

responsible for active uptake of salt in *Valonia*. A possible function of this effect of pressure would be to enable a growing cell to maintain a rather constant turgor pressure and, if other environmental factors were favorable, a constant rate of expansion. Cells subjected to changing salinities would especially benefit by having a feedback mechanism for controlling their turgor pressures. The mechanism by which pressure might affect salt transport is not clear, however, and offers an intriguing area for further study.

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12. The approximate concentrations (millimoles per liter) of major ions in the seawater were Na, 505; K, 11; and Cl, 600. The concentrations of major ions in the artificial sap were Na, 50; K, 615; and Cl, 650. The artificial sap contained 1 part seawater and 9 parts 0.67M KCl. Potassium fluxes were measured with K^{42} . Urea labeled with C^{14} was used in two experiments at an external concentration of 10 mmole/liter.
13. Pressures were applied and released gradually over a 10-minute period; sudden changes in pressure sometimes caused aggregation of the chloroplasts. The hydrostatic pressure of the perfusion system alone was about 15 cm of water, which was negligible compared to the experimental pressure of 1 atm.
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Ciguatoxin: More Than an Anticholinesterase

Abstract. *Anticholinesterase action of ciguatoxin has been reported. A study of the pharmacology of the action of the toxin on the respiratory system suggests that additional properties may be involved in the respiratory failure—the usual cause of death in ciguatera poisoning.*

Although anticholinesterase activity (1, 2) and the general parasympathomimetic properties of ciguatoxin (CT) have been described (3), there is some doubt that the entire action of this toxin can be explained so simply. Banner (4) has pointed out that, in some of the earlier reported cases, symptoms of ciguatera poisoning were alleviated when atropine or neostigmine was given (although the latter might be expected to potentiate the effects of an anticholinesterase). We now report on the effects of CT, with particular reference to the respiratory actions of the toxin which apparently cause death in ciguatera by asphyxia (1).

Female albino rats (180 to 250 g) were anesthetized with pentobarbital sodium (25 μ g per gram of body weight). Respiratory movements were recorded on a Grass polygraph with a thin wire hook in the diaphragm connected to a Grass force-displacement transducer (FTO3C); one jugular vein was cannulated for intravenous injection. The ani-

mal was prepared for artificial respiration by cannulation of the trachea; arterial blood pressure was monitored from one common carotid with a Statham P23 pressure transducer (5). Absolute quantities of toxin injected cannot be compared because the activity of the crude methanol extracts of CT varies considerably, depending on the toxicity of the fish from which it was extracted. Intravenous dosages were normally between one-tenth and one-half of the intraperitoneal LD₅₀ (lethal dose for 50 percent of animals tested) determined for each batch of extract used, and are thus in the low lethal range for intravenous injection. The toxic oil, prepared by the method of Scheuer *et al.* (2), was injected as an emulsion in human serum; equivalent quantities of human serum had no effect on respiration.

Within 5 to 10 seconds after CT was injected, a brief (2 to 5 seconds) increase in respiratory frequency occurred, and was followed by apnea lasting about 8 to 20 seconds (Fig.

1A), after which the amplitude of diaphragm movements sometimes increased again. With smaller doses, a transitory increase in frequency sometimes occurred, whereas with larger doses this phase was short or absent, and slowing of the respiratory rhythm was observed. As the rhythm slowed, the movements took on a gasping appearance, and the whole respiratory musculature became involved. The time from initial apnea to final respiratory failure depended on the dosage. With very large intravenous doses of CT there was no recovery from the initial apnea.

When the vagi were sectioned before injection of CT, the apneic phase did not occur. However, the initial increase in frequency, the subsequent slowing of the respiratory rhythm, and the time of final respiratory failure were not affected. The effects of prior vagotomy on tidal volume after CT injection were complex. At low doses, tidal volume decreased (Fig. 1B) rather than increased; when doses were increased this effect was reversed. Very large doses (twice the intraperitoneal LD₅₀) caused a marked increase in tidal volume within 5 seconds of injection, and respiration ceased 20 to 30 seconds later.

The pattern of respiratory failure caused by the anticholinesterases physostigmine (0.5 to 1.5 μ g/g) and paraxon (1.5 to 7.5 μ g/g) showed only the most superficial similarities to the effects of CT. The initial progressive reduction in tidal volume became, at the larger doses, so pronounced as to constitute an apneic period followed by gradually increasing, irregular gasping movements. However this apnea had a longer latency (25 to 35 seconds as opposed to 5 to 15 seconds with CT) and lacked the sudden onset characteristic of the initial apnea produced by CT. Bilateral vagotomy, rather than suppressing the apnea, enhanced the reduction in tidal volume, thereby producing an apneic phase at dosage (0.5 to 1.0 μ g of physostigmine per gram of body weight) at which this is not normally seen. Increasing the dosage did not reverse the apnea.

The final respiratory failure occurring after both CT and physostigmine is not a peripheral neuromuscular effect, as has been shown by stimulating the phrenic nerve with single maximum shocks at intervals during the experiment. There was some increase in twitch height, followed by reduction of the

