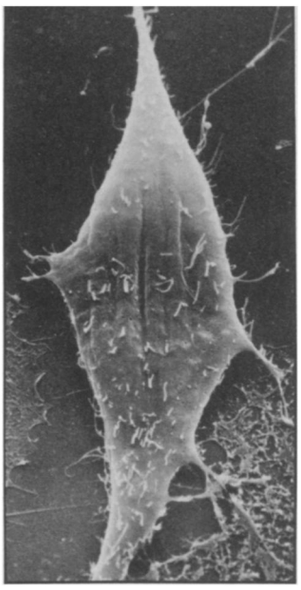
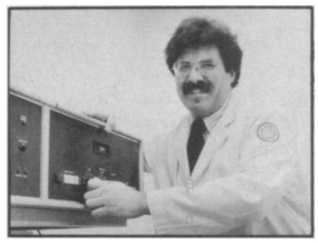


Unzipping Blood Vessel Linings

A technique for culturing cells that line blood vessels is shedding light on the vascular pathology of heart attacks, stroke, bleeding diseases and even of organ transplant rejection

BY JOAN AREHART-TREICHEL



Dick Nadel

Jaffe: The luck of a novice.

From the 1920s to the 1960s, scientists tried to grow endothelial cells — cells lining blood vessels — but failed. In 1963, for instance, a Japanese scientist named Maruyama came up with a culture technique that looked promising. He washed endothelial cells from human umbilical cord blood vessels free of blood, digested out the endothelial cells with the enzyme trypsin, spun them down in a centrifuge and put them into a nutrient medium in hopes that they would multiply. Maruyama, however, was unable either to get the cells to increase in number or to identify them unequivocally as endothelial cells.

Then in the summer of 1971, a Cornell University Medical Center hematologist, Ralph Nachman, challenged a young physician researcher in his lab, Eric Jaffe, to make Maruyama's technique work. Jaffe managed to bring off in four days what other scientists hadn't been able to achieve in 40 years. The reason? As Nachman recalls, "It was partly the luck of a novice. The experts thought it was too difficult." And as Jaffe recollects, "I just played around with different culture mediums until I found one that worked."

The real challenge, however, was to develop a means of identifying endothelial cells grown in tissue culture — something that had never been achieved before. And this is where Jaffe made a quantum leap forward. For a year and a half he persevered, and finally came up with a set of markers peculiar to endothelial cells. First, he noted that the cells are large, flat and polygonal and grow in only one layer. Later, he showed that they contain unique granules called Weibel-Palade bodies, blood group antigens appropriate to a person's blood type and a particular chemical called Factor VIII antigen. Jaffe's success in culturing endothelial cells and identifying the cultured cells was reported in the November 1973 JOURNAL OF CLINICAL INVESTIGATION. How did he feel about this coup? "It's nice being able to do something you want to do, that should be done, that allows you to ask questions worth asking," he says. "It happens to a lot of scientists besides me. And when you see one of these researchers, he glows."

Now, four years later, Jaffe is really beaming, because the culture and identification techniques he has perfected have been hailed around the world as valuable tools for studying healthy and diseased blood vessels and their roles in major diseases and conditions such as stroke, heart attacks, hemophilia and organ transplant rejection. Before, the participation of blood vessels in these pathologic processes was difficult to define. For his development of the techniques of endothelial cell culture, Jaffe was awarded, in the spring of 1977, the Passano Foundation $6,000 Distinguished Young Scientist Award for 1977. The foundation has as its purpose the encouragement of medical research, particularly that with a clinical application. Even more noteworthy, the techniques are already revealing some crucial insights into the role of endothelial cells in health and disease.

For one thing, endothelial cells appear to help prevent abnormal reactions of blood platelets with the blood vessel wall. These reactions are thought to be one of the prime causes of heart attacks and strokes because if they are severe they will lead to a blood clot. Two separate groups of investigators — Babette B. Weksler, Aaron Marcus and Eric Jaffe of Cornell University Medical Center and D. E. MacIntyre of Cambridge University in England and his co-workers — recently reported, in the September 1977 PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES and the Feb. 9 NATURE, that endothelial cells make a prostaglandin, called PGI_2, which is a ferocious inhibitor of platelet aggregation. Prostaglandins are fatty acids made by many tissues; they act primarily near their origins as "tissue hormones." The prostaglandin made by endothelial cells probably helps prevent abnormal or excessive platelet clustering that could damage a blood vessel wall.

