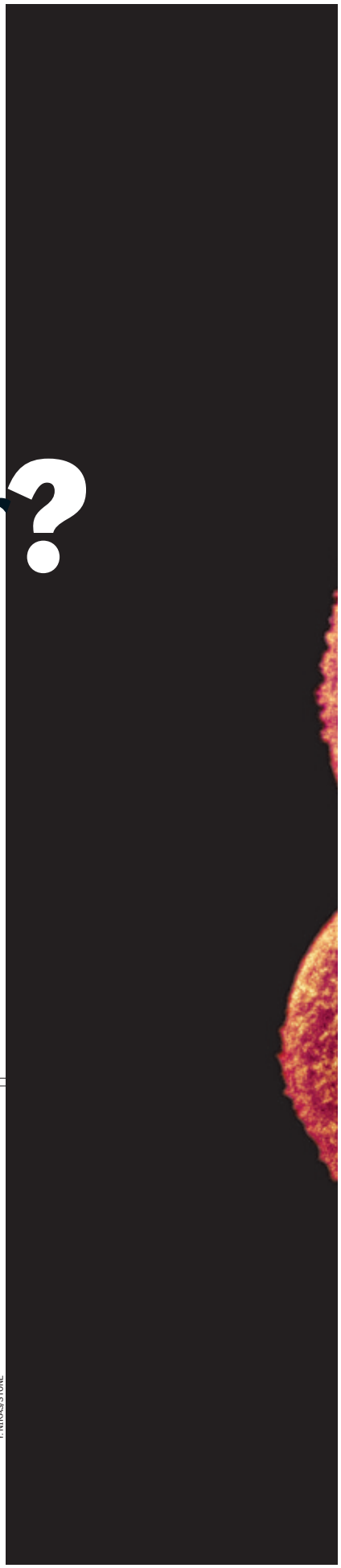
Are Stem Cells the Answer?

Researchers hope to coax human stem cells into becoming pancreatic cells to cure diabetes.

