Recombinant DNA: Fact and Fiction

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Almost 3 years ago, I joined with a group of scientific colleagues in publicly calling attention to possible biohazards of certain kinds of experiments that could be carried out with newly developed techniques for the propagation of genes from diverse sources in bacteria (*1*). Because of the newness and relative simplicity of these techniques (*2*), we were concerned that experiments involving certain genetic combinations that seemed to us to be hazardous might be performed before adequate consideration had been given to the potential dangers. Contrary to what was believed by many observers, our concerns pertained to a few very specific types of experiments that could be carried out with the new techniques, not to the techniques themselves.

Guidelines have long been available to protect laboratory workers and the general public against known hazards associated with the handling of certain chemicals, radioisotopes, and pathogenic microorganisms; but because of the newness of recombinant DNA techniques, no guidelines were yet available for this research. My colleagues and I wanted to be sure that these new techniques would not be used, for example, for the construction of streptococci or pneumococci resistant to penicillin, or for the creation of *Escherichia coli* capable of synthesizing botulinum toxin or diphtheria toxin. We asked that these experiments not be done, and also called for deferral of construction of bacterial recombinants containing tumor virus genes until the implications of such experiments could be given further consideration.

During the past 2 years, much fiction has been written about "recombinant DNA research." What began as an act of responsibility by scientists, including a number of those involved in the development of the new techniques, has become the breeding ground for a horde of publicists—most poorly informed, some well-meaning, some self-serving. In this article I attempt to inject some relevant facts into the extensive public discussion of recombinant DNA research.

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Some Basic Information

Recombinant DNA research is not a single entity, but rather it is a group of techniques that can be used for a wide variety of experiments. Much confusion has resulted from a lack of understanding of this point by many who have written about the subject. Recombinant DNA techniques, like chemicals on a shelf, are neither good nor bad per se. Certain experiments that can be done with these techniques are likely to be hazardous (just as certain experiments done with combinations of chemicals taken from the shelf will be hazardous), and there is universal agreement that such recombinant DNA experiments should not be done. Other experiments in which the very same techniques are used—such as taking apart a DNA molecule and putting segments of it back together again—are without conceivable hazard, and anyone who has looked into the matter has concluded that these experiments can be done without concern.

Then, there is the area "in between." For many experiments, there is no evidence of biohazard, but there is also no certainty that there is not a hazard. For these experiments, guidelines have been developed in an attempt to match a level of containment with a degree of hypothetical risk. Perhaps the single point that has been most misunderstood in the controversy about recombinant DNA research, is that discussion of "risk" in the middle category of experiments relates entirely to hypothetical and speculative possibilities, not expected consequences or even phenomena that seem likely to occur on the basis of what is known. Unfortunately, much of the speculation has been interpreted as fact.

There is nothing novel about the principle of matching containment with the level of anticipated hazard; the containment procedures used for pathogenic bacteria, toxic substances, and radioisotopes attempt to do this. However, the containment measures used in these areas address themselves only to known hazards and do not attempt to protect against the unknown. If the same principle of protecting only against known or expected hazards were followed in recombinant DNA research, there would be no containment whatsoever except for a very few experiments. In this instance, we are asking not only that there be no evidence of hazard, but that there be positive evidence that there is no hazard. In developing guidelines for recombinant DNA research, we have attempted to take precautionary steps to protect ourselves against hazards that are not known to exist—and this unprecedented act of caution is so novel that it has been widely misinterpreted as implying the imminence or at least the likelihood of danger.

Much has been made of the fact that, even if a particular recombinant DNA molecule shows no evidence of being hazardous at the present time, we are unable to say for certain that it will not devastate our planet some years hence. Of course this view is correct; similarly, we are unable to say for certain that the vaccines we are administering to millions of children do not contain agents that will produce contagious cancer some years hence, we are unable to say for certain that a virulent virus will not be brought to the United States next winter by a traveler from abroad, causing a nationwide fatal epidemic of a hitherto unknown disease—and we are unable to say for certain that novel hybrid plants being bred around the world will not suddenly become weeds that will overcome our major food crops and cause worldwide famine.

