

Working where the wind comes sweepin' down the plain, blood researcher Charles Esmon looks for connections that others might miss.

By NANCY ROSS-FLANIGAN

PHOTOGRAPHS BY ELI REICHMAN

# Synthesized Thinking

It's a spring day in Oklahoma, and the redbud trees are poised to burst into clouds of magenta. Against this backdrop, dressed in jeans and a plaid shirt, Charles T. Esmon looks like he'd be right at home stringing barbed wire on a ranch. But a few minutes into conversation with this easygoing HHMI investigator, it's clear that his home is in the lab, not on the range.

Moreover, the Illinois native says there's nowhere else he'd rather live and work than in his adopted state, a place better known for rodeo stars than for scientific standouts. To him, the setting is not the least bit stultifying—it is, in fact, downright stimulating.

Esmon's record stands as proof. In his 28 years at the University of Oklahoma Health Sciences Center and the Oklahoma Medical Research Foundation (OMRF), where he heads research in cardiovascular biology, Esmon has made major contributions to understanding blood clotting and associated disorders, uncovered links between the body's natural anticoagulant system and the immune system, and laid the foundation for the first drug to effectively treat septic shock—the most common cause of death in this country's intensive care units. In 2002, Esmon was elected to membership in the National Academy of Sciences, making him one of only four scientists with Oklahoma ties ever to be elected to that prestigious group. He's also the only HHMI investigator in the state.

## MORE CREATIVE

Being far from either coast, at an institution that's well-equipped but not wealthy, “we've had to be more creative,” says Naomi L. Esmon, who has been her husband's research partner for more than two decades. “We don't instantly look for a way to use the high-tech stuff because we figure the Harvards and the Johns Hopkins can afford all that equipment and they're going to go into it full-tilt. We would rather deal with the ideas than deal with the technology, even if that sometimes means doing things the old-fashioned way.”

Such circumstances “keep Chuck thinking strangely,” says Naomi, and she means that in a good way. When he is forced to come up with innova-

tive solutions to research problems, he's likely to notice connections that others might miss.

“He'll take information from biophysics—such as binding curves and enzyme kinetics—and just by listening to clinicians be able to put it all together, going from, say, crystal structures to biology and clinical applications,” says Naomi. “And it goes both ways—we'll see something in the biology that sends us back to doing the basic enzymology.”

Brown University professor Steven Opal, a physician and infectious disease specialist who has collaborated with Esmon, says he “is one of those rare individuals who can understand patterns within complex systems and integrate information in an orderly manner. He makes sense out of the intrinsic entropy that is human biology.”

In Esmon's early work, “thinking strangely,” along with a few fortunate twists of fate, led to the discovery that the blood vessel lining plays a key role in regulating blood clotting. At that time, in the mid-1970s, the idea that any-



thing in the lining might control what happened within the blood was unimaginable, he recalls. “It was somehow thought that a blood vessel was just a wonderful, Teflon tube—a magical, inert surface that blood just didn’t ‘see.’”

But something about that view didn’t make sense to Esmon: “Normally, blood doesn’t clot when it’s inside you. But if you shed blood into a tube, it all clots, and it clots solid. If you think about it, clotting to completion means it’s not regulated, so something is tremendously different in vivo than in vitro. The most obvious difference between what happens to blood in a tube and what happens in you is the ‘in you’ part, and the difference is the blood vessels.”

Esmon had become intrigued with protein C, a recently discovered anticoagulant that somehow prevented or limited blood clotting in the body but had no such effect in test tubes. Perhaps some process in the endothelial cells that line blood vessels spurred protein C into action, Esmon reasoned. But, although studying cultured endothelial cells might yield answers, “we didn’t have any endothelial cells and there was almost no cell culture being done here at the time,” he recalls.

Seeking a low-cost alternative, Esmon happened upon a paper by an East German researcher who collected pigs’ ears from a meatpacking house, connected tubes to blood vessels in the ears, and perfused the severed ears to study the workings of another type of activator. With no shortage of meatpacking plants in Oklahoma, Esmon got a slew of pigs’ ears and set up similar experiments to test the idea that thrombin—an enzyme that usually promotes clotting—might switch roles when bound to the endothelium and *prevent* clotting by activating protein C. Thrombin seemed a likely candidate, as researchers at the University of Iowa had recently found that it bound reversibly to endothelial cells.

