many other observations of the system. Furthermore, he adds, the uncouplers that Arnon says only transport protons across the membrane "have a lot of funny effects. It is more likely that they are inducing some kind of a bypass of plastoquinone." Dilley cites several other potential problems with Arnon's scheme. No one, he says, has ever been able to demonstrate reduction of ferredoxin by PSII in the absence of PSI—not even Arnon. Such a demonstration would be a convincing proof of Arnon's hypothesis. Furthermore, it has been shown by several investigators that tetramethylphenylene diamine can reverse inhibition of plastoquinone by DBMIB; that chemical, however, is a carrier of electrons rather than protons. And finally, DBMIB inhibition of plastoquinone blocks reduction of ferredoxin even when iodide ion is the source of electrons rather than water; when iodide ion is the electron source, no protons are generated, and Arnon's scheme suggests that ferredoxin reduction should not be affected. Were Arnon's data his, Good concludes, "I would say, 'Look boys, this observation doesn't fit the dogma. What do you make of it?' and leave it there."

Arnon is unfazed by the criticism: "Based on my past experience, I am not surprised by the skepticism with which the hypothesis has been received. It took years before we were able to get cyclic photophosphorylation accepted. The obstacles were formidable: the concept of cyclic electron transport flew in the face of theoretical arguments against the conversion of light energy into phosphate bond energy, and also challenged the then-entrenched view that mitochondria were the only organelles capable of ATP synthesis. There was even published evidence that chloroplasts could not form ATP unless mitochondria were present. When we discovered noncyclic photophosphorylation it was received with skepticism even by those who then accepted cyclic photophosphorylation. Likewise, our findings and concepts pertaining to ferredoxin have had tough sledding. In short, we have a long history of unpopular findings and theories that went counter to established doctrine. They've all met with great resistance, but have now become textbook facts." But despite Arnon's track record, says Dilley, "I would place my bet on the Z scheme at this time." Clearly, the last word on this subject has yet to be heard.—THOMAS H. MAUGH II

More Progress on Gene Transfer

Researchers appear to be on the verge of developing strains of animals that have functional foreign genes in their germ cells

The transfer of foreign genes into cultured cells, and their expression there, is becoming a common event (*Science*, 19 December 1980, p. 1334). In parallel with these in vitro experiments, investigators are also trying to introduce functional genes into the living animal, where the factors that control gene activities can be explored under more natural conditions. Ultimately they would like to produce strains of animals that carry foreign genes in their germ cells, the eggs and sperm. This would permit researchers to monitor the fate of an individual gene as a multicellular organism develops from a single fertilized egg, to see how a gene is turned on (or off) in the appropriate tissues during differentiation. Recent successes with egg injection experiments suggest that this goal may soon be achieved.

Recombinant DNA technology has made it possible to prepare purified genes in large enough quantities for injection into recently fertilized eggs (mouse eggs are generally used). As Beatrice Mintz of the Institute for Cancer Research in Philadelphia points out, "If you introduce recombinant DNA into the egg, you can get the DNA into all the the cells at once." Breeding the mice that develop from injected eggs would then provide large numbers of animals for use in studying the control of gene expression.

Mintz and her colleagues Erwin Wagner and Timothy Stewart now report* that a foreign gene, which was injected into fertilized mouse eggs, has produced a functional product in at least one of the embryos that subsequently developed. They injected a recombinant molecule (originally constructed by Tom Maniatis of Harvard University) consisting of bacterial plasmid DNA into which the thymidine kinase (*tk*) gene of herpes simplex virus (HSV) and the human β-globin gene had been inserted.

> According to Mintz, "One of the five animals was producing the herpes enzyme as well as its own . . . ."

Mintz, Wagner, and Stewart then implanted the injected eggs that survived without apparent damage in the oviducts of female mice which had been prepared for pregnancy by mating with vasectomized males. The investigators killed some of the females near the end of the gestation period and removed the individual fetuses. Each fetus and its placenta were separately analyzed for the two foreign genes and for the enzyme thymidine kinase, the product of the *tk* gene. There was not enough material to analyze for human β-globin.

"What we found was really very interesting," Mintz says. "An extremely high percentage—15 percent—of the developing mice had donor recombinant DNA. We found it in 5 animals out of 33, which is a very high take. Everyone of the five had both genes of interest in them. What is more important is that they had intact copies."

In similar experiments, Frank Ruddle and Jon Gordon of Yale University had previously shown that genetic material injected into mouse eggs would be retained in some of the animals that subsequently developed, but in rearranged form. The Mintz group also found evidence for rearranged viral *tk* and human β-globin genes in some of their animals.

Gordon and Ruddle have been continuing their egg injection work, but Gordon says, "We don't see intact genes yet." However, in a symposium last

*E. F. Wagner, T. A. Stewart, B. Mintz, *Proc. Natl. Acad. Sci. U.S.A.* (August 1981).

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March,† Karl Illmensee of the University of Geneva, Switzerland, reported that the human insulin gene appeared to remain intact in two mouse embryos that had developed from eggs injected with DNA carrying that gene. Illmensee's experiments were performed in collaboration with Axel Ullrich of Genentech, Incorporated, of San Francisco.

