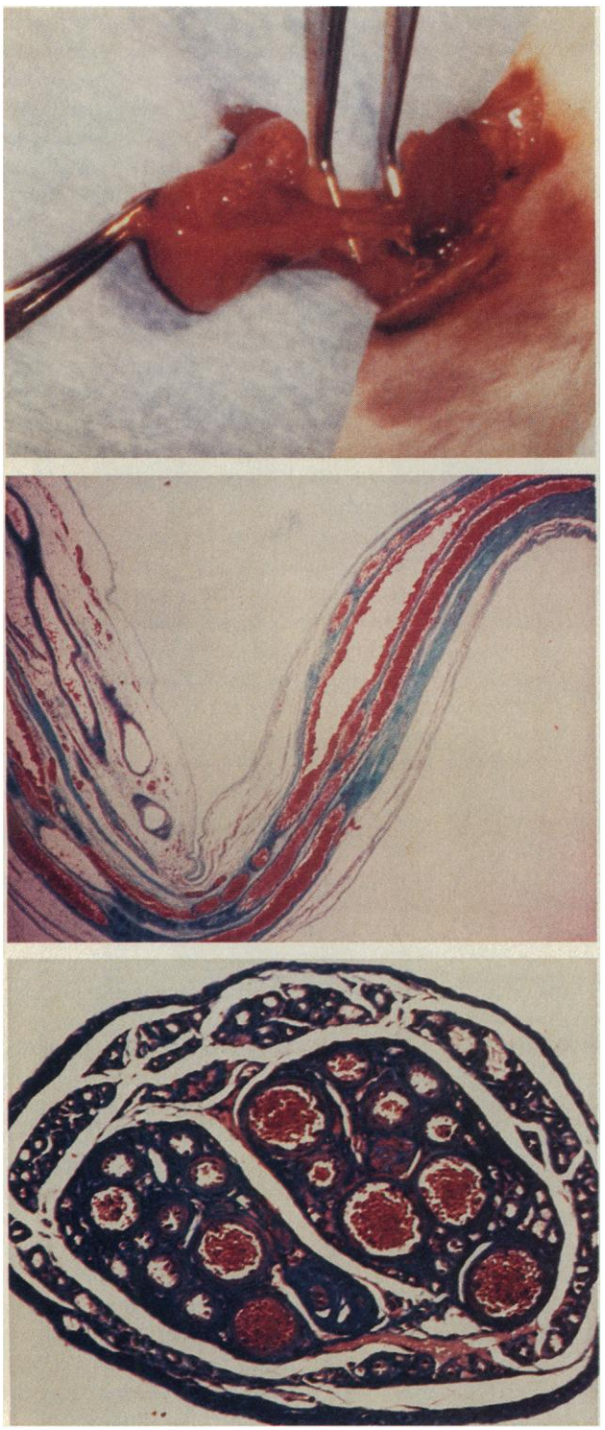


John A. Thompson

An organoid at work. *The addition of a growth factor that stimulates blood vessel formation was the key to creating a synthetic organ of fine Gore-Tex fibers coated with collagen. Here, vessels from an organoid that has been implanted in the peritoneal cavity of a rat extend to the animal's natural liver, thereby establishing a channel for the transfer of genetically modified proteins from the organoid to the rat's circulatory system.*
In the middle photograph, one can see that a vascular network of numerous individual vessels, with the structural properties of natural vessels, has been formed.
Below, a view in cross-section confirms the presence of abundant vessels lined with endothelial cells and surrounded by layers of smooth muscle. Two fascinating structures not surrounded by white vascular lumina appear to be nerve cells, but their precise identity remains unknown.

Gore Tex Organoids and Genetic Drugs

Researchers are creating implantable "neo-organs" to house cells engineered to produce missing proteins

Genes In Medicine

With little more than some strands of Gore-Tex finer than an angel's hair, a supply of collagen, and a dash of heparin-binding growth factor–1, John Thompson and Thomas Maciag have what Thompson calls "a cauldron of all the right stuff to create an organ." An "organoid," really. A man-made, biologically active ball of indestructible Gore-Tex (the fiber that skiers love) and cells that have two of the essential characteristics of real human organs: the capacity to make blood vessels and to secrete proteins.

The first working organoid, shown in the top picture on the left, is an artificial liver, surgically implanted in the peritoneal cavity of a Lewis rat. Blood vessels generated in the Gore-Tex liver stretch across the cavity and penetrate the animal's own liver. Tissue cannot live without blood.

Would-be gene therapists are creating these organoids as a way to get genetically altered cells into a patient's body, although the possibility of replacing diseased organs looms in the imagination. But the immediate challenge is to construct an organoid with cells containing genes that produce a protein that a patient either lacks or needs more of. Once implanted, the man-made organs would essentially become living factories, churning out a needed biological product.

