Genetic Sabotage in the Public Interest

Bioengineers construct finicky, debilitated organisms to make 'gene-grafting' safer

BY JANET L. HOPSON

It was only supposed to take them six weeks. That's what the molecular geneticists said at Asilomar, anyway. They were going to go back to their laboratories and in six weeks sabotage the emergency preparedness programs that took nature more than three billion years to build into bacteria. Their purpose was to build safety into a brand-new field of research known as recombinant genetic engineering.

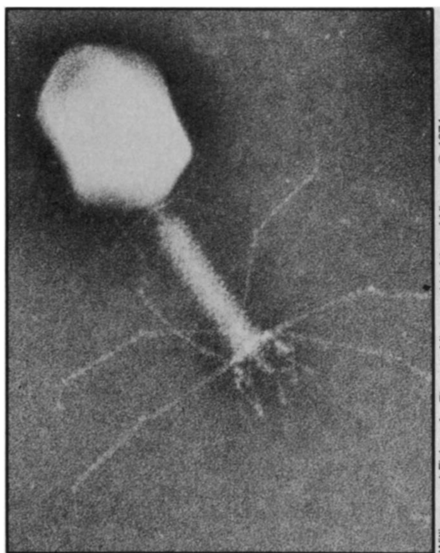
Science reporters, it seems, were the only ones to take that six-week figure seriously, though. "The tendency with experiments, very often, is to say, 'it's a piece of cake,' " one biologist said recently. "But most of us have been around long enough that if someone tells us it's gonna take 6 weeks, we multiply by a factor of 5 or 10." All right, 6 weeks times . . . lets say 8.5, brings us to just about now, one year after Asilomar. Exactly what, then, has been accomplished in that intervening time?

Biologists, the answer turns out, have not only carried out the biggest debilitation exercise in genetic history, but they did it right on schedule. And without cost overruns. A neat piece of targeted research, all in all.

"Asilomar," to backtrack for a moment, is shorthand for the International Conference on Recombinant DNA Molecules. Last February, about 150 biologists—mostly molecular geneticists—flew from different parts of the world to a sunny California state beach resort called Asilomar. There, with sufficient isolation and a naturally dramatic setting, they made scientific history by deciding to control research in a new field before it even started (SN: 3/8/75, p. 148).

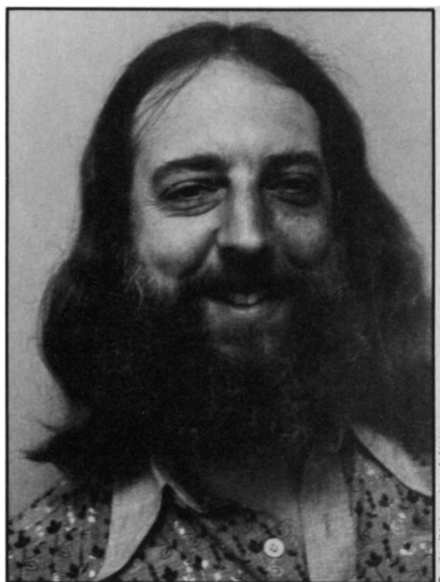
About five years ago, molecular biologists discovered a class of enzymes called restriction enzymes. Certain of these suddenly made it fairly easy to "graft" genes—to excise specific genes from an animal's DNA, splice it into a carrier molecule, send them both into a host organism, clone a batch of these hosts, then pick out the "recombinant" hosts with the new foreign genes. Genes from rabbits, toads, fruit flies and bacteria have thus far been spliced into other bacteria.

The potentials for these techniques were instantly recognized. Scientists had found not only a powerful tool for exploring the inner geography of minute organisms and life mechanisms on the molecular level, but also the means to a number of desirable ends. Corn and other crops could be fitted with nitrogen-fixing genes. Bacteria could be made to produce insulin or other drugs cheaply and easily. Genetic misprogrammings like cancer and diabetes might some day be correctable.



Williams and Fisher, An Electron Micrographic Atlas of Viruses, © 1974

E. coli bacteriophage: Packaged DNA.



Curtiss/University of Alabama

Curtiss: Building a sickly E. coli K-12.

But if these unnatural recombinants escaped from the laboratory, particularly during the experimental stages, they might tuck into some econiche where naturally evolved control mechanisms couldn't touch them. And the plasmids (small circular genetic elements) sometimes used to carry foreign genes into hosts often confer antibiotic resistance. These circular bits of DNA are notoriously promiscuous and could, if things got out of hand, hop into the wrong cells and transfer to them both antibiotic resistance and foreign genes. And besides all this, bacteria would just as happily grow diptheria toxin as insulin (although no one has yet persuaded them to grow either one) if an unscrupulous scientist were so inclined to use the technique. It was to discuss these risks and benefits and possible means of regulation that the Asilomar conference was convened.

