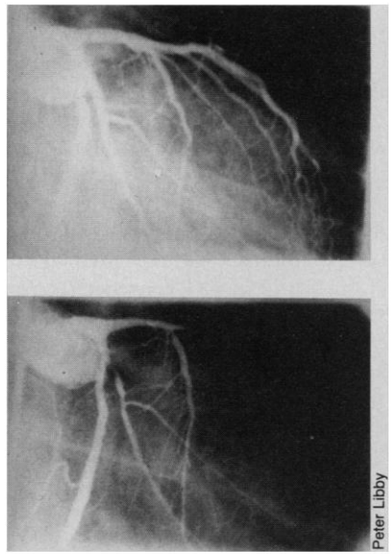
Holding the Line Against Heart Disease

The blood vessel linings constitute a major line of defense for the cardiovascular system. If it is breached, serious illness may result

BLOOD VESSELS, IT TURNS OUT, are more than mere plumbing. Researchers are discovering that they play a dynamic role in regulating blood pressure and blood clotting as well as their own growth. The key to these regulatory activities, according to a growing body of new findings, is the endothelial cells, which form the interface between the blood and the vessel walls. The endothelium is "the protector of the organism," says Victor J. Dzau of Harvard Medical School and Brigham and Women's Hospital in Boston, who chaired a symposium on vascular biology earlier this month for the National Heart, Lung, and Blood Institute. "You are dependent on the blood vessel—particularly the endothelium—to repair itself continuously."

If it doesn't, the consequences can be severe. Endothelial damage has been linked to vascular diseases, such as atherosclerosis and high blood pressure, with their attendant increased risk of having heart attack or stroke. Endothelial cell damage may also confound clinicians' efforts to repair the ravages of cardiovascular disease by surgical procedures such as heart transplantation or by balloon angioplasty, a procedure frequently used to open the blocked coronary arteries of heart patients.



Shut down. *Last October the arteries of a transplanted heart looked normal (top). By May major blockage had occurred.*

It's no wonder then that endothelial cell biology is emerging as "a major hot topic." It has been cited as such by *Science Watch*, a publication of the Institute for Scientific Information that tracks trends in basic research by counting the literature citations a particular area receives. It was also the featured topic in the heart institute's symposium, the latest in a continuing series dealing with "Frontiers in Basic Sciences That Relate to Heart, Lung, and Blood Diseases."

A better understanding of how endothelial cells work normally—and how they can malfunction—could give cardiovascular specialists new ways of counteracting the problems they are finding.

One of those problems has cropped up relatively recently in heart transplant recipients. Thanks to advances in suppressing the immune responses that can cause the rejection of foreign tissue, the recipients are living longer. But now many of the patients are developing an unusual form of atherosclerosis that can come on quickly and so completely fill the blood vessels of their new hearts that the only recourse is another transplant. "It's now the main problem limiting long-term survival of heart transplant patients," says cardiologist Peter Libby of Tufts University–New England Medical Center in Boston.

At the meeting, Libby presented evidence suggesting that damage to the endothelium of the blood vessels of the transplanted heart may be causing the trouble. He and his colleagues Jordan Pober of Harvard Medical School and Robert Salomon of New England Medical Center have found that the damage may be triggered by an immune reaction to the vessel lining, although not the same type that causes ordinary graft rejection. "One clue," Libby says, "is that it [the vessel blockage] affects transplanted vessels, but not those of the host."

The Boston workers suggest that when a patient's immune cells, particularly the T lymphocytes, come in contact with the inner wall of the foreign blood vessels, they are stimulated to release lymphokines, agents that act in several ways to mediate immune reactions. But the lymphokines' actions are not limited to immune cells; they also have several effects on the endothelial and smooth muscle cells of blood vessel walls. In particular, Libby says, the lymphokine gamma interferon can induce those cells to produce a type of immune molecule known as a class II major histocompatibility protein. Libby's group has detected the protein in the clogged coronary arteries of patients who died of transplant-associated atherosclerosis, but they have never detected it in normal human endothelia.

