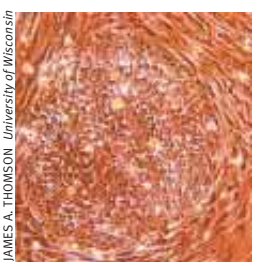
EMBRYONIC STEM CELLS FOR MEDICINE

Cells able to generate virtually all other cell types have recently been isolated. One day they could help repair a wide variety of damaged tissues. **By Roger A. Pedersen**

YOUR FRIEND has suffered a serious heart attack while hiking in a remote region of a national park. By the time he reaches a hospital, only one third of his heart is still working, and he seems unlikely to return to his formerly active life. Always the adventurer, though, he volunteers for an experimental treatment. He provides a small sample of skin cells. Technicians remove the genetic material from the cells and inject it into donated human eggs from which the nucleus, which houses the gene-bearing chromosomes, has been removed. These altered eggs are grown for a week in a laboratory, where they develop into early-stage embryos. The embryos yield cells that can be cultured to produce what are called embryonic stem cells. Such cells are able to form heart muscle cells, as well as other cell types.

The medical team therefore establishes a culture of embryonic stem cells and grows them under conditions that induce them to begin developing into heart cells. Being a perfect genetic match for your friend, these cells can be transplanted into his heart without causing his immune system to reject them. They grow and replace cells lost during the heart attack, returning him to health and strength.

This scenario is for now hypothetical, but it is not fantastic. Researchers already know of various types of stem cells. These are not themselves specialized to carry out the unique functions of particular organs, such as the heart, the liver or the brain. But when stem cells divide, some of the progeny "differentiate"—that is, they undergo changes that commit them to mature into cells of specific types. Other progeny remain as stem cells. Thus, intestinal stem cells continually regenerate the lining of the gut, skin stem cells make skin, and hematopoietic stem cells give rise to the range of cells found in blood. Stem cells enable our bodies to repair everyday wear and tear.



JAMES A. THOMSON *University of Wisconsin*

Human embryonic stem cells growing in culture (*central clump*) are maintained on a layer of mouse "feeder" cells (*background*).

Embryonic stem cells are even more extraordinary: they can give rise to essentially all cell types in the body. Human embryonic stem cells were first grown in culture just last year. In February 1998 James A. Thomson of the University of Wisconsin found the first candidates when he noted that certain human cells plucked from a group growing in culture resembled embryonic stem cells that he had earlier derived from rhesus monkey embryos. A thousand miles away in Baltimore, John D. Gearhart of Johns Hopkins University was isolating similar cells by culturing fragments of human fetal ovaries and testes. And in California, researchers at Geron Corporation in Menlo Park and in my laboratory at the University of California at San Francisco were carrying out related studies.

