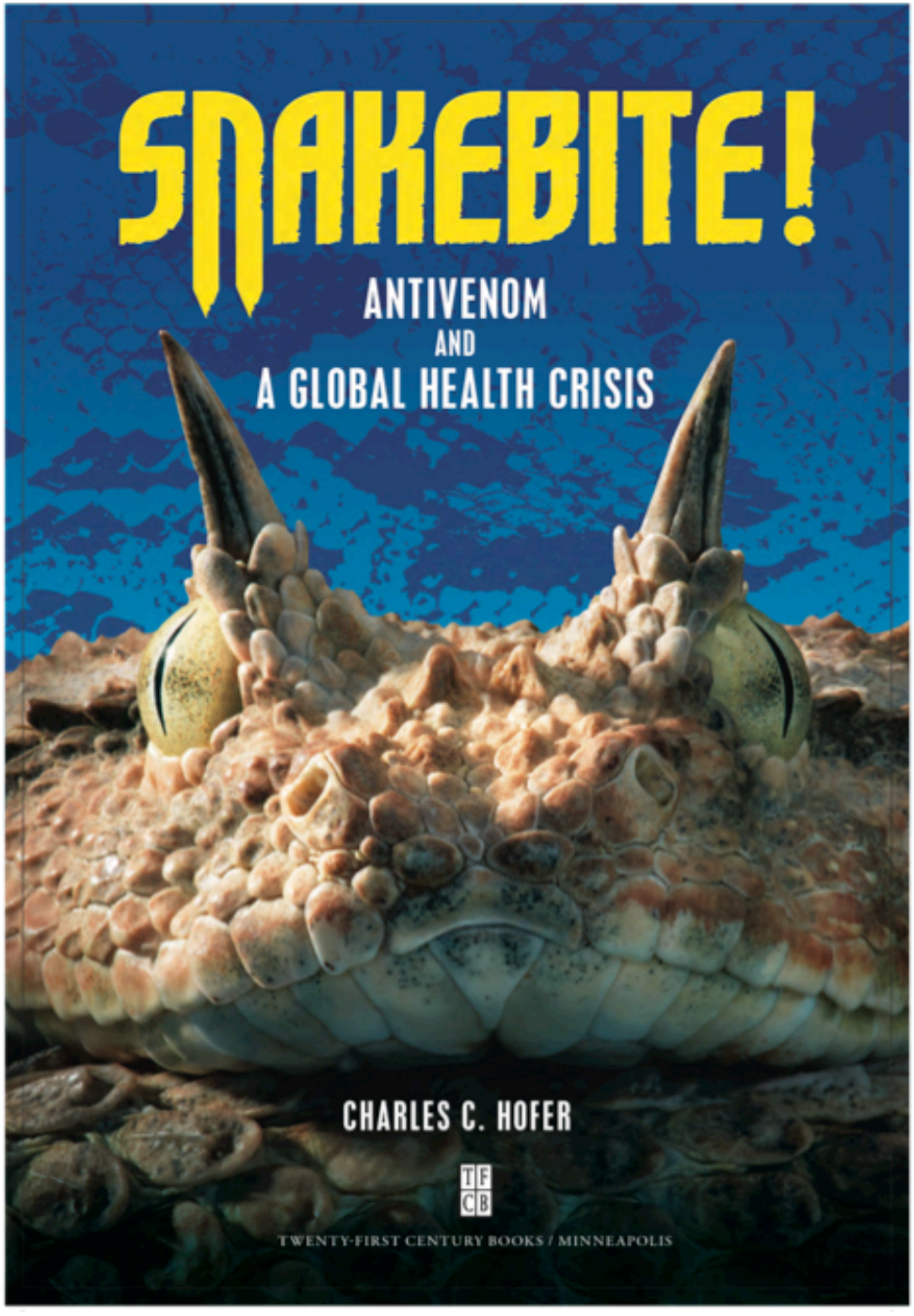


CHAPTER 4

THE LIFESAVER

Cuernavaca, Mexico, is a bustling city about two hours south of the nation's capital of Mexico City. In the center of Cuernavaca sits Barranca de Chapultepec, a zoo and park filled with waterfalls and towering Montezuma cypress trees. They display the lush, semitropical environment of the region.

At the zoo's herpetarium—the reptile and amphibian collection—it is venom extraction day. Edgar and Melisa, two students from the Universidad Nacional Autónoma de México (National Autonomous University of Mexico, or UNAM), are setting up equipment. Small glass aquariums, each containing a venomous serpent from a different region of the world, surround them. Among these snakes are a massive horned Gaboon viper from the rain forests of Africa, an 8-foot (2.4 m) spitting cobra from India, and a tropical rattlesnake from Mexico.