The statement that potential hazards could result from certain experiments involving recombinant DNA techniques is akin to the statement that a vaccine injected today into millions of people *could* lead to infectious cancer in 20 years, a pandemic caused by a traveler-borne virus *could* devastate the United States, or a new plant species *could* uncontrollably destroy the world's food supply. We have no reason to expect that any of these things will happen, but we are unable to say for certain that they will not happen. Similarly, we are unable to guarantee that any of man's efforts to influence the earth's weather, explore space, modify crops, or cure disease will not carry with them the seeds for the

we in fact point to one major area of human activity where one can say *for certain* that there is zero risk? Potentially, we could respond to such risks by taking measures such as prohibiting foreign travel to reduce the hazard of deadly virus importation and stopping experimentation with hybrid plants. It is possible to develop plausible "scare scenarios" involving virtually any activity or process, and these would have as much (or as little) basis in fact as most of the scenarios involving recombinant DNA. But we must distinguish fear of the unknown from fear that has some basis in fact; this appears to be the crux of the controversy surrounding recombinant DNA.

Unfortunately, the public has been led to believe that the biohazards described in various scenarios are likely or probable outcomes of recombinant DNA research. "If the scientists themselves are concerned enough to raise the issue," goes the fiction, "the problem is probably much worse than anyone will admit." However, the simple fact is that there is no evidence that a bacterium carrying any recombinant DNA molecule poses a hazard beyond the hazard that can be anticipated from the known properties of the components of the recombinant. And experiments involving genes that produce toxic substances or pose other known hazards are prohibited.

Freedom of Scientific Inquiry

This issue has been raised repeatedly during discussions of recombinant DNA research. "The time has come," the critics charge, "for scientists to abandon their long-held belief that they should be free to pursue the acquisition of new knowledge regardless of the consequences." The fact is that no one has proposed that freedom of inquiry should extend to scientific experiments that endanger public safety. Yet, "freedom of scientific inquiry" is repeatedly raised as a straw-man issue by critics who imply that somewhere there are those who argue that there should be no restraint whatsoever on research.

Instead, the history of this issue is one of self-imposed restraint by scientists from the very start. The scientific group that first raised the question of possible hazard in some kinds of recombinant DNA experiments included most of the scientists involved in the development of the techniques—and their concern was made public so that other investigators

ered the possibility of hazard could exercise appropriate restraint. While most scientists would defend their right to freedom of scientific thought and discourse, I do not know of anyone who has proposed that scientists should be free to do whatever experiments they choose regardless of the consequences.

Interference with "Evolutionary Wisdom"

Some critics of recombinant DNA research ask us to believe that the process of evolution of plants, animals, and microbes has remained delicately controlled for millions of years, and that the construction of recombinant DNA molecules now threatens the master plan of evolution. Such thinking, which requires a belief that nature is endowed with wisdom, intent, and foresight, is alien to most post-Darwinian biologists (*3*). Moreover, there is no evidence that the evolutionary process is delicately controlled by nature. To the contrary, man has long ago modified the process of evolution, and biological evolution continues to be influenced by man. Primitive man's domestication of animals and cultivation of crops provided an "unnatural" advantage to certain biological species and a consequent perturbation of evolution. The later creation by man of hybrid plants and animals has resulted in the propagation of new genetic combinations that are not the products of natural evolution. In the microbiological world, the use of antimicrobial agents to treat bacterial infections and the advent of mass immunization programs against viral disease has made untenable the thesis of delicate evolutionary control.

A recent letter (*4*) that has been widely quoted by critics of recombinant DNA research asks, "Have we the right to counteract irreversibly the evolutionary wisdom of millions of years . . .?" It is this so-called evolutionary wisdom that gave us the gene combinations for bubonic plague, smallpox, yellow fever, typhoid, polio, diabetes, and cancer. It is this wisdom that continues to give us uncontrollable diseases such as Lassa fever, Marburg virus, and very recently the Marburg-related hemorrhagic fever virus, which has resulted in nearly 100 percent mortality in infected individuals in Zaire and the Sudan. The acquisition and use of all biological and medical knowledge constitutes an intentional and continuing assault on evolutionary wisdom. Is this the "warfare against nature" that some critics fear from re-

How About the Benefits?

