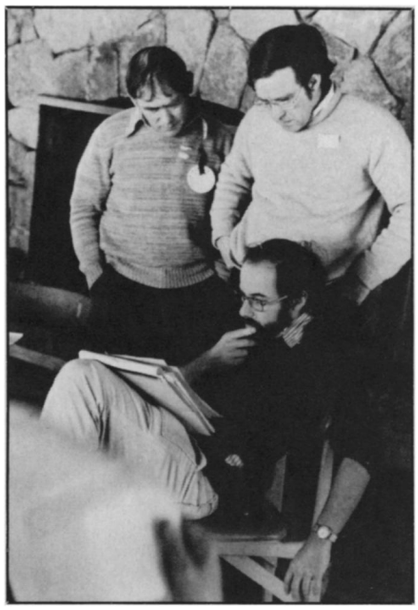
Asilomar decision: Unprecedented guidelines for gene-transplant research

SN Chemistry and Biology Editor Janet H. Weinberg attended last week's long-awaited Asilomar gene conference on the dangers of recombinant DNA *and wrote this news report. A subsequent article will explore the decision-making processes that led to the international group's recommendations.*

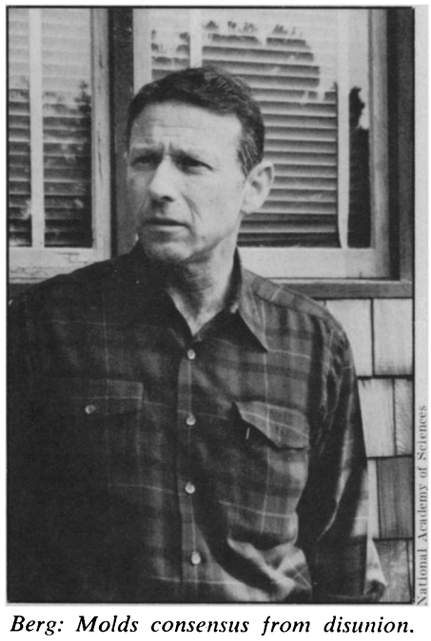
For the first time, scientists in a new and promising field have agreed to regulate and in some cases restrict their own basic investigations. Although new techniques of genetic manipulation offer great potential for basic and applied research, they also pose biological hazards. These hazards, the scientists decided, can be minimized only by self-regulation, even though such a step will restrict academic freedom and slow progress. This quiet piece of history took place last week as molecular biologists from 17 countries met in a secluded resort in Pacific Grove, Calif., to try to chart a safe course for the expanding field of genetic engineering.

Sophisticated biochemical techniques have been developed in the past year that enable scientists to recombine the genes of totally unrelated organisms and to essentially create new life forms. Such recombined organisms, outside the path of normal evolution, might also be outside the realm of natural control. Bacteria can be installed with new genes that make them resistant to antibiotics, or that can cause cancer. Tiny unknown sequences of DNA can be taken from a bacterial or animal cell, and when recombined with host cell DNA, code for unexpected and perhaps uncontrollable characteristics. Recognizing these possibilities, a prominent group of American biologists called last July for the deferment of certain types of potentially hazardous experiments (SN: 7/27/74, p. 52). The deferment was to last until this international meeting, sponsored by the National Academy of Sciences, could be held to devise a blueprint for the conduct of future research.

The principle of recombining DNA molecules is fairly simple. Special enzymes are used to excise portions of DNA from a bacterium, plant or animal, and to insert the new portions into the genetic code of living "vehicles." These are usually viruses, such as the bacterial virus phage lambda, or small rings of bacterial DNA called plasmids. The vehicle inserts its new message into the genetic complement of another cell, and that cell can start expressing the characteristic coded for by the gene.



Conference organizers draft proposal.



Berg: Molds consensus from disunion.

Members of the international conference on recombinant DNA molecules, including 86 scientists from the United States and 53 from other countries, decided after four difficult days of deliberation to end their voluntary deferral of certain types of experiments. They also decided, however, that some experiments should not be done until better containment techniques can be developed, and that some very risky experiments should not be done at all, under even the highest containment.

The original American group, headed by Paul Berg of Stanford University, called last summer for the deferral of experiments in two areas and the use of caution in a third: One, the introduction of genes for antibiotic resistance or toxin formation into bacterial plasmids without these characteristics; two, the linking of animal viruses, including cancer viruses, to recombinant vehicles; three, the linking of animal DNA to bacterial viruses or plasmids.

Working committees at the Asilomar conference (Asilomar is the name of the conference center at Pacific Grove) studied each of these types of experiments and considered the potential risks, benefits and available methods of control. They established a hierarchy of risks within each class of experiments and decided the type of containment commensurate with each level of risk. Containment includes physical barriers such as specially equipped laboratories, biological barriers in the form of organisms that can be easily controlled, the employment of good microbiological techniques and the proper training of personnel.

