

# Thicker Than Water

Biochemistry blends with fluid dynamics to yield a vascular science

By ELIZABETH PENNISI

**W**hen the space shuttle Columbia lifted off last June, cardiologist Drew Gaffney sat in the cabin with the rest of the crew, wearing a catheter in his arm to monitor his heart. Then, during the flight, a device attached to his leg measured the blood reaching his feet.

Gaffney, a payload specialist on the flight, conducted the experiments as part of Spacelab Life Sciences 1, a mission to study how the human body reacts to prolonged weightlessness. Some might say he went to extremes to collect his data.

"We measured blood pressure, heart rate and blood flow so we could get an integrated picture of what was happening [in space]," says Jay C. Buckey, who works with Gaffney at the University of Texas Southwestern Medical Center at Dallas.

For several hours after returning to Earth, astronauts tend to faint or get dizzy when they stand up. Their bodies cannot make the rapid adjustments needed to maintain blood flow in an upright position. Gaffney and Buckey want to know

why.

Although they still need to analyze their data, they suspect that the changes in the circulatory system's ability to respond depend on cellular and biochemical events that are influenced by blood flow.

Gaffney and Buckey belong to an expanding group of scientists who use fluid dynamics to learn how blood and its components keep arteries, veins and capillaries functioning properly. And while Gaffney may be unique in serving as his own guinea pig in the high-reaching shuttle experiment, many of his fellow researchers — including several engineers — have taken a down-to-earth approach to the same cellular and biochemical phenomena, directly linking them with blood flow for the first time.

**"T**he whole area of vascular biology is exploding these days," says Larry V. McIntire, a chemical engineer at Rice University in Houston.

McIntire is among a vanguard of investigators who emphasize the role of engineering components in circulation. These researchers seek to measure and understand the physical forces that determine how much a blood vessel will stretch and how fluid flow affects the endothelial cells lining the inner walls of blood vessels. Endothelial cells turn out to play key roles in maintaining the circulatory system. "They not only can sense flow, but they can do something about it," says John A. Frangos, a chemical engineer at Pennsylvania State University in University Park.

The dynamics of this flow can alter the circulatory system's architecture. Vessels dilate and constrict, add cells to widen their channels, or thicken their walls so that the channels narrow and clog. Flow properties also help determine where, and perhaps when, clots develop inside vessels, and flow provides the signal that prompts endothelial cells to make new proteins — the first step in a series of transformations that lead to vessel remodeling. In addition, blood — a complex slurry loaded with cells and substances — contributes to those changes through its chemical effects.

But it took a team effort by biologists and engineers to establish these connections and to convince skeptics of the importance of fluid dynamics in cardiovascular damage. An experiment five years ago drove the lesson home for Peter F. Davies, an experimental pathologist at the University of Chicago.

MIT engineer C. Forbes Dewey Jr. had built an apparatus that recreated not only smooth flow but also the irregular currents that develop where blood vessels branch. Davies, working with Harvard biologist Michael A. Gimbrone Jr., put endothelial cells into the flow chamber and observed many differences between cells subjected to smooth and turbulent flow.

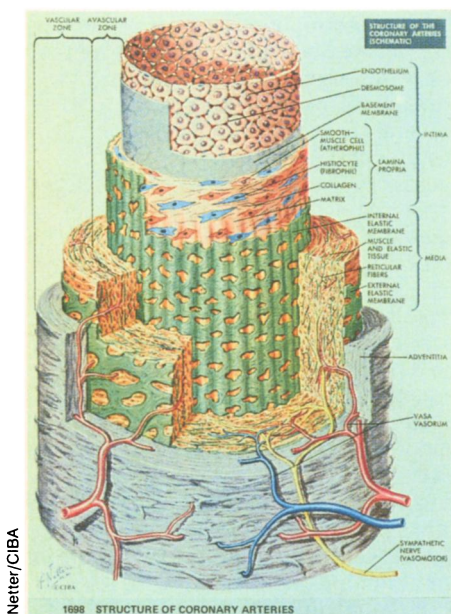
"We could not have done that experiment without the precision of the fluid dynamics folks," Davies says. "I don't think you can do this work without an appreciation of the mechanics involved."

He and many others now depend on cell cultures and flow chambers to make progress in their research. They grow batches of 100,000 to 1 million cells on small glass or plastic plates. The cells form a single sheet much like the monolayer lining of a blood vessel. The researchers then put the plate into a chamber or tube where they can regulate flow properties. Investigators have more control over variables with such devices than in experiments with live animals or animal tissues.

