Secrets of the K

iller Snails

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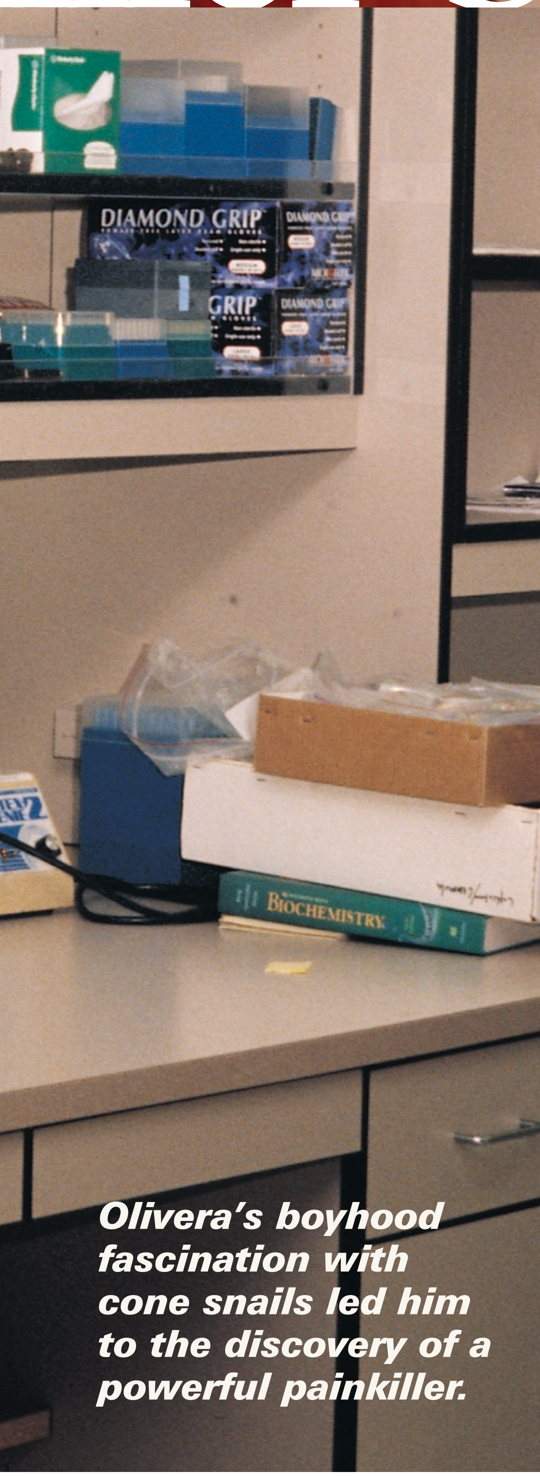
By Alisa Zapp Machalek



Kerry Matz

Olivera's boyhood fascination with cone snails led him to the discovery of a powerful painkiller.

Baldomero ("Toto") Olivera (left) is a biochemist at the University of Utah in Salt Lake City.

Olivera studies the venom of marine cone snails, which may be used to treat pain.

It started out as a short-term project—a hobby, almost—to fulfill childhood curiosity about a beautiful, but deadly, sea snail. Now, the research may lead to relief for thousands who suffer from intractable pain, epilepsy, or neurodegenerative disorders.

The source of this medicinal boon, amazingly, is a poisonous venom. Found in marine cone snails, this venom contains a cocktail of nerve toxins unmatched in nature—or, as scientists learned, in chemistry labs. But that's getting ahead of the story.

Back in the late 1960s, Baldomero Olivera needed a research project that was cheap and didn't require fancy machines. Known as "Toto" by all his friends, Olivera had left his native Philippines to pursue scientific training. (The nickname was coined decades ago by one of his young cousins who couldn't pronounce the "y" in "totoy," the Filipino nickname for "little boy.")

After earning a Ph.D. in biochemistry at the California Institute of Technology and working as a postdoctoral researcher at Stanford University, Olivera returned home flush with knowledge of the latest techniques in molecular biology. But his lab in Manila lacked the requisite funds and equipment to carry out these studies.

