TOXINS OF MARINE ORIGIN[1]

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Most major groups of marine animals include representatives that produce toxic secretions (1–3). Among multicellular animals, these secretions are generally employed either for defense or food capture. With a few noteworthy exceptions, marine toxins have not been critically analyzed, nor have searching pharmacological analyses been completed. For such studies the material should be available in relatively pure form and in some abundance. Many of the poisonous products attributed to marine organisms have not been isolated; most are unstable when separated from carrier substances; and nearly all are produced in small quantities by animals that are not, themselves, particularly abundant. This review will be concerned only with those toxic products of marine origin that have been studied in some detail, using modern analytical methods and instrumentation.

Dinoflagellate Toxins

The marine environment sustains a rate of organic productivity, measured by the photosynthetic fixation of carbon, more than twice as high as the land per unit of surface (4). By far the greater portion of this primary productivity is the work of microorganisms and nannoplankton that, together, form the base of the food pyramid in the sea. Prominent among this group of organisms are the diatoms, whose siliceous skeletons comprise the fossil deposits known as diatomaceous earth. Of only slightly less importance in the economy of the sea are the microscopic dinoflagellates. These organisms are either unarmored or they are covered with plates of cellulose. Dinoflagellates are generally not preserved in bottom sediments, nor do they appear to contribute to the fossil record. They reproduce rapidly by simple cell division when physical and nutritional conditions are optimal, often occurring in such concentration as to discolor the surface of the sea over considerable areas. Cell counts during such plankton blooms may reach 20 to 40 million per liter. These blooms, sometimes known as "red tides," may result in mass mortality of fish and other marine animals, although the evidence supporting a direct causal relationship appears equivocal (1, 5).

The principal organisms identified in toxic red tides are *Gonyaulax*

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catenella in California and the Pacific Northwest, *G. tamarensis* in the Bay of Fundy, and *Gymnodinium breve* in the Gulf of Mexico and the Atlantic coast of Florida. Sommer and his colleagues (6) reported that *G. catenella* contains about 85 per cent moisture; that the dry weight of 3000 organisms was about 15 micrograms, and that toxin represents about 6 per cent of the total dry weight.

Lamellibranch molluscs and other filter feeders remove most of the particulate matter from the considerable volumes of water they circulate over the gills in respiration (7). The mussel, for example, filters up to 20 liters of water per day (6), and an Alaskan butter clam of average size may filter 40 liters of water every 24 hours (8). Assuming total clearance, a California mussel of average size, bathed in a "red tide" of average intensity might thus ingest 3.0 g dry weight of *G. catenella* in 24 hours. If they were of average toxicity, the mussel might contain 180 mg of toxin. This considerable burden of toxin causes no overt pathology in the mollusc (6).

Toxin, which is not metabolized or excreted is sequestered in the hepatopancreas in *Mytilus californianus*, and in the siphons of the butter clam, *Saxidomus giganteus*, thus establishing the ecological foundation for "paralytic shellfish poisoning." Earlier investigators (6) reported that nontoxic *M. californianus* held in laboratory tanks could be made highly toxic by being fed a suspension of *G. catenella*. Burke et al. (5) isolated the toxin from axenic cultures of *G. catenella*, which proved identical to the substance that Schantz (9) had described from the mussel and from the Alaskan butter clam. The three extracts were identical in physical, chemical, and pharmacological properties.

Saxitoxin.—The compound responsible for the toxicity of poison shellfish, one of the most potent produced by marine organisms, was named saxitoxin, because of its association with the Alaskan Butter Clam, *Saxidomus giganteus*. This material has not yet been prepared in pure form, nor has it been successfully crystallized from solution. It is a basic substance that readily forms salts with mineral acids that are more soluble in water than in methanol and are insoluble in fat solvents. The molecular formula, $C_{10}H_{17}N_7O_4$, originally proposed for mussel poison by Schantz (9) has been questioned by Rapaport (10). Oxidation followed by reduction of saxitoxin yields dioxide and ammonia and a compound that has been identified as 2-imino-8-amino-6-methyl-3-β-carboxyethylpurine, $C_9H_{12}N_6O_2$. This reaction suggests that the original compound must have been $C_{10}H_{15}N_7O_4$. Kao (11) has speculated that the molecule probably contains two guanidinium residues and called attention to the similarity between this composition and that of the puffer poison, tetrodotoxin.

