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Cholesterol: From Biochemical Riddle to Blockbuster Drug for Heart Disease

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This article made possible through the generous support of the Endocrine Society.

COVER IMAGE: Decades of fundamental research on the mysterious and ubiquitous molecule cholesterol led scientists to understand its role in causing atherosclerosis and heart disease. This basic understanding allowed development of the statin drugs, which lower cholesterol production by inhibiting the biochemical process that produces it. Statin medications are now taken by millions of patients worldwide, and more recent research shows they may be useful in treatment of inflammatory and other disorders as well. The creation of cholesterol lowering drugs is an excellent example of how biochemists, endocrinologists, and physiologists were able to solve a basic scientific puzzle and bring that knowledge to bedside application. *Cholesterol molecule by Alfred Pasieka, Science Photo Library; heart and artery by John Bavosi / Photo Researchers. Design by Corporate Press.*

Cholesterol: From Biochemical Riddle to Blockbuster Drug for Heart Disease

Margie Patlak

Cholesterol. Probably no other biochemical term has penetrated the American vocabulary as much as this word. Most of us know our cholesterol levels as readily as we know our phone numbers. Even if we aren't keen on keeping track of how much cholesterol is traveling in our blood, our doctors certainly are, and for good reason: as an indicator of heart disease risk, blood cholesterol level has proven invaluable in preventive medicine. Indeed, before scientists discovered the role cholesterol played in heart attacks and chest pain, there was little doctors could do to prevent or forestall their patients from succumbing to the heart disease that killed their parents and grandparents.

But cholesterol hasn't been a household word for long. For much of the last century, cholesterol was merely the esoteric, favorite molecule of chemists, biochemists, and physiologists. Intrigued by its complex structure and the fact that it turned up in so many tissues in the body, these researchers spent most of their careers trying to figure out how the body creates cholesterol and what it does with it. A difficult puzzle to tackle, basic research on cholesterol has led to thousands of experiments, more than a dozen Nobel prizes, and helped uncover the key role this compound plays in the development of heart disease. Fortunately, it also led to the discovery of another group of molecules, known as statins, which greatly lower the amount of cholesterol cruising the blood. Clinical studies reveal that statins lower the risk of heart disease by as much as 40 percent and help explain, along with other new treatment and prevention strategies, why the number of deaths from heart disease has been slashed by close to 60 percent since 1950.

All of these benefits owe their genesis to dozens of researchers—in almost all the major disciplines of modern biology—who were intrigued by a mysteriously complex molecule omnipresent in the body. By pursuing answers to such basic questions as: How does the body make cholesterol?; What stops the body from making too much cholesterol?; and How does cholesterol cause heart disease?, these curious scientists have helped foster the development of powerful drugs that prevent a major killer. Thanks to their efforts, many more people will live long enough to become grandparents and enjoy their retirement, rather than dying in their forties or fifties from heart attacks as their parents did.

The Cholesterol Puzzle

Cholesterol was first isolated from gallstones close to the turn of the 19th century and soon "exerted a hypnotic fascination for scientists from the most diverse domains of science and medicine," noted cholesterol researchers Michael Brown and Joseph Goldstein in their 1985 Nobel prize acceptance speech. Researchers quickly discovered that wherever they looked for it, this complex compound would pop up. They found it not just in human gallstones, but in the blood, brain, and liver, too. Cholesterol was also widely distributed in the organs and tissues of animals. Any compound omnipresent in nature was bound to be an important player in key biological processes. But what cholesterol did was still a mystery.

To get a view of the role this important substance played in the body, chemists worked diligently to understand cholesterol better—to figure out its chemical make-up. By the beginning of the 20th century, they knew it consisted of

27 carbon atoms, 46 hydrogen atoms and a lone oxygen atom. But figuring out how exactly these elements were connected was a complex puzzle made up of atomic pieces. As one chemist noted, "there are a great many possible combinations for these atoms," so figuring out the structure of cholesterol posed "a fascinating game."

What made working on this puzzle even more intriguing was the fact that cholesterol's chemical formula (composition of atoms) didn't resemble that of any of the known common biological compounds, such as sugars, proteins or fats. Pioneering work by chemists Otto Diels, Heinrich Wieland and Adolf Windaus led to the discovery, in 1932, of the unique structure of cholesterol. They showed this molecule comprises 4 rings of carbon and hydrogen attached to an eight-carbon tail on one end and the lone oxygen on the other (Figure 1). All three chemists later won Nobel prizes in Chemistry for their efforts.

But as often is true in science, one problem solved leads to another problem created: how did the body synthesize such a magnificently complex molecule? As Windaus noted in his Nobel prize acceptance speech, many physiologists didn't think the body was capable of making cholesterol. But studies of rats and puppies deprived of cholesterol in their diets revealed they didn't suffer from a lack of the compound. Therefore, their bodies must have been able to create cholesterol.

These studies prompted biochemists to pursue how the body makes cholesterol. This was a formidable question to answer: "At first sight, the molecular architecture of cholesterol seemed enigmatic and devoid of any clues as to how this complex molecule might be constructed from the smaller molecules available in the cell," stated biochemist Konrad Bloch upon accepting his Nobel prize in 1964 (Figure 2). But the importance of figuring out this puzzle was underlined by the mounting evidence that cholesterol might foster heart disease.