By Maya Pines

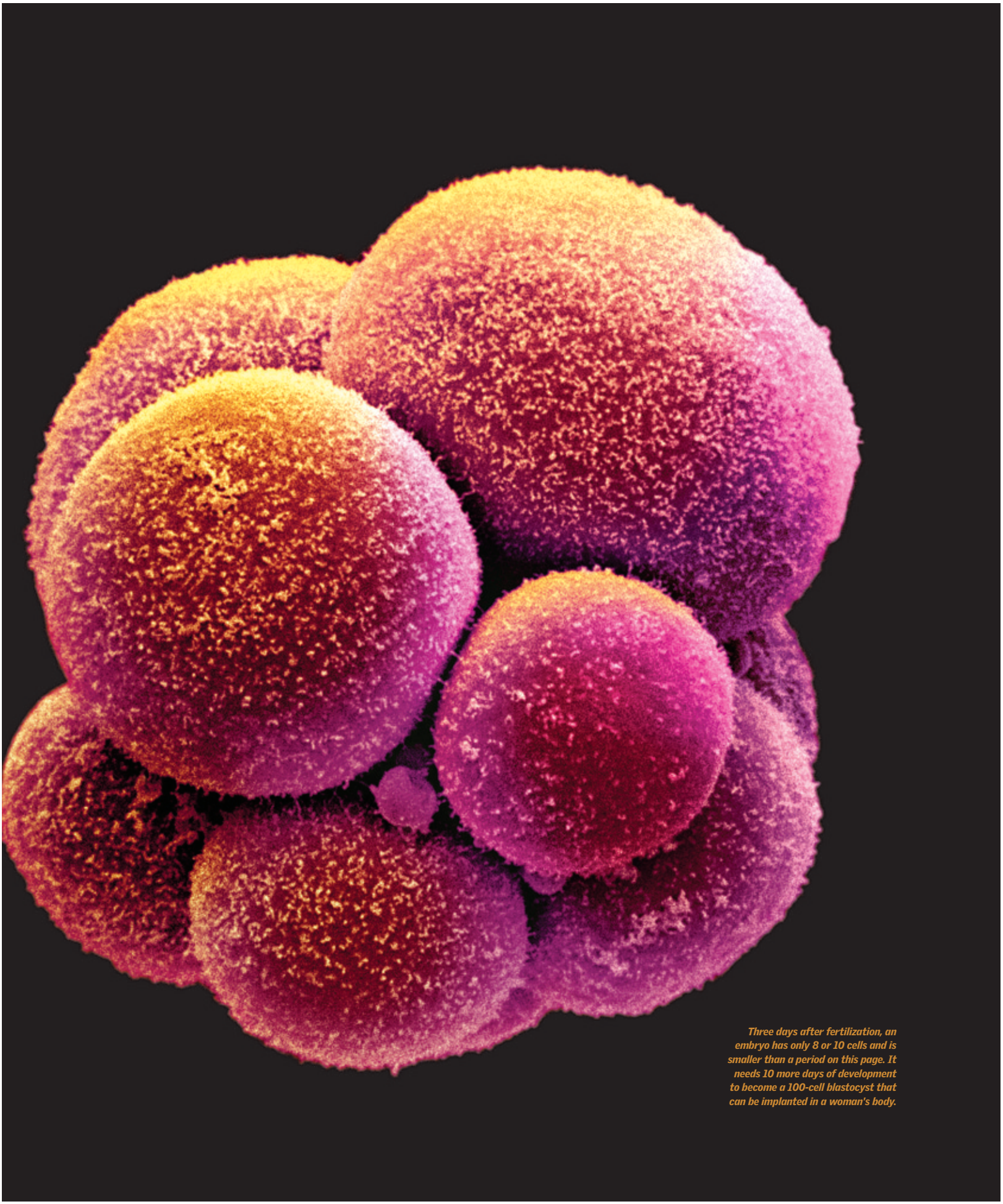


Y. NIKAS/SSTONE

DOUGLAS A. MELTON HAS A SINGLE, OVERRIDING goal: finding a cure for his nine-year-old son, Sam, and millions of others with type I (juvenile) diabetes. This is why Melton, an HHMI investigator at Harvard University, stopped focusing on the early development of frogs, in which he had done pioneering studies, and started research on mouse development. It's also why he is now leading a major drive to turn human embryonic stem (ES) cells into the special kind of pancreatic cells, called beta cells, that supply what diabetics lack: insulin.

First, of course, he needs human ES cells. As Melton explained during a September U.S. Senate committee hearing, only ES cells have "the remarkable capacity to make any kind of cell in the body"—skin, bone, brain, liver or other specialized cells, including pancreas. Because ES cells also reproduce themselves, they could actually become factories for specialized cells to replace those lost through disease or injury.

Millions of patients with conditions such as Alzheimer's disease,



Three days after fertilization, an embryo has only 8 or 10 cells and is smaller than a period on this page. It needs 10 more days of development to become a 100-cell blastocyst that can be implanted in a woman's body.

Parkinson's disease, cancer, osteoporosis and spinal injuries, as well as diabetes, might benefit from such therapy. "Every family in America has been touched by these diseases and conditions, and now we have the opportunity to offer them real hope," declared Representative James R. Langevin of Rhode Island, a quadriplegic with a damaged spine, when he testified before the same committee. Most scientists believe it will take at least 5 to 10 years, however, to solve the problems involved in translating such hopes into treatments.

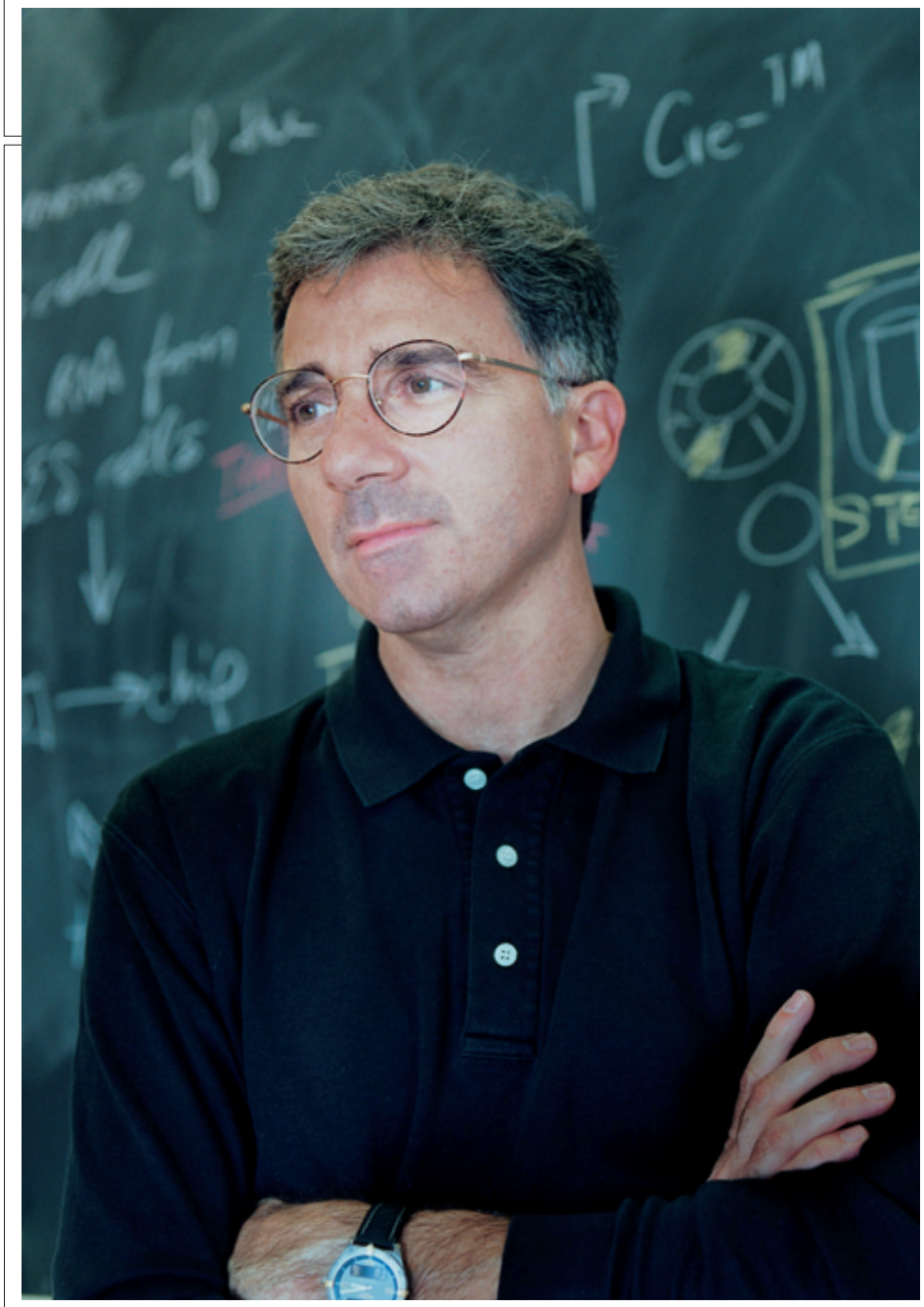
Douglas Melton embarked on this quest a decade ago, when his son was diagnosed with type I diabetes.

