The Toxins of Cyanobacteria

These poisons, which periodically and fatally contaminate the water supplies of wild and domestic animals, can also harm humans. But they are being coaxed into doing good

by Wayne W. Carmichael

On May 2, 1878, George Francis of Adelaide, Australia, published the first scholarly description of the potentially lethal effects produced by cyanobacteria—the microorganisms sometimes called blue-green algae or, more colloquially, pond scum. In a letter to *Nature* he noted that an alga he thought to be *Nodularia spumigena* had so proliferated in the estuary of the Murray River that it had formed a "thick scum like green oil paint, some two to six inches thick, and as thick and pasty as porridge." This growth had rendered the water "unwholesome" for cattle and other animals that drink at the surface, bringing on a rapid and sometimes terrible death:

> Symptoms—stupor and unconsciousness, falling and remaining quiet, as if asleep, unless touched, when convulsions come on, with head and neck drawn back by rigid spasm, which subsides before death. Time—sheep, from one to six or eight hours; horses, eight to twenty-four hours; dogs, four to five hours; pigs, three or four hours.

Since 1878, investigators have confirmed that *Nodularia* and many other genera of cyanobacteria include poisonous strains. Indeed, such microbes are known to account for spectacular die-offs of wild and domestic animals. In the midwestern U.S., for instance, migrating ducks and geese have perished by the thousands after consuming water contaminated by toxic cyanobacteria. In recent years, workers have identified the chemical structure of many cyanobacterial toxins and have also begun to decipher the steps by which the poisons can lead to suffering and death.

Such research is exciting interest today, in part because of worry over public health. No confirmed human death has yet been attributed to the poisons. But runoff from detergents and fertilizers is altering the chemistry of many municipal water supplies and swimming areas, increasing the concentration of nitrogen and phosphorus. These nutrients promote reproduction by dangerous cyanobacteria and thus foster formation of the dense growths, known as waterblooms, described by Francis. As cyanobacterial waterblooms become more common in reservoirs, rivers, lakes and ponds, the likelihood grows that people will be exposed to increased doses of toxins. (Water-treatment processes only partially filter out cyanobacteria and dilute their toxins.) The risk of animal die-offs grows as well.

The possibility of increased exposure has become particularly disturbing because some evidence suggests that certain cyanobacterial toxins might contribute to the development of cancer. Knowledge of the chemical structure and activity of the toxins should help scientists to devise more sensitive ways to measure the compounds in water and to develop antidotes to lethal doses. Improved understanding of how these chemicals function should also facilitate efforts to determine the long-term effects of exposure to nonlethal doses.

Research into the structure and activity of the toxins is sparking interest on other grounds as well. They and their derivatives are being considered as potential medicines for Alzheimer's disease and other disorders. The substances already serve as invaluable tools for exploration of questions in cell biology.

As worrisome and wonderful as the toxins are, other aspects of cyanobacteria are perhaps more familiar to many people. For example, textbooks often feature these bacteria as nitrogen fixers. The filamentous species (which consist of individual cells joined end to end, like beads on a string) convert atmospheric nitrogen into forms that plants and animals can use in their own life processes. In this way, they fertilize agricultural land throughout the world, most notably rice paddies, where they are often added to the soil.

Cyanobacteria are known, too, for the critical insights they have provided into the origins of life and into the origins of organelles in the cells of higher organisms. The fossil record shows that cyanobacteria already existed 3.3 to 3.5 billion years ago. Because they were the first organisms able to carry out oxygenic photosynthesis, and thus to convert carbon dioxide into oxygen, they undoubtedly played a major part in the oxygenation of the air [see "The Blue-Green Algae," by Patrick Echlin; SCIENTIFIC AMERICAN, June 1966]. Over time, their exertions probably helped to create the conditions needed for the emergence of aerobic organisms. At some point, theorists suggest, certain of the photosynthesizers were taken up permanently by other microbes. Eventually these cyanobacteria lost the ability to function independently and became chloroplasts: the bodies responsible for photosynthesis in plants.

It was the toxins, however, that sparked my own curiosity about cyanobacteria. That was back in the late 1960s, when I was an undergraduate majoring in botany at Oregon State University. At the time, I had the young student's usual fascination with the microscope and things microscopic. I was also intrigued by the question of how toxins—naturally produced poisons—

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damage the body. In biological circles, toxins are among the compounds referred to as secondary metabolites because they are produced by living organisms but are not known to be critically important to everyday survival.

I decided to pursue both of my interests by looking into the production and action of poisons made by cyanobacteria. In 1970 I therefore became a graduate student of Paul R. Gorham at the University of Alberta in Edmonton. Gorham was one of the first scientists to study the properties of toxic cyanobacteria and had been doing so since the 1950s. Researchers in South Africa, Australia and the U.S. were carrying out related investigations, but Gorham and his colleagues had already laid much groundwork for the kinds of studies I hoped to undertake.

When I joined Gorham's group, cyanobacteria were typically referred to as blue-green algae because of the turquoise coloring of most blooms and the similarity between the microbes and true algae (both carry out photosynthesis). But Roger Y. Stanier, then at the University of California at Berkeley, was beginning to reveal the "algae" part of the name to be a misnomer.

