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Algal Toxins

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INTRODUCTION

This is a literature review of the toxins produced by algae. For this paper, only toxicity to multicellular organisms was considered. This eliminated a large number of publications dealing with antibacterial and antiviral substances released by algae. Because the paper addresses those toxins produced by algae, the phenomenon of bioaccumulation of environmental contaminants is not discussed. Also not discussed is the one pathogenic algal genus, *Prototheca*, because there is no indication of a toxin being released (review by Sudman [165]).

What are discussed are a wide variety of toxins produced by five phyla of algae: Chlorophycophyta (green algae), Cyanophycophyta (blue-green algae [cyanobacteria]), Chrysophycophyta (diatoms, yellow-green and golden algae), Pyrrhophycophyta (dinoflagellates), and Rhodophycophyta (red algae). The types of molecules involved are diverse, going from simple ammonia to complicated polypeptides and polysaccharides. The physiological effects are also varied, ranging from the acute toxicity of paralytic shellfish poison of *Gonyaulax*, leading to death in a short period of time, to the chronic toxicity of carrageenans from red algae, which induce carcinogenic and ulcerative tissue changes over long periods of time. Perhaps the only link among this wide variety of toxins is that each is produced by some form of alga.

CHLOROPHYCOPHYTA

The Chlorophycophyta have been associated with toxicity only in rare instances. Aside from the three genera discussed below, Prescott's book *The Algae: a Review* (122) lists *Chlorella* and *Scenedesmus* as death-inducing algae, but information on these species is rare.

Caulerpa spp.

The marine benthic green alga *Caulerpa* is responsible for the production of two toxic substances, namely, caulerpicin and caulerpin (4, 38, 39). Both of these compounds have demonstrated toxicity in mice. They were originally isolated from *C. racemosa* but were also identified in *C. sertulariodes, C. lentillifera,* and *C. lamourouxii.* It is interesting to note that *Caulerpa* is probably the most popular edible alga in the Philippines but becomes toxic during the rainy months. The toxicity is believed to derive from the agitation of the plant during the rainy season (37).

The infrared spectrum of caulerpicin indicated that this compound was a long-chain saturated hydroxy amide. Aguilar-Santos and Doty (4) hypothesized from the spectral data that the structure was as follows:

$$CH_3-(CH_2)_{13}-\underset{\displaystyle CH_2OH}{\overset{|}{CH}}\!\!-NH-CO-(CH_2)_n-CH_3$$

(n = 23, 24, 25). Mass spectral information led them to believe that the actual substance was a mixture of these homologous molecules. This structure has not received full confirmation (144). The human physiological symptoms associated with caulerpicin ingestion include numbness and a cold sensation of the extremities, rapid and difficult breathing, slight depression, and eventually loss of balance. Depending on the dose, the effects are usually gone within a couple of hours to a day.

Caulerpin was found to be a heterocyclic, red substance after it was crystallized from ether extracts of the alga. It is a pyrazine derivative. Both spectral analysis and degradation reactions were used to determine the hypothesized structure of dimethyl 6,13-dihydrodibenzo[*b, i*] phenazine-5,12-dicarboxylate (Fig. 1) (144).

Cheatomorpha minima

The organic extract from the green alga *Cheatomorpha minima* has experimentally shown hemolytic activity and fish toxicity (ichthyotoxicity) (45). The infrared spectrum of the organic extract was typical for fatty acids, and the predominant species detected by gas-liquid chromatography were palmitic (33%); palmitoleic (12%); oleic, elaidic, and/or vaccenic (14%); and linoleic (10%) acids. The active, purified organic extract was obtained as a colorless solid which killed killifish (*Oryzias latipes*) in 120 min at 5 μg/ml and had a hemolytic activity of 1.99 saponin units (a quantitative measure of hemolytic activity for solid compounds) per mg (60). Eleven separate fatty acids from *C. minima* were tested for ichthyotoxicity and hemolytic activity (Table 1). The greater the saturation of the fatty acid, the lower the hemolytic activity; however, the saturation level did not correlate well with the ichthyotoxicity.

Ulva spp.

Another genus of the green algae which has hemolytic activity is the *Ulva* (activity found in three separate species in the nondialyzable fraction of a 70% ethanolic extract). After chemical separations, a total of three distinct hemolysins were isolated from *Ulva pertusa* (44). Of the three hemolysins isolated, two were water soluble and the third was fat soluble. The fat-soluble hemolysin was identified as palmitic acid (a C_{16} saturated fatty acid), with a hemolytic activity of 0.24 saponin unit per mg.

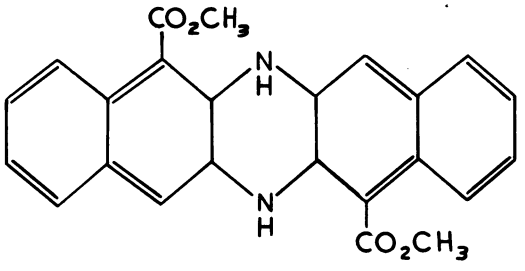


FIG. 1. *Caulerpin (from Scheuer [144]).*

TABLE 1. *Ichthyotoxic and hemolytic activities of fatty acids occurring in C. minima (from reference 45)*

Fatty acid	Hemolytic activity[a] (saponin units/mg)	Avg death time[b] 5 mg/100 ml		1 mg/100 ml	
8:0	1.37	11 min	(5)	44.6 min	(5)
9:0	0.73	12 min	(5)	34.2 min	(5)
10:0	0.49	13 min	(5)	6.6 h	(4)
12:0	0.52	16 min	(5)	20.2 min	(5)
14:0	2.48	9.4 h	(2)		(0)
16:0	3.01		(0)		(0)
16:1	7.17	1.2 h	(5)	2.4 h	(5)
18:0	0.35	15.2 h	(3)		(0)
18:1	4.52	2.5 h	(5)	1.9 h	(2)
18:2	9.49	2.8 h	(1)		(0)
20:0	0.26	14.3 h	(1)		(0)

[a] Estimated by the method of Oshima et al. (104) with 10% ethanol solution.

[b] Numbers in parentheses indicate number of fish in each group of five that died within 24 h.

Both of the water-soluble hemolysins were similar in chemical and physical parameters. Although the final chemical structure has not been elucidated for either of these substances, many of the chemical and biological properties have been determined. On the basis of infrared spectra and combustion data, one water-soluble substance is believed to be a galactolipid with a formula of $C_{31}H_{58}O_{14}$. This substance had a hemolytic activity of 1.44 saponin units per mg, as measured by the method of Hashimoto and Oshima (60). The second substance is believed to be a sulfolipid with a formula of $C_{25}H_{47}O_{11}SK$ and a hemolytic activity of 2.01 saponin units per mg.

Both of the water-soluble hemolysins were tested on sea urchin (*Hemicentrotus pulcherrimus*) eggs according to the procedures of Ruggieri and Nigrelli (133). This test is valuable for determining developmental modifications (i.e., animalization, fragmentation, radialized larvae, and abnormally formed plutei). At a concentration of 0.001 ml of either hemolysin per 10 ml of test solution (hemolysin plus seawater), both fertilized and unfertilized eggs were lysed. At a concentration of 0.0001 ml of hemolysin per 10 ml of test solution, neither fertilized nor unfertilized eggs were affected. Thus, the hemolysins

did not produce developmental modification, but they did induce toxicity.

CYANOPHYCOPHYTA

The Cyanophycophyta are one of the three phyla responsible for the majority of reported alga-caused deaths of fish, livestock, waterfowl, and humans (the other two are the Chrysophycophyta and the Pyrrhophycophyta). This phylum contains most of the genera of the freshwater toxic algae along with some of the toxic marine species. Of the more than 50 genera of blue-green algae, at least 8 have exhibited toxic characteristics; these include *Anabaena, Aphanizomenon, Coelosphaerium, Gloeotrichia, Lyngbea, Microcystis, Nodularia,* and *Nostoc.* The incidents of toxicity of these and other blue-greens have been collated by Schwimmer and Schwimmer (146, 147). The majority of reported deaths occur in the northern plains of North America. All of the documented cases of blue-green algal toxicity have occurred during the summer months, when concentrations of cyanophytes are sufficient to form blooms.

Aside from the lethal toxic effects of the blue-green algae, it is now believed that certain genera found in drinking waters are capable of inducing nonmortal gastrointestinal disorders in humans. Recently in Sewickley, Pa., 62% of the population contracted an illness with symptoms of diarrhea and abdominal cramps. This occurred at a time when one of the drinking water reservoirs was infested with a heavy growth of the blue-green alga *Schizothrix calcicola.* It is believed that this alga was responsible for the gastrointestinal tract disruption (82). Schwimmer and Schwimmer (147) also noted that *Oscillatoria* (from the same family as *Schizothrix*) was involved in a case of gastrointestinal problems. Similarly, a diarrhea-producing toxin was separated from the lethal toxin of *Microcystis aeruginosa* via dialysis (13). Although the algal component responsible for these problems has not been chemically identified, it seems possible that algae may be responsible for the degradation of drinking water quality in many instances.