The class II molecules on the transplant vessel lining could then draw in a second wave of lymphokine-producing immune cells, including the ones known as macrophages. In support of this idea, the Boston workers find large numbers of macrophages, as well as T cells, in the transplant lesions.

Because some of the lymphokines stimulate smooth muscle cell growth, the net result of all this, Libby says, could be a marked over-proliferation of the smooth muscle cells of the arterial lining, so much so that the arteries close down.

Heart transplants are relatively uncommon; only about 1700 were performed in the United States last year. But a procedure called balloon angioplasty, which is used to open the blocked coronary arteries of some heart patients, is done much more frequently. Some 250,000 of the operations were performed last year in the United States. For about 25% of the angioplasty patients, however, relief of the blockage is very temporary—their arteries close up again within 6 months—and endothelial cell damage may again be at fault.

In balloon angioplasty, a cardiologist threads a fine tube, tipped with a balloon, into a blocked coronary artery and then inflates the balloon to relieve the blockage. Researchers have long known that if they use a balloon catheter to rub away the arterial linings of experimental animals, they cause a persistent increase in the proliferation of the arterial smooth muscle cells. It can last as long as 12 months, notes Michael Reidy of the University of Washington School of Medicine in Seattle.

The same thing might happen to human arteries after angioplasty, contributing to their reclosure. "We're just doing balloon angioplasty in rats," says Reidy, who is studying how the injury stimulates smooth muscle cell proliferation. Results so far suggest that increased growth factor production may be involved.

Other influences may also contribute to the shutdown of arteries after angioplasty, and some of these would have immediate effects, not taking the days or weeks that growth stimulation would require to close off a blood vessel. Paul Vanhoutte of Baylor College of Medicine in Houston has evidence that when arteries are denuded of their endothelial cells, they lose one of their

New Genes for Ailing Hearts?

If all goes well, the first attempt to perform true gene therapy on a human patient will take place later this year (*Science*, 8 June, p. 1182). But at most, only a handful of children will be eligible for that particular therapy, which aims to treat a severe hereditary immunodeficiency. Meanwhile molecular biologists have been taking the first tentative steps toward devising gene therapies that can help the millions of people who suffer from the cardiovascular diseases.

Earlier this month at a symposium on "Vascular Biology and Medicine: The Next Frontier," James Wilson of the Howard Hughes Medical Institute at the University of Michigan School of Medicine in Ann Arbor reported that he and his colleagues have used gene therapy to lower blood cholesterol, albeit temporarily, in Watanabe rabbits. Because of a genetic defect, the cells of this strain of rabbits lack receptors for LDL (low-density lipoprotein) cholesterol, considered the bad form of cholesterol because it promotes atherosclerosis. As a result, the animals can't remove LDL cholesterol from their blood and readily develop atherosclerotic plaques.

In experiments begun when he worked with Richard Mulligan at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, Wilson attempted to correct the LDL receptor deficiency by introducing the receptor gene into the rabbits' liver cells. He performed the actual gene transfer on liver cells in culture and then injected the cells, with their new gene, back into live rabbits. He estimates that the injected cells could provide no more than 4% of the LDL receptor activity found in a normal liver, but even so, the animals' LDL cholesterol concentrations dropped about 35%. "That's the good news," Wilson says. "The bad news is that it's back up to normal in about 2 weeks." He does not yet know why that happened. One likely reason has been ruled out, Wilson says. The LDL receptor gene, which was introduced into the liver cells in a retrovirus vector, was stably integrated into the cellular DNA and should have remained there as long as the cells lived.

