Breaking the Sickle Cycle

Potential treatments emerge for sickle cell anemia

By DIANE E. LOUPE

Gaston

Sickled red blood cells.

Long ago, in the mosquito-infested tropics of Nigeria, traditional healers of the Ibo tribe devised strategies to prevent infant deaths. Tribe members believed some families were cursed with *ogbanje,* or repeater children – babies who die in infancy and are then reborn to the same parents. To persuade a child to stay when first born, some healers would offer sacrifices or make a small mark on the baby's face. Others, according to biochemist Stuart J. Edelstein, would remove the end of the little finger on the baby's left hand.

Edelstein, who documents the finger-cutting practice in *The Sickled Cell* (1986, Harvard University Press, Cambridge, Mass.), says many of the Ibo babies who died in infancy may actually have succumbed to infections as a result of sickle cell anemia.

Sickle cell disease stems from an inherited abnormality in hemoglobin – the oxygen-carrying pigment in blood – leading to elongated, or "sickled," red blood cells that clog the small blood vessels. Pneumonia and other infections often claim the lives of babies with the disease. In older children, it can stunt the growth of fingers and limbs. The fact that people with sickle cell anemia often display one or more shortened fingers could have led the Ibo to think a short finger might be necessary for survival beyond infancy, reasons Edelstein, of the University of Geneva in Switzerland.

Modern healers now think the key to a long life for sickle cell sufferers rests not in the fingers but in the womb. Fetuses in their last six months of gestation produce a form of hemoglobin that doesn't foster sickling, even in those who will later develop the disease. But shortly after birth, the body's hemoglobin-making system switches to produce adult hemoglobin, which *will* cause sickling in people with the disease. Researchers say recent progress in the search for ways to boost fetal hemoglobin production in afflicted children and adults offers hope of someday conquering sickle cell anemia, which today affects nearly 60,000 people in the United States and millions worldwide. Other promising approaches include gene therapy and the development of drugs to reduce the painful episodes triggered by sickling.

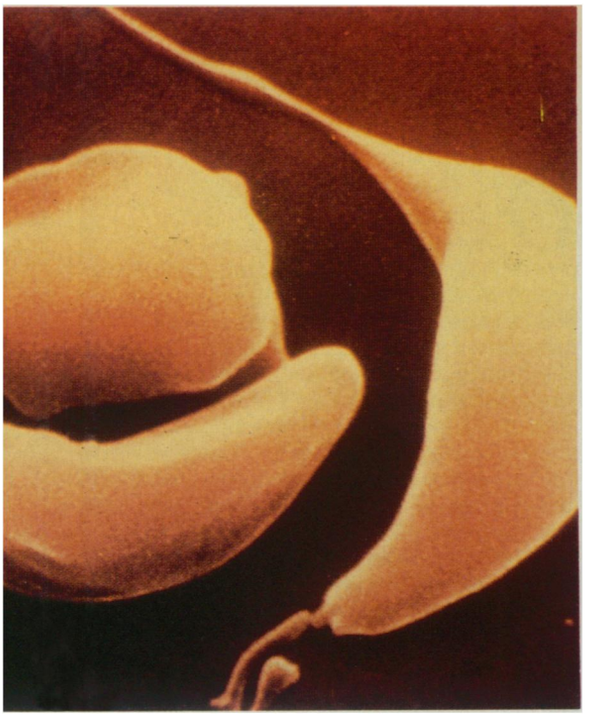
Sickle cell anemia primarily strikes the descendants of natives of equatorial Africa and some people in the Mediterranean, the Middle East, India and Spanish-speaking countries. In the United States, nearly one of every 400 black newborns develops the disease. Because the gene for sickling is recessive, a child must inherit it from both parents in order to have the full-blown disease. Scientists think the gene may have become widespread among black tribes in mosquito-ridden regions of Africa because people with sickle cell *trait,* who carry one normal and one sickling gene, have a slight resistance to malaria. These genetic carriers suffer disease symptoms only under extreme environmental stress.