"We have to make the big leap that isolated cells are behaving as they would in [real] blood vessels. But there is good, uniform consistency between our work and work *in vivo*," Davies says.

He and some 50 other researchers discussed their progress last April at the Workshop on the Mechanical Stress Effects on Vascular Cells. Results reported at the Atlanta workshop have boosted scientists' confidence about the vital interconnectedness of the physical and biochemical components of circulation.

"We're closer to showing that flow connects things," says Eugene C. Eckstein, a biomedical engineer at the University of Miami in Florida. Fluid flow helps determine how fast and how frequently blood-borne chemicals reach their targets. It also exerts stresses on the molecules that sit on the endothelial



Netter/CIBA

*Endothelial cells, which line blood vessels, respond to changes in blood flow and then pass on the appropriate signal to smooth muscle cells and other vessel components.*

cells.

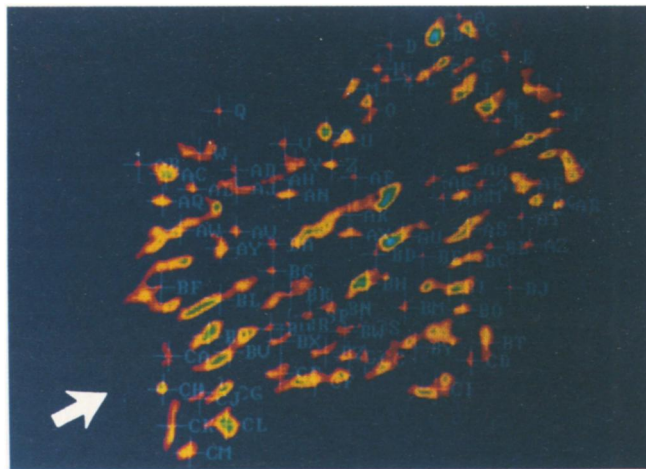
"It organizes and increases the rate [of chemical activity]," Eckstein says.

**S**tarting from zero at the vessel wall, the blood's velocity increases evenly so that the fluid at the center moves the fastest. Thus, one can envision flow as successive layers, or concentric tubes, arranged somewhat like the cylinders of a collapsed telescope. Each layer moves at a slower pace than the one inside it. Faster flow usually creates a larger differential between the speeds of adjacent layers, creating a steeper velocity gradient. The steeper gradient increases the pull, or shear, along the edge of the vessel. This shear, in turn, influences the endothelial cells.

The densely packed nature of blood makes the circulatory system seem like a major thoroughfare at rush hour. Various "vehicles" — cells and chemicals — travel along the tens of thousands of miles of blood vessels packed into the human body. Most are red blood cells, which weave across the crowded liquid highway like big tanker trucks, cutting off smaller cells and particles along the way. "These particles are jostling each other all the time," says Eckstein.

But unlike real highways, healthy blood vessels rarely develop traffic jams. For one thing, they can expand to accommodate heavy flow, dilating like elastic tubing. For another, red blood cells maneuver more like daredevil motorcycles than bulky trucks, flexing and deforming to squeeze through capillaries smaller than their own diameters. Without this cellular flexibility, blood would hardly flow at all, says physical chemist Harry L.

*Red and yellow blotches show where a single endothelial cell sticks to a surface. Observations of cells under various flow conditions, using confocal microscopy and digital image enhancement and processing, reveal that these adhesion sites change depending on flow direction (arrow).*



Davis, Andre Robotewskyj, Melvin Ghem/Univ

Goldsmith of McGill University in Montreal, Quebec. And even though red blood cells tend to convoy when flow slows — stacking up like a roll of coins — they tumble apart easily once things begin to speed up again.

While their role as oxygen carriers is well appreciated, red blood cells serve other functions as well. Red cells act as "little stirrers," says Kenneth H. Keller, a chemical engineer at the University of Minnesota in Minneapolis. By helping small molecules move more efficiently, they influence the rates at which these molecules can react with endothelial cells along the way. And flow, in turn, affects the mixing action.

As a red blood cell travels along through fluid layers that move at different speeds, each side of the cell encounters a different velocity. That difference causes the cell to spin. The greater the differential, the faster the spin and the greater the mixing action, says Keller. "So the motion of the red blood cell depends not so much on the flow as on the velocity gradient," he explains.