"The implantable organoid is an incredible delivery system," Thompson told *Science*. "We hope to use it first for patients with AIDS"—as a way to deliver steady doses of soluble CD4, the protein that keeps the AIDS virus from infecting cells.

"The whole thing is incredible," says Robert C. Gallo, co-discoverer of the AIDS virus. "To think that maybe we could implant a little pouch in patients and use it to deliver soluble CD4. If it really works it will be an amazing thing."

Gallo, chief of tumor cell biology at the National Cancer Institute (NCI), became part of the world of organoids a couple years ago when he called an NIH colleague, W. French Anderson, of the National Heart, Lung, and Blood Institute, to propose a collaborative effort to apply gene therapy to AIDS. Anderson was already working with Thompson, who was in Anderson's lab, and collaborating with Maciag. "A collaboration with Bob Gallo was a natural," says Anderson, whose collaborative network spreads not only throughout NIH but to university labs and biotechnology companies as well (*Science*, 8 September, p. 245).

Science, it is said, sometimes advances in unexpected directions as a result of experimental failures. The creation of organoids fits in that category, as does work on the use of endothelial cells as gene carriers in the lining of artificial blood vessels (see box, p. 749).

A decade's worth of what might be called "classic" research in gene therapy has been directed at inserting normal genes into bone marrow stem cells. The hope is that these engineered cells would become healthy red or white blood cells able to make a protein that a patient normally lacks because he has a defective or missing gene. But stem cells have proved an intractable target, driving scientists to look down other avenues of research. They turned, in particular, to lymphocytes and endothelial cells—the cells that line the vascular system as well as the airways in the lungs.

Preliminary success at getting genes into endothelial cells then prompted Anderson and his many colleagues to think about ways of delivering their potentially therapeutic cell/gene packages to the body. The idea of a vascular

implant was obvious, particularly to Anderson's wife, Kathryn D. Anderson, who is a pediatric surgeon at Washington's Children's Hospital National Medical Center. Could gene-bearing endothelial cells be delivered via implanted sponges, which are used routinely in surgery?

For that approach to work, a vascular network would have to be created to connect the sponges to the body's bloodstream. Here the phenomenon of angiogenesis comes into the picture.

Judah Folkman of Harvard's Children's Hospital observed years ago that solid tumors cannot grow without their own blood supply. He identified "tumor angiogenesis factor" as the chemical key to the phenomenon and has been working on blood vessel growth ever since. Maciag, another angiogenesis pioneer who is at the American Red Cross's Jerome H. Holland Laboratory in Rockville, Maryland, was called in as a consultant: How, he was asked by the Andersons and Thompson, can we get our Gelfoam collagen sponge to sprout blood vessels?

Maciag put them on to heparin-binding growth factor–1 (HBGF-1), an angiogenesis agent that binds to the collagen in the Gelfoam sponge. In an experiment with rats, reported in the 9 September 1988 issue of *Science*, the team implanted HBGF-1–impregnated sponges in the peritoneal cavity of rats. Just as they had hoped, new blood vessels grew from the implant over to the rat's liver, forming the necessary channel through which a gene product could be delivered.

The only drawback seemed to be the sponge. Surgical sponges left in the body after an operation are meant to dissolve within a month or so, which is just what happened in this case. As the Gelfoam sponge dissolved, the vascular bridge it was supporting collapsed, shutting off the channel between the implant and the natural liver.

Thompson called Maciag for further advice and the two met for dinner to contemplate what to do next. It was over a few drinks that the idea of using angel-hair Gore-Tex fibers came up—fibers that are known to be biologically compatible with living tissue and which do not disintegrate. Thompson and Maciag each credits the other with the pivotal insight.

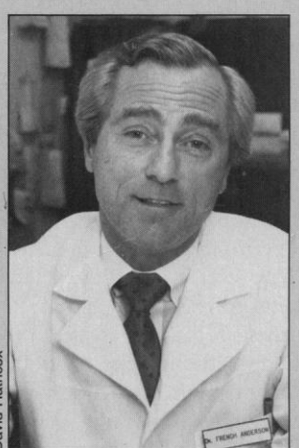
In collaborative research conducted at Genetic Therapy Inc., a biotechnology company affiliated with Anderson's lab and to which Thompson had moved, the experiment began. The "cauldron" of "angel-hair" fibers, collagen, and HBGF-1 was stirred. And, to the researchers' astonishment, the first "living" organoid was produced when the ball of tissue they created was implanted, like the Gelfoam sponge, in the peritoneal cavity of a rat.

