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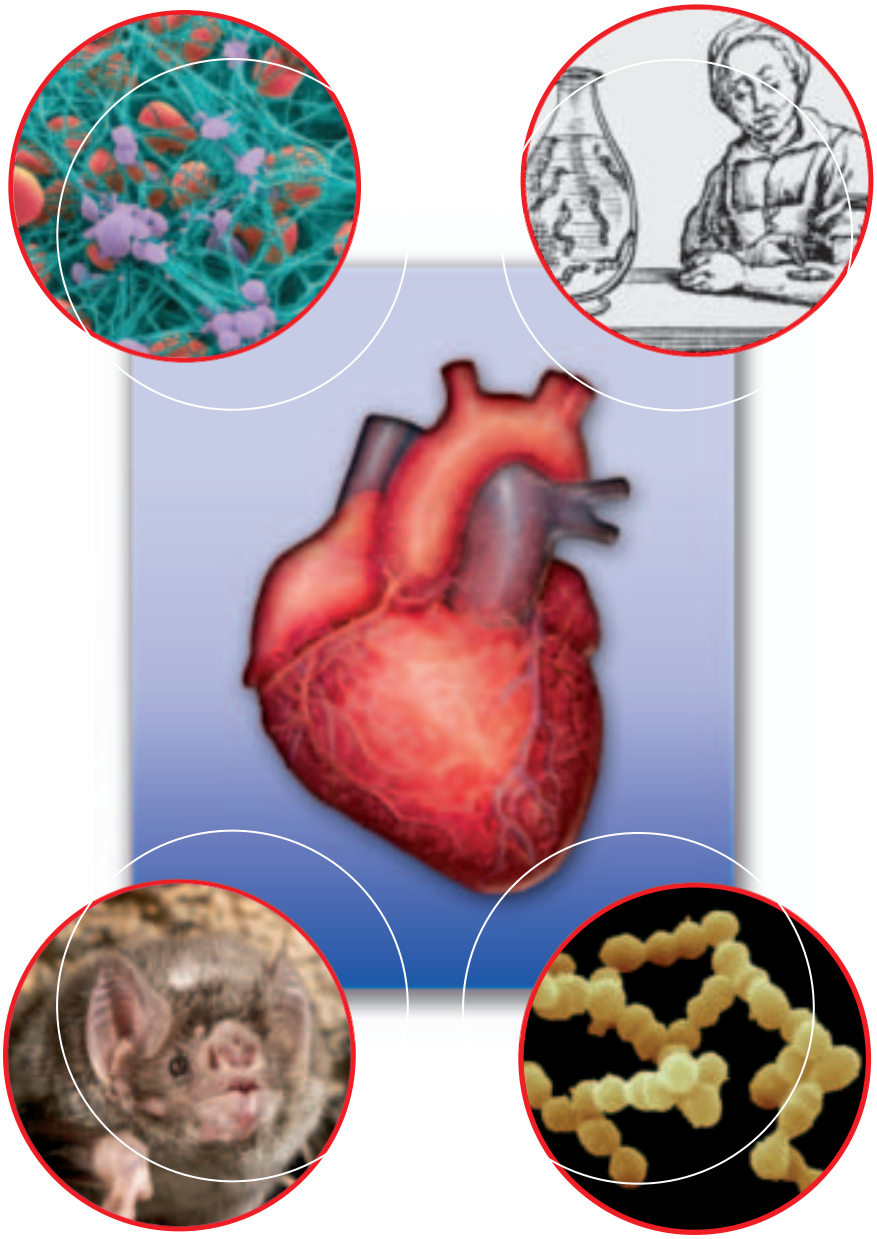

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CLOT BUSTERS!! - DISCOVERY OF THROMBOLYTIC THERAPY FOR HEART ATTACK & STROKE

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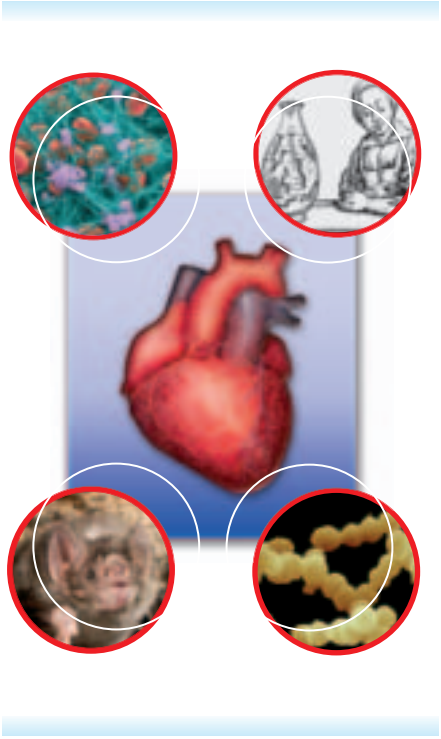


COVER IMAGE: The development of thrombolytics ("clot-busters") to treat heart attack and stroke followed a complex pathway of basic research and clinical observation. Natural clot busting agents, from human blood vessels, leeches, vampire bat saliva, and bacteria all played a role in helping scientists understand how to harness the power of thrombolytics to save lives. *Cover design by Corporate Press, photos courtesy of Science Photo Library, Photo Researchers, Inc. and the Organization for Bat Conservation.*

Clot-Busters!! - Discovery of Thrombolytic Therapy for Treating Heart Attack & Stroke

A Convergence of Pathways

What do the search for antibiotics in the 1930s, the discovery of the double helix in the 1950s, and an extremely aggressive case of melanoma skin cancer in the 1970s have in common? They all contributed a piece of the puzzle to a breakthrough treatment for heart attacks and the most common form of stroke.

That treatment, a class of "clot busting drugs" called thrombolytics, has saved untold lives. Yet as in so much of science, the introduction of thrombolytics took a long, circuitous route, with both head-scratching bewilderment and "aha!" moments along the way. The emerging, disparate pieces did not appear to belong to the same puzzle, much less to one pertaining to the heart or brain. A proliferation of new research specialties (molecular genetics, cancer biology, and biochemistry) co-evolved and turned up more pieces to contemplate. Budding technologies (surgical pathology, radiology, *in vitro* cell growth, and recombinant DNA) provided new ways to explore past and present discoveries, including clues about naturally occurring thrombolytic agents. Unrelated areas of research unexpectedly converged, and unsuccessful experiments in one area led to sudden advances in another.

Without any one of these separate advances, from microbiology to DNA technology, the puzzle would have remained incomplete. Instead, like the image on a Polaroid photo coming into focus, a new conceptual model of the nature of heart attacks (and then strokes) developed. That model showed that clots are the culprits behind acute heart attacks, known as acute myocardial infarction (AMI), as well as most strokes, and it vindicated a previously discarded notion of treating a heart attack by using clot-dissolving thrombolytics to open up the clogged arteries.

Because of decades of basic research and clinical studies, a new type of thrombolytic drug, known as t-PA for tissue plasminogen activator, appeared at the bedside in record time. Meanwhile, acceptance of the "open artery" theory of treating heart attacks led to still more life-saving techniques, including angioplasty, and preventive therapies, such as a daily dose of aspirin.

As a 2004 review article summarizing the history and development of thrombolytics stated, "Physicians trained after the advent of thrombolytic therapy may find it difficult to believe that this concept was one of the most hotly debated pathophysiological tenets of the 20th century. Yet, the development of thrombolytic agents for treatment of AMI remained a tortuous and stuttering process until this concept achieved widespread acceptance."[1]

In The Olden Days

While playing tag in the back yard of our Oklahoma home in 1959, my 53-year old grandfather, John Alonzo McCullough, felt chest pain, went to lie down, and waited for the house call from our family doctor, Charles Johnson. A slightly overweight Texan postal inspector and smoker, McCullough was not an unusually young heart attack victim in those days (Figure 2). At the dawn of the 1960s, when few people knew much about heart disease prevention and the average hospital had few cardiac procedures to offer, an even higher percentage of Americans who suffered heart attacks died from them than do so today.

In 1959, lying down was probably the best thing to do while

[1]Maroo A and Topol EJ. (2004) Journal of Thrombosis and Haemostasis. 2:1-4.