From Melton's point of view, the hardest part of the job will be learning how to coax primordial ES cells into becoming just one specific kind of cell—in this case, the beta cells of the pancreas that secrete insulin in response to blood sugar (glucose). Next, the new beta cells will have to be implanted into patients and continue functioning there. Finally, researchers must find ways to prevent a recipient's immune system from destroying the new cells. Only then can they hope "to transplant these cells into diabetics and effectively cure them by keeping their blood sugar under control," he says.

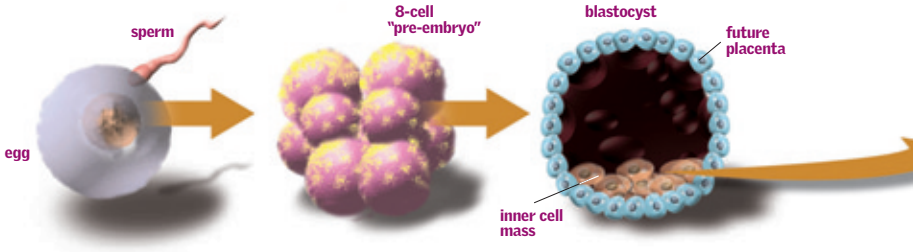
Melton embarked on this quest nearly a decade ago, when his son was diagnosed with type I diabetes—a debilitating disease in which the body's immune system destroys the insulin-producing beta cells. No one can live without insulin, which enables the body to use glucose as a basic fuel. People who cannot make their own insulin are totally dependent on daily injections of it. Melton's son, for example, routinely needs seven blood checks and insulin injections per day to maintain a safe balance between his food intake (which raises the level of glucose in the blood), physical activity (which lowers it) and insulin.

"Many times, particularly when he's playing soccer, we double that number of checks to avoid a crisis," says Melton. Diabetics suffer crises both when their glucose level is too high (this may cause lethargy or unconsciousness and may be life-threatening) and when it is too low (this "insulin shock" develops without warning; it may cause shakiness, confusion, seizures or unconsciousness). They may also face complications such as heart disease, stroke, blindness or kidney failure or require amputation, and their life spans are considerably shorter than average.

Before turning his attention to pancreatic development, Melton had won fame for his work on how the frog's body plan is established early in the life of the embryo. One of his best-known discoveries involved the frog's nervous system; he showed that this most complex part of the body forms simply by default, when a biochemical signal to make skin is lacking. Not surprising-



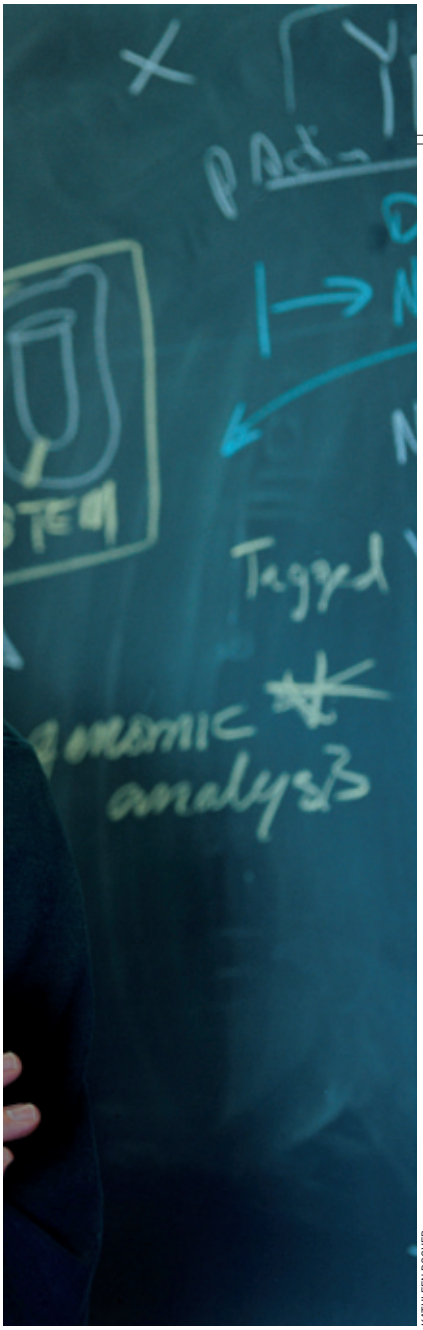
Harvesting Human ES Cells and Their S



1. A human sperm fertilizes an egg.

2. The fertilized egg grows into a ball of 8 uniform cells.

3. The clump of cells develops into a blastocyst containing about 100 cells (the inner cell mass) surrounded by a transparent shell (the future placenta).