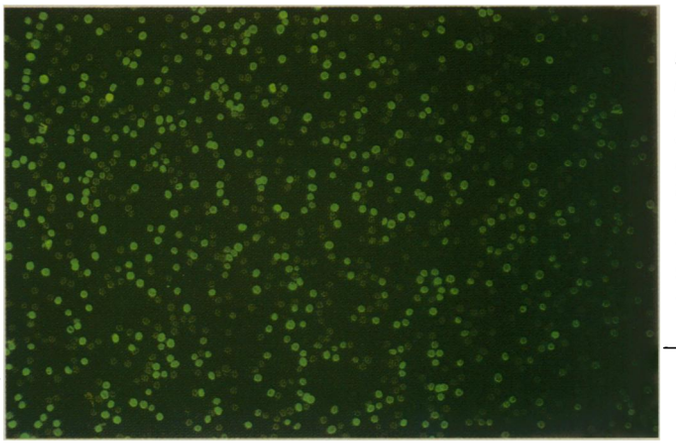
Fetal hemoglobin, normal adult hemoglobin and sickle hemoglobin differ in chemical structure, says Samuel Charache of the Johns Hopkins University School of Medicine in Baltimore, who directs research aimed at stimulating fetal hemoglobin production in sickle cell patients. Normal adult hemoglobin consists of four chains of amino acids – two alpha and two beta chains – linked by chemical bonds. Sickle hemoglobin substitutes the amino acid valine for a glutamic acid on a normal hemoglobin's beta chains. Fetal hemoglobin – produced in low levels by most adults – replaces the beta chains with gamma chains that contain a different number of amino acids.

All forms of hemoglobin bind oxygen molecules in the lungs and transport them to other parts of the body, where the oxygen gets unloaded. But when a sickle hemoglobin molecule unloads its oxygen, one of its beta chains is left with a surface protrusion that can lock into a complementary site on an adjacent beta chain. When the two chains lock together, the hemoglobin molecules form a rigid, polymeric rod.

"It's like children's building blocks with pegs and holes in them: You could make a big tall tower of them. That's just what happens with sickled hemoglobin: A whole string of them get stuck together," Charache says.

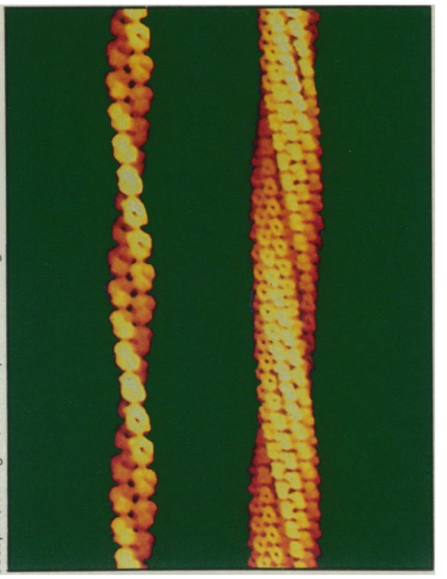
This transforms flexible, smooth, doughnut-shaped red blood cells into stiff, sticky, curved cells that clog small blood vessels. The circulatory traffic jam can lead to acute pain, organ damage, swollen or stunted hands and feet, and sometimes stroke due to blocked blood flow. In contrast, the gamma chains in fetal hemoglobin lack the surface struc-





Stamatoyannopoulos

Bright green dye highlights red blood cells from adult mice genetically engineered to produce human fetal hemoglobin. Unlike adult hemoglobin, the fetal substance does not cause sickling.



Josephs, Carragher, Bluemke/Univ. of Chicago

Computer-generated images of sickle hemoglobin molecules linked into rod-like strands. Left shows one pair of interlocking molecules — the strand's basic building blocks. Right shows seven pairs — the hemoglobin structure that makes blood cells sickle.

tures that foster polymerization. Or, as Charache puts it, fetal hemoglobin "doesn't have the peg or the hole."

In 1948, Janet Watson, a pediatrician at what was then called Kings County Hospital in New York City, reported that the red blood cells of infants who inherit sickle cell anemia don't sickle nearly as readily as those of adults with the disease. By the end of the 1970s, researchers noticed that people with sickle cell anemia who continue to produce substantial amounts of fetal hemoglobin after birth tend to suffer less severe symptoms of the disease. For example, sickle cell sufferers from eastern oases of Saudi Arabia produce about three times more fetal hemoglobin than do black sufferers in the United States, with fetal hemoglobin representing 18 to 25 percent of their total hemoglobin, compared with 5 to 10 percent in U.S. blacks with the disease. These individuals also have fewer episodes of pain and better spleen function while lacking other disease symptoms, reports a team of U.S. and Saudi researchers in the July 3 ANNALS OF THE NEW YORK ACADEMY OF SCIENCES.