Aside from the problems of acute toxicity, the Cyanophycophyta can be responsible for the induction of chronic health complications. The carcinogenic activity of a blue-green alga, *Nostoc rivulare,* was reported by Schwimmer and Schwimmer (146).

Microcystis aeruginosa

Of all the algae in the Cyanophycophyta with toxic properties, *M. aeruginosa* is probably the one that causes the most harm (46). The deaths of many poultry and cattle have occurred as results of blooms of this alga (99); however, a toxic culture of this alga was shown by Gorham (52) not to be the source of the poison(s) that causes waterfowl sickness. The alga is found most frequently in shallow freshwater lakes and ponds. Although an *M. aeruginosa* toxic compound was the first algal toxin to be chemically characterized (in terms of which amino acids were present in the polypeptide and the relative proportions of amino acids), the chemical structures of the toxins associated with this alga remain an enigma.

The NRC-1 strain of *M. aeruginosa,* isolated by Gorham (52) and fellow workers, proved to be toxic when administered either orally or intraperitoneally (i.p.). It was shown by this same group that the toxicity was dependent upon some process whereby the algal cells were disrupted and that the whole cells of this strain were not toxic. The conclusion was that the toxin or toxins were endotoxins. From mass cultures of *M. aeruginosa* NRC-1 two separate toxins were isolated. One of the toxins was labeled slow death factor (SDF) and produced death in mice in a period of 4 to 48 h; with this factor, death was preceded by piloerection, irregular breathing, and lethargy (64). The other toxin was named fast death factor (FDF) and produced death in 1 to 3 h; with this factor, death was preceded by pallor, violent convulsions, and prostration. An alternative name given to FDF was microcystin.

Many chemical experiments were done in an attempt to decipher the chemical and physical properties of FDF. It was found that the optimal temperature for growth of *M. aeruginosa* was 32.5°C; however, at this temperature very little toxin, if any, was produced. Through experiments it was found that the toxin production was optimum at a temperature of 25°C (R. Harris and P. Gorham, unpublished data). Microcystin was found to be a nonvolatile substance that was irreversibly adsorbed on activated carbon and water soluble. Gorham and co-workers also found that by feeding CO_2, they decreased cell yield and toxin production, and this was attributed to a drop in pH, even though the medium was relatively well buffered. It was determined that about one-third of the 28 strains of *M. aeruginosa* produced FDF (50–52, 158).

Eventually, FDF was found to be a polypeptide with seven different amino acids (20). The molecular weight of the substance was determined to be between 1,300 and 2,600. The compound was believed to be cyclic because the dinitrofluorobenzene test did not show any ter-

minal amino groups. The entire chemical structure consists of a cyclic compound of 10 amino acids (1 L-aspartic acid, 2 L-glutamic acid, 1 D-serine, 1 L-valine, 1 L-ornithine, 2 L-alanine, and 2 L-leucine [20]). The 50% lethal dose of FDF (administered i.p.) was found to be 0.466 mg/kg of body weight in mice, making FDF a moderately toxic substance. The toxicity of FDF was believed to be due to either the unnatural amino acid D-serine, the L-ornithine molecule, or the cyclic structure of the polypeptide, because these attributes have been associated with biological activity in other peptides (20).

SDF was found to be produced by bacteria that were associated with *M. aeruginosa*. The chemical composition of SDF has never been elucidated, but it has been found that there are two different types of bacteria affiliated with the alga and that they each produce a different SDF. One SDF is heat stable, and the other is heat labile. The heat-labile SDF was found to be more common than the other (168, 169). The heat-stable SDF was found to be a neurotoxin which produced slow deaths at a minimum concentration of 20 mg/kg in mice (i.p.) (46).

In the late sixties, Murthy and Capindale (99) isolated a different FDF from *M. aeruginosa* NRC-1. The 100% lethal dose (administered i.p.) for mice was 0.1 mg/kg, which means that it was about five times more toxic than the component described by Bishop et al. (20). Again, the substance was a polypeptide, but this peptide had a free primary amino group. The molecule consisted of 14 amino acids (L-aspartic acid, L-threonine, D-serine, L-glutamic acid, L-proline, L-glycine, L-alanine, L-valine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-ornithine, and L-arginine [99]), 7 of which are the same as the original microcystin. It is possible that the newly discovered FDF is merely a purification of the original toxin and that the original amino acid analysis was only able to pick up the dominant amino acids; however, it is also possible that these two toxins are separate entities.

Recently, a toxin has been isolated from an *M. aeruginosa* bloom by a group of Russian scientists (79). The molecular weight of this compound is approximately 19,400. Again, the toxin contains a polypeptide (16.6%), and acid hydrolysis has indicated the presence of 16 common amino acids.

Another toxin has been isolated from *M. aeruginosa* Kützing (13). This toxin produces diarrhea. The diarrhea toxin and FDF are separated via dialysis of the lysed whole cells. The dialyzable fraction contains FDF, whereas the nondialyzable fraction contains the diarrhea toxin.

Anabaena flos-aquae

The blue-green alga *Anabaena flos-aquae* has been responsible for several of the most severe incidents of algal poisoning (43, 131). This alga is usually associated with eutrophic freshwater lakes and sloughs during the summer months. The most frequent targets of *Anabaena* toxicity include ruminant livestock and waterfowl, and Carmichael and Gorham (29) have suggested that these species are more sensitive to the toxin than are other animals. The fact that this toxin kills waterfowl differentiates it from the FDF of *M. aeruginosa*, which is not toxic to waterfowl. The area in which *An. flos-aquae* blooms have been noticeably prevalent is the western region of Canada (95).

The toxin from *Anabaena* was named very fast death factor (VFDF) to distinguish it from the FDF of *Microcystis*. The amount of time necessary for FDF to cause death in animals is on the order of 1 to 3 h, whereas VFDF causes death in most laboratory animals in 1 to 20 min (52). For example, a minimal lethal dose administered intraperitoneally in mice killed them in 1 to 2 min, with the symptoms preceding death including paralysis, tremors, and mild convulsions. Death is usually due to respiratory arrest (28). Physiologically, this toxin acts as a depolarizing neuromuscular blocking agent, and because of a relatively small molecular weight it is very rapidly absorbed when taken by the oral route (24). The minimum lethal dose when given i.p. to mice is 0.25 mg/kg (35), which is within an order of magnitude of FDF.

Like *M. aeruginosa*, *An. flos-aquae* has both toxic and nontoxic strains. Gorham (52) found that out of 12 unialgal colony isolates (potentially genetically heterogeneous mixtures) of *An. flos-aquae*, 8 were toxic and 4 were nontoxic. He also showed that the toxicity of one of these strains was dependent upon environmental conditions; by altering the environment for three different cultures of the same strain, he found that one was toxic, whereas two were nontoxic. Later, the toxin production of the axenic clone *An. flos-aquae* NRC-44-1, which was stable under standard conditions, was shown to vary as a function of light and temperature (117). For example, toxin production was higher at 22.5 than at 15°C at all light intensities. The toxicity of a bloom in nature is contingent upon a large percentage of the bloom being of the toxic variety. It is estimated that 80 to 90% of the bloom must be toxic to induce deaths in cattle (29).

VFDF differs from FDF in that it is an exotoxin, which is excreted by *Anabaena* into the surrounding water (138). Chemically, VFDF was

found to be water soluble as well as heat, light, and alkaline labile. The hydrochloride of VFDF is heat stable and hydroscopic (B. Stavric and P. Gorham, Proc. Annu. Meet. Can. Soc. Plant Physiol., 1966, abstr. no. 21).

Recently, *Anabaena* VFDF has been renamed anatoxin A (34). The chemical structure for anatoxin A has been determined to be 2-acetyl-9-azabicyclo[4.2.1]non-2,3-ene (Fig. 2) (27, 35, 63). Several toxins from strains of *Anabaena* found in Alberta and Saskatchewan, Canada, have exhibited pharmacological and toxicological differences from anatoxin A. Carmichael and Gorham (30) have hypothesized that there are at least three other toxins (anatoxins B, C, and D) produced by *An. flos-aquae.*

Aphanizomenon flos-aquae

The blue-green alga *Aphanizomenon flos-aquae* is found in temperate lakes of the United States and Canada and is responsible for the production of a potent neurotoxin (134). The toxins from blooms of this alga have been implicated in the deaths of livestock and fish for a long time (69, 118, 120, 121, 136), but the actual isolation of toxins came in 1968 (137).

In 1964, at Lake Winnisquam, N.H., an algicide (copper sulfate) was added to a bloom of *Aph. flos-aquae* in an attempt to decrease algal growth. After the addition of the algicide, there were moderate fish mortalities. Two years later, in 1966, at Kezar Lake, N.H., the same treatment of a heavy bloom of *Aph. flos-aquae* resulted in a mass killing of more than 12,000 pounds (ca. 5,400 kg) of fish (137). Gentile and Maloney (47) found that the copper sulfate was not directly causing the fish deaths. These incidents indicate that the lethal algal substance is an endotoxin which is not released by the alga under normal growth conditions and/or that copper sulfate enhances the toxicity. Laboratory experiments with this toxin have also indicated the necessity of lysing the algal cells to release the endotoxin (137).