The best preparations of saxitoxin assay about 5500 mouse units of activity per milligram of dry toxin. The official method of assay and analysis was described by McFarren et al. (12) based on the procedure of Sommer & Meyer (13). The mouse unit is defined as the amount of saxitoxin required to kill a 20 g mouse in 14 min after intraperitoneal injection. Female mice are

significantly more sensitive than males. NaCl modifies the activity of the toxin; the higher the salt concentration, the lower the activity. The higher the pH, the lower the activity (14). There is considerable difference in sensitivity to saxitoxin among the common laboratory animals tested (15). The lethal dose for man is not known, but Meyer (16) estimated that the oral lethal dose would be about 40,000 mouse units from his study of three cases of paralytic shellfish poisoning. He estimated the amount of material eaten by counting the empty mussel shells remaining and determined the concentration of ingested toxin by analyzing uneaten portions of mussel. Death in man has followed the ingestion of as little as 1 mg of saxitoxin.

The toxin appears to be readily absorbed from most segments of the digestive tract and is excreted in the urine (17) at a rate that suggests that the kidney may be the principal site of its elimination from the body.

Early workers (17) reported that the poison from *M. californianus* depressed respiration, modified conduction in the myocardium, and depressed the cardioinhibitory and vasomotor centers. These effects were ascribed to direct actions on the central and peripheral nervous systems. The poison reduced systemic arterial pressure and depressed the breathing rate (18). Fingerman et al. (19) described a curare-like effect attributed to some interference with the response of the muscle to acetylcholine. Actually, as Kao has emphasized (11), the block in neuromuscular transmission is in the motor axon and on the muscle membrane with relatively little involvement of the motor end plate. The poison has a direct effect on the atrio-ventricular conduction system of the heart and on the heart muscle membrane. The hypotensive effect of saxitoxin is apparently not due to its influence on the heart or on the smooth muscle of the vascular system (20). Most clinical reports emphasize the serious depression of activity of the respiratory center in man. The precise mechanism of this depression is not yet clear.

On isolated frog sciatic nerve, on isolated nodes of Ranvier, and for a single-celled electroplax preparation of the electric eel, *Electrophorus electricus* (21), saxitoxin blocks propagated spikes without depolarization. Similar effects have been described (22) for skeletal muscle of the frog. Saxitoxin does not prevent normal depolarization by acetylcholine, nor does it influence the increased potassium efflux induced by acetylcholine and carbamylcholine. It is without effect on the potassium and chloride conductances of the excitable membrane, but appears to interfere specifically with the permeability of the membrane to the sodium ion. Kao (11) suggests that the guanidinium groups in the molecule may complex with some unspecified component of the membrane to close off sodium channels. Most gross pharmacological effects of saxitoxin, if this view be confirmed, may thus be ascribed to interference with active ion transport mechanisms in various biological membranes. The resemblance of this mode of action to that of holothurin, tetrodotoxin, and of the nematocyst toxin of *Physalia* is a striking illustration of the convergent evolution of molecules with similar biochemical activities in unrelated animals.

Sponges.—The phylum Porifera probably includes more than 5000 species. While many of these have been examined and numerous compounds isolated (1), none has been associated with specific toxicity in man or other test animals. It is of some interest to report our own failure with the fire sponge *Tedania ignis* (23). This organism is notorious for the intense burning sensation and urticarial reactions it produces in the hands of unwary collectors, reactions not generally thought to be induced by its spicules. Neither aqueous nor alcoholic extracts of this sponge showed significant biological activity when tested by intraperitoneal injection in mice, nor when injected directly into the hemocoele of the fiddler crab, *Uca pugilator*, in our standard bioassay procedure for other marine toxins (24).

Coelenterate Toxins

The phylum Cnidaria was so named by Hyman (25) because all members possess characteristic intracellular organelles, the cnidocysts or nematocysts. These are roughly spherical structures that range in size from about 5 microns to those of *Halistemma rubrum* that measure 1.12×0.12 mm (26), making them easily the largest nematocysts known. Essentially, the mature nematocyst consists of a thick transparent and smooth capsule, invaginated at one pole to form an extended coiled hollow tubule. The internal surface of the nematocyst tubule is armed with chitinous barbs arranged in spiral rows. The capsule also encloses a fluid rich in solutes, the staining reaction of which suggests that it contains considerable dissolved protein (27). The characteristic toxin that arms the nematocyst and augments its effectiveness is dissolved in this solution.