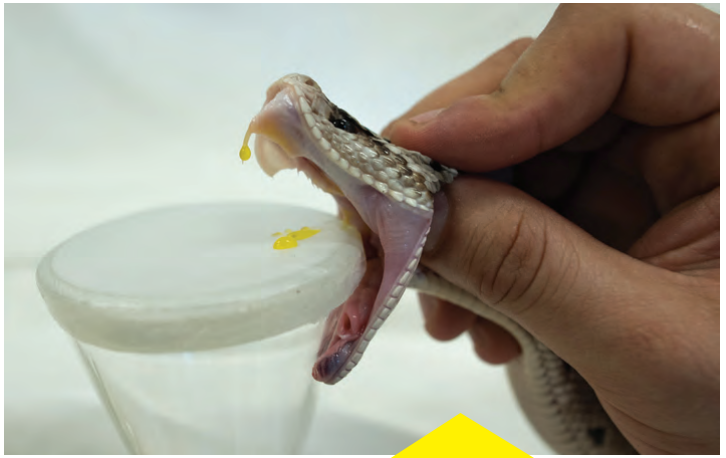
Fernando (*left*), the manager of the herpetarium at Barranca de Chapultepec, works with Edgar and Melisa, two students from the Universidad Nacional Autónoma de México. They carefully handle a spitting cobra as they prepare to collect its venom.

Edgar and Melisa have years of training in handling these snakes. Still, it takes a steady hand and serious concentration. Just one little slip, one small lapse in concentration, can result in a trip to the hospital.

Antivenom production begins at the herpetarium, and the first step is collecting venom from these dangerous snakes. Venomous snakes don't like being disturbed. And they especially don't like giving up their precious potion. But this work is important. Venom from these snakes will be used to produce antivenoms for Africa, Asia, and other parts of the world.

Soon Edgar and Melisa are ready to milk their first snake. Using long snake hooks, Edgar gently wrangles the spitting cobra from its glass cage. After some delicate maneuvering, he coaxes the slithering cobra into a long plastic tube. The tube is narrow enough so the snake cannot move, and only its head is exposed. Now they can handle the deadly cobra safely.

Melisa gently grabs the snake behind its head and places her fingers right over the cobra's venom glands. She gently guides the snake's head toward the collection jar, and the snake's mouth flares open to reveal its fangs. As the snake sinks its teeth into the film covering the mouth of the jar, Melisa gently massages the snake's cheeks, delicately squeezing the venom glands on each side. The flow of venom soon begins. A thick, yellow liquid oozes out of the snake's hollow fangs and drips down the inside of the glass jar, pooling at the bottom. Next, Edgar and Melisa delicately collect the precious venom and transfer it into small vials where it will be placed in cool storage before the venom embarks on the next stage of its epic journey to becoming lifesaving antivenom.



Melisa milks a snake by gently squeezing its venom glands to release the yellow venom that drips from the snake's fangs into the collection jar.

THE JOURNEY OF ANTIVENOM PRODUCTION

Edgar and Melisa are students of Dr. Alejandro Alagón, a professor at UNAM and a world leader in venom research. Students in his laboratory study all kinds of venoms from snakes, spiders, scorpions, and other venomous creatures. They examine the complex structures of venom molecules and their various toxins. By studying venoms at their most basic level, they can understand how to create antivenoms that are effective against a wide range of venoms. "We are generating basic knowledge of venoms," Alagón says. "And that knowledge immediately translates to applied knowledge [practical uses for that knowledge]."

Not all venoms are created equal. Out of the six hundred or so venomous snake species known worldwide, only about two hundred of these species are considered medically important. These highly venomous snake species cause many dangerous bites each year. While the United States hosts just a few medically important species, sub-Saharan Africa is home to dozens of these dangerous snake species. Creating antivenoms from these medically important species is critical to combating the snakebite crisis.

Dr. Alejandro Alagón, a professor at UNAM, works closely with Boyer to develop antivenoms that are used around the world. The collaboration between the two scientists and their teams is creating a wide range of antivenoms that are reliable and cost-effective.

IT'S IN THE BLOOD

The secret ingredient to antivenom is in blood, mostly from animals such as pigs, sheep, and horses. Mammal blood has solid parts and liquid parts. The solid parts are three types of blood cells. These include red blood cells that carry oxygen throughout the body and white blood cells that play a critical role in maintaining the immune system. Platelets, which cause clotting, are a third type of solid blood cell.