While trying to chemically characterize the *Aph. flos-aquae* toxin, it was noticed that the color reactions, the R_f values from paper and thin-layer chromatography, and the infrared spectra of the toxin were similar to those of saxitoxin (paralytic shellfish toxin), a toxin produced by the marine dinoflagellate *Gonyaulax catenella* (72). Further chemical and physiological work showed that *Aphanizomenon* toxin differed from saxitoxin (5). Recent work has demonstrated that the toxin from *Aphanizomenon* is actually composed of saxitoxin (Fig. 3) plus three other unknown toxic constituents (155). It is both interesting and unique to find an algal toxin that is produced by two algae which are from different phyla (Cyanophycophyta and Pyrrhophycophyta). It is also interesting to note that this toxin is produced by a freshwater alga and a marine species.

Like the toxins of the other blue-green algae, the production of *Aphanizomenon* toxin can be activated or inhibited by a variety of environmental parameters. Production of toxin was found to correlate with the age of the culture, the light intensity, and the temperature, but not with the nitrogen source (47). As an example, *Aph. flos-aquae* produced twice as much toxin at 26 as at 20°C, and at 30°C there was almost no toxin produced (46). The toxin was initially very difficult to detect because it is labile at culture temperatures and pH values. To make the toxin stable at a wide range of pH values, it was necessary to lower the temperature to 5°C (137). Like other blue-green algae, *Aph. flos-aquae* has both toxic and nontoxic strains (clones).

Chemically, the algal toxins are mostly heat and acid stable, but they are not stable in alkaline solutions (151). The toxins are very water

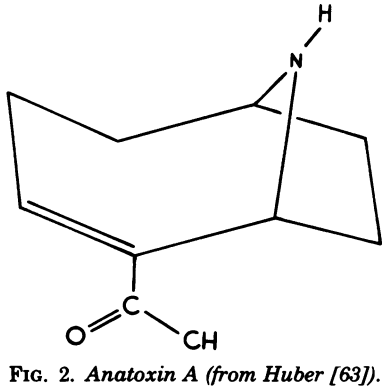


FIG. 2. *Anatoxin A (from Huber [63]).*

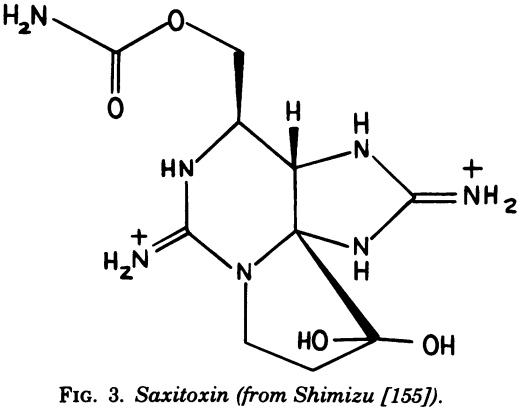


FIG. 3. *Saxitoxin (from Shimizu [155]).*

soluble (46) and dialyzable (72). They are also very hygroscopic and resistant to crystallization (72). Hydrogenation generally does not destroy their toxicities, although hydrogenation of saxitoxin does destroy its toxicity (142).

Aphanizomenon toxin was originally classified as another VFDF, even though it differed chemically from the *Anabaena* toxin, because it also acts rapidly (137). When injected i.p., the toxins usually act within 1 to 2 min, and the latent period is seldom more than 5 min (47, 137). The minimum lethal dose for the *Aphanizomenon* toxin has been reported by Gentile (46) to be from 0.05 to 0.10 mg/kg in mice. It is significant to note that this minimum lethal dose is less than that of either *Microcystis* toxin or *Anabaena* toxin (47, 137).

The symptoms and cause of death, when injected into mice, are similar for *Aphanizomenon* toxins and the other blue-green toxins. Mice exhibit irregular breathing, gaping mouth, spasms, loss of coordination, violent tremors, and death due to respiratory paralysis (5). Fish show an even greater susceptibility to toxicity and a significantly lower 100% lethal dose (47). When low doses are injected into the fish, the toxins initially cause a darker pigmentation in the head region. As the dark pigmentation spreads toward the tail, there is a loss of coordination, and death ensues within several minutes (72).

Physiologically, *Aphanizomenon* toxins are neuromuscular inhibitors that operate at the membrane level (137). The mechanism of action of these toxins is believed to be the blocking of action potentials by preventing the flow of sodium, thus preventing nerve conductance. The toxins have no apparent effects on the potassium-dependent transmembrane resting potential, and, consequently, there is no depolarizing of the nerve fibers (46). This mechanism is the same as those described for saxitoxin (95) and tetrodotoxin, a powerful toxin from the puffer fish (61, 73, 102). However, Gentile (45) notes that *Aphanizomenon* toxin blocks calcium-dependent action potentials, which are found in certain invertebrate muscles, but tetrodotoxin does not.

Toxic Marine Cyanophycophyta

As a result of the readily observable deaths of waterfowl, cattle, and fish, the toxicity of freshwater blue-green algae has received more attention then the toxicity of marine blue-greens. However, it seems well documented that the marine forms produce toxic compounds. The well-documented cases of marine blue-green-algal toxins have all been found in the family Oscillatoriaceae, which are unbranched filamentous algae that lack heterocysts.

The marine blue-green alga *S. calcicola* was believed to be responsible for the fish toxicity on Marakei in the Gilbert Islands. Subsequently, two lipid-soluble toxins were extracted from the alga (14). The chemical structures of these toxins have not been elucidated to date. As previously mentioned, a freshwater strain of this same species was believed to be responsible for the gastroenteritis in Sewickley, Pa.

From the Oscillatoriaceae, perhaps the most toxic alga is *Lyngbya-gracilis*. This alga has been isolated in relatively large quantities from deep water off the Marshall Islands. The toxic component has been determined to be debromoaplysiatoxin (Fig. 4) (100). Debromoaplysiatoxin has also been found in a mixture of *Oscillatoria nigroviridis* and *S. calcicola*. This compound has shown anti-leukemia activity (100).

As in all the other toxic algae, the environmental conditions appear to influence the pro-

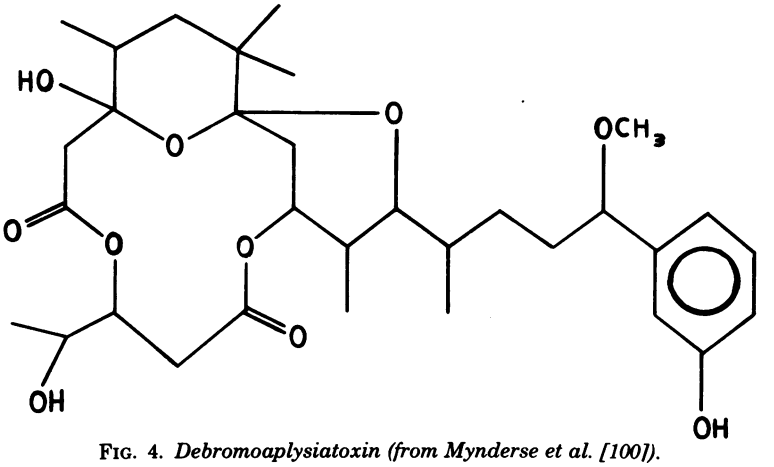


FIG. 4. *Debromoaplysiatoxin (from Mynderse et al. [100]).*

duction of debromoaplysiatoxin. Comparing the toxicity of an equal mixture of *O. nigroviridis* and *S. calcicola* from the seaward side of Enewetak Island with an equal mixture of the same algae from the lagoon side of the island showed that the extract from the seaward side of the island had a much more potent activity (100). The reason for the difference has not been explained.

The 100% lethal dose for a combination of aplysiatoxin and debromoaplysiatoxin was found to be 0.3 mg/kg when injected i.p. into mice (75). Kato and Scheuer (75) also noticed that accidental human contact with aplysiatoxins caused inflammation and swelling of mucous membranes in the eyes and nose and induced erythema and pus formation on the skin. The dermatological responses produced by these compounds are similar to the symptoms produced by dermatitis-producing *L. majuscula* (15, 93, 94). Analysis of the dermatitis-producing *L. majuscula* from Oahu, Hawaii, showed that debromoaplysiatoxin was present (100). The physiological and chemical characteristics of the dermatitis toxin from *L. majuscula,* isolated by the Hashimoto group (57), are consistent with the hypothesis that the dermatitis is produced by debromoaplysiatoxin (95).

Another toxin has been isolated from the nondematitis-producing type of *L. majuscula* (95) which is found in shallow water at Oahu. This toxin has been named lyngbyatoxin A. Lyngbyatoxin A is a nitrogenous toxin that is optically active and nonbasic. The minimum lethal dose in mice is 0.3 mg/kg. High-frequency nuclear magnetic resonance and mass spectral data indicate that the formula for the toxic substance is $C_{27}H_{39}N_3O_2$.