After the electron microscope was introduced in 1950, work by Stanier and others established that two radically different types of cells exist in the contemporary world. Prokaryotic varieties—those bearing the characteristics of bacteria—have no membrane enveloping their nuclear material and usually lack membrane-bound bodies in their interior. All other cells, including those of algae and more complex plants, are eukaryotic: they contain a definite nuclear membrane and have mitochondria as well as other organelles. Stanier's subsequent examinations of cyanobacteria prompted him to note in 1971 that "these organisms are not algae; their taxonomic association with eukaryotic groups is an anachronism.... Blue-green algae can now be recognized as a major group of bacteria."

Gorham's work, and later mine, extended the research begun when cyanobacteria were still thought to be algae. By the 1940s reports implicating the microorganisms in the poisoning of wild and domestic animals had accumulated from many parts of the world. The animals died after drinking from ponds or other waters partly covered by slimy carpets of what seemed to be algae, often in the dog days of late summer and early fall, when the temperature is high and the air is relatively still. Yet no firm link between specific genera of cyanobacteria and animal deaths had yet been established.

Theodore A. Olson, a microbiologist at the University of Minnesota, made that connection in the course of studies he carried out between 1948 and 1950. Olson collected samples of waterblooms in his state and determined that they contained copious amounts of species from the cyanobacterial genera *Microcystis* and *Anabaena* (common groups of planktonic cyanobacteria). By feeding cyanobacteria from those blooms to laboratory animals, he was able to demonstrate that certain water-dwelling forms can indeed be poisonous to animals.

POND IN BEIJING has been contaminated by an overgrowth, or waterbloom, of toxic cyanobacteria (*green scum*). These bacteria, flourishing in the Grandview Garden Park, are members of the widespread genus *Microcystis,* many species of which produce potent liver toxins. The toxins have killed animals, and the consumption of low doses in drinking water is suspected of contributing to a high rate of human liver cancer in certain parts of China.

MASS OF CYANOBACTERIA close to the shore of Balgavies Loch, near Dundee, Scotland, has the typical appearance of a waterbloom seen at short range: it resembles a thick pool of green oil paint. This bloom occurred in 1981 and was found to consist of species in the genus *Microcystis.*

This finding, in turn, raised new questions. Why, for example, were animals poisoned most often during the dog days of summer and fall? The answer now seems to be that cyanobacteria grow remarkably well and form waterblooms when four conditions converge: the wind is quiet or mild, and the water is a balmy temperature (15 to 30 degrees Celsius), is neutral to alkaline (having a pH of 6 to 9) and harbors an abundance of the nutrients nitrogen and phosphorus. Under such circumstances, cyanobacterial populations grow more successfully than do those of true algae. (True algae can also form waterblooms, but blooms in nutrient-rich water usually consist of toxic cyanobacteria.)

The cyanobacterial blooms by themselves probably would not harm animals if the microbes clustered far from shore. But cyanobacteria move up and down within the water to obtain light for photosynthesis and, in the process, often float to the surface. There, currents and any winds that arise can push the bacteria toward the land, causing poison-filled cells to accumulate in a thick layer near the leeward shore. Animals drinking such concentrated scum can readily consume a fatal dose.

Because the cells release toxins only when they themselves die or become old and leaky, animals usually have to ingest whole cells to be affected. They can, however, take in a fatal dose of toxins from cell-free water if someone has treated the water with a substance, such as copper sulfate, designed to break up waterblooms. The amount of cyanobacteria-tainted water needed to kill an animal depends on such factors as the type and amount of poison produced by the cells, the concentration of the cells, as well as the species, size, sex and age of the animal. Typically, though, the required volume ranges from a few millimeters (ounces) to several liters (a few gallons). Apparently, thirsty animals are often undeterred by the foul smell and taste of contaminated water.

The early demonstration that cyanobacterial toxins were responsible for animal kills in Minnesota also raised the questions that Gorham took up in the 1950s—namely, what is the chemical nature and modus operandi of the toxins? To find answers, he first had to develop methods for maintaining cultures of toxic cyanobacteria in the laboratory. In the 1950s and 1960s Gorham and his colleagues, then at the National Research Council in Ottawa, succeeded in establishing cultures for two of the most toxic cyanobacteria: *Anabaena flos-aquae* and *Microcystis aeruginosa.* With such cultures in hand, they were able to isolate poisons produced by the cells and identify their chemical makeup. A knowledge of chemical structure offers clues to how a molecule functions.

In 1972, soon after I arrived in Gorham's laboratory, Carol S. Huber and Oliver E. Edwards, working in Edwards's laboratory at the National Research Council, determined the chemical structure of a cyanobacterial toxin for the first time. Derived from *A. flos-aquae,* and named anatoxin-a, it turned out to be an alkaloid—one of thousands of nitrogen-rich compounds that have potent biological, usually neurological, effects. So far species from seven of 12 cyanobacterial genera involved in animal deaths have been cultured. Interestingly, none of the 12 genera grow attached to rocks or vegetation; all are planktonic, floating in water as single cells or filaments. Most produce more than one type of toxin.

The toxins that have been studied intensively to date belong to one of two groups, defined by the symptoms they have produced in animals. Some, such as anatoxin-a, are neurotoxins. They interfere with the functioning of the nervous system and often cause death within minutes, by leading to paralysis of the respiratory muscles.

Other cyanobacterial poisons, such as those produced by Francis's *N. spumigena,* are hepatotoxins. They damage the liver and kill animals by causing blood to pool in the liver. This pooling can lead to fatal circulatory shock within a few hours, or, by interfering with normal liver function, it can lead over several days to death by liver failure.

