions of the intestine could be observed (Fig. 2*A*, left). After the intestine was washed twice with Tyrode's solution, normal

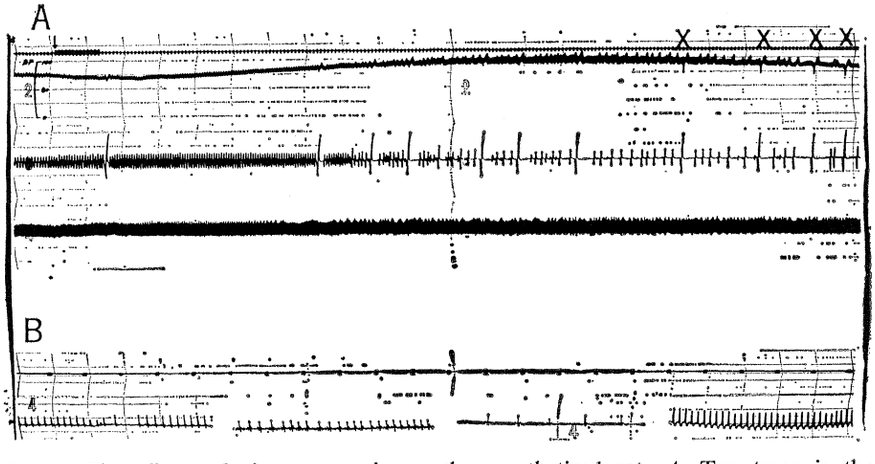

Fig. 1. The effects of ciguatera toxin on the anesthetized rat. *A*, Top trace is the time marker (each gradation = 1 second), second trace is blood pressure (in millimeters of mercury), third trace is respiration, bottom trace is the electrocardiogram (lead 1). Ciguatera toxin was injected intravenously at the arrow, in a dose of 4 mg/kg; *X* indicates ventricular block. *B*, Irregularities on the electrocardiogram, recorded with the chart moving at high speed (each gradation = 1 second).

isotonic contractions resumed. If the blood was first mixed with CT and allowed to stand for 20 minutes before the acetylcholine chloride was added (the CT being at a concentration of about 50 μg/ml), contractions of greater amplitude occurred, as shown in Fig. 2*A* (right). The hydrolytic effect of cholinesterase in blood upon acetylcholine was inhibited by the presence of CT. If the rabbit intestine was first treated with CT (50 μg/ml) for 2 minutes, the contractions caused by the additional acetylcholine chloride (0.25 μg/ml) were much greater than those elicited by acetylcholine chloride alone (Fig. 2*B*); the CT alone (50 μg/ml) caused no contractions.

When erythrocytes of human blood were treated with CT extract from shark liver, the cholinesterase enzyme activity was inhibited by 26 percent ($p < .001$) as determined by the electrometric method of Michel (*6*). In these experiments, 1 ml of buffer or of toxin solution (320 μg/ml or an estimated 0.42 μg/ml of pure toxin) was added to 1 ml of erythrocyte solution (0.02 ml of red blood cells) and 18 ml of buffer solution. Bovine erythrocyte cholinesterase (*7*) was then tested according to Ellman's method (*8*) with four different concentrations of CT (estimated as pure toxin) arranged logarithmically from 0.25 to 2 μg/ml. The cholinesterase activity was inhibited by 48 percent with CT at a concentration of 2 μg/ml. The data showed a linear relationship between the logarithmic concentration and the percentage of inhibition within the range studied. Our results are comparable with those of Winter (*9*) who used an automatic analyzer to demonstrate the effects of organic phosphate insecticides on bovine erythrocyte cholinesterase.

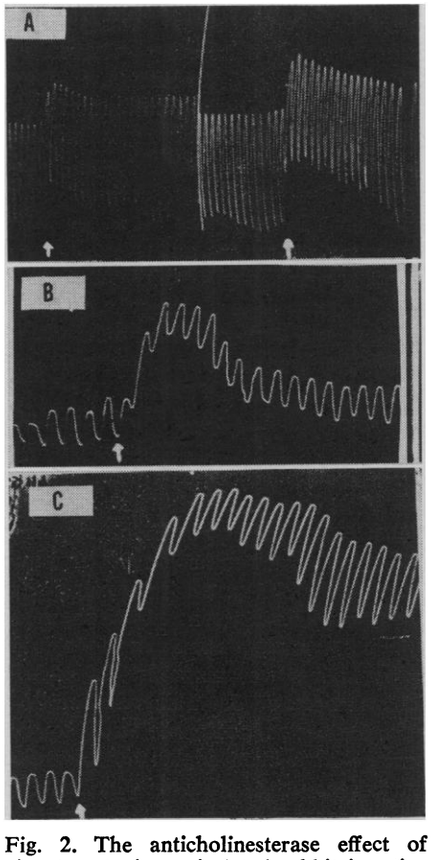

Fig. 2. The anticholinesterase effect of ciguatera toxin on isolated rabbit intestine in 25 ml Tyrode's solution bath. Acetylcholine chloride or mixed solution added at arrow. *A* (left), Acetylcholine chloride (0.1 μg/ml) and blood; (right), acetylcholine chloride and blood premixed with ciguatera toxin (50 μg/ml). *B*, Acetylcholine chloride (0.25 μg/ml). *C*, Intestine treated with ciguatera toxin (50 μg/ml) before the addition of acetylcholine chloride.

Various drugs were tested for their prophylactic and therapeutic action. Atropine (up to 5 mg/kg) injected before or after an injection of CT was effective in counteracting the muscarine-like action of CT. Atropine with magnesium sulphate (200 mg/kg) had the additional effect of abolishing the muscular paralysis and tremors due to the nicotine-like action of CT. The time that mice or rats given lethal doses of CT survived could be prolonged by giving successive doses of atropine, but death could not be prevented.

Physostigmine (*10*) injected with or without atropine before the administration of CT had little protective action on mice or rats (10 to 20 percent survived). Protopam chloride (2-formyl-1-methyl pyridinium chloride oxime), when administered by various routes with atropine, prevented death of both rats and mice injected with CT. Greater effectiveness was obtained by giving up to six successive doses of Protopam chloride (20 μg/g intravenously and 120 μg/g intramuscularly or intraperitoneally for the initial dose, reduced by 50 percent in successive doses) at 45-minute intervals, the number of doses given depending on the amount of CT injected. Although the effects of CT resembled those of the lipid-soluble organophosphorous compounds, the antagonistic action of Protopam chloride was not as great as against diisopropyl fluorophosphate and Paraoxon (*11*). It is not certain whether the mode of antagonistic action of Protopam to CT is comparable to that of the phosphate esters. Reactivation of cholinesterase inhibited by phosphate esters has been described by Nachmansohn (*11*) and O'Brien (*12*).

Since cyanosis was observed consistently before cessation of respiration, and since artificial respiration prevented death, the cause of death from ciguatera toxin is believed to be asphyxia.

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Glycerinated Skeletal and Smooth Muscle: Calcium and Magnesium Dependence

Abstract. *Contractions of glycerinated skeletal (striated) and vascular (smooth) muscles are similar in their calcium dependence but differ in their magnesium dependence. The threshold concentration of free Ca^{++} for contraction of either muscle was 1.8 × 10^{-7}M; maximum tension developed when the concentration of free Ca^{++} was slightly greater than 10^{-6}M. The Mg concentration required for contraction of smooth muscle was at least ten times as great as that for skeletal muscle.*

It is generally accepted that contraction and relaxation in striated muscle depend on the concentration of Ca^{++} in the sarcoplasm surrounding the myo-