particles and their behavior in order to deduce a flow velocity and direction.

The Voyager radio-wave sensor, much to the surprise of its designers, made some observations that may tip the balance on the corotation controversy. Joseph Alexander and Michael Kaiser of Goddard Space Flight Center, Greenbelt, Maryland, discovered a new type of radio emission from Jupiter's magnetosphere. And the source region seems to circle the planet 3 to 5 percent slower than the planet spins. Kaiser and Alexander think that the signal comes from the outer edge of Io's doughnut-shaped cloud. They conclude that the plasma that is producing the radio waves in this region is not quite keeping up with the planet's rotation—in agreement with Bridge's measurements of the flow velocity in the same area.

Krimigis's data indicate that the Jovian merry-go-round does not go on forever. On the nightside of Jupiter, between 9 million and 10 million kilometers from the planet, "the plasma can't hang on anymore—it streams off away from Jupiter." This flow, which Krimigis dubs the magnetospheric wind, may resemble the solar wind, a flow of particles away from the sun. By studying in detail the Jovian magnetospheric wind, Krimigis hopes to understand the physics that drives it and, by analogy, the solar wind and the winds emanating from many other stars.

While the two Voyagers and the two Pioneer spacecraft that preceded them have revealed much about the Jovian magnetosphere, many of the new findings are surprises or cannot be fathomed completely with available data. Scientists eagerly await the Galileo spacecraft, "may it fly," to test their speculations about how material from Io's volcanoes is distributed into the magnetosphere and to resolve the corotation controversy. Galileo is supposed to orbit Jupiter after it is launched in the mid-1980's by the problem-plagued Space Shuttle.

—BEVERLY KARPLUS HARTLINE

Gene Transfer Given a New Twist

The demonstration that genes introduced into bone marrow cells work in living mice is the most recent development in a series of advances in gene transfer.

Molecular biology has seen so many surprises in the past few years that the unexpected is becoming commonplace. Still, reports on television and in the newspapers that a "revolutionary" method of gene transfer had been discovered surprised many people, among them a number of scientists who are themselves studying methods of gene transfer between cells.

The reports described experiments in which a group of investigators* at the University of California at Los Angeles (UCLA) successfully introduced new genes into mouse cells and showed that the genes appeared to work when the cells were put back into living mice. Now this, in outline, is just what genetic engineering is all about.

Investigators have long sought a practical method of introducing new, functional genes into living organisms because such a technique might permit gene replacement therapy—a true cure for diseases, sickle-cell anemia, for example, which are caused by a single defective gene. And so the UCLA results were newsworthy.

Some news reports overstated their import, however, possibly aided by a press release from the university that hailed the techniques as "revolutionary," a word that subsequently appeared in the headline of a Washington *Post* story on the new developments. A more circumspect description was given by one researcher, who requested that he not be identified. "It is a nice experiment," he said, "but a logical extension of previous research." In other words, it fell something short of revolutionary.

In fact, investigators have been transferring genes between mammalian cells for years by a variety of techniques. Some of the earlier methods used cell fusion to produce hybrids bearing whole chromosomes or chromosome pieces from both cell types. More recently, investigators, with the aid of recombinant DNA technology, have devised more specific procedures for introducing individual genes into cells. During the past year and a half, for example, three groups of investigators reported the introduction of a globin gene from one animal species into cells from another. (Globin is the protein portion of the hemoglobin molecule.) In at least two of these cases, the transferred gene expressed itself in the synthesis of the appropriate globin protein.

The UCLA achievements grew out of this previous gene transfer work. According to Martin Cline, leader of the research group reporting the results, "We used established techniques for transferring the genes, but took them one step further—to an in vivo system."

In one series of experiments, described in the 3 April issue of *Nature*, the investigators induced resistance to the drug methotrexate in bone marrow cells by incubating them with DNA prepared from a line of methotrexate-resistant mouse cells. Methotrexate, which is used for cancer chemotherapy, kills cancer cells by inhibiting the enzyme dihydrofolate reductase (DHFR) and thus preventing the synthesis of the essential chemical folic acid.

Cancer cells may become resistant to methotrexate (or other cancer drugs, for that matter), in which case they are no longer killed by the drug, at least in concentrations that are tolerated by the rest of the body. Work from the laboratory of Robert Schimke, who is also at UCLA, has shown that methotrexate resistance is sometimes caused by amplification of the DHFR gene. Resistant cells, having many more copies of the gene than nonresistant ones, produce so much of the enzyme that they can live even in the presence of the drug.

The Cline group prepared DNA from methotrexate-resistant mouse cells having many extra copies of the DHFR gene. For the DNA preparation and subsequent gene transfer, they used meth-

*Martin Cline, Howard Stang, Karen Mercola, L. Morse, R. Ruprecht, Jeffrey Browne, and Winston Salser.

0036-8075/80/0425-0386$00.50/0

ods developed in the laboratory of Richard Axel, of Columbia University College of Physicians and Surgeons, that are themselves based on procedures devised by Silvia Bacchetti and Frank Graham of McMaster University in Hamilton, Ontario.

