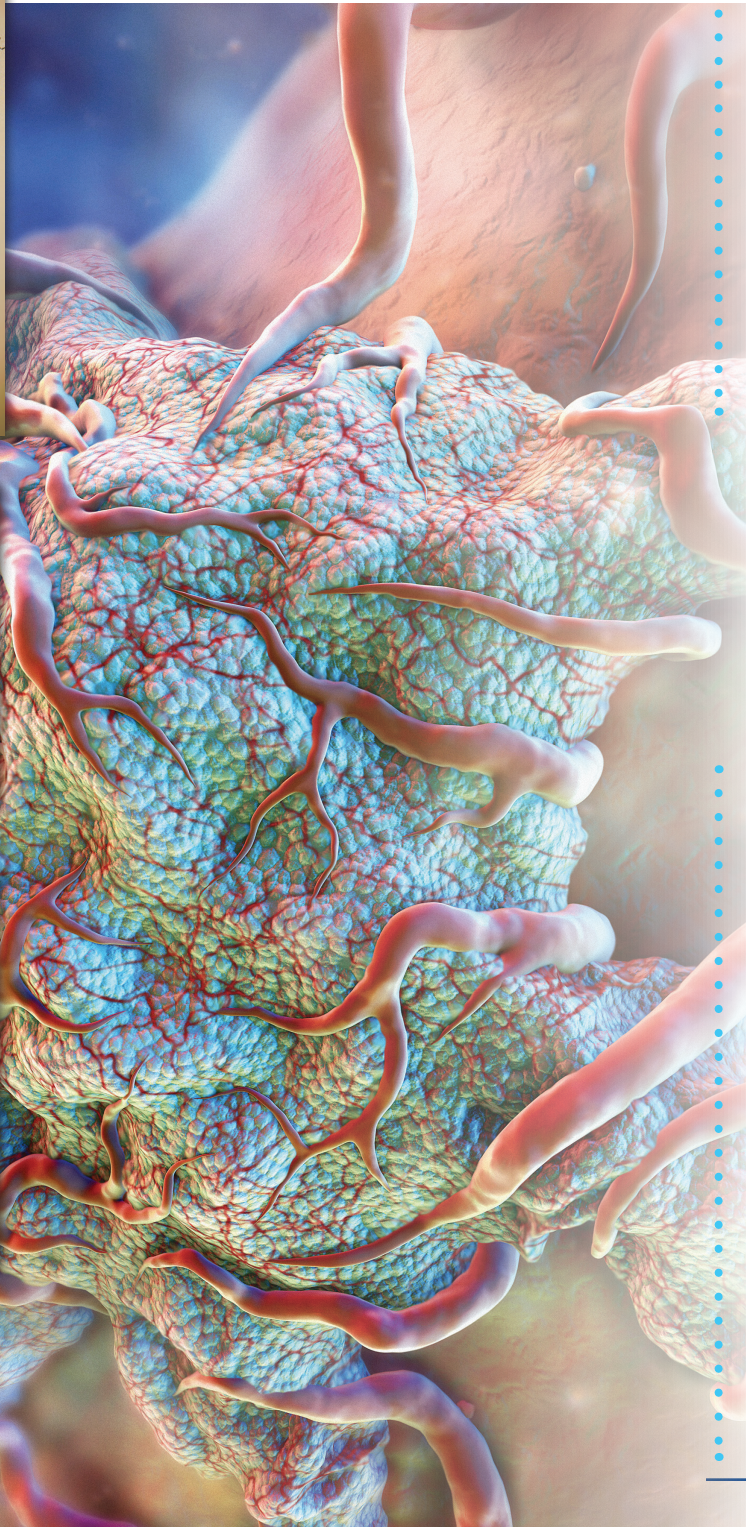
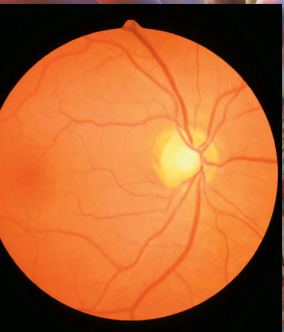
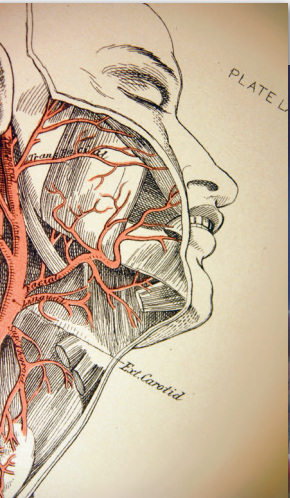


# Breakthroughs in Bioscience

Developed by the Federation of American Societies for Experimental Biology (FASEB) to educate the general public about the benefits of fundamental biomedical research.

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## Life's Blood: Angiogenesis in Health and Disease

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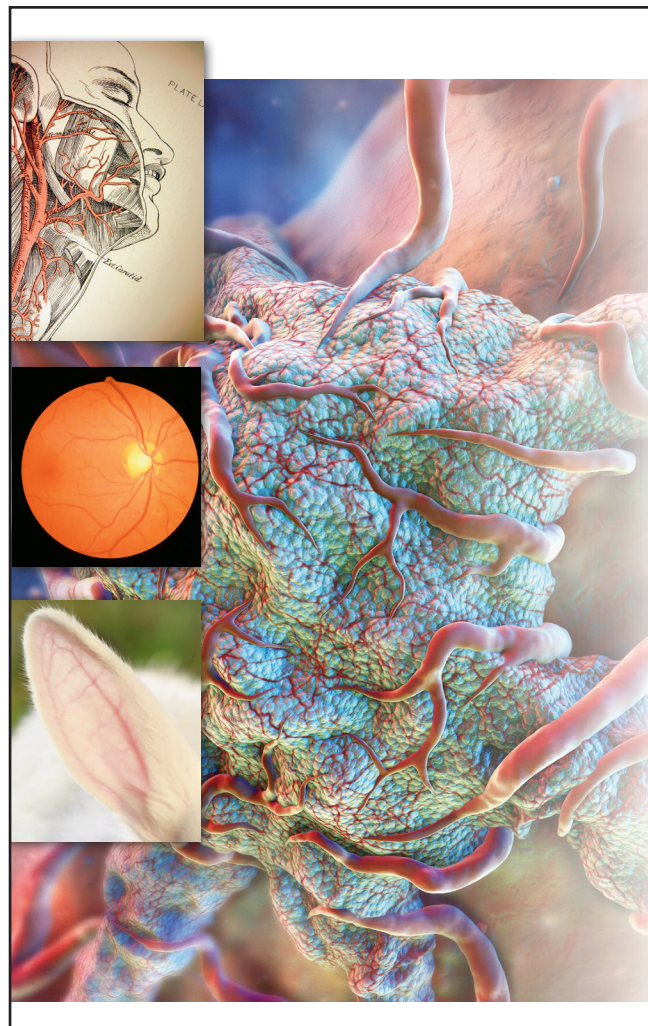
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**COVER:** Angiogenesis, or the formation of new blood vessels, plays a critical role in a number of diseases and conditions, including cancer, diabetic retinopathy, and wound healing. Decades of basic research discovery, from studying blood vessels through the thin membrane of a rabbit's ear to detailing the mechanism of how human blood vessels are formed, have contributed to a wide range of modern day therapies, based on increasing or decreasing angiogenesis. *Images from iStock Photo.*

# ANGIOGENESIS

## Life's Blood: Angiogenesis in Health and Disease

Discovery consists of seeing what everybody has seen, and thinking what nobody has thought.

*Albert Szent-Gyorgi, 1937 recipient of the Nobel Prize in Physiology or Medicine*

Our cells need oxygen and nutrients to survive. The cells of very small organisms can obtain what they need from the environment by simple diffusion, in which nutrients and gases pass from areas of high concentration to low concentration through cell membranes. But diffusion isn't very useful over distances greater than a millimeter or so. Larger animals, including humans, need a vascular system, a way for the oxygen and nutrients to get from the outside environment to every cell in the body (and for carbon dioxide and waste products to get back out to the environment).

Cells grow and multiply, and they die. Their metabolic rates can change, which changes their requirements for oxygen and nutrients. The vascular system can adapt to the body's changing needs by changing the amount of blood it delivers to the tissues. The formation of new blood vessels, a process called angiogenesis, needs to be highly regulated, and poorly regulated angiogenesis can have detrimental effects. Disease can occur if there are not enough blood vessels to feed the

tissues or, conversely, if blood vessels grow too exuberantly (Table 1).