"What we made resembles an electrical cable," Maciag told *Science*. "The cells in the matrix created real vascular lumina—blood vessels—as well as what we believe are nerve cells," says Thompson. Maciag says he is still somewhat "shocked" by it all. "How often do you design an experiment over dinner that works the first time?" he asks. (The experimental details are reported in the October issue of the *Proceedings of the National Academy of Sciences**)

The research team has imagined every possible use for these new synthetic organs

*John A. Thompson *et al.*, "Heparin-binding growth factor–1 induces the formation of organoid neovascular structures in vivo," *Proc. Natl. Acad. Sci. U.S.A.* **86**, 7928 (1989).

French Anderson's 20-Year Crusade



David Hathcox

Anderson. *Pushing gene therapy from the lab to the bedside.*

W. French Anderson has been daydreaming for the past two decades. As all his friends and competitors know, Anderson's life's ambition is to become the first "genetic surgeon." He is, depending upon one's point of view, committed to or obsessed with the idea of curing genetic disease by taking a broken gene out of a cell and replacing it with a whole one.

One early piece of evidence of Anderson's commitment is found in an article he submitted to *The New England Journal of Medicine* in 1968. Anderson, then newly arrived at the National Institutes of Health, foresaw the day when genetic defects could be corrected as an outgrowth of the phenomenal explosion of information about DNA and RNA. Recombinant DNA technology, with its restriction enzymes and capacity for cutting pieces of DNA apart and stitching them back together, had not been invented yet. But the molecular giants of the 1960s had revealed that the basic process of protein synthesis is the same in all living organisms.

In a paper titled "Current potential for modification of genetic defects," Anderson envisioned viruses carrying genetic information into cells and suggested that blood diseases such as sickle cell anemia and thalassemia might soon yield to the ministrations of the genetic surgeon.

The late Franz J. Ingelfinger, the imaginative and irreverent editor of the *New England Journal*, liked Anderson's "very erudite and fascinating" article and sympathized with one member of the editorial board who said it should be published as a "worthwhile adventure in pure speculation." But other, more conservative peer reviewers did not. It was, Ingelfinger wrote on 19 August 1968, "too speculative," or "as one reviewer said, 'Medical Prediction'. . . . " The article was rejected.

But Anderson's nature as a man willing to make bold if premature promises was clear. For 20 years, with each new development in the lab, Anderson has been predicting that the first experiment in human gene therapy is just around the corner. His competitors, none of whom is willing to be quoted, dislike it. "French is always raising expectations in the press, giving gene therapy a bad name," is a familiar refrain.

"People accuse me of jumping the gun and headline grabbing," says Anderson, who is chief of molecular hematology at the National Heart, Lung, and Blood Institute. Over the years, Anderson has written prolifically about gene therapy—about research and about the social and ethical issues that go with the territory. (Indeed, several papers from his lab have been published in *Science*.) He notes, with some justification, that when something he says makes news, it's been in the scientific literature first.

There is no arguing that Anderson, perhaps more than anyone else in the field, has pushed hard to get gene studies out of the lab and into the patient. After many predictions went unfulfilled, in the end it was Anderson and his colleagues who were the first to submit a gene transfer protocol for NIH approval.

A large body of researchers asks what's the rush? Why not wait until all of the science behind these experimental ventures is fully understood? Says Anderson, "If we did that, we'd never make any progress in medicine which, after all, is really what this research is all about." ■ B.J.C.

Endothelial Cells to the Rescue

James M. Wilson was having trouble getting retroviruses to infect hepatocytes, or liver cells, in culture. Wilson, working in Richard Mulligan's laboratory at the Whitehead Institute, wanted to use the viruses to carry foreign genes into the hepatocytes. The ultimate goal: to deliver missing or defective genes to patients with liver dysfunction.

But Wilson's cultures always seemed to be contaminated by endothelial cells—the cells that line blood vessels and the airways of the lungs. And for some reason, the retroviruses readily carried genes into the endothelial cells, while bypassing the hepatocytes.

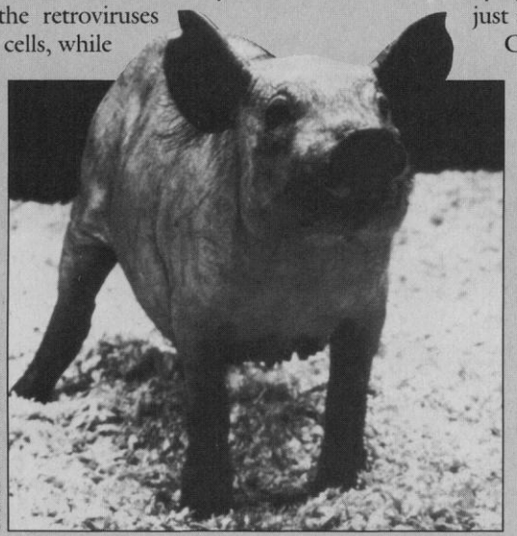
"Finally it occurred to me that there was a message in there," Wilson told *Science* as he recalled the origins of his interest in endothelial cells in gene therapy.