As early as the 19th century, the renowned German pathologist Rudolph Virchow had noted that the walls of the arteries of patients who died of heart attacks were often thickened and irregular, and contained plaques of a yellowish fatty substance, later identified by Windhaus as cholesterol. This pathological buildup on the walls of arteries was termed "atheroma," the Greek word for porridge, and the disease causing such buildups was called

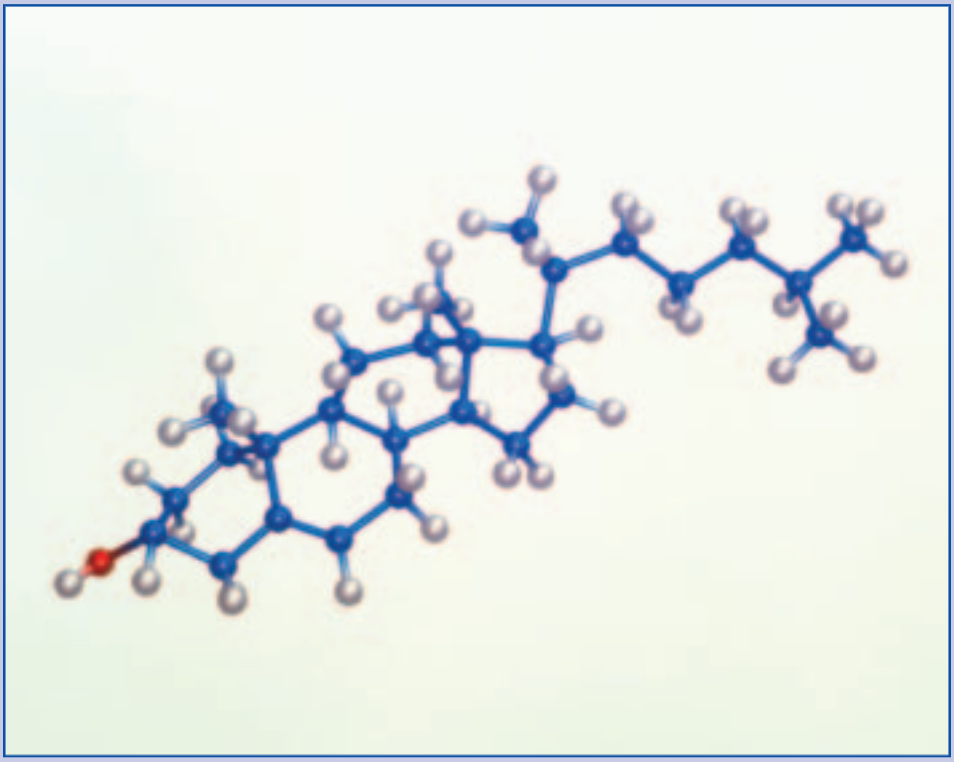


Figure 1: Chemical structure of cholesterol: In this drawing of the chemical structure of cholesterol, its carbon backbone is blue, attached hydrogen atoms are white, and its lone oxygen atom is red. Basic research on the structure and biosynthesis of cholesterol has led to more than a dozen Nobel prizes, and the development of statin drugs to prevent atherosclerosis. *Image by Laguna Design, Science Photo Library.*

Figure 2: Konrad Bloch: Biochemist Konrad Bloch shared the Nobel Prize for Physiology or Medicine in 1964 for his discovery of many of the 36 steps the body takes to make cholesterol. His research led researchers to uncover statins, cholesterol-lowering drugs that inhibit a key enzyme in the cholesterol biosynthesis pathway. *Image from the National Library of Medicine.*

"atherosclerosis." Then, in 1913, a Russian pathologist, Nikolaj Anitschkow, showed that a high-cholesterol diet led to the buildup of cholesterol-containing plaques in the rabbits' arteries, akin to the atherosclerosis seen in humans. And in the 1930s, researchers showed that people who inherited a tendency to suffer heart attacks at an extremely young age—as young as 2 years in some cases—had unusually high levels of cholesterol traveling in their blood.

These studies suggested that understanding how the body makes cholesterol might reward not only stymied chemists, but the millions of people who suffered from heart disease as well. However, finding a cure for heart disease was far from the mind of Bloch when he fled Nazi Germany in 1936 and joined up with Rudolph Schoenheimer, another German Jewish refugee, at Columbia University. These researchers were bent on succeeding where others had failed—solving the riddle of cholesterol biosynthesis.

Typically, biochemists figure out the body's instructions for making a complicated compound by looking at similar, simpler compounds and seeing how they could be changed into the molecule of interest through chemical embellishment (adding more pieces). But Schoenheimer had shown, while in Germany, that rabbits did not make cholesterol from similar plant compounds in their diet. Other efforts by many investigators to find intermediate compounds midway in the multi-step process of cholesterol synthesis had also failed miserably. As Bloch humbly noted later, "often the choices of nature are not those the chemist would predict."

Fortunately, a new research tool came to the rescue of the frustrated biochemists. After the discovery of radioactive radium by Marie and Pierre Curie in 1898, common simple compounds, such as water, were labeled with radioactive isotopes to trace whether they were incorporated into more complex molecules. (Radioactive isotopes are atoms, such as hydrogen, that have extra neutrons in their nuclei. These neutrons give off radiation as they decay.) The radioactive versions of common compounds continued to give off telltale signals that could be picked up by a detector, even after they were combined with other molecules. The strength of the signals given indicated the quantity of the compounds present.

German researchers were among the first groups of scientists to use radioisotopes in their chemical analyses. Robert Sonderhoff, a former student of Wieland, used radioisotopes to solve another chemical riddle, unrelated to cholesterol biosynthesis, in 1937. In the process, he also discovered that yeast fed a radioactive version of the two-carbon molecule acetate (a salt form of acetic acid or vinegar) produced a highly radioactive compound closely related to cholesterol, known as ergosterol. This suggested that acetate was a principle component of ergosterol and perhaps similar compounds, such as cholesterol.

Schoenheimer pioneered the use of radioisotopes in biochemical research in the United States. Before his untimely death in 1941, he put Bloch to work using the tracers to discover the basic building block(s) of cholesterol. Building on Sonderhoff's findings, Bloch and his colleague David Rittenberg fed a radioactive version of acetate to rodents and showed that it was a principle component of the cholesterol the animals generated. But to prove

that each of the 27 carbon atoms that comprised the backbone of cholesterol came from acetate, the researchers needed a simpler model.

A hot lead came in the 1940s from an unusual source—a mutant bread mold. Stanford University microbiologist E.L. Tatum discovered this strain, which was unable to make acetate. Bloch began a collaboration with Tatum that proved to be highly productive. The researchers fed the mutant mold a radioactive version of acetate and discovered that this compound was all it needed to build the complete carbon skeleton of ergosterol.

This suggested that the entire intricate carbon skeleton of cholesterol could be constructed from the simple 2-carbon acetate compound or a similar molecule. Experiments by German chemist Feodor Lynen (Wieland's son-in-law) and others soon confirmed this hypothesis and revealed that acetic acid, the chemical name for purified vinegar, is the basic building block of cholesterol. Bloch and Lynen shared the 1964 Nobel Prize in Physiology or Medicine for this research.