There is considerable evidence (28) suggesting that nematocysts may not be independent effectors, as they have been described, but may be subject to some kind of central control in certain Cnidaria. When activated by whatever mechanism, the nematocyst discharges with explosive force. During discharge the tubule everts progressively, bringing its internal armament to the definitive external surface to constitute an effective tangle for small prey organisms. Penetration of prey is facilitated by a continuously renewed crest of spines created at the advancing tip of the everting tubule when spines that were previously internal reach the tip and become superficial. This process was first described by Robson (29) in these terms, "the tip of the shaft is formed by a constantly renewed spearhead of opposed barbs. These flick out sharply and take their positions in each of the spiral rows." The eversion of the tubule in *Physalia physalis* is sufficiently forceful to penetrate a fish scale, the chitinous exoskeleton of crustacean prey, or even the skin of the hand of a human investigator protected by a surgical glove (24). The extruded tubule from a nematocyst of *Physalia* only 20 microns in diameter may exceed 3 mm in length. The everted tubule of the nematocyst of the Indo-Pacific sea wasp, *Chironex fleckeri* (30, 31) penetrates all layers of the human skin. The volume of the nematocyst after discharge is greater than the volume of the uneverted system (29, 32, 33).

The earlier work on coelenterate toxins has been well summarized (34–38). Whole animals, nematocyst-bearing tentacles, mesenteries or acontia alone were generally extracted by homogenizing living materials. Toxic symptoms evoked by injecting such extracts into experimental animals have been attributed to compounds variously called "hypnotoxine," "congestine," and "thalassine" (39–42). Tetramine, trigonelline, and homarine have been identified in homogenates of *Actinia equina* and of *Anemonia sulcata* (43, 44). Urocanylcholine and other quaternary amines have been described (45) from extracts of *Condylactis gigantea*, *Metridium dianthus*, and *Cyanea capillata*. Martin (46) described a heat-labile, nondialyzable, curare-like principle in the anemone *Rhodactis howesii*, to which he ascribed its considerable human toxicity. It should be emphasized that all of these investigations employed relatively nonspecific extracts of complex organs or tissues. In none of these studies could the biological activity of the extracts be ascribed exclusively to the nematocysts.

Modern-day studies of coelenterate toxins originated with Phillip's description (47) of a method for isolating clean nematocysts from surrounding tissue. His method, or modifications of it (24), have produced the extracts on which most of the specific observations of toxin pharmacology to be described in this survey have been made. Barnes' interesting membrane method (48) for collecting the venom of *Chironex fleckeri*, using the human amnion, provides samples of authentic nematocyst contents. Unlike toxin liberated by homogenization of isolated nematocysts, Barnes' preparation is quite stable. Clearly, the effectiveness of this procedure will vary with the activity and morphology of the nematocysts available. Nematocysts of *Aurelia aurita*, for example, rarely evoke local reactions in man; in our experience those of *Porpita* and *Velella* sp. never do. Nematocysts of these animals could not be studied by Barnes' procedure.

Nematocysts of coelenterates are primarily employed to capture prey. Since most coelenterates derive their nourishment from small, often planktonic, animals, the raptorial mechanisms must subdue all inhabitants of this stratum of the food pyramid. Several investigations (24, 28, 49–51) detail the effects of coelenterate toxins on crustacea. There is still little experimental information of the effects of these toxins on vertebrate prey animals.

There are even less clinical data describing the effects of coelenterate toxins on man. Southcott (52), Barnes (53, 54), and Cleland & Southcott (31), among others, have described human fatalities resulting from contact with the cubomedusae *Chironex fleckeri* and *Chiropsalmus quadrigatus*. Kingston & Southcott (55) describe the nematocysts of *C. fleckeri*. Their paper is illustrated with photomicrographs of sections of skin penetrated deeply by numerous nematocyst tubules of *Chironex* in a fatal human incident. Barnes (56) showed that the causative agent for "Irukandji stinging" of bathers in Queensland coastal waters was a small carybdeid medusa. Although many serious stingings by *Physalia* have been reported, there appear to be few authenticated instances of human deaths (57). These observations

suggest that all offensive nematocysts contain highly active toxins. Since most coelenterates are relatively innocuous toward man, there must be considerable variation in the capacity of the nematocysts to penetrate the skin.

Physalia toxin.—Our studies (24, 49, 51, 58–60) have established that the nematocyst toxin of *Physalia* is a complex protein consisting of several peptides; that it is thermolabile, and nondialyzable, with an LD_{50} for mice of approximately 200μg lyophilized toxin per kg when injected intraperitoneally. The LD_{50} for the rat is 100μg/kg, and 200μg/kg intravenously is lethal for the dog (61). Our crude toxin preparations have been lethal to all metazoan organisms tested. These have included various molluscs, crustaceans, fishes, amphibia, and common laboratory mammals. The toxin stimulates growth in *Paramecium caudatum* and in *Tetrahymena* sp., and apparently serves as an acceptable carbon source for these protozoa. Erythrocytes of the mullet, *Mugil cephalus*, were not hemolyzed when incubated at 37° C with several dilutions of toxin *in vitro* (24). Extensive hemolysis, however, followed intravenous injection of toxin in the anaesthetized dog (61). Serum potassium was elevated by about 22 per cent, while serum sodium was only slightly reduced (<5 per cent).