What happens when circulation is insufficient to provide the nec-

essary oxygen and nutrients to the cells? Decreased blood flow to the legs and feet is a symptom of diabetes or the buildup of atherosclerotic plaques in the arter-

Diseases related to insufficient angiogenesis	Diseases related to excessive angiogenesis
<ul style="list-style-type: none"><li>• Alzheimer's disease</li><li>• Amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease)</li><li>• Diabetes</li><li>• Atherosclerosis</li><li>• Hypertension</li><li>• Crohn's disease</li><li>• Lupus</li><li>• Preeclampsia</li><li>• Nephropathy</li><li>• Chronic wounds</li><li>• Coronary artery disease</li><li>• Diabetic ulcers</li></ul>	<ul style="list-style-type: none"><li>• Cancer</li><li>• Multiple sclerosis</li><li>• Vascular malformations</li><li>• Obesity</li><li>• Psoriasis</li><li>• Warts</li><li>• Allergic dermatitis</li><li>• Kaposi's sarcoma in AIDS</li><li>• Diabetic retinopathy</li><li>• Primary pulmonary hypertension</li><li>• Asthma</li><li>• Cystic fibrosis</li><li>• Inflammatory bowel disease</li><li>• Periodontal disease</li><li>• Liver cirrhosis</li><li>• Endometriosis</li><li>• Ovarian cysts</li><li>• Uterine bleeding</li><li>• Arthritis</li><li>• Osteomyelitis</li><li>• Diabetic nephropathy</li></ul>

Table 1 – Examples of diseases related to problems with angiogenesis: The loss of control over angiogenesis (the formation of new blood vessels) can lead to a large number of disease states. This is true both when insufficient angiogenesis occurs or when blood vessels grow excessively. Many scientists and physicians believe that understanding and controlling angiogenesis is a unifying approach to treating a wide variety of diseases. Adapted from Carmeliet, P. 2005. Nature 438, 932-936.

ies of the legs. Insufficient blood supply can result in delayed healing of wounds as well as pain, numbness, or leg weakness, and the affected limb may ultimately have to be amputated.

Decreased blood flow to the heart due to atherosclerosis or a clot can damage or kill heart muscle, resulting in symptoms such as angina (chest pain) and shortness of breath. In its most dire consequence, cellular damage due to insufficient oxygen may lead to a myocardial infarction, otherwise known as a heart attack. Decreased blood flow to the brain may have similarly dire consequences, including stroke.

On the other hand, what happens if the formation of blood vessels is too exuberant? One consequence is blindness. In the U.S., diabetic retinopathy is the leading cause of blindness in adults of working age, and age-related macular degeneration is the leading cause of blindness in elderly adults. Both are the result of abnormal growth of new blood vessels in the eye. In these neovascular diseases, the new blood vessels that form beneath the retina (the back of the eye, where light is converted, via electrical/chemical signals, to nerve impulses that then go to the brain) are fragile and often leak, disrupting and damaging the eye tissue, resulting in vision loss (Figure 1). We also now know that cancerous tumors are dependent upon angiogenesis for growth and metastasis (spreading).

Recent breakthroughs in our understanding of angiogenesis

are leading to new strategies for treating these diseases. But these breakthroughs have been built on nearly four centuries of fundamental research on angiogenesis, as generations of scientists have asked the question: how do blood vessels grow?

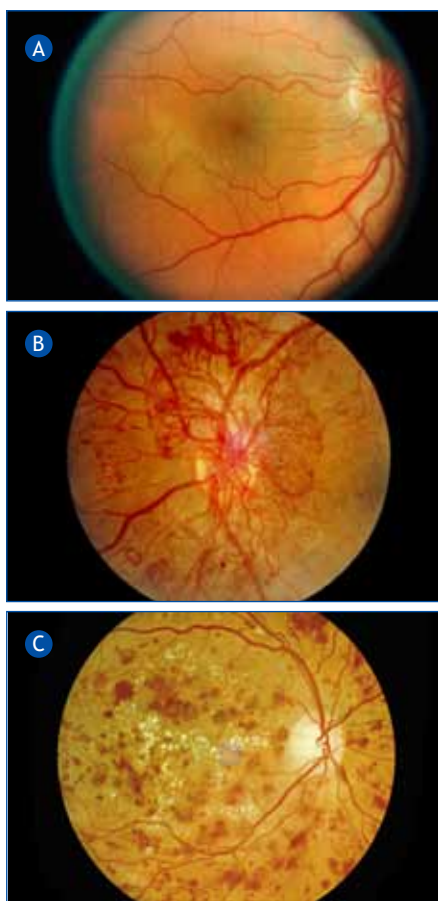
## Pumps and Pipes, Frogs and Fish

In 1628, William Harvey proposed the idea that blood circulated, with the heart acting as a

pump, propelling blood to the tissues via the arteries and back to the heart via the veins, much as water is transported through pipes. How the outgoing pipes were connected to the incoming pipes remained a mystery until 1661, when Marcello Malpighi used the newly invented microscope to observe tiny capillaries connecting the smallest arteries and veins. At around the same time, Antony van Leeuwenhoek examined the circulatory systems of tadpoles, fish, rooster combs, rabbit ears, and bat wings. In a letter, he noted that “the passage of the blood from the arteries into the veins of the tadpole, is not performed in any other than those vessels, which are so minute as only to admit the passage of a single globule at a time, we may conclude that the same is performed in like manner in our own bodies, and in those of other animals.”

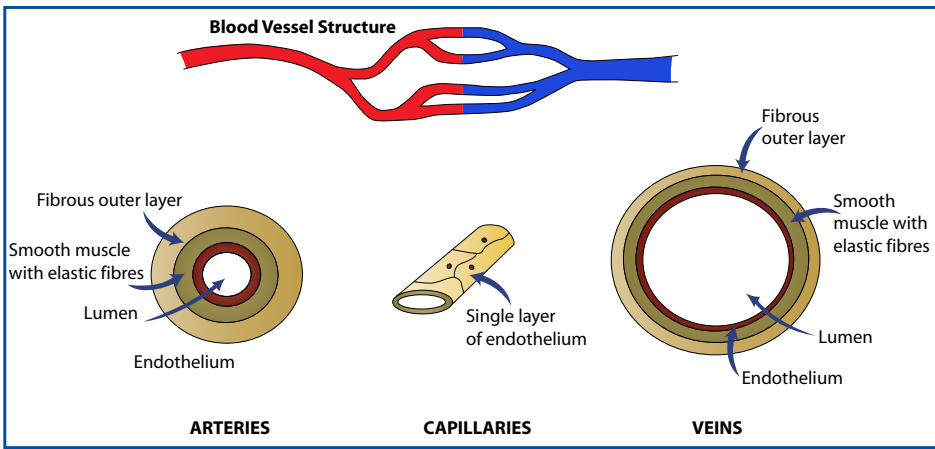
As the quality and power of microscopes increased, blood vessels could be observed in greater detail. It wasn't until late in the 19th century that it was confirmed that capillaries had walls consisting of a single layer of cells and a basement membrane (Figure 2). It is through the thin walls of the capillaries that the exchange of material between the blood and the tissues occurs. The vascular endothelial cells that make up the walls of the capillaries also line the insides of larger vessels and the inner surface of the heart.

Techniques for visualizing tissues under the microscope continued to advance. By inject-

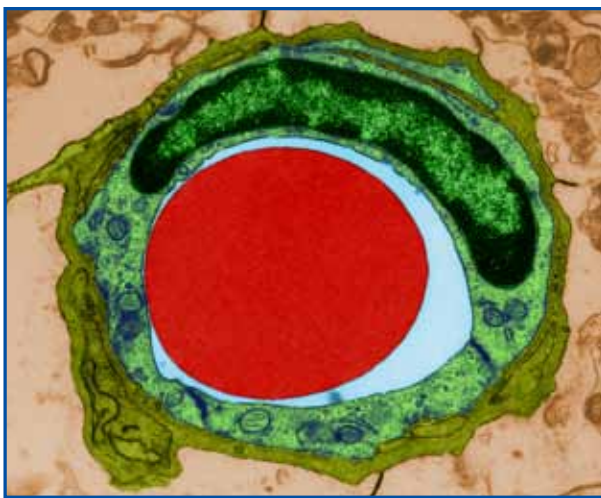


**Figure 1 – Diabetic retinopathy:** Ophthalmoscope views of a normal retina (A) and those from patients suffering from diabetic retinopathy (B, C). Retinopathy, or abnormal angiogenesis (blood vessel growth) in the retina, is a symptom of diabetes. The retina develops an excess of blood vessels (B), which are fragile and leak, causing hemorrhages (red spots seen in C) that can lead to blindness. Photo credit: Science Photo Library.

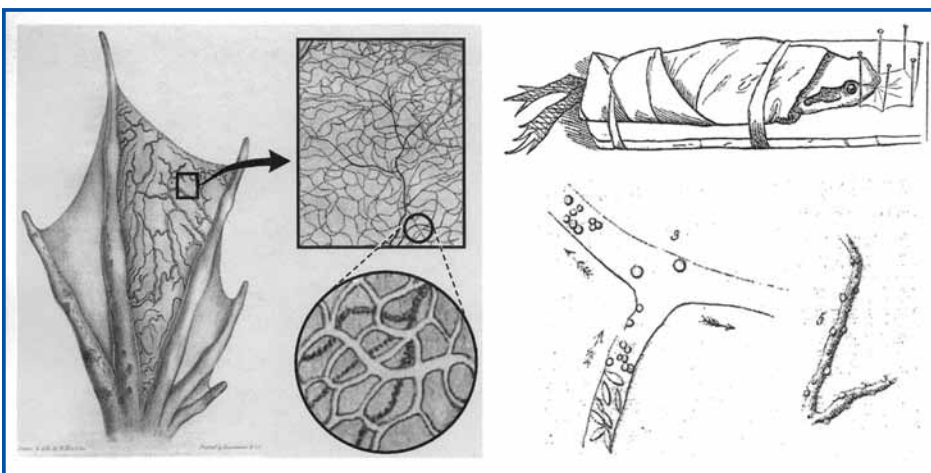




**Figure 2 – Structure of a blood vessel:** Arteries, which carry blood away from the heart, and veins, which return blood to the heart, are connected to each other and other tissues by very small blood vessels called capillaries. Capillaries are made of single layer of cells, called endothelium (singular = endothelial), and a basement membrane. Figure design by Corporate Press.



