Gene Injection Remedies Cell Defect

Gene-splicing and micromanipulation techniques have been combined to correct a genetic flaw in specific mouse cells. While any clinical application of the method remains remote, the experimental possibilities for examining action of selected genes in a mammalian cell excited scientists at a meeting last week at the University of Chicago's Comprehensive Sickle Cell Center. W. French Anderson of the National Institutes of Health reported that success of the gene transplant technique had been confirmed just days before.

Previous experiments with transplanted genes bathed cells in media containing viruses or rings of genetic material (plasmids) and then selected for further study those cells that took up new genetic material. In their recent experiments Anderson and collaborators instead injected genes from thin hollow needles directly into specific mouse cells growing in laboratory tissue culture. A microscope was used to guide the needle, called a micropipette, which is approximately 1 micron in diameter.

In several instances, Anderson now reports, a mouse cell not only accepted the foreign genetic material but, 30 cell generations later, the transplanted genes were still functioning in the original cell's almost one billion descendants. In one experiment mouse cells incorporated a viral gene that produces an enzyme the cells originally lacked. In another experiment the mouse cells were made to produce small amounts of a human blood protein.

Genes were prepared for the transplant with recombinant DNA methods. Each gene, with all its necessary regulatory stretches, was spliced into a ring of bacterial DNA. To maximize their chance of success, Anderson and collaborators injected about 20 copies of the foreign gene into each cell. Some of the transplanted genes were in closed DNA rings and others were in linear forms, which had been snipped open at one site by an enzyme. Many aspects of the procedure still need to be evaluated to determine the best method for future use. The investigators do not yet know, for instance, whether cut or uncut plasmids are most successful or whether the material is most effective when it is injected into the cell nucleus, near the nucleus or into the cytoplasm. "We went so long with everything negative," Anderson explained in a telephone interview. "Now it's a matter of sorting it all out."

"Clearly the gene we gave was replicating in the cell," Anderson says. The descendants make the product of the transplanted gene. Mouse cells deficient in the gene for the enzyme thymidine kinase were given a thymidine kinase gene taken from a herpes simplex virus. The recipient cells and their descendants make the essential enzyme and thus survive, and the enzyme they produce is of the viral form. Similarly, when mouse cells were injected with human beta-globin gene, one of a group of genes required for hemoglobin synthesis, the descendant cells made low levels of human, not mouse, globin.

Does the functioning, transplanted DNA insert itself into the mouse cell chromosomes or does it remain as an independent, replicating ring? The scientists are investigating that question with a probe molecule that binds to the human beta-globin gene. Preliminary results indicate that the transplanted genes either go into only one site in the mouse chromosomes or they remain as a plasmid. To distinguish between the two possibilities the scientists will attempt to "rescue" plasmids from the mammalian cells and move them back into bacteria. "If a bacterial plasmid can replicate as a plasmid in mammalian cells, it would be an extraordinary finding," Anderson says.

Anderson is not disturbed that the mouse cells containing human beta-globin gene only make low levels of the human protein. The cellular conditions within those cells are not optimal for expression of that gene, he says. The mouse cells he uses are from a line of undifferentiated cells (L cells) that do not normally manufacture hemoglobin. The next major research step will be to implant the human gene in mouse cells that do make blood proteins. Anderson plans to use mouse erythroleukemia cells, which grow in culture and can be induced to synthesize hemoglobin and turn red. He will attempt to "cure" genetically defective hemoglobin-forming cells by injecting the gene they lack.

The opportunity for studying gene control is the greatest immediate scientific significance of the recent achievement, Anderson says. For instance, scientists may soon be able to compare under identical conditions the operation of a gene transplanted into different types of mammalian cells or of different genes transplanted into the same cell type. In addition, researchers can alter the genetic material before implanting it into a recipient cell. To find the regions of DNA necessary to initiate gene expression, investigators could trim portions of the DNA until the gene no longer functions. Such research is expected to offer insights into how genes are turned on and off during development and later cell operation. This information would be necessary for the long-term goal of making genetic repairs in human cells, Anderson says.

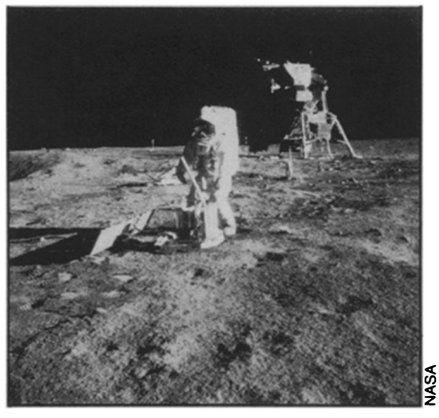
Collaborators in the research are Elaine Diacumakos of Rockefeller University and Lillian Killos, Linda Sanders-Haigh and Peter Kretschmer of NIH. □

Whereabouts of moonrocks called 'uncertain'

The pieces of the moon carried to earth by the astronauts of the Apollo program are often described as priceless resources, whether as tools of science or as symbols of a significant human endeavor. As such, they are stored in special facilities, constantly guarded, heavily documented, weighed sometimes to within millionths of a gram and subjected to elaborate security procedures when they are sent out for study or exhibition. Every trace is theoretically monitored wherever it goes, and scientists examining the material in laboratories away from the National Aeronautics and Space Administration's Johnson Space Center sign written agreements about storing the samples in locked safes and following other stringent guidelines.

And yet, charges a NASA audit report, "substantial quantities are unaccounted for or missing."