Both the neurogenic heart of crustacea (49) and the myogenic heart of the rat (60) and the dog (61) show dose-dependent damage. Less that 60 sec after the intravenous injection of as little as 12μg toxin/kg body weight, the P-R interval in the ECG of the dog was reduced, then the P wave was suppressed with the activation of an ectopic pacemaker site in the vicinity of the A-V node. This ectopic center dominated the heart for variable periods up to one minute, after which normal sinus rhythm was restored spontaneously. Toxin doses of 50 to 100μg/kg elicited coupled extrasystoles similar to those in ouabain-induced arrhythmias (62). These abnormalities were corrected and normal sinus rhythm restored by the intravenous administration of isotonic KCl. 200μg/kg quickly caused cardiovascular collapse. The ECG of the rat shows a series of similar effects after toxin injection, including prolongation of the Q-T interval at low dose levels (60 to 90 μg/kg), shortened P-R intervals at medium dose levels (100 to 300μg/kg). High dose levels (500 to 1500μg/kg) suppressed the P wave, caused A-V block, ventricular insufficiency, fibrillation, and death. This sequence of changes may be completed within 10 seconds in the rat when the dose level is 100 μg/kg or higher.

The neurogenic heart of a crustacean responds to *Physalia* toxin somewhat differently (49). In these forms, the heart is immediately arrested. The output of bioelectric potentials by cardiac ganglion cells is exaggerated but uncoordinated and without effect on the contractile mechanism of the heart. After administration of sublethal doses, the cardiac effects may often be detected for an hour or more. At higher dose levels the cardiac arrest is irreversible. If, however, the heart be driven by stimuli supplied directly through indwelling electrodes, it responds with a normal beat. This observation suggests that conduction along the axons of the cardiac ganglion cells has been

prevented, or that the terminations of these processes on the muscle cells have been altered.

Toxin applied to a segment of the isolated desheathed sciatic nerve of the frog (Lane, unpublished data) blocks conduction through the treated segment without affecting conduction in untreated portions of the nerve trunk. Neither the end-plate nor the muscle membrane was affected under the conditions of this experiment.

Unpublished observations by James Larsen in this laboratory strongly suggest that *Physalia* toxin modifies the permeability of the surviving frog skin. D. J. Quinn has recently shown (unpublished data) that *Physalia* toxin, like ouabain, may inhibit ATPases in the gill epithelium of the land crab, *Cardisoma guanhumi*, reducing active ion transport. These observations suggest that *Physalia* toxin may cause a generalized depolarization of cell membranes by interference with the activities of ion pumps on which the normal transmembrane potential depends. This proposed mechanism of action clarifies the hemolysis observed in the dog; inhibition of normal ion transport process apparently changed intracellular osmolarity, permitting imbition of water and cellular destruction. Consistent with this interpretation is the observation that hematocrit values were elevated with no appreciable increase in numbers of erythrocytes in peripheral blood.

Chironex fleckeri.—Clinical descriptions of human fatalities from stingings by the sea wasp *C. fleckeri* (31) emphasize how quickly the victims may die. Barnes (54) says, "From the moment of stinging to apparent death, the interval may be but a few minutes. Three minutes is a common assessment, but lesser periods are well authenticated. . ." The victims show no generalized paralysis, no respiratory failure, no cyanosis. Rather they are grey, apprehensive, and conscious until the final moment of collapse. "The overall impression is that death results from sudden cardiac arrest of either myocardial or central origin" (34). There is a striking similarity between these observations in man and our own in a variety of test animals that suggest a similarity between the nematocyst toxin in *Physalia* and that of *Chironex*. Studies presently in progress promise to extend this resemblance to still other nematocyst toxins.

Echinoderm Toxins

All major divisions of the ancient phylum Echinodermata are represented by Cambrian fossils (63), so these exclusively marine animals must have had a long pre-Cambrian history. Modern echinoderms collectively present a baffling assemblage of primitive and advanced morphological and physiological characteristics that prompted Hyman (64) to characterize them as a "noble group especially designed to puzzle the zoologist."

The class Holothuroidea, the sea cucumbers, contains many representatives of nutritional and pharmacologic importance; 30 toxic species belonging to five orders are listed by Nigrelli & Jakowska (65). Frey (66) described the

use of holothurians by Indo-Pacific natives to stun fish, and suggested that this might be a comparatively recent practice because of its restricted use and the scarcity of references to it in the literature. In the western Pacific several species of sea cucumber are converted to bêche de mer, or trepang, and are used as food by man. Preparation of these animals for the market includes repeated boiling, bleaching, and treatment with digestive enzymes. *Thelanota ananas*, *Stichopus variegatus*, and *Holothuria atra* are all eaten as trepang, and each is toxic when fresh material is assayed on fish (65, 67).

