A Chance Discovery

Hobart Harris

"Doctors are always looking for ways to push back the frontier."

Chris T. Anderson

Hobart Harris (left) is a physician-scientist at the University of California, San Francisco.

Harris grows liver cells (top) in the lab to study the body-wide infection called sepsis.

By Dan Hogan

Growing up, Hobart Harris dreamed that he would one day become a doctor.

Driven by his thirst for knowledge and his determination to become a physician, Harris, now 43, graduated from high school a year early, at age 16.

With little invested in the outcome, he decided to apply to Harvard College. According to Harris, up until that time in the mid-1970s, schools like Harvard seemed alien to many African Americans like himself who had struggled against formidable social and economic odds.

He got in.

"I had no awareness of what an Ivy League college was like," Harris remembers. "What impacted me most was the environment—my fellow students and professors. It was a wonderfully diverse and incredibly challenging environment that opened my eyes to other possibilities."

"I was not born into a family of scientists," says Harris, now a surgeon at the University of California, San Francisco. "My father was born just a few miles outside of Boston, but he might as well have been born on another planet when it came to the idea of attending Harvard."

At Harvard, Harris began to consider the idea of becoming not just a physician, but a physician-scientist. "I was gradually drawn to science," he explains, "because it allows you to ask questions, to be creative, and to contribute to a greater body of knowledge."

During college and medical school (also at Harvard), Harris got involved in many different research projects that piqued his curiosity—everything from how drugs called opiates affect the human brain to how people's diets play a role in heart disease.

After earning his M.D., he did an internship and residency at the University of California, San Francisco Medical Center. There, he began a research project investigating the causes of heart disease. Unexpectedly, the project changed course rather suddenly and led Harris to explore an entirely different area of science, the human immune system.

Germ Wars

Our immune systems are constantly protecting our bodies from invading germs—bacteria, viruses, fungi, and parasites—that can make us sick and sometimes even kill us. Waging this war on our behalf is an army called the immune system. On the

front line are cells called white blood cells, which travel throughout the body fighting infection.

Yet sometimes, our natural defenses are not enough. This is especially true in the case of a potentially fatal body-wide infection called sepsis, one of the leading causes of death in hospital intensive care units today. Sepsis can follow traumatic injury or other serious infections.

In the United States alone, sepsis strikes approximately 750,000 people every year, killing some 200,000. Symptoms can progress from fever and chills to severe inflammation, and ultimately, multiple organ failure and death. The most deadly form of sepsis is caused by "Gram-negative" bacteria, a class of bacteria that get their name from a staining technique that microbiologists use to distinguish bacteria based on the structure of their cell walls. The cell walls of Gram-negative bacteria contain molecules of a toxic substance called endotoxin. When these cell walls break down, the endotoxin molecules are released into the bloodstream, spreading the deadly poison throughout the body.

Aero Photo

Harris attended both college and medical school at Harvard (right), where he became interested in pursuing a research career.

Fat Fights Infection

Researchers have long suspected that fat molecules in our blood play some role in fighting infection. "For many years, scientists thought high levels of fat-containing molecules called lipoproteins were the body's way of mobilizing its stores of fat to provide energy to fight infection," Harris explains, adding that as early as the late 1950s, doctors had noticed that patients suffering from deadly bacterial outbreaks of cholera had unusually large amounts of lipoproteins coursing through their blood.

When you eat fatty foods, the lipid components (fats) in those foods are transported throughout your body via your bloodstream. Just as oil does not mix well with water, lipids do not readily dissolve in blood, which consists mostly of water and blood cells. The body solves this problem by coating small droplets of fat with other molecules that are able to mix with both fat and water. These coated fat droplets are called lipoproteins, and they come in a variety of molecular flavors. The lipid part of lipoproteins can contain different types of fats, such as cholesterol or triglycerides.

So-called high-density and low-density lipoproteins (HDLs and LDLs) are rich in cholesterol. Other types of lipoproteins, such as very-low-density lipoproteins (VLDLs), contain triglycerides instead.

By the 1970s, scientists had figured out that cholesterol-rich lipoproteins could react with endotoxins, shielding the endotoxins and making them less toxic. "But no one had ever examined whether lipoproteins containing triglycerides could interact with endotoxins as well," Harris notes.

"Many discoveries in science are made by serendipity."