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ly, his first studies of pancreatic development were carried out in frogs. Then he moved on to mice, which are genetically much closer to humans. Scientists already had accumulated decades of experience with mouse ES cells, and Melton expected to limit his studies to these. The plan changed in the late 1990s, when James A. Thompson of the University of Wisconsin, Madison, and others devised ways to make human ES cells grow in the lab almost as well as mouse ES cells did.

The news of this achievement galvanized Melton. He knew what he had to do: work with human ES cells. But how? Only a few self-perpetuating colonies, or "lines," of human ES cells had been reported in scientific papers, and most of them belonged to private companies that held the patents for them.

At first he collaborated with other scientists on experiments with human ES cells. "This [work] showed that, like mouse ES cells, the human ES cells respond to various growth factors and differentiate," Melton says. "But we could not find a growth factor that made all the ES cells differentiate into a single type of cell. They would differentiate willy-nilly. This implies that we will not find a growth factor, or even a cocktail of factors, that will cause them all to become beta cells. We will need a different method."

Finding this method will require great effort and, most likely, many different ES cell lines, he believes. "I'm especially concerned because we know from mouse work that some ES cell lines are better than others for making endoderm, the embryonic layer from which the pancreas develops. We don't want to take the chance of being restricted to just a few lines, some of which clearly don't grow well."

A NEW PARTNERSHIP

At a friend's barbecue about four years ago, Melton, who was then chair of Harvard's department of molecular and cellular biology, met R. Douglas Powers, a professor at Boston College and the scientific and laboratory director for Boston IVF (an in vitro fertilization clinic). Melton told Powers about his work with mouse ES cells and his attempts to learn how these cells make a pancreas. "It's a kind of decision tree," he explained. "We want to know what genes and cells are involved in each decision so we can learn how to direct the cells' differentiation down that pathway."

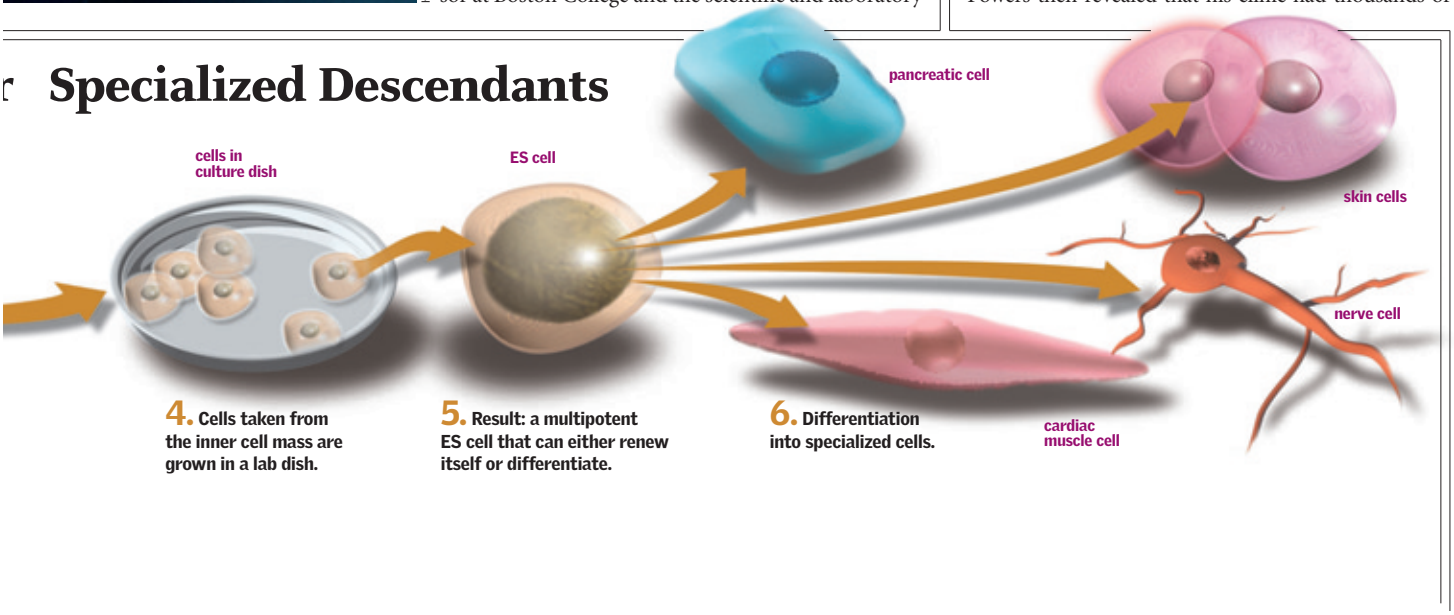
They also talked about the then-recent discovery that human ES cells could be grown in culture. This led to a discussion of the need for more human ES cell lines. Powers then revealed that his clinic had thousands of

Panel Says More ES Cell Colonies Are Needed

Additional colonies of human embryonic stem (ES) cells will be needed if "regenerative medical therapies" are to fulfill their promise, a panel of the National Academy of Sciences recently concluded. "The human ES cell lines that are already available should be very useful for learning more about the biology of stem cells and answering basic questions about them," commented Bert Vogelstein, an HHMI investigator at The Johns Hopkins University School of Medicine, who chaired the panel. "But in the long run they won't be enough. New lines will need to be developed to replace existing ones that become compromised by age, and to address concerns about [growing the stem cells] with animal cells and serum that could result in risks for humans, as well as to fully explore potential medical applications."