But it took scientists about two decades to discover exactly how the body transformed vinegar into cholesterol. Thanks to federal funding of their research, they were able to uncover all 36 steps in this complex process. These steps were shepherded by 30 different enzymes, many newly discovered in the process of deciphering cholesterol biosynthesis. One of those enzymes, known as HMG-CoA reductase, went on to play a starring role in future efforts to develop drugs that combat heart disease.

The Cholesterol Debate

While Bloch and others were busy trying to decipher how the body concocts cholesterol, a charged debate about the role this compound played in heart disease was taking place in the biomedical community. It was well accepted that cholesterol accumulated in the plaque that narrowed the arteries of people with atherosclerosis. Yet some people thought this cholesterol buildup was not the cause of the disease, but merely an innocent bystander in the disease process. Many people thought the cholesterol-enhanced narrowing of the arteries was an inevitable consequence of aging and not a disease, per se.

These skeptics pointed out that if a high level of blood cholesterol was a cause of heart disease, then why didn't most people who suffered heart attacks have this telltale abnormality? Studies at the time showed that most people with heart disease had "normal" levels of blood cholesterol. However, the 240-300 mg/dL level of blood cholesterol considered "normal" was not normal at all, but rather typical when people consume a high-cholesterol, high-fat diet. In those days, high-fat dairy products, beef, and eggs, all of which are high in cholesterol, were the staples of the typical American diet. So the average blood cholesterol level used to define what was considered "normal" was quite high compared to today's standards.

Intrigued by this debate, medical physicist John Gofman of the University of California in Berkeley dove into the fray. Maybe it wasn't the total amount of cholesterol in the blood that affected the development of atherosclerosis, he reasoned, but how exactly the cholesterol was transported. He decided to study that transport with the aid of a new tool, the ultracentrifuge. The Donner laboratory where he worked had recently acquired the second ultracentrifuge to be built in the United States. This spinning device was amazingly adept at separating out various components of a solution based on their densities.

Cholesterol is a type of fat, and fat and water don't mix. Cholesterol has to be encased in a water-friendly structure called a lipoprotein to be easily transported in the watery avenues of blood that traverse the body. Due to their varied densities, Gofman was able to use the ultracentrifuge to discover different types of lipoproteins carrying cholesterol in the blood.

With funding from the National Heart Institute, (which later became the National, Heart, Lung and Blood Institute), Gofman and others performed experiments on animals and humans that revealed elevated blood levels of cholesterol carried in low-density

lipoproteins (LDL) were linked to a high-cholesterol diet and heightened risk of atherosclerosis. Cholesterol carried in high-density lipoproteins (HDL) was not linked to atherosclerosis risk at all, but rather seemed to protect against the disease. It wasn't the total level of cholesterol in the blood that was so important for coronary heart disease, Gofman concluded, but rather how the cholesterol was distributed among the different particles that can carry it in the bloodstream.

Gofman's findings gave birth to the popularized notion of "good" (HDL) and "bad" (LDL) cholesterol, but the role that both played in atherosclerosis was still hotly debated during the 1950s. The more definitive evidence implicating blood cholesterol levels with coronary heart disease came from the monumental Framingham study. In 1948, when this study began, there were no treatments for coronary heart disease. Preventing this disorder, therefore, was an obvious priority, but a difficult one to pursue because the cause was unknown.

To help discern this, researchers funded by the National Heart Institute decided to look for factors that heightened risk of developing heart attacks or strokes amongst more than 5,000 Framingham, Massachusetts residents. These medical detectives had the Framingham volunteers report their smoking, eating, drinking, and exercise habits as well as undergoing frequent physical exams and extensive blood tests on a regular basis for decades.

This still ongoing project has looked at dozens of potential heart disease-causing culprits, from smoking and lack of exercise to having diabetes or an enlarged heart. But one of the first factors that surfaced as being strongly linked to the risk of developing a heart attack was blood cholesterol level. In the early 1960s, the Framingham study researchers reported that men who had total cholesterol levels greater than 245 mg/dL at the start of the study were three times more likely to develop heart disease 10 years later than those with lower levels. (Subsequent studies showed similar findings in women, and moreover that elevated levels of LDL cholesterol, in particular, were linked to heightened risk of developing coronary heart disease.)

Bolstering the putative role of cholesterol in causing heart disease, the researchers noted that the risk of heart disease rose neck and neck with the amount of cholesterol circulating in the blood. Such a consistently close relationship, added to the fact that the high cholesterol levels *preceded* the development of heart attacks or other signs of heart disease, strongly suggested cholesterol fostered heart disease.

With a smoking gun in hand, researchers conducted hundreds of federally supported fundamental research studies aimed at understanding how exactly cholesterol might cause heart disease. Done by a host of curious scientists, none of this research was aimed at finding a way to prevent heart disease as an immediate goal. The researchers were more focused on teasing apart the complex scenario that underlies the disorder and finding out why a seemingly innocuous component of diet could lead to a heart attack. Rabbits, pigs, non-human primates and rodents were particularly invaluable as animal models for this research. By feeding these animals a high cholesterol diet and then examining their arteries at various intervals thereafter, investigators pieced together a detailed, play-by-play account of how blood cholesterol fosters atherosclerosis.