Other cells passing through the fluid roadways exert their own effects. White blood cells are larger and less flexible than red cells, notes Shu Chien, who has tracked the motions of these immune-system cells through the bloodstream. Spherical and stiffer, white cells create more resistance and are much more likely to jam up than red blood cells.

"Fortunately, we have 700 red blood cells to one white blood cell, or we'd

be in trouble," says Chien, a biomedical engineer at the University of California, San Diego.

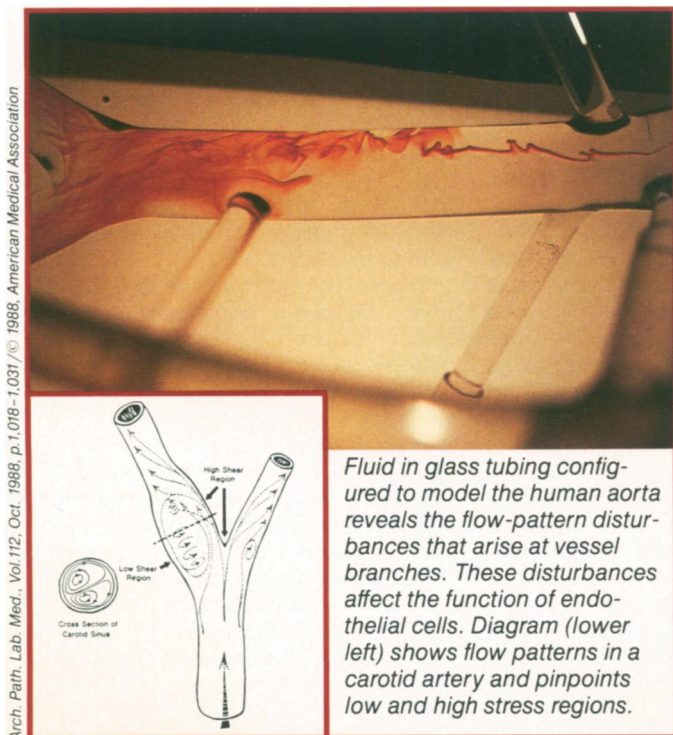
Normally, white cells stay on the bloodstream's main highways, avoiding the narrow capillaries that red blood cells so adeptly squeeze through. But illness can make the white cells "sticky" and even more likely to impede smooth circulation. "This may contribute to the exaggeration of a [heart] condition," possibly leading to a second heart attack, Chien says.

**I**rregularities in flow represent a second important fluid condition. These develop when the blood reaches a branch or rounds a curve in the circulatory system. It "wants" to go straight, fails to follow the vessel channel, and so leaves a void where it pulls away from the edge, explains David N. Ku, a biomedical engineer at Georgia Institute of Technology in Atlanta. Blood downstream then reverses direction and rushes back to fill the void, creating an "eddy" with very low shear. This eddying presents a complex picture to researchers seeking to describe the fluid dynamics of blood flow. That blood flow pulsates with each contraction of the heart adds to the complexity.

At these spots, "the flow is very uneven, almost chaotic," Chien says. The endothelial cells beneath the eddies become rounded instead of elongated. The flow seems to make them bulge out into the bloodstream. "This kind of lifting force tends to kill the [endothelial] cell," Chien adds. In large arteries, where curves and branches break up blood flow, that force may play a significant part in cardiovascular disease.

For several decades, researchers have recognized that damage to the endothelial lining leaves the blood vessel vulnerable. "It's sort of like scratching the paint on your automobile: It basically rusts," says Dewey. But only recently have investigators begun to uncover the details of this vulnerability.

Endothelial damage may set the stage



*Fluid in glass tubing configured to model the human aorta reveals the flow-pattern disturbances that arise at vessel branches. These disturbances affect the function of endothelial cells. Diagram (lower left) shows flow patterns in a carotid artery and pinpoints low and high stress regions.*

Ku/Georgia Tech

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for the development of atherosclerotic plaques. Chien theorizes, based on his experiments in animals, cultured cells and human blood samples, that irregular flow patterns accelerate the life cycle of the endothelial cells. As the cells prepare to divide, they change their shape somewhat so that the inner lining they form gets "leaky." The gaps between the endothelial cells allow low-density lipoproteins (LDLs), which carry cholesterol in the blood, to sneak into the vessel wall.