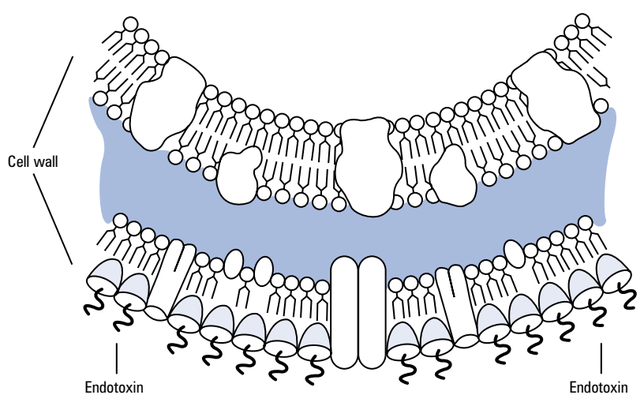
Troubling Mystery

It was 1988, and Harris was in the middle of conducting a set of experiments designed to shed light on why people develop atherosclerosis—a narrowing of the arteries that is caused by the buildup of cholesterol and fat. Atherosclerosis often leads to heart disease.

Harris and his coworkers had been particularly interested in how the human body naturally clears fat from the bloodstream. The researchers designed experiments to track the movement of lipoproteins in the body by first removing the lipoprotein molecules from the blood of healthy volunteers.

The carefully designed experiment involved drawing blood from a group of people who had agreed to participate in Harris' research study, mixing each person's blood

Long, jagged molecules of a poison called endotoxin jut out from the cell walls of Gram-negative bacteria.



A Lucky Horseshoe

George Lower/Marine Biological Laboratory

Strolling along the eastern shores of the United States, you may find a disk-shaped shell with a spiked tail that resembles the remnants of some prehistoric creature. In all probability, the shell came from a horseshoe crab, whose scientific name is *Limulus polyphemus*.

Horseshoe crab shells cover East Coast beaches, but these marine organisms have also proven to be an important medical tool. Researchers use horseshoe crab blood to screen for the presence of dangerous bacterial poisons called endotoxins.

In 1988, when Hobart Harris was studying atherosclerosis at the Department of Veterans Affairs Medical Center in San Francisco, the blood of one of Harris' research participants was tested for the presence of endotoxin using horseshoe crab blood, a method that is now called the "Limulus amoebocyte lysate (LAL) assay." Ironically, the physician-scientist whose discovery originally led to the development of that test, Jack Levin, was at the time working two floors down from Harris, and it was Levin's laboratory that tested Harris' samples.

Just as Harris' finding that triglyceride-rich lipoproteins could mask endotoxins was made by accident, so was Levin's initial discovery that led to the development of the LAL assay. Working with scientist Fred Bang of the Marine Biological Laboratory in Woods Hole, Massachusetts, Levin discovered in the early 1970s that horseshoe crab blood exposed to the bacterium *E. coli* would quickly clot, forming a mushy lump. Further experiments confirmed that endotoxins from the specialized cells called "amoebocytes" in the bacteria are what cause the gel-like clot to form.

The LAL test, a widely used medical tool and a multi-billion dollar enterprise, arose out of those early experiments. The test is routinely used to rapidly and efficiently detect the presence of potentially deadly endotoxins in medicines, blood products, and medical devices such as pacemakers and catheters. —*D.H.*

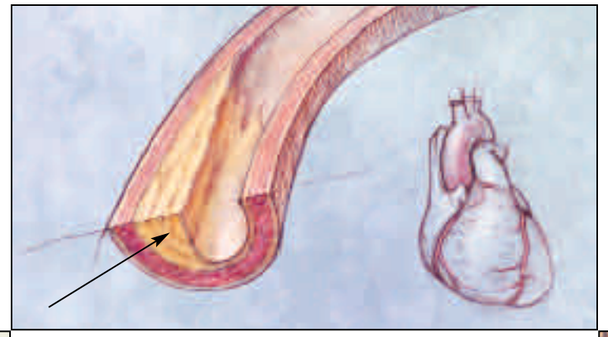
with a natural chemical label, then re-infusing the blood back into that person.

Then, something terrible happened.

Suddenly, one of the study volunteers became sick, as if his blood had been exposed to the endotoxin poison.

Harris rushed to intervene, but fortunately the volunteer's reaction was relatively mild, and he soon recovered.

But for Harris, the reaction remained a troubling mystery. As researchers learned years later, endotoxin is everywhere; small traces can even survive harsh sterilization procedures. Likely, Harris suspected, a small amount of endotoxin got into the study volunteer's blood before it was infused back into him.



Fat-containing molecules called lipoproteins are an important part of the blood vessel blockages (right, arrow) associated with atherosclerosis.

Surprisingly, however, the man's blood had tested negative for bacterial endotoxin in a separate test (see sidebar).

So what had gone wrong? Did something else cause the man to become sick? Or, Harris wondered, perhaps the endotoxin was there but was somehow hidden and undetectable?

"We began to think that maybe lipoproteins could somehow attach themselves to the endotoxin molecules, making the endotoxin undetectable but still active," Harris explains.

The man's reaction, and Harris' possible explanation for why it happened, ended up steering Harris' research career in an entirely new direction—the human immune system.