On the political front, the assembled scientists agreed to regulate each other's experiments with voluntary guidelines. They have by now spent the better part of a year getting the specifics down on paper (SN: 12/13/75, p. 372). An integral part of those guidelines, however, created a challenge of a different sort on the scientific front: the development of new techniques designed to keep genetically recombined organisms in the laboratory and away from those niches they might fill with unpredictable consequences.

Many of the experiments, they decided, would have to be done with physical containment equipment—glove boxes, ventilation hoods, negative air pressure—to prevent the escape of novel organisms and to protect laboratory workers. But, a few "bioengineers" told the others at Asilomar, if the normal survival programming of these experimental organisms were genetically altered, scientists could have "biological containment" as well. They could have bacteria, plasmids and viruses as debilitated and dependent as toy Pekinese. The former, theoretically, would provide safety rather than amusement, but would be no less an inversion of nature's survival ethic.

The organisms used most often to carry and receive grafted genes—*Escherichia coli* K-12, bacterial viruses, plasmids—are about as stripped down a collection

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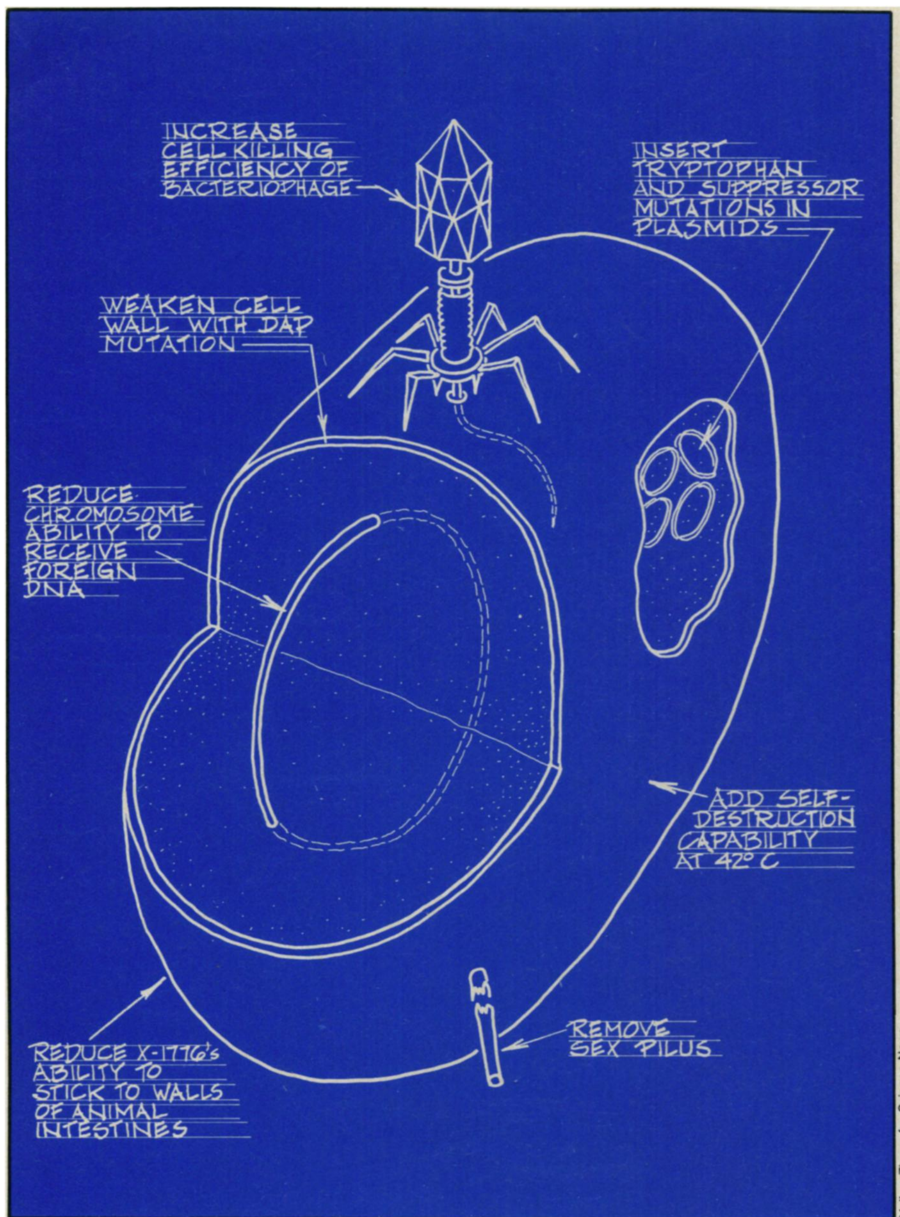