**Figure 3 – Single blood cell moving through a capillary:** This electron micrograph shows a cross section of a capillary that is just wide enough to let the red blood cell shown to pass through. The green, single layer of epithelial cells make up the capillary wall. Photo credit: Dennis Kunkel Microscopy, Inc.



**Figure 4 – Early studies of blood vessel development in frogs:** In the 1830s and 40s, scientists Marshall Hall and Augustus Waller observed and recorded capillary structure and function in the transparent tissue found in the feet and tongues of frogs. By observing these tiny vessels in living animals, researchers were able to see blood cells moving through the capillaries and the formation of new vessels over time. Source: Hwa, C. and Aird, W.C., 2007, *Am J Physiology Heart Circ Physiol*, 293. Used with permission.

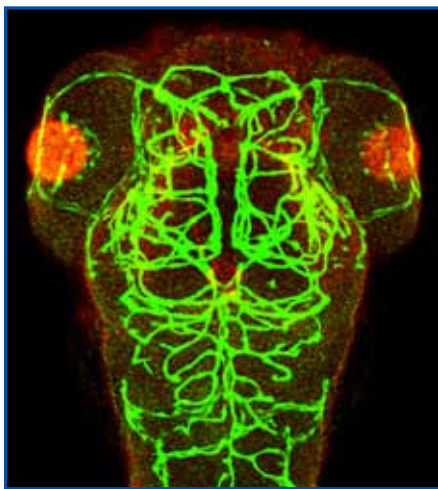
ing dyes or other chemicals into an animal’s circulatory system, researchers could trace the circuitous routes taken by the smaller blood vessels under the microscope. Eventually, the electron microscope provided even more detail. However, even the most sophisticated of these techniques required tissue to be removed from the animal after death.

By choosing relatively transparent tissues as a sort of living microscope slide, investigators were able to observe blood cells tumbling in single file through the capillaries (Figure 3). In the middle of the 19th century, scientists like Marshall Hall and Augustus Waller watched capillaries in action in frogs’ feet and tongues (Figure 4). An advantage to studying the living animal is that you can observe changes in the same animal over time. A microscope slide is just a snapshot of what we now know is a highly dynamic process. Not only did scientists see blood cells move through the capillaries, but they found that capillaries themselves can change over time. As early as 1844, Platner observed capillaries in the tails of tadpoles and newts sprouting from existing blood vessels and joining with other capillaries.

In 1918, Eliot Clark published a detailed study of the capillaries in the tails of individual tadpoles over the course of several weeks. As Clark observed his tadpoles, he made careful drawings of the changes he saw. Vessels sprouted, grew, and atrophied and Clark concluded that “devel-

opment into the complicated and nicely balanced system of the adult animal comes to be dependent upon the mechanical factors concerned with the pull and push of outside tissues, with blood-pressure and blood-circulation, and with the interchange of substances through the wall.”

The metamorphosis of a larval tadpole into an adult frog requires reorganization of many body systems, including the circulatory system. Any vertebrate animal, be it a frog or a human, undergoes massive changes in the process of changing from a fertilized egg to an adult, a process



**Figure 5 – Zebrafish:** A number of animal models have been used to study angiogenesis in normal physiology and disease. Recently, scientists have inserted genes into zebrafish which cause blood vessels to fluoresce green, allowing scientists to observe formation of new blood vessels and changes in response to stimuli in live embryos. Figure courtesy of Drs. Yung-Shu Kuan and Marnie Halpern.

that has been studied extensively throughout the last century. In a modern version of the tadpole tail, investigators have recently

inserted genes into zebrafish that cause the blood vessels to fluoresce. These fish are used to monitor how blood vessels form over time in a live embryo (Figure 5).

An early embryo is small enough for its cells to get the necessary oxygen and nutrients through diffusion. As it grows larger, too large for diffusion to be effective, a circulatory system begins to form, assembling the plumbing network required to transport what needs to be moved throughout the body. Embryonic endothelial cells lay down a network of capillaries. Over time, the embryonic vascular system sprouts new capillaries where needed and prunes capillaries away where they are not needed. Vessels enlarge and mature, adding layers of cells to become arteries and veins, supplying the expanding organism with a corresponding expanding vasculature in a process called vasculogenesis.

In a normal healthy adult, major changes in the vascular system only occur in specific circumstances, such as during the female reproductive cycle, or in the case of tissue injury, when new blood vessels are needed to replace damaged ones. Changes can also occur when the demand for oxygen increases (with athletic training, for example) or the availability of oxygen decreases (such as at high altitude). These changes are known as angiogenesis, and our understanding of how this process works and how we could exploit it to treat disease

## Three Ways to Build Blood Vessels

New blood vessels can be formed under many different circumstances—during embryonic development, in healing wounds, and in reproduction. Vessel growth involves at least three processes:

- Vasculogenesis is the formation of blood vessels from embryonic or bone-marrow-derived circulating endothelial precursor cells or other cell types, usually during embryonic development.
- Angiogenesis (sometimes called neovascularization) is the formation of new vessels, usually capillaries, by sprouting from existing vessels. Angiogenesis is thought to be driven by low oxygen levels.
- Arteriogenesis is blood vessel maturation by the addition of cell layers to make a larger vessel. Arteriogenesis is thought to be driven by mechanical factors, such as those Eliot Clark described in tadpole tails.

would not come to fruition until decades more of careful observation by scientists.

## Watching through Windows

At the University of Pennsylvania, Eliot Clark, who had so painstakingly described the capillaries in tadpole tails in 1918, worked side-by-side in his lab with his wife, Eleanor, an “unpaid volunteer.” One day, Eleanor accidentally pierced her finger with a glass tube as fine as a strand of hair. After several





**Figure 6 – Rabbits:** Eliot and Eleanor Clark (not pictured) from the University of Pennsylvania developed a method to study blood vessel development in the rabbit’s ear. Although a 1939 *Time Magazine* article described the experiments in a tongue-in-cheek fashion - “the rabbits are placid and happy, wear warm grey flannel pajamas, take vacations in Europe, occasionally feast on ice cream and cake” - this procedure allowed scientists to study angiogenesis in living mammals without the need for major surgery or heavy sedation. Although rabbits and other animals remain critical models in modern biomedical research, like the Clarks, today’s researchers continually seek new methods to refine experiments, reduce the number of animals used, and replace animal models with alternative methods. Photo credit: Science Photo Library

days, her finger swelled up, and they removed the glass. Being the meticulous scientists they were, the Clarks looked inside the glass tube with a microscope and saw capillaries growing there. This gave them the idea to make a “window” on the vascular system of a rabbit’s ear. In 1924, they, along with Clark’s student, J. Calvin Sandison, designed what was thereafter to be called the Sandison-Clark chamber, in which a small hole is punched in the ear of a rabbit, and the area is placed between two transparent plates. The tissue grows back to fill the area, which can then be observed by placing the chamber, still attached to the living rabbit’s ear, under a microscope (Figure 6).

The technique was widely adopted and the chamber became



**Figure 7 – John Hunter:** An 18th century Scottish surgeon and naturalist, John Hunter was one of the foremost scientists of his day and an early advocate of the scientific method. He was the first person to coin the term “angiogenesis” and conducted a number of important studies looking at the growth of new vasculature in the antlers of deer. Source: National Portrait Gallery, London.

a workhorse in the scientific community for decades. It was even featured in a 1939 issue of *Time Magazine*, which highlighted the Clark’s important work on vascular development. Later adapted to the skin on the backs of mice and to the cheek pouches of hamsters, the chamber created a window on the inner workings of living animals and has been used to study a variety of processes, such as inflammation and tissue transplantation, and to see how chemical and physical treatments affect these processes.

The Sandison-Clark chamber allowed up-close visualization of the healing process and of the formation of new blood vessels as tissue refilled the space in the chamber. The process begins as bleeding stops and a clot forms. Infiltrating cells replace the clot with a sort of temporary filler called “granulation tissue.” New blood vessels also appear in the wound at this time, sprouting off of the remaining uninjured vessels and reestablishing blood flow so that healing can continue. As the wound continues to heal, new cells enter and produce stronger, more permanent tissue. Scientists were now able to observe, and begin to understand, the formation of new blood vessels following damage or injury: angiogenesis.

## Dynamic Plumbing: Angiogenesis and Adaptation

Healing an injured part of the body requires rebuilding the

vascular system, starting from scratch. But the body can also remodel its plumbing, in other words undergo angiogenesis, in response to changing needs. It is especially responsive to oxygen (or the lack thereof). Reduced levels of oxygen (hypoxia) or reduced blood flow (ischemia) results in reduced oxygen availability for tissues. The noted Scottish physician, John Hunter (Figure 7) was an early investigator in this field, and conducted a classic experiment involving the antlers of male fallow deer.

