More Than a Hand to Hold

When Dr. Fletcher Taylor's mother needed knee surgery two years ago, he asked her to have it done in Oklahoma City, despite the inconvenience of having to leave her home in San Francisco.

Fletcher Taylor lives in Oklahoma City, but his request wasn't based solely on his desire to be nearby during her convalescence; nor was there a dearth of capable orthopedic surgeons in the Bay Area.

Taylor was concerned that his mother's age and the type of surgery she was to undergo placed her at high risk for developing a potentially dangerous blood clot following the operation.

Mrs. Taylor already knew that her son was one of the world's top experts in blood clotting, or thrombosis. Since 1975, Taylor had directed a multi-million dollar thrombosis research program at the University of Oklahoma. Still, no one not even her son, could prevent a post-operative clot from forming — either it does or it doesn't — but Taylor's research group had developed both standard and experimental blood tests that allow for early diagnosis and effective treatment. It was reasonable and proper for Taylor to ask his mother to fly to Oklahoma City.

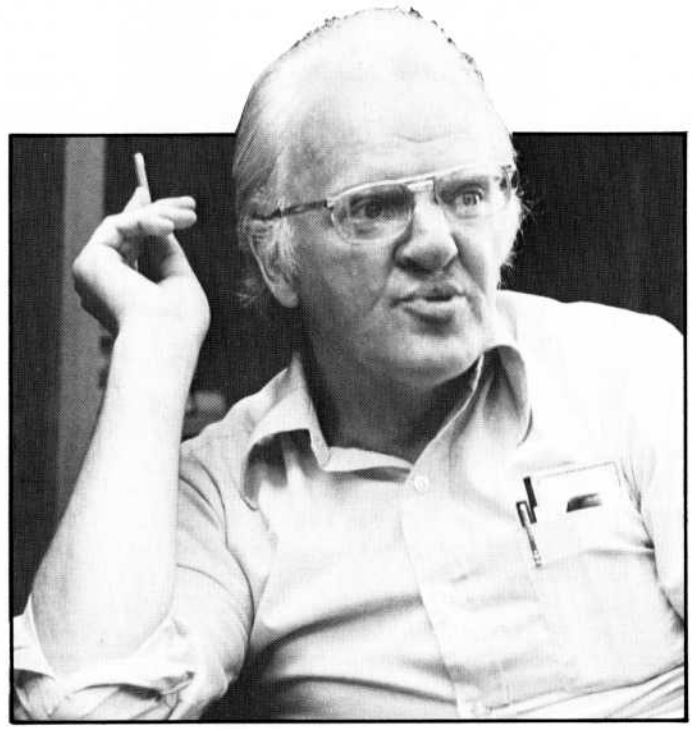
A few days after successful knee surgery, Mrs. Taylor developed a blood clot in a vein in her thigh. There had been no advance signs, no present symptoms (which is true in two-thirds of all cases), but the blood tests indicated the presence of a clot. A fibrinogen scan showed its location.

Two potential dangers existed: the clot could travel upstream and lodge in a lung forming a potentially lethal pulmonary embolus, or additional clots could form in the vein and threaten the kidneys.

While Mrs. Taylor was receiving anti-coagulant medication to prevent further clotting, additional blood tests indicated that her body's clot dissolving elements were working, and finally that the clot was gone. Eliminated at an early stage, a recurrence of the clot was unlikely.

According to the World Health Organization, thrombosis is the cause of 60 percent of all deaths. Clots blocking arteries can cause heart attack or stroke depending on where the vessel leads; obstructed veins may lead to potentially lethal pulmonary emboli; and clots in small vessels can result in kidney damage and high blood pressure. *Continued on Next Page*

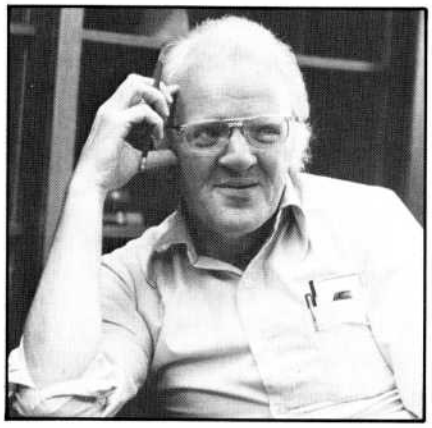
Medical science had little hope to offer potential stroke victims until Dr. Fletcher Taylor turned from internal medicine to research.