Damage to endothelial cells, on the other hand, may encourage heart disease and strokes under certain conditions. How? By making arteries harden. Hardening of the arteries is a major risk factor in heart disease and strokes. If endothelial cells in some area of an artery are damaged, the prostaglandin they make that normally inhibits platelet aggregation is not produced in its usual amounts and a layer of aggregated platelets forms over the damaged area. The platelets then release a growth factor that causes smooth muscle cells underlying the endothelial cells to proliferate. Eventually this process, if prolonged or repeated, leads to thickening or hardening of the arteries.

Endothelial cells also appear to be intimately involved in homophilia and von Willebrand's disease, in which victims experience life-threatening hemorrhage from trivial injuries because they lack one or more protein factors that help blood coagulate at a wound site and heal the wound. Specifically, Jaffe, Nachman and Leon W. Hoyer of the University of Connecticut School of Medicine have found that cultured endothelial cells make Factor VIII antigen, which is decreased in the blood of patients with von Willebrand's disease, but which is normal or elevated in the blood of hemophiliacs, and that cultured endothelial cells also make von Willebrand Factor, which is lacking in von Willebrand victims, but normal in hemophiliacs. However, endothelial cells do not make Factor VIII clot-promoting function, a protein absent in the blood of both hemophiliacs and von Willebrand patients.

These findings are "very exciting," Jaffe wrote in the Feb. 17, 1977 NEW ENGLAND JOURNAL OF MEDICINE, and "should lead to a more complete understanding of the pathophysiology of hemophilia and von Willebrand's disease."

A fourth role for endothelial cells is emerging from endothelial cell culturing — involvement in organ transplant acceptance or rejection. Organ transplant rejection continues to be a major obstacle to transplant success.

The success of an organ transplant depends primarily on the degree of im-

munologic similarity between donor and recipient, that is, on whether certain antigens on a grafted organ are similar to antigens of the recipient's own organs. If the antigens aren't alike, the person's immune system will recognize the strange antigen and attack or reject as a foreign body the organ containing the strange antigen.

Some of the antigens involved in organ transplant acceptance or rejection are so-called HL-A antigens. Blood vessels in a grafted organ are the first tissue in the organ to be attacked if a recipient's immune system decides the organ is an enemy. Now Allan Gibofsky of the City University of New York and his colleagues have found that cultured endothelial cells also contain HL-A antigens. So it's quite possible, then, that endothelial cells lining the blood vessels of a grafted organ are the first cells in the organ to be either accepted or rejected during organ transplantation. Or as Gibofsky and team put it in the September 1975 JOURNAL OF IMMUNOLOGY: "The endothelial cells of a grafted organ are the first cell types contacted by the immunocytes of the recipient and thus are the first cells that may be recognized as 'non-self.'"

These researchers have also found that if endothelial cells are kept in culture for a long period of time, they lose some of their HL-A antigens. This finding, the investigators believe, may provide some leads to how HL-A antigens might be snuffed out in tissue culture, and then in humans, so that they cannot lead to organ transplant rejection.

Thanks to endothelial cell culturing, endothelial cell repair is also being understood better. First, endothelial cells near a wound in endothelial tissue move into the wound site, as if to provide a supportive lattice, Michael Gimbrone and colleagues at Harvard Medical School have found. Then endothelial cells in the wound start to replicate. After that, cell migration and cell division continue simultaneously until a complete layer of endothelial cells has been regenerated.