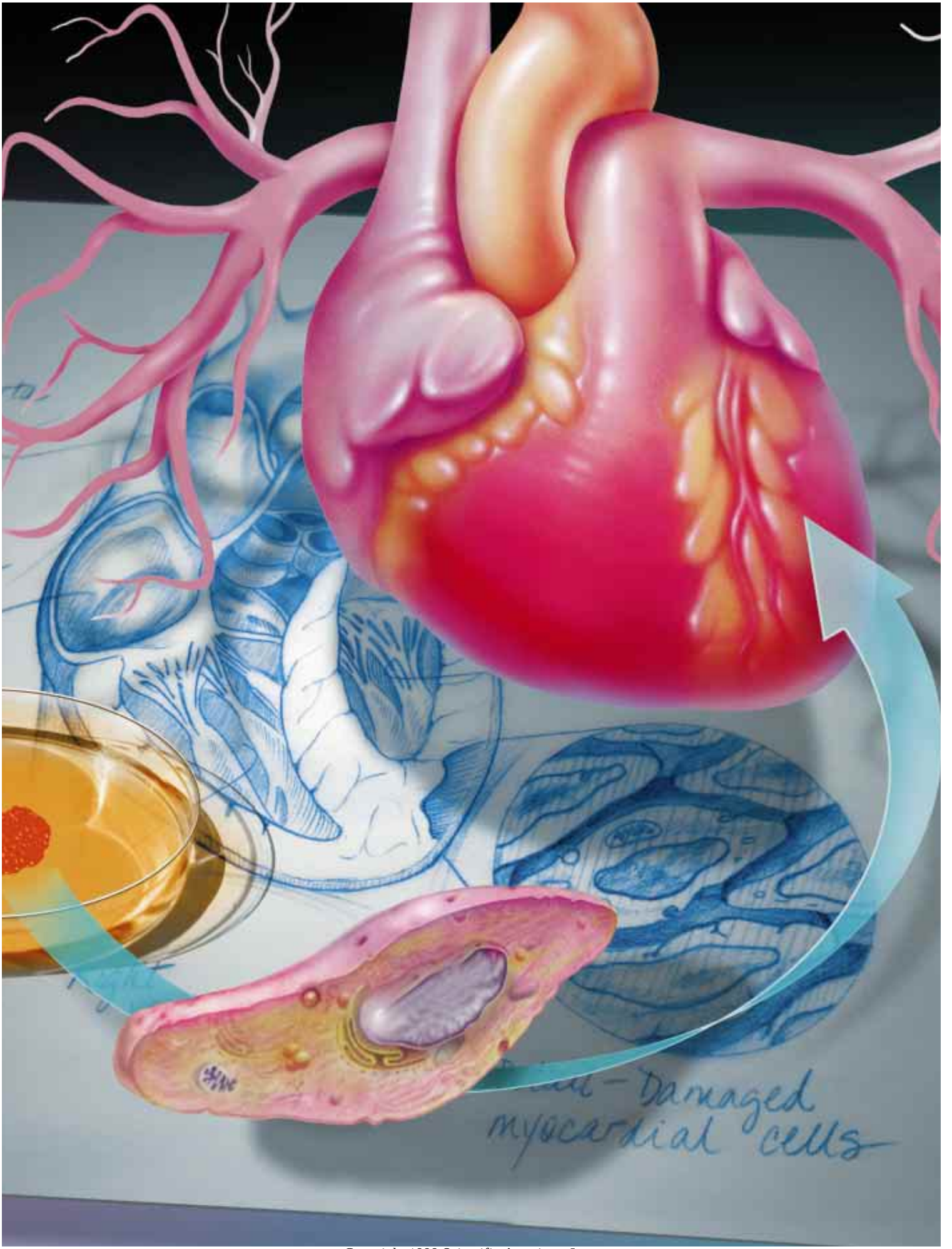
But Thomson was well served by his previous experience with embryonic stem cells of rhesus monkeys and marmosets, which—like humans—are primates. In the following months he pulled ahead of the rest of us in the difficult task of inducing the fragile human cells to grow in culture, and he confirmed that they were indeed embryonic stem cells.

FAR-REACHING POTENTIAL

In studies reported in the November 6, 1998, issue of *Science*, Thomson demonstrated that the human cells formed a wide variety of recognizable tissues when transplanted under the skin of mice. Discussing his results before an inquisitive subcommittee of the U.S. Senate, Thomson described how the cells gave rise to tissue like that lining the gut as well as to cartilage, bone, muscle and neural epithelium (precursor tissue of the nervous system), among other types. What is more, descendants of all three fun-

CYNTHIA TURNER

Cultured cells that have been derived from early human embryos may eventually be coaxed to develop into replacement tissue for a variety of damaged organs, including the heart.