Volker Zinser for Science News

Genetic blueprint: 'Specifications' for safer E. coli, virus and plasmid construction.

of vehicles as one could find. In an attempt to list bottom line life criteria (how does one tell a living thing from a rock?), biologists have focused on simple, one-celled organisms such as the intestinal resident *E. coli.* This lowest common denominator respires, assimilates nutrients, excretes, grows, reproduces and reacts to its surroundings as surely as a red tailed hawk or a sea otter.

Viruses and plasmids, on the other hand, strain that life definition. They are clearly negentropic (expend energy to keep themselves in order) but are essentially just packaged DNA—molecular parasites that need a host cell for survival (SN: 6/21/75, p. 404).

While evolution has not dealt these organisms swift legs or protective coloration, it has made them survivors nonetheless, through two billion years of environmental testing. Even with such abbreviated genetic messages as one finds in *E. coli* cells or virus particles, space has been made on their circular chromosomes for a tangle of survival genes. Each organism has a series of fail-safe survival systems that allow it to withstand environmental stresses and to ensure the successful passage of its DNA through time. There are, in fact, so many gene-coded biochemical systems backed up by alternative systems, that, in the words of one researcher, "*E. coli* makes NASA look sick!"

That enteric bacterium, the modern biologist's basic off-the-shelf laboratory animal, or the common soil bacterium *Bacillus subtilis,* can induce or repress literally dozens of enzyme systems in response to environmental cues. They can grow or stop growing. Differentiate or form endospores. Mate sexually or divide vegetatively. Use lactose or 100 other substrates, depending on external conditions and the chance each alternative affords for survival.

Viruses such as bacteriophage lambda, or plasmids such as "ColE-1," are metabolically inert semilife forms, which concentrate their survival energies on reproduction. Their genes and back-up genes ensure the ability to infect host cells and merge with the host's genome.

All these fail-safe systems, from the organism's viewpoint, are defenses against a hostile world in which only one of several will survive for as long as five minutes. But for the molecular biologist, these systems are armaments against safe containment. Several researchers, therefore, in the year since Asilomar, have learned how to disarm the recombinant hosts and vectors genetically, and how to give them finicky appetites that can only be satisfied on a laboratory diet.

Roy Curtiss III, a lanky, long-haired microbiologist from the University of Alabama Medical Center at Birmingham, has had probably the greatest success thus far at disarming the *E. coli* K-12 strain. He and 10 students worked overtime for months to construct what Curtiss calls "X-1776." (Curtiss names all his organisms X [chi] followed by some number or letter, in honor of his days at the University of Chicago.)

By growing bacteria, then isolating, characterizing and selecting the individuals with the desired mutations, Curtiss managed to sculpture X-1776 to fit a predetermined set of safety criteria. Five mutations now make it more useful for recombinant research than normal *E. coli* K-12. Two mutations make it nearly impossible for the bacterium to colonize the intestinal tracts of animals. It cannot manufacture its own cell wall unless supplied with biochemicals it is unlikely to encounter in nature. It is temperature sensitive and will self-destruct at moderately high (42°C) temperatures. It has a reduced ability to receive or transmit foreign DNA. And it is unable to exchange genetic material (conjugate) with most other bacteria or be infected by most bacterial viruses.

X-1776 is so sickly and dependent that if it were somehow to escape the laboratory, its chances of surviving would be just one in a billion. Curtiss will send reports on the construction of X-1776 to a journal within a few weeks, he says, and to his fellow members of the National Institutes of Health Advisory Committee on Recombinant DNA Molecules. The committee will review the report to determine whether X-1776 is an "EK-2" host, according to the post-Asilomar safety guidelines (SN: 12/13/75, p. 372). Many experiments cannot proceed without EK-2 hosts and vectors, and researchers have waited impatiently for their construction and approval. "I have been getting several calls a week for a year," Curtiss says, "from scientists who want the safer *E. coli.*"

Safer vectors—viruses and plasmids that carry foreign DNA into host cells—are under construction, too. Donald Helinski of the University of California at San

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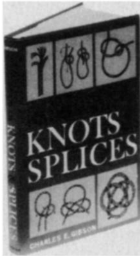

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Diego, Herbert Boyer of UC San Francisco and Charles Yanofsky of Stanford, among others, are working with plasmids.