For all but a very few experiments, the risks of recombinant DNA research are speculative. Are the benefits equally speculative or is there some factual basis for expecting that benefits will occur from this technique? I believe that the anticipation of benefits has a substantial basis in fact, and that the benefits fall into two principal categories: (i) advancement of fundamental scientific and medical knowledge, and (ii) possible practical applications.

In the short space of 3½ years, the use of the recombinant DNA technology has already been of major importance in the advancement of fundamental knowledge. We need to understand the structure and function of genes, and this methodology provides a way to isolate large quantities of specific segments of DNA in pure form. For example, recombinant DNA methodology has provided us with much information about the structure of plasmids that cause antibiotic resistance in bacteria, and has given us insights into how these elements propagate themselves, how they evolve, and how their genes are regulated. In the past, our inability to isolate specific genetic regions of the chromosomes of higher organisms has limited our understanding of the genes of complex cells. Now use of recombinant DNA techniques has provided knowledge about how genes are organized into chromosomes and how gene expression is controlled. With such knowledge we can begin to learn how defects in the structure of such genes alter their function.

On a more practical level, recombinant DNA techniques potentially permit the construction of bacterial strains that can produce biologically important substances such as antibodies and hormones. Although the full expression of higher organism DNA that is necessary to accomplish such production has not yet been achieved in bacteria, the steps that need to be taken to reach this goal are defined, and we can reasonably expect that the introduction of appropriate "start" and "stop" control signals into recombinant DNA molecules will enable the expression of animal cell genes. On an even shorter time scale, we can expect recombinant DNA techniques to revolutionize the production of antibiotics, vitamins, and medically and industrially useful chemicals by eliminating the need to grow and process the often exotic bacterial and fungal strains currently used as sources for such agents. We can anticipate the construction of

not destroyed by the antibiotic inactivating enzymes responsible for drug resistance in bacteria.

In the area of vaccine production, we can anticipate the construction of specific bacterial strains able to produce desired antigenic products, eliminating the present need for immunization with killed or attenuated specimens of disease-causing viruses.

One practical application of recombinant DNA technology in the area of vaccine production is already close to being realized. An *E. coli* plasmid coding for an enteric toxin fatal to livestock has been taken apart, and the toxin gene has been separated from the remainder of the plasmid. The next step is to cut away a small segment of the toxin-producing gene so that the substance produced by the resulting gene in *E. coli* will not have toxic properties but will be immunologically active in stimulating antibody production.

Other benefits from recombinant DNA research in the areas of food and energy production are more speculative. However, even in these areas there is a scientific basis for expecting that the benefits will someday be realized. The limited availability of fertilizers and the potential hazards associated with excessive use of nitrogen fertilizers now limits the yields of grain and other crops, but agricultural experts suggest that transplantation of the nitrogenase system from the chromosomes of certain bacteria into plants or into other bacteria that live symbiotically with food crop plants may eliminate the need for fertilizers. For many years, scientists have modified the heredity of plants by comparatively primitive techniques. Now there is a means of doing this with greater precision than has been possible previously.

Certain algae are known to produce hydrogen from water, using sunlight as energy. This process potentially can yield a virtually limitless source of pollution-free energy if technical and biochemical problems indigenous to the known hydrogen-producing organisms can be solved. Recombinant DNA techniques offer a possible means of solution to these problems.

It is ironic that some of the most vocal opposition to recombinant DNA research has come from those most concerned about the environment. The ability to manipulate microbial genes offers the promise of more effective utilization of renewable resources for mankind's food and energy needs; the status quo offers the prospect of progressive and continuing devastation of the environ-

been misled into taking what I believe to be an antienvironmental position on the issue of recombinant DNA.

The NIH Guidelines

Even if hazards are speculative and the potential benefits are significant and convincing, wouldn't it still be better to carry out recombinant DNA experiments under conditions that provide an added measure of safety—just in case some of the conjectural hazards prove to be real?

This is exactly what is required under the NIH (National Institutes of Health) guidelines (*5*) for recombinant DNA research:

1) These guidelines prohibit experiments in which there is some scientific basis for anticipating that a hazard will occur. In addition, they prohibit experiments in which a hazard, although it might be entirely speculative, was judged by NIH to be potentially serious enough to warrant prohibition of the experiment. The types of experiment that were the basis of the initial "moratorium" are included in this category; contrary to the statements of some who have written about recombinant DNA research, there has in fact been no lifting of the original restrictions on such experiments.