The panel, which strongly endorsed federal financing of research and the government oversight that comes with it, consisted of senior biomedical researchers who were not personally involved in stem cell research and had no conflicts of interest. Vogelstein was joined by Barry R. Bloom, dean of the Harvard School of Public Health; Corey Goodman, a neuroscientist and now president and CEO of Renovis; Patricia King, a medical ethicist at the Georgetown University Law Center; Myron Weisfeldt, chairman of the department of medicine at The John Hopkins University School of Medicine; and Guy McKhann, professor of neurology at The Johns Hopkins University School of Medicine. **—MP**

Specialized Descendants



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frozen preimplantation embryos left over from couples' efforts to produce a pregnancy and that these extra embryos were slated for destruction. When Melton asked Powers whether he would be willing to collaborate with him in using such embryos to produce new human ES cell lines for his research, Powers readily agreed.

Last year, Melton approached Tom Cech, the new president of HHMI, with the proposal he had discussed with Powers. Boston IVF, one of the nation's largest fertility clinics, would supply frozen embryos that were left over from fertility treatments, with the donors' consent. The Boston IVF scientists would then gently thaw these very early embryos, still at the eight-cell stage, and prepare them to be grown in a lab dish.

Then, a few days later, when the embryos have grown into slightly larger blastocysts with a hollow core, Andrew McMahon, newly appointed chair of the department of molecular and cellular biology at Harvard, would do his part. Using his experience in deriving many lines of mouse ES cells, he would tease out some cells from the inner cell mass of the human blastocysts and try to turn them into new lines of self-reproducing human ES cells. This process would take at least six months, including a stage during which the cells' biological characteristics would be identified and confirmed. Finally, Melton would experiment with various combinations of growth factors or other molecules in an effort to prod the ES cells into becoming active beta cells that churn out insulin.

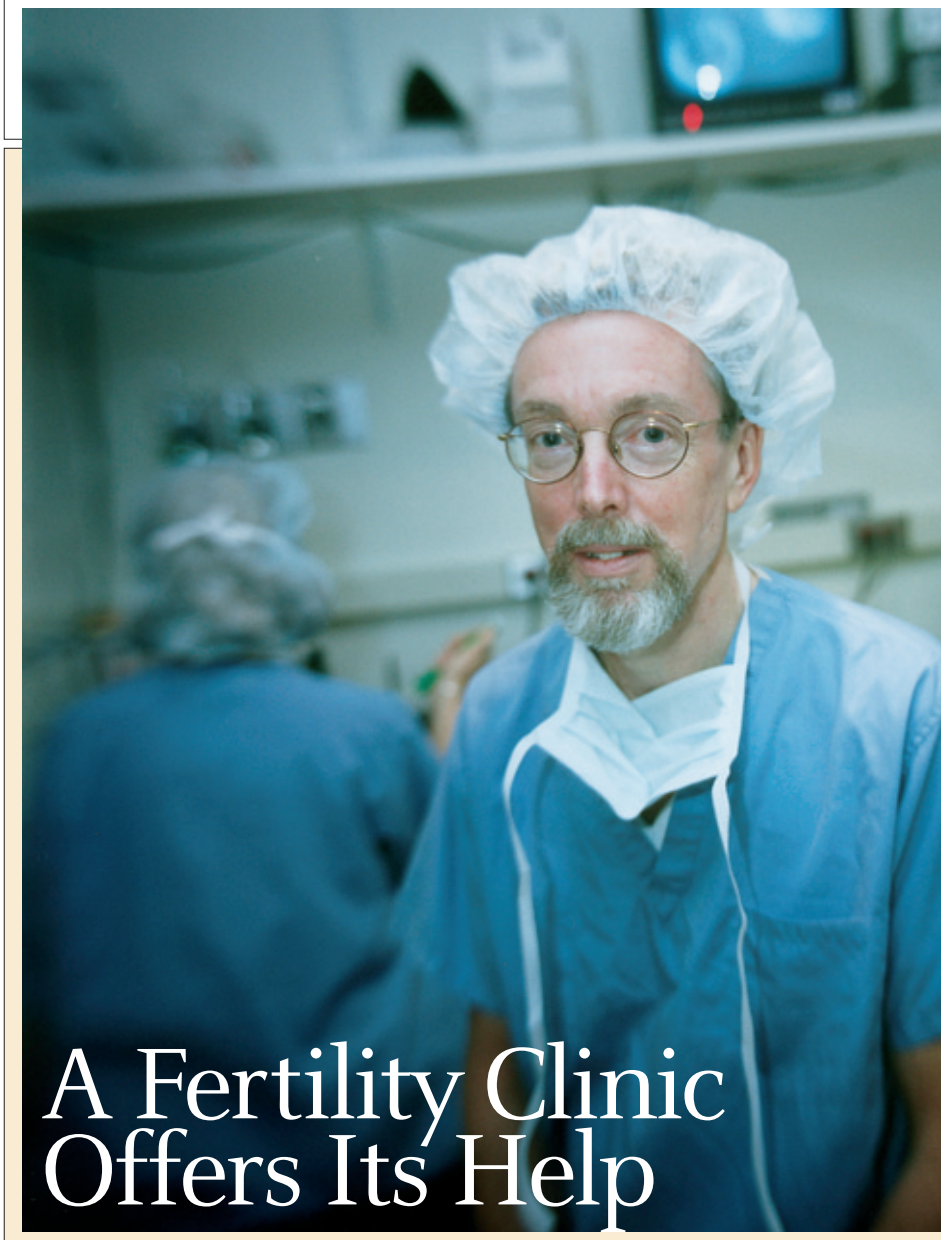
At HHMI, Melton found a receptive audience. "Our primary mission is to carry out the very best in biomedical research, and Doug is one of our researchers," says Cech. "He came to us and said he had the opportunity to do some very exciting and potentially very important research. We evaluated it carefully. Then we decided to fund it, as long as it remained legal and passed review by the Harvard Institutional Review Board. We also entered into an agreement with Harvard and Boston IVF covering the proposal.