Four neurotoxins have been studied in detail. Of these, anatoxin-a and anatoxin-a(s) seem unique to cyanobacteria. The other two—saxitoxin and neosaxitoxin—arise in certain marine algae as well. I had the good fortune of being able to explore the activity of anatoxin-a soon after its structure was deciphered. This compound, made by various strains of the freshwater genera *Anabaena* and *Oscillatoria,* mimics the neurotransmitter acetylcholine.

When acetylcholine is released by neurons (nerve cells) that impinge on muscle cells, it binds to receptor molecules containing both a neurotransmitter binding site and an ion channel that spans the cell membrane. As acetylcholine attaches to the receptors, the channel opens, triggering the ionic movement that induces muscle cells to contract. Soon after, the channel closes, and the receptors ready themselves to respond to new signals. Meanwhile an enzyme called acetylcholinesterase de-

grades the acetylcholine, thereby preventing overstimulation of the muscle cells.

Anatoxin-a is deadly because it cannot be degraded by acetylcholinesterase or by any other enzyme in eukaryotic cells. Consequently, it remains available to overstimulate muscle. It can induce muscle twitching and cramping, followed by fatigue and paralysis. If respiratory muscles are affected, the animal may suffer convulsions (from lack of oxygen to the brain) and die of suffocation. Unfortunately, no one has succeeded in producing an antidote to anatoxin-a. Hence, the only practical way for farmers or other concerned individuals to prevent deaths is to recognize that a toxic waterbloom may be developing and to find an alternative water supply for the animals until the bloom is eliminated.

For animals, anatoxin-a is an anathema, but for scientists it is a blessing. As a mimic of acetylcholine, anatoxin-a makes a fine research tool. For example, because it resists breakdown by acetylcholinesterase, the toxin and its derivatives can be used in place of acetylcholine in experiments examining how acetylcholine binds to and influences the activity of acetylcholine receptors (especially the so-called nicotinic acetylcholine receptors in the peripheral and central nervous system).

Edson X. Albuquerque and his colleagues at the University of Maryland School of Medicine are looking at anatoxin-a in other ways as well. The researchers are in the early stages of exploring the intriguing possibility that a modified version might one day be administered to slow the mental degeneration of Alzheimer's disease. In many patients, such deterioration results in part from destruction of neurons that produce acetylcholine. Acetylcholine itself cannot be administered to replace the lost neurotransmitter because it disappears too quickly. But a version of anatoxin-a that has been modified to reduce its toxicity might work in its place. Derivatives of anatoxin-a could also conceivably prove useful for other disorders in which acetylcholine is deficient or is prevented from acting effectively, such as myasthenia gravis (a degenerative disorder that causes muscle weakness).

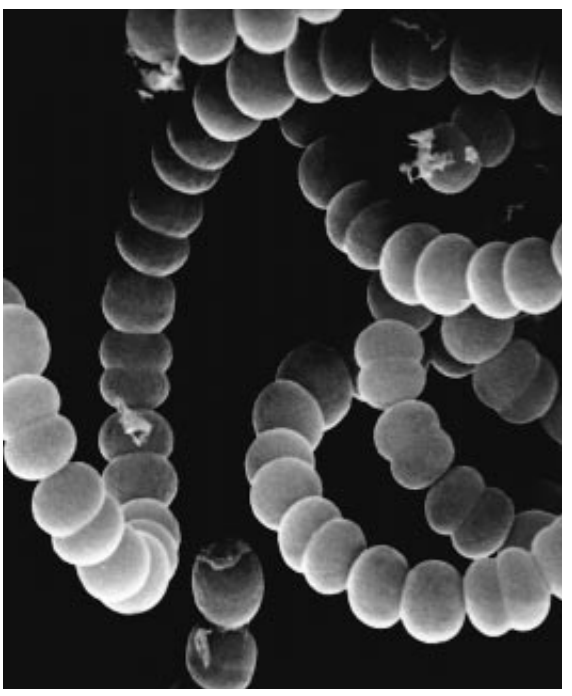
The other neurotoxin unique to cyanobacteria, anatoxin-a(s), is made by strains of *Anabaena*. It produces many of the same symptoms as anatoxin-a—which is how it came to have such a similar name. The letter "s" was appended because anatoxin-a(s) seemed to be a variant of anatoxin-a that caused vertebrates to salivate excessively. Recently, however, my students and I at Wright State University, together with Shigeki Matsunaga and Richard E. Moore of the University of Hawaii, have shown that anatoxin-a(s) differs chemically from anatoxin-a and elicits symptoms by other means.

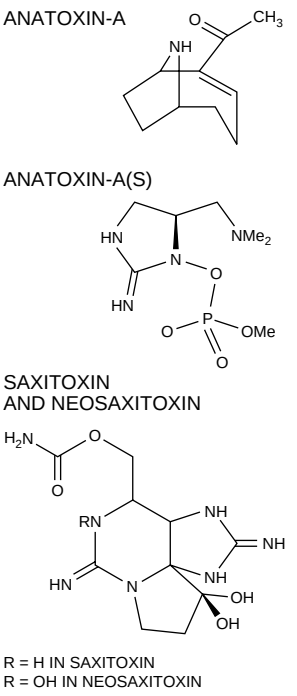
Anatoxin-a(s) is a naturally occurring organic phosphate that functions much like synthetic organophosphate insecticides, such as parathion and malathion. To my knowledge, it is the only natural organophosphate yet discovered. Even though its structure differs from that of the synthetic compounds, its killing power, like theirs, stems from its ability to inhibit acetylcholinesterase. By impeding acetylcholinesterase from degrading acetylcholine, it ensures that acetylcholine remains continuously available to stimulate—and overstimulate—muscle cells.