The report, prepared by the Office of Audit of NASA's Southwest Region, does not assert that some specific quantity of lunar material has disappeared from JSC. It does, however, allege numerous cases in which the documentation procedures at



NASA

the center's Lunar Curatorial Facility are inadequate to say for certain whether the material is missing or not. "A typical example," says the report, "was lunar sample 10084,19 weighing 100.2 grams transferred to a P.I. [principal investigator] on September 13, 1969. A report, dated April 11, 1977, showed that the P.I. had consumed 97.683 grams, 2.484 grams had either been returned to the Curator or

transferred to another P.I. and the balance of 0.33 grams still in possession of the P.I. There were no details as to how, for what, or when the 97.683 grams were consumed. There was also nothing to indicate why the P.I. chose to keep 0.33 grams of the sample in his possession for over nine years."

Other cited examples include cases where documentation was inconsistent, changed over time, or failed to properly indicate such problems as samples that never reached their intended recipients. Even the total cited weight of the whole moonrock collection has changed in various accounts, the report alleges, ranging from 384,900 grams (listed in a 1974 Lunar Sample Security Report as the weight "reported by astronauts from the lunar surface") to as little as 381,792 grams (from the Curator's records as of Nov. 13, 1978).

In a response to the report, NASA officials at JSC maintain that many of the cited uncertainties in fact stemmed from inventory and documentation procedures that were used early in the lunar sample program but which were substantially improved in 1972. Individual weighing tolerances, says the JSC response, add up to an overall uncertainty of only plus or minus 26 grams, "as well as an estimated possible total error from initial inventory and early processing of ±490 grams." Changes (due to various factors) in the total estimated sample weight, says JSC, have only made a difference of 0.07 percent from 1974 to 1978.

A letter written by the Southwest Region's director to NASA's newly installed Inspector General since the report came out, however, makes a stronger claim: that the samples whose status, as of the report, was in "the uncertain category" amounted to at least 11,279 grams (about 25 pounds), or 24.6 percent of the portion of the moonrock collection that, according to the letter, has ever been released for distribution.

NASA is now preparing additional response to the audit report's charges, as requested by Sen. William Proxmire (D-Wis.), chairman of the Senate Appropriations Subcommittee that funds the agency. But that is not necessarily going to be the end of the matter. The report covers numerous subjects besides the whereabouts of the moonrocks themselves, ranging from possible conflicts of interest within the panel that selects the sample investigators to free-spending junkets by the contractor employee who manages the curatorial facility. In addition, the report's author has claimed in a letter to Proxmire that previous audits were suppressed by a NASA official, and that "in the past few years both the Regional Director and I were severely harassed about findings put into reports," to the point where the report's author opted for early retirement. It is a thorny matter, with strong language in documents from both sides, and a hearing could conceivably result. For NASA, meanwhile, it's budget time. □

Nobel Prizes: Emphasis on applications

Medicine


Susan Lapides

Cormack


World Wide Photos

Hounsfield

Two researchers who have never met shared the Nobel Prize in Medicine for their work in computer-assisted tomography, a two-dimensional X-ray technique that allows physicians to visualize specific slices of the anatomy in great detail.

Allan M. Cormack of Tufts University in Medford, Mass., and Godfrey N. Hounsfield, an electronics engineer at the British company EMI, received the award for their contributions to what has been described as the biggest advance in medical technology since the invention of the X-ray machine.

The Nobel Committee said that Cormack was the first to "analyze from a theoretical standpoint the conditions for accurate X-ray pictures of entire biological systems." Hounsfield, the Committee said, "made the major contribution to introducing computer tomography into medicine by constructing the first practical system applied to general health care."

The award is unusual in that neither recipient has a doctoral degree in medicine or in any field of science.

Chemistry


WWP

Wittig


WWP

Brown

New vistas in the synthesis of organic chemicals were recognized in the choice for the 1979 Nobel Prize in Chemistry. Herbert C. Brown, now at Purdue University, and Georg Wittig, professor-emeritus at the University of Heidelberg in West Germany, shared the prize for work that has facilitated large-scale manufacture of biologically active materials and that has opened an area of research expected to take another generation of chemists to fully explore.

Brown's research has centered on the chemistry of boron, the element that sits next to carbon in the periodic table. Early in his career Brown, with H. I. Schlesinger, discovered compounds containing only boron and hydrogen, and later Brown discovered important uses for those reactive chemicals. In synthesis of a variety of organic compounds, for instance, he used the boron-hydrogen molecules to temporarily link complex organic molecules until the desired chemical bonds were formed. Boron compounds and related chemical agents are now used in manufacture of cortisone and other drugs as well as in a new class of pesticides. Brown also has investigated steric strain, a force dictated by the spatial arrangement of atoms in a molecule. Synthetic chemists had always considered the strain a hindrance, but Brown demonstrated that it can actually assist some reactions. Brown holds many patents important to synthetic chemistry and is currently a consultant to Exxon.

Wittig is the developer of a widely used step in chemical synthesis known as the "Wittig Reaction." It allows chemists to create almost any olefin — a straight chain hydrocarbon containing a double bond — with the double bond in a desired location. One practical application of this reaction is the synthesis of vitamin A. Wittig also has used phosphorus-containing compounds as a link for synthesizing a variety of complex molecules.

Physics

Glashow, Weinberg and Salam


WWP

The award of the 1979 Nobel Prize for Physics could easily serve as a commentary on the growing unity of physics. It is given for a major step toward the unification of physics into a single theory embodied in the work of the recipients, Steven Weinberg and Sheldon Glashow (who won the 1950 Westinghouse Science Talent Search contest) of Harvard University and Abdus Salam of the International Center for Theoretical Physics at Trieste. In a way it also reflects the rather clear experimental showing in favor of the theory and the