GENETIC ENGINEERIN
NEW STRAINS OF

Scientists have recently succeeded in re-arranging the basic genetic material of living things, and so have opened an exciting new research frontier. But recombinant DNA technology, warns an eminent biologist in this article, 'is so far-reaching in its potential for harm that decisions on how to handle it must not be left to the scientists alone.'

Early in July 1976, the 10-man City Council of Cambridge, Massachusetts, at the urging of some concerned Harvard scientists, voted to ask Harvard University to halt temporarily the construction of a new $500,000 laboratory for specialized genetics research. This move on the part of the Mayor and other local elected officials against the scientific decisions of the university was unprecedented, but so was the dramatic reason for it—the fear that the biologists, who propose to tamper with the genetic apparatus of microorganisms, would create a new Andromeda-like strain that might escape their control and spread an incurable disease to the population.

The Cambridge Council's intervention in Harvard affairs cannot be dismissed as an overreaction of ignorant laymen to the esoteric pursuits of science, for it has been spurred by the carefully considered opinions of some distinguished workers in biological research—Nobelist George Wald is one of the leaders. They are concerned with the recently attained power of biology to alter the genes of living things and create new and possibly dangerous hybrids of animals, plants and viruses. Of course such alarms have been raised before: The A-bomb, nerve gas, biological warfare, the destruction of the stratospheric ozone layer by fluorocarbon sprays—all have been held up as threats to human existence. But all of these dangers can, in theory if not in practice, be limited or controlled. The threat of a new form of life is more compelling, for once released, it cannot be controlled, and its effects cannot be reversed. A new disease may simply have to run its course, attacking millions in its path. The Cambridge Council undoubtedly had some stark vision of such a biological holocaust when it made its decision. (Harvard responded to the council's request by setting up a committee to consider the city's concern about the laboratory.)

The scientists who alerted the Cambridge City Council are not typical. Most of those not directly involved in the new genetic research take a hands-off position, perhaps out of reluctance to interfere with the venerated "right" of scientists to free inquiry. They may also fear that negative publicity associated with biological research will disillusion the public and diminish the funds so necessary for all types of research. Meanwhile, scientists working in the controversial areas are motivated by their own intellectual curiosity and the powerful drive for success and recognition.

Recent discoveries in molecular genetics have provided spectacular new techniques whose exploitation is difficult to resist so long as scientists continue to focus on innovation rather than social benefit. The involved scientists are aware that their experimentation entails some risks to the public, but they argue that adequate precautions can be taken to make the risks acceptably small. At a major international conference held early in 1975 at the Asilomar conference center in Pacific Grove, California, leading molecular biologists took the rare step of proposing rules to limit genetic research. More recently, they have been instrumental in drawing up a similar set of guidelines that has just been issued by the Director of the U.S. National Institutes of Health.

The guidelines may alleviate the nervousness of some scientists, but—as a researcher in molecular biology for 25 years—my own view is that they will not effectively reduce the danger. Indeed, they may actually lull us into a false sense of security.

The danger has developed with the discovery of a special form of DNA, the substance that controls the growth and reproduction of all living cells. Ordinary DNA is a large molecule shaped like a double helix, or spiral staircase; it is found in the nucleus of every living cell. We now know that the arrangement of atoms in the helix reflects the code, or set of instructions, that guides the development of every cell in the fulfillment of its genetic destiny. The new form of DNA, known as recombinant DNA, is simply a mosaic of DNA fragments obtained from different types of cells. These patchwork molecules, man-made in the laboratory, have the power to enter a host cell and become a part ot its permanent genetic complement. What they may do to the cell we do not know.

