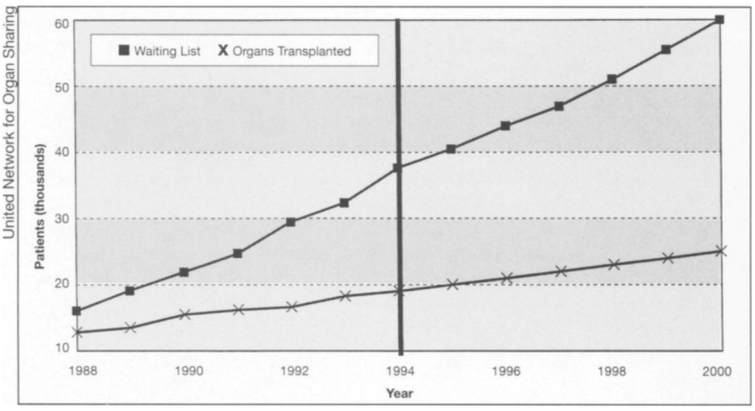
The Xeno-Solution

Perils and promise of transplanting animal organs into people

By JOHN TRAVIS

Over the next year, 10 patients whose livers have failed will pin their hopes for a new life on a pig. As they wait for human donor organs to be found, surgeons at Duke University Medical Center in Durham, N.C., will try to fend off death by connecting each patient's circulation to a pig liver kept alive outside the animal.

The unusual operations may foreshadow the return of permanent animal-to-human transplants, a procedure called xenotransplantation after *xenos,* the Greek word for strange or foreign. Such extreme efforts date back at least to the turn of the century. In 1905, for example, a French surgeon transplanted slices of a rabbit kidney into a child. Lamb was the animal of choice in 1923, when another kidney xenotransplant was tried.



Xenotransplantation offers one potential solution to the disparity between the number of potential organ recipients and the number of actual transplants. That gap is projected to grow.

The most notorious case of xenotransplantation may have been Baby Fae, an infant born with a heart defect that would kill her within a month. In the fall of 1984, physicians operated on the 2-week-old baby, replacing her heart with one from a young baboon. Three weeks later, her immune system having successfully waged war on the foreign organ, Baby Fae died.

Most of the enthusiasm for xenotransplantation died along with Baby Fae. Yet in the last few years, as investigators have slowly begun to understand why all past xenotransplant attempts failed, that enthusiasm has started to resurface. Researchers now believe they have a much clearer idea of why the human body fiercely rejects organs from other animals and how to circumvent that destructive process.

Several groups, for example, have recently altered the genes of pigs in order to make porcine organs more like human organs and thus less likely to be immediately rejected. Investigators are also exploring strategies to combat delayed organ rejection.

These efforts have convinced many xenotransplant researchers that their dream is possible. "We're flushed with excitement, but it's a long road ahead," cautions Fritz H. Bach of Harvard Medical School's Deaconess Hospital in Boston.

That road was laid out at the recent Third International Congress for Xenotransplantation in Boston, a meeting that drew more than 700 investigators. Motivating that crowd of scientists and physicians is a simple reality: There is a desperate need for replacement organs.

In the United States alone, 40,000 people languish on the waiting lists for organ transplants, according to the United Network for Organ Sharing, and the number is growing steadily. "There are eight people who die every day in the U.S. because of organ shortages," says Bach, who chaired the Boston meeting.

Though offering a solution to those shortages, xenotransplantation presents a number of difficulties that human-to-human transplants avoid. The most severe obstacle is hyperacute rejection, an immune response that within a few minutes to a few hours kills the healthy animal organ by cutting off its supply of blood. "The transplant turns black," says David H. Sachs, who directs the Transplantation Biology Research Center at Massachusetts General Hospital (MGH) in Boston.

In past xenotransplants, surgeons could avoid hyperacute rejection by using organs from chimpanzees, the primate genetically most like humans. Chimpanzees are now an endangered species, however, so researchers have turned to another close relative of humans, the baboon. In 1992, for example, pioneering transplant surgeon Thomas E. Starzl of the University of Pittsburgh Medical Center led unsuccessful efforts to transplant baboon livers into two dying patients.