In more recent experiments, Wilson's group, in collaboration with researchers at Albert Einstein Medical Center in New York City and the University of Connecticut in Farmington, has been attempting to get the LDL receptor gene directly into the livers of live animals without having to use a viral vector. The researchers inject Watanabe rabbits with a complex of the gene and a protein that targets it to liver cells. As expected, the gene was active only in the liver cells, but the activity lasted no more than 3 days, possibly because the foreign gene didn't integrate into the cellular DNA.

The animals' LDL cholesterol concentrations dropped about 35%.

Researchers are also exploring different approaches to gene therapy for atherosclerosis and other cardiovascular diseases. Endothelial cells make particularly attractive targets for gene therapy because they are in intimate contact with the blood and play such an important regulatory role in maintaining the normal functions of the cardiovascular system (see accompanying story). About a year ago, Wilson and Mulligan reported that genetically modified endothelial cells could be impregnated on artificial arterial grafts, which were then introduced into dogs. What's more, the foreign gene the endothelial cells were carrying was active for at least 5 weeks. In another experiment reported at the same time, Elizabeth Nabel and Gary Nabel of the University of Michigan Medical Center showed that, when genetically altered endothelial cells were introduced into minipigs, the cells would attach to an artery from which the endothelial cells had been removed and the gene would be active.

But these successes do not mean that all the problems are solved. Mulligan notes that only a small number of modified cells were introduced in the arterial grafts. Moreover, clinical practice has shown that blood flow through vessel grafts frequently becomes blocked off, either by blood clots or by tissue growth. And Una Ryan, who is doing gene therapy research at Monsanto Company in St. Louis, pointed to another problem that could haunt efforts to apply the technology to cardiovascular genes. The products encoded by many of the genes one might want to use often have several effects—some beneficial, but others not. "We're going to have to understand all of them before we can undertake gene therapy," she warns. ■ J. M.

defenses against blood platelets.

Platelets are an important part of the body's defenses against injury. If a blood vessel is cut, they congregate at the site and prevent hemorrhaging by releasing agents that constrict the vessel and form a blood clot. But if this were to happen abnormally—say, in a coronary artery—the result would be disaster. So the normal endothelium has defenses to keep platelets from working at the wrong time and place.

One way it does this is by producing a blood vessel dilator called endothelial-derived relaxing factor (EDRF) in response to the clotting and vasoconstrictive agents that platelets release. But Vanhoutte and his colleagues found that when they rubbed away the endothelial linings of animal arteries with a balloon, that ability was lost.

And it was not regained even after the endothelial cells grew back, although the experiment was continued for 6 months. "Somehow in the regeneration process they [the endothial cells] have lost this pathway for releasing EDRF," Vanhoutte says. "The consequence is clinically important because it implies that the cells don't have to be dead [for the response to be lost]."

Removing the endothelium could foster clot formation as well as vasoconstriction. The endothelial lining is a major site for the conversion of plasminogen to plasmin, a clot-dissolving enzyme. So with the cells gone, that clot protection would be greatly reduced.

Many of the same mechanisms that researchers are identifying as contributing to transplant-associated atherosclerosis and the reclosure of arteries opened by balloon angioplasty may also be operating in typical atherosclerosis. Platelets, for example, are present in ordinary atherosclerotic plaques, where they contribute to the lesion formation and can also produce blood clots that precipitate heart attacks.

And as Libby points out, macrophages and T cells are found in typical atherosclerotic lesions as well as in the transplant-associated kind, although there are other differences between the two types of atherosclerosis. "The transplant-associated lesion may represent an extreme case of the same mechanism found in the usual form of atherosclerosis," Libby says.