By RICHARD GREEN

Illustrated by Brent Purdy

When Dr. Taylor got his first big thrombosis grant at OU six years ago, no reliable blood assays existed for the early detection of blood clots. Nor was there a safe or effective way to monitor treatment with the unpredictable blood thinners like heparin and coumadin. Treatment sometimes was little more than a shot in the dark, and tended to be hazardous. The



doctor might administer too little medication, and the clotting would remain or worsen, or he might use too much causing the patient to hemorrhage internally.

The reason for this relatively primitive state of affairs stemmed, in large part, from the fact that scientists had an awful time getting blood clots to dissolve in test-tube solutions. So many unnatural chemicals had to be added to dissolve the clots that the results were equivocal at best.

Despite working with this handicap, Taylor and relatively few others in the field began to understand that clotting and clot dissolving both are chain reactions of proteins and enzymes in the blood. These chain reactions, or cascades, as Taylor calls them, seemed to result from the action of blood platelets.

"When we get cut and start to bleed, platelets accumulate at the injured site and begin to clump together," Taylor says. "This causes the elements in the clotting process to become activated, to help the platelets form a stable clot, so the bleeding will stop. Hemophiliacs have a deficit in one of these clotting factors."

Simultaneously, the elements of the dissolving cascade become activated, Taylor says. "This action in normal people keeps their blood from turning into a mass of jelly."

Though most of his fellow investigators were studying the clotting cascade, Taylor concentrated on the elements of clot dissolution. Before coming to OU he spent 15 years isolating and studying these elements. The effort, he says, was not unlike disassembling a car engine to identify and study each part, to see how they all worked together to make the car go.

"The difference in the analogy is that we couldn't see the parts in the blood, so we had to devise other techniques to demonstrate their existence. The other difference is that in 1958, when I became interested in thrombosis, I was starting at ground zero."

In 1958 when Taylor was an internal medicine resident at the University of California at San Francisco, two events changed the course of his professional career. "During the two months that I was on stroke service," he explains, "I got tired of holding the hands of people who were dying of stroke."

About the time Taylor was going

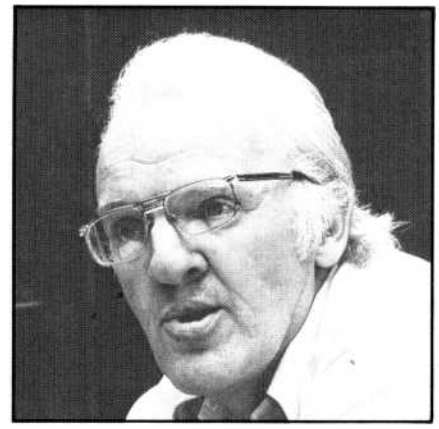
through this almost daily emotional turmoil, he met two men interested in basic information on clot dissolution. Drs. Julius Conroe and D. Kline were also interested in helping an intelligent and eager young man to get ahead. Through them, Taylor received his first research support to study blood clots.

By 1962, Taylor had a major part of his professional career mapped out.

With mixed emotions, Taylor told his father, a San Francisco internist, about his plans. "He was very disappointed when I told him that I couldn't go into practice with him. I said I would be spending 15 years in laboratories studying blood clotting at the molecular level, and trying to adapt this knowledge to potentially useful clinical tests."

In that way, Taylor told his father, he could return to thrombosis patients to offer them more than just a hand to hold.

Twelve years later, in 1974, Taylor had a clot lysis (dissolving) test that looked "very promising clinically." He believed he was ready to launch a major thrombosis program combining basic laboratory research with clinical studies.



To have any chance to acquire the necessary federal funding, Taylor needed more support in terms of laboratory space and funding from the University of Pennsylvania where he headed the coagulation section and had conducted thrombosis research since 1964. When university officials told him that their priorities unfortunately didn't include substantial increased support for his activities, Taylor knew he would have to look elsewhere.

Simultaneously, the University of Oklahoma's Health Sciences Center campus in Oklahoma City was emerging from a period of financial crisis. Only two years before — to avert a possible financial disaster at the HSC — the University had relinquished administrative and fiscal control of its two teaching hospitals. But by late 1974, with new leadership and more help from the legislature, the corner had been turned at the HSC; the emphasis switched from survival to expansion.