The experiment yielded encouraging results, but it scared the daylights out of visitors, Esmon recalls. “The pigs’ ears were kept in a room with a glass door, near the entrance to the research area. So when you’d walk by, you’d see 20 pigs’ ears lined up with these things going in and coming out and dripping—it really looked like something out of a Frankenstein movie.”

## FACTOR V

The macabre setup soon could be dismantled, however, thanks to a serendipitous event. Hedging his bets against failure in the riskier protein C work, Esmon also was studying factor V, a protein critical for blood clotting. He wanted to follow the process by which subunits of the protein separate and come back together, and he thought a CD/ORD (circular dichroism and optical rotary dispersion) machine would reveal the details. He had no such instrument, but Whyte G. Owen, a friend from graduate school days, had access to one at the University of Iowa, so Esmon scheduled a visit.

“Whyte called me just before I flew off and said the machine was working,” Esmon recalls. “By the time I landed, the machine was broken.” So much for factor V. But as luck would have it, Esmon had tucked a vial of protein C into his pocket, just in case there was time after the CD/ORD experiments to test his thrombin-activation hypothesis with a model Owen was using to study a control mechanism in the heart.

Using a technique first described in 1897, Esmon and Owen flowed protein C and thrombin, separately and together, through vessels in a beating rabbit heart. “If we incubated thrombin with protein C outside the heart, nothing happened,” says Esmon. “If we put protein C through the heart, nothing happened, and if we put thrombin through the heart, nothing happened. But if we put the combination through, we had a really, really potent anticoagu-

lant. . . . We got about a 20,000-fold rate enhancement,” suggesting that something in the blood vessels made the difference. Later work by Esmon, Owen, and Naomi Esmon confirmed that thrombin binds to a specific receptor in the vessel wall, thrombomodulin, which speeds up protein C activation.

Details of the protein C pathway were falling into place, but the researchers wondered just how important the pathway was to human health. The first hints came from a baby born completely deficient in protein C. Almost immediately after birth, the infant developed large clots in the small blood vessels where protein C activation normally occurs. Doctors tried to intervene with clotting inhibitors, but nothing worked, and the baby died.

“With that patient discovery, we knew darn well that protein C was physiologically very important to humans,” says Esmon.

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Another medical observation helped round out the protein C picture: Healthy people rarely develop blood clots, even after surgery, but surgical patients with cancer, atherosclerosis, diabetes, and certain other diseases must be treated aggressively to prevent life-threatening clots.

“The one thing that’s common in these conditions is some sort of underlying inflammation—an infection or disease process, for example,” notes Esmon. Perhaps clots are more common in people with these diseases because inflammatory mediators turn off the clot-preventing system, he and his colleagues speculated, and their hypothesis turned out to be true.

“Over a number of years, with a lot of collaborators,” he says, “we’ve been able to show that the system shuts down in diabetes, over atherosclerotic plaques, in vein-bypass grafts (at least, in experimental animals), and in some types of autoimmune disease.”

That body of research has influenced other scientists as well, says Opal. “Esmon’s work has made us all refocus our attention on the mechanisms of inflammation and cellular signaling induced by the coagulation system and on the role of endogenous anticoagulants as immune modulators.”

Finding a link with inflammation prompted Esmon and OMRF colleague Fletcher B. Taylor to perform a set of experiments with implications for treating septic shock, a response to massive bacterial infection that sends blood pressure plummeting and can lead to organ failure and death.

Esmon and Taylor tried infusing lab animals with small amounts of thrombin, in hopes of activating the natural anticoagulant system, and then injecting them with *Escherichia coli* bacteria in amounts that normally would produce lethal septic shock. The procedure was not without its critics: In spite of the earlier work showing how thrombin activates protein C, the idea of preventing the widespread clotting associated with septic shock by squirting in an enzyme that ordinarily *promotes* clotting was “by no means well-accepted,” Esmon recalls. But the experiment worked. “Most of the animals lived, and most didn’t even get sick.”

Esmon and Taylor went on to show that the same effects—keeping inflammation at bay as well as preventing clotting—could be achieved by treating the animals with activated protein C extracts. This work set the stage

for Eli Lilly and Company to develop the drug Xigris, which is widely used today to treat severe sepsis.