In 1978, hematologists Joseph DeSimone and Paul Heller of the University of Illinois College of Medicine in Chicago detected elevated levels of fetal hemoglobin in baboons recovering from experimentally induced anemia and oxygen starvation. That same year, medical geneticist George Stamatoyannopoulos and hematologist Thalia Papayannopoulou of the University of Washington in Seattle reported *in vitro* results indicating fetal hemoglobin production is stimulated in human adults by the destruction of erythroblasts, a type of bone marrow cell that begets red blood cells. A loss of erythrocytes, they suggested, would force the bone marrow to use even younger cells — erythroid progenitors — to make blood. Using this "premature recruitment" hypothesis, the Seattle researchers predicted that other medications, including the oral anticancer drug hydroxyurea, might also stimulate production of fetal hemoglobin in adults.

This September, at a Washington, D.C., symposium on sickle cell disease, Charache reported preliminary results from a multicenter clinical study of hydroxyurea's efficacy. Many of the sickle cell patients in that study have responded to the drug by producing enough fetal hemoglobin to alleviate their symptoms, he says. Yet one-quarter to one-third of the participants show no significant improvement. Charache hopes to learn why by the time the three-year study ends next September.

"We know physicians are giving [hydroxyurea] to [sickle cell] patients without knowing if it really does anything," Charache says. "It's a drug that has to be used very carefully. . . . Instead of killing only the cells you want to kill off, you could kill them all off. That could be fatal."

Hydroxyurea studies have prompted researchers to investigate other possible fetal hemoglobin stimulators. Stamatoyannopoulos and his colleagues have observed increased production of fetal reticulocytes — young red cells that contain fetal hemoglobin — in four of 10 sickle cell patients treated with erythropoietin, an engineered version of a kidney-released hormone that promotes red blood cell proliferation. But Stamatoyannopoulos says he remains unsure whether this drug will have practical applications in treating sickle cell anemia.

For many sickle cell investigators, the most exciting quest is the search for the molecular mechanisms that normally trigger fetal hemoglobin production. Stamatoyannopoulos says dozens of researchers are trying to pinpoint the DNA region that switches the fetal hemoglobin gene on and off early in life.

"This is the real competition in the laboratory," he says. "Who will be first?"

Stamatoyannopoulos says the progenitors of cells producing fetal hemoglobin may contain proteins, called trans-acting factors, that interact with genes to express the fetal hemoglobin. As they mature, he suggests, these cells lose those proteins and produce adult hemoglobin.

Another avenue of research seeks treatments to ease the symptoms of sickling. Among the more promising are vaso-erythroactive agents, drugs that modify red blood cell membranes to prevent their dehydration and to lower cell concentrations of hemo-

globin. Lennette J. Benjamin, codirector of the Comprehensive Sickle Cell Center at Montefiore Hospital Medical Center in New York City, led a 1986 study showing that an experimental drug called cetiedil reduced patients' painful episodes of blood vessel blockage. Benjamin told participants at the September symposium that she seeks to improve cetiedil's effectiveness by administering it more frequently to patients. In lab tests, commercially available calcium-channel blockers such as verapamil and diltiazem also show promise of inhibiting sickling, Benjamin says.

Biochemists James M. Manning and Anthony Cerami of Rockefeller University in New York City discovered almost 20 years ago that cyanate salts could inhibit sickling *in vitro* by bonding to a site on the hemoglobin molecule, preventing the hemoglobin from taking on the rod configuration when deoxygenated. But other researchers who used sodium cyanate in sickle cell patients found it reacted with other proteins, producing toxic neurological effects. Manning then searched for other drugs that might bind more specifically to the hemoglobin molecule to prevent sickling. The most promising, he says, is methyl acetyl phosphate, an experimental drug that inhibits polymerization of hemoglobin *in vitro*. Manning is now planning preliminary tests of methyl acetyl phosphate in sickle cell patients.

But even the best of these therapies would leave patients dependent on medication to prevent the sickling; they cannot offer a cure. While bone marrow transplantation *has* cured a few sickle cell patients, this procedure carries a higher mortality risk than the disease itself. In 1984, researchers at St. Jude Children's Research Hospital in Memphis, Tenn., and others reported in the NEW ENGLAND JOURNAL OF MEDICINE that they had cured an 8-year-old girl of both sickle cell anemia and leukemia with a bone marrow transplant. And Belgian researchers reported in the June 25, 1988 LANCET that they used marrow transplants to cure sickle cell anemia in five children from Zaire. But physicians consider the procedure too risky and expensive for widespread use in treating sickle cell disease, Charache says, and some researchers have criticized the Belgian team for using it.