The HSC medical research effort

was miniscule; most faculty members had their hands full with teaching obligations alone. At almost precisely this point, two influential HSC faculty members, the late Dr. Robert Byrd and Dr. Robert Rogers, enticed Fletcher Taylor to visit Oklahoma.

It wasn't long before Taylor and the HSC knew their interests and needs coincided. Everything Taylor needed to write a competitive grant proposal was present: faculty, facilities and the willingness to make a long-range commitment.

He came to OU without public fanfare, but a few months later in April 1975, he was the subject of a press conference in Oklahoma City. Taylor just had been notified by the National Institutes of Health that his thrombosis grant proposal had been funded for $3.2 million for five years — the largest grant ever awarded to the University and among the largest federal grants awarded that year.

The funding provided for two major components: in federalese what is called a "program project," involving several basic science projects conducted by investigators in association with Dr. Taylor; and the establishment of a thrombosis and coagulation service. In that service, located in Oklahoma Memorial Hospital, the usefulness of the dilute clot lysis test was evaluated.

Here's how the test works: Blood is drawn from a person judged to be at high risk for developing a blood clot in a vein. The blood sample is diluted with a phosphate buffer and placed, along with a natural clotting substance, into a solution in a test tube. After the blood forms a clot in about 30 minutes, the test tube is placed in a bath warmed to body temperature.

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The time required for the clot to dissolve is of the essence. If the clot takes longer than about 12 hours to dissolve in each of three tests, the patient has an 80 per cent chance of having a blood clot in a vein. To gauge the test's accuracy, the patient receives a fibrinogen scan and/or a venogram, a special x-ray that Taylor calls "the gold standard for locating blood clots."

Over the service's first four years, the lysis test and the fibrinogen scan were administrated to 199 high-risk patients. The lysis test indicated that 40 had clots while only eight displayed any symptoms. Results were confirmed by the fibrinogen scan. But if a method of evaluating the blood test already exists, then of what clinical usefulness is the new test?

"A fibrinogen scan costs $200, a venogram costs just as much and is painful," Taylor says. "The clot lysis test involves only drawing blood and costs about $15.

But the test had an apparent drawback. According to the evaluation, the clot lysis test indicated 20 false positives, 20 people who didn't have a clot, contrary to the results of the test. How could that mistake be explained?

Taylor speculated that the positive test results might indicate that those people had an increased tendency to form clots; maybe they were on the verge of developing blood clots. But, he couldn't be sure. Perhaps the test was missing something. It was, an entire stage of clot dissolution. But not until 1978 would the missing stage be identified by some of Taylor's OU colleagues.

A key to the eventual discovery was provided through the case of Richard Smalley, a man with a unique blood clotting disorder. Following an abrasion and normal bleeding, a clot would form at the injured site; but as the bleeding eased, the clot suddenly would begin dissolving, and the bleeding would begin again. The clot would dissolve even more rapidly if Smalley had been active prior to the injury.

Though Smalley clearly had a bleeding disorder, it couldn't be a type of hemophilia because a normal clot formed. Something in Smalley's blood apparently was chewing up the clot prematurely, probably some sort of defect in his clot dissolving system.

To find the defect and study it, Taylor recruited "one of the best protein chemists available, Dr. Robert Radcliffe." At the end of Radcliffe's

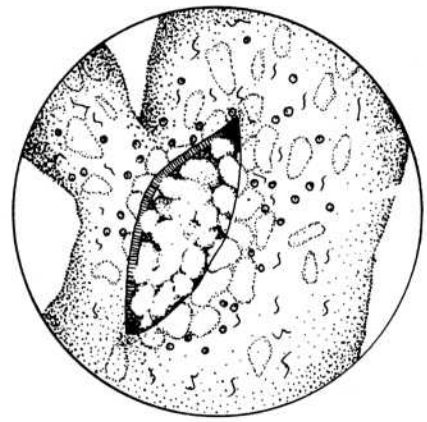


Figure I.
In a normal clotting process, platelets clump together to stop bleeding.