If the blood contains high levels of LDLs, the lipoproteins overwhelm the vessel wall's ability to dispose of them, Chien suggests. At this point, white blood cells called monocytes may get involved. As part of their conversion to macrophages — another type of white cell — monocytes ingest lipoproteins and their oxidized products. The cells, which now resemble bits of foam, remain in the vessel wall. Thus a plaque begins to take shape.

In addition, Chien and his co-workers have shown that the endothelial cells of rats that develop high blood pressure tend to divide and die more quickly than those of normal rats. This may help explain why high blood pressure increases an individual's risk of developing clogged arteries, he says.

Vessel-wall plaques represent only a first step toward the eventual closing of an artery — a process that may take decades, says Vincent T. Turitto, a chemical engineer at Memphis (Tenn.) State University. Turitto's scenario for this process also involves the formation of clumps of platelets — circulating bits of cytoplasm that help plug damaged vessels — and the deposition of fibrin, a clotting protein.

The bulging plaque, like a stone in a stream, alters fluid flow nearby. As blood passes by the plaque, its flow speeds up dramatically, creating a much higher than normal drag along the endothelial cells covering and opposite the plaque. Then, downstream, an eddy forms. Each of these conditions exerts an effect on the endothelial cells, making them more prone to the buildup of fibrin and platelets, Turitto says. Thus, the altered flow conditions can activate mechanisms that hasten or worsen congestion and may eventually cut off flow.

Turitto says his experiments demonstrate that platelets concentrate in the high-flow areas. And because fibrin is abundant in blood vessels exposed to low-flow conditions, he speculates that the protein settles into the vessel wall where eddies form. There, fibrin contributes to artery clogging by stabilizing the blockage that builds up in the artery.

"What's important and needs to be appreciated is that there are physical factors that influence many of the cells in or on the vessel wall," Turitto says. "Physical stress initiates a whole host of chemical events. [This concept] is only really coming to fruition in the last few years."

**B**ecause of their role in clogged arteries, platelets have captured the interest of many researchers. Miami's Eckstein, for instance, studies platelet distribution in the bloodstream. While red blood cells try to avoid the vessel wall, with some drifting toward the center, platelets seek out their own particular positions, he reports in the July *BIOPHYSICAL JOURNAL*. Eckstein's theoretical calculations and experimental results indicate that platelets quickly concentrate near — but not next to — the vessel wall.

Eckstein is now taking "snapshots" of blood flow to determine the paths platelets take through vessels. First he lets platelet-free blood pass through a flow chamber's tube. After at least 10 minutes, he lets blood containing platelet-sized, fluorescing beads seep into this stream. He then puts the tube in liquid nitrogen, freezing all the fluids. Successive slices of the tube let him map how the glowing beads — which simulate platelets — move in the fluid flow. In other experiments, he adds various concentrations of red blood cells to the mixture.

From his snapshots, Eckstein knows that the large red blood cells "kick the beads," he says. "It's like driving down a gravel street. The gravel ends up at the sides; so do the platelets." Greater concentrations of red blood cells lead to higher densities of platelets near the vessel wall. Eckstein speculates that platelets near the vessel wall can respond quite quickly to nicks and damage in the wall, rapidly plugging up the leaks.

Red cells "disturb the flow [through a

blood vessel] because they are present in enormous numbers," Goldsmith adds. Platelets constitute less than 1 percent of blood's volume, while red blood cells account for 40 to 45 percent. An abundance of red blood cells may increase the rate at which platelets collide with each other or with the vessel wall; the red cells may also release a chemical that makes these platelets stickier, he suggests.

Goldsmith studies platelet aggregation in a flow chamber, counting how many congregate when subjected to different flow conditions for specific lengths of time. He finds that the higher the flow rate, the greater the buildup. Again, he says, either of two factors may be at work here: Increased flow may bring the vessel wall or the platelets into closer contact with an activating agent, or it may knock more platelets against the vessel wall, where they stick.

In his experiments, Goldsmith has demonstrated that even with very low amounts of activating agent, high shear can cause substantial platelet aggregation. This further implicates fluid as a key factor in platelet pileups, he says, adding that the shear force he observed is "quite typical of ones that are seen in the body."