CHRYSOPHYCOPHYTA

The phylum Chrysophycophyta is composed of three different subphyla or classes of algae. These three are: Xanthophyceae (Heterokontae or yellow-green algae); Chrysophyceae (golden algae); and Bacillariophyceae (diatoms). There is no documented case of a toxic member of the Xanthophyceae. Almost all of the published work on toxins from this phylum is related to the single species *Prymnesium parvum,* which is a member of the Chrysophyceae. Ergo, most of this section will address *P. parvum.* Another genus of the Chrysophyceae, *Ochromonas,* has been shown to contain extractable toxins.

There is only one documented case of health impairment due to an alga from the Bacillariophyceae (diatoms) (186). In this case, a white sucker (*Catostomus commersonii*) had granulomatous enteritis with a number of genera of diatoms present in the lesions. The lesions in this case were similar to tuberculosis lesions in fish, except that giant cells were found in the lesions. Wolke and Trainor (186) hypothesize two possible mechanisms for the formation of the granulomas. The first mechanism is the reaction of tissues to the silicon dioxide from the cell wall of the diatom. The second mechanism is a foreign body reaction to the mineral exoskeleton of the diatom. These authors also hypothesize a need for a preexisting lesion to allow the diatoms to penetrate the epithelium.

Prymnesium parvum

P. parvum is a marine alga of the family Chrysophyceae. *P. parvum* is a relatively small alga (10 μm in diameter) that has two flagella, a haptonema, and a scaly surface (151). This alga has been associated with ichthyotoxicity from northern Europe down to the Middle East for many years (26, 33, 80, 81, 106, 127, 178). These instances of fish kills were sporadic, but the introduction of inland brackish water breeding ponds for fish in Israel caused a continual problem with *Prymnesium.* Many of these ponds had severe fish deaths because they provided the perfect ecological niche for *P. parvum,* including standing marine waters with a high concentrations of nutrients coupled with a mild climate. The alga became endemic in these ponds and remains in them at low levels (150, 151).

There is evidence to show that several different toxic components are present in *P. parvum.* This idea gained credence when it was shown that different activities were predominant when using different toxin preparations (88). These toxins are capable of eliciting three separate physiological responses which are used to characterize the toxins; namely, ichthyotoxic activity, hemolytic activity, and antispasmodic effects on smooth muscle (107, 138, 149, 176). By thin-layer silica gel chromatography, the purified toxic principle was separated into six toxic components (176). All six of the components were shown to have both ichthyotoxic and hemolytic activities, but the ratio of hemolytic activity to ichthyotoxic activity was different for each of the components. Another indication of the heterogeneity of the *Prymnesium* toxin is the fact that alkaline treatment (0.5 N NaOH) inactivates the hemolytic activity of the toxin but does not decrease the ichthyotoxic activity. Also, the antispasmodic activity is decreased at a faster rate than the ichthyotoxic or hemolytic activity when the toxin is exposed to heat and/or light (128).

As with other toxin-producing algae, environmental conditions influence the growth of *P.*

parvum and the production of its toxins. Salinity is a critical factor for growth and toxin production in ponds or cultures. *Prymnesium* is never found in waters that contain less than 0.12% sodium chloride (129, 150), and the optimum concentration for growth and toxin production is from 0.30 to 5.0% (84). The maximum production of ichthyotoxin and hemolysin was found at salinities around 2.0% (107, 153). *Prymnesium* is not found in waters with temperatures greater than 30°C (151), and the production of blooms of the alga is dependent upon the presence of vitamin B_{12} and thiamine (40, 84, 123, 124). It has been shown that a low inorganic phosphate content in the water increases the toxicity of the cells 10 to 20 times (149). Several other environmental parameters that have been linked with the events of *Prymnesium* toxicity include the level and percentage of illumination (114); the pH of the water, which determines the level of cation complexing with the toxins (174); the binding of colloids in the water with the toxins (152); and the inactivation of toxins by various chemical, physical, and biological agents (149).

As in the case of the blue-green and dinoflagellate toxins, there is no simple numerical correlation between the number of algal cells and the amount of toxins produced (150). Biosynthesis of the toxic products of the alga is at a maximum during the latter portion of the logarithmic growth phase and is maintained for a portion of the stationary growth phase (149). By growing *P. parvum* cultures in different media and under different environmental conditions, it has been shown that cell growth and toxin synthesis require different conditions for optimization. As a matter of fact, *Prymnesium* hemolysin was produced by resting cells in a mineral medium that was unable to sustain growth (153).

Many chemical and physical properties were determined for the toxins produced by *Prymnesium*. These toxins were found to be exotoxins (114, 150, 151) albeit a percentage of the toxins remain intracellular until death and disintegration of the alga. Therefore, blooms of the dying alga tend to increase toxicity by releasing the intracellular fraction of the toxins. The toxins have an amphiphatic nature, which means they form micelles (hydrophobic end of the molecule on the inside and hydrophilic end on the outside) in solution when present above the critical concentration (0.6 ng/ml) in isotonic sodium chloride solution. This phenomenon allows the toxins to behave like surface-active agents (173).

The toxins of *P. parvum* are very labile compounds (112, 128, 153). These toxins are quickly inactivated by visible light in the 400- to 510-nm range and by ultraviolet light in the 255-nm range (128, 130). The ichthyotoxic components are inactivated by heating to 70°C (152), but the hemolytic components have a complicated relationship with temperature (114). The toxins are very hygroscopic. They are not dialyzable. Although it has been shown that the pH of the medium has an effect on the toxins, the nature of the effect remains nebulous because the results are determined by the method of chemical purification. Paster (113) isolated a glycolipid that was biologically alkaline labile (at pH 8 for 24 h) and was stable under acidic conditions (pH 5) and low temperatures (114). Ulitzur and Shilo (176) isolated a lipoprotein–carbohydrate molecule which had stable or slightly activated ichthyotoxicity under alkaline conditions (0.5 N NaOH) (151). Consequently, the environmental conditions also determine the extracellular stability of the toxins, in addition to affecting biosynthesis.

As mentioned, the toxic compound (prymnesin) isolated by Paster (113) was a glycolipid. Ultracentrifugation showed the molecular weight to be 23,000 ± 1,800, and this compound was the only one found in both ultracentrifugation and electrophoresis. The lipid content was found to be approximately 30% of the compound, and the carbohydrate content made up the other 70%. The basic structure of the molecule was a long-chain polysaccharide consisting of about 100 hexose sugars, including glucose, galactose, and mannose in a ratio of 2:1:1 mol. Twenty-six of the hydroxyl groups on the polysaccharide chain had ester linkages with long-chain fatty acids. The fatty acids were myristic, palmitic, stearic, and oleic acids in the ratio of 2:2:8:1 mol. Element analysis showed the presence of only carbon (42.5%), hydrogen (6.95%), and oxygen (50.55%), whereas nitrogen, phosphorus, and sulfur were not present. As for biological activity, the 50% lethal dose for minnows (*Gambusia affinis*) (i.p.) was 1.8 ± 0.4 μg/300 mg of body weight, that for mice (i.p.) was 1.4 ± 0.3 mg/kg, the 50% hemolytic dose for rabbit erythrocytes was 25 ng/ml, and the 50% inhibition of acetylcholine-induced contraction for guinea pig ileum was 30 ng/ml (113).

Alternatively, the compound isolated by Ulitzur and Shilo (176) was a lipoprotein–carbohydrate molecule. This compound was named toxin B and consists of all six of the components that were separated via thin-layer chromatography. Of the six separate components, only three showed any protein content, whereas all six of the toxins contained phosphate. The entire six-component toxin was comprised of 22% protein, 0.47% phosphate, and 10 to 12% hexose sugars. Analysis of the protein portion showed the presence of 15 different amino acids (Table 2), which accounted for

TABLE 2. *Amino acid composition of toxin B protein[a] (from Ulitzur and Shilo [176]).*

Residue	No./100 residues
Lysine	6.8
Histidine	4.0
Arginine	1.0
Aspartate	6.8
Glutamate	6.8
Tyrosine	1.0
Threonine	5.0
Serine	13.3
Glycine	11.7
Alanine	11.7
Valine	7.2
Leucine	10.2
Isoleucine	5.6
Phenylalanine	4.5
Proline	5.0

[a] The toxin B preparation (165 μg) was hydrolyzed for 22 h with 6 M HCl in sealed ampoules at 120°C. The amino acids in the hydrolysate were found and identified by using a Beckman amino acid analyzer.

20.4% of the molecule (leaving 1.6% of the molecule unaccounted for in terms of protein content). In terms of chemical partitioning, toxin B was found to be similar to acidic polar lipids (151); however, in terms of many solubility characteristics, the molecule appeared to behave like a proteolipid compound. Proteolipids are differentiated from lipoproteins because they are soluble in organic solvents but not soluble in water (150). Toxin B was found to differ from proteolipids because it was soluble in dimethyl sulfoxide and methanol (151). In essence, the heterogeneous compound seemed to differ from all well-distinguished classes of compounds, and the structure will just have to await further chemical work.