Thanks to their findings, it is now generally understood that the first step in the development of heart disease is an excess of LDL cholesterol traveling in the blood. This excess, which could be diet-induced or could stem from a genetic tendency, makes it more likely that some of the LDL cholesterol from the blood will slip in between the cells that comprise the inner walls of arteries. Not easily broken down, the LDL cholesterol remains lodged in between these cells, causing a local irritation. To deal with this irritating intruder, the garbage-eating cells of the immune system (macrophages) are drawn to the cholesterol lodged in the artery and ingest it. Eventually the macrophages die, spilling their cholesterol contents into the arte-

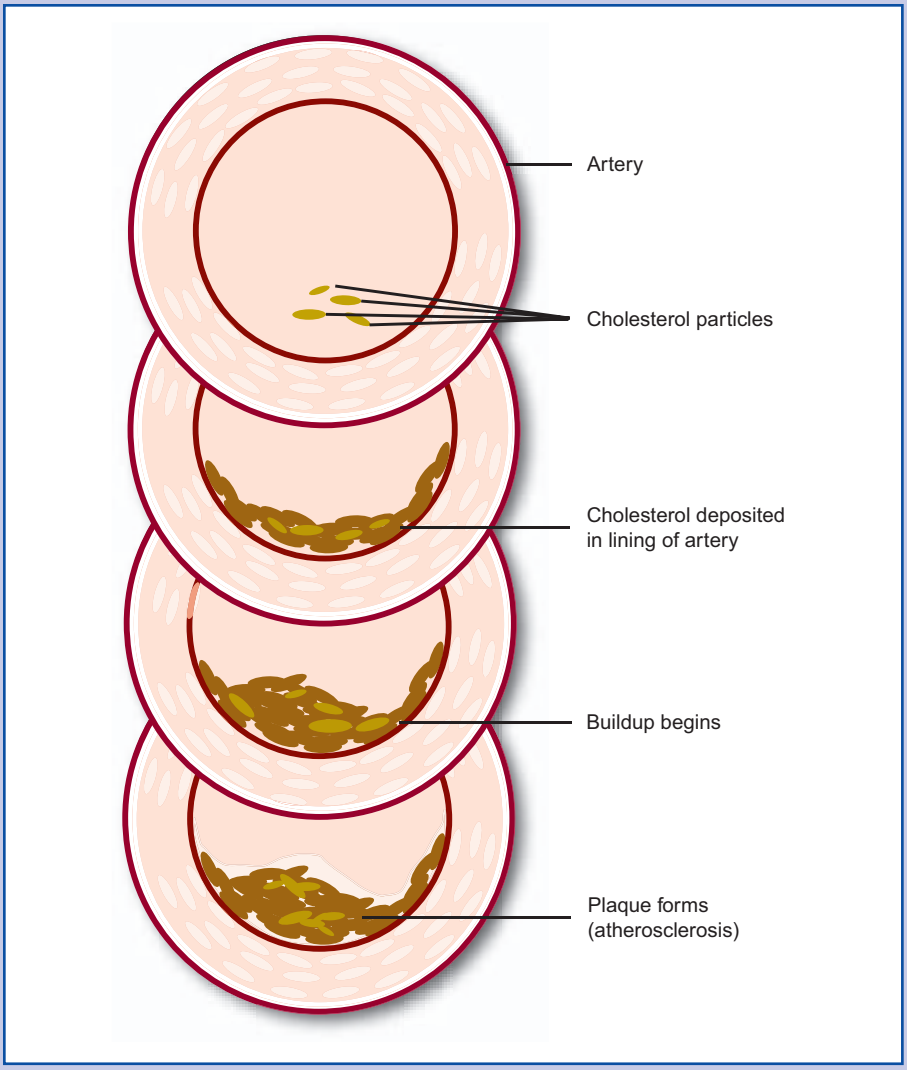


Figure 3: Cholesterol and Atherosclerosis: This drawing illustrates the development of atherosclerosis in an artery. This process is triggered by an excess of LDL cholesterol traveling in the blood, which can gather along the walls of the artery forming fatty streaks. Fibrous muscle cells eventually surround these streaks, forming an artery-narrowing plaque. *Figure designed by Corporate Press.*

rial wall. This cholesterol, combined with the intact cholesterol-engorged macrophages, form the fatty streaks in the arteries that Virchow first observed.

Fibrous muscle cells are often drawn to a wound, which they surround, creating a tough cellular patch. These muscle cells are also drawn to cholesterol-laden fatty streaks, which they encase with fibrous plaques that narrow the arteries. Such narrowing can limit the flow of oxygen-laden blood to the heart, resulting in chest pain known as angina. Over time, the plaques also tend to rupture and form blood clots that can obstruct the flow of blood to the heart, prompting angina or a heart attack (Figure 3).

Thus, atherosclerosis can be seen as the artery's response to injury by LDL cholesterol. HDL is the "good guy" in this story because research reveals it ferries cholesterol out of the cholesterol-injured regions in the arteries and into the liver where it is destroyed or safely stored. There's also evidence that HDL helps prevent cholesterol lodged in the artery from prompting the influx of macrophages and muscle cells that form plaque.

Pursuit of a Cholesterol Lowering Drug

Although all the details of how blood cholesterol leads to atherosclerosis weren't filled in by the 1960s, enough was known to suggest that limiting blood cholesterol via a low-cholesterol diet or medications might prevent heart disease. At this point, Bloch and other basic researchers had discovered that the body used cholesterol to make its cell membranes as well as key compounds, including hormones, vitamin D, and the bile acids that aid digestion. But the amount of blood LDL cholesterol needed to meet those bodily needs is one-fifth the level typically seen in people from Western nations. It stood to reason that Americans could stand to benefit by lowering their blood cholesterol levels.

As early as 1961, the American Heart Association endorsed a low-fat, low-cholesterol diet. But using diet to lower blood cholesterol levels has had limited success because many people have difficulty adhering to such a diet. Plus, dietary intervention doesn't significantly lower the blood cholesterol levels of many individuals who have a genetically encod-

ed tendency to make excessive quantities of cholesterol.

In the 1960s, drugs such as resins, niacin and fibrates were first used to lower blood cholesterol levels. These drugs do not directly limit the body's synthesis of cholesterol. Some lower blood cholesterol levels by limiting the production of cholesterol-carrying lipoproteins, or by inhibiting dietary cholesterol absorption from the intestines. Others work by increasing the liver's need for cholesterol. But fibrates, resins and niacin are only modestly effective—each facilitating a small (15 to 20 percent) drop in serum LDL cholesterol level. For most people, these medicines also have side effects or other limitations that make them difficult to take on a regular basis.

By 1970, there was still a need for an effective cholesterol-lowering drug. Efforts to find one were aided by basic research that had uncovered how the body regulates its production of cholesterol. Liver cells are the workhorses for the bulk of such production, probably because they are also the main consumers of cholesterol. Because making cholesterol is so energy-intensive, liver cells have a biochemical feedback mechanism to ensure that they don't make more cholesterol than they need. Ten years earlier, research on yeast and rats had revealed that a key component of this feedback mechanism is the enzyme HMG CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase.