Not many researchers view baboons as a good source of donor organs. The animals take a long time to raise, have organs that are often too small for human use, and perhaps most important, may harbor infectious agents that present a threat to humans (see sidebar).

Many investigators believe the future of xenotransplantation may dwell in pigpens. Pigs are easy to raise, mature quickly, produce large litters, and have organs comparable in size to humans'. Since pigs are widely raised for food, using their organs for xenotransplantation should raise fewer ethical concerns than using nonhuman primates' organs.

Despite these advantages, pig organs offer a major drawback. They provoke hyperacute rejection. That's because the surfaces of porcine endothelial cells, which line the blood vessels of the donar organs, sport molecules that their human counterparts don't.

One particular molecule, a sugar that

investigators call alpha-gal, poses the largest problem. Coursing through human blood, as well as that of many nonhuman primates, are natural antibodies that view alpha-gal molecules as evidence of a foreign invasion.

"The xenoreactive natural antibodies, as soon as blood flows into that organ, will recognize the pig endothelial cells and bind to them," says Bach.

That binding sets off a cascade of complex interactions among a large number of blood proteins known collectively as complement. This complement cascade eventually results in the formation of a membrane attack complex, a multiprotein assemblage that latches onto the surface of endothelial cells, killing them directly or, more often, activating them.

This activation has disastrous consequences, says Bach. The densely packed endothelial cells shrink away from one another, causing blood vessels to leak. Furthermore, the activated endothelial cells encourage proteins and platelets in the blood to forms dense clots, obstructing the vessels. Both actions quickly starve the transplanted organ of blood.

Investigators placing pig organs in other animal species have stemmed endothelial cell activation and the resulting hyperacute rejection by depleting a prospective recipient's store of alpha-gal antibodies before the xenotransplant.


Imutran

The blood vessels in the organs of these genetically engineered pigs display human proteins.

One method involves first filtering blood through an external pig organ, to sop up the alpha-gal antibodies, and then returning the blood.

Besides being cumbersome, such techniques deplete alpha-gal antibodies only temporarily: The host simply makes new ones. Furthermore, through what's called the alternate pathway, the complement cascade can sometimes proceed even without alpha-gal antibodies. As a result, some investigators have decided to focus more directly on preventing the actions of complement.

In one study, investigators flooded the blood of monkeys with soluble molecules that bind to and block the action of one of the complement proteins. This enabled pig hearts to survive in the primates for as long as 6 days, reported Fred Sanfilippo of the Johns Hopkins University School of Medicine in Baltimore. In contrast, untreated monkeys usually rejected pig hearts in under 8 hours.

Yet widely inhibiting complement may not be safe. "Complement is a terribly important part of our body. We probably need it to survive," says Bach.

The most promising approach to vanquishing hyperacute rejection may be not to treat the recipient, but to change the donor organ. Investigators have recently transplanted into nonhuman primates the organs of pigs genetically engineered to avoid the complement cascade. Human organs aren't vulnerable to their own complement because proteins that inhibit the cascade stud their endothelial cells. The pig endothelium carries similar protectors, but they're effective only against pig complement.

That realization prompted a number of academic groups and biotech firms to pop genes with the instructions for human complement inhibitors into the genomes of pigs. Talks in Boston suggested that initial experiments with these

Would xenotransplants produce epidemics worse than AIDS?

The most pointed question about xenotransplantation may not be whether it *can* be done but whether it *should* be done. Married to the hope of alleviating the desperate organ shortage and curing illnesses such as diabetes is the real, but unquantifiable, risk of introducing deadly new viruses to the human population, says Jonathan S. Allan, a virologist at the Southwest Foundation for Biomedical Research in San Antonio.

Investigators now believe that HIV, the virus that causes AIDS, originated as a monkey virus that somehow infected humans. Animals are also thought to host other viruses, such as Ebola, that can infect and kill humans. Allan fears that baboons harbor comparable viruses that researchers can't eliminate from donor animals.