Many effects of *Prymnesium* toxins, including ichthyotoxicity, cytotoxicity, and hemolytic activity, are predicated on a single mechanism. This mechanism is the change in the permeability of cell membranes (66, 70, 96) which causes the membranes to become cation permselective. As a result of this change, certain cells are lysed, which results in many of the different biological activities of the toxin.

A second important biological activity of the toxin, which may or may not be related to the change in membrane permeability, is the blockage of the neuromotor impulse at the postsynaptic membrane of the neuromuscular junction (111). This is the mechanism that leads to the deaths of various laboratory animals when the toxin is injected either i.p. or intravenously. The toxin blocks neuromuscular transmission while not actually impairing the nerve or muscle cells. For the laboratory animals tested, the blocking of the nerve-muscle transmission has caused death from respiratory paralysis (110). The 50% lethal dose for mice given an i.p. dose of *Prymnesium* toxin was found to be 1.4 mg/kg (37).

Although deaths of laboratory animals by respiratory paralysis are common for many different algal toxins, the toxins of *Prymnesium* are hypothesized to operate on a different principle. *Prymnesium* toxins block the neuromuscular junction without causing depolarization (111). The *Gymnodinium veneficum* toxin increases neurotransmission before blockage and causes depolarization (1). Saxitoxin, which has been isolated from both *Gonyaulax* and *Aphanizomenon,* produces its effects through a change in sodium conductance (150), whereas the toxin from *Prymnesium* has no effect on sodium conductance.

The ichthyotoxicity caused by the *Prymnesium* toxin appears to have a different mechanism of action than that of the induced deaths which occur as a result of either i.p. or intravenous injections. Although i.p. injections of toxins cause rapid deaths in fish (18), the i.p. and oral routes of administration require much larger doses to induce lethal effects than that required when the fish is submerged in the toxin (175). Also, when the fish is immersed there is a synergistic relationship between the ichthyotoxin and a variety of cationic cofactors (calcium ion, magnesium ion, streptomycin, polyamines, neomycin, and cationic detergents) which reduce the amount of toxin needed to cause mortality (174, 188). It is suggested that the ichthyotoxin and the cation are linked to form an active complex (150). When the toxin is administered i.p., cation activation does not occur (150). Hence, the injection and immersion modes of administration seem to measure two different toxic activities.

Only gill-breathing animals are receptive to the toxic effects of *P. parvum* when immersed. As an example, when gill-breathing amphibians (*Rana pipiens* and *Bufo* sp.) are exposed to the ichthyotoxin, they develop tail curving and paralysis and eventually die. However, after metamorphosis, these same animals are refractory to the ichthyotoxin when immersed in it (152). The mechanism of toxic action on gill-breathing animals is believed to consist of two separate steps (150). First, the gill tissues are reversibly damaged, which results in the loss of the selective permeability of the gills. Second, the fish is exposed to all the toxicants present in the water which can now enter the gills, including the ichthyotoxin (151). Thus, ichthyotoxicity seems to involve a different mechanism of action than the generally measured i.p. 50% lethal dose.

In Israel, the method of controlling *P. parvum* is to use a low level of aqueous ammonia, which

lyses the cells. This method has the advantages of having low toxicity to other aquatic forms and being inexpensive. A drawback is that ammonia control is of short duration, thus requiring constant vigilance (154).

Ochromonas spp.

Certain species of *Ochromonas*, which is another genus of the algal family Chrysophyceae, have been found to be toxic (*O. malhamensis, O. danica*, and *O. minuta*) (54, 55, 161). Spiegelstein extracted two separate fractions from *Ochromonas* (150), which he labeled T_a and T_m. T_a was extracted with acetone and was found to be soluble in the water portion of a benzene–water mixture. T_m was extracted with methanol from the residue found to be insoluble in acetone. Both T_a and T_m are soluble in a variety of polar substances (methanol, ethanol, butanol, and water), and both are of low molecular weight. Because they are of low molecular weight, both are easily dialyzed.

Both T_a and T_m show ichthyotoxic, hemolytic, and antispasmodic activities, like *P. parvum* toxin. However, in contrast to the *Prymnesium* toxin, these toxins are not inactivated by either visible or ultraviolet light. T_a is heat, alkaline, and acid labile, whereas T_m is heat, alkaline, and acid stable.

The fish lethality for these compounds occurs for both i.p. administration and immersion. As with the *Prymnesium* toxin, cation activation when the fish are immersed has been reported for both of these substances.

PYRRHOPHYCOPHYTA

The algal members of the Pyrrhophycophyta are also known as the dinoflagellates. This phylum has a unique basic morphology that differentiates its members from those of the other phyla. The algae are divided into two sections (the epitheca and the hypotheca) by a groove that circumscribes the algal cell (the transverse furrow) (Fig. 5). In the most general case, the cells have two flagella; one is directed longitudinally and provides the general directional locomotion, whereas the other circles around the transverse furrow and causes the cell to rotate.

Although there are many species of dinoflagellates discussed in this section, there are several other species that should be mentioned. Most of the documented causes of paralytic shellfish poisoning have involved the *Gonyaulax* genus, but another dinoflagellate, *Pyrodinium phoneus*, has also produced this type of poisoning in the North Sea (139). There is another species of dinoflagellate, found in Japan, which has been shown to be hepatotoxic and nephrotoxic in animals, namely, *Exuviella mariae-lebouriae* (139).

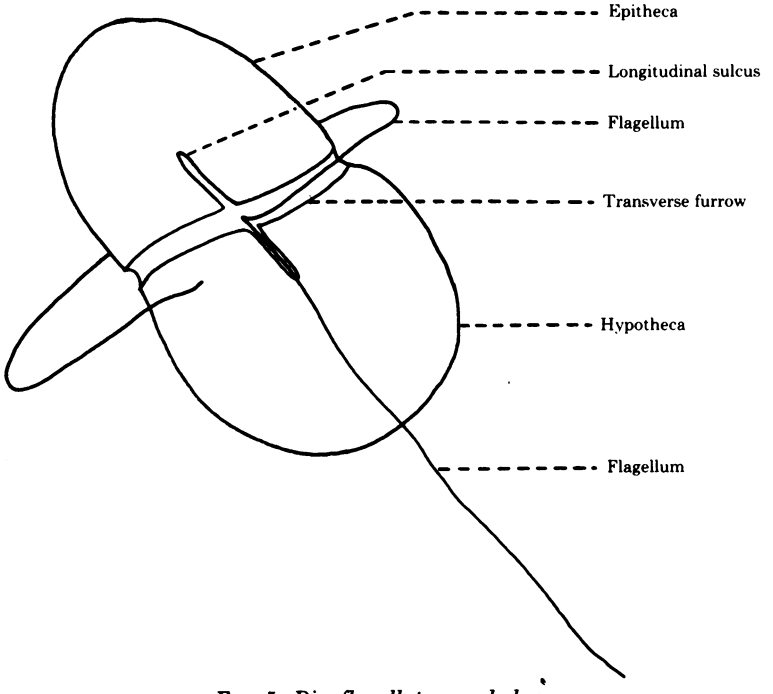


FIG. 5. *Dinoflagellate morphology.*

Most of toxic dinoflagellates are marine organisms, and they are generally within the range of 40 to 60 μm in diameter. Blooms are usually dependent upon an annual cycle of environmental parameters to produce the right temperature, salinity, illumination, pH, and concentrations of trace elements and growth factors. Generally, toxic species are found greater than 30° latitude (139).

Recently, there has been work to substantiate the hypothesis that ciguatera (a type of fish poisoning) is due to a toxic dinoflagellate (95). A single investigation has concluded that *Diplosalis* sp. nov. is very likely to be the cause of ciguatera (189).

Peridinium polonicum

There has been evidence to incriminate the freshwater dinoflagellate *Peridinium polonicum* as an ichthyotoxic species (59, 91). It was originally isolated from Lake Sagani, Tokyo, Japan, and identified as *Glenodinium gymnodinium*, but further investigation showed that it was actually *P. polonicum* (3). Maximum concentrations of this alga (about 5,000 cells per ml) occurred in September and October, when the water temperatures were from 20 to 23°C.

As with all algal toxins, environmental conditions were critical in determining the extent of toxicity. Bioassays using the killifish (*O. latipes*) showed that the toxin was more active at higher pH values (59); therefore, fish kills occurred in the late afternoon, when the lake alkalinity was at a maximum. The minimum lethal dose for killifish when immersed in the ichthyotoxin was 0.132 mg/liter, with death coming after 40 min. The minimum lethal dose for mice when injected i.p. was 2.5 mg/kg, with death following injection by 2 min (59).

The hydrochloride of the toxin was found to be pure on both paper chromatography and thin-layer chromatography. The initial chemical characterization indicated the presence of a secondary and a tertiary amino group, as well as a sulfhydryl radical. The compound was found to have no ultraviolet absorption. Mosher et al. (97) published that the compound was an alkaloid. About the same time, the mass spectrum of the purified hydrochloride was found to be very similar to the mass spectrum of 12-methoxyibogamine (Fig. 6) (25). No further chemical analysis has been published.