As a structurally novel organophosphate, anatoxin-a(s) could in theory form the basis for new pesticides. Synthetic organophosphates are widely used because they are more toxic to insects than to humans. They are, however, under some fire. Soluble in lipids (fats), they tend to accumulate in cell membranes and other lipid-rich parts of humans and other vertebrates. Anatoxin-a(s), in contrast, is more soluble in water and, hence, more biodegradable. So it could be safer. On the other hand, it might also be less able to cross the lipid-rich cuticles, or exoskeletons, of insects. By tinkering with the structure of anatoxin-a(s), investigators might be able to design a compound that would minimize accumulation in tissues of vertebrates but continue to kill agricultural pests.

As is true of anatoxin-a and anatoxin-a(s), the neurotoxins saxitoxin and neosaxitoxin disrupt communication between neurons and muscle cells. But they do so by preventing acetylcholine from being released by neurons. In order to secrete acetylcholine or other neurotransmitters, neurons must first generate an electrical impulse. Then the impulse must propagate along the length of a projection called an axon—an activity that depends on the flow of sodium and potassium ions across channels in the axonal membrane. When the impulse reaches an axon terminal, the terminal releases stores of acetylcholine. Saxitoxin and neosaxitoxin block the inward flow of sodium ions across the membrane channels; in so doing, they snuff out any impulses and forestall the secretion of acetylcholine.

Although saxitoxin and neosaxitoxin occur in some strains of the cyanobacterial genera *Anabaena* and *Aphanizomenon,* these poisons are actually better known as products of dinoflagel-

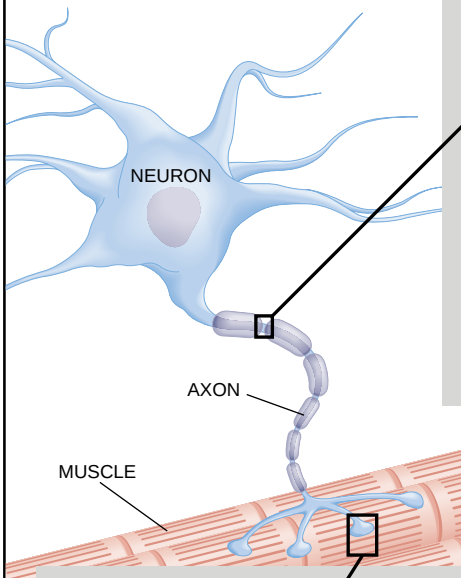


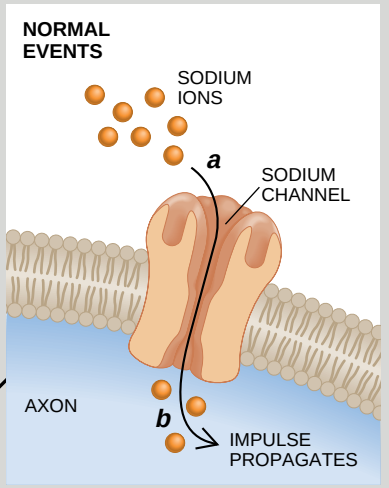


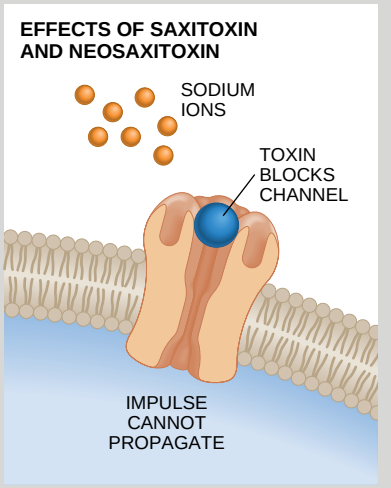
BEADS ON A STRING (*micrograph*) are actually cells of the cyanobacterium *Anabaena flos-aquae,* magnified some 2,500 times. *A. flos-aquae* is a major producer of neurotoxins, poisons that interfere with the functioning of the nervous system. The strain shown here was responsible for the death of hogs in Griggsville, Ill. The chemical structures at the right represent toxins made by strains of *Anabaena;* all except anatoxin-a(s) also occur in other cyanobacteria.

How Neurotoxins Kill

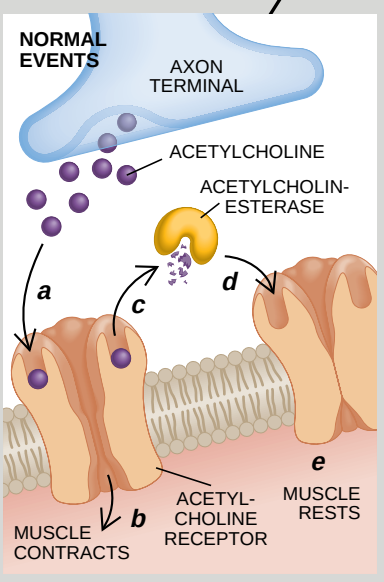
Neurotoxins produced by cyanobacteria can disrupt normal signaling between neurons and muscles in several ways. All of them lead to death by causing paralysis of respiratory muscles, followed by suffocation.