In July 1785, Hunter tied off the artery that supplied one of a deer's antlers. The affected antler became cooler, indicating that it was not getting enough blood. Hunter expected the antler, deprived of blood, to die and fall off. In a week or two, however, the antler warmed up again. Thinking his procedure may have failed, he investigated further. The artery he had tied off was still blocked, but around that blockage he observed a network of new vessels that had grown, enlarged, and connected with other vessels to supply the antler. This is now called collateral circulation. Hunter applied his findings to vascular surgery in humans, devising a groundbreaking procedure to treat aneurysms in leg arteries, thus avoiding the need to amputate.

Blood vessels are not merely pipes through which blood flows. They are dynamic and able to adapt to changing conditions. When one route is blocked, alternate routes can form. Hunter may

not have been the first to observe collateral circulation, but he may have been the first to recognize its clinical significance. When Harvey first described the circulation of blood, he described the heart as a pump that circulated blood throughout the body. But the cells of the heart are just as reliant on blood as any other cell in the body. Lack of oxygen can damage the cells of the heart muscle, and they may function ineffectively, or even die. If the pump fails for lack of oxygen, the blood doesn't circulate, and the cells in the rest of the body will quickly fail; brain cells can only live a few minutes without blood.

The heart has a rich supply of blood vessels to keep the heart muscle supplied with oxygen. Not surprisingly, the heart has a backup, or alternate routes, in case the main supply is interrupted. In describing this widespread cardiac collateral circulation, Charles L. Hudson, Alan R. Moritz, and Joseph T. Wearn suggested, in 1932, that collaterals are "probably of significance in compensating for sclerosis [thickening or hardening] of the large trunks of the coronary arteries." Indeed, animal experiments and human postmortems have demonstrated that when coronary vessels are blocked, existing collateral vessels enlarge and additional collaterals form.

When the demand for oxygen increases, as with prolonged, intense athletic training, new capillaries can form to deliver more blood, and more oxygen, to

cells. Extensive physical training can promote the growth of collateral vessels in the skeletal muscles, heart, and parts of the brain. The increased demand for oxygen may be a trigger for vessel growth. In addition, mechanical forces are generated by increased blood flow in collaterals when flow is restricted elsewhere and this may also contribute to the growth of collaterals. Collateral growth is the body's clever way of using angiogenesis to adapt to new challenges. But scientists were soon to discover that our body's tissues were not the only ones using angiogenesis for survival.



**Figure 8 – Judah Folkman:** A pioneering scientist who proposed targeting angiogenesis as an approach for cancer therapy, Folkman first became interested in medicine when accompanying his father, who was a rabbi, on visits to patients in the hospital. Folkman strongly believed in angiogenesis as a unifying concept in developing new disease therapies, a vision which is being realized with an array of angiogenesis-related drugs on the market. Courtesy of the Children's Hospital, Boston.



During his stint in the navy, Judah Folkman and his colleague David Long encased substances within a synthetic silicone rubber polymer (a very large molecule composed of numerous repeated subunits). When this was implanted into an animal, the substance leached out of the polymer, allowing slow, sustained delivery into the tissue. Twenty years later, with the participation of Sheldon Segal of the Rockefeller Foundation's Population Council, this technology was used in the highly successful implantable contraceptive Norplant®.

Later, while studying angiogenesis at Harvard, Folkman needed a way to let suspected angiogenic factors leach slowly into the corneas of his experimental animals. The silicone rubber he had used previously had worked with small molecules, but was not useful with larger ones, like proteins. Folkman asked Robert Langer, an engineer from MIT who was working as a post-doc in his lab, to design a system to allow proteins to be released slowly, and Langer devised a method of encasing the experimental proteins in polymers that allowed them to be released over a long period of time. They published a description of the procedure in *Nature* in 1976.

Langer saw the potential for the sustained release of drugs in other applications and went on to devote his career to developing new methods to control the release of drugs. He was a pioneer in the development of transdermal patches, which are now used to deliver such diverse substances as nicotine for smoking cessation, estrogen for menopause symptoms, and nitroglycerine for angina.

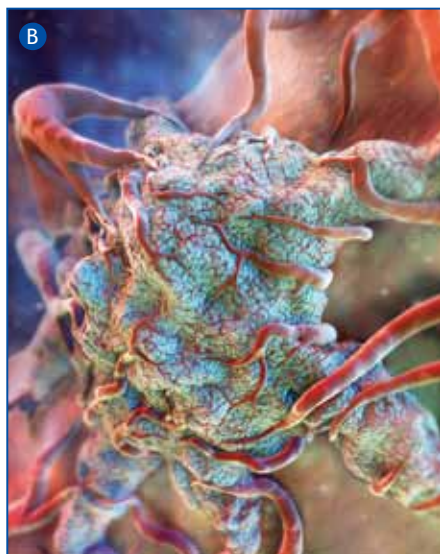
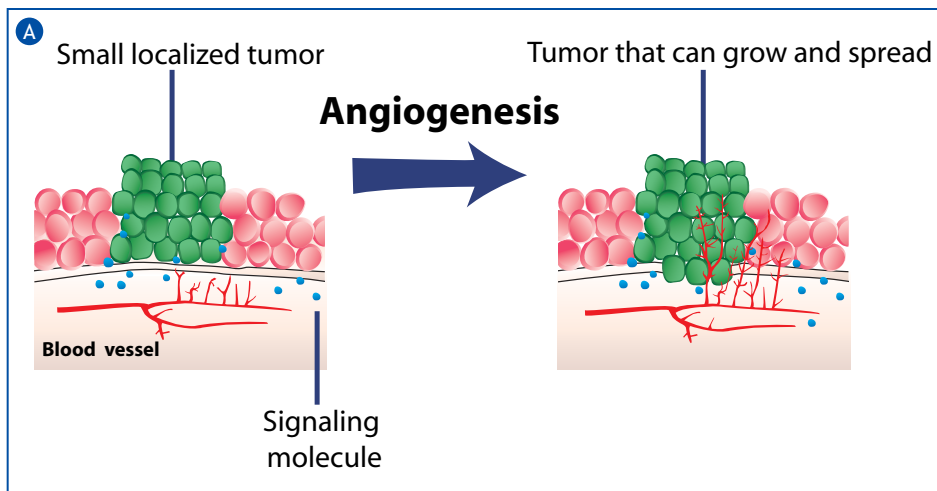
## Blood and Tumors

In 1960, during his surgical residency at Massachusetts General Hospital in Boston, Judah Folkman (Figure 8) was drafted by the military. While serving his two-year stint in the navy, Folkman worked at the Naval Medical Research Institute in Bethesda, Maryland. He and NYU pathologist Fred Becker were assigned a project to come up with a cell-free blood substitute for transfusing into patients when fresh blood was not available. The idea was to dry hemoglobin, the oxygen-carrying protein in red blood cells, so that it could be reconstituted and keep a patient's cells alive until fresh blood could be obtained.

Folkman and Becker infused thyroid glands from dogs with various solutions to keep the organs alive outside the body. Their system could keep the thyroid cells alive, but to determine whether the system would allow cells to grow—instead of merely surviving—they transferred mouse tumor cells into the glands, cells they knew could multiply rapidly. The tumors grew, but only to the size of a pin head. If the same tumors were implanted into live mice, they grew like wildfire. When Folkman removed the tumors and examined them, he noted that the tumors that had grown were filled with blood vessels, while the tiny tumors were not. This planted the seed of an idea into Folkman's brain: tumor growth depended on the growth of blood vessels.

This was not an entirely novel observation. As early as 1865, German physician Rudolf Virchow, often called “The Father of Pathology,” observed that tumors were filled with blood vessels. In 1913, James Murphy placed rat tumor cells onto the membranes of developing chick embryos and noted that “numerous dilated vessels are seen coursing through the membrane and penetrating the semitranslucent grayish tissue of the tumor itself.” A quarter of a century later, Gordon Ide used the Sandison-Clark rabbit ear chambers to study the vascular reaction to tumor cells and proposed that tumors need new blood vessels to provide them with the oxygen and nutrients they needed for growth. In 1941, Harry Greene transplanted rabbit tumors into the eyes of guinea pigs, noting that “the tumors obtained a blood supply from the foreign host.” Glenn Algire and his colleagues at the National Cancer Institute adapted the rabbit ear window to the skin on the backs of mice in 1945 and suggested that “the change in the tumor cell that enables it to evoke capillary proliferation is the only change necessary to give the tumor cell its increased autonomy of growth relative to the normal cell from which it arose.” (Figure 9)

Although Folkman wasn't the first to note the connection between tumors and vascularity, he was the first to expand the idea further and propose a clinical application. Just as small



**Figure 9 – Tumor angiogenesis:** Tumor angiogenesis refers to the growth of new blood vessels that infiltrate cancerous tumors, supplying the tumor with nutrients and oxygen. Angiogenesis occurs in response to the tumor sending out signals to the tissues surrounding them, which triggers blood vessel proliferation (A). Blood supply is important to tumor growth and metastasis (spread), and tumors are closely associated with their blood supply, as seen in this computer stimulation (B). Image credit: National Cancer Institute, Corporate Press, and Science Photo Library

organisms can survive without a vascular system by exchanging materials with the outside environment by diffusion, Folkman reasoned that the tumors were only able to grow to a small size without a vascular system. He suspected that the tumors did something to encourage the growth of the blood vessels they needed, but he had to do more experiments to confirm his speculation. After his service to the military ended, Folkman returned to Massachusetts General Hospital and continued his research. Meanwhile, two groups of investigators, one at Chicago Medical School and another at

Harvard Medical School, adapted the Sandison-Clark rabbit ear chamber to the cheek pouches of hamsters. By placing a membrane between the tumor and the tissue, they showed that the tumor produced a factor that could pass through the membrane to promote angiogenesis on the other side. Direct contact wasn't required; the factor could diffuse from the tumor to the vessels it affected.