What attracts immune cells such as macrophages to ordinary atherosclerotic sites is still a question, however, since it couldn't be the presence of foreign tissue. But new findings by Michael Gimbrone and Myron Cybulski of Harvard Medical School may provide a clue. They have found that the edges of early atherosclerotic plaques in rabbits have an adhesion molecule that can latch on to macrophages. The Harvard

Animal Cells Transformed in Vivo

Researchers at Duke University have developed what they believe is a versatile and simple method of inserting novel genes into the somatic cells of live animals: shoot them in with a biological version of a BB gun. Conventional means for transforming such cells are complex, indirect endeavors. Typically, the cells targeted for transformation are drawn out of the organism, transformed with a retrovirus, and then reintroduced. Indeed, in what will be the first clinical trial for human gene therapy later this year, National Institutes of Health researchers will use that approach with bone marrow cells to correct adenosine deaminase deficiency (ADA) in a select group of patients (*Science*, 8 June, p. 1182).

But at the 13 June meeting of the Tissue Culture Association in Houston, Stephen A. Johnston of Duke described his simplified technique. He uses a redesigned version of the so-called gene gun, first developed at Cornell University, which sold the rights to it to DuPont last year. The gene gun shoots DNA-coated, gold microprojectiles into living cells. The gun was first used successfully to transform algae and yeast in 1988 and is now used routinely to transform plants. In April, for example, Monsanto used the gene gun to transform the recalcitrant crop, corn.

But the gene gun, as it was originally designed, used a gunpowder-like explosion to shoot the DNA into cells, and it damaged the more delicate animal cells. The new model uses high-pressure gas to propel the DNA. "We can shoot tiny BBs at high velocity, with little trauma to the tissue," Johnston says. Indeed, Johnston and his collaborators at Cornell and Dupont have just shown that the redesigned gun can insert novel genes into the ear, skin, and surgically exposed liver cells of mice.

Johnston is confident that the redesigned gene gun, also known as a biolistics device, for biological and ballistic, can be developed for medical uses as well. "Our goal is to design an instrument to do transformations on somatic cells in a surgical setting," says Johnston. "We have it down to almost a hand-held instrument. We are trying to keep the idea simple." What's more, he adds, "the versatility of the gun allows us to transform various tissues." Muscle or skin cells might be modified to provide circulating factors, such as insulin, says Johnston. Or the endothelial cells in the blood vessels of heart attack victims might be engineered to express the clot-buster TPA, tissue plasminogen activator. But first, Johnston admits, he and other researchers must demonstrate that this technique can be used to achieve stable expression of inserted genes and that it can transform an adequate number of cells.

"People say, 'It is a bizarre system. But it works.' "

Within the last few months other researchers have also announced new techniques for inserting genes into live animal cells. Christine E. Holt of the University of California, San Diego, inserted genes into the neuroepithelium of frog embryos using lipofectin, a synthetic lipid that entraps DNA much as liposomes do. And Jon A. Wolff of the Waisman Center at the University of Wisconsin injected genes into the skeletal muscle of young mice (see *Science*, 23 March, p. 1465). But Wolff's technique didn't work well with brain, blood, liver, or spleen tissue. Thus, neither of these techniques appears as versatile as the gene gun.

How have Johnston's colleagues reacted to the news of the first successful use of the gene gun in live animal cells? Much as plant biologists did earlier, says Johnston. "People say, 'It is a bizarre system. But it works.' " ■ ANNE SIMON MOFFAT

Anne Simon Moffat is a free-lance writer in Ithaca, New York.

workers are now trying to determine whether the adhesion molecule initiates plaque formation by attracting the immune cells. At the very least, Gimbrone says, the new adhesion molecule should serve as a marker for early detection of atherosclerosis, and if it is involved in plaque initiation, it may also provide a point of attack for preventing the buildup of the artery-clogging deposits.

However macrophages get into plaques, they may have several effects in addition to stimulating the proliferation of arterial smooth muscle cells by secreting lymphokines. According to Daniel Steinberg of the University of California, San Diego, they may also contribute to the cholesterol deposition that is one of the hallmarks of typical atherosclerosis lesions.

Macrophages are notorious for producing strong chemical oxidizing agents such as the superoxide anion. That helps them kill foreign bacteria and clean up debris at inflammatory sites. But it can also have a less desirable effect, Steinberg says, namely, the oxidation of LDL (low-density lipoprotein) cholesterol, known as the bad form of cholesterol because it promotes atherosclerosis.