"I really wanted to continue my postdoc work," Olivera confesses. "But it just wasn't possible." He describes this research as "working with" DNA ligase, an enzyme that seals together two pieces of DNA. Actually, he *discovered* this important enzyme, which now plays a key role in recombinant DNA technology.

In his fledgling lab, he chose instead a project inspired by his boyhood days of collecting sea shells. He remembered one shell in particular, that of the cone snail species *Conus geographus (C. geographus)*, an animal so lethal that one sting kills an adult within hours. *C. geographus* also used to be called the "cigarette snail," because when stung, its victims were said to have enough time to smoke a cigarette, but little else, before they died.

"My initial goal was to purify whatever caused human fatalities from *C. geographus*," Olivera says. "It had local advantages and required little equipment."

Olivera's boyhood fascination with cone snails led him to discover that the snail venoms contain exquisitely specific and powerful nerve toxins. Today, research on the venoms spills out from dozens of laboratories worldwide and is the centerpiece of three pharmaceutical companies.

Shake, Scratch, and Roll

Olivera began his experiments years ago by injecting *C. geographus* venom into the abdomens of mice. Immediately, the mice were paralyzed—just as are the fish that swim too close to a hungry snail. To find the secret paralyzing ingredient in snail venom, Olivera and his research team chemically divided the venom into a series of different fractions and injected them one by one into mice.

The researchers discovered, to their surprise, that the venom contains not one, but many different nerve toxins. And the toxins turned out to be peptides—small, protein-like molecules. Olivera set out to uncover how the toxins do their deadly work. He and his coworkers soon learned that a sting from *C. geographus* is equivalent to eating a lethal dose of badly prepared Japanese puffer fish while a cobra is biting you.

Kerry Matz

Conus geographus ("geography cone") gets its name from map-like markings on its shell. It is one of the few snails that can kill a human.

That's interesting, Olivera reasoned, but if the peptides merely mimicked the actions of other known toxins, they weren't unique enough to hold his interest. Yearning for the thrill of discovery, Olivera returned to doing molecular biology and nearly abandoned the cone snail research.

In 1972, he moved back to the United States and ended up at the University of Utah. Two or three years later, a 19-year-old undergraduate student named Craig Clark injected life into the nearly forgotten project, forever changing the course of Olivera's research.

"Craig got the brilliant idea of injecting components of the venom directly into the central nervous systems [instead of into the abdomens] of mice," explains Olivera. The results stunned them all.

Depending on which peptide the researchers injected, the mice would shake, sleep, scratch, convulse, or become sluggish. One of the peptides even caused different reactions depending on the age of the mouse—it put newborn mice to sleep, but whipped adult mice into a hyperactive frenzy. To a neuroscientist like Olivera, this was like discovering a sunken treasure ship. What were the peptides doing? How did they work? Could he find which ingredient caused the weird behaviors? Could it be harnessed as a medicine? What was once dismissed as blasé became a bounty ripe for discovery.

Now, 30 years later, the project has taken over Olivera's lab ("I gave up doing molecular biology about 10 years ago," he says). He even co-founded a company called Cognetix, Inc. in Salt Lake City, Utah, to tap the pharmaceutical potential of cone snail toxins, which he calls "conotoxins."

"It Gets Pretty Interesting in There"

Olivera is not only interested in how snail toxins work, he is also intrigued with snail behavior. "Knowing how the snails actually use their venom is really useful," he explains. "It helps us make sense of what different toxins are doing."

Olivera keeps cone snails and fish in eight large aquarium tanks in his laboratory. "It gets pretty interesting in there," he chuckles. Once, he saw a *C. geographus* levitating in the middle of a tank and lowering itself like a spider down a thread. On another occasion, he saw a group of small, aggressive snails abandon their meal upon the arrival of a larger species of snail, leading Olivera to believe that the snails may use their venom to deter competition, as well as to kill prey.

And then there was the time that surface-swimming fish were mysteriously disappearing from the tank at night. Eventually, he witnessed a snail "extending its mouth like the barrel of a gun, clearly aiming at a fish." Then the fish started acting strangely. It was moving its fins, but not getting anywhere—as if it was swimming in place. The snail must have released some chemical that made the fish stay in place, Olivera concluded.