Holothurin.—The Bahamian sea cucumber, *Actinopyga agassizi*, the principal source of the active steroid saponin holothurin, has been studied by Nigrelli and his associates (65, 68–71). In this species holothurin occurs most abundantly in the Cuvierian tubules attached to the common stem of the respiratory tree near its junction with the cloaca. These reddish, branching filaments contain large numbers of cells filled with characteristic granules that are thought to be the source of the active material. More recently, holothurin has been found in species that lack Cuvierian tubules (Nigrelli, personal communication, 1967) and may, indeed, be a characteristic component of the body wall of all echinoderms.

The chemistry and pharmacology of holothurin has been reviewed recently (1, 2, 71–73). It is a steroid glycoside with four simple sugars attached to the steroid nucleus. The sequence of the monoses is quinovose, 3-0-methylglucose, glucose, and xylose (73). The molecular weight is 1150. Crude holothurin is lethal to marine fish and many fresh-water invertebrates in concentrations of 1 to 100 mg/l (65). At dose levels of approximately 1g/l, crude holothurin suppresses root hair development in water cress, causes necrosis of onion root tips; retards pupation in *Drosophila*; causes abnormalities in protein synthesis during the early development of the sea urchin, *Arbacia punctulata*; inhibits growth in the protozoa *Euglena* and *Tetrahymena*; prevents regeneration in the planarian, *Dugesia tigrinum*; and reduces the growth of sarcoma 180 and of Krebs-2 ascites tumor cells *in vitro* (67, 68). Crude holothurin is also somewhat more hemolytic than a reference saponin (65).

When holothurin is purified by ethanolic extraction and subsequent crystallization from ethanol, the LD_{50} for mice is 8 to 10 mg/kg. This material, called holothurin A (70), has marked neurotoxic properties (69), reducing the height of the spike potential without reducing the speed of conduction through the desheathed sciatic nerve of the frog. This effect is concentration-dependent, and irreversible. Treatment of single fiber-single node preparations with 8.7×10^{-6} M holothurin (74) reduced the amount of axoplasmic, macromolecular basophilic material in the vicinity of the Node of Ranvier.

The spectrum of effects attributed to holothurin suggests that it prevents intracellular protein synthesis in a wide range of biological materials. Conceivably, holothurin could influence the rate of RNA synthesis or replication. These results, however, were achieved with relatively impure material at

high concentrations so some caution should be exercised in their interpretation.

Ecologically, holothurin in Cuvierian tubules *in situ* has no effect on the sea cucumber, the intact Cuvierian tubules apparently limiting the solubility of the toxin. In the undisturbed animal, these structures are bathed by coelomic fluid. In echinoids and holothuria, this resembles sea water in total osmolarity. It differs from sea water, however, in ionic composition, containing from 103 per cent to 160 per cent of normal sea water potassium. The pH of coelomic fluid may be lower than sea water (74a). When the Cuvierian tubules, along with other viscera, are ejected by the animal in the autotomy reaction, they are suddenly bathed by a different ionic environment and a lower pH. These factors could promote the breakdown of the Cuvierian tubules with release of their toxin. This aspect of the biology of autotomy has apparently not been investigated. Whatever may ultimately be established as the mechanism, holothurin is released after autotomy, poisoning fish, invertebrates, and even other *A. agassizi* in the neighborhood (65). Autotomy may be induced by excessive handling, in or out of the water, by oxygen deficiency, or it can follow exposure of the sea cucumber to dilute solutions of holothurin. In nature, autotomy and evisceration are the ultimate defensive gesture of the holothuria. Recovery from this extreme reaction is accomplished in time, only by the slow processes of regeneration.

An inquiline fish *Carpus bermudensis*, lives in the cloaca and respiratory trees of *A. agassizi*. It is unaffected by the burden of holothurin contained in the normal Cuvierian tubules but is quickly killed when autotomy releases the toxin into the water surrounding the sea cucumber.

Various workers have prepared active extracts from the body wall and tube feet of asteroids (75–78). In general, although these differed from holothurin in the carbohydrate moiety, they were all steroid saponins and displayed the same qualitative activity as holothurin.

Echinoids cause injury to man and to other predators with their movable spines, but there is little evidence to support the envenomation of prey by this means. In man, the pain caused by penetration of the spines, and the foreign body reaction that sometimes follows, are probably elicited by the broken tips of the calcareous spines or by their epidermal investment. Extracts of certain spines of two diadematid sea urchins contained norepinephrine (79), but no significant amounts of histamine or acetylcholine.