Fungus Brings Breakthrough

HMG-CoA reductase surfaces early-on in the cholesterol synthesis process to carry out a chemical conversion that irreversibly jumpstarts the production of cholesterol. Sufficient quantities of cholesterol in the liver trigger this key enzyme to shut down its activity, thereby shutting down cholesterol synthesis. HMG-CoA reductase was an obvious target, therefore, for drug makers trying to concoct a cholesterol-lowering drug.

But discovery of the first effective inhibitor of this enzyme actually came from Japanese microbiologists searching for a new antibiotic. The microscopic world of bacteria and fungi is very competitive. To win the battle for limited resources, many strains are armed with compounds that inhibit the production of key molecules others need to survive. Microbiologist Akira Endo of Sankyo Pharmaceuticals knew that most bacteria and fungi required cholesterol-like compounds to grow. He reasoned that it was highly likely that there were microbes that produced an inhibitor of HMG-CoA reductase. Such an inhibitor would be a lethal weapon for the organism.

For two years, Endo and his colleagues screened about 6,000 microbe strains for their ability to produce a HMG-CoA reductase inhibitor. In 1973, they struck pay dirt. From more than 800 gallons filled with a penicillin mold, they isolated about a teaspoonful of a substance that inhibited HMG-CoA reductase. This inhibitor, called mevastatin, works by latching on to the enzyme and preventing it from binding to its target molecule in the cholesterol synthesis pathway. Many medicines work in a similar manner, including blood pressure drugs known as ACE (angiotensin-converting enzyme) inhibitors, and clot-busting drugs used to treat heart attack and stroke patients. These medicines all inhibit key enzymes that trigger important biochemical pathways in the body. (For more information on this see "From Viper's Venom to Drug Design: Treating Hypertension," and "Clot Busters!: Discovery of Thrombolytic Therapy for Heart Attack and Stroke" in the Breakthroughs in Bioscience series.)

Mevastatin didn't prove to be a good antibiotic, as Endo intended. But he didn't abandon study of the compound because, around this same time, Michael Brown and Joseph Goldstein (Figure 4) at the University of Texas in Dallas were reporting revolutionary discoveries on how the body regulates its production of cholesterol. Their findings suggested a compound such as mevastatin would be the ideal cholesterol-lowering drug for people prone to heart disease.

Uncovering the Keys to Cholesterol Production

Both Brown and Goldstein

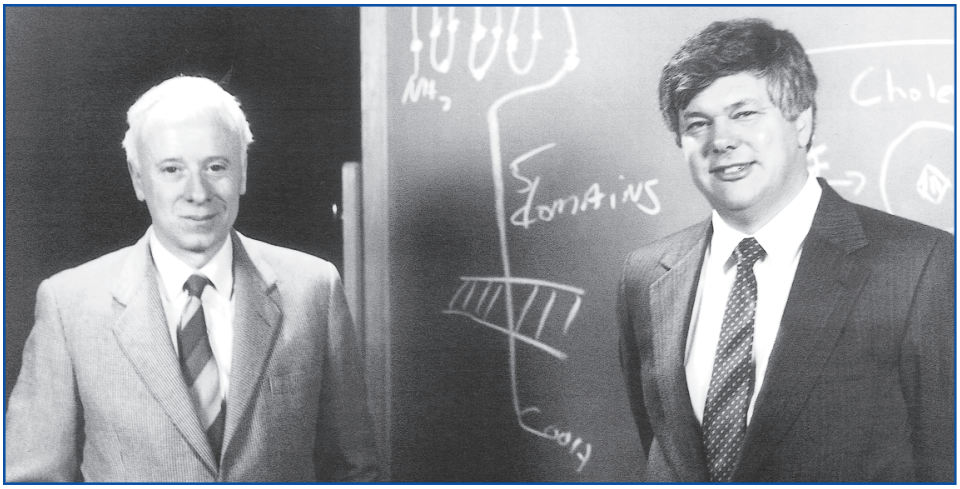


Figure 4: Brown and Goldstein: Long-time friends, Michael Brown (right) and Joseph Goldstein won the Nobel Prize for Physiology or Medicine in 1985 for their discovery of the LDL receptor and the role it plays in regulating how much cholesterol is in the bloodstream. Their research suggested a drug, such as a statin, that inhibited production of the HMG CoA reductase enzyme would provide a safe and effective way to lower blood LDL cholesterol levels. *Photo courtesy of Dr. Joseph Goldstein.*

completed their undergraduate degrees in chemistry and then went on to receive medical degrees. They forged a friendship while working together as interns at Massachusetts General Hospital. After his internship, Brown worked in a biochemistry lab at the NationaI Institutes of Health (NIH), where he investigated how enzymes regulate the production of key compounds in the body. He then joined the staff of the University of Texas Medical School where he worked on purifying HMG-CoA reductase.

Goldstein, in the interim, also spent several years at NIH and the University of Washington in Seattle, where he focused on uncovering genetic explanations for various diseases, including familial hypercholesterolemia (FH). Patients with the most severe form of this disease have 5 times the normal level of cholesterol traveling in their blood and develop heart attack-triggering atherosclerosis during childhood. While working at NIH, Goldstein cared for patients with FH and this prompted his interest in uncovering why people with this debilitating disease had such excessive blood cholesterol levels.

For many years after completing their medical internships, Brown and Goldstein lived more than 2,000 miles apart. But they kept in regular contact while Goldstein lived in Seattle. During that time, they developed the hypothesis that faulty regulation of HMG CoA reductase was the cause of FH. Goldstein was then reunited with Brown, taking a position at the University of Texas where the two conducted landmark studies on cultured cells from normal individuals and those with FH. This revealed fundamental cholesterol-regulating processes that not only go awry in FH patients, but in people who have too much cholesterol in their diets.

As Brown and Goldstein noted in their 1985 Nobel prize acceptance speech, the careful packaging of cholesterol into lipoproteins so it can easily be transported in the blood also creates a problem: how can cholesterol be delivered into cells so they can use it to build their membranes, hormones, or bile acids? Within lipoproteins, cholesterol is stored in a form that the cell cannot use.