The liquid portion of blood is called plasma. It helps protect the delicate solid blood cells and is the fluid in which blood cells move freely throughout the body, getting quickly to the places they need to go. Plasma is mostly composed of water, but it is also made of several types of proteins known as antibodies. Like the protein inhibitors in mongooses and king snakes, antibodies render venom useless.

Lab workers produce antivenom using these powerful antibodies in mammal blood. It starts with hyperimmunization. During hyperimmunization, a worker will inject a series of small, harmless doses of venom into a horse or other large mammal over weeks or months. The animal's body goes to work fighting the venom. Gradually, as more venom is injected, the body increases its defenses against the venom. The animal's immune system will eventually produce enough antibodies to overwhelm the venom and make it useless. Once the animal's blood has produced a significant amount of antibodies, the blood can be collected to make antivenom.

COWBOYS AND ANTIVENOM

Just outside the small village of Agua Fria in the state of Puebla, Mexico, roosters announce that the day is beginning. The sun has yet to rise, and it is still too dark to see, but activity is well under way. A few voices in the distance give instructions, and an old gate creaks open. From somewhere in the darkness comes the gentle sound of hooves meandering into a corral. At first, just a few, then many more. As first light breaks, dozens of horses are wandering into the corral.

VACCINES AND ANTIVENOM

In 1796 an English doctor named Edward Jenner launched a medical revolution. People in many parts of the world were suffering outbreaks of smallpox. This virus was killing thousands of children each year in Europe alone. Meanwhile, in the rural, agricultural areas of England, a similar but far less deadly disease called cowpox was affecting local milkmaids, the young women who worked closely with cows and other livestock on farms. Doctors began to notice that women affected by the nuisance cowpox virus never contracted the deadly smallpox virus.

Jenner decided to perform a risky experiment. The doctor took a small amount of the cowpox virus and introduced it to a small child who was otherwise healthy. After a few days, Jenner exposed the child to the harmful smallpox virus. Jenner found that the boy did not contract the smallpox. The boy's immune system had learned to fight back against the virus. With this groundbreaking discovery, Jenner created the world's first vaccine.

Vaccines work by introducing a dead or otherwise harmless form of a virus or bacterium into the body. The vaccination triggers an immune response that destroys the virus or bacterium before it has a chance to infect cells, replicate, and spread inside the body. Later, if the virus or bacterium attacks the body again, T cells and B cells will remember the specific invader. These cells will produce antibodies that specifically target and destroy the invader.

In the United States, young people are regularly vaccinated against once-common diseases such as mumps, measles, and rubella. These diseases, which can be deadly, have been almost wiped out through widespread public vaccination programs. As for smallpox, the disease has all but vanished, thanks to vaccines.

Edward Jenner (1749–1823) was the first to vaccinate a patient against smallpox. This illustration shows an expreiment in which he infected his infant son wth smallpox.

Horses feed at Ojo de Agua Ranch in Agua Fria, Mexico. These horses are a key part of producing the raw materials for antivenom.

It is breakfast time at the ranch. For some of the horses, it is also blood collection day.

This is the Ojo de Agua Ranch. It has been in Alagón's family for generations. Once it operated as a dairy farm, housing dozens of milk cows. These days, the ranch is home to more than 120 horses that play an important role in the production of antivenom. Each year, Alagón's horses help create more than twenty thousand vials of antivenom. The pharmaceutical company Inosan Biopharma will sell them in Europe and Africa.

The horses at the ranch regularly have their blood drawn. Their antibodies are the raw material for antivenom. The horses are well fed and cared for, spending most days lazily grazing in the ranch's rolling pastures. It's a good life for a horse. "The trick is you need to treat the horses well," Alagón says, giving away the secret behind his successful antivenom production. Managing more than a hundred horses requires

many ranch hands and cowboys who constantly care for the horses—they clean, feed, and move the horses to and from the picturesque pastures in which they spend their days. "We have spoiled horses here at the ranch," Alagón says.

The horses are also here to work. About fifteen ranch hands and managers keep the horses on a strict schedule. Every few weeks, each horse receives a series of injections containing tiny, harmless doses of venom from snakes, scorpions, or another venomous source. During hyperimmunization, each horse's robust immune system goes to work fighting the introduced toxins. Over six months, each horse's immune system will produce a rich supply of antibodies.

Then it is time to collect blood from the horses. Ranch hands lead each horse into a small holding pen. There they insert a large needle into the horse's jugular vein, the large blood vessel that runs along each side of the neck in mammals. The needle is connected to a long tube, and soon blood begins to flow. The tube runs from the pen into a sterile room in a nearby building where the blood flows into 1.3-gallon (5 L) plastic bags. No bacteria or other harmful agents can get inside the room to contaminate the blood.