The DNA preparation was then incubated with bone marrow cells taken from a strain of mice whose cells all bear a distinctive and easily recognizable marker chromosome. During the incubation, some of the cells took up the DNA, a process called transformation. (This kind of transformation, simply meaning the acquisition of new genes, is different from the malignant transformation by which normal cells become cancerous.)

One of the major problems in gene transfer experiments is identifying the cells bearing the desired new gene. Drug resistance provides a solution to the problem because only cells with the resistance gene can multiply in the presence of the drug and, consequently, they can be selected out of a mixture of cells, most of which lack the resistance gene.

Usually selection is carried out on cells in culture. And it is here that the Cline group added a new twist to gene transfer because they injected the transformed bone marrow cells, together with an equal number of "mock transformed" cells, back into living mice for the selection procedure. Cells were mock transformed by incubating them with DNA not containing multiple DHFR genes; they did not carry the chromosome marker and so could be distinguished from the transformed variety. The mice, which were genetically and immunologically compatible with both types of injected cells, had been previously irradiated to destroy their own bone marrow.

To select for resistant bone marrow cells, Cline and his colleagues then treated the mice with methotrexate. This drug, like most of the others used for cancer therapy, may also kill normal cells, especially those, including bone marrow cells, that divide rapidly. The methotrexate dose was carefully chosen to depress the division of nonresistant bone marrow cells without being so high that it killed the mice.

Under this selective pressure, the bone marrow of the animals became enriched with cells bearing the marker chromosome—that is, with cells that had been transformed by exposure to DNA carrying multiple DHFR genes. This result indicates that the methotrexate-resistance genes entered the cells and were working there. In support of this hypothesis, Cline points out that cells taken from the animals had two to four times as much DHFR activity as cells taken from controls that had not received transformed cells. In addition, the blood status of methotrexate-treated mice that had been injected with transformed cells was better than that of drug-treated controls. For example, the former had a higher percentage of red cells in their blood than the latter.

All in all, the evidence strongly suggests that genes for methotrexate resistance were transferred into the bone marrow cells, but a direct demonstration of the presence there of the transferred genes was not possible. The DHFR genes obtained from methotrexate-resistant mouse cells are not distinguishable from those already present, although in smaller numbers, in all mouse cells, Cline explains.

For this reason, the UCLA workers undertook a second series of experiments in which they transferred a viral gene, the thymidine kinase gene of herpes simplex virus, into murine bone marrow cells. Because the viral DNA can be distinguished from that of mice, the investigators could detect the viral material in the transformed cells, thus obtaining direct evidence for gene transfer. In these experiments, as in the case of the DHFR gene transfer, the investigators selected for the cells bearing the viral thymidine kinase gene in living mice. A description of the thymidine kinase work is currently in press at *Science*.

Although the UCLA group has taken another step on the road to a practical method for genetic engineering, a number of barriers, some formidable, will have to be surmounted before there is a cure for sickle-cell anemia or any other genetic disease. "The principal obstacle," says Cline, "is the need to control the expression of the gene."

Simply transferring the gene and finding a way to select for the cells bearing it is not enough. The gene product must also be made only in the appropriate cells, at the right time, and in the correct amount. Bob Williamson of St. Mary's Hospital Medical School in London, writing in the News and Views section of the same issue of *Nature* in which the UCLA work was reported, says, "Expression in inappropriate tissue or abnormal control in the cell in which they [the transferred genes] are normally expressed could be disastrous." Unfortunately for genetic engineers, gene control in mammalian cells is one of the bigger mysteries remaining to molecular biologists.

One manipulation that might help to bring a transferred gene under appropriate control is to introduce it into the chromosome at the site where it normally resides. At present, there is no way to direct a gene to a specific chromosomal site or to ensure that it is integrated into a chromosome at all. It is even difficult to determine whether or not a transferred gene has been integrated, and this has not yet been accomplished for the DHFR and thymidine kinase genes transferred by the UCLA group.

"Expression in inappropriate tissue or abnormal control in the cell in which they [the transferred genes] are normally expressed could be disastrous."

Cure of genetic diseases is not the only potential application of genetic engineering, however. Cline suggests that the techniques developed at UCLA might be of value in cancer chemotherapy. The harmful effects on the bone marrow of many anticancer drugs often limit the treatment received by a cancer patient. Cline's idea is to remove bone marrow cells from a patient who is about to undergo chemotherapy. After they are transformed by genes conferring resistance to whatever drug is to be used, the cells can be given back to the patient. If these transformed cells establish themselves in the patient's bone marrow, as the methotrexate-resistant cells did in the mice, then the patient might be able to tolerate higher doses of the drug or a longer course of treatment with it than might otherwise be possible. Cline suggests that an application such as this might be tested in human patients within "3 to 5 years." Time, as they say, will tell.—JEAN L. MARX