To solve this dilemma, a cell in need of cholesterol creates specialized proteins, known as LDL receptors, which jut out from pits on the surface of its cell membrane. Discovered by Brown and Goldstein in 1973, these receptors are especially suited for nabbing LDL cholesterol molecules and can pick one out of more than a billion molecules of water. When the LDL receptors bind to a passing LDL cholesterol molecule, the edges of the pit merge and the entire spherical sac pinches off inside the cells (Figure 5A).

Now the LDL cholesterol and attached receptor are completely encapsulated in a bubble-like structure. This vesicle delivers its contents to a specialized organelle (lysosome), which biochemically frees the cholesterol into a form that can be used by the cell. Once released of its contents, the LDL receptor travels back to the surface of the cell where it ushers in other LDL cholesterol molecules. The LDL receptor makes an impressive total of several hundred trips into and out of the cell during its 20-hour lifespan, thereby helping to lower the concentra-

The LDL Receptor Pathway

Figure 5a: LDL Receptor Pathway: When Cholesterol is Needed by the Cell: Cells need cholesterol to build their membranes, steroid hormones, vitamin D, and bile acids. To nab such cholesterol carried by LDL particles in the bloodstream, they create LDL receptors. These receptors are lodged in pits on the surface of the cells. When they bind to LDL cholesterol molecules, the edges of the pit merge, totally encapsulating the LDL cholesterol. This vesicle delivers its contents to a specialized organelle (lysosome), which biochemically frees the cholesterol into a form that can be used by the cell. Once released of its contents, the LDL receptor travels back to the surface of the cell, where it ushers in other LDL cholesterol molecules. The cell also boosts its own production of cholesterol by increasing its production of HMG CoA reductase, an enzymatic trigger for cholesterol production. *Figure designed by Corporate Press, adapted from Nobel Lecture, 1985, MS Brown and JL Goldstein.*

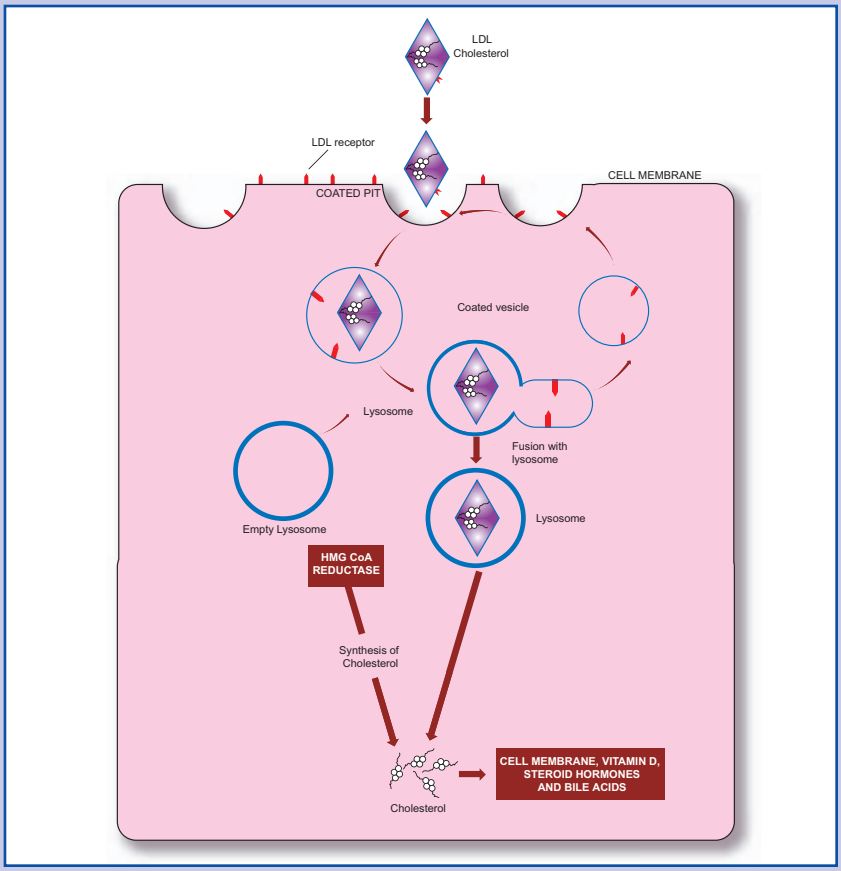
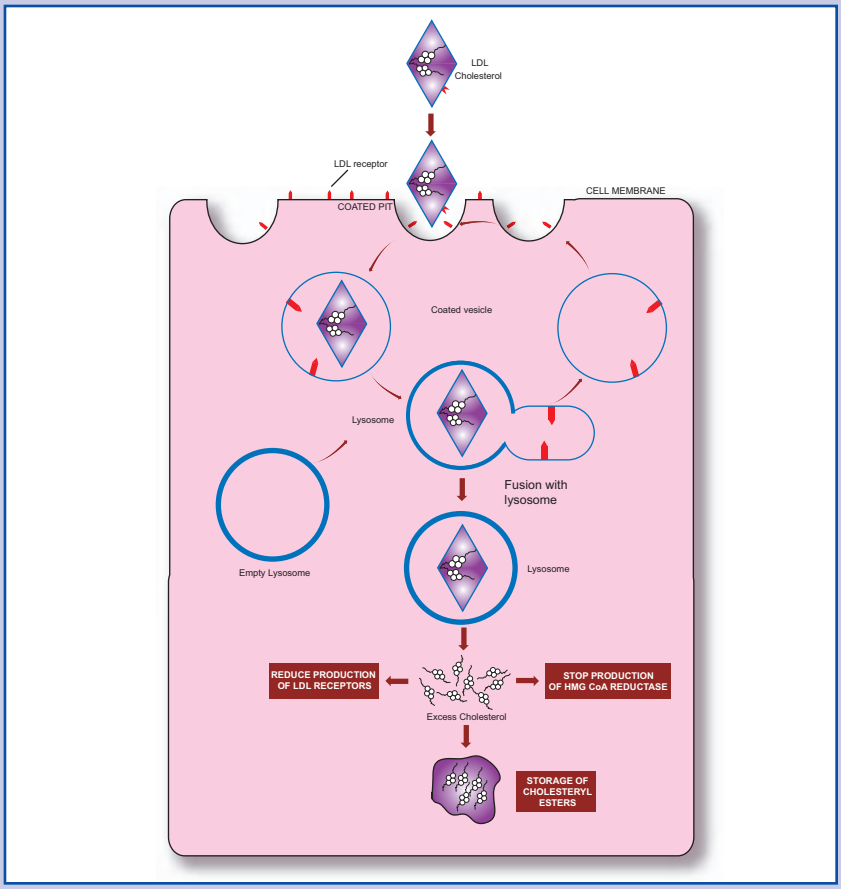