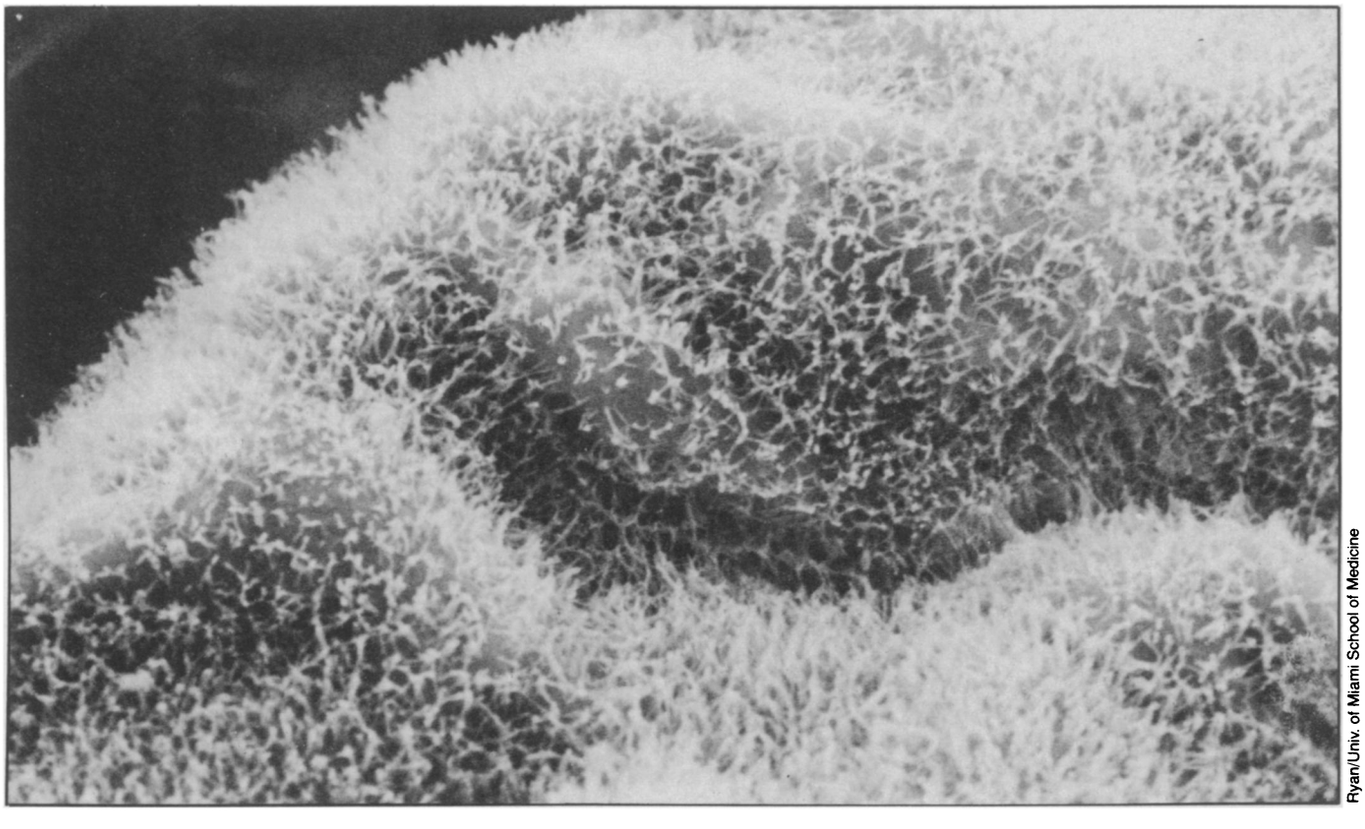
Still more insights into endothelial cells — how they use protein hormones in the bloodstream — are coming from endothelial cell culture studies. For instance, Alice R. Johnson and co-workers at the University of Texas Health Science Center at Dallas cultured endothelial cells, then studied the activities of some enzymes in the cells that act on the protein hormones bradykinin, angiotensin II and Substance P. Angiotensin II constricts blood vessels, and hence encourages high blood pressure. Substance P and bradykinin, on the other hand, are potent blood vessel expanders, and thus lower blood pressure. So it appears that endothelial cells contain several enzymes that can participate in blood pressure regulation.

And more spin-offs from endothelial cell culturing are in the works. For example, Gimbrone and colleagues have found that both endothelial cells and smooth muscle cells lining blood vessel walls make prostaglandin E, a prostaglandin vital to blood pressure regulation and to blood vessel tone and permeability. Thus, prostaglandin E may play a role in these cells' responses to various stimuli.

Endothelial cell culturing, in fact, has been taken a step further by Una S. Ryan and her colleagues at the University of Miami School of Medicine. They have adapted the Maruyama-Jaffe technique so that they can study endothelial cells that line blood vessels in lungs rather than in umbilical cords. A report on their adaptation of the technique is in press with TISSUE AND CELL. Some noteworthy characteristics of lung endothelial cells that they have found are that the cells are very thin, being thickest in the region of the nucleus, and have on their surfaces lots of projections called caveolae yet only a few Weible-Palade bodies, compared with the many found on umbilical cord endothelial cells. However, lung endothelial cells, like umbilical endothelial cells, do make prostaglandins and enzymes that act on bradykinin and angiotensin II.

Thus, the endothelial cell culturing technique initiated by Maruyama, perfected by Jaffe and further adapted by other researchers has already yielded numerous insights into the crucial world of blood vessel linings. Undoubtedly, more such glimpses will come in the near future as well, such as which activities are unique to endothelial cells and which are also common to other kinds of cells, precisely what roles endothelial cells play in various diseases, how endothelial cell damage might be corrected, and so forth. In fact, one can easily see the culturing technique being extended to tissues other than umbilical cords and lungs. That way, endothelial cells from many areas of the body could be scrutinized and compared. □

Scanning electron micrograph of the surface of lung artery. It is now possible to grow such cells and to study them in culture.



Ryan/Univ. of Miami School of Medicine