Folkman set about trying to identify that diffusible factor. He and his coworkers ground up rat tumors and made a crude preparation of its proteins, which he passed over a column that

separated the proteins by size into a series of test tubes. He injected the fluid from each tube under the skin of rats to see if it induced blood vessel growth in the skin in order to find which tube contained the mysterious factor. The factor itself was not purified or characterized in any detail, but because of its ability to induce blood vessel growth, Folkman dubbed it “tumor angiogenesis factor,” or TAF.

The identification of TAF—and the discovery that tumors require a new blood supply to grow bigger than a pin head—prompted Folkman to hypothesize that interference with angiogenesis, or with TAF in particular, might rob a tumor of its blood supply, and thus inhibit its growth. He proposed anti-angiogenesis as a novel treatment for cancer in a talk in October 1971. A transcript was published in the *New England Journal of Medicine* a few weeks later.

His hypothesis was met with indifference, skepticism, and even ridicule.

Despite a lack of support from his peers—and funding agencies—Folkman persisted with his experiments. He placed tumor cells in a lab dish, where they grew unrestricted when lying flat against the bottom, bathed in nutritive medium. When the cells were grown in solid medium, in three dimensions, they only grew to spheroids of about 3 or 4 mm in diameter--about a million cells. Folkman hypothesized that that was the limit beyond which cells



could obtain the nutrients they needed through simple diffusion—without a vascular system. He also looked to the eye, and its unusual blood supply. Folkman and a post-doctoral fellow, Michael Gimbrone, found that tumors placed near the iris of a rabbit’s eye, where there was a rich blood supply, grew quickly and to quite a large size. In contrast, tumors placed in the cornea, where there were fewer blood vessels, did not die, but failed to grow to a size bigger than a millimeter or so. All of the evidence seemed to confirm that angiogenesis was important to tumor growth. But how did this process work? What were the factors involved? And how could scientists use this information to halt the growth of cancer cells?

## The Search for Factors: Alphabet Soup

In the 1970s, Michael Gimbrone came up with a way to grow vascular endothelial cells in lab dishes, a feat that was once thought impossible. This particular advance expedited the search for angiogenic factors, reducing the need for experiments using live animals or chick embryos. The advent of molecular biology and other technical advances allowed the identification, isolation, and characterization of numerous angiogenic factors over the next few decades. (Table 2)

Some factors were isolated from tumors, but others were isolated from normal tissues. Two angiogenesis-promoting

<b>Angiogenic factors</b> <i>(promote angiogenesis)</i>	<b>Angiogenesis inhibitors</b> <i>(interfere with angiogenesis)</i>
<ul style="list-style-type: none"> <li>• Basic fibroblast growth factor (bFGF)</li> <li>• Acidic fibroblast growth factor (aFGF)</li> <li>• Vascular endothelial growth factor (VEGF)</li> <li>• Angiogenin</li> <li>• Epidermal growth factor</li> <li>• Interleukin 8</li> <li>• Granulocyte colony-stimulating factor</li> <li>• Placental growth factor</li> <li>• Platelet-derived endothelial growth factor</li> <li>• Tumor necrosis factor alpha (TNF-<math>\alpha</math>)</li> </ul>	<ul style="list-style-type: none"> <li>• Angiostatin</li> <li>• Endostatin</li> <li>• Interferons</li> <li>• Platelet factor 4</li> <li>• Thrombospondin</li> <li>• Tissue inhibitor of metalloproteinase (TIMP) 1, 2, and 3</li> <li>• Troponin 1</li> <li>• Angiopoeitin-2</li> </ul>

**Table 2 – Factors that stimulate or inhibit angiogenesis:** A number of naturally-occurring factors have been identified that either promote or inhibit growth of new blood vessels. (Note: List is not comprehensive.)

factors, called acidic and basic fibroblast growth factors (aFGF and bFGF), were first isolated from pituitary glands and brains. By the mid-1980s, they had been purified, their genetic sequences determined, and the genes were cloned, that is, their genetic sequences were inserted into bacteria so that large quantities of the proteins could be produced in the lab for further study.

At Beth Israel Hospital in Boston, in 1983, Harold Dvorak isolated a factor that made it easier for certain proteins to cross the vascular endothelium in such tissues as skin, lungs, muscles, and the retina. Dvorak called it vascular permeability factor (VPF). Meanwhile, at the University of California, San Francisco, postdoctoral fellow Napoleone Ferrara discovered a novel growth factor activity that stimulated the proliferation

of vascular endothelial cells. In 1989, while working in the department of cardiovascular research at Genentech, Ferrara purified the protein and cloned the gene, naming its product vascular endothelial growth factor—VEGF (pronounced VEJ-eff). Further characterization revealed that VEGF was identical to Dvorak’s VPF. In fact, in the early days, many of the same factors were given different names because investigators isolated them from different tissues or measured different activities, such as the abilities of VPF/VEGF to both stimulate vascular endothelium and make it more permeable.

It soon became clear that some factors, like VEGF, have multiple functions, and that many known factors also had activities that affected angiogenesis (Figure 10). Other multifunctional growth

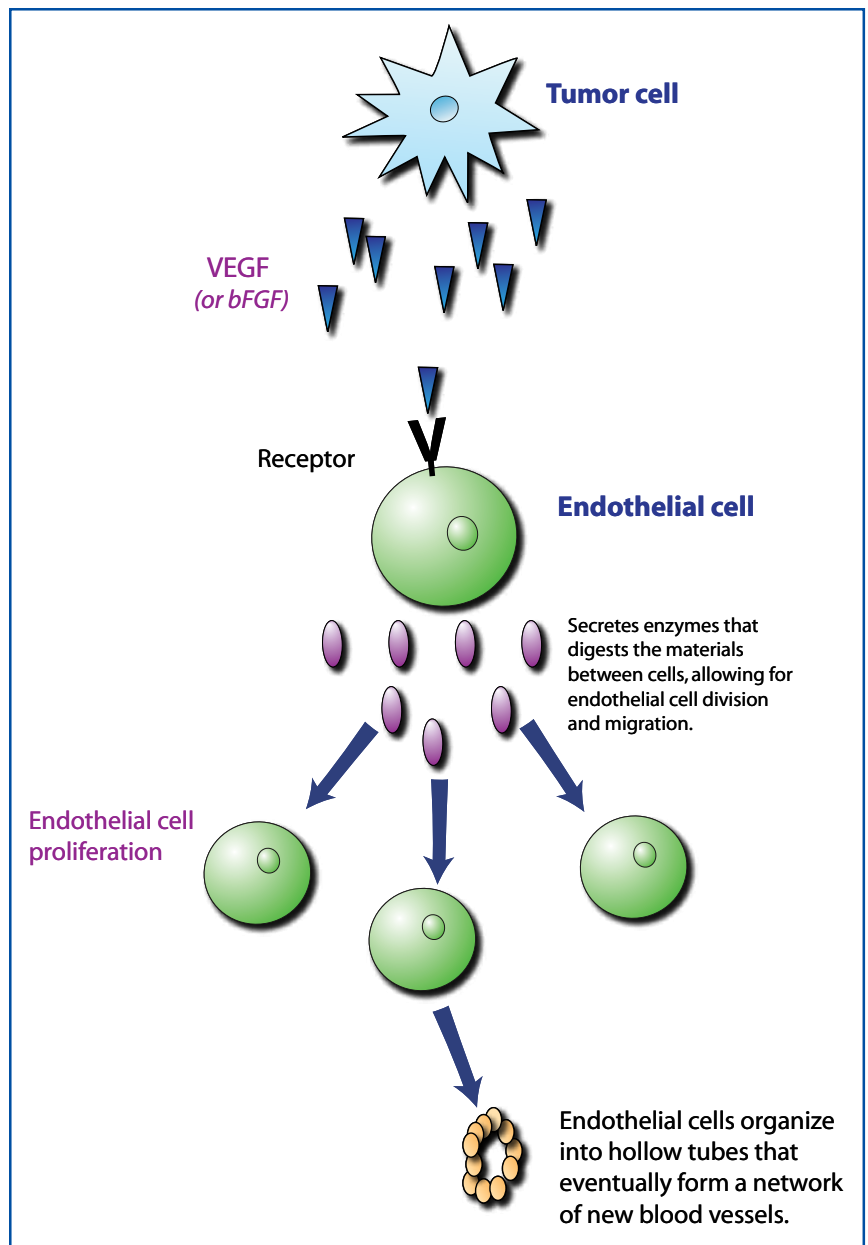
**Figure 10 – How angiogenic factors affect angiogenesis:** Tumors release angiogenic (promoting blood vessel growth) factors, such as vascular endothelial growth factor (VEGF) or basic fibroblast growth factor (bFGF). These interact with receptors on endothelial cells, setting off a series of events that lead to new growth of blood vessels. Endothelial cells release enzymes which degrade the proteins and complex sugars that are found in the matrix between cells in the surrounding tissues. This degradation gives the endothelial cells room to migrate and multiply (proliferate). Over time they will begin to self-organize into hollow tubes and then continue to grow into a mature, new network of blood vessels. Adapted from the National Cancer Institute, National Institutes of Health by Corporate Press.

factors with angiogenic properties include epidermal growth factor (EGF), tumor necrosis factor (TNF), platelet-derived growth factor (PDGF), and angiogenin.

