# Sickle Cells: The Molecular Attack

Isabel Tellez-Nagel, Albert Einstein

by Joan Arehart-Treichel

Sickle-cell anemia research has in the last two years been one of the few glamour fields of biomedical research other than cancer and heart disease. Now, according to a Government-sponsored study, one of the most promising research paths against the disease has been proved to be a dead end. The pathologist who discovered the route disagrees. Whatever the truth of the matter, the effort has opened the door to several other promising approaches.

Sickle-cell anemia is an inherited disorder of hemoglobin molecules in red blood cells. It is largely, although not exclusively, a black problem. About 10 percent of American blacks carry a gene for sickled hemoglobin. About one out of every 500 American blacks actually has the disease (carries two genes for sickled hemoglobin). Sickle-cell anemia takes its toll by producing so-called sickling crises. During a crisis, not enough oxygen reaches a person's red blood cells locally, such as in the heart or brain, and the red cells take on a sickled shape. The sickled cells then clog blood vessels, causing excruciating pain and tissue damage.

Sickle-cell anemia was first described in 1910 by a Chicago cardiologist. Some work was done on the disease in subsequent years, but the major step forward came in 1949 when Linus Pauling (a later Nobel Prize winner and a current vitamin C proponent) proposed the concept of molecular diseases by taking sickle-cell anemia as a model. Sickling, Pauling suggested, could be due to the presence of abnormal hemoglobin molecules in a red blood cell. These molecules might aggregate into rods and distort the cell into a crescent shape. Pauling and his colleagues showed that there is indeed a molecular difference between sickled hemoglobin and normal hemoglobin, but they did not pinpoint its nature.

The molecular basis of sickled hemoglobin was further revealed in the 1950's when Makio Murayama, a physical chemist working with Pauling at the California Institute of Technology, discovered that a type of chemical bonding known as hydrophobic bonding was responsible for the sickling behavior of sickled hemoglobin. Then during the 1960's Robert M. Nalbandian, a pathologist at Wayne State University, became an avid student of Murayama's work. Nalbandian deduced, from Murayama's theories and other information about the structure of sickled hemoglobin, the kind of chemical it would take to desickle the hemoglobin. Out of some million organic compounds to chose from, Nalbandian selected urea. And to his and Murayama's great delight, the chemical desickled sickled hemoglobin in the test tube.

Nalbandian's discovery was the first promising therapy for sickle-cell anemia. And when he and his colleagues successfully used urea to reverse sickling crises on a few patients in 1970, they received worldwide publicity. In February 1971 President Nixon named sickle-cell anemia a priority disease for research money. So during 1972 and 1973 the National Institutes of Health funded various research centers around the United States to test urea clinically, to see whether it was indeed the ideal treatment for sickle-cell anemia.

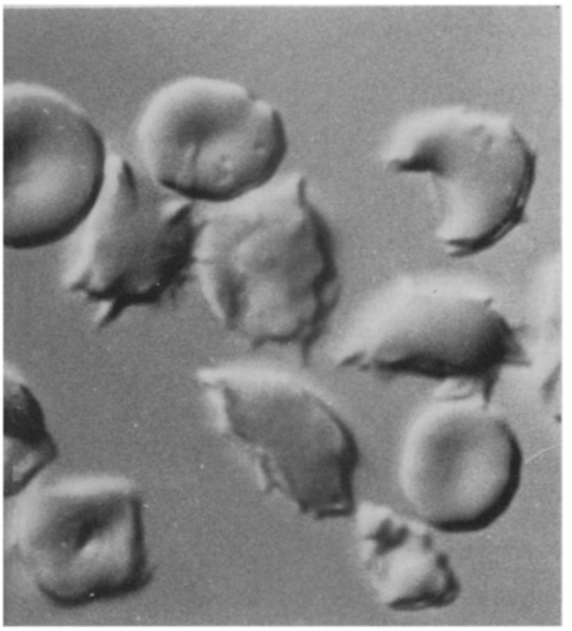
Two years and some million research dollars later, the NIH returns on urea are in. They were reported in December at the annual meeting of the American Society for Hematologists by Alfred P. Kraus of the University of Tennessee. Kraus was spokesman for the entire NIH-sponsored group that had tested urea. The consensus: Urea is ineffective.

Nalbandian recalls the situation painfully: "When Dr. Kraus was through in front of, my God, 4,000 sophisticated hematology researchers, the nation's cream of the crop, I got up and told Dr. Kraus I had no quarrel with his data. But I did criticize the NIH for not using the protocol specified by myself and my co-workers. There has never been a therapeutic failure, a medical misadventure or a death where our protocol has been used in faithful compliance."

Nalbandian charges, for example, that the NIH-sponsored investigators gave urea to patients in a much weaker solution than is necessary to achieve desirable effects. To which Kraus replies: "We did not give as strong a urea solution, but the important thing is the level of urea reached in the patient, and we did reach the urea levels he specified. In fact, we even exceeded them and still got disappointing results."