Amphidinium spp.

Several species within the dinoflagellate genus *Amphidinium* have been implicated as ichthyotoxic. The supernatant from a culture of *A. klebsii* and *A. rhynchocephalum*, containing about 1,500,000 cells per ml, caused fish mortality in 15 to 25 min (85). The culture supernatant from *A. carteri* was also found to be ichthyotoxic (65). Ikawa and Taylor (65) hypothesized that the toxin was choline-like, and, consequently, they assayed *A. carteri* cells and found that 0.36 to 0.41% of the dry weight was choline chloride equivalent. This assay included pharmacologically inactive forms of choline as well as pharmacologically active forms. For this reason, they assayed *Amphidinium* cells which were processed by a different method to find the fraction of the total amount of choline-like molecules that were in the water-insoluble, inactive form. It was found that 0.07 to 0.10% of the dry weight was in the inactive form of cholines, which means that 0.26 to 0.34% of the dry weight was in the active form of the choline-like compounds.

By combining the use of assay procedures and chromatographic work, it was determined that *A. carteri* has three nonlipoidal choline derivatives. One of the substances has been identified as choline *O*-sulfate (Fig. 7), and another behaves physiologically, chemically, and nutritionally like a choline ester. At least one of the unknown compounds exhibited acetylcholine-like activity on a number of heart systems (65). The toxicological properties of these choline-like algal extracts were not tested.

Noctiluca miliaris

The dinoflagellate *Noctiluca miliaris* is responsible for many red tides from spring to summer in the Seto Inland Sea, Japan, and the organisms can cover an area of several square

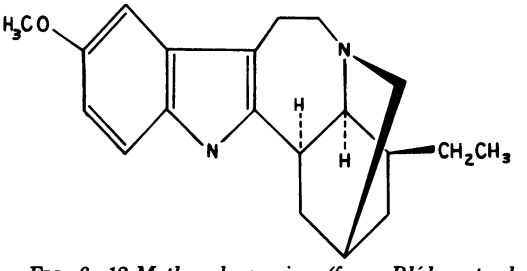
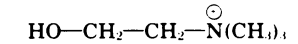


FIG. 6. *12-Methoxybogamine (from Bláha et al. [21]).*

A



B

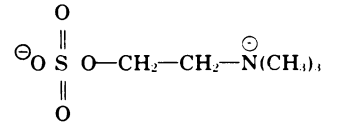


FIG. 7. *Choline (A) and choline O-sulfate (B).*

kilometers. The *N. miliaris* red tide along the coast of Madras, Japan, has led to the poisoning of many fish and invertebrates. By taking acidic extracts of *N. miliaris*, the toxicity of this algal species has been shown both by i.p. injection into mice and by immersion of fish.

The purification of the toxic compound was attained by successive treatments with charcoal, Sephadex G-10, and Cu-Chelex-100 resin (103). Amino acid liquid chromatography showed that the toxic compound was ammonia (NH_3). A series of samples were collected from the red tides along the coast of Japan in 1972 and 1975. The ammonia contents of the *Noctiluca* samples varied from 6.6 to 68.9 mg of ammonia as nitrogen per g of material. It was found that the ichthyotoxicity was closely related to the ammonia content of the alga. According to Okaichi and Nishio (103), the toxicity of *Noctiluca* is due to ammonia.

Gymnodinium spp.

The nonthecate marine dinoflagellate *Gymnodinium* has two different species which have exhibited toxic properties. *G. breve* is found predominantly in the Gulf of Mexico and is responsible for the red tides in this area, whereas with *G. veneficum* of the English Channel, which has been found to contain toxic substances, a naturally occurring bloom resulting in toxicity has not been documented (139). The red tides due to *G. breve* are lethal to a large variety of aquatic and terrestrial biota (Table 3). In 1947, a particularly severe outbreak of red tide, which was due to *G. breve* Davis, plagued the Gulf Coast for a period of 9 months (151). The concentration of organisms for this red tide varied from 400,000 to 60,000,000 organisms per liter. During the 1971 red tide, caused by *G. breve*, it was estimated that 100 tons (ca. 90 t) of fish died per day (134).

It has been discovered that the larger a particular test species, the greater the resistance to the toxic components of *G. breve*. Also, vertebrates are more susceptible than invertebrates because the toxin acts on the nervous system, which is more specifically defined in vertebrates. Humans are susceptible to the toxic effects of *G. breve* red tides primarily through the food chain (especially shellfish). The toxic effects produced in humans are neither severe nor of long duration. No human deaths have been reported as a result of ingestion of seafood exposed to *G. breve* (164). *Gymnodinium* is not the alga that is generally associated with paralytic shellfish poison. Another type of human health problem occurs when *G. breve* cells are lysed and become airborne due to sea spray. In this instance the cells are responsible for an odorless eye and respiratory irritant (67, 151, 164, 187).

Several basic parameters differentiate *G. breve* toxin from the paralytic shellfish poison that is generally attributed to *Gonyaulax*. The *Gymnodinium* toxin is an endotoxin, whereas the *Gonyaulax* toxin is an exotoxin. Also, the *Gymnodinium* toxin is not water soluble, but the *Gonyaulax* toxin is (139, 164). The differences in the chemical structures of the two toxins will have to await the elucidation of the *Gymnodinium* structure.

As is true with all algae, the environmental conditions are critical in determining when a red tide will occur. It has been found that the best salinity content for *G. breve* blooms is from 3.1 to 3.7% (132). Salinities below 2.4% are inhibitory (9). Since most Gulf Coast estuaries do not have the proper salinity, the red tides are not usually found closer to the shore than the outer reaches of the bays. Another environmental criterion of importance in culturing *Gymnodinium* has been the requirement for inorganic salts and the three vitamins biotin, thiamine, and vitamin B_{12}. A factor which has been responsible for a large amount of growth stimulation is gibberellic acid (116). The purpose of mentioning these various environmental factors is to note the many diversified parameters which can affect blooms of the various algae.

Rainfall and land runoff have also been correlated with *G. breve* red tides (159, 177). The factor which induces increased *Gymnodinium* growth is chelated iron (185), and it has been hypothesized that the humic acids in land runoff are responsible for chelating enough iron to induce blooms of *G. breve*. The precision of this estimation has been worked out to the point that if more than 235,000 pounds (ca. 106,600 kg) of iron is in the Peace River over a 3-month period, then a red tide is forecast in the Charlotte Harbor area of Florida (68).

There have been reports to indicate that *G. breve* toxin concentration correlates with the number of algal cells. Many, if not all, toxic algae have been tested for such a correlation, and it was found that no such relationship exists in other species. However, one study showed a direct correlation between the average death time of a laboratory animal and *G. breve* cell density (163). Another study found a relationship between the number of *G. breve* cells and the potency of the toxin (162).

In terms of determining the chemical structure of the *G. breve* toxin, a large amount of contradictory work has been published (7, 34, 89, 135, 160, 170). The different chemical separations used to isolate the toxin and the unstable

TABLE 3. *Species list of dead animals found during red tide outbreaks due to G. breve (from Steidinger et al. [164])*