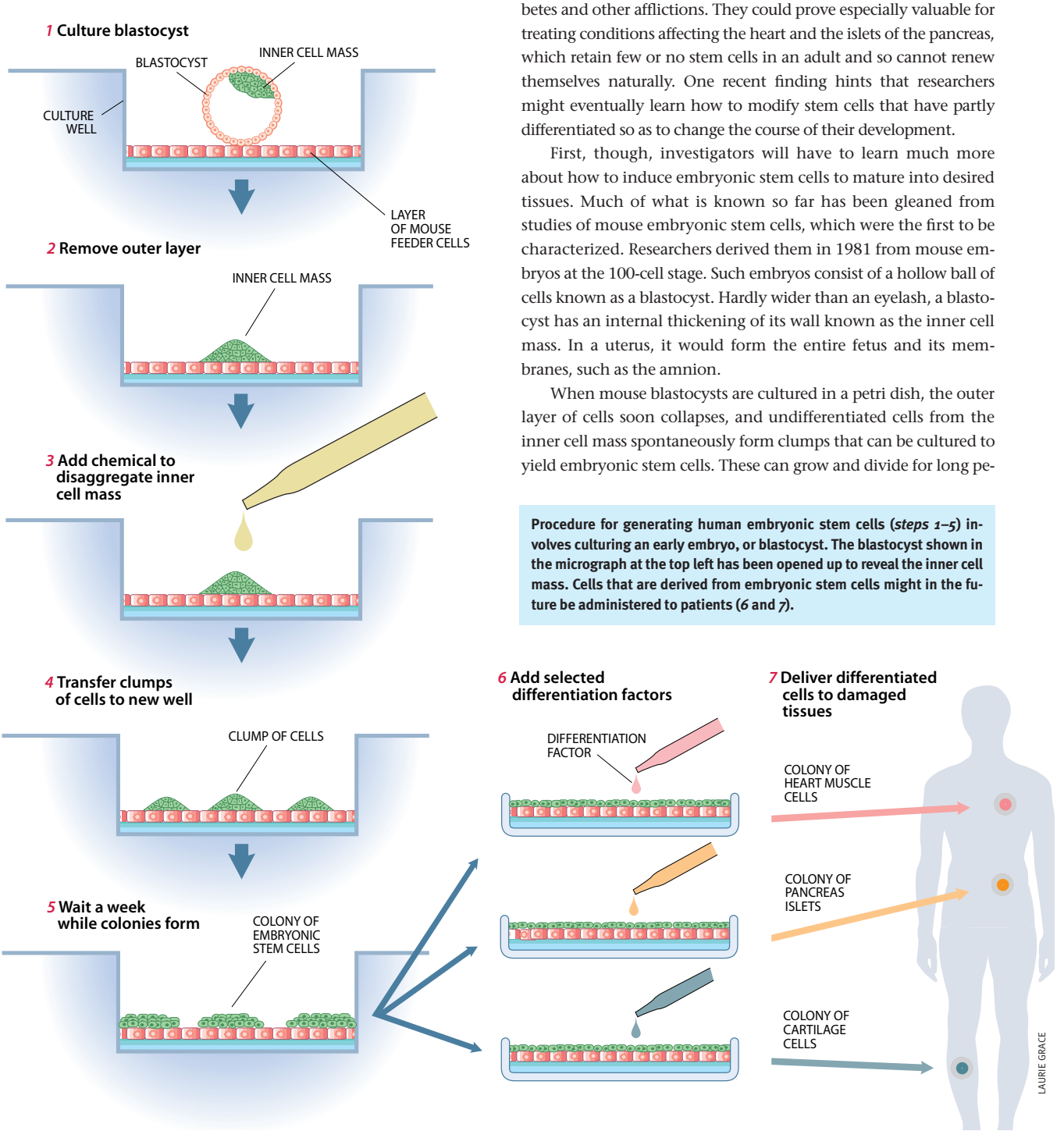
YORGOS NIKAS, *Hammersmith Hospital, London*

damental body layers of a mammalian embryo were represented. Some normally derive from the outermost layer (the ectoderm), others from the innermost or middle layers (the endoderm or mesoderm). This variety offered further evidence of the cells' developmental flexibility. Such results encourage hope that research on embryonic stem cells will ultimately lead to techniques for generating cells that can be employed in therapies for many conditions in which tissue is damaged.

If it were possible to control the differentiation of human embryonic stem cells in culture, the resulting cells could help repair damage caused by congestive heart failure, Parkinson's disease, diabetes and other afflictions. They could prove especially valuable for treating conditions affecting the heart and the islets of the pancreas, which retain few or no stem cells in an adult and so cannot renew themselves naturally. One recent finding hints that researchers might eventually learn how to modify stem cells that have partly differentiated so as to change the course of their development.