"We are comfortable with our decision on all grounds—medical, scientific, ethical," Cech declares. "In fact, considering the potential for human health, we think it would be unethical not to proceed."

REASONS FOR OPTIMISM

The collaboration between Harvard, Boston IVF and HHMI is just beginning. Renovation of a laboratory dedicated to the stem cell project has just been completed. It will enable Melton to study, in great detail, the various steps in the development of insulin-producing beta cells in humans.

Melton points out that he'll benefit from the experience of National Institutes of Health (NIH) scientists who recently succeeded in coaxing mouse ES cells to develop



A Fertility Clinic Offers Its Help

Thousands of frozen human embryos, floating in culture fluid in hundreds of thin plastic vials, are stored in insulated tanks at Boston IVF, one of the nation's largest fertility clinics. Each embryo consists of only eight cells and is considerably smaller than a period on this page.

HHMI investigator Douglas Melton refers to the eight-cell clumps as "pre-embryos." At the time they are frozen, three days after in vitro fertilization, they are still several steps away from becoming the 100-cell blastocysts that, under the right circumstances, are implanted in a woman's body about 10 days later. Yet these microscopic balls of cells are an essential resource for Melton and his colleagues at Harvard, who plan to produce new colonies of human embryonic stem (ES) cells for research. Melton hopes to turn such ES cells into pancreatic beta cells, which secrete insulin, to replace those that are missing in people with juvenile diabetes.

The embryos come from the many eager couples, about 20 a day, who stream into Boston IVF's clinic in Waltham, Massachusetts, for help in having a baby—specifically, by the in vitro fertilization process from which the clinic takes its name. Despite repeated attempts, all these couples have failed to achieve a pregnancy "the old-fashioned way," as R. Douglas Powers, Boston IVF's scientific and laboratory director, puts it. Now they rely on the clinic to unite their sperm and eggs in a dish.

The couples are greeted by a team of blue-smocked young technicians and embryologists who pad about in blue paper shoe covers and caps as they move in and out of labs and operating rooms. One of the most important procedures the

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R. Douglas Powers, scientific and lab director of Boston IVF, gives couples the option to donate their extra embryos for research on stem cells.

lab carries out is semen analysis. If the semen proves suitable, the husband's sperm may be processed.

First, however, the woman's eggs must be removed from her ovaries, which requires a minor operation. Only one egg normally matures in the ovaries every month, but a single egg is extremely unlikely to result in a successful pregnancy. Thus, to avoid repeat operations, the woman receives hormones that stimulate her ovaries to produce many more eggs. The physicians can then collect 10 to 20 eggs at one time.

"Assume we have obtained 15 eggs," says Powers. "We put the eggs in a culture medium in a dish, and then we drop the sperm on top of the eggs. The sperm swim around; about 80 percent of the time they actually attach to the eggs and penetrate them. So maybe 13 eggs will get fertilized in vitro. Any eggs that were not fertilized are discarded right away.

"Probably 10 of the 13 eggs will go on to develop into fairly good looking embryos," says Powers, explaining that he chose the term "good looking" deliberately. "We have no test that can tell us reliably which embryos are the best," he says. "So we just look at them with a microscope and try to judge them on their appearance."

Several of these 10 embryos will then be put into the woman's uterus. "With the current state of infertility treatments," Powers explains, "we can't guarantee that if you put only one embryo back she'll have a high probability of getting pregnant. So usually, depending on her age, somewhere between two and four embryos are placed back—fewer embryos for younger women and more for older women.

"Assuming you put 3 of the 10 embryos back, you've still got 7 left over," he says. "That's where the freezing comes in. If the woman doesn't get pregnant the first time, she can come back for another attempt. At that point, we thaw out some more of her embryos and try again."

If the couple is lucky, the woman will get pregnant after one or two attempts of in vitro fertilization and have a healthy baby. She might even have a second child later. But eventually the couple may not want any more children, even though some of their frozen embryos remain stored in liquid nitrogen. Or, if the woman does not get pregnant, the couple may sooner or later cease trying. "That's why the number of embryos we have in storage slowly grows and grows," Powers explains. "We freeze about 8 embryos a day, and we probably thaw out 3 embryos every day. Since 1989, we have built up a large bank of embryos." In fact, he says, the clinic now has about 3,000 frozen embryos under lock and key.