Scientific name	Common name
Vertebrates	
Achirus lineatus	Lined sole
Aluterus schoepfi	Orange filefish
Anchoa mitchelli	Bay anchovy
Archosargus probatocephalus	Sheepshead
Arius felis	Sea catfish
Astroscopus y-graccum	Southern stargazer
Bagre marina	Gaff-topsail catfish
Bairdiella chrysura	Silver perch
Balistes capriscus	Gray triggerfish
Balistes vetula	Queen triggerfish
Bascanichthys teres	Sooty eel
Brevoortia patronus	Gulf menhaden
Calamus sp.	Porgy family
Caranx crysos	Blue runner
Caranx hippos	Crevalle jack
Centropomus undecimalis	Snook
Chaetodipterus faber	Atlantic spadefish
Chilomycterus schoepfi	Striped burrfish
Chlorscombrus chrysurus	Atlantic bumper
Cynoscion arcnarius	Sand sea trout
Cynoscion nebulosus	Spotted sea trout
Decapterus punctatus	Round scad
Diodon holocanthus	Balloonfish
Diplectrum formosum	Sand perch
Echeneis naucrates	Shark sucker
Elops saurus	Ladyfish
Epinephelus morio	Red grouper
Epinephelus nigritus	Warsaw grouper
Eucinostomus gula	Silver jenny
Floridichthys carpio	Gold-spotted killifish
Fundulus similis	Longnose killifish
?Gymnothorax moringa	Spotted moray
?Gymnothorax vicinus	Purple-mouth moray
Haemulon aurolineatum	Tomtate
Haemulon parra	Sailor's-choice
Haemulon plumieri	White grunt
Haemulon sciurus	Blue-striped grunt
Harengula pensacolae	Scaled sardine
Hemiramphus sp.	Halfbeak family
Hyporhamphus unifasciatus	Halfbeak
Istiophorus platypterus	Sailfish
Lactophrys trigonus	Trunkfish
Lactophrys quadricornis	Scrawled cowfish
Lagodon rhomboides	Pinfish
Leiostomus xanthurus	Spot
Lepisosteus sp.	Gar family
Lutjanus griseus	Gray snapper
Megalops atlanticus	Tarpon
Menidia beryllina	Tidewater silverside
Menticirrhus americanus	Southern kingfish
Menticirrhus littoralis	Gulf kingfish
Monocanthus hispidus	Planehead filefish
Mugil cephalus	Striped mullet
Mycteroperca bonaci	Black grouper
Myrophis punctatus	Speckled worm eel
Mystriophis intertinctus	Spotted spoon-nose eel
Ogcocephalus vespertilio	Longnose batfish
Oligoplites saurus	Leatherjacket
Ophichthys gomesi	Shrimp eel
Ophichthys ocellatus	Pale-spotted eel
Ophidion holbrooki	Bank cusk-eel
Opisthonema oglinum	Atlantic thread herring
Opsanus tau	Oyster toadfish
Orthopristis chrysopterus	Pigfish
Paralichthys albiguttus	Gulf flounder
Peprilus alepidotus	Harvest fish
Peprilus burti	Gulf butterfish
Peprilus triacanthus	Butterfish
Pogonias cromis	Black drum
Pomacanthus arcuatus	Gray angelfish
Porichthys porosissimus	Atlantic midshipman
Prionotus scitulus	Leopard sea robin
Pristigenys alta	Short bigeye
Sciaenops ocellatus	Red drum
Scomberomorus maculatus	Spanish mackerel
Scorpaena sp.	Scorpion fish family
Sphoeroides maculatus	Northern puffer
Sphoeroides nephelus	Southern puffer
Sphoeroides testudineus	Checkered puffer
Sphyraena barracuda	Great barracuda
Strongylura marina	Atlantic needlefish
Strongylura notata	Redfin needlefish
Symphurus plagiusa	Blackcheek tonguefish
Synodus foetens	Inshore lizard fish
Trachinotus carolinus	Florida pompano
	Porpoise
	Turtles
	Cormorant[a]
	Ducks[a]
	Frigate bird[a]
	Gulls[a]
	Terns[a]
	Vultures[a]
Invertebrates	
Arbacia sp.[b]	Sea urchin
Balanus sp.[b]	Barnacle
Callinectes sapidus[b]	Blue crab
Crassostrea virginica[b]	American oyster
Donax variabilis[b]	Coquina
Ircinia sp.[b]	Sponge
Limulus polyphemus	Horseshoe crab
Penaeus sp.[b]	Shrimp

[a] Certain animals may be affected via the food chain or by taking in water containing *G. breve*, e.g., birds with salt glands.

[b] Species may suffer mortality not attributable directly to toxin, but rather to associated adverse conditions, e.g., oxygen depletion.

nature of the toxic compound have combined to yield a variety of results. The recent use of high-pressure liquid chromatography for the analysis of biological compounds (24, 180) has led to the conclusion that there is actually only a single ichthyotoxic component (77). The chemical structure of this component has not been elucidated because of its instability. Kim and Padilla (77) have found that the original toxin is broken down to a more stable toxic component, as well as to other stable but nontoxic components. The purified toxin was found to be unstable when stored in the dark at 4°C in the dry state. G. Padilla believes that the toxic molecule has a

molecular weight of approximately 400 and that there is no sulfur or phosphorus in the toxin and very little nitrogen, if any (personal conversation). *G. breve* produces a separate hemolytic component which has been separated from the ichthyotoxic component (78, 109, 115).

The physiological action of the *G. breve* toxin is as much of an enigma as the chemical structure of this compound. The dominant hypothesis as to the mode of action of *G. breve* toxin is that it acts as a depolarizing agent, similar to the toxin from *G. veneficum* (1, 164). Depolarizing agents block nerve transmission and are responsible for decreasing or eliminating the resting membrane potentials. These agents can also reduce the abilities of the nerves and muscles to transmit action potentials. *G. breve* toxin has been responsible for reducing the resting membrane potentials and the amplitudes of action potentials for nerves, muscles, and skin tissue (J. Sasner, Ph.D. thesis, University of California at Los Angeles, Los Angeles, 1965). More recently, Sasner et al. (135) showed that a *G. breve* extraction did not induce generalized depolarization but that it acted as a synaptic blocking agent (probably at the end plate). This anticholinesterase-like activity was different from the depolarizing activity previously demonstrated. Perhaps the different mechanisms of action are due to the various methods of purification and the unstable nature of the molecule. Abbott and Ballantine (1) showed that the interpretation of the mechanism of action was contingent upon the pH of extraction.

The systemic pharmacological effects of the *G. breve* extracts are likewise somewhat nebulous. Humans who are exposed to *G. breve* toxins via ingestion develop symptoms of central nervous system poisoning (hot and cold reversals, vertigo, and slowed pulse), as well as symptoms of peripheral nervous system toxicity (ataxia, dilated pupils, mild diarrhea, and tingling sensations) (164). According to Steidinger et al. (164), animals that were susceptible to the lethal effects of the toxin were initially paralyzed and then died from respiratory failure. This is the mechanism of lethality which has been reported most often for toxic algae. However, according to Spiegelstein et al. (160), the behavior of mice after the injection of *Gymnodinium* extract indicated cardiovascular failure. Trieff et al. (172) found that mortality of mice occurred either rapidly (0 to 2 h) or relatively slow (24 to 48 h). The rapid mechanism was of the neuromuscular type, which ended with respiratory failure, but the slow mechanism was characterized by a distended bladder and the dilation of capillaries. The actual physiological and pharmacological occurrences will have to await further research. The hemolytic fraction of the toxin does not exhibit any lethal activity (2).

Gonyaulax spp.

There are six different species of the marine dinoflagellate *Gonyaulax* which have been documented as toxic species. The three species *G. catenella, G. acatenella,* and *G. tamarensis* have been shown to cause paralytic shellfish poisoning, whereas the species *G. monilata* and *G. polygramma* have been related to ichthyotoxic incidents. *G. catenella* is found on the northern Pacific coasts of North America and Japan, *G. acatenella* is found on the Pacific coast of North America, and *G. tamarensis* is found off the North American Atlantic coast and in the North Sea. *G. polygramma* has been associated with red tides and has caused fish toxicity off Massachusetts and oyster deaths off Japan (151). *G. polyedra* is found along the coast of southern California and contains a poison, but it has not caused shellfish or fish poisoning (145). *G. monilata* inhabits the Gulf of Mexico and causes fish mortalities, but it does not affect warm-blooded animals (78, 126). The *Gonyaulax* species associated with paralytic shellfish poisoning have received more experimental attention than have the other species.

Paralytic shellfish poisoning occurs when an organism feeds on a shellfish that has filtered a toxic species of the dinoflagellates (most frequently *Gonyaulax*). The shellfish are not adversely affected by the alga. There are more than 220 human fatalities which have occurred as results of paralytic shellfish poisoning (56). Whereas the majority of shellfish have a toxin half-life in terms of days, certain species (e.g., Alaska butter clams) can maintain the toxin for a period of years (125). The heat-stable nature of these toxins allows them to avoid damage in food preparation steps which would degrade heat-labile toxins. The fact that there is no effective antidote for the poison means that a victim's prognosis is dependent upon the quantity ingested. Mussels along the California coast were not safe for human consumption when there were more than 200 *G. catenella* cells per ml of water.

Most species of shellfish bind the *Gonyaulax* toxins in the hepatopancreas, where it does not cause any harm to the shellfish. Apparently, there is a mechanism in the hepatopancreas that releases the bound toxins or destroys them. The bound toxins are released in a weak acid solution (about pH 2), so the binding is not extremely strong. The shellfish that operate in the foregoing manner are usually detoxified by 1 or 2 weeks in a nontoxic environment. Some clams (e.g., Alaska butter clams) bind the toxins in the si-

phon. In the siphon, the rate of toxin release or destruction is low, so these clams may remain toxic for 1 year or more after the environment is cleared of the *Gonyaulax* (139).

Most chemical isolations have been done on *G. catenella* and *G. tamarensis*. It was found that the poisons extracted from clams, mussels, and *G. catenella* were chemically approximately the same (143). *G. tamarensis* is considered a more severe practical problem, because the red tides of this alga occur more frequently and cover a larger area than the tides of *G. catenella* (48). Recent work with thin-layer and high-pressure liquid chromatography and electrophoresis has indicated that the toxic constituents of these two algal species are relatively similar (105). There have been seven separate toxins isolated from *G. tamarensis*: saxitoxin, gonyautoxin 1, gonyautoxin 2, gonyautoxin 3, gonyautoxin 4, gonyautoxin 5, and neosaxitoxin. For only three of these toxins have the chemical structures been fully described (Fig. 8) (one of the three is the compound saxitoxin, which was previously described as being isolated from *Aph. flos-aquae*); however, the other four toxins are hypothesized to have similar structures (104).