Oxidized LDL cholesterol not only attracts more macrophages to a growing atherosclerotic site, but it is also taken up by the cells much more readily than native LDL cholesterol. As a result the site becomes clogged with cholesterol-laden macrophages, leading to further progression of atherosclerosis.

Finally, LDL cholesterol is not the only blood lipid that is a risk factor for heart attacks. Epidemiological studies have shown that lipoprotein(a) is a risk factor, too, although how the molecule might predispose to heart attacks has been unclear. New findings from Ralph Nachman's lab at Cornell Medical College in New York City now suggest that it may work by interfering chemically with endothelial defenses against clot formation, much as the damage caused by angioplasty interferes physically.

About 2 years ago, researchers learned that the protein component of lipoprotein(a) has a structure similar to plasminogen's. That suggested that the lipoprotein might somehow contribute to blood clot formation. Nachman now reports that it may do this by interfering with plasminogen binding to the endothelial lining. As a result of the inhibition of binding, he says, the release of plasmin from tissue-bound plasminogen goes down 80 to 90%, and "over a long period, this may contribute to atherogenesis." Indeed, when Nachman and his colleagues looked for lipoprotein(a) deposition on the endothelium of coronary arteries, they found it on diseased arteries, but not on normal ones.

The reactions that contribute to the development of arterial lesions, both the ordinary atherosclerotic type and those seen in transplanted hearts and after balloon angioplasty, are by and large the same reactions that the body uses to defend itself against injury and invading pathogens. As atherosclerosis expert Russell Ross of the University of Washington School of Medicine in Seattle points out, for example, "Atherosclerosis is wound-healing gone wrong." A good deal of evidence now indicates that an intact endothelium is needed to keep those reactions from going wrong. ■ JEAN MARX

Animal Cells Transformed in Vivo

Researchers at Duke University have developed what they believe is a versatile and simple method of inserting novel genes into the somatic cells of live animals: shoot them in with a biological version of a BB gun. Conventional means for transforming such cells are complex, indirect endeavors. Typically, the cells targeted for transformation are drawn out of the organism, transformed with a retrovirus, and then reintroduced. Indeed, in what will be the first clinical trial for human gene therapy later this year, National Institutes of Health researchers will use that approach with bone marrow cells to correct adenosine deaminase deficiency (ADA) in a select group of patients (*Science*, 8 June, p. 1182).

"People say, 'It is a bizarre system. But it works.' "

But at the 13 June meeting of the Tissue Culture Association in Houston, Stephen A. Johnston of Duke described his simplified technique. He uses a redesigned version of the so-called gene gun, first developed at Cornell University, which sold the rights to it to DuPont last year. The gene gun shoots DNA-coated, gold microprojectiles into living cells. The gun was first used successfully to transform algae and yeast in 1988 and is now used routinely to transform plants. In April, for example, Monsanto used the gene gun to transform the recalcitrant crop, corn.

But the gene gun, as it was originally designed, used a gunpowder-like explosion to shoot the DNA into cells, and it damaged the more delicate animal cells. The new model uses high-pressure gas to propel the DNA. "We can shoot tiny BBs at high velocity, with little trauma to the tissue," Johnston says. Indeed, Johnston and his collaborators at Cornell and Dupont have just shown that the redesigned gun can insert novel genes into the ear, skin, and surgically exposed liver cells of mice.

Johnston is confident that the redesigned gene gun, also known as a biolistics device, for biological and ballistic, can be developed for medical uses as well. "Our goal is to design an instrument to do transformations on somatic cells in a surgical setting," says Johnston. "We have it down to almost a hand-held instrument. We are trying to keep the idea simple." What's more, he adds, "the versatility of the gun allows us to transform various tissues." Muscle or skin cells might be modified to provide circulating factors, such as insulin, says Johnston. Or the endothelial cells in the blood vessels of heart attack victims might be engineered to express the clot-buster TPA, tissue plasminogen activator. But first, Johnston admits, he and other researchers must demonstrate that this technique can be used to achieve stable expression of inserted genes and that it can transform an adequate number of cells.