The discovery of recombinant DNA is one of the more striking technological achievements of our century. The story began in 1944 when a team of scientists, Oswald T. Avery, Colin MacCloud and Maclyn McCarty, at the Rockefeller Institute (now Rockefeller University) showed for the first time that DNA is the hereditary substance of living cells. Later work showed that the long DNA molecule is composed of sections called genes. Each gene determines a characteristic—hair color, for example. Another discovery important to the development of recombinant DNA was made by William Hayes and Joshua Lederberg in 1952. They showed that bacterial cells contain circular DNA molecules, called plasmids, in addition to the main DNA molecule. The plasmids are small, easy to handle in the laboratory and can enter other bacteria with ease. The plasmids also contain a series of genes, linked together in the form of a circle. In 1962, W. Arber and D. Dussoix showed that bacterial cells contain a substance, called a restriction enzyme, that acts as a fine chemical scalpel to split foreign DNA molecules into specific fragments. This process, part of the bacteria's defense mechanism, occurs when a bacterial virus infects a bacterium. The enzyme was purified from bacteria by H. Boyer and his coworkers, and in 1972 it was shown by J. Mertz and R. Davis of Stanford University School of Medicine that the split DNA fragments have "sticky ends"—when the ends touch they stick to each other. This astonishing characteristic of the DNA fragments makes genetic engineering possible.

Recombinant DNA is actually very easy to make. Any high school student can do it. Restriction enzymes are available commercially and may be used to split DNA molecules from any source—man, cancer viruses, bacteria, plants, insects—to produce fragment with sticky ends. When a split plasmid DNA from a harmless bacterium is mixed with another split DNA, say from a cancer viru their sticky ends join, and a new, hybrid plasmid is formed. Th new form of DNA has the characteristics of both DNAs—bacter and cancer—from which it was made. To make vast quantitie this new entity, it is only necessary to mix the new plasmid with bacteria. The bacteria absorb them and then manu exact copies in limitless number. Using bacteria as factories i way, any kind of recombinant DNA can be made in large qu

LIFE—OR DEATH

by LIEBE F. CAVALIERI

is possibility is so attractive that both Stanford University and e University of California have applied for patents for DNA combinant technology.

This technology is, of course, very exciting to molecular biogists. Its application to basic research promises to provide answers o a number of fundamental biological questions. With this desirable nd result in mind, scientists feel justified in taking the risks inherent n the technique.

For example, Paul Berg, who directs a large facility at Stanford University School of Medicine, has stated that the ability to produce arge quantities of gene fragments with recombinant DNA procedures permits expanded study of the structure of genes and how they work to produce enzymes that influence an organism's development. David Hogness, also of Stanford, has shown that the methodology provides a way to find the location of specific genes within the chromosome (a chromosome is a very long molecule of DNA). This may give scientists deeper insight into when and where enzymes are made. These matters are worthy of attention, but I am confident that these as well as other problems of molecular biology can be solved by using other, safer experimental procedures—albeit over a longer period of time.

We have also been told that recombinant DNA research may offer us a number of practical benefits. Paul Berg has stated that "if the genes that produce the enzymes that make vitamins, antibiotics or hormones can be made to function in *E. coli*, then this bacterium, which can be grown in large quantity in the laboratory, could be used to produce these materials."