Some multifunctional factors have activities that interfere with angiogenesis. Interferon, a protein released by the immune system, for example, was first identified as an inhibitor of virus growth in cells. Bruce Zetter, a member of the Folkman lab, showed that one kind of interferon, interferon- $\alpha$ , interferes with the proliferation and migration of endothelial cells, which is an important step in the formation of new capillaries. In other words, interferon has anti-angiogenic function in addition to its antiviral function.

### Switch Off, Switch On

Some naturally-occurring factors—like interferon- $\alpha$ —inhibit the growth of new blood vessels. Another rich source of inhibitory factors turned out to be the tumors themselves. By chance, Noël Bouck, a researcher at Northwestern University



School of Medicine found herself listening to one of Folkman's talks in 1987. (Later, Bouck would reveal that she ended up in Folkman's lecture because she had worn a new pair of shoes to the conference and needed a place to sit down to rest her aching feet!) She was inspired by what she had seen and subsequently went on to show that thrombospondin, a factor first isolated from blood platelets, was also released by tumors and had

anti-angiogenic properties.

After reading Bouck's thrombospondin paper, Folkman formed a new hypothesis to explain a vexing problem for cancer surgeons—removal of some tumors causes tumors in other sites of the body to grow more rapidly. Folkman hypothesized that cells from the main tumor metastasize, or spread, to other parts of the body, but that the distant tumors only grow to a limited size. The main tumor prevents



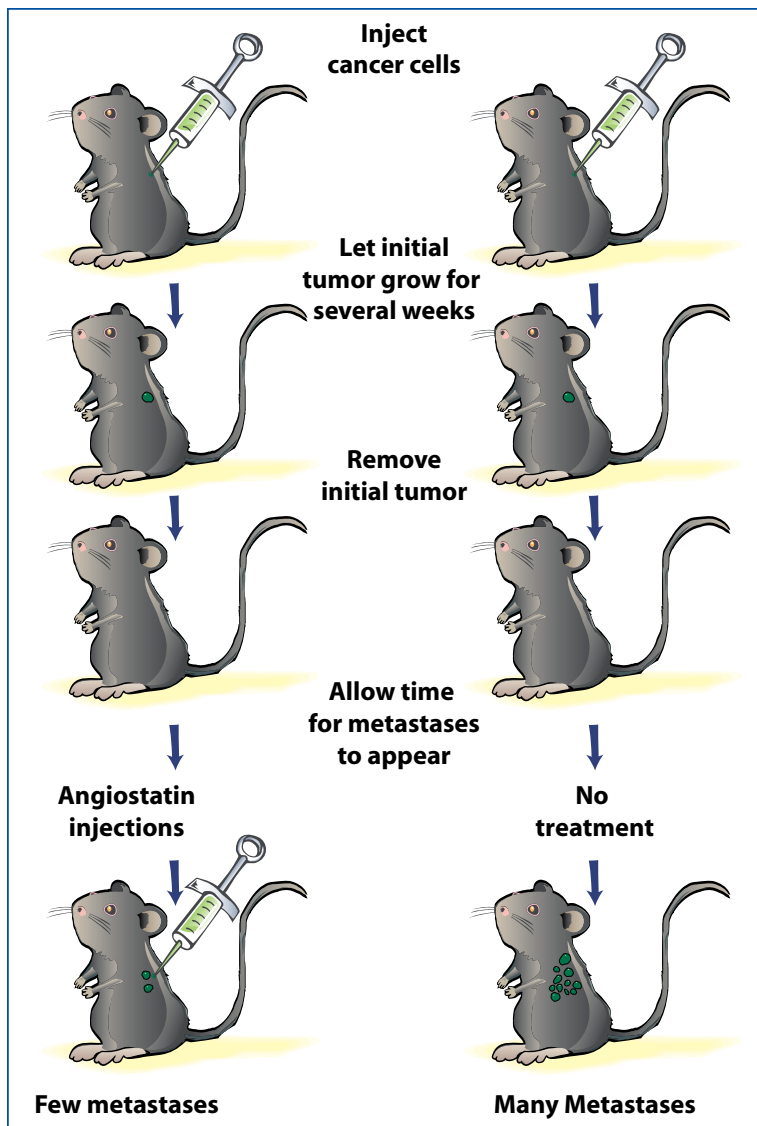


Figure 11 – Angiogenesis inhibitors and metastasis: Michael O’Reilly, a postdoctoral fellow working with Judah Folkman, first isolated angiostatin, a naturally occurring protein that inhibits angiogenesis. Angiostatin is produced by primary tumors and prevents the growth of metastases, or secondary tumors that occur when cancer cells spread. Scientists have shown that if the primary tumor is removed, as shown in this diagram, metastasis may occur unless an inhibitor, such as angiostatin, is administered. This helped explain a problem that had baffled cancer surgeons: why removal of a tumor seemed to promote cancer spread to other tissues. The pursuit of angiogenesis inhibitors is a major area of research in the quest for new cancer treatments, and a number of angiogenesis inhibitors have already been approved as cancer drugs. Adapted from the National Cancer Institute, National Institutes of Health, by Corporate Press.

them from forming their own vascular systems by producing angiogenesis inhibitors. When the main tumor is removed, that inhibition is removed as well, and the distant tumors grow larger. Folkman compared it to throwing a switch; the balance of

pro- and anti-angiogenic factors determined whether angiogenesis was stimulated or inhibited. When the main tumor was present, it switched angiogenesis off in the other tumors, but once it was removed, the distant tumors switched angiogenesis on.

*Thalidomide: New Use for a Notorious Drug*

Introduced in the 1950s in Europe, thalidomide was used to treat insomnia and morning sickness. Although not approved by the FDA, it was used widely in Europe and Canada until 1962, when it was associated with birth defects in at least 8,000 (and as many as 20,000) babies of women who had taken the drug while pregnant. The defects included missing digits, stunted arms and legs, and deformed internal organs (see figure). The drug was taken off the market in most countries.

In the search for angiogenesis inhibitors, Robert D’Amato, who joined Folkman’s lab in 1992, decided to take another look at thalidomide. D’Amato found that the reason it caused birth defects was that it was interfering with angiogenesis. Although it took some doing to get approval for this controversial drug associated in so many people’s minds with tragic birth defects, the first human trials began in 2000. By 2006, thalidomide was approved by the FDA for the treatment of multiple myeloma.



Folkman set a new post-doc, Michael O’Reilly, to the task of isolating more angiogenesis inhibitors from tumors. O’Reilly overcame some technical hurdles and finally, after collecting 18 liters of urine from mice with tumors, he isolated an inhibitor, which they called angiostatin (Figure 11). A few years later, using a similar strategy, they came up with another inhibitor, called endostatin.

Not all inhibitors are found in humans, and some of them were discovered serendipitously. One example was TNP-470. An investigator in Folkman’s lab, Don