Powers teaches embryology at Boston College and was one of the founders of Boston IVF. "We've been responsible for the birth of 9,000 babies," he notes proudly. In the past, when a couple decided they had finished their fertility treatments, they were given the choice of leaving the remaining embryos in storage or discarding them. Now, Powers says with satisfaction, "we can offer them an additional choice: donating their embryos for research." **—MP**

into pancreatic cells that make insulin. "The cells self-assemble to form three-dimensional clusters similar… to normal pancreatic islets," reported Ron McKay, chief of the Laboratory of Molecular Biology at the National Institute of Neurological Disorders and Stroke, and his associates in the May 18, 2001, issue of *Science*. "When these cell clusters were exposed to glucose, they released small amounts of insulin. They continued to function even when injected into diabetic mice"—although at levels too low to make a real difference in the health of the mice.

Melton intends to reproduce these findings and extend them to human ES cells. He is convinced that if he succeeds in producing human beta cells that pump out enough insulin, he'll be able to implant these cells into diabetics and cure them. The evidence comes from Canada, he says. Last year, a group of physicians in Edmonton took pancreases from cadavers, obtained islets from them and then transplanted the islets (which contain beta cells as well as three other kinds of pancreatic cells) into patients who had very hard to control diabetes. "The results are extremely encouraging," says Melton. "All of the patients are doing well. They are no longer taking insulin injections."

The procedure is not perfect, however. "The patients have to be kept on immunosuppressant drugs so they won't reject the foreign islets," Melton explains. "For this reason, you would not at this point treat a child with such implants, since it's unknown whether taking daily insulin injections and having imperfect glucose control is better or worse than having perfect glucose control but taking immunosuppressants all your life."

The problem of immunity will be solved, Melton believes, when "some clever immunologists figure out how to induce tolerance and block rejection. Or maybe scientists will develop a special net to enclose the transplanted beta cells and keep them away from the body's immune system."

But there is another big problem: supply. "There just aren't enough cadavers to treat the one million type I diabetics in the U.S., plus another million type II [adult-onset] diabetics who take insulin," he says. "The very best estimates say that there are only 1,000 to 2,000 pancreases available from cadavers in any one year. Why? Because the pancreas is exquisitely sensitive to the loss of oxygen …. You only really have access to patients who first of all are organ donors and, secondly, who you know are about to die."

For this reason, Melton places his hopes on the production of fresh beta cells from a renewable source, human ES cells. He envisions two promising outcomes. First, if functioning beta cells are implanted into type I diabetics, such as his son, the cells will squirt out just the amount of insulin each patient needs. This will occur internally, elim-

inating both the need for injections and the fear of crises.

Second, as scientists learn which signals tell the ES cells to become beta cells, they may be able to mimic these signals with drugs and stimulate patients' own stem cells to make more beta cells. If this is possible in type I diabetes—if these patients still have some pancreatic precursor cells to stimulate—"you would need to combine such therapies with some block to the immune system," Melton warns. "Otherwise, the person would get more beta cells, but the immune system would be there saying 'whack, whack,' and just killing them off. But in type II patients, where there is no autoimmune attack, a stimulus to the patients' own cells might provide a cure."

A NEED FOR ADDITIONAL ES CELL LINES

Melton knows that the clock is ticking, that he must work rapidly and that the hunt for a cure would be speeded up considerably if he and other scientists were studying human ES cells. Until recently (August 2001), however, the majority of U.S. bioscientists—those who receive funds from government agencies—were forbidden to use the funds to work with human ES cells; some legislators were concerned that human embryos, which are potential life, would be destroyed in the process of deriving these cells.

There was much discussion about possible alternatives—using stem cells from adult tissue such as blood or bone marrow, for instance, or those derived from umbilical cords or placentas. Such cells can generate several kinds of specialized cells and are certainly worth pursuing, Melton says, but he warns that because these cells have already started down a particular path, they cannot generate all of the estimated 200 types of cells of the human body. Besides, he points out, adult stem cells "are very rare and difficult to find, and in most cases, no one can get them to grow outside the body. An ES cell, by contrast, has no trouble growing in a culture dish."

On August 9, 2001, President Bush tried to settle the controversy about federal financing of research on human ES cells by allowing it to proceed only as long as the cells came from human embryos that were left over from fertility treatments and that were already destroyed by the day of his speech. These constraints were apparently meant to ensure that government funding of stem cell research was kept separate from, and did not encourage, the further destruction of human embryos.

NIH then produced a list of 64 human ES cell lines that already existed or were in various stages of development in the United States, Australia, Sweden, Israel and India. At the Senate hearing called by Senator Edward M. Kennedy a few weeks later, however, new issues were raised.

Senator Arlen Specter argued, "many of the lines cited are not robust, viable or usable." He also pointed out that because all the human ES cell lines produced so far were grown on layers of mouse feeder cells, they may be unsafe for human use. Melton added that the August 9 cutoff date "was not chosen for scientific reasons, and its arbitrary selection will have an effect on the progress of research."

Although ES cell lines may live indefinitely, Melton said, "decades of experience with mouse ES cells" have shown they can lose their full potential with increasing age. For example, the cells can lose their ability to remain in the undifferentiated state. In addition, they can become contaminated in the lab or accumulate harmful mutations. Thus, under the President's restrictions, Melton told the Senate committee, by the time research on human ES cells advances to the point at which clinical applications can begin, "the viability of the existing cell lines will have been exhausted.

"If we can turn human ES cells into pancreatic beta cells, we would want to use additional, new ES cell lines," he emphasized. In that quest, he hopes that the privately funded HHMI-Harvard-Boston IVF collaboration can help him along. H

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Krissy Jones, an embryologist at Boston IVF, adds liquid nitrogen to a tank containing hundreds of frozen embryos.