"Baboons carry viruses that could lead to another AIDS epidemic," asserts Allan. "[Xenotransplanters] are saying that lightning can't strike twice."

Baboons concern Allan because they are so similar to humans. That makes them ideal organ donors, but it also means that viruses which infect baboons are likely to view humans as inviting hosts. "If you have to use a species, pigs will be much less of a pathological nightmare," says Allan.

Allan's concerns have caught the attention of other researchers and federal officials, who openly acknowledge that xenotransplants do pose a risk of transmitting infectious organisms. In a June meeting, the Institute of Medicine, which plans to release a report on xenotransplantation next year, addressed the issue of infectious risks.

The Food and Drug Administration recently convened an advisory panel to examine the dangers posed by an experiment designed to transplant baboon cells into a dying AIDS patient (see main story). With Allan abstaining, the panel unanimously approved the single experiment, even though a member of the research team tells SCIENCE NEWS the operation will probably transfer one known virus from the baboon into the patient. Allan notes that the transfer of baboon viruses to a patient who will probably die does not present a serious threat to the overall population.

Nonetheless, he points out that investigators plan to transplant baboon hearts temporarily into young patients awaiting human organs. These patients are likely to survive their xenotransplants and may spread viruses that go unrecognized for years, says Allan. "You're going to pull the baboon heart out, but you're not pulling the baboon viruses out," he warns.

Investigators have little solid data on how easy or difficult it is to transfer viruses, bacteria, or parasites between species during transplants. "In a few years, we may have the ability to be more quantitative about the risk," says Louisa Chapman of the Centers for Disease Control and Prevention (CDC) in Atlanta.

Placing a moratorium on xenotransplants or restricting them severely while researchers determine that risk "could put a stranglehold on an area of great medical promise," says Chapman.

The FDA and CDC will soon publish guidelines designed to reduce the risk of transmitting infectious agents but will leave the approval and monitoring of xenotransplant experiments to local review boards, says Philip D. Noguchi, head of FDA's division of cellular and gene therapies.

Allan has been sharply critical of the proposed guidelines. "There's no federal regulation or oversight for the future of xenotransplantation. It's almost irresponsible," he says.

"We're not just leaving it up to the local community. We're demanding they take responsibility," protests Noguchi. Yet Noguchi admits that he does worry about the dangers of xenotransplants. "It's a chance. It could blow up in our faces. Some nights I sleep better than other nights," he says. —*J. Travis*

transgenic pigs have been more successful than investigators had expected.

Since they wanted only to examine the survival of these engineered pig organs, investigators either connected an external donor heart to the primate's circulation or implanted the heart without removing the primate's own organ. Pig hearts that display human complement inhibitors called CD59 and DAF can survive for more than 30 hours in baboons; unmodified organs last about an hour, reported investigators from Duke Medical Center and Nextran, a biotech firm in Princeton, N.J.

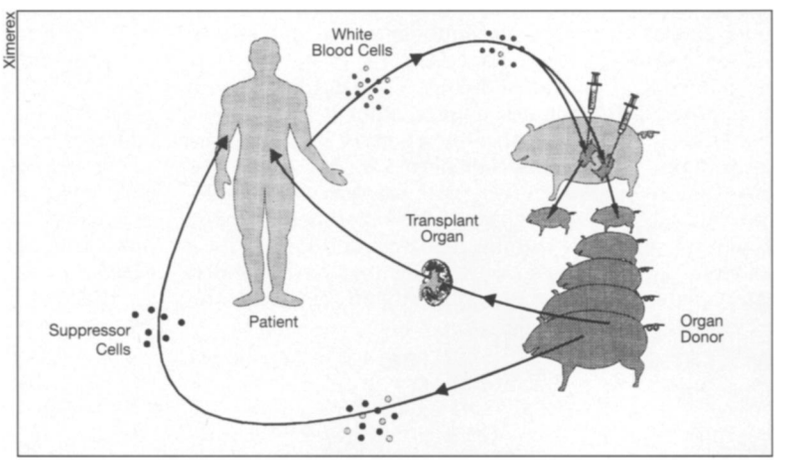
An even bigger stir was generated by data on human DAF-producing pigs created by scientists at the University of Cambridge and Imutran, a biotech company in Cambridge, England. Some hearts from these animals lasted more than 5 days in monkeys. Moreover, when investigators also used large doses of immunosuppressive drugs, some DAF-laden pig hearts survived more than 2 months.