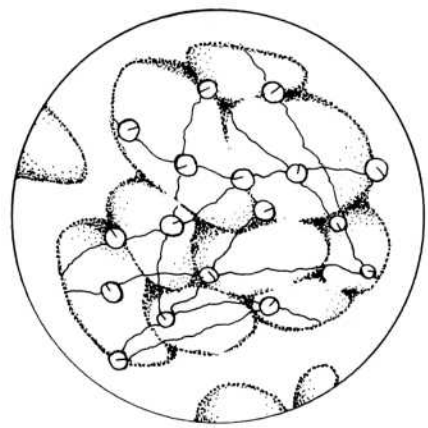


Figure II.
Enzymes react with fibrinogen to form a network to stabilize the clot.

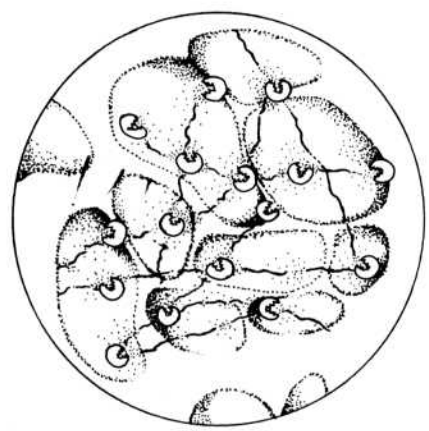


Figure III.
As a wound heals, plasmin enzymes react with other enzymes and begin to virtually chew up the fibrinogen network, dissolving the clot.

first year he could name many factors not responsible for Smalley's problem. "It was a question of botching and bungling in the dark till we narrowed the possibilities to one enzyme, called a plasminogen activator."

The enzyme didn't attack the clot, Radcliffe found, but as the name says, it activated plasminogen which activated another enzyme (in the clot dissolving cascade) called plasmin that chewed up the clot's superstructure. Smalley had a much higher level of plasminogen activator than other people, particularly following exercise, although everyone's P.A. level increases following exercise. Levels also increase as a response to factors such as trauma, emotional upset, and a host of drugs including nicotine.

Radcliffe was joined in the study of plasminogen activator by an M.D., Philip Comp, who had come with Taylor from Pennsylvania. During the program's first two years, Dr. Comp had worked on a Ph.D. in biochemistry at OU so he could later join the group as a bona fide research physician.

Comp and Radcliffe studied a group of patients with cirrhosis of the liver who tended to bleed abnormally, and found high levels of plasminogen activator. They speculate that a damaged liver isn't removing plasminogen activator from the blood, hence the increased tendency to bleed.

Contained in the vessel walls, the plasminogen activator is released normally into the blood at a certain rate, Comp believes, except when some other factors intervene such as surgery or pregnancy.

"About three-fourths of all blood clot patients I see develop the clots following surgery," says Comp, who heads the program's thrombosis and coagulation service. "The fact that P.A. is pretty well cleared from the blood following surgery may have something to do with the development of blood clots."

Pregnant women have an increased tendency to develop blood clots, he says. "Deep in my heart I know at least part of the reason: P.A. levels decrease. I think that is a predisposing cause. After the baby is delivered, the P.A. levels rise and the tendency for blood to clot lessens. "Although I can't prove it," Comp says, "I like to think that blood thinners, like heparin, increase the levels of plasminogen activator which trigger the enzymes that dissolve the clot."

Radcliffe was the first person to isolate plasminogen activator, and he has been laboriously identifying all of its parts since. When that work is completed soon, he'll turn to characterizing the things that control P.A., so that "we eventually can control it. Maybe then, it can be used to dissolve clots very quickly and safely."