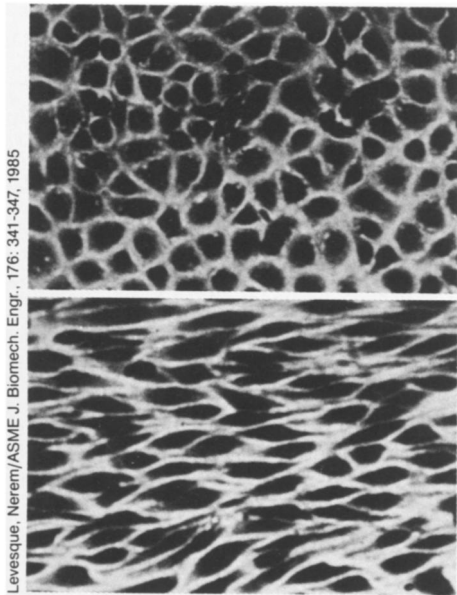
Aaron L. Fogelson, a mathematician at the University of Utah in Salt Lake City, takes a numerical approach to understanding clot formation within vessels. He has developed a series of differential equations to describe blood flow, the movement of cells in blood and the motions of chemicals secreted by those cells. The equations simplify what happens in nature, giving researchers a chance to gauge the relative importance of various flow components.

Fogelson's work stands out because his equations take into consideration how flow changes as each platelet joins the growing blockage, says Eckstein. Fogelson is developing new computational techniques for working through these equations, and he plans to use them to simulate the behavior of the circulatory system as blockages form. He also plans to hook up with experimentalists who track the motion of individual platelets with video microscopy.

"There's lots of evidence that the motion of the blood is somehow important in how [platelet] aggregations start, but nobody has a clue to how it works," Fogelson says.

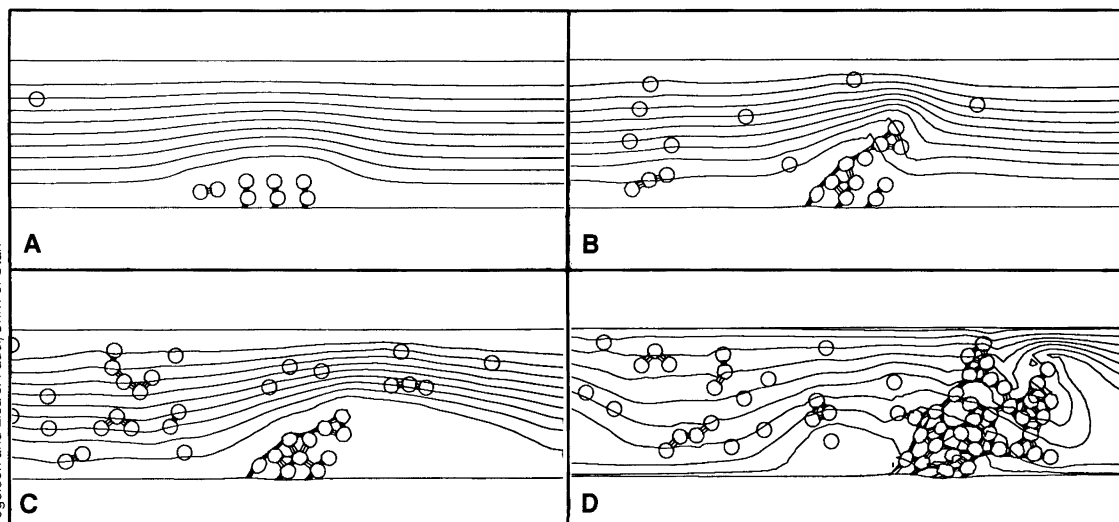
**W**hile these researchers study how blood-flow patterns lead to traffic jams and pileups, others are delving into biochemical details of the endothelial cell itself, focusing on the effects flow can have on its metabolism.

"The thing that's been very interesting is the joining of this really deep understanding of fluid mechanics and transport phenomena with biochemistry," says Minnesota's Keller. "People are beginning



Flow conditions can remodel endothelial cells. Under low-shear conditions, the cells tend to be round (top). In high-flow regions, they become elongated, with their long axes lined up along the direction of flow.

Levesque, Nerem/ASME J. Biomech. Engr., 176: 341-347, 1985



Computer simulations show how clumps of platelets build up in arteries. Platelets (circles) become sticky and interact with the fluid, the vessel wall and each other (diagram A). Platelets quickly attach, altering flow (B). The fluid then tends to push the platelets downstream, rounding the growing clump (C). But as platelets continue to stick, flow becomes quite disrupted (D).

to look quite specifically at the protein components. Rather than having separate people looking at these questions, we are beginning to recognize we have to treat these at the same time."