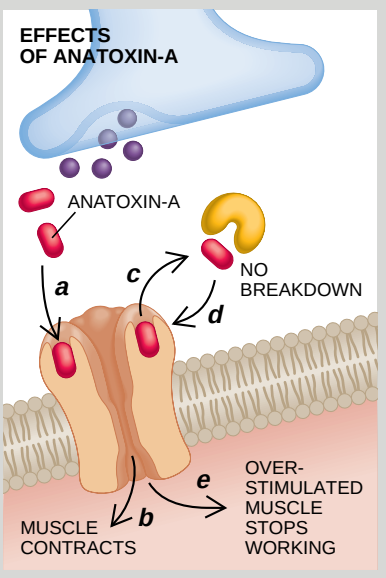


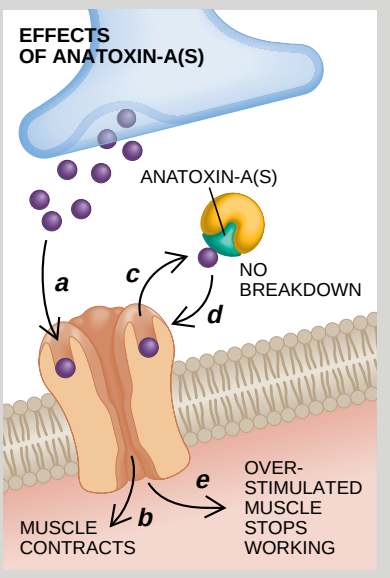




Saxitoxin and neosaxitoxin silence the neurons that act on muscle cells. Sodium ions (*gold*) must flow into neurons (*a at left*) in order for the neurons to relay impulses (*b*) to other cells. Saxitoxin and neosaxitoxin (*blue sphere at right*) halt impulse propagation by preventing the ions from passing into the neurons. When the nerve cells are thus quieted, muscle cells receive no stimulation and become paralyzed.







Anatoxin-a and anatoxin-a(s) (*center and right panels*) overexcite muscle cells by disrupting the functioning of the neurotransmitter acetylcholine. Normally, acetylcholine molecules (*purple*) bind to acetylcholine receptors on muscle cells (*a in left panel*), thereby inducing the cells to contract (*b*). Then the enzyme acetylcholinesterase (*yellow*) degrades acetylcholine (*c*), allowing its receptors and hence the muscle cells to return to their resting state (*d* and *e*). Anatoxin-a (*red in center panel*) is a mimic of acetylcholine. It, too, binds to acetylcholine receptors (*a*), triggering contraction (*b*), but it cannot be degraded by acetylcholinesterase (*c*). Consequently, it continues to act on muscle cells (*d*). The cells then become so exhausted from contracting that they stop operating (*e*). Anatoxin-a(s) (*green in right panel*) acts more indirectly. It allows acetylcholine to bind to its receptors and induce contraction as usual (*a* and *b*), but it blocks acetylcholinesterase from degrading acetylcholine (*c*). As a result, the neurotransmitter persists and overstimulates respiratory muscles (*d*), which once again eventually become too fatigued to operate (*e*).

lates—the marine algae that have caused "red tides" (red waterblooms) in several coastal areas of the world. These red tides have led to repeated outbreaks of paralytic shellfish poisoning and to the closure of shellfish beds in those areas.

The discovery of saxitoxin and neosaxitoxin in cyanobacteria added few new ideas for drugs or insecticides or for ways to solve problems in cell biology, since the chemicals were already known entities. The finding did pose a fascinating riddle, however. What would cause freshwater cyanobacteria to produce the same chemicals made by marine eukaryotes? Did these disparate groups evolve the same pathways of synthesis independently, or did they perhaps share a common ancestor?

That particular puzzle remains unsolved, but the realization that cyanobacteria produce saxitoxin and neosaxitoxin has made it possible to unravel another scientific knot. For years, the biosynthetic pathway for production of the toxins was unknown because dinoflagellates were difficult to cultivate in the laboratory. Studies of more readily grown species of *Aphanizomenon* allowed Yuzuru Shimizu and his students at the University of Rhode Island to work out the pathway in 1984.

Cyanobacterial neurotoxins, then, are both deadly and potentially valuable, but they are not as ubiquitous as the other major class of cyanobacterial poisons: the hepatotoxins. Whereas neurotoxins have been blamed for kills mainly in North America (with some in Great Britain, Australia and Scandinavia), hepatotoxins have been implicated in incidents occurring in virtually every corner of the earth. For this reason, great excitement ensued in the early 1980s, when a group headed by Dawie P. Botes, then at the Council for Scientific and Industrial Research in Pretoria, determined the chemical structure of a liver toxin. Such toxins were long known to be peptides (small chains of amino acids), but the technological advances needed for determining the precise structures of the toxins did not occur until the 1970s.

Soon after Botes established the chemical identity of the first few hepatotoxins, my laboratory and others confirmed his results and began identifying the chemical makeup of other hepatotoxins. Extensive structural analyses, mainly in the laboratory of Kenneth L. Rinehart of the University of Illinois, have now established that the liver toxins form a family of at least 53 related cyclic, or ringed, peptides. Those consisting of seven amino acids are called microcystins; those consisting of five amino acids are called nodularins. The names reflect the fact that the toxins were originally isolated from members of the genera *Microcystis* and *Nodularia*.

Research into the hepatotoxins—much of which is carried out at other laboratories with toxins supplied by my group—is directed primarily at understanding how the compounds affect the body. Investigators know that the peptides cause hepatocytes, the functional cells of the liver, to shrink. In consequence, the cells, which are normally packed tightly together, separate. When the cells separate, other cells forming the so-called sinusoidal capillaries of the liver also separate [*see illustration on page 86*]. Then the blood carried by the vessels seeps into liver tissue and accumulates there, leading to local tissue damage and, often, to shock.