The conference members, by majority vote, agreed that the experiments can be divided into low-, moderate- and high-risk categories and should have corresponding containment. Berg explains that many type one, two and three experiments are now designated low risk, and can be resumed in normally equipped laboratories. The conference members refused to list specific experiments that would fall under each designation, preferring instead to leave such assignment up to the individual investigator. In general, however, genes for certain types of antibiotic resistance that occur in nature and controllable viruses can be inserted under low-risk containment.

Moderate-risk experiments should not be done until safer experimental organisms are developed, the conference decided, and until the interested investigator's laboratory has undergone some expensive modifications. These modifications include addition of biological safety cabinets and negative air pressure and could cost upwards of $40,000,

Berg says. Safer organisms are being developed but are not yet available, so moderate-risk exeriments probably will have to be deferred for at least several months. Many experiments using DNA from animal and plant viruses would require moderate containment.

A few experiments, designated high risk, can proceed only in very elaborate, high-containment facilities, only six of which now exist in the United States. The work is done in biological safety cabinets, in a room with negative pressure and air locks, and the personnel must be decontaminated when leaving. High-risk experiments such as those employing cancer-producing viruses or inserting genes for lethal toxins into widely found bacteria will by the nature of the containment requirements proceed very slowly, Berg says. This slowdown will be a built-in safety factor. Most of the conference members agreed that combining toxin genes with common bacteria, especially for biological warfare, would be morally indefensible under any containment regime.

The conference was not intended to set up specific guidelines with binding enforcement, Berg says, but rather to establish a moral climate. The general categories of risk and containment were designed to help each individual country set up its own guidelines and to help investigators weigh the risks and safeguards needed for their own experiments. The outstanding researchers from the field of genetic engineering were invited and their prominence and authority will add considerable moral force to the Asilomar guidelines.

Self-regulation by peer pressure, however, will not prevent scientific agencies from setting up more specific standards. An advisory committee of the National Institutes of Health met the day after the conference to guide NIH in the publication of more specific containment criteria. Researchers probably will have to include certification of their laboratory's containment level with requests for funds, and granting agencies will consider the risks and containment before awarding funds. In England, the Medical Research Council will reconsider laws passed there last summer prohibiting many type one, two and three experiments. In addition to the Asilomar proposal, the MRC will evaluate recommendations in the Ashby report, issued by a British study committee on genetic engineering in December.

Several Asilomar conference attendees from abroad indicated that comparable agencies in their countries will consider the guidelines. A delegation of Soviet biologists, including perhaps the Soviet Union's most influential scientist, W. A. Englehardt of the Institute of Molecular Biology, observed the conference proceedings silently. Afterwards, A. A. Bayev of the same institute called the provisional statement "reasonable" and "acceptable" and said it would be useful for discussions in the Soviet Union.

One rationale for keeping the guidelines general was expressed by Stanford molecular biologists Stanley Cohen and Nobel laureate Joshua Lederberg. They worry that any rules might be crystallized into law by legislators eyeing the field with distrust. But despite the desire for academic freedom, British bacteriologist Mark H. Richmond of the University of Bristol says, scientists are realizing that they must answer to society for exposing innocents to the chance of "non-trival" risks. The conference marks, Richmond says, "the end of the age of innocence" for basic research and the growing acceptance of less-than-complete academic freedom.

Discussions at Asilomar were not limited to potential catastrophes and uncomfortable regulations. Genetic recombination offers a powerful research and development tool. Judging from the long scientific sessions in apparent oblivion to the physical beauty of the Monterey peninsula, the new tool can clearly become a passion. "Problems people were interested in 100 years ago are starting to be tractable now with these methods," says Sydney Brenner, from the MRC in Cambridge. For example, the complete sequencing of genes is "just around the corner," and with it, an understanding of gene function, regulation and expression.

Besides basic research, the technique looks promising for medicine, agriculture and industry. One of the handiest features of recombination is what scientists call "amplification." One can, for example, snip out a segment of DNA, insert it into a plasmid molecule, then grow large quantities of this vector and the new gene. Brenner calls this "a multiple Xerox machine, stamping out multiple copies of the blueprints." After the vector is sent to deliver its molecular message, the receptor cell and the gene product can be grown in quantity. If the gene product happens to be a protein like insulin, one has a microbial drug machine. This amplification tech-