"This was an extraordinary insight, because it shows us there's a substance [in the venom] that doesn't have to be injected—it must be absorbed by the gills," he says.

Now, in addition to injecting the toxins, Olivera will also squirt them into the aquarium and watch for unusual fish behaviors. This experiment never would have occurred to him if he hadn't been observing live snails in action, he says.

Pinpoint Accuracy

To really understand how the venoms work, Olivera's group isolates and characterizes individual toxins in the deadly potions. The scientists discovered that each toxin hones in on just one type of molecule. In many cases, these molecules are "channel" proteins that control the flow of electrically charged particles such as calcium, sodium, and potassium into and out of cells. By blocking these channels, the toxins shut down messages between the brain and muscles, causing paralysis or electrical shock in a snail's prey.

Kerry Matz

Cone snails have strikingly beautiful shells.

Olivera discovered that the peptide that puts newborn mice to sleep locks onto a corner of one type of brain protein. That's about as specific as you can get. In fact, these peptides are so accurate in pinpointing their targets that they are now used by neuroscientists to identify and study specific brain proteins.

It's like identifying one child from a crowd of kids who all have the same color hair, eyes, and skin, says Olivera. "If you were a parent of one of those kids, you'd have no trouble in picking your child out from the group," he says. "In a sense, that's what the toxins do."

Such specificity is irresistible to designers of new medicines. It holds the tantalizing promise of leading to a highly effective medication with very few side effects. For example, most "calcium channel blockers," which are medications used to treat high blood pressure, plug up calcium channels throughout the body, not just in the heart, where the drugs are needed. Conotoxins, on the other hand, seem to block only the calcium channels found in nerves, and not those in heart or other tissues. In this way, conotoxins could act as "smart" drugs that exert their effects only where they're needed, without spilling over to other bodily systems and potentially causing unwanted side effects.

Already, pharmaceutical companies are tapping the potential of dozens of cone snail peptides to treat disorders including pain, epilepsy, cardiovascular disease, and various neurological disorders. In addition to Cognetix, two other companies focus their business around the toxins—Elan Corporation, plc, in Dublin, Ireland, and Xenome Ltd. in Brisbane, Australia.

The clinical applications of *Conus* toxins are inspired by the snail's own biology. Paralyzing peptides might be used as anesthetics. "Sleepy" or "sluggish" peptides could be used as anti-epilepsy medications to tame nerve cells that fire out of control during seizures.

Olivera's long-term goal is to use the peptides to treat even more elusive conditions such as Alzheimer's, Parkinson's, schizophrenia, and depression. "I'd like to make a contribution [to the treatment of] mental illness and neurodegenerative diseases," he says.

A Poison for Pain

One *Conus* peptide is already well on its journey to becoming a useful drug. Olivera originally called it omega-conotoxin MVIIA. Elan, which hopes to market the molecule, calls it by the generic name ziconotide. The peptide blocks calcium channels in one area of the spinal cord, preventing certain pain signals from reaching the brain. By testing the molecule in animals, scientists discovered that it is 1,000 times more powerful than morphine in treating certain types of pain. Even more exciting, it alleviates one type of pain, called neuropathic pain, for which morphine is inadequate.

Finally, it appears that ziconotide is free of morphine's fatal flaw—the development of tolerance. When people are given morphine for long periods of time, their bodies grow to "tolerate" the drug, requiring them to take more and more of it to provide pain relief. Ziconotide causes no such trouble. It appears to retain its potency without causing tolerance, even after prolonged use.

The peptide was tested initially on people with terminal cancer or AIDS. These trials were so successful that they were expanded to include other patients with severe, untreatable pain. Now the molecule is in phase III clinical trials—the last set required before requesting approval by the Food and Drug Administration (FDA).

Ziconotide is a rare—and possibly unique—example of a molecule used unaltered from a creature's chemistry. Usually, pharmaceutical chemists try to improve upon natural compounds by designing molecules with the same action, but with better pharmaceutical qualities, such as how well the molecules are absorbed by the body. In the case of ziconotide, after hundreds of attempts to design a "better" drug, the scientists returned to the original conotoxin.