Pedicellarian toxins.—Some asteroids and all echinoids develop small, pincer-like structures called pedicellariae on the surface of the test. These serve both as offensive and defensive weapons, mechanically trapping small food organisms and discouraging larger predators by inflicting painful nips. The globiferous pedicellariae of many echinoids house "poison glands" draining their secretions through ducts terminating in hollow teeth on the pincers (64). The globiferous pedicellariae of *Tripneustes gratilla* have been studied by Alender (79). Single pedicellaria of this form, when applied di-

rectly to the skin, causes a painful sting resembling that of the bee. Systemic symptoms appeared after multiple stingings. A complex, nondialyzable, heat-labile protein was extracted with saline (80). The LD_{50} for mice was 10 to 16μg protein/kg; 22μg/kg was lethal for rabbits. The material was hemolytic, hypotensive, and caused irreversible A-V block in the isolated rabbit heart at high dose levels. No further information is available on the chemical composition of this echinoderm material, but the need for chemical and pharmacological characterization is apparent. These specialized organs of the echinoids, are clearly equipped chemically to perform their protective function in the animal. The small size of the pedicellariae and their location on the test between the spines would probably deny them any significant role in predation.

Molluscan Toxins

Excluding the pelecypods that have been implicated in paralytic shellfish poisoning, representatives of at least two other molluscan classes, the Gastropoda and the Cephalopoda, also secrete toxins that have been studied in some detail.

Conus toxin.—The genus *Conus* (Gastropoda) occurs in all the warm seas of the world. The rarity and beauty of the shells of those found in shallow water in the western Pacific aroused the interest and cupidity of shell collectors from whom came the earliest intimations of the toxic properties of the animal. Cones immobilize their prey by injecting secretions of their venom glands with specially modified radular teeth. The venom is sufficiently virulent to kill children (81, 82, 84).

The morphology and mode of action of the venom apparatus, as far as is presently known (1–2), are described by Kohn (81) and Endean & Izatt (83). These descriptions leave many questions unanswered. What is the chemistry of the venom? Where is it produced in the venom apparatus? By what mechanism is it produced? Endean (82) described large numbers of $8 \times 15\mu$ vesicles in the venom expressed from the venom duct of *Conus striatus*. Nothing is known of their fine structure, mode of formation, or their function. The venom of some spiders and snakes includes similar packets of material surrounded by boundary membranes. It is tempting to speculate that these membranes may prevent premature mixing of the complex ingredients of the venom. This would be particularly effective if the components thus sequestered were active hydrolytic enzymes. The precise function of these formed elements of the cone venom can only be established by further experimental studies.

The function of the sturdy, muscular "venom bulb" should also be clarified. The histology of this structure certainly does not suggest a secretory function. A more probable function for this structure would be to pump the contents of the venom duct into the hollow radula tooth. The histogenesis of

the radula and the mechanism of migration and orientation of the individual radula teeth are apparently still unknown.

Cone venom is a viscous fluid, containing proteins and carbohydrates, that may be white, yellow, grey, or black depending on the species from which it is taken. The secretions within the venom duct have been shown to contain homarine, γ-butyrobetaine, N-methyl-pyridinium, indole amines and 5-hydroxytryptamine, but the component responsible for lethality has not yet been identified. The venom of certain cones is lethal for molluscs, others for polychaete worms, and still others affect vertebrates including man. Among these latter species are *C. geographus*, *C. catus*, and *C. tulipa* (82).

Venoms toxic to mice elicit hypotension, reduced heart rate, and hyperpnea when administered in sublethal concentrations. Larger doses cause respiratory arrest. Fishes injected with cone venoms toxic to vertebrates display aberrations in orientation, erection of fin rays, contraction of chromatophores, and changes in breathing rate and rhythm.

Other carnivorous gastropods elaborate toxic secretions in their salivary glands. Noteworthy among such animals is *Neptunea arthritica*, widely distributed in the northwestern Pacific where it is commonly used as food by man. Asano and his colleagues (85) have shown that the salivary glands of this species produce 2.5 to 7 mg tetramine/gram of gland. In *Neptunea antiqua* in the North Sea, Fange (86) showed that tetramine may constitute nearly 1 per cent of the weight of the salivary gland (9 mg/g). Although there is some reluctance to accept tetramine (tetramethyl ammonium hydroxide) as an offensive secretion (38), the saliva of these animals contains little else that could immobilize its prey.