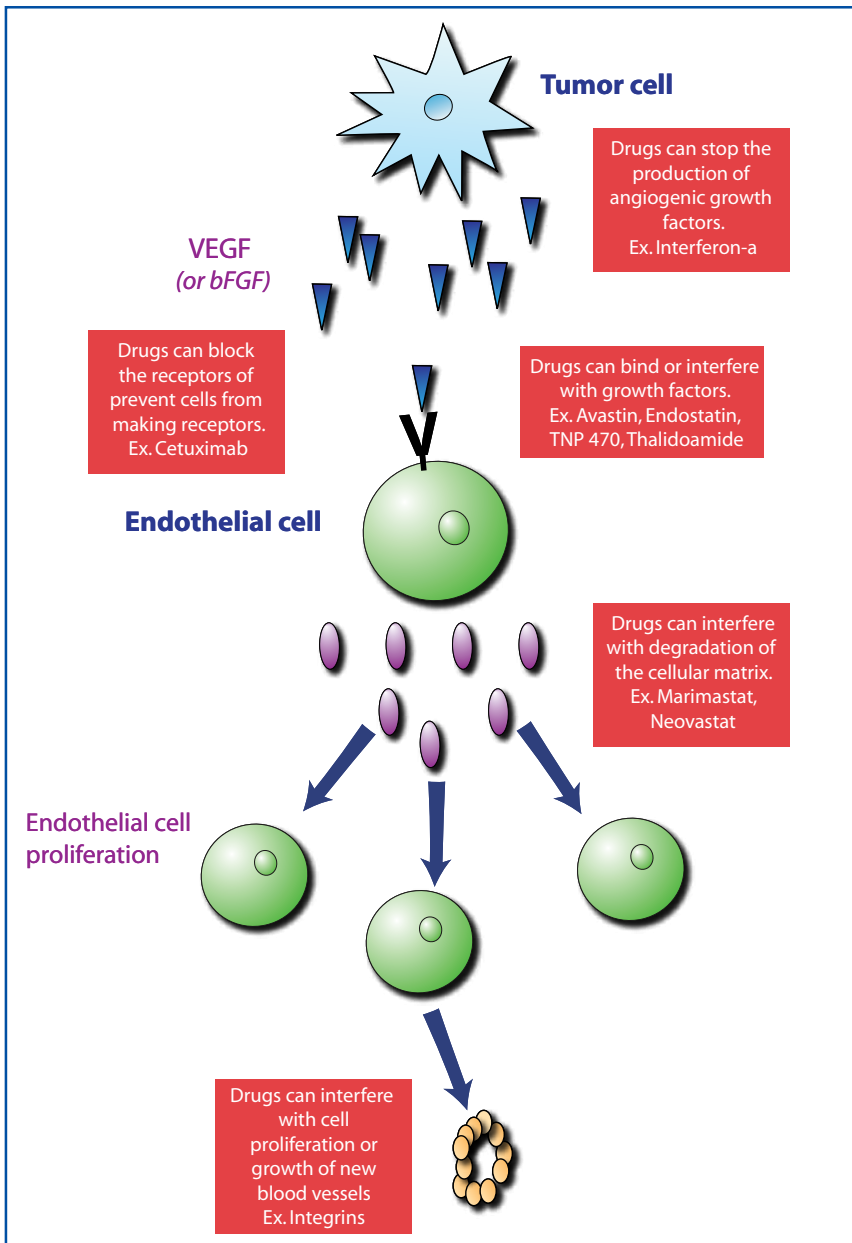


Figure 12 – Drugs target different stages of angiogenesis: There are a number of stages of angiogenesis that can be stopped by drugs to inhibit blood vessel growth associated with disease such as cancer or macular degeneration. Adapted from the National Cancer Institute, National Institutes of Health, by Corporate Press.

Ingber discovered a fungus contaminating his cultures of epithelial cells. The cells near the fungus were all rounded up, much different from the normally flat appearance of endothelial cells in culture. Highly reminiscent of Alexander Fleming’s serendipitous discovery of penicillin as a fungal contaminant in 1929, the fungus was isolated and cultured

in large batches to examine its potential to stimulate angiogenesis. The factor isolated from the culture, fumagillin, was indeed angiogenic, but unsuitable for use as a drug, so an altered synthetic form called TNP-470 was synthesized (and later adapted into a drug called caplostatin).

The search for angiogenesis inhibitors continues today.

Inhibitors have been synthesized in the laboratory, but also found in tree bark, garlic, ginseng, and shark cartilage (see also thalidoamide sidebar). Today there are at least 300 known inhibitors, at least thirty of which are found naturally in the body (endogenous inhibitors).

## From Finding Factors to Drug Development

But how does it all fit together? With at least 20 different endogenous angiogenic growth factors and at least 30 endogenous inhibitors constantly in balance, what is the signal that tips the scale and flips the angiogenic switch? One important signal is oxygen, or a lack thereof. We have seen how reduced oxygen levels can promote blood vessel growth in skeletal and heart muscles. This also appears to be true in tumors. In 1992, two groups of scientists, one from Israel and one from Germany, published back-to-back papers in the journal *Nature* showing that areas of a tumor with the lowest levels of oxygen made high levels of VEGF. The tumors respond to hypoxia by switching angiogenesis on.

Analysis of the mechanism of hypoxia-induced angiogenesis has implicated several intermediates that signal low oxygen and result in the production of angiogenic factors. Mechanical forces associated with increased blood flow, such as those described by Eliot Clark in his observations of the tadpole tail, also seem to stimulate angiogenesis by causing cells to produce angiogenic



factors, although the mechanism is less well understood.

The cellular and molecular processes that lead low oxygen (or other signals) to stimulate vascular endothelial cells are being elucidated in greater detail, revealing new targets for intervention. In addition to all the factors themselves, there are molecules that stimulate cells to make the factors, like mTOR (mammalian target of rapamycin) and HIFs (hypoxia-inducible transcription factors). There are also the receptors on the endothelial cells that respond to the factors, and the molecules within the cells that take the signal from the receptor and tell the endothelial cell to start (or stop) making more capillaries. The more we know about the mechanisms of angiogenesis and the participants in the process, the more targets we have available for potential exploitation in drug development (Figure 12).

Back at Genentech, Napoleone Ferrara had been developing a drug to inhibit the actions of VEGF. He and his team made a lab-produced version of antibodies, the proteins the immune system secretes to protect against disease-causing microorganisms (See the *Breakthroughs in Bioscience* article on monoclonal antibodies). Ferrara made antibodies that bind to VEGF and prevent it from signaling angiogenesis. That antibody, after much testing, eventually became the first angiogenesis inhibitor to be approved by the FDA for treating human cancer, bevacizumab (or the more easily-pro-

nounceable, commercial name, Avastin, (Figure 13).

Using an antibody to bind to and block a growth factor is only one way to inhibit angiogenesis. Drugs have been developed that block early participants in the angiogenesis process, such as mTOR, and inhibit the produc-



**Figure 13 – Avastin:** Bevacizumab, whose trade name is Avastin, was the first angiogenesis inhibitor approved to treat cancer in the United States. It is a monoclonal antibody that specifically blocks vascular endothelial growth factor (VEGF), which is produced by tumors to promote angiogenesis.

tion of growth factors. Other drugs block the growth factor receptors, or keep endothelial cells from making the receptors, so that growth factors have no way to exert their effect on the endothelial cell. Still others interfere with the function of the growth factor receptors, keeping them from signaling the cell to tell it to grow, or inhibit multiple steps of the angiogenic process. And for some drugs that inhibit angiogenesis, the mechanism is unknown.

## Into the Clinic and the Headlines

By the 1990s, control of angiogenesis was making its way into the clinic. The first clinical trial for cancer began in 1992, with TNP-470, the precursor to caplostatin that was inspired by a fungal contaminant. Angiogenesis burst into the nation's consciousness on Sunday, May 3rd, 1998, when an article on the front page of the *New York Times*, proclaimed, "HOPE IN THE LAB... A Cautious Awe Greets Drugs That Eradicate Tumors in Mice." This was soon followed by cover stories in *Newsweek*, *Time*, and *U.S. News and World Report* (Figure 14). The press buzz made a cure for cancer seem imminent.



**Figure 14 – Angiogenesis makes the headlines:** In 1998, tremendous excitement was generated about the prospects of controlling angiogenesis to treat cancer after a *New York Times* article describing Judah Folkman's work made a cure for cancer seem imminent. Similar stories graced the covers of *Time*, *Newsweek*, and *U.S. News and World Report*. Folkman himself was more cautious in promoting his breakthrough discoveries, warning that cancer was a complex disease and stating, "We never use the word 'cure' because it is far away." Used with permission.

“If you have cancer and you are a mouse, we can take good care of you,” cautioned Folkman. Translating laboratory findings in mice—no matter how dramatic—to clinically useful treatments is a long road fraught with numerous dead ends. Clinical trials for safety and efficacy may yield disappointing results.

Despite the caveats of Folkman and other researchers, the enthusiasm of the general public for antiangiogenic therapy was mirrored by the NIH. In 1999, Richard Klausner, head of the National Cancer Institute, made antiangiogenic therapies of cancer a national priority. In 1999 alone, 48 antiangiogenic drugs

entered clinical trials as cancer treatments. Today, around one dozen antiangiogenic drugs are approved by the FDA to treat cancer (Table 3).

Research into antiangiogenic therapies continues and even with encouraging results and successful treatments, challenges remain. The biggest problem is the potential for the development of resistance to antiangiogenic drugs. Mutations in tumor cells may produce additional growth factors, or increase their production of growth factors, overcoming the antiangiogenic effects of the drugs. Other mutations may increase tumor cells’ ability to survive in low levels

of oxygen, decreasing the need for new blood vessel formation. “Vascular mimicry” refers to the fact that vessels within tumors may be lined with tumor cells, instead of endothelial cells, limiting the effectiveness of drugs that interfere with endothelial cell function. Our understanding of the molecular events associated with angiogenesis is incomplete, especially in the relationship between the mechanical and metabolic factors that promote angiogenesis. Research is also aimed at finding ways to affect angiogenesis using the body’s own, endogenous factors instead of introducing foreign substances.

Generic Name	Brand Name	Inhibits or Promotes Angiogenesis	Approved to Treat
Bevacizumab	<i>Avastin</i>	Inhibits	Metastatic colorectal cancer, lung cancer, advanced breast cancer, kidney cancer
Becaplerman	<i>Regranex</i>	Promotes	Ulcers caused by diabetes
Cetuximab	<i>Erbix</i>	Inhibits	Head and neck cancers
Erlotinib	<i>Tarveca</i>	Inhibits	Lung cancer, pancreatic cancer
Everolimus	<i>Afinitor</i>	Inhibits	Advanced kidney cancer
Lenalidomide	<i>Revlimid</i>	Inhibits	Myelodysplastic syndrome, multiple myeloma
Panitumumab	<i>Vectibix</i>	Inhibits	Metastatic colorectal cancer
Pegaptanib	<i>Macugen</i>	Inhibits	Wet macular degeneration
Ranibizumab	<i>Luncentis</i>	Inhibits	Wet macular degeneration
Sorafenib	<i>Nexavar</i>	Inhibits	Kidney and liver cancer
Sunitinib	<i>Sutent</i>	Inhibits	Kidney cancer, gastrointestinal stromal tumors
Tensirolimus	<i>Torisel</i>	Inhibits	Advanced kidney cancer
Thalidomide	<i>Thalomid</i>	Inhibits	Multiple myeloma

**Table 3—** FDA-Approved Drugs Based on Angiogenesis: A number of anti-angiogenic drugs, primarily aimed at treating cancer and eye disease, have been approved for use in the United States, as has one angiogenic drug for treatment of diabetic ulcers. Many more promising drugs related to angiogenesis are currently undergoing clinical trials for a variety of diseases, but it remains to be seen if they will prove effective.