Helinski combined Curtiss's X-1776 with two plasmids called "ColE1-kan" and "ColE1-trp." The former confers resistance to the seldom-used antibiotic kanomycin. This "marker" makes it possible to weed out the X-1776 cells that have picked up foreign genes during cloning. Cultures are treated with kanomycin after gene insertion and cloning. Those that have taken up ColE1-kan and the attached foreign gene do not die off and can thus be recognized. ColE1-trp is probably safer, though, Helinski says, because it does not make X-1776 antibiotic resistant. It is, rather, a "nutritional marker." Only X-1776 cells containing the ColE1-trp plasmid (and attached foreign gene) can grow on culture plates that lack the amino acid tryptophan, and, thus, they can be recognized.

But neither of these plasmids is itself a disabled vector. "We hope to have one within this year," Helinski told SCIENCE NEWS. Ideally, it would have four mutations—"trp," a "temperature sensitivity" mutation so it stops replicating when inside warmblooded animals, and two "suppressor" mutations. These would prevent the plasmid from replicating itself inside any host cell but X-1776, minimizing the danger that the plasmid could carry a foreign gene into a new cell and there make more copies of itself.

Other researchers are disarming viruses. Frederick R. Blattner and Bill G. Williams at the University of Wisconsin at Madison have engineered a series of bacteriophages (bacterial viruses) they call, appropriately, "Charon phages." (Charon, in Greek mythology, ferried the souls of the dead safely across the river Styx.) Charon 3 and 4, both mutations of bacteriophage lambda, can hold large pieces of foreign DNA, and the success of the foreign gene splicing can be monitored with dye indicator plates.

One potential worry over phage vectors is that when they infect bacterial cells (a necessary step in the cloning procedure) they will "lysogenize" or become part of a cell's chromosome, rather than killing the cell. This "lysogen" could then survive and possibly transfer its newly incorporated foreign gene to an unwitting animal host with unforeseeable consequences. The Charon 3 and 4 phages have been engineered to kill cells so efficiently that only one surviving cell with the foreign gene can be found during the production of 1,000 billion phages. This is 1,000 times more deadly than the NIH guidelines require of an EK-2 vector. An additional safety feature, Williams explains, is that a large number of phage particles can be grown in a small volume of culture medium. Thus less spillable liquid must be handled. The team will submit a report on the Charon phages to SCIENCE within a few days, Williams says.

Some recombinant researchers are quite opposed to the idea of using a human pathogen such as *E. coli* and its plasmids and viruses for experiments. During a workshop on the design and testing of safer biological systems at La Jolla, Calif., last November, several biologists lobbied for substitute cells. Frank Young, an amiable microbiologist from the University of Rochester in New York, stood up during the meeting and pulled off his green sweater. Underneath, emblazoned on a yellow T-shirt, was a chromosome map of *Bacillus subtilis,* Young's personal favorite. *B. subtilis,* he explained, is generally nonpathogenic and, unlike *E. coli,* does not reside in the human gut. "It likes rotten hay and dirt better," he said. Others at La Jolla advocated various *Bacillus* and nonpathogenic *Pseudomonas* species.

"But," Curtiss says, "the particular strain of *E. coli* we are using, *E. coli K-12,* has become something of a hot-house organism. We've used it in the laboratory for so many years that it really likes laboratories better than nature." And, University of Washington microbiologist Stanley Falkow adds, "*E. coli* K-12 does not successfully colonize the normal intestine of man, pigs, calves or mice." Even when infected with certain plasmids that give the bacterium the ability to adhere to the small intestine and to form diarrheal toxins, Falkow says, *E. coli* K-12 doesn't survive for long in the gut.

"And besides all this," Fred Blattner says, "*E. coli* is the best known organism on the planet. We know far more about the genetics and ecology of *E. coli* K-12 than about *Pseudomonas* or *B. subtilis.* It just makes better sense to use something you understand. The really exciting thing," Blattner says, "is that we know so much about the bacteriophages and *E. coli* that it was possible for us to sit down and predict, 'If we put in this gene and that, we should be able to construct organisms that are 10 to the X power safer.' And that's exactly what happened."

This year-long exercise in survival sabotage has been successful as far as targeted biology goes. The big promises of genetic manipulation—medicines, super crops, cures, basic understanding—cannot be realized without these safer constructs, and their approval as EK-2 systems is under way.

But, unlike, say, the Viking Lander speeding toward Mars, these organisms are headed for an amorphous target—safety. And bioengineers, unlike space engineers, will never know precisely when they hit that target. "The construction of safer hosts and vectors has really just started," Curtiss says, "and these systems will have to be continually improved and retested. The more we use them, though, the more confident I think we will all be that recombinant experiments can be done safely." □