Octopus toxin.—The chemistry and pharmacology of the secretions of the posterior salivary glands of various octopods have been described by Ghiretti (87). The viscous saliva of these animals contains many compounds of pharmacologic interest including tyramine, histamine, acetylcholine, taurine, serotonin, proteolytic enzymes, and a hyaluronidase. In addition, however, Ghiretti described another component that he called cephalotoxin. This very toxic substance originates in the posterior salivary glands of several octopods, and is principally effective against various crustaceans. Ghiretti described the responses of a crab injected with cephalotoxin in these terms, "the legs immediately contract and the animal remains in this position for about one minute. The righting reaction is abolished, the appendages begin to tremble, the pincers open and close without external stimulation and tetanic spasms of the body at intervals are seen. Little by little the animal becomes quiet; spontaneous movements as well as provoked reactions disappear and the aggressive animal of a few minutes before is changed to an inert limp organism. The heart beats as before and no apparent modification of the blood or of its circulation has occurred." Cephalotoxin has no effect on neuromuscular preparations of Crustacea *in vitro*. Isolated perfused hearts of

the crabs *Maja* and *Eriphia*, as well as the isolated heart of *Octopus*, were arrested in diastole. Oxygen uptake by various Crustacea was decreased as much as 70 per cent after cephalotoxin administration. Regrettably, the chemical composition of cephalotoxin is still unknown.

Toxins of Marine Fish

Several families of fish include species that are injurious to man (1, 2). Some of these, such as the stonefish, *Synanceja horrida*, the zebrafish, *Pterois volitans*, the scorpionfish, *Scorpaenopsis diabolus*, the stingrays, and the weeverfish, inflict injury by modified fin rays or special spines. These structures are clothed in life by epithelia containing glandular tissue that is thought to be the source of the venom. What is known of the chemistry and pharmacology of fish venoms is reviewed by Russell (1).

Apart from the large number of venomous fish, the flesh of which is often prized as food, are other fish which contain toxins that are distributed throughout the viscera and body musculature. This group has been termed ichthyosarcotoxic (88) and includes those forms responsible for ciguatera and for tetrodon poisoning.

Ciguatoxin.—Ciguatera, or tropical fish poisoning, probably occurs in warm water areas around the globe. Approximately 300 species of fish have been implicated in this poisoning, among which are many common food fishes such as the jacks, snappers, porgies, moray eels, and the barracudas. Ciguatera was first named in the Caribbean and is widespread in the central and western Pacific islands. Symptoms of ciguatera are nausea, paresthesia generally limited to the mouth, tongue, and throat, weakness, abdominal pain, vomiting, diarrhea, chills, muscular weakness and incoordination. Severe poisoning may be fatal.

Reef fish are thought to acquire their burden of ciguatoxin from food organisms. Randall (89) has speculated that the toxin is first synthesized by a benthic blue-green alga and is subsequently concentrated in the viscera and flesh of herbivorous acanthurids or surgeonfish. In turn, these are eaten by larger carnivorous fish such as the snappers, groupers, and barracudas, where further concentration of the toxin occurs in viscera and flesh. Banner et al. (90) showed that the toxin persisted in *Lutjanus bohar* for at least 18 months when toxic fish from the Line Islands were transplanted to laboratory pens at the Hawaii Marine Laboratory and fed on nontoxic herring from Puget Sound. No method is known by which toxic fish may be identified before they are eaten. Unfortunately, the toxicity of fish resident on a reef may vary unpredictably with time and microhabitat.

The chemistry of ciguatoxin extracted from the flesh of the moray eel, *Gymnothorax javanicus*, has been described recently by Scheuer and his colleagues (91). Their results suggest that ciguatoxin is a lipid containing a quaternary nitrogen atom, one or more hydroxyl groups, and a cyclopentanone moiety. It is probably not a phosphatidic ester,

The anticholinesterase activity of ciguatoxin was first demonstrated by Li (92), using an impure preparation, whose LD_{50} for mice after intraperitoneal injection was only 12 mg/kg. This material inhibited the normal cholinesterase activity of whole blood, caused miosis after topical application in the rabbit, and elicited cardiac arrhythmias ranging in severity from simple prolongation of the P-R interval to various degrees of A-V blockade.

Tetrodotoxin.—The poisonous nature of fish of the suborder Tetraodontoidae has been recognized since ancient times. Halstead (2) reproduces a drawing of the poisonous pufferfish *Tetraodon stellatus* that decorated the tomb of the Egyptian Pharaoh Ti, Fifth Dynasty (ca. 2700 B.C.) and includes the hieroglyphic inscription that represents the puffer. Kao (11) quotes descriptions of the fish and of its toxicity that appeared in the first Chinese pharmacopeia, The Book of Herbs, that he assigns to the first or second century B.C. Recently (93), the poison produced by salamanders of the genus *Taricha* was shown to be identical chemically, physically, and pharmacologically with the toxin secreted by tetraodontoid fish. This was first described by Twitty and his colleagues (94, 95) after interspecific transplantation of organ primordia between embryos of *Ambystoma tigrinum* and *Taricha torosa*. *Ambystoma* hosts were paralyzed by the implantation of *Taricha* eyestalks. The distribution of tarichatoxin (= tetrodotoxin) among amphibia has recently been described by Wakely et al. (98). There is no satisfactory evolutionary explanation for the occurrence of this toxin in such widely separated taxa as the tetraodontoid fishes and the salamandridae.

