Sickle Cell (II): Many Agents Near Trials

A better understanding of the molecular basis of sickle cell disease leads to new approaches to therapy

The prospects for a successful therapy for sickle cell disease have never looked brighter. This assessment is not based on the presence of any medical miracle in the pipeline since most investigators agree that there probably is none. Rather, it is based on a new approach to the problem. For the first time, there is now a comprehensive understanding of the molecular and atomic interactions that trigger a sickle cell crisis (*Science*, 16 January, p. 265), an understanding that suggests new therapeutic approaches. For the first time, there are fast, accurate techniques for assaying the potential therapeutic effects of a new drug in vitro. For the first time, there is an organized program to test the potential efficacy of new agents.

There are, moreover, a number of potential new drugs that give indications of having therapeutic efficacy. Most are first generation agents that will probably be supplanted by more specific, more effective agents as knowledge of their effects is refined, and none is likely to be completely effective by itself. Nonetheless, a combination of new agents will probably, within a period of another 10 years, make it possible to prevent the recurrent crises that are responsible for the most debilitating effects of sickle cell disease.

Current therapies for sickle cell disease are extremely limited. They are designed primarily for supportive care during painful crises, treatment of infections, and care of affected organs. The primary agents used are analgesics, plasma and water to increase the volume of blood, and antibiotics. Transfusions of whole blood are occasionally used for treatment of severe crises, certain pregnancies, and, in some groups of children, for prevention of stroke. Some investigators have advocated more frequent, prophylactic blood transfusions, but this approach is limited by side effects that include iron overload, hepatitis, and allergic responses.

New therapeutic agents can be divided into three major categories: (i) those that inhibit polymerization of sickle hemoglobin (HbS) by disrupting intermolecular bonding, (ii) those that inhibit polymerization by decreasing the concentration of deoxygenated HbS (deoxy HbS), and (iii) those that interact with erythrocyte membranes.

One of the most common approaches to sickle cell therapy during the past decade has been the use of so-called chaotropic agents to disrupt the hydrophobic bonding responsible for polymerization of HbS. Urea was the first chemical to be used for this purpose, but clinical trials during the early 1970's showed that it was not sufficiently effective at the concentrations that could be achieved with tolerable doses. Many other chaotropic agents have been studied, including alkyl ureas, organic solvents, ethanol, and detergents.

Perhaps the most promising of the chaotropic agents are peptides that can bind to the surface of the HbS molecule to prevent the intermolecular contacts necessary for polymerization. Among the investigators using this approach are Alan N. Schechter, Constance T. Noguchi, and their colleagues at the National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD) and a team headed by Alexander Rich of the Massachusetts Institute of Technology and Marian Gorecki of the Weizmann Institute in Rehovot, Israel. Schechter has attempted to design short peptides that should bind specifically to the known contact region on the surface of the HbS molecule, while Rich and Gorecki have used a more general approach that nonetheless recognizes the hydrophobic nature of the binding site. A major advantage of the use of peptides is that the component amino acids are naturally occurring compounds, and thus should have very low toxicities.

Schechter has found that L-phenylalanine and peptides containing it are effective in increasing the delay time before polymerization in vitro and thus should retard sickling in vivo. But the efficacy of even the best compounds he has studied, he says, needs to be improved by another factor of 50 to 100 before it would be really useful in humans; their ability to pass through the red cell membrane also needs to be improved. He estimates that clinical trials are still 5 years away. Rich and Gorecki have obtained good results with, among other things, the benzyl esters of L-phenylalanine and L-tryptophan. They are studying various modifications of these parent compounds to improve efficacy, and have already conducted toxicity trials in rodents; Rich hopes to begin trials in humans within 6 to 9 months. "Sickle cell disease has been studied to death," he says, and it is time to begin applying some of the new knowledge.

Covalent bonding may also be used to prevent polymerization. If a bifunctional molecule reacts with HbS while it is oxygenated, cross-linking might prevent the conformational changes that normally occur when HbS releases oxygen, thereby preventing the molecule from assuming the conformation necessary for intermolecular bonding. Among such reagents are the imidates developed by Lester Packer of the Lawrence Berkeley Laboratory. Bertram Lubin of the Children's Hospital Medical Center in Oakland and William Mentzer of the University of California at San Francisco are now studying one of these compounds, dimethyl adipimidate. They have found that this agent can pass through the erythrocyte membrane in vitro and completely prevent the cells from sickling, even when they are fully deoxygenated.