Mintz, Wagner, and Stewart found significant quantities of HSV thymidine kinase activity in one of the five fetuses that carried the intact *tk* gene. A second fetus may have had small amounts of the viral enzyme. According to Mintz, "One of the five animals was producing the herpes enzyme as well as its own. A second case is a pretty good candidate for gene function."

The presence of HSV thymidine kinase in one fetus was independently confirmed by an immunological assay performed in the laboratory of Saul Silverstein at Columbia University. Not enough tissue was available to perform this assay for the second fetus.

Showing that foreign DNA is maintained in a functional form in the animals that develop from injected eggs is a significant achievement, but it is only one requirement that must be met if investigators are to study gene control in living animals. The DNA must be incorporated into the host cell chromosomes and transmitted through the egg and sperm to future generations of animals. Breeding is the only way to generate sufficient numbers of animals carrying the foreign gene to do the desired developmental studies.

According to Mintz, the evidence, although not ironclad, strongly supports the idea that the viral *tk* and human β-globin genes were integrated in the mouse chromosomes. Meanwhile, Ruddle and Gordon have determined that injected DNA, even though it is rearranged, can be integrated and transmitted through the germ cells to a second generation of mice.

The Yale workers allowed some of the animals that developed from injected eggs to grow to adulthood and then surgically removed their spleens to test for the presence of the foreign DNA. Gordon says, "Some of the foreign DNA was retained without integration into the recipient cell DNA. But in three other animals there was clear evidence for integration; gene sequences of the host DNA were attached to the transferred gene."

† The symposium, Developmental Biology Using Purified Genes, was sponsored by ICN Pharmaceuticals and the University of California at Los Angeles and held in Keystone, Colorado, on 15 to 20 March.

Starship Capricorn

The starship Enterprise may have had a 5-year mission to explore strange new worlds, but little was ever said about the arrangements for sanitation and garbage disposal.

Hoping to help fill this gap in the literature, a team of scientists headed by Cornell University chemical engineer Michael L. Shuler has done a computer simulation study of regenerative life support systems for long space voyages. In their report to the Intersociety Conference on Environmental Systems, held on 15 July in San Francisco, they concluded that the systems should be optimized by including a pair of goats.

The simulation was for a 10-year mission with a crew of 24 people. Certain foods would be stored in dried form, but grains, fruits, and vegetables would be grown hydroponically. Inedible plant material and human wastes would be decomposed in aerobic bacterial digesters, which produce a nitrogen-rich liquid suitable for fertilizer in the hydroponic gardens and a sludge that could be stored in the emptied food bins.

The goats would fine-tune the system. Since they are ruminants "with very nonfastidious feed requirements," according to the study team, their digestive systems could do the initial decomposing of stems, stalks, and roots, thus making it faster and easier for bacteria to complete the job. They would also consume a small part of the sludge as a nitrogen supplement.

An additional advantage, according to the study team, is that the goats would reduce the need for stored food supplements by providing meat and "large quantities of milk per unit body weight."—M. MITCHELL WALDROP

One of the three animals was a female, and she has now been bred several times. Six of the ten progeny tested thus far carried the foreign DNA in the same form as the mother. Gordon concludes, "It appears that we have integrated material in the chromosome; it was transmitted to about half the progeny, which is about what you would expect from normal patterns of inheritance."

The Mintz group also has adult mice that developed from some of their injected eggs. The results of studies of these animals are not yet available.

Why intact genes are transferred in some cases but not in others is not known. Ruddle and Gordon had originally hypothesized that maintenance of an intact viral *tk* gene and its expression might prove harmful to normal development, in which case they might never see the viral enzyme in any of the mice that survived the fetal period. Gordon explains, "The *tk* gene product generates some of the subunits for making DNA. If you alter the activity of enzymes crucial for this pathway you could disrupt development."

The Yale workers examined only newborn or older mice whereas the Mintz group and Illmensee and Ullrich studied fetuses, which might have eventually succumbed to the deleterious effects of viral thymidine kinase production. Militating against this possibility, however, is the fact that all the fetuses studied by the Mintz group appeared normal just before the end of gestation, when many cell divisions had already occurred. Mintz notes, incidentally, that the total amount of thymidine kinase activity in the mouse fetus that was producing both viral and murine enzyme was the same as in the other fetuses. This suggests that the animal may have been regulating the two genes in a coordinate fashion with the result that the total enzyme activity was not excessive.

A second, and perhaps more likely, explanation of why the integrity of transferred genes was maintained in some cases but not others is that plasmid composition, which differed among the three laboratories, might influence the manner in which DNA is maintained in the cell. Not much information is available on how the plasmid might affect gene transfer, although there is preliminary evidence that some plasmid sequences are not tolerated by the cell. As a result they may have to be rearranged if they are to be retained at all. Other DNA carried by the plasmid might be altered at the same time. More work is clearly required to clarify the role of the plasmid.

In any event, all the steps necessary for studying gene expression in the living animal have now been taken, even if not in the same laboratory. It should be only a matter of time before investigators can breed mice that carry functional foreign genes.—JEAN L. MARX

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