One of the more vivid early descriptions of the effects of puffer poison on man is that of the legendary Captain James Cook in the journal of his second voyage around the world in the Resolution (96). An unfamiliar fish was obtained from the natives of newly discovered New Caledonia. Before it was cooked for dinner a drawing and description of the fish was prepared by Mr. Forster, the ship's naturalist. Captain Cook's journal then continues, " . . . luckily for us the operation of drawing and describing the fish took so much time that it was too late so that only the liver and roe was dressed of which Mr. Forster and myself did but taste. About three to four o'clock in the morning, we were seized with most extraordinary weakness in all our limbs attended with numbness of sensation like to that caused by exposing one's hands and feet to a fire after having been pinched much by frost. I had almost lost the sense of feeling nor could I distinguish between light and heavy objects, a quart pot full of water and a feather was the same in my hand. We each took a vomit and after that a sweat which gave great relief. In the morning one of the pigs which had eaten the entrails was found dead."

Tetrodotoxin, the compound responsible for toxicity in tetraodontoid fish, is an amino perhydroquinazoline compound, molecular formula $C_{11}H_{17}N_3O_8$; mol wt 319 (Fig. 1). The chemistry of the compound as recently reviewed (11, 93, 97) clarifies some of the earlier difficulties encountered with establishing the elemental analysis of the material (93). The abundance of

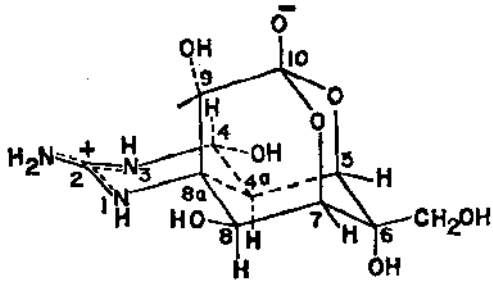

FIG. 1. Structure of Tetradotoxin, from Mosher et al. (93)

OH groups attached to the extremely polar molecule complicated early attempts at purification. The structure of the molecule was established by Mosher et al. (93). Narahashi et al. (99) have studied the activity of derivatives of tetrodotoxin. Their results generally confirm the observations of Tsuda et al. (100) which suggest that any substitution at C_4 reduces the activity of the toxin. Apparently, both the hydroxyl group on C_4 and the hemilactal oxygen link between C_5 and C_{10} are essential for optimal activity. Opening the latter and reducing the former eliminates all toxicity. Camougis et al. (101) emphasize the significance of the guanidinium end of the molecule while confirming the importance of the hemilactal. Kao (11) suggests a structural similarity between tetrodotoxin and saxitoxin based, on their similar pharmacological activity. Regrettably, no evidence exists to suggest that tetrodotoxin in the puffer fish originates from dietary sources like saxitoxin in the butter clam. Indeed, the analyses of Tani [quoted in ref. (11)] show clearly that the concentration of tetrodotoxin in the ovaries and liver of the puffer increases with the onset of the spawning season, both in absolute amount and in concentration, strongly suggesting that the rate of synthesis of the toxin varies with the reproductive state of the animal.

Tetrodotoxin is one of the most potent, nonprotein toxins known, the intraperitoneal LD_{50} for mice being 10μg/kg. The dose-response curve is extremely steep, the minimal effective dose being about 8μg/kg, and the LD_{100}, 12μg/kg. The toxin is effective against all common laboratory mammals, fish, except the puffer group, amphibia except for salamanders of the genus *Taricha*, and many invertebrates. In the intact laboratory mammal tetrodotoxin produces a prompt progressive reduction in activity of voluntary muscles, hypotension, respiratory depression and a reduction in body temperature.

Recent papers (101, 102) have confirmed Kao's conclusion (11) that the primary target of tetrodotoxin is the sodium permeability of nerve axon and of muscle membrane. Tetrodotoxin, apparently, does not affect active transport of sodium through the frog skin (103), and is ineffective on potassium or chloride conductance. To this degree, tetrodotoxin provides a precision tool for those studying the role of ion movements in biological systems.

Marine toxins include some of the most virulent known. Both protein and nonprotein types are recognized. Study of their mode of action at the cellular level has clarified normal metabolic functional processes in cells and tissues. As purification and characterization of further marine toxins is accomplished, additional pharmacological tools will be provided to study basic cellular processes.

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CONTENTS