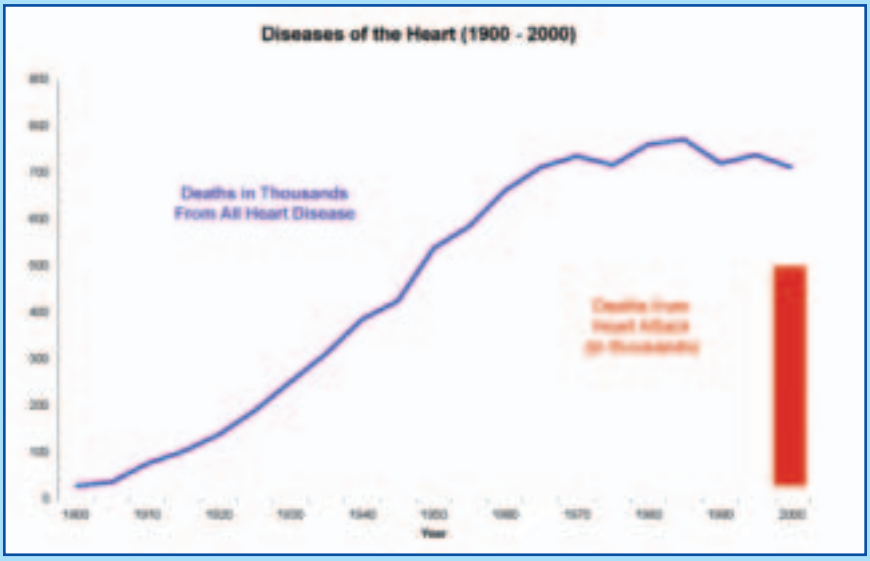


Figure 1: The Toll of Clots. As leading causes of death in the United States, heart attack ranks first and stroke third (second, world-wide). The total number of heart disease deaths per year increased throughout the 20th century, along with the growing population and the long term toll of smoking and a more sedentary lifestyle. Fewer and fewer heart attacks are fatal, in part due to thrombolytics, resulting in a dramatically declining death rate over thirty years (from 25% to 8%). *Sources: CDC/NCHS, American Heart Association 2004 Update, and National Hospital Discharge Survey, NHLBI.*

Figure 2: A lucky heart attack survivor c. 1955: The longer a heart muscle goes without oxygen, the more damage it sustains. Resting the heart limits the damage and before the discovery of thrombolytics was the recommended therapy. *Photo courtesy of John McCullough and Cathryn Delude.*

waiting for a doctor or ambulance. Dr. Johnson did not yet know to give my grandfather a few aspirin to prevent further growth of the clot that—unbeknownst to either man—was blocking the flow of blood in a coronary artery and depriving the heart muscle of oxygen. The longer the heart muscle went without oxygen, the more damage it sustained. Resting the heart muscle reduced its oxygen demand and limited the damage. Yet Dr. Johnson probably had this prognosis for my grandfather: either he dies, or he does not. (Luckily, he did not, and he lived another non-smoking 35 years on a lean diet and long daily walks.)

Changing Therapeutic Directions

Strangely enough, had this heart attack happened in 1899, before my grandfather was born, his doctor might have read the just-published article, "Infarction of the Heart." It proposed that acute heart attacks are frequently caused by thrombosis, or clotting, in the coronary arteries. Other scientists had observed clots in the blood vessels for several centuries. One scientist called the clots seen in arteries at autopsy "chicken fat," because that was what they resembled. Their pale yellow color stems from the fact that post-mortem arterial clots contain few red blood cells, consisting mainly of coagulated blood plasma. Another scientist called the clots in veins, which do contain red blood cells, "currant

jelly" for their red, gelatinous appearance. Had they been able to observe the blood vessels prior to death at this time, researchers might have described white clots, as well, made up of clumps of platelets.

While doctors began to accept the theory that clots cause heart attacks at the turn of the 20th century, they assumed that AMIs were inevitably fatal. With that pessimistic assumption, there was little impetus to develop therapies. During the 1910s, however, some researchers challenged the expectation that heart attacks were always fatal, reporting that many patients did survive and recover. That realization spawned a new therapeutic model: the "open artery hypothesis" proposed that restoring the blood flow to the heart muscle could improve the survival and recovery rate for heart attack victims.

The field changed directions again during the 1940s. Prominent medical experts, including some at the National Institutes of Health (NIH), held that coronary thrombosis was the result, not the cause of heart muscle damage. Electrocardiograms (EKGs) enabled doctors to observe that patients undergoing heart attacks had irregular EKG readings, indicating heart muscle spasms. On the other hand, autopsies revealed arterial clots in only about a third of heart attack victims. Thus, many experts concluded that heart spasms could cause the clotting, but that the reverse was not true. The drive to develop thrombolytic drugs to treat heart attacks slackened.

The "open artery" model for heart attacks proposed that restoring blood flow to the heart muscles could prevent damage and improve survival chances. But this theory took several decades to become widely accepted.

Undaunted, Dr. Sol Sherry was convinced that a thrombolytic agent called streptokinase (SK), produced by streptococcal bacteria, could treat unwelcome clots. Without knowing exactly how SK worked, Sherry used it on a heart attack patient (who survived) in 1947 and later demonstrated that it dissolved clots in rabbit ear veins. In the 1950s, he and collaborators treated more heart attack patients with SK, using massive doses that were much higher than the therapeutic dose given today.[2]

Looking back, the results of Sherry's studies showed what has since become the cardinal rule of heart attack therapy: the sooner the better. Patients who received SK treatment within 14 hours of their heart attack had a lower mortality rate in the hospital. Those who received delayed treatment fared no better than untreated patients.

At the time, no one considered the potential importance of early treatment with thrombolytics. If they had analyzed the data according to how early patients received treatment, they would have noted clear-cut benefits for patients who received SK in the first few hours. As it was, pooling all the results showed an insignificant overall benefit compared to the elevated risk of bleeding as a side effect of the intervention in the body's blood-clotting system. Thus, many researchers reached erroneous conclusions, dismissed the benefits of thrombolytics, and questioned the clot theory of the disease.

"We were dissolving clots in people whose heart muscles were already dead. We didn't realize we had to treat early to receive benefits," recalls Dr. Burton Sobel of his days as a young researcher investigating thrombolytic therapy for heart attacks. "We were all confused and we thought either something was wrong with our hypothesis that heart attacks were caused by clots or something else was wrong. It turned out to be something else, the need for very early treatment. Our lack of subsidiary knowledge discouraged progress and the field dried up."

The situation changed again in 1980, when new technologies began to converge with clinical research and Dr. Marcus DeWood finally caught clots at the scene of the crime. He used

[2]Tillet WS and Sherry S. (1949) Journal of Clinical Investigation 28:172-190; Fletcher AP, Alkjersig N, Smyrniotis FE and Sherry S. (1958) Trans. Assoc. Am. Phys. 71:287-296.

angiography, the technique of inserting a catheter for an X-ray examination of the blood vessels connected to the heart, on 126 patients within 24 hours of the onset of symptoms. Of those patients, almost 50% showed evidence of thrombi. DeWood could even retrieve the thrombi from 52 patients, an amazing feat at that time. During the next 24 hours, many fewer clots were observed. The decrease in thrombi over time resulted from the body's natural clot-dissolving process (see "Fibrinolysis," page 11), which continues post-mortem and accounts for the absence of clots in the autopsies of many heart attack victims.

DeWood's finding helped reaffirm the open-artery hypothesis for treating acute myocardial infarction. Now the medical community had an acute need for a thrombolytic drug that could immediately begin dissolving the clot and quickly restore blood flow to endangered heart muscle. "The hope was we could have a therapy that was like the additive you put in car radiators that plugs up only the holes," recalls FASEB Executive Director and medical researcher Dr. Frederick R. Rickles. "Only thrombolytics would work in reverse. It would open up the plugs, and only the plugs, so that it would not cause bleeding. But in practice that's difficult to do."

> It took new medical technology to finally establish scientifically the true cause of heart attacks: thrombi, also known as blood clots, in the coronary arteries.