Within the last few months other researchers have also announced new techniques for inserting genes into live animal cells. Christine E. Holt of the University of California, San Diego, inserted genes into the neuroepithelium of frog embryos using lipofectin, a synthetic lipid that entraps DNA much as liposomes do. And Jon A. Wolff of the Waisman Center at the University of Wisconsin injected genes into the skeletal muscle of young mice (see *Science*, 23 March, p. 1465). But Wolff's technique didn't work well with brain, blood, liver, or spleen tissue. Thus, neither of these techniques appears as versatile as the gene gun.

How have Johnston's colleagues reacted to the news of the first successful use of the gene gun in live animal cells? Much as plant biologists did earlier, says Johnston. "People say, 'It is a bizarre system. But it works.' " ■ ANNE SIMON MOFFAT

Anne Simon Moffat is a free-lance writer in Ithaca, New York.

workers are now trying to determine whether the adhesion molecule initiates plaque formation by attracting the immune cells. At the very least, Gimbrone says, the new adhesion molecule should serve as a marker for early detection of atherosclerosis, and if it is involved in plaque initiation, it may also provide a point of attack for preventing the buildup of the artery-clogging deposits.

However macrophages get into plaques, they may have several effects in addition to stimulating the proliferation of arterial smooth muscle cells by secreting lymphokines. According to Daniel Steinberg of the University of California, San Diego, they may also contribute to the cholesterol deposition that is one of the hallmarks of typical atherosclerosis lesions.

Macrophages are notorious for producing strong chemical oxidizing agents such as the superoxide anion. That helps them kill foreign bacteria and clean up debris at inflammatory sites. But it can also have a less desirable effect, Steinberg says, namely, the oxidation of LDL (low-density lipoprotein) cholesterol, known as the bad form of cholesterol because it promotes atherosclerosis.

Oxidized LDL cholesterol not only attracts more macrophages to a growing atherosclerotic site, but it is also taken up by the cells much more readily than native LDL cholesterol. As a result the site becomes clogged with cholesterol-laden macrophages, leading to further progression of atherosclerosis.

Finally, LDL cholesterol is not the only blood lipid that is a risk factor for heart attacks. Epidemiological studies have shown that lipoprotein(a) is a risk factor, too, although how the molecule might predispose to heart attacks has been unclear. New findings from Ralph Nachman's lab at Cornell Medical College in New York City now suggest that it may work by interfering chemically with endothelial defenses against clot formation, much as the damage caused by angioplasty interferes physically.

About 2 years ago, researchers learned that the protein component of lipoprotein(a) has a structure similar to plasminogen's. That suggested that the lipoprotein might somehow contribute to blood clot formation. Nachman now reports that it may do this by interfering with plasminogen binding to the endothelial lining. As a result of the inhibition of binding, he says, the release of plasmin from tissue-bound plasminogen goes down 80 to 90%, and "over a long period, this may contribute to atherogenesis." Indeed, when Nachman and his colleagues looked for lipoprotein(a) deposition on the endothelium of coronary arteries, they found it on diseased arteries, but not on normal ones.

The reactions that contribute to the development of arterial lesions, both the ordinary atherosclerotic type and those seen in transplanted hearts and after balloon angioplasty, are by and large the same reactions that the body uses to defend itself against injury and invading pathogens. As atherosclerosis expert Russell Ross of the University of Washington School of Medicine in Seattle points out, for example, "Atherosclerosis is wound-healing gone wrong." A good deal of evidence now indicates that an intact endothelium is needed to keep those reactions from going wrong. ■ JEAN MARX