Starzl called those results a "landmark" in xenotransplantation.

The long-lasting hearts still looked perfect when examined after the monkeys' deaths, says David J.G. White of the University of Cambridge. Animal regulations in the United Kingdom forced the investigators to kill many of the monkeys before organ rejection set in because the drugs had made the primates sick. "Hyperacute rejection is dead. I announce its death," White told SCIENCE NEWS.

Since activated endothelial cells are common to both forms of rejection, Bach and other investigators are exploring ways to create transgenic pigs whose endothelial cells ignore the body's activating signals. Even if the cells do become activated, Bach believes, there are ways to prevent the genes that cause blood clotting and other rejection-related events from turning on.

One certain way of defeating delayed xenograft rejection is to use potent immunosuppressants to check the cells mobilized by a host's immune system. These drugs can also overcome the last apparent barrier to xenotransplantation—a delayed rejection driven by immune cells known as T cells. This T cell response, which may take days or months to emerge, represents the primary hurdle in human-to-human transplants.



In one proposed strategy to persuade the human body to tolerate pig organs, researchers would gather immature white blood cells from a potential organ recipient and inject them into pig fetuses. As the fetuses develop, the human cells should learn to tolerate pig cells. Investigators would then harvest these cells from the pigs after birth and return some of them, the suppressor cells, to the potential organ recipient. Researchers hope the pig-tolerant cells will suppress the recipient's normal rejection response when the pig organ is transplanted.

Many xenotransplant investigators are skeptical of White's brash statement. What's more, they fear that overcoming hyperacute rejection isn't the end of their job.

An equally destructive immune response, known as delayed xenograft rejection, can occur in the days after a transplant. Until recently, researchers had rarely seen an organ survive long enough to undergo it.

Investigators now report that delayed xenograft rejection appears to begin when macrophages and natural killer cells infiltrate the organ. These two classes of immune cells respond to any foreign object, whether a virus or an organ. The immune cells then secrete chemical signals that activate endothelial cells, leading ultimately to organ rejection. Delayed rejection occurs without the complement cascade that defines hyperacute rejection.

For some time, says Hugh Auchincloss Jr. of MGH, investigators had hoped that T cells would prove less troublesome in xenotransplantation than in human-to-human operations. That optimism is fading, he explains, as growing amounts of data suggest that this cellular response is even stronger when animal organs are used.

Though immunosuppressive drugs can often stymie the T cell response, they may leave patients vulnerable to equally deadly cancers and infections. Consequently, investigators would like to educate the host immune system into accepting the transplant.

"The creation of tolerance is how we must go. How to do it is the $64 question. A lot of people says it's impossible. I say it's not," says Starzl.

Starzl's optimism stems from the fact that some people have received human organs and successfully weaned themselves off immunosuppressive drugs after a few years. When a solid organ is transplanted, Starzl says, immature immune cells from the donor inadvertently go along for the ride. These cells spread throughout the host body and mature, a process Starzl thinks helps induce tolerance. "When you transplant an organ, it's as if you're accidentally transplanting a bit of bone marrow," he says.

Investigators are pursuing deliberate strategies for creating tolerance as well. One approach involves augmenting a recipient's immune system with cells from the immune system of the organ donor. Sachs, who leads a team of investigators from MGH and the Boston firm BioTransplant, has completed experiments in which researchers transferred the bone marrow of pigs into monkeys.