Lubin and Mentzer have just completed toxicity studies in rodents and have found that the compound and all its hydrolysis products are nontoxic; they are now applying for permission to perform clinical trials. Dimethyl adipimidate is not specific for hemoglobin, and therefore treatment of sickle cell erythrocytes would have to be extracorporeal—that is, blood would be withdrawn and treated, cleansed of reaction products, and returned to the patient. This process can be performed automatically by adapting a machine developed by Albert L. Babb of the University of Washington Medical School. Extracorporeal therapy would not, of course, be practical for all sickle cell patients, says Lubin, but it would be useful for the 20 to 30 percent whose disease is most severe.

The second major approach is to decrease the concentration of deoxy HbS. Kinetic studies by William A. Eaton and James Hofrichter of NIAMDD have shown that even small decreases in the

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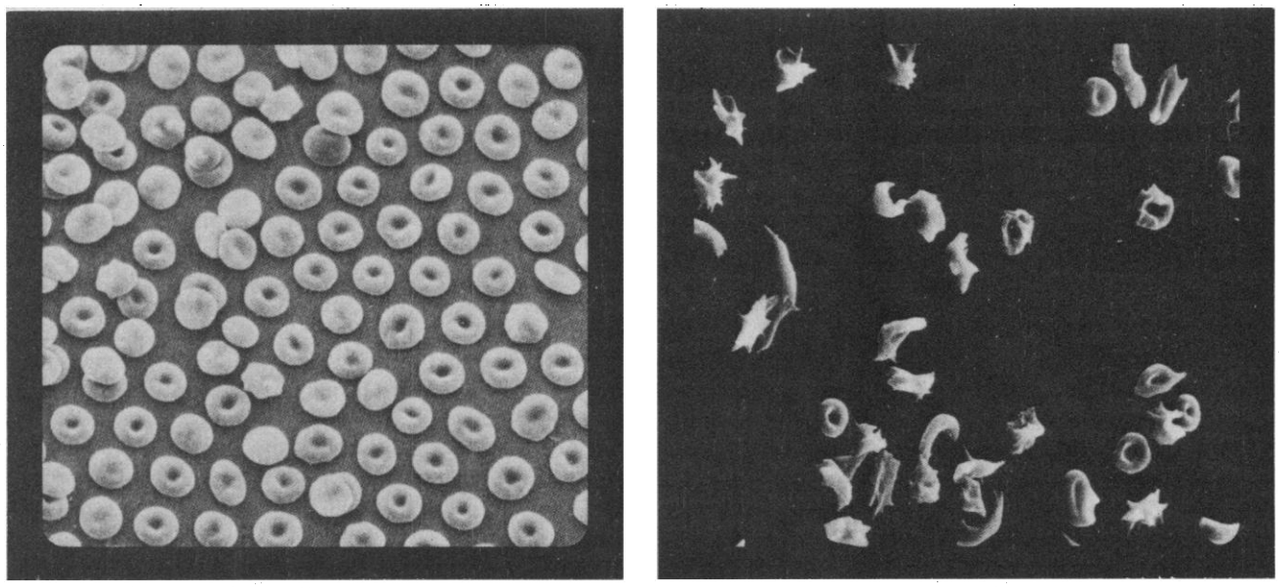

The morphology of sickling

Normal erythrocytes have a uniform appearance (left), but sickled erythrocytes (right) assume a variety of bizarre shapes.

concentration of deoxy HbS can produce large increases in the delay time for polymerization, thereby presumably alleviating disease symptoms. One way to do this is to increase the oxygen affinity of HbS; if it is harder for HbS to release oxygen, there will be less deoxy HbS than normal under any given set of physiological conditions. (Studies of individuals with naturally occurring increased oxygen affinity show that there are few ill effects associated with it.)

One way to increase oxygen affinity is to react HbS with reagents that alter its conformation so that it binds oxygen more tightly. The best known such reagent is cyanate. Cyanate reacts with free amino groups on the α chains of HbS, thereby increasing oxygen affinity. Clinical trials of cyanate were discontinued, however, when it was observed that the drug produced peripheral nerve damage. One possible way around this problem is extracorporeal carbamoylation of sickle erythrocytes by means of Babb's machine. A group of physicians headed by Dennis A. Diederich of the University of Kansas Medical Center has recently completed a clinical trial using cyanate and is expected to report a modest therapeutic efficacy.