Figure 5b: LDL Receptor Pathway: When There is an Excess of Cholesterol: When cells have enough cholesterol, the excess is stored. This excess cholesterol also stops the cell from making HMG CoA reductase, the enzymatic trigger for producing cholesterol. Cholesterol content is further limited by the excess cholesterol shutting down production of LDL receptors. This stops LDL cholesterol from passing from the bloodstream into cells. The end result is an elevated level of the cholesterol in the blood, which triggers atherosclerosis. *Figure designed by Corporate Press, adapted from Nobel Lecture, 1985, MS Brown and JL Goldstein.*



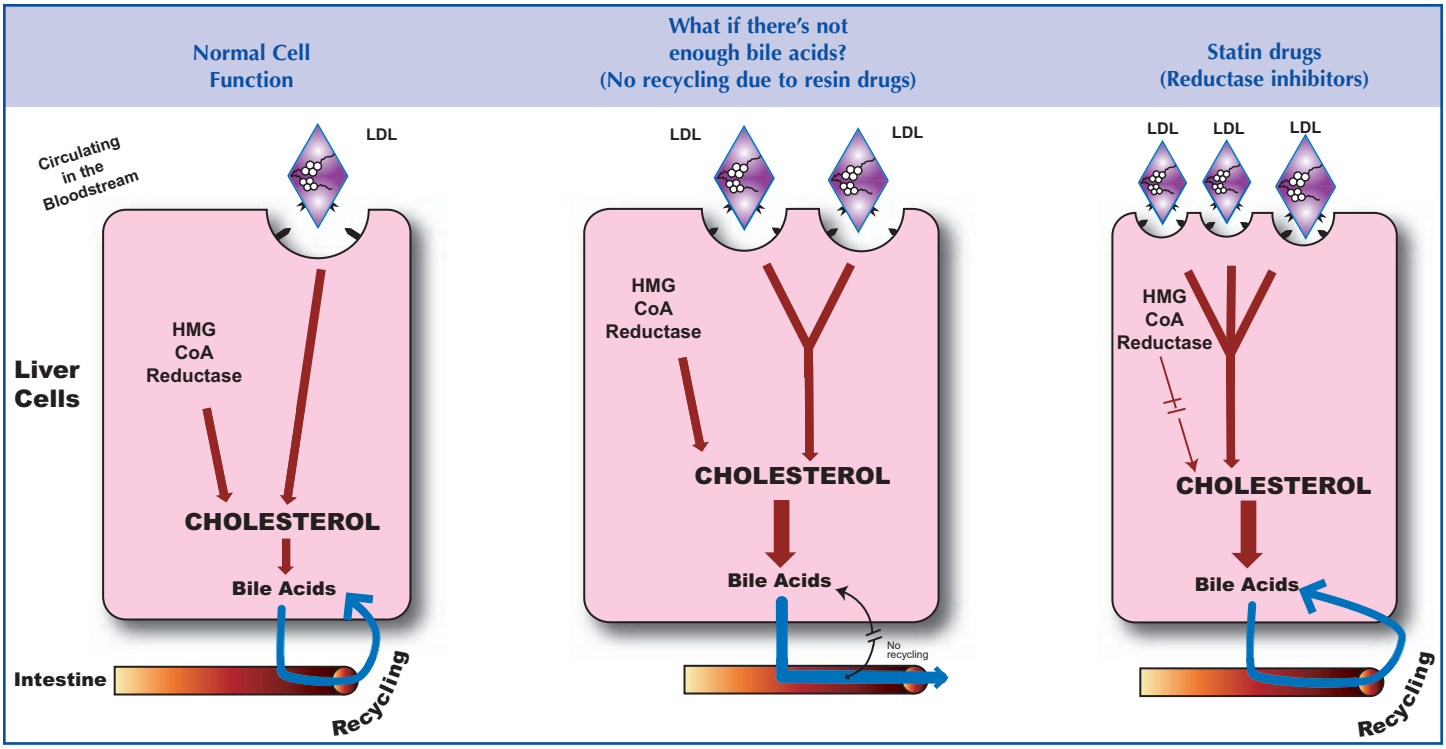


Figure 6: How Resin and Statin Drugs Lower Blood Cholesterol: Resins, commonly used cholesterol-lowering drugs in the 1970's, work by preventing the reabsorption and subsequent recycling of bile acids to aid digestion. To make new bile aids, the liver increases its production of HMG CoA reductase, the enzymatic trigger for cholesterol production. It also gathers cholesterol from the bloodstream by slightly boosting its production of LDL receptors on its cells. Statins, by contrast, limit cells' production of cholesterol by directly inhibiting HMG-CoA reductase. This prompts cells to gather more cholesterol from the blood by making substantially more LDL receptors. The end result is more effective lowering of blood LDL cholesterol level. *Figure designed by Corporate Press, adapted from Nobel Lecture, 1985, MS Brown and JL Goldstein.*

tion of LDL cholesterol in the blood and the subsequent buildup of atherosclerotic plaques.

Another way the body ensures that it doesn't build up too much cholesterol is by shutting off its own cholesterol production when high enough levels are achieved. Brown and Goldstein discovered that the signal that shuts off such cholesterol production was the lysosome-liberated form of cholesterol. Cells that accumulated this cholesterol stopped producing HMG CoA reductase, the enzymatic trigger for making cholesterol. These cells also limited their cholesterol intake by shutting down their production of LDL receptors. Conversely, when cellular demands for cholesterol are high, cells upped their production of LDL receptors so they drew in more of the compound from the bloodstream (Figure 5B). For example, cells that are actively dividing and need cholesterol to build their new cell membranes have 10 times as many LDL receptors as nondividing cells.