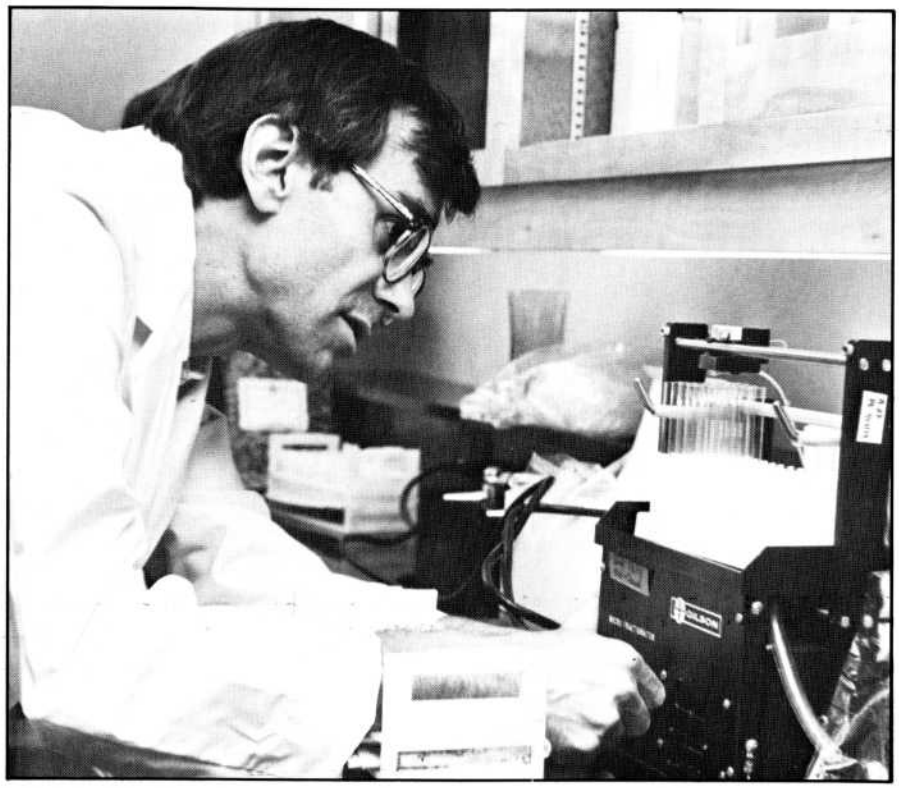
For now, Taylor and Comp have an experimental assay of P.A. that they are applying to patients to gauge the correlation between P.A. levels and thrombosis. All the data hasn't been analyzed yet, but Taylor calls the preliminary results "very encouraging."

An even more preliminary study involves a blood test that demonstrates a crucial connection between P.A. and another element in the blood. Called protein C, it was first isolated by another of Taylor's recruits, Dr. Chuck Esmon. When he discovered protein C, Esmon said it looked like a clotting factor. But when he added it to a test tube, clotting either didn't occur or took longer than normal. "It inhibits clotting factor five, so the clotting cascade is disrupted."

When Esmon added protein C to a test tube containing a clot, he found to his amazement that the clot would break apart. But protein C, he discovered, wasn't acting directly on the clot. It was reacting with white blood cells to prolong the life of the normally short-lived plasminogen activator, causing the clots to dissolve relatively quickly in test tubes and in animal models.

Clearly this white blood cell/protein C interaction was another stage of clot dissolution, which the dilute clot lysis test hadn't picked up. Why? Apparently because in that test, remember, an unnatural substance was added to get the clot to dissolve; it must have masked this interaction.

This discovery, made only about two years ago, led to the development of the whole blood lysis test, which Taylor predicts will replace the dilute clot lysis test as a diagnostic tool. Already preliminary data indicate the new test produces a much lower percentage of false positives, from 30 down to eight per cent.



In order to be a full-fledged member of Taylor's research team, Dr. Philip Comp, already a M.D., spent two years getting his Ph.D. in biochemistry.

Though clotting and clot dissolving aren't fully understood on the molecular level, certain people under certain conditions are susceptible to developing thrombosis. Dr. Comp can't explain precisely why patients bedridden for several days or weeks are at increased risk; nor does he know why increased movement reduces the risk. But Comp says, "If I ever have surgery, I'm going to be up the first day if at all possible. At least I'm going to be wiggling my toes."

In one case, Richard Wilson, a Maud, Oklahoma, high school counselor developed blood clots in the veins of his thighs after a strenuous session of weight lifting, including leg pressing 450 pounds. Two mornings later, he couldn't get out of bed. His legs were painful, badly swollen and had a murky gray pallor. The clotting was spreading upstream.

Patients bedridden for several days or weeks are at increased risk. Increased movement reduces risk.

By the time Wilson had been hospitalized, the clotting nearly had reached the veins that drain the kidneys. Because Taylor had reliable tests, he could deliver an effective supply of heparin, which helped to stop the spread to allow Wilson's clot dissolving enzymes to work.

Heavy tobacco smoking is associated with thrombosis, although the cause and effect mechanism isn't known. Taylor tells about an Oklahoma City business executive whose doctor advised him to stop smoking cigarettes and take up cigars, thinking the man wouldn't inhale cigar smoke.