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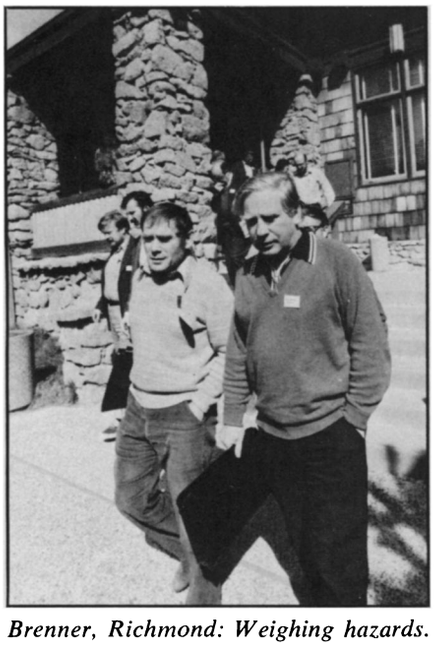
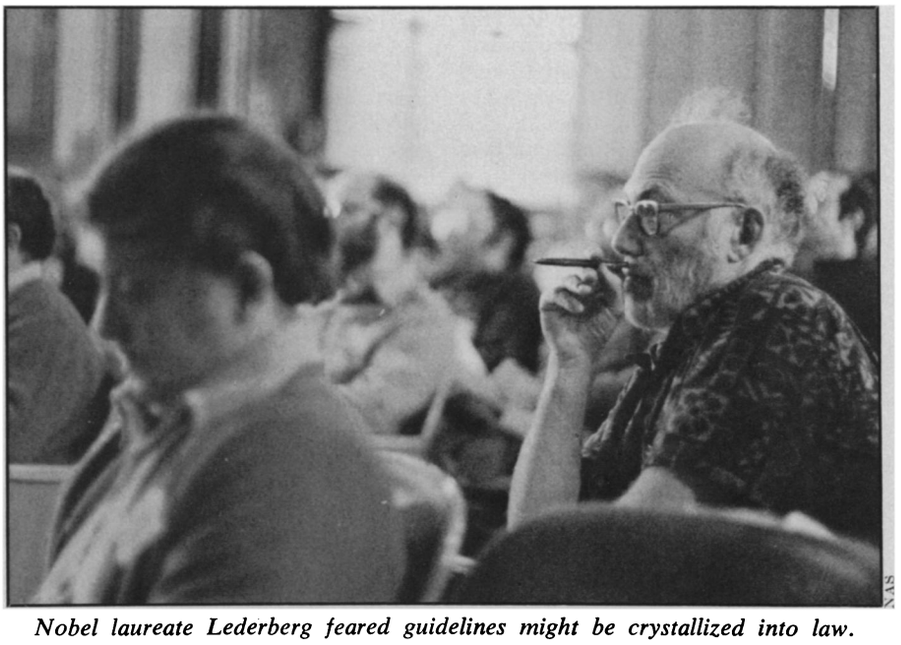
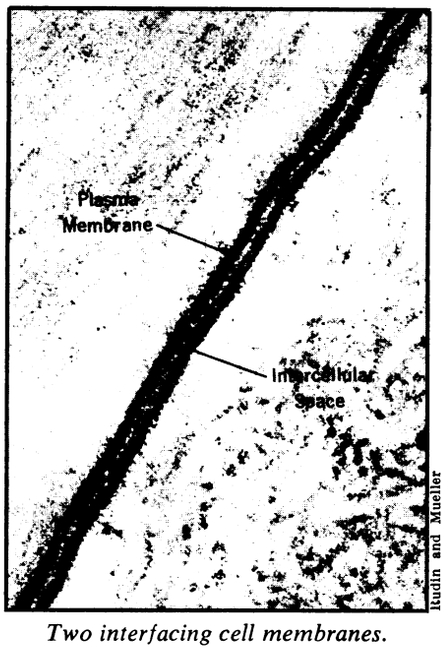
Gene researchers from around the world have replaced a self-imposed ban on their work with guidelines to reduce experimental risks



Brenner, Richmond: Weighing hazards.



Nobel laureate Lederberg feared guidelines might be crystallized into law.



Two interfacing cell membranes.

they found another protein, protamine, that charged the alamethicin channel, thus permitting only chloride ions to pass through the membrane. Thus they incorporated both alamethicin and protamine-alamethicin channels into the membrane, and the membrane had two kinds of ion gates.

Now the artificial membrane was, for all practical purposes, a synthetic membrane, both in structure and in function. What's more, it could produce nerve impulses indistinguishable from those of living nerve cells.

Rudin, Mueller and their co-workers have used their synthetic membrane to confirm the well-established theory of the nerve impulse, proposed by Nobel laureates Alan L. Hodgkin and Andrew F. Huxley of Cambridge University. The theory states that the nerve impulse—action potential—consists of the transport of ions of opposite charges through the nerve cell membrane, and this transport is controlled by electric current.