Other details of the poisoning process are only now becoming clear. For instance, scientists have wondered why the toxins act most powerfully on the liver. The answer probably is that they are moved into hepatocytes by the transport system, found only in hepatocytes, that carries bile salts into the cells.

Maria T. C. Runnegar of the University of Southern California and Ian R. Falconer of the University of Adelaide in Australia have taken the lead in addressing the related problem of how the toxins deform hepatocytes. They, and more recently Val R. Beasley of the University of Illinois and John E. Eriksson of the Finland-Swedish University of Åbo, have found that the poisons distort liver cells by acting on the cytoskeleton: the gridwork of protein strands that, among other functions, gives shape to cells.

The cytoskeletal components most affected by the toxins are the protein polymers known as intermediate filaments and microfilaments. Subunits are continually added to and lost from the intermediate filaments, and the protein strands forming the microfilaments continually associate and dissociate. The net sizes of the intermediate filaments and of the microfilaments change little over time, however. Microcystins and nodularins seem to tilt the balance toward subunit loss and dissociation. The intermediate filaments apparently undergo change first, followed by the microfilaments. As the cytoskeleton shrinks, the fingerlike projections through which hepatocytes interact with neighboring cells withdraw, breaking the cell's contact with other hepatocytes and with sinusoidal capillaries.

Still more recent work in many laboratories offers some insight into how the toxins manage to disrupt cytoskeletal components. In studies of microcystins and nodularins, researchers have found that the toxins are potent inhibitors of enzymes known as protein phosphatases. These enzymes work in concert with other enzymes—protein kinases—to regulate the number of phosphate groups on proteins. The kinases add phosphate groups, and the phosphatases remove them.

Such phosphorylation and dephosphorylation reactions have long been known to influence the structure and function of intermediate filaments and microfilaments. It seems, therefore, that the toxins disrupt the fibers by upsetting the normal regulatory balance between phosphorylation and dephosphorylation. More specifically, it is thought that the unchecked activity of the kinases and the resulting excessive phosphorylation of the intermediate filaments and the microfilaments (or of proteins that act on them) increase the rate of subunit loss and dissociation.

The revelation that cyanobacterial hepatotoxins can inhibit protein phosphatases has raised the disturbing possibility that human exposure to nonlethal doses might contribute to the development of cancer. Beyond influencing the structure and function of cytoskeletal fibers, protein kinases and protein phosphatases play a major part in regulating cell division. Protein kinases, which themselves are regulated by various proteins, promote movement of cells through the cell division cycle. Protein phosphatases help to check cell division by quieting the activity of the regulators. The toxins, by inhibiting the phosphatases, probably give the upper hand to the proteins that activate kinases; they may thus help release the normal brakes on cell proliferation.

Studies by Hirota Fujiki and his colleagues at the Saitama Cancer Center in Japan have now shown in cultured cells and in whole animals that microcystins and nodularins can indeed hasten tumor development. These toxins do not seem to initiate a cell's progression toward becoming cancerous; however, once something else has triggered early changes, the hepatotoxins promote development of further carcinogenic alterations. My group in Ohio and our colleagues at the Academy of Sciences in Wuhan, China, and at Shanghai Medical University are attempting to find out whether such activity might contribute to malignancy in humans. To do so, we are carrying out a long-term study of people in China who are exposed repeatedly to microcystins in

their drinking water. We suspect that the extraordinarily high rates of liver cancer in parts of China may be tied to the cyanobacterial toxins in such water.

Some evidence also suggests that exposure to sublethal levels of cyanobacterial hepatotoxins have caused temporary stomach, intestinal and liver dysfunction in human populations. In several instances in which many people were afflicted simultaneously, circumstantial evidence implicated toxic cyanobacteria in drinking water as the cause. It seems reasonable to guess that repeated low-level exposure to the toxins could favor the development of chronic disorders of the gastrointestinal tract and liver. If cancer and other chronic illnesses are indeed a danger, then drinking-water supplies may need closer monitoring in many places.

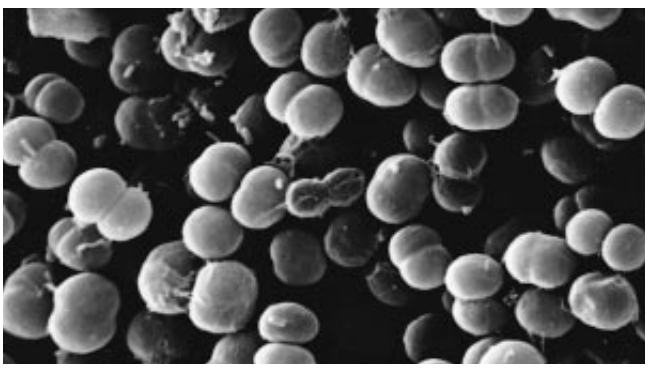
As is true of the neurotoxins, the hepatotoxins are not all bad. Because they affect the cytoskeleton, they are now being used as tools to probe the workings of this cellular scaffolding. Certain mushroom poisons are also applied in this way. And because microcystins impede protein phosphatases, they are aiding investigators in the effort to understand how those enzymes work. For example, because certain microcystins bind strongly to protein phosphatases 1 and 2A, they are being used as probes to isolate the enzymes from tissue preparations. By then determining the amino acid sequences of the enzymes, workers should be able to deduce the nucleotide sequences of the corresponding genes. They should also be able to isolate the genes to explore their regulation.