The man, who was middle aged and apparently healthy, began smoking six to eight cigars daily, but he was inhaling. "Suddenly, he developed a large clot in an artery that supplies blood to a foot," Taylor says. "It completely shut off blood flow to the foot, which became gangrenous and had to be amputated."

Taylor thinks the man developed a high level of tobacco glycoprotein through inhaling cigar smoke and that this activated blood platelets—the first stage of clot formation.

Biochemist Chad Cox was studying platelets even before Taylor's University of Oklahoma thrombosis program began. Cox's research was included as one of the original program projects.

Cox says evidence suggests that platelets are involved in heart attack and stroke. He provides this scenario: "Plaque building up in an artery releases a substance that 'tells' the platelets to activate at that site. When that occurs, all of the clotting factors are recruited onto specific receptor sites on the platelet surface and the clot is formed in the artery.

"If it occludes a coronary artery, you have a heart attack. If it plugs an artery to the brain, you have a stroke," he says. "The same series of events could occur if the plaque tore loose from the vessel wall, and the platelets recognized it as a wound site."

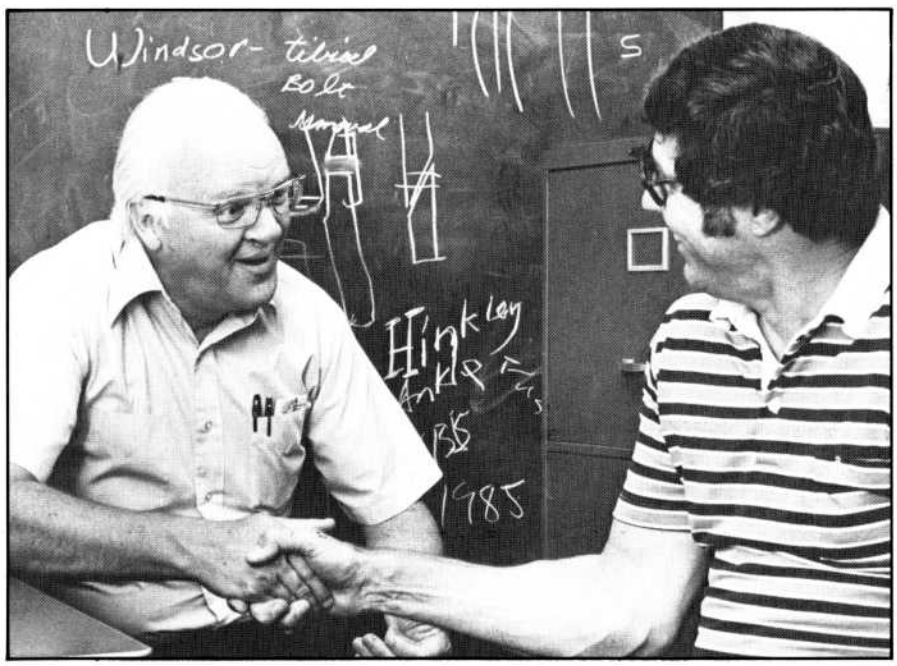
Cox is interested in determining signs of platelet activation. Generally, activated platelets have changed shape. They secrete substances what make them stick together and other compounds that "tell" vessel walls to constrict. Cox thinks it will be several years before all components of platelet activation are understood.

One reason is that many different substances have been implicated. In 1964 when a bad case of Hong Kong flu put Taylor to bed for a week, he found that his platelet count had dropped. Likewise, kidney biopsies he had had performed on himself in a hospital showed platelets were accumulating in the organ's capillaries.

He theorizes that the platelets were activated by a flu virus, causing a sub-clinical form of thrombosis. Even this can damage blood vessels, which can lead to plaque formation and eventually hardening of the arteries. In other words, it may be that hardening of the arteries is another example of platelet activation. If we can understand the mechanism and the elements that turn platelets on and off, then we can go about controlling them."

In Taylor's office a large pyramid of empty Falstaff beer cans stands on more or less permanent display. A hand-made sign taped to the cans reads, "Welcome, you missed a hell of a party!"

The sign must be intended for people outside the thrombosis program. It couldn't apply to the scientists, technicians and students who have contributed so much to thrombosis research and treatment. They haven't missed the party; in fact, Taylor says, "We've just gotten warmed up."



Thrombosis victim Richard Wilson, right, a high school counselor, thanks Taylor for the treament discoveries which probably saved his life.