Changes in blood flow cause immediate and long-term changes in the chemistry of the endothelial cells, scientists find. "Fluid flow is a real potent actor," says MIT's Dewey. "[Endothelial cells] are really major players in this whole thing, and the fluid flow modulates their activity."

No one knows how this occurs, however. The flow may simply alter the rate at which small molecules hit the endothelial cell surface, where they can stimulate or inhibit cell function. "The faster the flow, the greater the rate of collision," notes Goldsmith.

But flow may also exert a more complex effect, somehow stimulating the cell and making it more appealing to passing substances such as platelets. Some researchers think endothelial cells contain mechanosensors that act as stretch receptors or, as Dewey puts it, "biological strain gauges." In this scenario, the push or pull of fluid sets off a response in the cell membrane.

McIntire, however, doesn't think the flow deforms the membrane enough to activate a stretch receptor. Instead, he theorizes that large molecules at the cell surface stick out and unravel or undergo some other change in response to flow, and that this change may trigger a response. That response may involve the movement of calcium — or, more likely, potassium — ions through the membrane, says McIntire, who bases his theory on his own studies as well as on research by others.

Davies has found that the flow-induced drag, if powerful enough, can cause the endothelial cell membrane to let potassium through. It does this by activating molecules that form pore-like ion channels through the membrane. Certain substances that dilate blood vessels also activate these channels, he says. Once the pores open, they let potassium out of

the cell, increasing the difference between the electrical charges inside and outside the cell. This "hyperpolarization" may kickstart other chemical reactions that send signals throughout the cell, but no one knows for sure what is really happening.

"If we can find out what the mechanism is, there's some hope that we may find some way of intervening in hypertension," Davies asserts.

Whatever the trigger, something about fast flow sets off a chemical cascade that ultimately leads to the release of a dilating substance, which oozes out to the smooth muscle cells that make up the vessel wall. "It's what the endothelial cell is actually making and secreting that is important," McIntire says. "It's important in communicating to the cells in the blood and to the cells underneath it."

That communication could affect the immune response or make some parts of the body more susceptible to cancer, he suggests, because the vessel wall may become more receptive to latching on to white cells or cancer cells flowing past.

**T**he chemical messages also affect vessel tone and blood pressure. For example, the cascade can cause the endothelial cell to reduce its normal production of vessel-constricting chemicals, says Frangos. As a result, the blood vessels of athletes — who often call on their circulatory system to increase blood flow to active muscles — tend to have larger diameters than those of non-athletes. "Apparently the endothelial cells change the production rate of growth factors for local cells," Frangos adds. "They basically tell the cells to grow and remodel so that when you do need blood, it's easier to deliver."

McIntire has demonstrated that shear stress, for example, causes the endothelial cell to increase production of tissue plasminogen activator, a chemical that breaks up clots that might otherwise build up along the vessel wall. At the same time, rapid flow leads to lower

levels of endothelin, a substance that stimulates cell division in smooth muscle. In low-shear conditions, platelets get activated and may release another substance that stimulates cell division in smooth muscle, says McIntire. This proliferation could thicken the vessel wall, eventually narrowing its channel.

In pioneering experiments conducted at Rice with Matthias U. Nollert and N.J. Panaro, McIntire has looked at the details of fluid flow's effects on this chemical cascade, called signal transduction. Chemical signals direct enzymes and other molecules to either rev up or dampen genetic activity in endothelial cells. In the upcoming *Annals of the New York Academy of Sciences: Biochemical Engineering VII*, McIntire and his co-workers describe the genetic events they observed inside cultured endothelial cells.

The genes activated seem to vary depending on the type of stress on the endothelial cell. Some genes "turn on" in a matter of minutes, initiating the production of proteins that then bind to other genes, triggering additional protein production, they report. These genes seem to remain active for only a few hours, while others seem to turn on later and remain active longer. Thus the cells can begin releasing tissue plasminogen activator, endothelin, growth factors or other proteins in a matter of hours. These chemicals, in turn, affect other cells in the vessel.

The new gene findings will help Gaffney understand how the changes in his blood flow allowed him to stand up comfortably in space but not on the ground. On a broader scale, they should help propel an abstract engineering science into mainstream biology.

"Once you can show that it [fluid dynamics] has quite large effects on metabolism, you get biologists interested," says McIntire. "You now see people talking about the necessity of doing [flow] experiments and considering the potential role of fluid flow [in the circulatory system]." □