A worker at Ojo de Agua Ranch injects a horse to collect its blood. Antibodies in this blood will go on to become antivenom.

A technician at Ojo de Agua Ranch checks a bag of horse blood as the plasma and blood cells separate. After processing and purifying the plasma, it is bottled and distributed as antivenom.

After a few minutes, the bag is full. Technicians hang the bag of blood from a pole, and gravity goes to work. After a few hours, the horse blood has naturally separated, with the solid, heavy blood cells sinking to the bottom of the bag. The watery plasma remains on top. Workers pump the solid blood cells back into the horse. They save the plasma and store it in a refrigerator to move to the next step in creating antivenom.

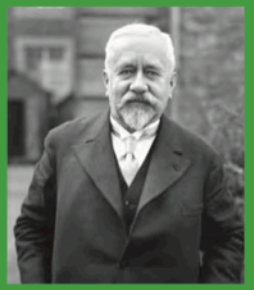
PURIFICATION

The workers send the separated horse plasma to a pharmaceutical lab in Mexico City. Then the plasma goes through a few more steps to ensure the antibodies are refined and safe for use.

The venom antibody molecule is shaped like a Y. The two arms, known as Fab fragments, are the parts of the antibody that bind to the

THE FATHER OF ANTIVENOM

In October 1891, a young French physician named Albert Calmette (*pictured*) arrived in Saigon, the capital city of the French colony Cochinchina (modern-day Vietnam). The twenty-eight-year-old Calmette was there to help open a new branch of the Pasteur Institute, a world-renowned facility dedicated to the study of diseases. The institute was named after its founder, French chemist Louis Pasteur, a pioneer in vaccines.



Calmette's initial mission in Cochinchina was to fight diseases such as rabies and smallpox that were ravaging this part of the world. However, Calmette was also interested in other health issues specific to the region. He was particularly intrigued with the study of harmful toxins such as those in snake venom.

Cochinchina had no shortage of venomous serpents. Aggressive elapid snakes, including cobras and kraits, often terrorized the region. Shortly after Calmette arrived in Cochinchina, monsoon floods caused deadly cobras to flush from their normal habitats and invade a small village. The sudden influx of snakes among humans led to forty snakebites. In just a few days, several people died.

When Calmette arrived in Asia, no cure for venomous snakebites existed. Once envenomated, a victim could turn only to traditional medicines to ease the pain of what could eventually become a slow and painful death. The young Calmette wanted to solve this problem.

By 1894 Calmette developed the first promising snake antivenom. Calmette borrowed methods developed by a former mentor, a physician at Pasteur Institute named Emile Roux. Using hyperimmunization, Roux developed the world's first medicine to fight diphtheria, a terrible disease that affects the nose and throat.

Two years later, Calmette's antivenomous serum was saving lives in Southeast Asia and beyond. Since the days of Calmette's efforts, the field of toxicology—the study of poisonous chemicals and their effects—has come a long way. The work of many toxicologists has resulted in reliable antivenoms that fight the effects of snakes as well as scorpions, spiders, and other toxic animals. Because of his groundbreaking work, Calmette is called the Father of Antivenom.

toxin molecules, neutralizing the venom. The stem of the Y is made of horse proteins. This protein-rich stem can trigger dangerous allergic reactions if the body of a snakebite patient who receives the antivenom rejects the proteins.

For several decades, manufacturers used whole antibody molecules to make antivenoms. Up to 40 percent of antivenom patients experienced allergic reactions to the medicine. But during the 1980s, antivenom production made a giant leap forward. Scientists added an enzyme called pepsin to the antibodies. Pepsin is found in stomach acid and is good at digesting things—especially other protein molecules. Scientists discovered that pepsin could eat away the stem of the antibody protein, leaving behind the Fab fragments, the parts of the antibodies most important in fighting the effects of venom toxins. This discovery revolutionized antivenom production, and the number of patients experiencing allergic reactions plummeted during the 1990s.

After lab workers remove the protein-rich stems with the pepsin treatment, they clean and filter the antibodies several times to remove any foreign substances that may weaken the medicine. From there, they freeze-dry and bottle what is now considered antivenom. Then it is ready to ship to hospitals and medical facilities around the world.

Producing antivenom is one thing. Getting the precious drug to the communities that need it—and at a realistic cost—is another process altogether.

Once the antibodies are filtered and purified, the product is considered antivenom. The antivenom may be freeze-dried or concentrated into a powder or liquid form. It is then bottled and shipped around the world to be injected into snakebite patients.