First, though, investigators will have to learn much more about how to induce embryonic stem cells to mature into desired tissues. Much of what is known so far has been gleaned from studies of mouse embryonic stem cells, which were the first to be characterized. Researchers derived them in 1981 from mouse embryos at the 100-cell stage. Such embryos consist of a hollow ball of cells known as a blastocyst. Hardly wider than an eyelash, a blastocyst has an internal thickening of its wall known as the inner cell mass. In a uterus, it would form the entire fetus and its membranes, such as the amnion.

When mouse blastocysts are cultured in a petri dish, the outer layer of cells soon collapses, and undifferentiated cells from the inner cell mass spontaneously form clumps that can be cultured to yield embryonic stem cells. These can grow and divide for long pe-



Procedure for generating human embryonic stem cells (*steps 1–5*) involves culturing an early embryo, or blastocyst. The blastocyst shown in the micrograph at the top left has been opened up to reveal the inner cell mass. Cells that are derived from embryonic stem cells might in the future be administered to patients (*6* and *7*).

riods in an undifferentiated state. Yet when injected back into a mouse blastocyst, they respond to physiological cues, and mature cells derived from those stem cells appear in virtually the full range of the embryo's tissues. For this reason embryonic stem cells are termed pluripotent, from the Latin for "many capabilities." (Mouse embryonic stem cells are sometimes described as totipotent, implying that they can form all tissues, although they do not form placenta.) Embryonic stem cells thus have a lot in common with cells in the inner cell mass, the mothers of all cells in the body, but are not identical to them: subtle changes occur in culture that slightly limit their potential.

As investigators experimented with different culture conditions, they found that if a key biological chemical, known as leukemia inhibitory factor, is not supplied, the cells start differentiating in an unpredictable way. Interestingly, though, the repertoire of cell types that have arisen in this way is much smaller than that seen when the cells are injected into a blastocyst—probably because vital biological chemicals present in the embryo are not in the culture medium. This contrast raised the question of whether artificial conditions could be found that would mimic those in the embryo.

DIRECTING DEVELOPMENT

Such manipulations are possible. Gerard Bain and David I. Gottlieb and their associates at the Washington University School of Medicine have shown that treating mouse embryonic stem cells with the vitamin A derivative retinoic acid can stimulate them to produce neurons (nerve cells). That simple chemical seems to achieve this dramatic effect on the cells by activating a set of genes used only by neurons while inhibiting genes expressed in cells differentiating along other pathways.

My colleague Meri Firpo and her former co-workers in Gordon Keller's laboratory at the National Jewish Medical and Research Center in Denver had comparable success deriving blood cells. They discovered that specific growth factors stimulated cells derived from embryonic stem cells to produce the complete range of cells found in blood.

Embryonic **stem cells** can give rise to **essentially all cell types** in the body.

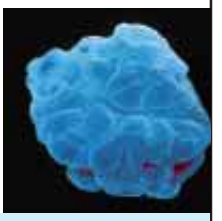
Embryonic stem cells might even generate some useful tissues without special treatment. I never cease to be amazed, when looking through a microscope at cultures derived from embryonic stem cells, to see spontaneously differentiating clumps beating with the rhythm of a heart. Investigators could potentially allow such transformations to occur and then select out, and propagate, the cell types they need.

Loren J. Field and his associates at the Indiana University School of Medicine have done just that. Employing a simple but elegant method, they enriched the yield of spontaneously differentiating heart muscle cells, or cardiomyocytes, to greater than 99 percent purity. To achieve that goal, they first introduced into mouse embryonic stem cells an antibiotic-resistance gene that had

THE ETHICS OF USING EMBRYONIC CELLS

THE FULL POTENTIAL of recent discoveries on embryonic stem cells will be realized only if society deems this research worthy of support. Many people feel that human embryos growing in laboratory dishes, even at the earliest stages of development (between fertilization and the 100-cell blastocyst stage), warrant special moral consideration, because they can grow into human beings if returned to a uterus for gestation. In 1994 an expert panel of ethicists and researchers convened by the U.S. National Institutes of Health studied the issue. It recommended that some embryo research, including the derivation and analysis of human embryonic stem cells, was ethically justifiable and merited consideration for federal funding.