2) The NIH guidelines require that a large class of other experiments be carried out in P4 (high level) containment facilities of the type designed for work with the most hazardous naturally occurring microorganisms known to man (such as Lassa fever virus, Marburg virus, and Zaire hemorrhagic fever virus). It is difficult to imagine more hazardous self-propagating biological agents than such viruses, some of which lead to nearly 100 percent mortality in infected individuals. The P4 containment requires a specially built laboratory with airlocks and filters, biological safety cabinets, clothing changes for personnel, autoclaves within the facility, and the like. This level of containment is required for recombinant DNA experiments for which there is at present no evidence of hazard, but for which it is perceived that the hazard might be potentially serious if conjectural fears prove to be real. There are at present only four or five installations in the United States where P4 experiments could be carried out.

3) Experiments associated with a still lesser degree of hypothetical risk can be conducted in P3 containment facilities. These are also specially constructed lab-

trances, negative air pressure, and special air filtration devices. Facilities where P3 experiments can be performed are limited in number, but they exist at some universities.

4) Experiments in which the hazard is considered unlikely to be serious even if it occurs still require laboratory procedures (P2 containment) that have for years been considered sufficient for research with such pathogenic bacteria as *Salmonella typhosa*, *Clostridium botulinum*, and *Cholera vibrio*. The NIH guidelines require that P2 facilities be used for work with bacteria carrying interspecies recombinant DNA molecules that have shown no evidence of being hazardous—and even for some recombinant DNA experiments in which there is substantial evidence of lack of hazard.

5) The P1 (lowest) level of containment can be used only for recombinant DNA molecules that potentially can be made by ordinary biological gene exchange in bacteria. Conformity to even this lowest level of containment in the laboratory requires decontamination of work surfaces daily and after spills of biological materials, the use of mechanical pipetting devices or cotton plugged pipettes by workers, a pest control program, and decontamination of liquid and solid waste leaving the laboratory.

In other areas of actual or potential biological hazard, physical containment is all that microbiologists have had to rely upon; if the Lassa fever virus were to be released inadvertently from a P4 facility, there would be no further barrier to prevent the propagation of this virus which is known to be deadly and for which no specific therapy exists. However, the NIH guidelines for recombinant DNA research have provided for an additional level of safety for workers and the public: This is a system of biological containment that is designed to reduce by many orders of magnitude the chance of propagation outside the laboratory of microorganisms used as hosts for recombinant DNA molecules.

An inevitable consequence of these containment procedures is that they have made it difficult for the public to appreciate that most of the hazards under discussion are conjectural. Because in the past, governmental agencies have often been slow to respond to clear and definite dangers in other areas of technology, it has been inconceivable to scientists working in other fields and to the public at large that an extensive and costly federal machinery would have been established to provide protection in this area of research unless severe haz-

recombinant DNA research has prompted international meetings, extensive coverage in the news media, and governmental intervention at the federal level has been perceived by the public as prima facie evidence that this research must be more dangerous than all the rest. The scientific community's response has been to establish increasingly elaborate procedures to police itself—but these very acts of scientific caution and responsibility have only served to perpetuate and strengthen the general belief that the hazards under discussion must be clear-cut and imminent in order for such steps to be necessary.

It is worth pointing out that despite predictions of imminent disaster from recombinant DNA experiments, the fact remains that during the past 3½ years, many billions of bacteria containing a wide variety of recombinant DNA molecules have been grown and propagated in the United States and abroad, incorporating DNA from viruses, protozoa, insects, sea urchins, frogs, yeast, mammals, and unrelated bacterial species into *E. coli*, without hazardous consequences so far as I am aware. And the majority of these experiments were carried out prior to the strict containment procedures specified in the current federal guidelines.