Other compounds have been studied for their effects on oxygen affinity, but one family of chemicals deserves comment because it represents a new approach to the problem. Joseph A. Walder and Arthur Arnone of the University of Iowa College of Medicine have synthesized a family of bifunctional reagents typified by bis(3,5-dibromosalicyl) fumarate. This reagent cross-links hemoglobin chains in the same manner as the imidates, but is much more specific since it forms complexes with the 2,3-diphosphoglycerate binding site of HbS. Walder and Arnone have found that this chemical increases the delay time for polymerization in vitro by a factor of 35,000 (cyanate, in comparison, increases it by a factor of 30), one of the largest increases observed for any new agent. They have conducted toxicity studies of several of the compounds in rodents and are now conducting studies in other animals. Clinical trials could follow soon.

Another way to decrease the concentration of deoxy HbS is to increase the volume of the erythrocyte. A team headed by H. Franklin Bunn of the Harvard Medical School and Robert M. Rosa of Beth Israel Hospital in Boston reported in November that they had induced hyponatremia (low concentrations of sodium in the blood) in three patients who had severe, frequent crises. This was achieved by high intake of fluids, severe restrictions on salt intake, and administration of a hormone that retards elimination of urine. Hyponatremia causes erythrocytes to swell, thereby diluting the HbS contained within. The Boston group found that chronic hyponatremia reduced the frequency of painful crises and short-term induced hyponatremia shortened existing crises. This method is an extreme form of therapy with a potential for water intoxication and should be used only for the most severely affected patients, but it does demonstrate that dilution of HbS is effective therapy.

Another way to dilute HbS is to induce bone marrow to produce either fetal hemoglobin (HbF) from genes that are already in the cell or normal hemoglobin (HbA) from newly inserted genes. For the long term, this represents the most promising form of therapy because it is a potential "cure" that does not require daily ingestion of drugs. Joseph De Simone of the University of Illinois Medical Center, for example, has been able to induce erythrocytes in baboons to produce HbF in amounts up to 40 percent of the total cellular hemoglobin. He achieves this by destroying a substantial fraction of erythrocytes so that the baboon's bone marrow must produce new ones rapidly. Under conditions of rapid maturation, the cells produce more HbF. Unfortunately, the high levels of HbF could not be maintained, but his work demonstrates that production of HbF can be switched on in mature animals.

Other investigators, such as Arthur W. Nienhuis and M.-Y. Chin of the National Heart, Lung, and Blood Institute, Thomas Maniatis of the California Institute of Technology, and Richard Axel of Columbia University, hope eventually to insert a gene coding for the normal β chain of HbA into bone marrow cells of sickle cell patients. Nienhuis and Chin inserted human globin genes into cultured mouse cells. They found that five copies of the gene were inserted into each cell but that only about 100 copies of messenger RNA (mRNA) were produced from them. The two globin genes in normal bone marrow cells, in contrast, produce about 50,000 copies of mRNA, and therefore the inserted genes are just barely being expressed. Maniatis and Axel have inserted human globin genes into cultured mouse bone marrow cells, but they find that there are only about 200 copies of mRNA per gene, and that the genes do

not respond to agents that normally induce gene expression. Both groups are now searching for ways to get the genes to produce much larger quantities of mRNA. In a very controversial experiment, Martin Cline of the University of California at Los Angeles has attempted much the same feat in humans (*Science*, 19 December 1980, p. 1334).

The final category of potential therapeutic agents includes those that act on the erythrocyte membrane. One of these is Cetiedil, a local anaesthetic that is used clinically in Europe for treating chronic cardiovascular disease. Physicians there observed that it also inhibits sickling. Charles M. Peterson of Rockefeller University has examined Cetiedil and found that it does not interfere with polymerization or oxygen affinity. The lipophilic molecule does insert itself in the erythrocyte membrane and stays there for a long time, but no one knows what it does there. Preliminary uncontrolled studies in the Ivory Coast have shown that the drug alleviates sickle cell crises, and Peterson hopes to begin controlled clinical trials within the year.