The Road to Thrombolytics

Luckily, a virtually ready-made drug was already on the shelf: streptokinase. Sherry and his protégés had accumulated evidence showing that SK indeed did save the lives of heart attack victims. Other researchers were forging ahead with another naturally occurring thrombolytic, urokinase, which is produced by kidney cells and is used mainly for treatment of pulmonary embolisms [blockages in the lungs]. The introduction of streptokinase into medical practice for acute heart attacks in the 1970s began to make its mark on the declining death rates from acute heart attack. (Figure 5)

Just as SK was gaining glory, a new and glamorous kid arrived on the block: recombinant-PA. While some researchers were investigating SK as a therapy for heart attack, others, following independent paths in the basic research arena, had already discovered a naturally occurring human thrombolytic agent, called tissue plasminogen activator or t-PA, that circulates in the human bloodstream. Newly available

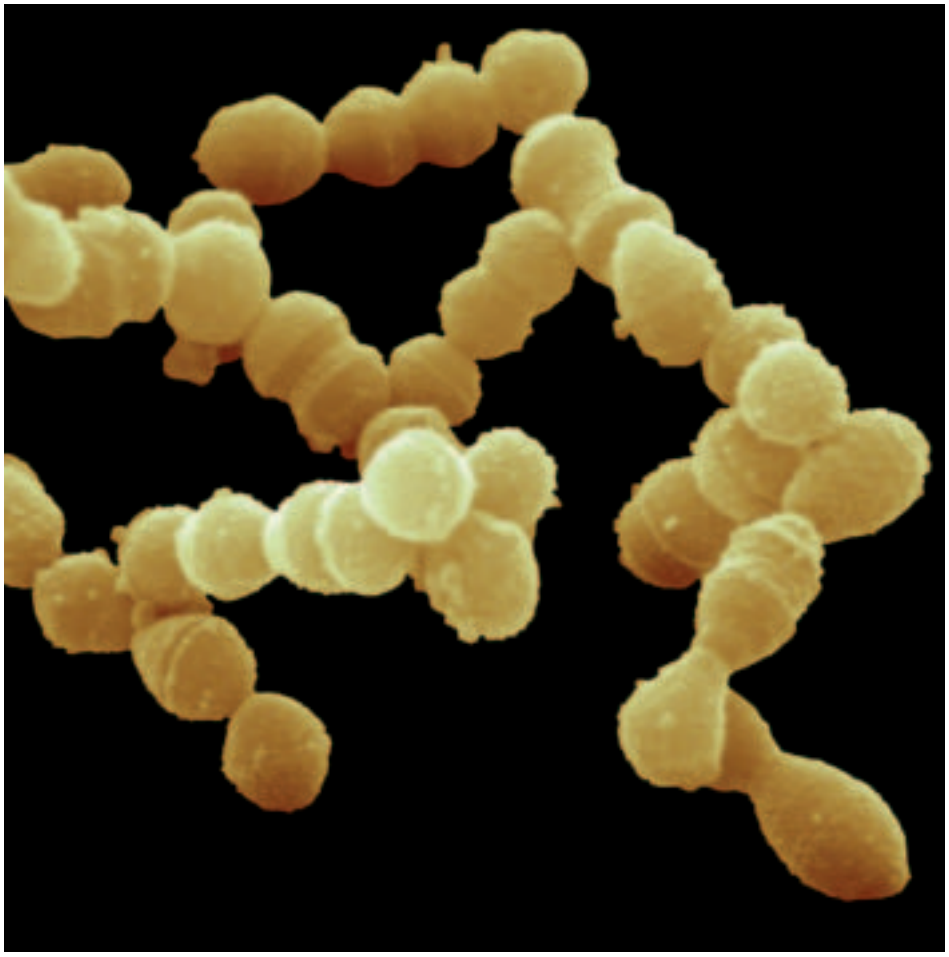


Figure 3: Colored scanning electron micrograph (SEM) of Streptococcus. Streptococcal bacteria produce a molecule, streptokinase, to gain access to the human blood stream and to penetrate tissues. Scientists isolated SK to use as the first thrombolytic drug to treat acute heart attack. *Science Photo Library/Photo Researchers, Inc.*

molecular techniques for exploring the biochemistry of t-PA's interaction with various blood proteins coincided with newly minted DNA technologies. These technologies enabled scientists to identify the genetic sequences and then clone important blood proteins and enzymes for further study or therapeutic applications. Proteins produced by this method are called *recombinant* proteins. In just seven years, researchers would use t-PA for therapy and introduce the first recombinant drug, rt-PA, to the pharmacy shelves.

The Raison d'Etre for Natural Clot Busters

Why are there naturally occurring thrombolytic agents? The human blood system is an intricately complicated field of checks and balances, defenses and attacks. Take, for instance, a finger prick. A dot of blood appears but then, normally, stops flowing. The slight break in the blood vessel wall sets in motion a cascade of events that coagulate blood and prevent uncontrolled bleeding, a natural clotting process called hemostasis. The excessive and sometimes life-threatening bleeding in hemophilia, for example, results from a deficiency in just one of many blood proteins involved in the clotting cascade. Bacteria, bats, leeches, and snakes long ago figured out the benefits of overriding the human body's blood clotting system. To gain access to the blood stream or to invade human tissue, these organisms secrete molecules that break down clots or prevent them from forming.

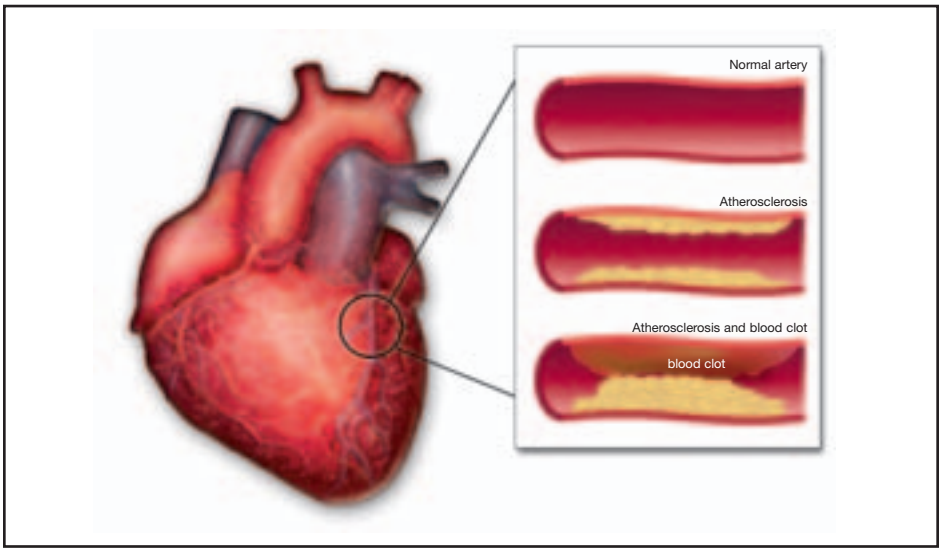


Figure 4: When the lining of the coronary artery is clear and healthy, blood flows easily to the heart, bringing it the oxygen it needs to function. Atherosclerosis, or hardening of the arteries, is caused by a build up of plaque (fatty material) along the walls of the artery, and can narrow the passageway bringing blood to the heart. It is thought that atherosclerosis is caused by a response to damage to the artery walls from high cholesterol, high blood pressure, and cigarette smoking. A blood clot can plug the opening narrowed by the atherosclerosis, ceasing all blood flow to the heart muscle. When this happens, the heart muscle does not receive oxygen, is damaged, stops working, and eventually dies. This is a heart attack, or myocardial infarction. *Designed by Corporate Press.*

Streptococcal bacteria, or *Streptococci*, for instance, inflict numerous types of infection on humans, ranging from sore throat to rheumatic fever, and they double as the notorious "flesh-eating bacteria." Doctors had noticed that some patients with streptococcal infections developed clots that then spontaneously dissolved. In 1933, around the time of growing skepticism about the open artery model of treating heart attacks, bacterial infections were a huge menace to public health. No antibiotics were yet available, although a fervent search was underway, a search that later turned out to be fortuitous yet critical for the development of thrombolytic therapy. In pursuing antibiotics, researchers were studying *Streptococci* to understand the disease process and find ways to subdue infections. In the process, they isolated the agent that dissolves clots and called it streptokinase.

Eventually, scientists came to understand that *Streptococci* produce streptokinase as part of an evolutionary one-upmanship with the human immune system. Immune cells recognize when *Streptococci* are trying to penetrate various tissues. In defense, the immune cells instruct the body to build roadblocks, in the form of clots, which also help stop the spread of infection to other parts of the body. In a counter attack,

the *Streptococci* secrete streptokinase, which dissolves the clots and enables the bacteria to enjoy the nutrients in human tissue and to spread infection.