Cell Cultures

If fatty lipoproteins really were interacting with endotoxin, Harris and his colleagues reasoned at the time, then perhaps they were acting like molecular "sponges." Such sponges would "soak up" the endotoxin molecules and shield the body from the endotoxin's poisonous effects. If that were true, the result would be a lipoprotein-endotoxin duo that has the poisonous part neatly hidden inside the fat droplet, invisible to the immune system.

19th-century chemist Louis Pasteur coined one of Harris' favorite quotes, "Chance favors only the prepared mind."

Harris' thought was just a hunch, and it needed testing.

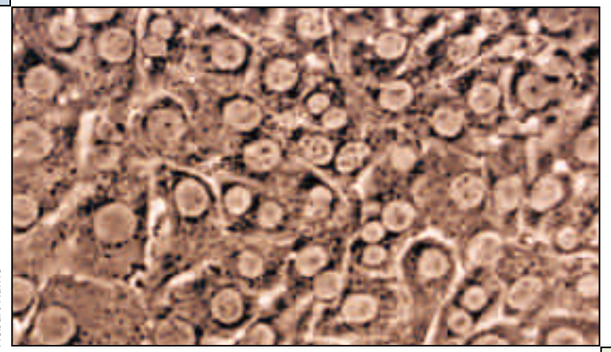
To test his molecular-sponge idea, Harris began a series of experiments using lab mice to model what happens in the human body. His hunch proved correct: The researchers discovered that when endotoxin was tied to a lipoprotein, it was in fact much less toxic to mice than was endotoxin by itself.

In his next set of experiments, Harris wanted to know exactly what happened to endotoxin molecules stuck to lipoproteins. He found that the lipoprotein-endotoxin complexes are processed by specialized liver cells called hepatocytes. Since the liver also contains many other types of cells besides hepatocytes, Harris decided to grow the hepatocytes in plastic lab culture dishes. "We do virtually all our work in cell cultures now," Harris notes, explaining that cell cultures offer a more controlled environment than whole organs, with fewer variables that can interfere with the experiments.

From these cell culture studies, Harris discovered that when endotoxins are bound to triglyceride-rich lipoproteins, the hepatocytes become less responsive to molecules called cytokines. One job of these small hormone-like molecules is to help bring about the process of inflammation, a normal part of the way our bodies deal with infection or injury.

Better Treatments?

Harris is now trying to gain a better understanding of how this process works, but many pieces of the puzzle still need to be found before better sepsis treatments can be developed.



Hobart Harris

Harris performs many of his experiments with liver cells called hepatocytes, which are grown in laboratory culture dishes.

When the body is exposed to dangerous bacteria, Harris explains, a couple of things can happen. The body assembles certain molecules to cause inflammation, and other molecules to shut off inflammation.

"What we think happens in sepsis is an upsetting of this balance. The body overreacts, and the [inflammation-causing] responses overwhelm the [inflammation-stopping] responses." Harris' research might also explain why previous attempts to treat sepsis have been largely unsuccessful. One early approach was to develop anti-endotoxin therapies, using antibodies and chemical absorbents to soak up the rogue endotoxin molecules. These therapies did not work.

"The problem is that by the time you try to treat the patient, the horse is already out of the barn," Harris notes. "The endotoxin that you're trying to counteract has already had its effects." Similar therapies designed to block the cytokine molecules—produced by the body in response to endotoxins—have also failed, Harris adds.

So, instead of trying to block the molecules that stimulate inflammation, Harris suggests, perhaps sepsis could be better treated by encouraging those processes that help stop inflammation.

"Doctors are always looking for ways to push back the frontier, to redefine what is lethal and to find ways for people to survive events or diseases that they would never have been able to survive before," Harris says. "Organ transplantation and open-heart surgery are examples of this. And perhaps we'll be able to conquer sepsis one day as well."

Science and Serendipity

Looking back to that fateful day 12 years ago when the participant in Harris' research study suddenly got sick, Harris is thankful, in a sense, that events turned out the way they did.

"That day was both the worst and the best experience of my scientific career," Harris recalls.

"It was the worst, because we had a complication in a human subject who was also a friend of mine. But at the same time, it was the best—because it opened up a whole new area of research that has become very productive."

That day also reminds Harris of a famous quote, engraved in stone in his dormitory at Harvard: "Chance favors only the prepared mind." The phrase was coined by the great 19th-century French chemist Louis Pasteur, whose germ theory of disease laid the foundation for modern microbiology.

"What Pasteur was referring to was the fact that many discoveries in science are made by serendipity," Harris explains. "And, if you're too focused on finding one thing, you may be unprepared for finding something completely different."

Harris often shares the lesson with the many students he now mentors.

"Young people often seem to be overwhelmed by the prospect of becoming a scientist. They say, 'How could I ever get there from here?' In actuality, it's a day-by-day, step-by-step process. … One of the things that I try to encourage people to do is not to limit themselves, but to be daring and to be willing to experience new environments and new challenges," Harris says. ■