At about the same time, researchers in the NIH lab of W. French Anderson also took an interest in endothelial cells as vehicles for carrying therapeutic genes into the body.

As Una Ryan of the University of Miami says, "endothelial cells are ideal vehicles for the delivery of genes" because of their capacity to interact with all kinds of vasoactive substances in the circulation. Ryan, an endothelial cell expert, has joined the vast network of researchers collaborating with Anderson's lab. That team reported successful gene expression in rabbit endothelial cells in the 13 January issue of *Science*.

"When you think about it," says Wilson, who is now at the University of Michigan at Ann Arbor, "endothelial cells are a natural." They are receptive to retroviruses. They come in direct contact with the blood, providing a natural route for the delivery of a therapeutic gene product.

So far, Wilson has tested endothelial cells in dogs. In the 16 June issue of *Science*, he, Mulligan, and their colleagues reported a successful experiment in which endothelial cells bearing the *lacZ* gene were seeded onto the interior lining of an artificial, Dacron blood vessel. *LacZ*, which produces beta-galactosidase, was chosen as a marker gene for this experiment because beta-galactosidase is readily identifiable by a blue stain. Five weeks after the artificial vessels were implanted, it was evident that endothelial cells carrying *lacZ* had completely lined the vessels, just as predicted.



Charles River Laboratories

Yucatan minipig. *Genetically modified endothelial cells that produce TPA may keep this pig from getting blood clots and a heart attack.*

Colleagues of Wilson's at Ann Arbor, led by Gary Nabel, had similar success in an experiment on recombinant gene expression in endothelial cells in the Yucatan minipig. This animal is a favorite of heart researchers because it gets a form of atherosclerosis that closely resembles the disease in people. Nabel also reported in the 16 June *Science*.

The next step will be to try a similar experiment with a therapeutically important gene—most likely the gene for TPA, or tissue plasminogen activator, which breaks up blood clots that can cause fatal heart attacks. "It might be possible to insert the gene for TPA into endothelial cells along with appropriate regulatory sequences, theoretically allowing the gene to be turned on and off to prevent acute myocardial infarction," says geneticist William N. Kelley,* who until recently was chief of medicine and head of the fledgling Human Gene Therapy Institute at the University of Michigan. "This is an exciting new technology for the delivery of recombinant genes to sites of vascular injury in vivo," says Kelley.

Beyond that, researchers are studying the possibility of using engineered endothelial cells to deliver clotting factor to hemophiliacs or von Willebrand's factor to people who suffer from that relatively common genetic bleeding disorder. They could also be useful for treating diabetes. "The possibilities one can imagine are limitless," says Kelley. ■ **B.J.C**

*Kelley is now at the University of Pennsylvania.

whose existence owes a lot to serendipity. Anderson thinks that, because they can grow blood vessels into whatever natural organ they are near, the possibilities are endless: liver, spleen, coronary arteries.

Thompson, who has just moved from Genetic Therapy Inc. to the University of Alabama at Birmingham, is particularly intrigued by the thought that they may have hit upon something that will be useful for neurological diseases like Alzheimer's. "In addition to making blood vessels, the organoids produce what look like nerve cells," Thompson told *Science*. This was really unexpected. "Does it mean we might be able to grow new nerves for Alzheimer's victims? Our neuroscience friends are really turned on by this."

But that is for the future. First on the agenda is soluble CD4 and AIDS. In that regard, one might think of the organoids as a delivery system for a delivery system.

Convinced that, for now, it is not feasible to actually integrate a cloned CD4 gene into the DNA of an AIDS patient, the Anderson group is experimenting with lymphocytes as a carrier cell, much the way lymphocytes are being used in the gene transfer studies that began last spring in terminally ill melanoma patients.

One reason for choosing lymphocytes is that the melanoma study has already shown that they can survive and express a gene product for as long as 64 days. "Right now, we're putting the cloned CD4 gene into human lymphocytes and then infusing them into SCID [immune-deficient] mice. Don Mosher at Medical Biology Institute in La Jolla is doing that part of the experiment for us," Anderson reports.

The cells do secrete CD4 but, thus far, at concentrations far too low to be of any therapeutic benefit. If the cells can be manipulated to put out higher levels of CD4, the next step will be to expose the mice to the AIDS virus to see whether the CD4 can prevent it from infecting normal cells.

At the end of the experimental line, CD4-secreting lymphocytes would be put in an organoid that could be implanted in the peritoneum of an AIDS patient, where it would secrete the life-saving agent for months at a time. That's the long-term plan. ■ **BARBARA J. CULLITON**