Open-Artery Model for Treating Acute Heart Attack

- Blood clots (thrombi) block the blood supply to the heart muscles.
- Without oxygen from the blood, the heart muscles suffer damage.
- The longer a muscle goes without oxygen, the more severe the damage.
- Restoring the blood flow reduces damage to heart muscles.
- There are three main ways to restore blood flow:
    1. Clot-busting drugs (thrombolytics) can dissolve clots immediately.
    2. Angioplasty, a surgical procedure, can open the artery with a balloon and then, if indicated, install a stent (a scaffold-like device) that helps keep the vessel open.
    3. Bypass surgery provides an alternate route around the blockage, using a blood vessel from the calf or elsewhere in the body.

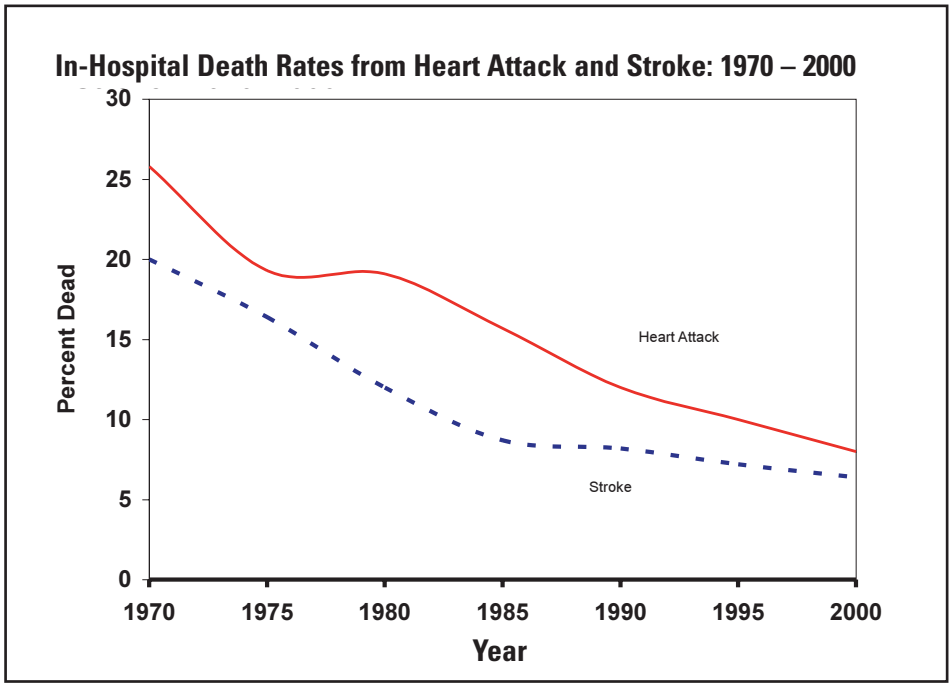


Figure 5: The in-hospital death rate for acute myocardial infarction fell from 25% in 1970 (when early thrombolytics were becoming marginally accepted) to 10% in 1989 (when thrombolytic use had doubled and a new recombinant form of tissue plasminogen activator [t-PA] was introduced) to 8% in 2002 (after angioplasty was more widely used), for a total decline of 68%. *National Hospital Discharge Survey Annual Reports; National Heart, Lung and Blood Institute.*

Discovering t-PA and its Surprising Application[3]

In 1947, researchers reported finding an anti-clotting agent that human cells naturally produce to clear up clotting, later called tissue plasminogen activator or t-PA. Meanwhile, cancer researchers were studying an unidentified agent's suspected role in cancer-forming cells. They knew the agent degraded a fiber-like substance called fibrin that helps build blood clots, and the more of it a cell made, the more cancerous the cell looked. Researchers speculated that the same agent responsible for chewing up fibrin also helped the cancer cell chew through the extra-cellular matrix of tissues, recalls one of those researchers, the Belgian biochemist, Dr. Desire Collen. His team was looking for an inhibitor to that agent in order to interfere with the cancer cells' advance.

In 1979, Collen received a line of aggressive melanoma cells derived from a patient named Bowes. These melanoma cells showed an unusual amount of fibrinolytic activity, and Collen and colleagues worked to purify the fibrin-digesting agent. Within the year, he demonstrated that the agent was t-PA. It turned out that the Bowes cell line produced 100 to 1000 times more t-PA than any other known source at the time. "We realized we had a cell line that everyone had been looking for," he says. He began to turn

[3]Collen D and Lijnen HR. (2004) Tissue-type plasminogen activator: a historical perspective and personal account. Journal of Thrombosis and Haemostasis. 2(4): 541-546.

Figure 6: The widely practiced technique of bloodletting throughout history unwittingly relied on the potential of a leech protein to override the human body's natural blood clotting system. This woodcut is from Historia Medica by W. van den Bossche, which was published in 1638. *Science Photo Library/Photo Researchers, Inc.*

his attention to using t-PA to dissolve clots.

In the first half of 1980, Collen and colleagues used t-PA to dissolve clots in rabbits with pulmonary embolus and later reported their results in *Nature*[4]. Before publication, Collen presented his findings at a conference on June 12, 1980. "I still remember it," he says. "After the presentation a young lady, Dr. Diane Pennica, came up to me and said she was interested in cloning t-PA and then to make a recombinant form."

Although the Bowes cell line was prolific compared to other cell lines, it could not produce enough t-PA for a marketable new drug. Making larger quantities of a cellular product required coaxing another type of cell, grown in tissue cultures, to churn out the protein like a mini factory. The new biotechnology industry envisioned using recombinant DNA technology to identify, clone, and sometimes fine-tune genes for specific products, and then insert the genes in the DNA of cells that could mass-produce those products. In this case, they would insert the gene for t-PA in a cell line that would then "manufacture" the t-PA as a recombinant protein.

As Collen explains, Pennica had just joined the very small biotech firm Genentech, which had fewer than 100 employees. When he agreed to collaborate with the company to work on a recombinant t-PA (rt-PA), they embarked on a new model for drug development: the academic and biotech collaboration that is routine today. Within a year, working 80 to 90 hour-weeks, Pennica had cloned the gene for human t-PA and was producing rt-PA in a tissue culture of Chinese hamster ovary cells. That was a speedy accomplishment in the early 1980s, but with today's technology, "a graduate student should be able to do this work in a week," Collen says.

A serendipitous discovery by cancer researchers had unanticipated benefits in improved thrombolytic therapy for heart attack and stroke. "We realized we had a cell line that everyone had been looking for."

Collen and U.S. collaborators, including Sobel, next demonstrated in animal experiments that t-PA could dissolve coronary clots without inducing systemic bleeding, followed by a similar "proof of concept" study involving seven patients with coronary thrombosis. After testing the recombinant form, rt-PA, in experimental animals, promising results from a small, placebo-controlled trial later in 1984 set the stage for large-scale randomized multi-center clinical trial.

In just three more years, rt-PA had received FDA approval for treating acute myocardial

[4]Matsuo O, Rijken DC, and Collen D. (1981) Thrombolysis by human tissue plasminogen activator and urokinase in rabbits with experimental pulmonary embolus. Nature. 291 (5816):590-591.

infarction and soon hit the market. Seven years from bench to bedside (1980-1987) still stands as one of the fastest drug development processes in history, in spite of its occurrence during biotechnology's infancy.

The Life and Death of a Clot[5]

The blood clotting system is a good example of how the body usually maintains a delicate balance between opposing actions. In this case, the forces leading to a blood clot are opposed by those that dissolve the clot, which are countered by another process that slows down the dissolution of the clot to give time for the healing process to work. When those forces get out of balance, or when one of the players is missing or defective, diseases and disorders rear their ugly heads.

The key players in clot-building system are the proteins thrombin and fibrin, and special blood cells, the platelets. Think back on the finger prick and its tiny vessel break. Numerous round, white platelets gather and then adhere to the tissue below the vessel surface. The clump of platelets forms a plug that begins to prevent blood loss.

Throughout the body, cells are constantly "talking" to each other, sending molecular signals near and far that trigger various types of activity. At the finger prick, such signals trigger the formation of thrombin, which summons up more clot-building activity.

Meanwhile, an inactive protein called fibrinogen circulates in the bloodstream, but does not interact with other blood proteins. At the site of the finger prick, however, fibrinogen encounters thrombin, which activates the fibrinogen, transforming it into fibrin. Fibrin proteins coalesce into long, ropey

Fibrin is a key player in building a blood clot. Plasmin and tissue plasminogen activator (t-PA) are the key players in dissolving a clot.

fibers that settle around the platelets, reinforcing and anchoring the plug to prevent blood loss while other systems kick into gear to repair the damage. Fibrin fibers function in a clot like the supporting meshwork holding boulders along a highway or the rocks of a dike. This meshwork stops blood loss, but allows some blood to pass through to the tissues, providing nourishment while the wound is under repair.