Even so, a congressional ban has ensured that no federal monies have yet been appropriated for research on human embryos. (The work of James A. Thomson and John D. Gearhart mentioned in this article, as well as my own work on related cells, was all supported by Geron Corporation in Menlo Park, Calif.) Some countries, notably the U.K., have concluded that research on human embryos does warrant governmental review and support, whereas a few, such as Germany, have decided otherwise.



JASON BURNS *Phototake*

A human embryo five days after fertilization.

Together with most of my colleagues, I consider laboratory research on human embryos a legitimate scientific activity because of the work's enormous medical promise. Of course, informed consent must be obtained from the donors of any human materials used for research. Embryos are now routinely created in clinics to treat infertility, and those not implanted in a uterus are destroyed if they are not donated for research.

The transfer of experimental embryos to a uterus, however, must meet a different standard of ethics and safety, because that act opens up their potential to develop into human beings. Any manipulations on an embryo that is to develop must be demonstrably safe and bring unambiguous benefits for the resulting person.

It is clear that cloning human beings would not meet this standard, and I seriously doubt that it ever will. [*Editors' note:* Others disagree. See "I, Clone," on page 80.] That is why I spearheaded a voluntary moratorium on reproductive cloning of humans, a policy that has been endorsed by essentially all U.S. scientists who could credibly consider such an activity.

Early this year the NIH announced that it favors supporting research on lines of embryonic stem cells that scientists establish using funds from other sources. It did so after considering the biological potential of these cells. Once they are derived, either from a natural embryo or possibly from one produced through somatic cell nuclear transfer (as described in the main text), embryonic stem cells are no longer equivalent to an embryo in their developmental power.

Specifically, to grow stem cells in the test tube, researchers must remove the outer layer of cells in the originating blastocyst. These excised cells are essential to the development of the placenta, which normally nourishes the product of conception and protects it from rejection by the mother's immune system. By stripping them away, a researcher eliminates any possibility that the remaining inner cells can develop in a uterus. Embryonic stem cells provide a source of medically useful differentiating tissues that lack the awesome potential of an intact embryo. —*R.A.P.*

Researchers should be able to make perfectly **matched tissues** for **transplantation.**

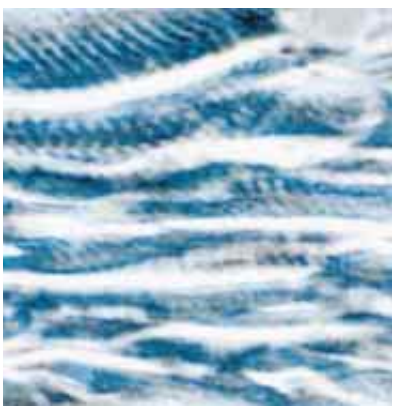
been engineered to express itself only in cardiomyocytes. After allowing the cells to differentiate and exposing them to enough antibiotic to kill cells that lacked the resistance gene, Field's team was able to recover essentially pure cardiomyocytes. Remarkably, when the cells were transplanted into the hearts of adult mice, the cardiomyocytes engrafted and remained viable for as long as seven weeks, the longest period the researchers analyzed.