Through their federally funded research, Brown and Goldstein not only discovered this whole exquisite means of controlling cholesterol production, but why patients with FH have such an excessive amount of cholesterol in their blood. These patients lack the ability to make enough functioning LDL receptors. Consequently, large amounts of LDL cholesterol stay in the bloodstream, causing premature atherosclerosis. The regulatory mechanism Brown and Goldstein uncovered also suggested a way to use drugs so that "it may one day be possible for many people to have their steak and live to enjoy it too," the Texan researchers wrote in a *Scientific American* article they authored in 1984.

Statins Make Their Mark

As Brown and Goldstein noted, resins (the cholesterol drugs commonly used in the 1970s) work by preventing the reabsorption and subsequent recycling of bile acids to aid digestion. As a result, the liver needs more cholesterol to make new bile acids and does so by boosting its production of LDL receptors on its cells. These receptors take up more cholesterol from the blood. But the liver also satisfies its hunger for more cholesterol by upping its production of HMG-CoA reductase, triggering its cells to produce more cholesterol. This self-synthesis of cholesterol means that the liver only has to increase its production of LDL receptors by 15 to 20 percent to restore adequate cholesterol levels (Figure 6). Therefore, blood levels of cholesterol only fall by an equal percentage in patients that take resins to lower their cholesterol.

A much more effective drug, the researchers reasoned, would be one that directly limits cells' production of cholesterol via HMG-CoA reductase inhibition. This would prompt cells to gather more cholesterol from the blood by making more LDL receptors. The end result would be lower LDL blood cholesterol levels without seriously limiting liver cells' access to this vital substance.

Aware of Brown and Goldstein's findings, Endo, the microbiologist in Japan, rightfully assumed that the mevastatin he discovered could be useful in lowering blood levels of LDL cholesterol without lowering levels of HDL, the "good" cholesterol. He and others at Sankyo studied its ability to do so in cell cultures and several animal species. When studies of mevastatin at extremely high doses in dogs caused toxic reactions, Sankyo abandoned development of the compound into a cholesterol-lowering drug. But the findings of Brown, Goldstein, and Endo still inspired other drug companies to discover and test HMG-CoA inhibitors, collectively called statins. Merck produced lovastatin, the first statin to enter the market (under the brand name Mevacor) in 1987. That drug was soon followed by six other statins.

Clinical studies show all these statins are remarkably effective at lowering blood LDL cholesterol levels—they cause a 35 to 55 percent drop, on average. This translates into about a 25 to 40 percent reduction in deaths from heart disease. Also remarkable is the fact that statins do not cause significant side effects in the vast majority of patients who take them, typically in a one-pill-a-day regimen that is easy to follow. Although one statin was withdrawn from the market because it caused serious muscle and kidney damage in a small number of patients, this side effect is extremely rare for the other statins. In the United Kingdom, statins are considered safe enough to be sold over-the-counter.

Clinical studies reveal that statins not only lower the risk of developing and dying from heart disease, but some may also lower the risk of stroke by 20 percent, presumably by preventing the build-up of clot-producing plaques in brain arteries. One study found that administering a statin reduced the numbers of treated people from dying from all causes by 30 percent. The benefits of statins seem to be so great that some doctors joke these medicines should be put in the drinking water, along with fluoride.

In addition, federally funded basic research in recent years has revealed other potential benefits of statins, beyond lowering blood levels of cholesterol. Laboratory studies suggest that HMG CoA reductase influences a number of other processes in the body besides cholesterol production, including inflammation, the breakdown of bone, and the growth of tumors. Thus, by inhibiting this powerful enzyme, statins might have the potential to aid treatment of a wide range of disorders including inflammatory diseases such as rheumatoid arthritis and multiple sclerosis, bone deteriorating disorders such as osteoporosis, and a number of cancers. Researchers are just beginning to test the effectiveness of statins in treating these diseases.

Researchers also continue to explore, in a more detailed fashion, the biochemical processes that underlie atherosclerosis. These investigators are trying to decipher what chemical signals draw macrophages and muscle cells to cholesterol deposits in the wall of the artery, as well as what factors trigger the rupture of plaque in arteries. If history can be a guide, such fundamental research is likely to suggest new targets for drugs to prevent or treat heart disease.

After all, statins would not have been discovered and used to prevent heart disease if basic researchers such as Wieland and Bloch had not bothered pursuing an enigmatic molecule. Or if scores of other curious scientists hadn't tried to figure out the various steps involved in atherosclerosis. By uncovering the intricate mechanisms involved in the production of cholesterol and its deposition in the walls of arteries, basic scientists have given us the medical tools to stave off coronary heart disease. Thanks to their efforts, more than 25 million people worldwide now take statins and will have many more years added on to their lives as a result. These drugs prevent more than 125,000 people in this country of dying from heart attacks each year.

Cholesterol. Who would have thought that the study of just one molecule would have such widespread biomedical beneficial effects?

Biographies

Margie Patlak writes about biomedical research and health from the Philadelphia region. She has written for *Discover, Glamour, Physician's Weekly, Consumer Reports on Health,* the *Washington Post, Los Angeles Times, Dallas Morning News* and numerous other publications. She also writes frequently for the National Institutes of Health and the National Academy of Sciences. This is her fifth article for the *Breakthroughs in Bioscience* series.

Mason Wright Freeman, M.D., is founder and Chief of the Lipid Metabolism Unit at Massachusetts General Hospital (MGH) and Associate Professor of Medicine at Harvard University Medical School, where his research interests encompass the genetics of cholesterol disorders and the role of lipoproteins in atherosclerosis. He also serves as Director of both the Nessel Gene Therapy Center and Microarray Facility at MGH. Dr. Freeman is board certified in Endocrinology, Diabetes, Metabolism, and Internal Medicine, and sees hundreds of patients per year. He is author of numerous professional publications and books, including *The Harvard Medical School Guide to Lowering Your Cholesterol*, with Christine Junge.

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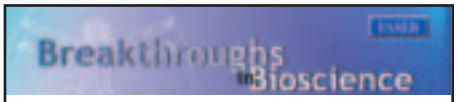

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Published
2005

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This article made possible through the generous support of the Endocrine Society

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