Ordinarily there are more positive potassium ions inside a cell than outside. Thus the potassium ions tend to leak through the membrane. This leakage of positive potassium ions produces the resting potential of the membrane. The same situation exists for the synthetic membrane of the Philadelphia team. Then the team applied current to their membrane. The current caused the faster chloride channel in the membrane to open. Negative chloride ions roared through the channel and outside the membrane. Now the outside of the membrane was more negative than positive, producing the peak of the nerve impulse (action potential). But the excess negative ions now lured more positive potassium ions out of the membrane, thus returning the membrane to its resting potential, and a closing of the ion gates.

"So," Rudin says, "we have synthesized nerve cell firing in our synthetic membrane. The firings can beat spontaneously like heart beats or we can control the frequency of the firings."

Only recently have the Philadelphia molecular biologists started looking at the effects of drugs on their synthetic membranes. They have found that the local anesthetic procaine blocks the nerve impulse in a synthetic membrane by blocking the chloride channel. On the other hand, one of the major tranquillizers used to treat schizophrenia—chloropromazine—reduces the nerve impulse in the membrane by blocking the potassium channel. Such action may explain how the drug helps schizophrenics. Nerve impulses in the brain lead to the production of the nerve transmitter dopamine, and an excess of dopamine, the result of a genetic defect, has been linked to schizophrenia.

Such discoveries, Rudin, Mueller and their colleagues believe, represent a first step toward molecular pharmacology—understanding how drugs work at the molecular level, and thus tailoring drug therapy accordingly.

The molecular biologists suspect that one or several ion channels in the membranes of the brains of schizophrenics may be faulty and hence trigger or at least exacerbate the disease. Thus they hope to use a synthetic membrane to probe for such defects. They would isolate and purify some of the ion channels in the membranes of the brains of schizophrenics. They would insert the channels into their synthetic membrane to see whether the channels work normally or not. "Extracting and purifying such channels will be tough business, though," Rudin admits. "To do so we'll have to devise some new techniques in lipid phase biochemistry."

The Philadelphia molecular biologists are also interested in synthesizing membranes that reside inside the cell instead of around it. These membranes include the nuclear membrane (the membrane that surrounds the nucleus), the mitochondrial membrane (the membrane that surrounds the mitochondrion) and the endoplasmic reticulum (a tubular membrane network that contains ribosomes, on which proteins are packaged).

On the whole, the Philadelphia biologists' research is helping set the stage for two new fields—molecular pharmacology and molecular psychiatry. It is also helping set the stage for a new era—biological synthesis. "The age of biological synthesis is in its infancy, but it is clearly discernible," James Danielli, a theoretical biologist with the State University of New York, has declared. □

. . . Gene Conference

nique allows more time for experiments and less time doing tedious extractions or slowly assembling individual nucleotides or amino acids.

Researchers plan to use recombinant DNA to modify normal organisms and correct defective ones. Plant physiologists envision the day when staple food crops can be fitted with all of the most efficient plant equipment—genes for fixing nitrogen, for resisting diseases, for producing essential amino acids and for stepped-up carbohydrate production. And medical researchers would like someday to correct genetic errors that cause human and animal diseases by excising defective genes and inserting operative ones.

Perhaps the most immediate application, and a very appropriate one, is the development of a safety vector. Brenner, like a fast-talking salesman, dazzles the ear with descriptions of recombinant vehicles with "self-destruction capability" and the "built-in assurance of add-on safety devices." Brenner and others are developing safe vectors now. Those planning moderate- and high-risk experiments are cheering from the sidelines, since they cannot proceed under the guidelines without such vectors.

Brenner says the danger of an unnatural, recombined organism escaping the laboratory (with unforeseen consequences) can be slashed to perhaps one chance in 10^{60} if enough safety devices are added to make it impossible for the organism to live outside of highly artificial conditions. One could, for example, take a bacterium that has lost the ability, through mutation or genetic manipulation, to manufacture an amino acid essential to the growth and repair of its cell wall. The amino acid would be available only if provided in the culture medium. If such an organism were to escape containment, it would "self destruct" since the researcher would no longer be providing the missing amino acid. One such safety feature might reduce the chances of escape to one in a million, Brenner says, so adding 10 features would make the chances incredibly remote.

Commenting on the conference, Berg voiced pleasure that guidelines were reached. It appeared up until the last session that some members might not agree to the general guidelines or even to the need for any self-regulation. Berg feared that such a position might appear self-serving to legislators and bring swift controls upon the research. A by-product as beneficial as the guidelines, he says, was that the conference "raised the level of discussion in the field." No one will go into this field without thinking about the risks and benefits "and I couldn't have said that eight months ago." □