“Now we’re interested in understanding more about how this system works,” says Esmon. Xigris is the only effective drug for treating sepsis, but it doesn’t rescue all patients—about half of those treated with the drug still die.

Typically, the drug is given for four days, after which the patient’s protein-C-activating mechanisms should be able to take over. But that doesn’t always happen. “It turns out that some patients have almost no capacity to activate endogenous protein C, while in others that system is more or less okay,” says Esmon. So he and colleagues are developing an assay for circulating activated protein C that they hope will pinpoint patients who need prolonged treatment with Xigris. They’re also trying to document the extent to which the protein C system is compromised in a wide range of inflammatory conditions, from atherosclerosis and diabetes to inflammatory bowel disease. They plan to use that information to predict which patients are at greatest risk for developing blood clots and to monitor the effectiveness of prevention and treatment efforts.

### RECEPTOR’S TRICKS

The latest chapter in the protein C story revolves around a new character, the endothelial protein C receptor (EPCR), which Esmon’s lab identified and continues to study. Originally found on the surface of vascular endothelial cells, EPCR enhances protein C activation by snaring the protein and making it more accessible to the thrombin-thrombomodulin complex. But EPCR has a few other tricks in its repertoire. One of them, discovered by Esmon’s postdoctoral fellow Jun Xu, is its unusual ability to travel from the cell surface to the nucleus, toting activated protein C and altering the expression of certain genes in the process. This behavior suggests that EPCR may have a developmental func-

tion, which current experiments with mice are aimed at clarifying.

Studying the structure of EPCR has yielded still more surprises. The versatile molecule turns out to look a lot like the major histocompatibility complex class I family of molecules, which play a major role in the inflammatory response. “So it begins to look like EPCR might be involved in the immune system directly,” says Esmon.

In a project headed by Naomi Esmon, researchers are exploring the involvement of protein C and EPCR in a condition known as lupus anticoagulant/antiphospholipid antibody syndrome, which occurs in some people with autoimmune diseases such as systemic lupus erythematosus. Patients who have the syndrome produce antibodies that inhibit blood clotting in a test tube but increase the risk of blood clots in the veins, arteries, and placenta (thereby causing recurrent miscarriages). The researchers have shown that this happens because these patients produce antibodies that prevent activated protein C from doing its anticoagulant work, particularly on membranes that have been oxidized—a common occurrence in inflammation. Current work focuses on developing an assay for the inhibitory antibodies, which could help identify lupus patients at risk for developing clots and be used to monitor their treatment, and on probing the role of EPCR in the syndrome.

Earlier in life, Esmon thought about going to medical school. He elected instead to pursue research. On the whole, though, his career can be characterized as interlinking both interests. From crystal structures and mouse models to the bedside and back, the Esmons’ projects weave disparate pieces into ever-clearer pictures of complex physiological processes, just as Naomi Esmon described in explaining how her husband’s need for “thinking strangely” takes their work in so many interesting directions. With a mind that darts here and there like the bright fish he keeps in saltwater aquariums at home, Esmon never lacks inspiration. But it’s not just his brilliance that has made him successful, says

Tim Mather, who worked in Esmon’s lab as a graduate student and now is a faculty member in the cardiovascular biology research program at OMRF.

“He’s not only a fountain of ideas, but he’s also very generous with his ideas,” Mather says. And Esmon encourages the same attitude in his lab group by welcoming suggestions from everyone and never shooting them down. “He’s like a very indulgent older brother,” Mather observes. “He wants to see you succeed.”

As a result, there’s a spirit of openness and cooperation in the Esmon lab that surprises some postdocs who’ve worked in more cutthroat environments. “There’s just enough competition to keep things interesting, but you never feel that you have to lock up your notebooks,” says Deborah Stearns-Kurosawa, who was a postdoctoral fellow in Esmon’s lab and is now on the OMRF faculty.

For Esmon, the work is not only interesting but also immensely satisfying, he says. “If you start out doing the basic biochemistry, and you get some people’s lives saved, it’s a very good feeling.” **H**

*Naomi and Charles Esmon—partners in marriage as well as in the lab—with Shadow (L.) and Lilly.*