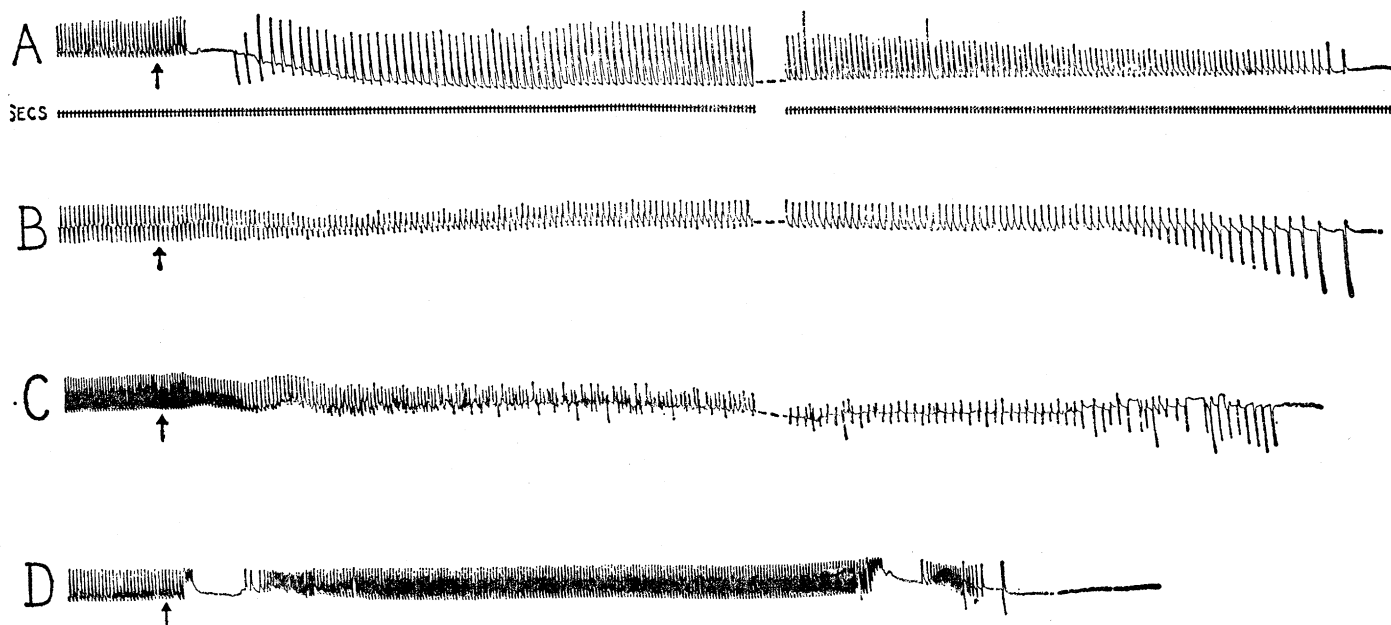


Fig. 1. A comparison of the respiratory action of ciguatoxin, physostigmine, and acetylcholine in four rats. The time of injection is marked with an arrow in each case. (A) Ciguatoxin (one-tenth of the intraperitoneal LD_{50}) injected; (B) ciguatoxin (one-tenth of the intraperitoneal LD_{50}) injected after cutting both vagi; (C) physostigmine ($1 \mu\text{g/g}$) injected; (D) acetylcholine ($1 \mu\text{g/g}$) injected. Record A was interrupted for $3\frac{1}{2}$ minutes; records B and C were interrupted for $2\frac{1}{2}$ minutes.

twitch and then by neuromuscular block. However, in the ten physostigmine and eight CT experiments in which the phrenic nerve was stimulated, the final respiratory failure preceded the maximum neuromuscular facilitation and occurred while the twitch height was still relatively little affected.

Intravenous injection of acetylcholine (0.5 to $1.5 \mu\text{g/g}$) produced an initial apnea similar to that resulting from CT. Vagal sectioning prevented this apnea. With large doses ($>1.5 \mu\text{g/g}$), as with CT, there may be no recovery from the initial apnea. At lower doses (Fig. 1D), the initial apnea was followed by a period in which the tidal volume was unaltered but frequency was increased; this led to respiratory failure without the characteristic slowing of respiratory frequency and increase in tidal volume (which follows CT). At doses of less than $1 \mu\text{g/g}$, there was usually no final respiratory failure after injection of acetylcholine.

In view of the beneficial effects of atropine therapy in the treatment of ciguatera poisoning (4), it was surprising that even prior injection of atropine, at doses ($40 \mu\text{g/g}$) which protected against more than ten times the normally effective dose of acetylcholine or physostigmine, did not prevent the initial apnea or significantly delay the time of final respiratory failure after the standard dose of CT (one-tenth of the intraperitoneal LD_{50}). Some pro-

TECTIVE action has been reported (1) for the cholinesterase reactivator pralidoxime methiodide (PAM), although the mechanism of this action is not clear since CT is not an organophosphorus compound (2). However, we did not find any protective action against the respiratory effects of the standard dose of CT even after prior intravenous injection of up to $150 \mu\text{g}$ of PAM per gram of body weight.

The absence of any detailed correspondence between the respiratory effects of CT and physostigmine (or paraoxon) suggests that the respiratory effects of CT are not primarily caused by its previously reported anticholinesterase action. The apparent correspondence between the initial respiratory effects of CT and physostigmine suggests that these result from a transmitter-like cholinomimetic action. The actions of CT may be more complex, as indicated by the lack of antagonism by atropine, which is an effective antagonist of the respiratory actions of acetylcholine and even of the peripheral actions of CT (1).

The fact that atropine cannot block the major respiratory effects of CT makes it doubtful that the toxin is purely an anticholinesterase in its action. An anticholinesterase can act only through its effect on the concentration of acetylcholine; at muscarinic junctions, the effects of anticholinesterase action must be blockable by atropine.

The junctions in question may be atropine-sensitive, since atropine did block the respiratory effects of acetylcholine and physostigmine or paraoxon. We therefore suggest either that CT may have a greater affinity for the muscarinic receptor site than atropine or acetylcholine, or that its effects may not involve these receptors. Unless cholinergic receptors are somehow affected, it is difficult to see why the actions of CT should be so apparently confined to cholinergic systems (1, 3). The first of these hypotheses thus seems the more acceptable.

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