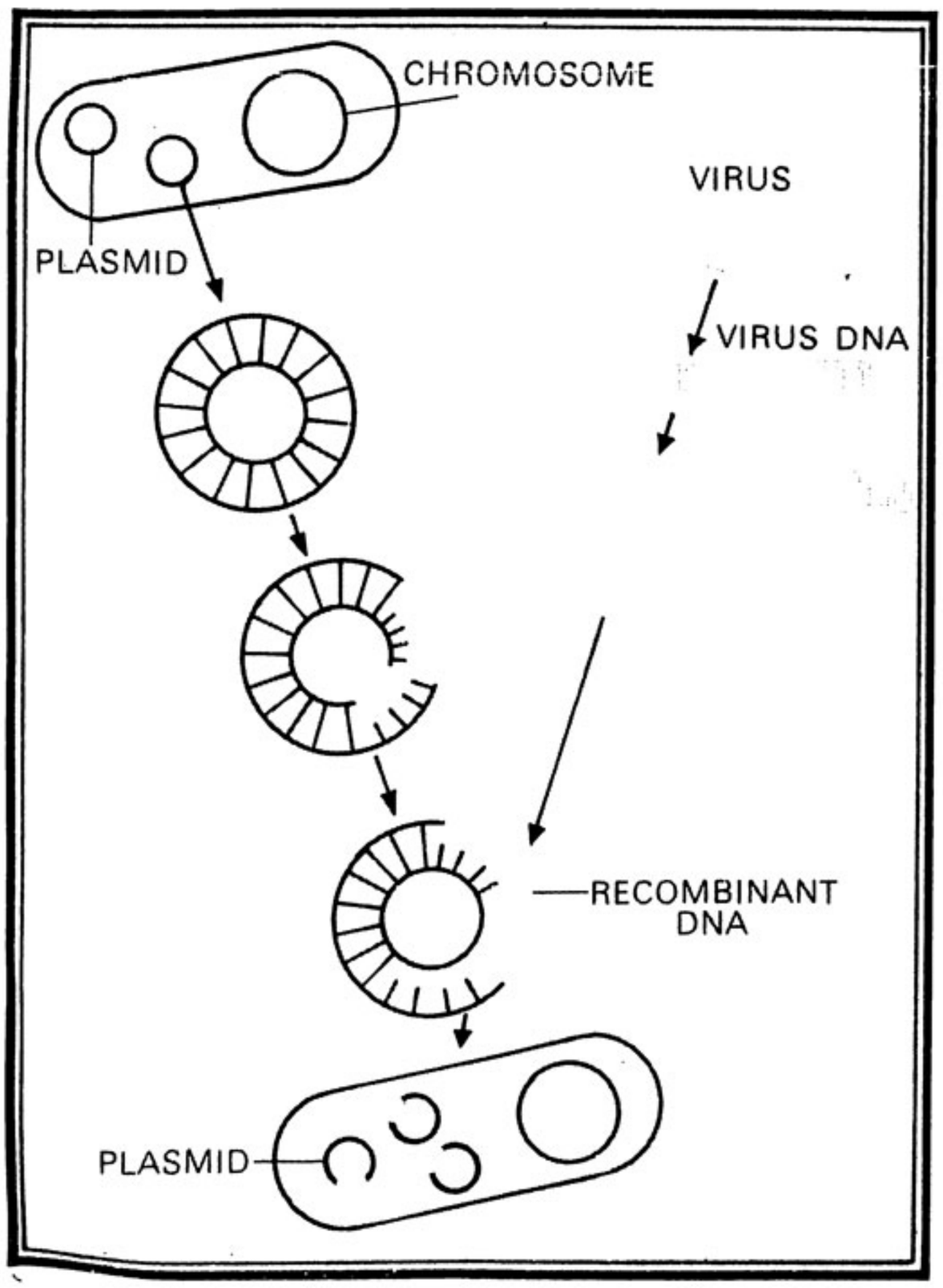
Berg has also said the research could yield "important benefits to expanding the world food supply." Our food supply depends upon the availability of fertilizer, which provides the nitrogen essential to plant growth. But abundant nitrogen exists in the atmosphere, and certain plants—legumes—are able to take advantage of it; they contain bacteria that convert the atmospheric nitrogen to forms plants can use. Recombinant DNA technology, according to its proponents, might make it possible to create major food crops with a similar ability.

In another area, Berg has asserted that "the isolation of genes put us at the threshold of a new form of medicine, gene therapy, to treat crippling genetic diseases."

These claims, however, may be overstated. It may well be possible to produce vitamins or hormones such as insulin by means of genetic engineering, but commercial production of such biological chemicals has been possible for years. The idea of creating food crops nourished by atmospheric nitrogen is intriguing, but at the 1976 International Symposium on recombinant molecules, held at the Massachusetts Institute of Technology, plant scientists reported not only that goals such as these are difficult and distant, but they are more likely to be achieved by the traditional methods of genetics rather than by the new molecular recombinant techniques. As for gene therapy, Berg himself says that "though simple and attractive in principle, this step has many pitfalls and unknowns, and these still have to be examined carefully before such therapy could be considered."

But to measure the true value of such potential benefits, we must weigh them against the hazards of this technology. The research involves many unknown factors beyond the control of the scientist. Since the plasmid and nonplasmid DNA fragments may combine in many different ways in a given recombinant experiment, it is necessary to create vast numbers of cells with unknown genetic alterations in order to obtain a cell containing a specific recombinant DNA. The probability of creating a dangerous genetic agent in the process is real, and there is no way to test for the danger. The scientist does not know what he has done until he has analyzed the newly created cells—at which point it may be too late.