At the molecular level, the system works essentially the same whether a clot forms in response to an injury to prevent blood loss (hemostasis) or in a misguided response (thrombosis) to a misinterpreted threat, such as arterial plaque. Thrombosis is any unwarranted response, in the absence of imminent blood loss, that blocks a blood vessel.

Even while a clot forms, other molecules begin to dissolve it, a counter-process called fibrinolysis. The cast of characters for this biological drama includes plasmin, its precursor plasminogen, and plasminogen activators, such as t-PA.

Figure 7: The molecular structure of tissue plasminogen activator (t-PA).

Plasminogen flows ubiquitously through the circulatory system but, like fibrinogen, does not interact with other molecules until activated. In fibrinolysis, the presence of fibrin in a clot alerts the plasminogen activator protein t-PA to its upcoming role. When plasminogen encounters t-PA near the clot, the plasminogen is converted into plasmin. Plasmin snips apart the fibrin mesh supporting the clot. As fibrin degrades, the platelets are removed and the clot dissolves.

To prevent the natural fibrinolytic activity from happening too quickly, molecular inhibitors normally slow down the activation of plasmin, allowing the clot to linger as long as it is needed. When the inhibitors, for either genetic or environmental

[5]Jackson CM. (2002) Biochemistry of Haemostasis, in Bhagavan, N.V. Medical Biochemistry, 4th Edition. Academy Press. San Diego. 840-872.

(lifestyle) reasons, get out of balance and overpower the plasmin activation, fibrinolysis cannot function normally. Such an imbalance can lead to the thrombi that cause heart attacks and strokes.

When renegade clots form in the coronary arteries, they overwhelm the natural fibrinolytic process. Thrombolytic drugs are basically pharmaceutical interventions that provide a souped-up version of fibrinolysis.

How Streptokinase and t-PA Work

With the refined understanding of how plasmin becomes active at the site of a clot, researchers gained new insights into how streptokinase works as a thrombolytic. Scientists realized that SK bypasses the intermediary plasminogen activator (t-PA) and binds directly to plasminogen in a way that activates it. In addition, SK is unaffected by the natural inhibitors of plasmin, so it works quickly when applied as a thrombolytic drug.

However, SK activates not only the plasminogen that is bound to a fibrin clot. It also activates the normally inactive plasminogen that courses throughout the bloodstream, forming plasmin throughout the body. This widespread plasmin can degrade diverse molecules involved in coagulation. As a result, SK not only dissolves clots, it also predisposes bleeding in other organs and tissues.

Building a Clot

Hemostasis:
A Defensive Maneuver

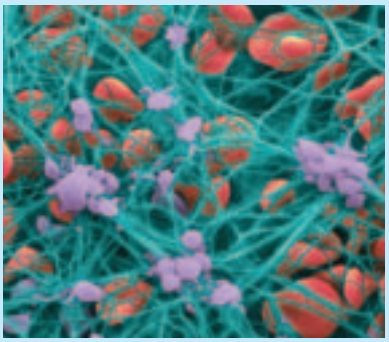
- Platelets gather around an injured vessel and form a plug.
- The injury triggers the creation of thrombin at the site of the wound.
- Thrombin activates fibrinogen at that site, transforming it into fibrin.
- Fibrin fibers cast a reinforcing net around the platelets, creating a clot that prevents blood loss.
- The fibrin clot prevents blood loss, while other systems in the body repair the wound.

Thrombosis:
A Biological Mistake

- Damage inside a blood vessel tricks the blood system into creating thrombin.
- Thrombin sets in motion the same process, forming a fibrin clot.
- The clot blocks the artery and shuts off the supply of blood and oxygen to the heart.

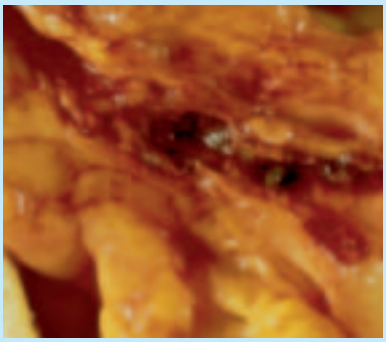
Clots Gone Wild

Like many of the body's intricate biological systems, what can help in one context can harm under other conditions. The normal blood clotting system is defensive, intended to prevent blood loss, a normal process called hemostasis. At other times, the body aggressively over-responds when there is not really a danger of blood loss, a disease process called thrombosis. In heart attacks and strokes, damage inside a blood vessel, often caused by cholesterol deposits, can trigger the same molecular reactions and produce an undesirable clot, called a thrombus. Unlike a normal clot, thrombi cut off essential blood flow to vital organs.



Colorized scanning electron micrograph of whole clot, showing fibrin (blue), platelets (purple) and red blood cells (red). *Photo courtesy of Dr. John Weisel, published in* Nature, *Vol. 413 (6855), used with permission. (Y. Veklich/J.Weisel)*

Colorized scanning electron micrograph of platelets. *Photo courtesy of Dr. John Weisel, published in* Journal of Biological Chemistry, *Vol. 278 (51) used with permission.)*



Gross specimen of tissue from a heart, showing a blood clot (thrombus, dark red, center) in one of the coronary arteries. These are the arteries that supply blood to the heart muscle itself. A blood clot in one of these can block or reduce the blood supply, cutting the oxygen supply that keeps the muscle functioning, which can cause a heart attack. *CNRI/Photo Researchers, Inc.*

Streptokinase's systemic activity is responsible for its major drawback as a thrombolytic drug: unwanted and potentially lethal bleeding. The risk of bleeding stems from the fact that heart attack victims generally have vascular damage elsewhere in the body in addition to the coronary arteries, and those damaged vessels are susceptible to bleeding.

Another drawback of SK relates to the human body's ability to recognize it as the product of a bacterial invader. The body produces antibodies to SK, and the antibodies can thwart the drug in subsequent doses. Additionally, a small minority of people can have a severe allergic reaction.

Like SK, t-PA activates plasminogen, which generates the fibrin-degrading plasmin. Unlike SK, however, human t-PA acts almost exclusively on the plasminogen that is bound to a fibrin clot. Thus, t-PA's activity is considered relatively "fibrin specific," meaning it only dissolves clots and carries a lower risk of systemic bleeding. Moreover, rt-PA is engineered to jump into high gear when sitting on a strand of fibrin. Because rt-PA derives from a human protein, it does not arouse the suspicion of the immune system, instigate an allergic reaction, or lose its effectiveness with a repeated dose.

Door-to-Needle Time

Practicing physicians wanted to see rigorous proof of clear-cut clinical benefits to patients before adopting thrombolytics as a treatment practice on a more widespread basis. Early promising evidence of SK's benefits came from studies using dogs with coronary thrombosis during the 1970s. After Dr. Marcus DeWood (mentioned previously) advanced his results establishing the causal link between clots and heart attacks, the NIH began planning a large-scale clinical trial for the early 1980s to test the safety and efficacy of SK for acute myocardial infarction. When researchers learned about t-PA, they added it, and later rt-PA, to the trials. Based on numerous studies that showed rt-PA's generally higher efficiency and its lower risk of bleeding or adverse immune response, it came to be viewed by many in the United States as the drug of choice, despite its high cost.

The clinical trial phase clearly established that time is of the essence when treating acute heart attack victims. The concept "door-to-needle time" emerged, indicating the time between when a patient first has symptoms and when he or she receives the first dose of the thrombolytic.

One trial showed that patients who receive thrombolytics—and successfully had their arteries opened—within one hour have only a 1% mortality rate during the next 30 days. Waiting another half hour raises the 30-day mortality rate to 6.4%. After 3 hours, 80% of the benefits were lost, and after 4 hours thrombolytics have virtually no effect in reducing mortality.[6] Thus, the practical clinical goal is to administer t-PA or SK within 90 (plus or minus 30) minutes of the onset of symptoms. A similar temporal window of opportunity was established for stroke in later trials.

Achieving the rapid door-to-needle time, however, requires that the patient recognize the early onset of symptoms and call for help immediately. Even with rapid treatment with thrombolytics, about 60% of heart attack patients still need more intervention to completely restore blood flow. Many of these are candidates for angioplasty.

