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.

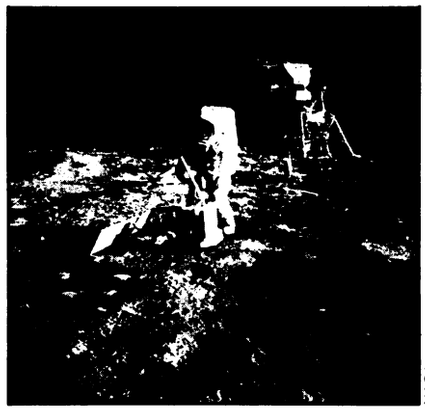
Collaborators in the research are Elaine Diacumakos of Rockefeller University and Lillian Killos, Linda Sanders-Haigh and Peter Kretschmer of NIH. □

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



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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

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