Nalbandian also objects that the NIH-sponsored investigators dispensed with using a central venous catheter for fluid replacement. Counters Kraus: "The central venous catheter is a complex thing to put in a patient; it would cost hundreds of dollars to do. Also, considering that patients have crises frequently, if you use a central venous catheter for all crises, you will soon use up all of a patient's veins."

Scientists in NIH's Sickle Cell Branch tend to side with the NIH-sponsored in-



James Jamieson, Rockefeller Univ.

*A red blood cell clogged with chains of sickled hemoglobin molecules has taken on a sickled shape (far left). Sickled cells are mixed with a few normal (round) blood cells (center). Lorraine and Alfred Kraus hope that carbamyl phosphate will be the ideal treatment for sickle-cell anemia.*

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vestigators. "Scientists determined that urea was not the answer," states John Hercules. "There are a whole lot of side effects, and it doesn't alter the sickling state in patients as it does in culture." Says George Riley: "We spent two years and about a million dollars and have found it [urea] completely ineffective."

Murayama sides with Nalbandian in that he doesn't believe that the NIH has given urea a fair shake. "If you want to reproduce investigators' findings," he says, "you should do the experiment as they did it originally. In this instance NIH broke this unwritten law."

Whether urea is finished or not, it has pointed the way to several other promising desickling agents. Anthony Cerami and James M. Manning, biochemists at Rockefeller University, got the idea that the positive effect of urea as a desickling agent might be due to cyanate because urea breaks down to cyanate. So they decided to shake up some sickled cells with cyanate and see whether it would inhibit the sickling. To their satisfaction, the cyanate worked, but not as urea does. Urea attacks the hydrophobic bonds that allow deoxygenated hemoglobin molecules to stack up and clog red blood cells. Cyanate hooks onto the hemoglobin molecules (carbamylates them). Cerami and Manning also found that cyanate extends the life of red blood cells in patients. Red cells die quicker in patients with sickle-cell disease than they do in healthy persons.

Cerami and his co-workers have been trying sodium cyanate on sickle-cell patients for about two years now. "We have some evidence that we can actually lessen the number of painful episodes people have," reports Charles M. Peterson, the clinician in charge of the research, "and we're working on setting up a double-blind study through the NIH to prove that."

While Cerami and his team have been exploring cyanate, Kraus and his biochemist wife, Lorraine, have been studying a related promising chemical —carbamyl phosphate. In 1971 they reported that carbamyl phosphate breaks down to cyanate in red blood cells and carbamylates sickled hemoglobin in a manner similar to sodium cyanate. Carbamyl phosphate also extends the life of red blood cells in patients. They are now exploring different ways of giving the drug to patients.

Sodium cyanate and carbamyl phosphate are two of the drugs NIH is now most interested in. "At the present time we're supporting a large effort in sodium cyanate," says Hercules. "We think it might work better than urea because of its chemical configuration."

But it is too soon to tell whether either of these drugs will turn out to be an effective and safe treatment. "Carbamyl phosphate looks very promising," Lorraine Kraus says. Says Roland D. Scott, in charge of sickle-cell research at Howard University, "I think this whole thing [work on cyanate and carbamyl phosphate] is exciting, but we must be cautious in announcing a treatment to the public until we have competent evaluation." For example, sodium cyanate weakens muscles if given at high doses. "We haven't seen this side effect in low doses," says Peterson, 'but we want to better define the side effects before the drug is marketed."

Some scientists think more will have to be learned about the molecular quirks of sickled hemoglobin before the ideal desickling drug is found. Ronald L. Nagel of the Albert Einstein College of Medicine and his team are trying to better define the molecular phenomenon behind sickled hemoglobin. Then, says Nagel, "there may be pharmacological ways of modifying the properties of the molecule as a form of treatment." James Hofrichter, a physical chemist at NIH, is trying to determine the structure of sickled hemoglobin at atomic resolution so that an organic chemist might then design a molecule to upset the energy holding sickled hemoglobin molecules together. Hofrichter hopes that a cool, rational approach to finding a treatment will pay off, but he doesn't rule out the possibility that rational science will be outscooped by serendipity.

"It is possible," he says, "that the disease will be solved not by this method at all, but by some guy throwing chemicals into sickled blood and finding something that stops it."

Whether sickle-cell research will lead to effective treatment any time soon or not, it will probably pay off in other ways. "Sickle-cell anemia has had an enormous impact in biology because it was the first disease for which an exact molecular basis was found," says Nagel. "It has had an enormous impact in genetics. And now it will probably have an enormous impact in the way of modifying a protein to treat a disease. So we're not at the physiological-pharmacological level, we're at the molecular-pharmacological level, which is new and will have an impact on other things." □