In angioplasty, a catheter carrying a deflated balloon is inserted into the closed artery. The catheter injects a dye that enables real-time X-ray imaging of the heart's arteries. Depending on what these images reveal, the balloon may be inflated to open the artery and/or a stent placed to keep the vessel open. Less frequently, the clot can be removed or otherwise cut apart. The term percutaneous coronary intervention (PCI) covers all these intervention options. Like thrombolytics, PCI's success requires a rapid "door to balloon" time: 90 (± 30) minutes. If the surgery is delayed three hours, 80% of the benefit is lost.

[6]Bergman SR, Lerch RA, Fox KA, Ludbrook PA, Welch MJ, Ter-Pogossian MM and Sobel BE. (1982) Temporal dependence of beneficial effects of coronary thrombolysis characterized by positron tomography. American Journal of Medicine. 73:573-581.

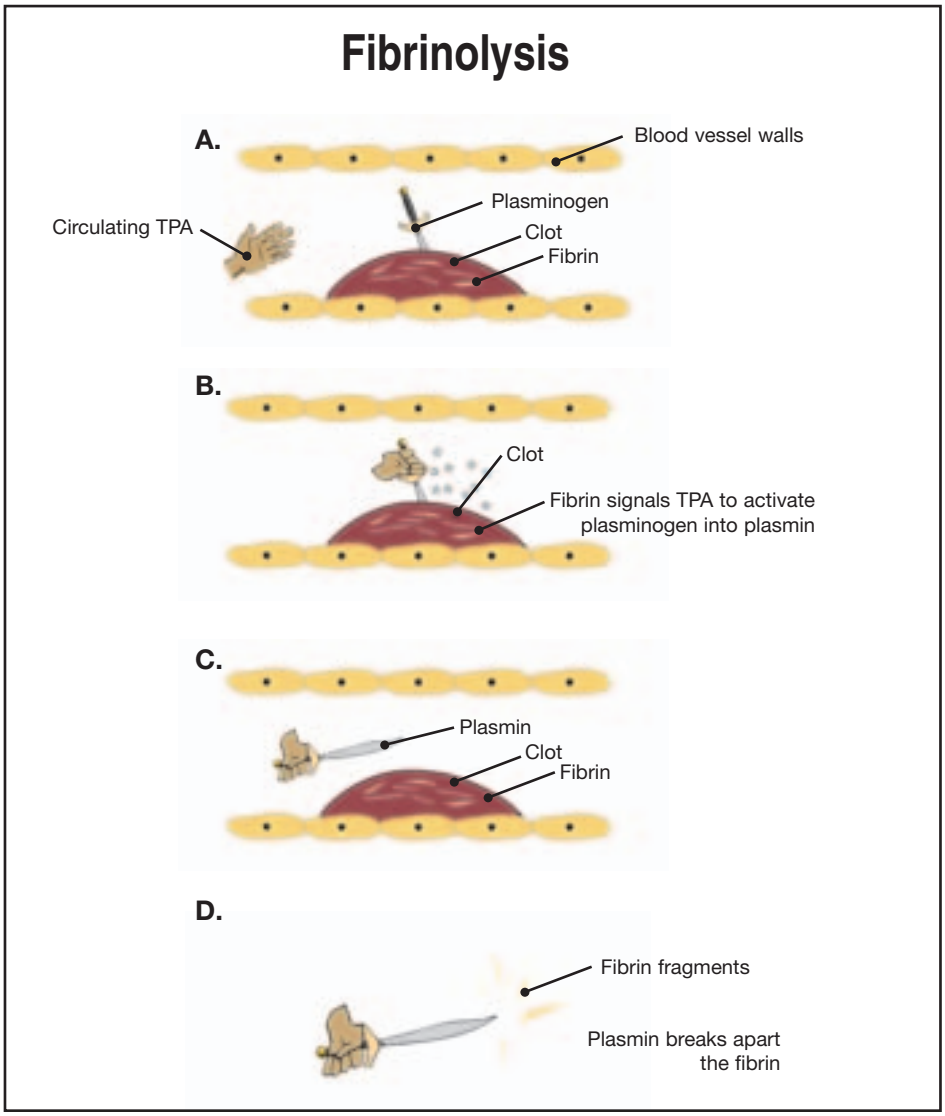


Figure 8: "Sword in the stone" analogy of fibrinolysis. The inactive substance, plasminogen, binds to fibrin (shown as yellow fibers within the clot) becoming incorporated into the clot as it forms. Tissue plasminogen activator (t-PA) circulates freely in the blood. (**A**) Fibrin within the clot sends our signals which activates t-PA, causing it to bind to plasminogen. (**B**) In turn, t-PA activates plasminogen, converting it to the enzyme, plasmin. (**C**) Plasmin cleaves through the fibrin fibers in the clot. The clot dissolves. (**D**) *Figure designed by Dr. Carrie Wolinetz and Corporate Press.*

In a typical community, however, there are practical barriers to using PCI.[7] Only 20-30% of U.S. hospitals (and fewer still in Europe) have the capacity (emergency cardiac units and catheterzion laboratories) to perform emergency PCIs in the narrow window of time required for best results. Despite increased

Most researchers came to see recombinant t-PA as the thrombolytic of choice because of its higher efficiency and generally lower risk of adverse side effects.

Thrombolytic Treatment for Heart Attack

Benefits:

- Thrombolytic agents alone reduce early mortality 30%, and they are even more effective (40%) when administered with the anticoagulant, heparin.
- Thrombolytics provide longer-term benefits for survivors, who have just a 5% mortality rate at one year.

Risk:

- The risk of a major bleed or brain hemorrhage generally affects 2-3% of heart attack victims. Minor bleeds, such as nosebleeds, are more common.
- The risk particularly affects patients with other health complications, those over the age of 80, and those being treated for strokes (who run, on average, a 6% risk).

Source: Fibrinolytic Therapy Trialists' (FTT) Collaboration; Fuster and Verstraete, "Thrombolysis in the Treatment of Acute Myocardial Infarction," 1992, p. 299.

[7]Pharmacoinvasive Therapy in Acute Myocardial Infarction. Edited by Harold L. Dauerman, M.D. and Burton E. Sobel, M.D., Marcel Dekker, Inc., New York, January, 2005. (Pre-publication)

Early Recognition of Heart Attack & Stroke Saves Lives

Heart Attack

- Pain, discomfort, pressure or numbness in chest or upper body.
- Pain may feel like indigestion or heartburn.
- Shortness of breath.
- Cold sweat, nausea, vomiting or feeling light-headed.

Stroke (all symptoms are SUDDEN!)

- Numbness or weakness, especially on one side of the body.
- Confusion or troubled speech, or trouble understanding speech.
- Trouble seeing with one or both eyes.
- Dizziness, difficulty walking, loss of balance or coordination.
- Severe headache with no known cause.

emphasis on the need for speed, hospitals reduced the door-to-balloon time by an average of only eight minutes during the 1990s. Thus, even though PCI can generally open up the blood vessels even more effectively than thrombolytics *when all other things are equal,* in most communities PCI comes too late to fully benefit.

The American College of Cardiology's guidelines now recommend that if transferring patients to an angioplasty facility delays treatment by one hour, doctors should consider first offering thrombolytics to open the arteries immediately, at least partially. Then, those patients who need further intervention can receive angioplasty or other procedures. That way, patients will benefit from both t-PA's rapid rescue of blood flow and from PCI's complete reopening of the artery.

Stroke Therapy Leaps Forward[8]

While thrombolytic therapy for heart attacks was advancing, so too was interest in using thrombolytics to treat strokes. One doctor calls the arrival of t-PA a "leap forward" in treating ischemic strokes.

It was previously thought that there was nothing doctors could do to stop a stroke in process. That view began to change during the second half of the 20th century, in part because scientists better understood the nature of this violent event deep in the inaccessible brain. As with heart attack researchers, that better understanding also raised the possibility that strokes could be treated.

Strokes come in two types. More than 80% are ischemic strokes caused by clots blocking blood flow in a vessel in the brain. The remainder, hemorrhagic strokes, result from bursting of a blood vessel that causes bleeding into the brain.

Research showed that quickly restoring the blood flow to the area of the brain blocked by a clot could salvage the tissue and avert brain damage. However, the medical decision to give a stroke patient a thrombolytic drug is more complicated than it is for cardiologists. Since some strokes are hemorrhagic, doctors must use a brain scan, CAT scan, or MRI to clearly exclude the possibility that the stroke is the bleeding type.