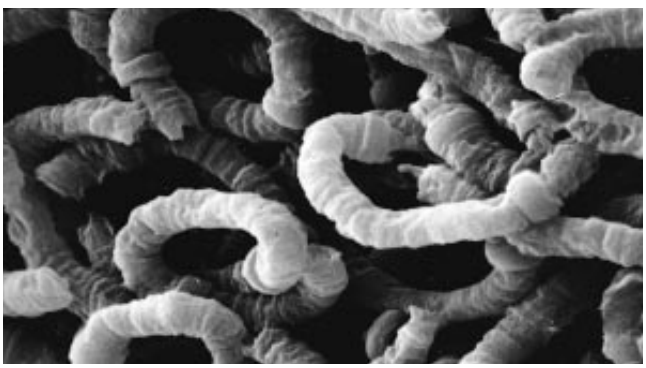
The neurotoxins and hepatotoxins are certainly the most dangerous cyanobacterial compounds, but they are by no means the only bioactive chemicals made by these bacteria. For example, the microbes produce an array of cytotoxins: substances that can harm cells but do not kill multicellular organisms. Studies carried out mainly by Moore and Gregory M. L. Patterson, also at the University of Hawaii, indicate that several cytotoxins show promise as killers of algae and bacteria. Some may even serve as agents for attacking tumor cells and the human immunodeficiency virus, the cause of AIDS.

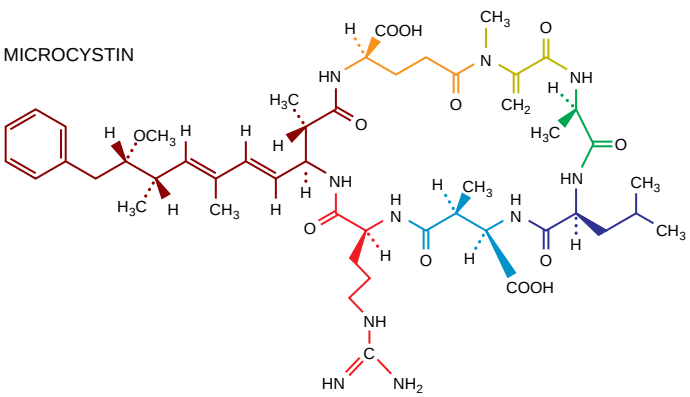
To what purpose are all these chemicals made? They may enhance cyanobacterial defense against attack by other organisms in the everyday environment. But why would cyanobacteria produce substances capable of killing livestock and other large animals? After all, livestock have never been the primary predators of those microbes.

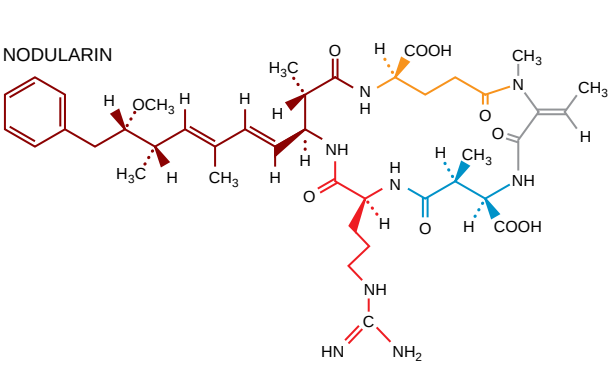
Some recent work in my laboratory, done in collaboration with William R. DeMott of Indiana University–Purdue University at Fort Wayne, is providing clues. It turns out that cyanobacterial neurotoxins and hepatotoxins can be extremely harmful not only to birds, cows, horses and the like but also to the minute animals (zooplankton) living in lakes and ponds. The toxins may be directly lethal (especially the neurotoxins), or they may reduce the number and size of offspring produced by the creatures that feed on cyanobacteria. In other words, just as vascular plants make tannins, phenols, sterols and alkaloids to defend against predation, it is likely that cyanobacteria synthesize poisons to ward off attack by fellow planktonic species.

In support of this suggestion, we have found that zooplankton species generally do not eat cyanobacteria capable of producing toxins unless there is no other food around; then, they often attempt to modulate the amount they take in to ensure that they avoid a lethal dose. Those who walk this aquatic tightrope successfully pay a price, of course, in fewer offspring, but at least they survive to reproduce.