Despite the experience thus far, it will always be valid to argue that recombinant DNA molecules that seem safe today may prove hazardous tomorrow. One can no more prove the safety of a particular genetic combination under all imaginable circumstances than one can prove that currently administered vaccines do not contain an undetected self-propagating agent capable of producing cancer in the future, or that a hybrid plant created today will not lead to disastrous consequences some years hence. No matter what evidence is collected to document the safety of a new therapeutic agent, a vaccine, a process, or a particular kind of recombinant DNA molecule, one can always conjure up the possibility of future hazards that cannot be disproved. When one deals with conjecture, the number of possible hazards is unlimited; the experiments that can be done to establish the absence of hazard are finite in number.

Those who argue that we should not use recombinant DNA techniques until or unless we are absolutely certain that there is zero risk fail to recognize that no one will ever be able to guarantee total freedom from risk in any significant human activity. All that we can reasonably expect is a mechanism for dealing responsibly with hazards that are known to exist or which appear likely on the basis of information that is known. Beyond this, we can and should exercise caution in any activity that carries us into previously uncharted territory, whether it is recombinant DNA research, creation of a new drug or vaccine, or bringing a spaceship back to Earth from the moon.

Today, as in the past, there are those who would like to think that there is freedom from risk in the status quo. However, humanity continues to be buffeted by ancient and new diseases, and by malnutrition and pollution; recombinant DNA techniques offer a reasonable expectation for a partial solution to some of these problems. Thus, we must ask whether we can afford to allow preoccupation with and conjecture about hazards that are not known to exist, to limit our ability to deal with hazards that do exist. Is there in fact greater risk in proceeding judiciously, or in not proceeding at all? We must ask whether there is any rational basis for predicting the dire consequences of recombinant DNA research portrayed in the scenarios proposed by some. We must then examine the "benefit" side of the picture and weigh the already realized benefits and the reasonable expectation of additional benefits, against the vague fear of the unknown that has in my opinion been the focal point of this controversy.

References and Notes

1. P. Berg, D. Baltimore, H. W. Boyer, S. N. Cohen, R. W. Davis, D. S. Hogness, D. Nathans, R. Roblin, J. D. Watson, S. Weissman, N. D. Zinder, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 2593 (1974).
2. S. N. Cohen, A. C. Y. Chang, H. W. Boyer, R. B. Helling, *ibid.* **70**, 3240 (1973); S. N. Cohen, *Sci. Am.* **233** (No. 7), 24 (1975).
3. If we accept the view that any natural barriers to the propagation of genetic material derived from unrelated species do not owe their existence to the intent of nature, we can reason that evolution has created and maintained such barriers because opportunities for genetic mixing occur in nature. Furthermore, we must conclude that limitations to gene exchange have evolved because the mixing of genes from diverse organisms is biologically undesirable—not in a moral or theological sense as some observers would have us believe—but to those organisms involved.
4. E. Chargaff, *Science* **192**, 938 (1976).
5. *Fed. Reg.* **41**(176) (9 September 1976), pp. 38426–38483.

NEWS AND COMMENT

Brazil's Nuclear Program: Carter's Nonproliferation Policy Backfires

Brasilia. The Carter Administration's attempt to convince West Germany to renege on its controversial agreement with Brazil for supplying nuclear technology has created a major furor here. Vice President Mondale's discussion of the matter with West German officials on his first foreign mission, before any consultation with Brazil, has fanned an earlier but muted concern into a nationwide outpouring of resentment at what is seen as U.S. interference with Brazil's efforts

affair seems likely to further damage U.S.–Brazilian relations, which were already deteriorating, and to accelerate a discernible tilt toward Europe and Japan as the favored partners for cooperative development projects and trade deals.

The resentment expressed here is not confined to government officials but comes from many disparate elements of Brazilian society and seems to have had the effect of strengthening political support for President Ernesto Geisel and his

men for the opposition party, the Brazilian Democratic Movement (MDB), have publicly condemned the U.S. moves and defended the West German agreement. In December a leading MDB figure, Senator Paulo Brossard of Rio Grande do Sul, said in response to then President-elect Carter's call for cancellation of the agreement that while he respected Carter's position, "it is not possible to accept it without protesting the interference in matters that are the exclusive competence of my country and its own interests." The tone of the rhetoric has become harsher in recent weeks. There has been heavy press coverage in Brazil of the Mondale trip, and editorial opinion has been overwhelmingly anti-American. Even university scientists who had been openly critical of the nuclear deal on technical grounds have closed ranks behind the government