With the help of immunosuppressive drugs administered for about a month after the transplants, the bone marrow survived for more than 300 days in some of the primates. Since bone marrow is the birthplace of immune cells, these monkeys had a part pig, part monkey immune system. As a result, their immune cells did not treat pig cells as foreign, reported Sachs. He hopes this strategy may someday enable scientists to transplant pig organs safely into monkeys—and eventually into humans.

William E. Beschorner, president of Ximerex, a small company based in Baldwin, Md., presented a tolerance-inducing strategy that, if successful, might create less stress for the organ recipient. Beschorner suggests that investigators obtain white blood cells from a prospective organ recipient and inject them into developing pig fetuses (see diagram).

During the fetal period, the pig teaches its immune cells not to recognize its own tissue as foreign. Beschorner hypothesizes that if transplanted human immune cells were educated along with the pig cells, investigators could harvest pig-tolerant human immune cells from newborn pigs and return them to the patient. These cells might suppress the normal immune response of uneducated human cells, he says.

Auchincloss calls Beschorner's proposal "a bit of a long shot" but one worth pursuing. He notes that the idea of "suppressor" immune cells has recently come back into fashion after being a "dirty word" in the 1980s.

Xenotransplant researchers are divided on how quickly they should return to the clinic. Since cells don't face the same risk of hyperacute rejection as whole organs—blood vessels from donor animals do not accompany the cells—a number of groups today are already trying cellular xenotransplants.

As SCIENCE NEWS went to press, investigators at the University of Pittsburgh and the University of California, San Francisco, were making final preparations for an attempt to rebuild the devastated immune system of an AIDS patient with cells derived from the bone marrow of a baboon. HIV, the virus that causes AIDS, does not infect baboon immune cells.

Researchers in Sweden have transplanted, with the aid of immunosuppressive drugs, islet cells from the pig pancreas into human diabetics. The scientists want to see how well the porcine islets, which have survived for several months, secrete insulin and what effect this insulin will have on the patients' diabetes.

Some neurodegenerative diseases, notably Huntington's and Parkinson's, may be treated experimentally with transplants of human fetal cells into the brain. But obtaining such tissue is difficult and controversial, prompting some investigators to try porcine fetal cells instead. Xenotransplants into Parkinson's patients are already under way, and several people with Huntington's are slated to undergo the procedure in the next few months (SN: 10/7/95, p.230).

A growing number of investigators expect transplant surgeons to begin using animal organs as bridges—interim transplants while a patient waits for a human organ. In addition to Duke's bridge experiments with livers from Nextran's pigs, other physicians are prepared to transplant baboon hearts into children temporarily.

The most heated controversy among xenotransplanters centers on the question of when pigs or baboons may provide "destination" organs, permanent replacements of failing hearts, kidneys, livers, and other organs. White argues that the success of the transgenic pigs suggests that a trial with a few human patients might be feasible within a year or two.

Auchincloss, who told SCIENCE NEWS he was delighted by the success of transgenic pig organs, nevertheless challenges White's optimism. He argues that investigators must demonstrate at least a year's survival of an organ, with tolerable drug regimens, in large animals before moving ahead to humans. White's experiments, he says, depend on unacceptable doses of drugs.

"If you give enough immunosuppression to kill the patient, the xenograft will survive. I don't think these results justify clinical xenotransplants," he told the Boston audience after White's talk.

Another important caveat, researchers note, is that the xenotransplants with transgenic pig hearts did not establish how functional the organs could be, because the primates' own hearts weren't removed for the experiments.

"It's easy to argue both sides, whether to move quickly or be cautious. But the worst thing that can happen is a negative public reaction to an approach tried too quickly. That could set the field back," says Sanfilippo.

The thrilling scientific advances reported at the Boston meeting may blind some researchers to the clinical difficulties ahead, adds Columbia University's Robert E. Michler, who heads one of the most active transplant clinics in the nation.

"I don't share the same enthusiasm as others that we will permanently put animal organs into people within 1 to 3 years," he says. "You have to understand that there are scientists out there who are in a race right now, and that race may cloud their vision. All they want to do is be the first to put a pig organ into a human. I want to be the first to put a pig organ into a human and have him go home." □