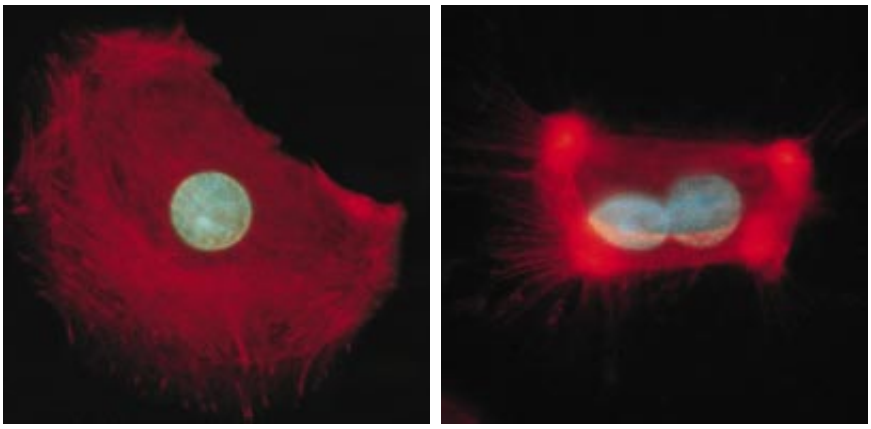




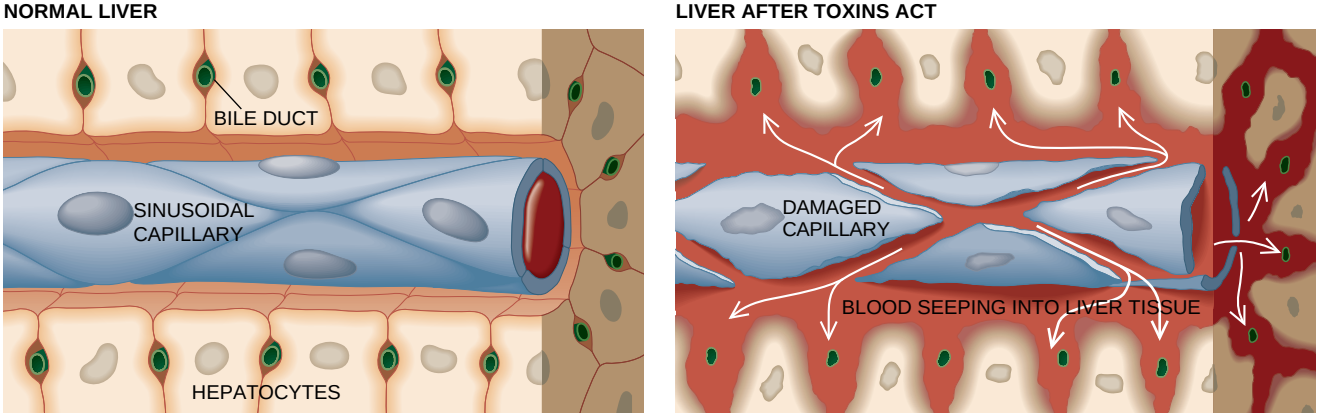




CYANOBACTERIAL SPECIES *Microcystis aeruginosa* (*left micrograph*) and *Nodularia spumigena* (*right micrograph*), shown enlarged some 2,500 and 1,250 times, respectively, are among the many forms that synthesize toxins destructive to liver cells known as hepatocytes. The poisons, including the two varieties for which chemical structures are shown at the bottom, are peptides. Those consisting of seven amino acids (distinguished by color) are called microcystins (because they were first discovered in a strain of *Microcystis*); those consisting of five amino acids are called nodularins.



MICROFILAMENTS (*red threads in micrographs*), structural components of cells, are usually quite long, as in the rat hepatocyte at the left. But after exposure to microcystins (*right*), microfilaments collapse toward the nucleus (*blue*). (This cell, like many healthy hepatocytes, happens to have two nuclei.) Such collapse helps to shrink hepatocytes—which normally touch one another and touch sinusoidal capillaries (*left drawing*). Then the shrunken cells separate from one another and from the sinusoids (*right drawing*). The cells of the sinusoids separate as well, causing blood to spill into liver tissue. This bleeding can lead swiftly to death.



It is possible, though, that the protective effect is incidental. The toxins may once have had some critical function that they have since lost. This likelihood is suggested by the fact that microcystins and nodularins act on the protein phosphatases that regulate the proliferation of eukaryotic cells. The hepatotoxins do not now seem to participate in cell function and cell division in cyanobacteria, but they may have played such a role early in the evolution of these organisms (and of other microbes as well).

Regardless of their intended purpose, the toxicity of many chemicals produced by cyanobacteria is undeniable. For this reason, I am becoming increasingly worried by a modern fad: the eating of cyanobacteria from the genus *Spirulina* as a health food. Certain tribes in Chad and many peoples in Mexico have consumed two closely related species of *Spirulina* for hundreds of years. When world health officials and scientists began looking for new high-protein food sources in the mid-1960s, many of them turned to *Spirulina* because of its high protein content. Beginning in the late 1970s certain producers and distributors of *Spirulina* began promoting it throughout large parts of the U.S., Canada and Europe as a nutritious food for humans. It has also been marketed as a diet pill, because anecdotal reports, as yet unconfirmed, indicated that a few grams taken before meals dulled the appetite.

Spirulina itself is not harmful. The danger arises because there are no guidelines requiring those marketing *Spirulina* to monitor their products for contamination by potentially toxic cyanobacteria or by cyanobacterial toxins. Moreover, the general public is ill equipped to distinguish *Spirulina* and other benign cyanobacterial products from poisonous forms of cyanobacteria.

My worry has recently intensified because the popularity of *Spirulina* has led to the production and marketing of such cyanobacteria as *Anabaena* and *Aphanizomenon*—genera that contain highly toxic strains. Some promotional material for cyanobacteria-containing products even claims that the items being sold can moderate some disease symptoms, including those of debilitating neuromuscular disorders. Yet this literature does not provide a listing of all microbial species in the marketed products, nor does it indicate that anyone is monitoring the products to ensure they are pure and nontoxic. Because cyanobacteria are often collected simply from the surface of an open body of water and because neither sellers nor buyers can distinguish toxic from nontoxic strains without applying sophisticated biochemical tests, the safety of these items is questionable.

All told, the cyanobacteria constitute a small taxonomic group, containing perhaps 500 to 1,500 species. But their power to harm and to help animals and humankind is great. Investigated and exploited responsibly, they can provide valuable tools for basic research in the life sciences and may one day participate in the treatment of disease.

FURTHER READING

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