Another attack on membranes has been conducted by George J. Brewer and his colleagues at the University of Michigan. They have treated a small number of patients with zinc acetate and thioridazine, a tranquilizer. Both thioridazine and zinc are inhibitors of calmodulin, a naturally occurring substance that, in response to the sickle erythrocytes' increased concentration of calcium, causes the cell membrane to stiffen so that the cell becomes permanently sickled. The combination of reagents increases the half-life of sickle erythrocytes by as much as 75 percent, but there is no evidence yet that they decrease the frequency or severity of crises. Like Cetiedil, though, these agents could prove very useful in conjunction with antipolymerization agents.

Two points should be made about prospective agents. They must almost certainly be prophylactic rather than curative: It is substantially more difficult to depolymerize HbS than it is to prevent it from polymerizing in the first place. In fact, there are very few agents that have been shown to depolymerize gelled HbS. A potential new antisickling agent must thus be very safe since it will be used on a daily basis throughout the patient's life.— THOMAS H. MAUGH II

Massive Neutrinos: Masters of the Universe?

Neutrinos with mass would explain a lot about the distribution and dynamics of galaxies; they might even suffice to close the universe

For cosmologists, the idea of a universe filled with massive neutrinos carries the Copernican principle to its extreme: first the earth was not at the center of the solar system; then the sun was not at the center of the galaxy; and finally the galaxy was not at the center of the universe. Now, says University of Chicago astrophysicist David N. Schramm, it seems that the stuff we are made of may not even be the dominant kind of matter in the universe.

Relic neutrinos—those left over from the Big Bang—are thought to outnumber the protons, neutrons, and electrons of ordinary matter by about 10 billion to 1, he said last month at the Tenth Texas Symposium on Relativistic Astrophysics in Baltimore. On the average, every cubic centimeter in the universe contains some 450 of them. Their ghostly indifference to ordinary matter is famous: supposedly massless, moving at the speed of light, they are capable of passing through the earth as if it weren't there.

But if 40 years of neutrino theory is wrong, if those 450 particles per cubic centimeter have even a tiny mass, then their cumulative gravitational pull would be immensely greater than theorists have imagined. Massive neutrinos, in fact, could explain a number of cosmological puzzles related to the structure and expansion of the universe as a whole and to the formation and mass distribution of the galaxies.

While such speculations are more than a dozen years old, the revived interest in massive neutrinos so apparent at the Texas Symposium was sparked in part by two independent experiments last year, one by Soviet physicists and the other by a group from the University of California, Irvine. These experiments produced the first direct, albeit controversial, evidence that even the lightest neutrinos have masses of a few tens of electron volts (*Science*, 16 May 1980, p. 697). For comparison, 10 electron volts is 0.002 percent of the mass of the electron.

But physicists and astrophysicists would be taking the idea of neutrino mass very seriously in any case, because such masses are also predicted in many of the so-called Grand Unified Theories of elementary particle interaction. Such theories typically give a different mass to each of the three known types of neutrinos; some of the particles may even be unstable. Floyd W. Stecker of NASA's Goddard Spaceflight Center in Greenbelt, Maryland, has suggested, for example, that a feature in the ultraviolet spectrum of the sky near the north galactic pole may arise from the decay of heavy neutrinos in a neutrino halo surrounding the Milky Way galaxy. He estimates a mass of 14 electron volts for the particle, consistent with the laboratory experiments.

There is a vast difference between neutrinos having even the tiniest mass, and neutrinos having zero mass. Zero-mass particles, such as photons, are constrained by relativity to move only at the speed of light. At that speed, they can carry energy even without mass—in fact, according to Einstein, their energy content alone would allow them to exert gravitational forces on other bodies—but they could never slow down.

On the other hand, relativity dictates that massive particles must never travel at the speed of light. Like protons or electrons, massive neutrinos could indeed slow down and come to rest. And therein lies the difference for cosmology. Not only would a tiny mass increment enhance the gravitational effect of the relic neutrinos enormously, but those moving slowly enough could become gravitationally bound in galaxies or clusters of galaxies, and thereby participate in the evolution of these objects. The only thing that could bind a massless neutrino, by contrast, is a black hole.

Schramm told the symposium that massive relic neutrinos bound in large clusters of galaxies might in fact offer a straightforward resolution of the "miss-

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