The most promising – and the most distant – prospect for a cure would involve replacing or altering the defective hemoglobin gene with a normally functioning one. Bone marrow cells appear the best candidates for gene therapy because they can be removed, treated and reinserted without perishing. Arthur W. Nienhuis, chief of clinical hematology at the National Heart, Lung, and Blood Institute (NHLBI) in Bethesda, Md., and others are working with laboratory animals to find ways to alter stem cells – extremely rare bone marrow cells that seem to provide the marrow with a blueprint to produce hemoglobin.

"We hope ultimately to cure sickle cell disease and other bone marrow diseases by inserting [a normally functioning] gene into the stem cells . . . thereby replacing the function of the defective gene product," Nienhuis says. He is studying whether a genetically altered viral particle can be fashioned to carry a normal gene into stem cells of patients with sickle cell disease to make them produce normal adult hemoglobin.

Already, scientists have succeeded in inserting a gene for human adult hemoglobin into mice, which then produced large quantities of the substance (SN: 9/2/89, p.149). And Stamatoyannopoulos, working with University of Washington colleague Tariq Enver and others, has managed to insert a human fetal hemoglobin gene into fertilized mouse eggs. After birth, the genetically altered mice expressed the human gene, the researchers report in the September PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES (Vol.86, No.18).

Tim M. Townes, a molecular biologist at the University of Alabama at Birmingham, says he and his collaborators at the University of Pennsylvania in Philadelphia are close to creating mice that produce human sickle hemoglobin. Such mice could provide a valuable animal model for studying the disease, he says.

One researcher expressing both optimism and caution about the new developments in sickle cell research is Marilyn H. Gaston of the Health Resources Services Administration in Washington, D.C. In 1986, while serving as deputy chief of NHLBI's Sickle Cell Branch, Gaston led a study demonstrating that penicillin could prolong the lives of sickle-cell-afflicted children, who are especially susceptible to bacterial infections until age 5. That finding has led many states to require or recommend that hospitals screen all newborns for sickle cell disease so that afflicted infants can receive preventive treatment with penicillin.

Gaston says she considers hydroxyurea the most immediately promising treatment and gene therapy the most hopeful long-term strategy. But she warns that sickle cell anemia has a tendency to baffle researchers.

"I'm kind of jaded," she confesses. "I've gotten so close to this illness, then it moves away. It's elusive. Now we're trying to bridge the gap between theory and therapy." □

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and-marketing program is flawed. When the fate of a program involving legally harvested elephants relies too heavily on the presence of an ivory market, Mr. Miller might ponder whether his solution is actually part of the problem.

Phillip K. Bigelow
Bellingham, Wash.

Musings on membrane DNA

I read with fascination the article on cell-surface-bound DNA ("DNA's Extended Domain," SN: 10/7/89, p.234). What puzzles me is why many eukaryotic biologists have ignored or doubted this concept for so long, since prokaryotes (i.e., bacteria) have been doing the same thing for eons.

Both gram-positive and gram-negative bacteria have specific DNA-binding proteins or "receptors" that bind to and promote the uptake of naked DNA molecules. In fact, the first genetic exchange mechanism to be discovered in bacteria, namely transformation, is based on this phenomenon.

Although I am unaware of any immunity provided to a bacterium with DNA bound to it (perhaps this should be an area of future investigation), this process definitely represents a conservation as well as a recycling mechanism for discarded DNA molecules.

Francis X. Steiner
Assistant Professor of Biology
Hillsdale College
Hillsdale, Mich.

Your excellent article on membrane-bound DNA has led me to exercise my scientific imagination.

The purpose of DNA receptors may initially have been to act as a primitive means of sharing genetic information between cells. Their function as a salvage pathway for conserving DNA precursors could be more coincidental.

A more recent function could be to serve as a mechanism to stimulate interferon production; viral or cellular DNA that is bound and internalized could trigger production of protective interferon molecules. (Can nonimmunogenic strands of DNA be used to stimulate interferon production *in vivo* and provide therapeutic benefit?) Further, membrane DNA receptors could serve as convenient entry sites for viral genomes, validating this mode of pathogenic attack.

As to how membrane-bound DNA survives, perhaps it doesn't. It's true that it faces a hostile environment. Quite possibly the DNA visualized by staining has been damaged by reaction with oxidizing radicals and is irreversibly bound to its receptor until other enzymes or processes can clear the blocked site.

James Huttner
Sylvania, Ohio

Address communications to:
Editor, **SCIENCE NEWS**
1719 N St., NW
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