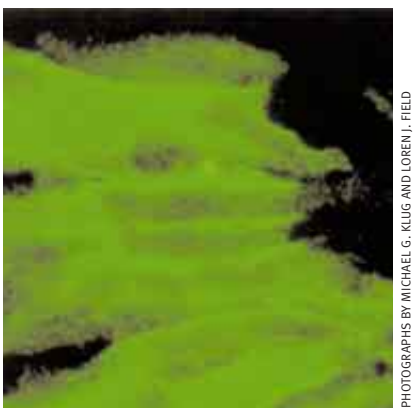
Likewise, Terrence Deacon of Harvard Medical School and his co-workers have transplanted embryonic stem cells into a particular region in the brains of adult mice. They observed that many of the engrafted cells assumed the typical shape of neurons. Some of those cells produced an enzyme that is needed to make the neurotransmitter dopamine and occurs in quantity in dopamine-secreting neurons. Others produced a chemical found in a different class of neurons. What is more, the nervelike cells in the grafts elaborated projections that resembled the long, signal-carrying neuronal branches known as axons; in the brain, some of these extended into the surrounding tissue. Whether such cells not only look normal but also function normally has not yet been assessed. Nor is it clear which (if any) growth factors in the mice stimulated the transplants to form neurons: surprisingly, nervelike cells also developed in grafts placed adjacent to the kidney.

The technique for establishing a culture of embryonic stem cells is more involved when primate embryos are the source, rather than mouse embryos. The outer cell layer of the primate blastocyst does not fall apart so readily in culture, so researchers must remove it, or the cells of the inner cell mass will die. But the results from the mouse studies suggest that as researchers gain experience with human embryonic stem cells, it will become possible to stimulate them to produce, at least, blood cells, heart muscle cells and neurons. Other medically valuable types might be achievable, such as pancreatic islet cells, for treatment of diabetes; skin fibroblasts, for treatment of burns or wounds; chondrocytes, for regenerating cartilage lost in arthritis; and endothelial (blood vessel–forming) cells, to repair blood vessels damaged by atherosclerosis.

Unfortunately, embryonic stem cells also have a dark side. The jumble of cell types they form when injected into mature mice constitutes a type of tumor, known as a teratoma. Researchers will have to be sure, before using cells therapeutically, that they have all differentiated enough to be incapable of spreading inappropriately or forming unwanted tissue. Rigorous purification of such cells will be required to safeguard the recipients.

The cells that Gearhart obtained from developing ovaries and testes also show medical promise. They are called embryonic germ cells, because they are derived from the ancestors of sperm and eggs, which are together referred to as germ cells. Gearhart has shown that his cells, too, are pluripotent: in the petri dish they can give rise to cells characteristic of each of the embryo's basic layers. As of this writing, however, Gearhart has not published details of what happens when embryonic germ cells are placed under the skin of mice, so information about their potential for tissue formation is still somewhat limited.



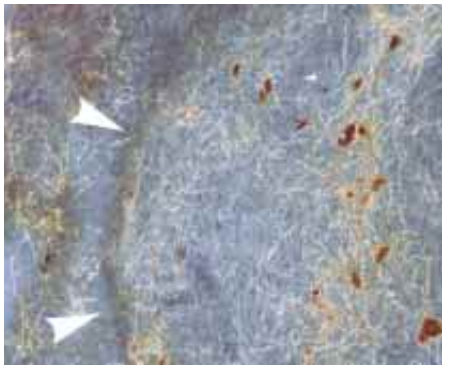


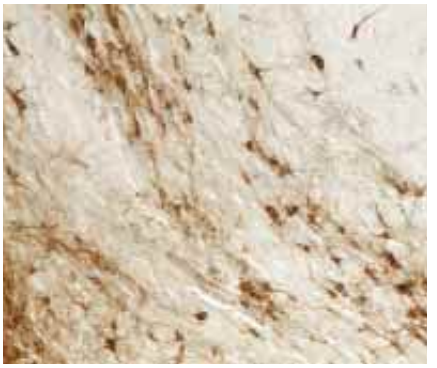
PHOTOGRAPHS BY MICHAEL G. KLUG AND LOREN J. FIELD *Indiana University School of Medicine*

Myosin, a protein found mainly in muscle, fluoresces red in cells derived from mouse embryonic stem cells (*left*). Transplanted into a mouse's heart, the cells become enmeshed with heart muscle (*center*). The donated cells can be distinguished by green fluorescence (*right*).