A Global Struggle to Deal with Human ES Cells

Policies on human embryonic stem (ES) cell research are as diverse as the global community itself. Some countries' regulations are highly restrictive toward such research, while others allow almost total freedom. This is an area of rapid change, however, and many governments are reviewing their policies. What follows is a brief status report on eight countries and two international bodies, compiled with the help of LeRoy B. Walters, a bioethicist at Georgetown University and a member of the HHMI Bioethics Advisory Board. Web site addresses are provided, where available, for obtaining updates.

AUSTRALIA The law regarding human embryo research varies from state to state, but that situation may soon change. In August 2001, a federal parliamentary committee called for the legalization of research using ES cells derived from spare embryos and for the creation of a national licensing body to regulate all ES cell research. In the meantime, the committee proposed a three-year moratorium on therapeutic cloning (the use of ES cells created through nuclear transfer for research or transplantation). Six cell lines in Australia meet President Bush's criteria, making them eligible for research supported by U.S. federal funds.

» Australian House of Representatives Standing Committee on Legal and Constitutional Affairs: *www.aph.gov.au/house/committee/laca*

FRANCE Although human embryo research is currently prohibited in France, in January 2002 the Assembly of the French Parliament passed a bill that would permit research on leftover embryos from fertility clinics. Prime Minister M. Lionel Jospin had initially advocated the creation of embryos through nuclear transfer for such purposes, but negative feedback from two advisory groups showed that legalizing research on spare in vitro fertilization (IVF) embryos alone had a far greater chance of legislative success.

GERMANY The Nazi era haunted discussions of bioethics in Germany for many years, causing a public reaction against anything that hinted at eugenics. Thus, existing embryo-protection legislation (1990) bans fertilizing an ovum for any purpose other than reproduction. Public sentiment is changing, however, and Chancellor Gerhard Schroeder supports a liberalization of the current law. In January 2002, the German Bundestag voted to allow the importation of stem cells derived from embryos that had already been produced by the time of the vote.

ISRAEL A 1998 law prohibits reproductive cloning but allows leeway for human embryo research. Talmudic law places distinct value on the embryo only after implantation and deems it to have achieved the status of a "formed" human only after 40 days. In August 2001, a national bioethics committee approved the derivation of ES cells and research into therapeutic cloning. Israel has four cell lines that meet U.S. government guidelines for federal funding.

» Israel Academy of Sciences and Humanities: *www.academy.ac.il*

JAPAN A law passed in November 2000 allows experiments with human embryos and cloned embryos in vitro, but it prohibits placing any cloned embryos into a uterus. An expert panel on bioethics recommended in August 2001 that research to derive human ES cell lines from spare embryos be permitted. In September 2001, the Ministry of Education, Science & Technology released guidelines to implement the panel's recommendations.

» English translation of the law passed in 2000: *www.mext.go.jp/english/shinkou/index.htm*

THE NETHERLANDS The lower house of the Dutch parliament approved an Embryo Bill in October 2001 that would allow the use of left-over human embryos for research. The creation of embryos for research purposes would be permitted only through a royal decree and concurrence by the parliament. Action by the upper house is expected early in 2002.

» Ministry of Health, Welfare and Sport: *www.minvws.nl/english/index.html?folder=3*

SWEDEN In December 2001, the Swedish Research Council published guidelines that reaffirm Sweden's traditional policy of permitting research on surplus embryos. Although the Council did not approve the creation of embryos for research through IVF, it declared that creating embryos through somatic cell nuclear transfer "can be ethically defensible." However, this step would require that the Swedish government take steps to ensure it is legally permissible. Sweden is home to the largest number of ES cell lines (24) that meet Bush administration criteria for federal funding.

» English translation of the Swedish Research Council's report: *www.vr.se/medicin/index.asp*

UNITED KINGDOM For the past decade, British researchers have been allowed to use spare human embryos, and also create embryos, for research purposes. A 1990 law initially limited such work to contraception, infertility and congenital diseases. But its scope was extended in early 2001, when Parliament adopted regulations that permit human embryo research for "developing treatments for serious disease." A clear goal of these regulations was to permit the creation of embryos for research through somatic cell nuclear transfer, and in January 2002, the Court of Appeal ruled that the 1990 law does in fact cover embryos created in that way. Since 1990, more than 53,000 human embryos have been used in research in the United Kingdom.

» Human Fertilisation and Embryology Authority: *www.hfea.gov.uk/*

EUROPEAN UNION AND COUNCIL OF EUROPE Trans-European bodies have consistently supported the use of leftover embryos in research but have opposed the creation of human embryos for research purposes. In November 2000, the European Commission's European Group on Ethics in Science and New Technology concluded that using spare human embryos from IVF clinics for stem cell research is acceptable, but that creating embryos for research was not currently necessary. The European Parliament's Temporary Committee on Human Genetics recommended in November 2001 that the European Union ban all EU funding for human ES cell research; however, this recommendation was decisively rejected by the Parliament, which has budgeted substantial funds for such research from 2002 through 2006.

» European Group on Ethics in Science and New Technology: *europa.eu.int/comm/european_group_ethics/*

» Temporary Committee on Human Genetics: *www.europarl.eu.int/committees/genetics_home.htm*

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