Stroke treatment is most effective when started within three hours of the onset of the stroke.

Recombinant t-PA received approval for treatment of ischemic stroke in 1996, following an NIH trial indicating that treatment with rt-PA could reduce long-term disability by 33% over untreated patients. The drug is currently being used in the U.S., Canada, Australia, and some European countries, and approval for use throughout Europe is expected soon.

In spite of the potential of rt-PA to reduce death and disability, only about 3% – 5% of stroke patients receive it. Door-to-needle time is equally critical in stroke as in acute myocardial infarction. Treatment with rt-PA is most effective when used as soon as possible after the stroke happens, preferably within the first 3 hours after stroke onset.

[8]Bousser, MG. (2004) Antithrombic Strategy in Stroke. Journal of Thrombosis and Haemostasis. 86:1-7.; Silliman S. (1998) Acute stroke therapy with intravenous tissue plasminogen activator. Jacksonville Medicine. 492-494.

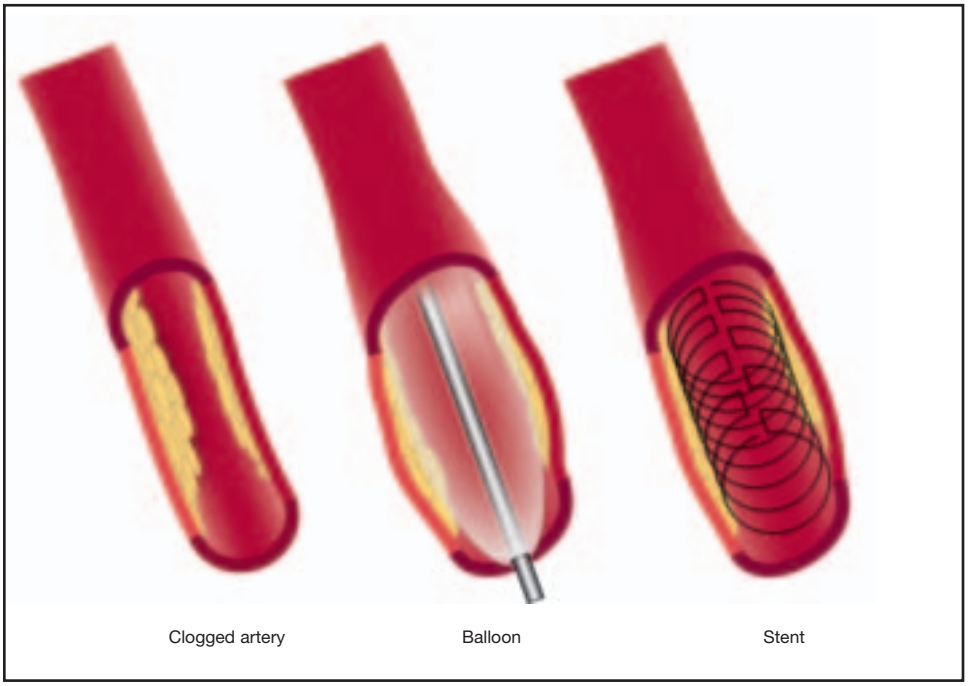


Figure 9: Percutaneous Transluminal Coronary Angioplasty (PTCA), often referred to as simply angioplasty, is used to widen narrowed or blocked arteries. It involves inserting a catheter through the patient's leg, arm or groin and moving it to the blocked artery. A balloon at the tip of the catheter is inflated, compressing the plaque on the walls of the blood vessel so that blood can flow more easily. Then the balloon is deflated and the catheter is removed. Many of these procedures also involve placing a stent. Stents are wire mesh tubes used to permanently hold the artery open to improve blood flow. *Designed by Corporate Press.*

Many patients do not seek treatment within this time to receive maximum benefits from thrombolytics, largely because of a low public awareness (17%) about the warning signs and symptoms of stroke. There also remains the fear of bleeding complications associated with thrombolytics, which discourages some doctors from prescribing them.

Good news may be on the horizon. Some early evidence suggests that some stroke patients may benefit from treatment with thrombolytics beyond three hours from stroke onset. Doctors are using new brain imaging techniques to identify and select those patients. Studies are also ongoing to examine new thrombolytic products that may have fewer risky side effects.

In other patients, however, studies indicate that after three hours of stroke onset, the blood vessels below the clot become so weakened that the blood-brain barrier is easily breached. Administering t-PA after that time frame can allow t-PA to seep through the vessel wall into the brain and increase the damage being done to the already-stressed neurons. Very recent research conducted by neuroscientists found that another drug, Activated Protein C (APC), protects neurons from the same cell-death programs that t-PA accelerates. APC is approved for use in sepsis, in which the toxins produced by infectious microorganisms poison cells. Depending on the outcome of safety and efficacy trials in humans, this combination could potentially extend the window of time for delivering t-PA safely and make t-PA beneficial effects available for many more patients.

With more patients surviving acute heart attacks and strokes, there is also more focus on how to maintain the survivor's long-term cardiovascular health. Breaking up the clot can resolve the immediate, acute problem, but not the underlying problem of vascular disease. Along with treatment by thrombolytics or angioplasty, all heart attack patients receive an anti-coagulant that prevents new clots from forming, a combination termed adjuvant therapy. In addition, a heart attack or a stroke is not a one-time event; they often recur. To prevent recurrences, survivors continue taking anti-coagulants and/or anti-platelets long-term. Anti-coagulants target and inhibit thrombin, thus preventing the formation of the fibrin that reinforces a clot. Anti-platelets

Thrombolytic Benefits for Stroke

- Early treatment with t-PA reduces long-term disability by 33% to 55% and saves an estimated $4 million for every 1,000 patients treated.
- Patient delay in seeking treatment prevents widespread use of thrombolytics.

prevent platelets from clumping together and forming the initial plug for a clot.

Coming Attractions

Three quarters of the world cannot afford rt-PA, comments rt-PA's co-creator Dr. Desire Collen, so most heart attack and stroke patients receive the less desirable streptokinase, if they receive therapy. Collen and others are now working on a microbial relative of streptokinase, called staphylokinase, that elicits a much weaker and slower immune response than SK. Produced by the staphylococcal bacterium, staphylokinase is fibrin specific, like t-PA, so it does not pose as great a risk of bleeding as SK. Because it is the natural product of bacteria, like SK, it should not be as expensive as rt-PA. As Collen explains, staphylokinase could have the safety and efficiency of rt-PA, with the low cost of SK.

In addition to staphylokinase, several new modifications of rt-PA have entered the market or are in the drug pipeline. One drug, known as TNK-rt-PA, a modification of the initial recombinant drug, remains in the bloodstream longer and is more specific in its activity. Researchers continue using molecular genetics and biochemistry to develop new thrombolytics that activate plasmin

Figure 10: The saliva from vampire bats serves as the basis for a new candidate for a thrombolytic therapeutic. *Photo courtesy of the Organization for Bat Conservation.*

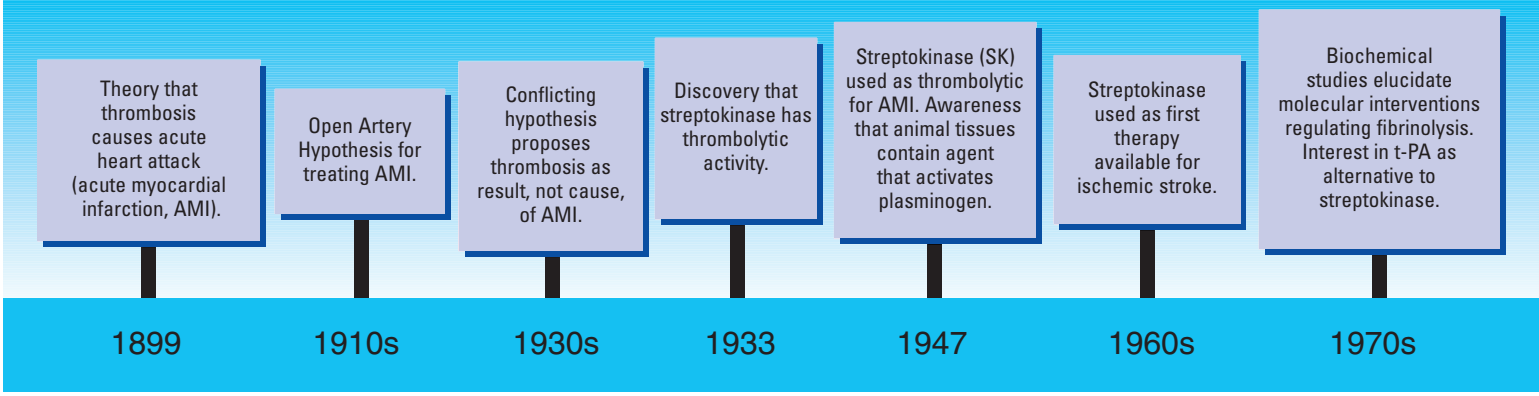
more efficiently with fewer negative side effects (such as bleeding and immune reactions) that currently exclude many heart attack and stroke patients.