CHALLENGES AND OPPORTUNITIES

All the differentiated cells discussed so far would probably be useful in medicine as isolated cells or as suspensions; they do not have to organize themselves into precisely structured, multicellular tissues to serve a valuable function in the body. That is good news, because organ formation is a complex, three-dimensional process. Organs generally result from interactions between embryonic tissues derived from two distinct sources. Lungs, for example, form when cells derived from the middle layer of the embryo interact with those of the embryonic foregut, which is derived from the inner layer. The process stimulates embryonic foregut cells to form branches that eventually become the lungs. For would-be tissue engineers, learning how to direct pluripotent stem cells through similar interactions with the goal of building entire organs will be





TERRENCE DEACON *Harvard Medical School*

Cells resembling nerve cells (*brown and gold in image at right*) form when mouse embryonic stem cells are placed in a mouse brain (*blue background*). Indications that the cells may indeed be nerve cells include the extension of projections into the surrounding tissue (*arrows*) and the production of an enzyme (*brown in far right image*) made by certain nerve cells in the brain.

hugely difficult. Nevertheless, some researchers are working on solutions to those very problems.

Another challenge is to create cells for transplantation that are not recognized as foreign by the recipient's immune system. This end could be achieved in principle by genetically altering human embryonic stem cells so they function as "universal donors" compatible with any recipient. Alternatively, embryonic stem cells genetically identical to the patient's cells could be created, as in the scenario of the heart attack victim described earlier.

The first option, creating a universal donor cell type, would involve disrupting or altering a substantial number of genes in cells. The changes would prevent the cells from displaying proteins on their outer surface that label them as foreign for the immune system. Yet bringing about this alteration could be hard, because it would require growing embryonic stem cells under harsh conditions, in particular exposing them to multiple rounds of selection with different drugs.

The second option, making cells that are genetically identical to the patient's tissues, involves combining embryonic stem cell technology and a fundamental step in cloning, as described in the vignette opening this article. Using a hollow glass needle one tenth of the diameter of a human hair, a researcher would transfer a somatic (nonreproductive) cell—or just its gene-containing nucleus—into an unfertilized egg whose chromosomes have been removed. The egg would then be activated by an electrical shock, launching it on its developmental journey with only the genetic information of the transferred, or donor, cell.

In several animal studies on nuclear transfer, cells from existing adult animals have been used as the gene donors, and the altered cells have been implanted into the uterus of a living animal. These experiments gave rise to Dolly the sheep and to some mice and cattle as well. To create cells for transplantation with this combination of approaches, an investigator would use a cell from the patient as a donor but would culture the resulting embryo only until it reached the blastocyst stage. Then the embryo would be used to produce embryonic stem cells that were genetically identical to a patient's own cells.

Human embryonic stem cells could have other applications, too. Because the cells could generate human cells in basically unlimited amounts, they should be extremely useful in research efforts designed for discovering rare human proteins. These programs need great quantities of cells in order to produce identifiable amounts of normally scarce proteins. And because embryonic stem cells resemble cells in early embryos, they could be employed to flag drugs that might interfere with development and cause birth defects.

Finally, such cells offer an approach to studying the earliest events in human development at the cellular and molecular levels in a way that is ethically acceptable. The moral issues associated with experiments on embryos should not arise because embryonic stem cells lack the ability to form an embryo by themselves [*see box on page 21*]. Research on the cells could provide insights into fundamental questions that have puzzled embryologists for decades, such as how embryonic cells become different from one another, and what causes them to organize into organs and tissues. The lessons learned from mice, frogs, fish and fruit flies on these subjects are highly germane to humans. Yet understanding these processes in our own species will ultimately provide us with the greatest benefits and the deepest satisfaction.

As **researchers** gain experience with **human embryonic stem cells**, it will become possible to stimulate them to produce, at least, blood cells, **heart muscle cells** and neurons.

ABOUT THE AUTHOR

ROGER A. PEDERSEN is professor of obstetrics, gynecology and reproductive sciences at the University of California, San Francisco. His moratorium on cloning of human beings can be read at www.faseb.org/opar/cloning.moratorium.html on the World Wide Web. This article also appeared in *Scientific American* in April 1999.