Olivera has a large collection of ancient pottery found in the Philippines.

Perhaps the most endearing part of the story is that the molecule was discovered not by a professional scientist, but by a young student, Michael McIntosh, just a few days after he graduated from high school. McIntosh went on to earn an M.D. and now, more than 20 years later, still works with cone snails and collaborates with Olivera. He is a research psychiatrist in the Department of Psychiatry and Biology at the University of Utah and uses *Conus* peptides to uncover the biochemical basis of mental illness. He also oversees the research of undergraduates, many of whom purify new peptides from cone snail venoms.

Olivera won't profit from ziconotide's success, because he never patented the discovery. But for this one fish that got away, there remains a school left to catch.

One pharmaceutical researcher calls the field an "ocean of opportunity." There are 500 different cone snails, and each produces on average 100 different toxins. That means 50,000 possible nerve toxins—and 50,000 starting points for new medicines.

Compared to this, current knowledge is just a ripple on the surface. "We probably know the [peptide] sequence of more than 1,000 toxins," Olivera says. He estimates that his research group has chemically manufactured about 100 toxins, but clearly understands the biochemical workings of only 10 to 20.

Another Type of Treasure

In addition to his quest for future medicines from the cone snail venoms, Olivera is on a more literal treasure hunt. He collects ancient pottery. These pieces, originally from China and the Far East, were used as ritual burial vessels in the Philippines from the 10th to the 16th centuries.

Years ago, the pottery was so plentiful that children would play with pieces that washed up in the floods. "You could choose what you wanted—it was a little bit like cone snails," Olivera says.

He has accumulated an impressive collection of plates, bowls, and vessels, usually paying under $20 for each. Some years ago, the pottery piqued the interest of wealthy collectors and prices skyrocketed.

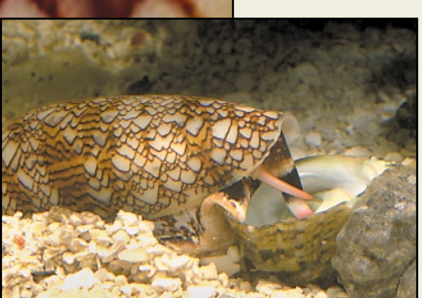
"We don't have any big, famous objects—just a lot of small, pretty ones," Olivera says. For him, the fun part is buying pieces he likes, then learning more about them.

Olivera's world seems filled with researching things new and collecting things old. In either pursuit, he has a remarkable knack for discovering hidden treasure. In a way, isn't that what science is all about? ■

Kerry Matz

Deadly Treasures

What are the most poisonous creatures you can think of? Cobras? Scorpions? Japanese puffer fish? Now mix all these together and add 100 or so other nerve toxins. It sounds like a black magic witch's brew straight out of a fairy tale. Shockingly, it's a potion actually found in nature—in the venom of marine cone snails.



These snails live in the coral reefs surrounding Australia, Indonesia, and the Philippines. They use their venoms to hunt worms, other snails, or fish—some larger than themselves.

Each species of cone snail concocts its own unique venom containing dozens of nerve toxins. Some of these toxins instantly shock the prey, as does the sting of an electric eel or the poisons of scorpions and sea anemones. Others cause paralysis, like the venoms of cobras and puffer fish.

Cone snails use a variety of different hunting strategies. Some snails bury themselves in the sand and, when they smell a meal nearby, they extend a long, fleshy lure that attracts fish. Hidden in this wriggling, worm-like appendage is a sharp, barbed dart the snail uses to harpoon the prey and inject its venom. The snail then reels in the paralyzed fish and extends its mouth to engulf its catch.

Other snails open their mouths wide to capture entire schools of small fish. Then, at their leisure, the snails stab each of the unlucky swimmers with venom-filled darts. An hour or two later, the snails spit out all that remains of their meals—bones, scales, and the used harpoons.

In addition to their vast promise as a source of new drugs (see main story), cone snails are valued by collectors for their beautiful, intricately patterned shells. Some cone snail shells sell for thousands of dollars. According to one story, in 1796, a 2-inch-long shell fetched more at an auction than a painting by the famous Dutch artist Vermeer.—*A.Z.M.*