On another front, researchers continue to study naturally produced thrombolytics. One new drug candidate derives from an enzyme in vampire bat saliva and may be effective as long as 9 hours after the onset of symptoms. This drug, desmoteplase (DSPA), is in clinical trails for stroke in Europe, Asia, and Australia. Extending the narrow window of time allotted to most existing thrombolytics would allow more patients benefit from the treatment.

As mentioned earlier, basic research in neuroscience suggests that combining t-PA with an already approved drug for sepsis, APC, may one day increase the benefits of thrombolytic therapy for a broader segment of stroke patients while decreasing the risks. This apparent coalescence of different lines of research is quite fitting for the field of thrombolytics, with its long and rich tradition of the intertwining of interdisciplinary fields.

Developing new and better drugs for adjuvant therapy is also a "hot field," focusing on drugs to prevent clots from forming in the first place, or from recurring after treatment for a heart attack or stroke. Currently, much of the research on anti-coagulants is studying how snake venom enzymes that dissolve clots work. That approach seems appropriate, since one widely used anti-coagulant, hirudin, is also derived from a naturally occuring protein from

Timeline: 100+ Years of Thrombolytics Development



a leech. Researchers are also introducing thrombolytics and related agents for the treatment of deep vein thrombosis and other cardiovascular conditions.

Half a century of basic research led to unanticipated new therapies and significant improvements in human health.

Before new drugs are introduced, a great deal of basic research still needs to be done, and then each drug candidate needs to pass the hurdles of clinical trials. Introducing a new drug that will be used by large numbers of people requires large trials, which have become prohibitively expensive. Dr. Desire Collen suggests that thrombolytics have become a victim of their own success; they have lowered mortality rates so significantly that it is difficult to measure incremental improvements in new modifications.

Out of Basic Research, A Life-Saving Drug

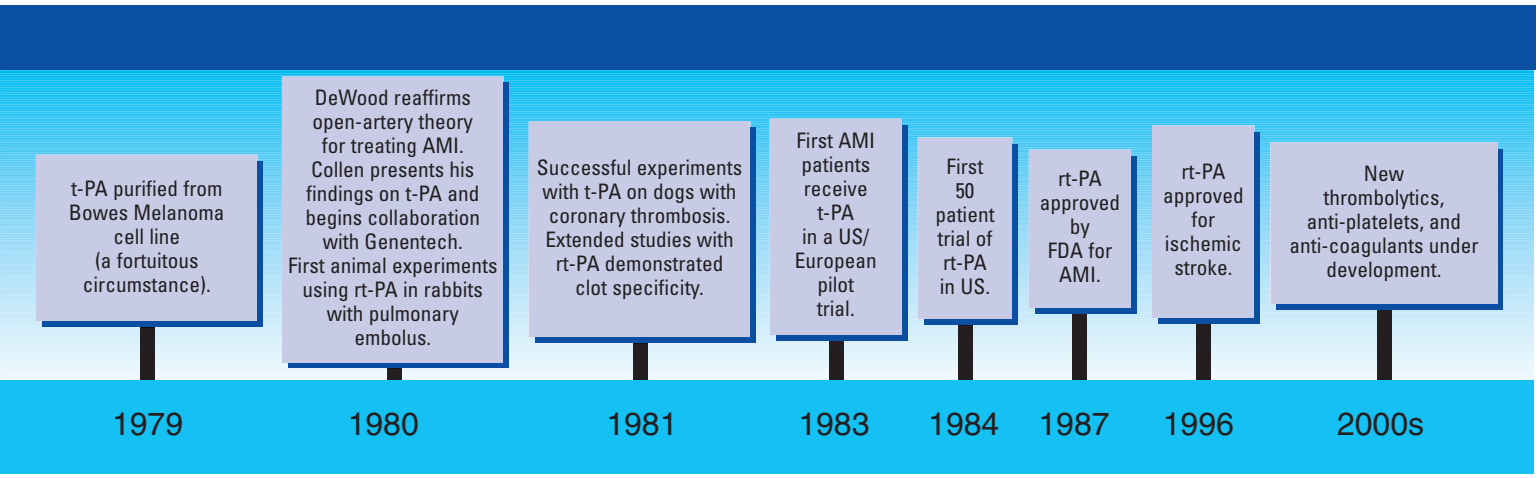
Over the past century, basic physiological studies led to a new understanding of the disease processes involved in heart attacks and strokes. Yet the steps from research to therapy did not follow a straightforward path.

Figure 11: Researcher milks venom from a Malaysian pit viper *(Calloselasma rhodostoma)*. The venom contains an anticoagulant drug which is being studied for use in treating stroke patients. This drug improves blood flow by reducing the amount of fibrinogen (a clotting protein) in the blood plasma. Strokes occur when the blood supply to part of the brain is cut off by a blockage or bleeding in the brain. They can lead to brain damage and are a leading cause of death. *Volker Steger / Photo Researchers, Inc.*

Science and medicine first needed to establish the cause and effect relationship between blood clots and disease. Only then would they realize the need for thrombolytic drugs and other interventions that could remove the endangering clots. Haltingly, the outcomes of basic research and clinical trials overturned one model of disease, and replaced it with a model that links the biochemical reactions of blood clotting to heart attack and stroke.

In the end, the contributions of medical researchers, microbiologists, cancer researchers, biochemists, biotech scientists, and cardiac specialists gave rise to the world's first widely used drug made from recombinant proteins, rt-PA. That work is not complete, however. There are still many refinements needed to reduce side effects, increase speed and effectiveness, decrease cost, and make the benefits available worldwide.

All together, curiosity, passion, and scientific and medical rigor brought about fundamental improvement in therapy for heart attack and stroke victims. Millions of lives are saved and prolonged by these developments. Serendipity and luck played a role in redirecting basic research towards thrombolytics, says Desire Collen, a principal figure in the clot busting story. "But the rest was blood, sweat, and tears."



Biographies

Cathryn Delude writes about science, medicine, biotechnology, water, and energy from Andover, Massachusetts. She has written for FASEB in the past, and also for *Scientific American, The Scientist,* the *Harvard School of Public Health Review,* Howard Hughes Medical Institute, the *Boston Globe,* and other publications, and she develops science education materials with The Writing Company.

Kenneth Mann, Ph.D., professor and chair of biochemistry at the University of Vermont, College of Medicine, is an international leader in the field of blood clotting research and holds several patents related to the diagnosis and study of functions that are critical in normal blood coagulation. He is chair of the National Hemophilia Foundation Research Working Group, a past vice-chair of medicine at the Mayo Clinic, past associate editor of *Blood* and presently associate editor of *The Journal of Thrombosis and Hemostasis* and *The Journal of Atherosclerosis and Thrombosis*. Mann has held volunteer positions with the National Institutes of Health, the American Heart Association, the American Society of Hematology, the International Society on Thrombosis and Hemostasis and the American Society of Biochemistry and Molecular Biology. He is the recipient of numerous national and international awards.

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Additional Resources

American Heart Association
7272 Greenville Avenue
Dallas, TX 75231
800-242-8721
http://www.heart.org

National Heart, Lung, and Blood Institute (NHLBI)
National Institutes of Health
P.O. Box 30105
Bethesda, MD 20824-0105
301 592 8573
http://www.nhlbi.nih.gov/index.htm

Stroke: Hope through Research.
National of Neurological Disorders and Stroke (NINDS)
National Institutes of Health
P.O. Box 5801
Bethesda, MD 20824
http://www.ninds.gov
800-352-9424

MayoClinic.com Heart Center/Brain & Nervous System Center
http://www.mayoclinic.com/

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