## Beyond Cancer

Cancer was not the only disease on the government's angiogenesis radar. A few months after the *New York Times* article was published, NIH released a request for research proposals aimed at translating angiogenesis research into clinical treatments for a variety of diseases, stating that "controlling the angiogenic process may prove effective as a treatment paradigm for a wide range of cardiovascular and pulmonary diseases, for inhibiting tumor growth and metastasis, and for preventing and treating certain eye diseases."

Despite the prodigious output of Folkman's lab, he was not the only investigator interested in angiogenesis, nor was cancer the only clinical application of experimental findings about angiogenesis. Folkman, himself, knew this and proposed angiogenesis as an "organizing principle" for drug development, writing, in 2007, "the discovery of a molecular mechanism for one phenomenon might be more rap-

idly demonstrated for a second phenomenon if one understands *a priori* that the two are connected. Furthermore, when the mechanisms underlying different diseases can be related in this way, the development of therapeutics for one disease could aid the development of therapeutics for others." The search for factors affecting angiogenesis may have been started with cancer in mind, but the benefits of that research have far-reaching consequences.

In 1948, Isaac Michaelson suggested that a diffusible factor, which he called "Factor X," promoted the abnormal revascularization associated with eye diseases like diabetic retinopathy. Half a century later, ophthalmologist Anthony Adamis, in collaboration with investigators including Jan Miller and Lloyd Paul Aiello, began exploring the basis of abnormal capillary growth. Together, they showed that the vessels in the eyes of diabetics become clogged by the exceptionally "sticky" cells flowing through them. That produces

hypoxia in the eye, which in turn prompts the secretion of VEGF (which may be Michaelson's elusive "Factor X"). As has been shown for so many other diseases associated with angiogenesis, low oxygen promotes new capillary growth. In this case, the growth of capillaries is overzealous and the capillaries are abnormally fragile, which ultimately results in retinal damage and vision loss.

Age-related macular degeneration, too, has VEGF as a player in disease promotion. In fact, one of the first antiangiogenic drugs, Avastin, which blocks VEGF and was originally approved for cancer, is also effective against macular degeneration. Antiangiogenic treatments continue to be studied. Today, there are two FDA-approved antiangiogenic drugs for eye diseases. Both of them inhibit VEGF.

## Promoting Angiogenesis to Save Legs and Lives

Encouraging angiogenesis is not always a bad thing. While most of the research on angiogenesis



**Figure 15 – Promoting angiogenesis for wound healing:** As the Clarks discovered in the 1930s, angiogenesis is an important part of wound healing. Therapeutic angiogenesis, or the promotion of blood vessel growth to treat disease, is currently being used to treat chronic wounds (A), associated with diseases like diabetes or multiple sclerosis, in which prolongation of the wound may be due to abnormalities in vasculature. Treatment with angiogenic factors, like those found in Regranex, an angiogenesis-stimulating ointment, can lead to healing of chronic wounds and ulcers (B). Courtesy of the Angiogenesis Foundation.

has focused on inhibiting the process in tumors and neovascular eye diseases, other investigators have been looking at it from the other direction. Therapeutic angiogenesis, or the promotion of angiogenesis to treat disease, is also an active area of clinical research.

The process of wound healing, for example, involves the same pro-angiogenic factors as those used by tumors, including VEGF and FGF. In fact, Harold Dvorak noted the similarities between tumor angiogenesis and wound healing, calling tumors “wounds that do not heal,” meaning that both tumors and healing wounds make VEGF, but the tissue in a healed wound stops producing VEGF, while a tumor does not. More than 30 factors have been identified as participating in the wound healing process.

When wound healing is inhibited, as often occurs in diabetics, augmentation of the angiogenic process helps promote wound healing (Figure 15). In 1997, the FDA approved becaplermin (Regranex), an ointment containing a genetically-engineered form of the angiogenic platelet-derived growth factor (PDGF), for treatment of diabetic foot ulcers. In 1999 alone, ten clinical trials were conducted on therapeutic angiogenesis for wound healing and similar research continues.

In the mid-1990s, Jeffrey Isner, a cardiologist at Tufts University in Boston, began to think that therapeutic angiogenesis might be applied to the cardiovascular

system. Patients with peripheral arterial disease have limited circulation in the legs, which may be painful, and eventually require amputation. Since angiogenic growth factors are involved in the development of collateral circulation, he decided that therapeutic angiogenesis might be an effective treatment for this insufficient blood flow.

He and post-doctoral fellow, Satoshi Takeshita, performed experiments reminiscent of John Hunter’s deer antler experiment, tying off an artery to block blood flow to the legs of rabbits. Before tying off the artery, however, Isner and Takeshita injected the angiogenic factor, FGF. The injection enhanced the formation of collateral blood vessels. They repeated the experiment with VEGF, again resulting in increased formation of collateral circulation compared with rabbits injected with an inert placebo.

Since growth factors lose activity shortly after being injected, Isner looked to gene therapy. He injected the gene for VEGF into experimental animals with great success. Eventually he used the procedure on humans, and treated over a hundred patients with poor leg circulation over the course of three years.

Encouraged by the improved circulation in his patients’ legs, Isner turned his attention to the heart. The arteries that supply the heart with blood and oxygen can become blocked. When that happens, cells of the heart muscle may die, producing pain, short-

ness of breath, or even a heart attack. Collateral circulation can compensate, but it may not be enough. One way to overcome this problem is by angioplasty, surgery to open up the vessels. Another is bypass surgery, where vessels from other parts of the body are grafted into the heart to replace the blocked vessels. But bypass surgery is invasive surgery that may not be appropriate or sufficient to increase the blood supply to a satisfactory level. Isner adapted his gene therapy treatment to the heart. Between 1997 and 1999, Isner treated several dozen patients by injecting VEGF DNA into their hearts. Ninety percent of the patients showed improvement in their heart symptoms.

Although there was some controversy over Isner’s technique—and gene therapy in general—the idea of pro-angiogenic treatment of peripheral and coronary artery disease caught on. More clinical trials were initiated, some using growth factors and some using gene therapy. Clinical trials in therapeutic angiogenesis have also used immature endothelial cells (endothelial progenitor cells and stem cells) to promote angiogenesis. Although it was anticipated that the cells themselves would become part of the new vasculature, the role of the introduced cells seems to be one of a growth factor factory, churning out the factors the vessels that need to sprout new capillaries and improve circulation of ischemic tissues.



## More to be Done

Cardiovascular disease and cancer are the two biggest killers in the developed world, and the requirement for blood ties these two seemingly unrelated diseases together. Angiogenesis research is providing new ways to deprive cancer cells of their blood and

to improve blood flow to tissues that need more blood.

Jeffrey Isner died suddenly of a heart attack in 2001. The cells of his own heart, deprived of oxygen, did not survive long enough for him to see his ideas about therapeutic angiogenesis expand into the diverse assortment of

clinical trials that may lead to new treatments for cardiovascular disease.

Judah Folkman, too, died suddenly of an apparent heart attack in 2008, in the Denver Airport on his way to a meeting of the Angiogenesis Foundation. At the time of his death, there were over 1,000 laboratories studying angiogenesis worldwide, more than 50 angiogenesis inhibitors in clinical trials, and more than a million patients being treated with antiangiogenic therapy. While Folkman himself said, “Science isn’t one success after another. It’s mostly one success in a desert of failure,” clearly the work on angiogenesis by Folkman and the scientists who came before and after can be counted as one such success.

### Further Reading:

<http://www.cancer.gov/cancertopics/understandingcancer/angiogenesis>

<http://www.pbs.org/wgbh/nova/cancer/>

[http://www.contemporarysurgery.com/uploadedFiles/CS1103\\_angiogenesis.pdf](http://www.contemporarysurgery.com/uploadedFiles/CS1103_angiogenesis.pdf)

<http://www.angio.org/ua.php>

<http://www.pbs.org/saf/1202/segments/1202-6.htm>

Dr. Folkman’s War: Angiogenesis and the Defeat of Cancer (Random House, 2001), by Robert Cooke

### Biographies:

*Jacqueline Jaeger Houtman, Ph.D.* writes about biomedical science from Madison, Wisconsin. She enjoys writing for physicians, scientists, middle school students, and the general public. Dr. Houtman is the author of the book, *The Reinvention of Thomas Edison*, and has also written for The Dana Foundation, World Book, and scientific journals, including *Clinical and Experimental Allergy* and *Journal of NeuroVirology*. She can be reached at via her website: <http://www.jhoutman.com/index.html>

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