The chemical characteristics of all seven of these toxins are very similar. They are all very hygroscopic and highly water soluble. They were all difficult to classify chemically because they are noncrystalline, highly polar, and nonvolatile. Many of the chemical properties of these compounds have been reported by various sources (23, 104, 141, 157). The 50% lethal doses for saxitoxin are 5 to 10 μg/kg when administered to a mouse i.p. (141) and 3.4 μg/kg when administered intravenously (184). This means that saxitoxin is the most potent algal toxin on record.

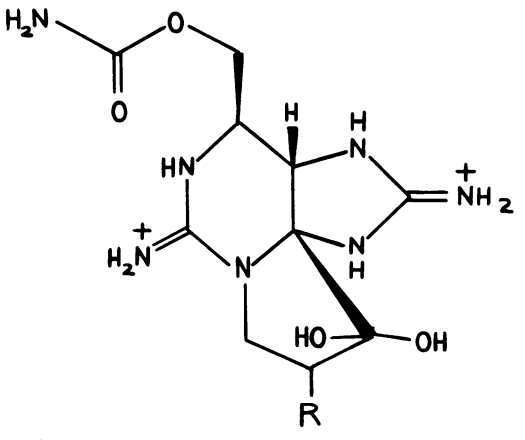


FIG. 8. *Gonyaulax toxins: saxitoxin (R = H), gonyautoxin 2 (R = α-OH), and gonyautoxin 3 (R = β-OH) (from Shimizu et al. [157]).*

On a cellular level, the physiological action of *Gonyaulax* toxins is the blocking of sodium molecules that would passively cross excited membranes, essentially preventing action potentials. The toxins have a negligible effect on potassium and chloride permeabilities (49, 101, 102). This effectively blocks axonal and muscular conduction while having a minimal effect on the neuromuscular synapse (74). This type of block occurs without depolarization.

The pharmacological activity of the *Gonyaulax* toxins includes potent central and peripheral neurotoxic actions. Central nervous system actions include cardiovascular and respiratory effects, whereas peripheral nervous system actions are effects at the neuromuscular synapses and sensory nerve endings (151). Schantz (139) describes the human symptoms of paralytic shellfish poisoning as follows.

> The symptoms of shellfish poisoning begin with a numbness in the lips, tongue and finger-tips and may be apparent within a few minutes after eating poisonous shellfish. This sensation is followed by a feeling of numbness in the legs, arms, and neck with general muscular incoordination. A feeling of lightness, as though floating on air, is often described by the afflicted persons. Other associated symptoms may be listed as dizziness, weakness, drowsiness, incoherence, headache, and the like. The mental symptoms vary, but most patients appear calm and remain conscious during the illness. As the illness progresses, respiratory distress and muscular paralysis become more and more severe and death results from respiratory paralysis within 2 to 12 hours, depending upon the magnitude of the dose. If one survives 24 hours the prognosis is good, and there appear to be no lasting effects. [139]

RHODOPHYCOPHYTA

The carrageenans are heterogenous mixtures of anionic polysaccharides which are extracted from certain algae of the Rhodophycophyta. The chemical compositions of these molecules (Fig. 9) include alternating derivatives of galactose, 3,6-anhydrogalactose, and sulfated galactose. Molecules with the basic structure of carrageenan have been isolated from the following genera of the Rhodophycophyta: *Chondrus, Gigartina, Hypnea, Rhodymenia, Irideae, Gracilaria, Fucellaria, Polydes,* and *Eucheuma* (36).

There are essentially two types of carrageenans, which are differentiated according to molecular weights. The native, undegraded, food-grade carrageenan has a high molecular weight (100,000 to 800,000), includes both kappa and lambda chains, and is derived primarily from the alga *Chondrus crispus*. This type of carrageenan is used as an emulsifier, stabilizer, or thickener in many types of foods because of its ability to bind casein. The carrageenan used in the ther-

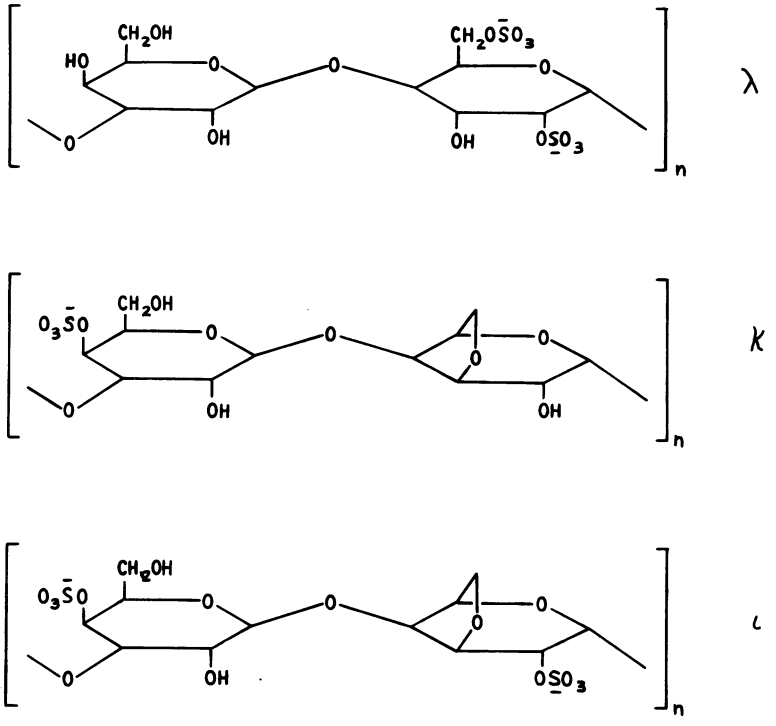


FIG. 9. *Idealized structures of the different carrageenans (from Mueller and Rees [98]).*

apy of peptic ulcers has a comparatively low molecular weight (5,000 to 30,000), consists of only iota chains, and is derived primarily from the alga *Eucheuma spinosum*. The iota, kappa, and lambda galactose units differ in the number of sulfate groups present and in the types of chemical linkages (41).

The reason for mentioning the carrageenans in this paper is that they represent an algal polysaccharide with the capability to induce serious physiological effects. They have a wide spectrum of biological actions (36) which include the induction of acute (edema) and chronic (granuloma) inflammatory responses, inhibition of the C1 component of the complement sequence, activation of Hagemann factor, selective toxicity to macrophages (10, 31, 166) and strong immunosuppression (12, 19). The many physiological effects of carrageenan coupled with its widespread use as a food additive and as a drug have led to a series of experiments to determine the health implications of its use.

Until recently, the only deaths produced by carrageenans were through the intravenous route of administration (36). At 15 mg/kg, the carrageenan fraction from *Gigartina acicularis* caused two out of three dogs to die within 24 h (62). Similarly, in a rabbit study the minimum lethal dose, when administered intravenously, to cause death within 24 h was 1 to 5 mg/kg for lambda-carrageenan and 3 to 15 mg/kg for kappa-carrageenan; these fractions were taken from *C. crispis* (11). Thomson and Horne (166) have also produced lethality by i.p. injection of 1 to 25 mg of potassium carrageenan in 20- to 25-g mice. In their experiments, i.p. administration produced death much more frequently with lambda-carrageenan than with the iota or kappa chain. Recently, Wakabayashi et al. (179) have caused colorectal tumors in rats via oral administration of degraded iota-carrageenan.

Thomson and Horne (166) also made several observations concerning the toxic mechanisms of carrageenans. Since carrageenans activate Hagemann factor, they also activate kinins, which enhance thrombosis. This is consistent with one of the findings of Thomson and Horne, namely, that the carrageenans cause disseminated intravascular coagulation which may eventually produce infarctions of internal organs and extremities. They also found that the carrageenans were hepatotoxic.

It was known about 20 years ago that a subcutaneous injection of carrageenan caused collagenous granuloma in guinea pigs, but this information did not prevent it from being listed on the first GRAS (Generally Recognized As Safe) list put out by the U.S. Food and Drug Administration in 1959 (71). The subcutaneous route of administration was not considered applicable

for this food additive because the high-molecular-weight carrageenans are not absorbed in the gastrointestinal tract. Several studies have shown that the high-molecular-weight species do not produce major tissue disruption (i.e., ulceration) (16, 22, 86, 119, 148). Alternatively, the low-molecular-weight carrageenans have produced ulcerative colitis, mucosal erosions, and other tissue changes in laboratory animals (17, 41, 42, 88, 181–183). The prevailing theory is that the critical factor in determining whether a carrageenan will produce ulceration or similar tissue phenomena is whether or not it has the ability to be absorbed by the gastrointestinal tract. Herbivores absorb all molecular weights of carrageenans, whereas primates only absorb the low-molecular-weight fraction.

Carrageenan subcutaneously administered to rats in a single dose of 50 mg produced a significant number (11 out of 39) of sarcomas over a 2-year period (32). Granulomatous tumors have been produced by i.p. administration of carrageenans many times (146). The mechanism of cancer production in this instance may be an example of foreign-body oncogenesis as opposed to chemical carcinogenesis (92). As a result of the health implications presented by carrageenans, they were removed from the GRAS list, and, albeit they continue to be a widely used food additive, the molecular weight is stringently regulated (71).

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