Furthermore, because recombinant DNAs can reproduce themselves in their host cells, they can become permanent residents wherever the host cells are found, and once released into the world, it would be impossible to control them. In this respect, they are quite unlike any other man-made hazard. If we stop manufacturing DDT or Red Dye No. 2, they will cease to be problems. The air and waters of the earth will gradually return to normal if we stop pouring wastes into them. Not so with genetically altered bacteria; a single unrecognized accident could contaminate the entire earth with an ineradicable and dangerous agent that might not reveal its presence until its deadly work was done.



The recombinant process: *In the diagram at left, the elliptical figure at top represents a bacterium containing one chromosome (a large DNA molecule and two plasmids (smaller circular DNA molecules). The large circle represents a virus with chainlike DNA molecules. After both plasmid and viral DNA molecules are isolated and treated with restriction enzyme, they break apart. A fragment of viral DNA joins the plasmid ring to form a new recombined molecule the plasmid is re-absorbed by the bacterium, which multiplies without*

Recombinant DNA technology has 'put us at the threshold of a new form of medicine, gene therapy, to treat crippling genetic diseases.' In agriculture, the new technology could yield 'important benefits to expanding the world's food supply' by creating new crops.

Dr. Robert Sinsheimer, chairman of the Biology Division of the California Institute of Technology and chairman of the editorial board of the Proceedings of the National Academy of Sciences, has warned that "what we are doing is almost certainly irreversible. Knowing human frailty, these structures will escape, and there is no way to recapture them." Erwin Chargaff, Professor Emeritus of Columbia University and a recipient of the U.S. National Medal of Science and other honors for his work on DNA, has written, "I should say that the spreading of experimental cancer may be confidently expected."

The widespread use of *E. coli* bacteria in this new genetic research increases its dangers. From the point of view of public health, this bacterium is the worst of all possible choices. It is a normal inhabitant of the human digestive tract and can easily enter the body through the mouth or nose. Once there, it can multiply and remain permanently. Thus every laboratory working with *E. coli* recombinants is staffed by potential carriers who could spread a dangerous recombinant to the rest of the world. *E. coli* is found in the sewage that flows through all of our communities; it lives in all warm-blooded animals, in insects and in fish; it is present on grass and vegetables and in water.

In addition to its ubiquitousness, *E. coli* is very promiscuous and during mating has a formidable propensity to transfer from one cell to another plasmid containing genetic information. This exchange can occur even with some other types of bacteria. Dead *E. coli* can also transfer their genes. Even weakened strains of *E. coli*, which require special laboratory conditions for growth, are able to pass on dangerous recombinant genes to healthier bacteria. Scientists choose *E. coli* for use in genetic engineering research because more is known about its genetics than about those of any other cell, and they do not wish to spend the time necessary to study the genetics of a substitute. The few years required to study such an alternate loom large to the scientist with his eye on the Nobel Prize.

For the public at large, however, the difference between 20 or 22 years in the achievement of some hypothetical benefit cannot justify the risks. Consider, for example, the fact that DNA from cancer viruses has already been introduced into a weakened form of *E. coli* (the ability of this form to survive in the human intestines is disputed at present). Suppose a laboratory technician accidentally pours a culture of these bacteria into a sink—not an unlikely occurrence. In the trap of the sink, or farther on in the sewage system, malignant properties are transferred from the laboratory bacteria to normal *E. coli*. The sewage, with the bacteria containing cancer genes, is eventually discharged into the sea near a shellfish bed. People in distant places eat the shellfish and the bacteria take up residence in their intestines and are spread from person to person. In the individual the bacteria can transfer recombinant DNA containing cancer genes to human cells. The result—cancer, normally not infectious, is spread in epidemic proportions by normally harmless bacteria.

The introduction of genes from cancer viruses into *E. coli* is only one of a number of ways in which cancer might be spread by recombinant DNA. Biologists suspect that the DNA of all normal animal cells contains inactive cancer genes. Cancer may normally arise when these genes are activated by some unusual disturbance such as tobacco tar or air pollutants. Similarly, the transfer of supposedly harmless recombinant DNA from *E. coli* to a human cell could act as a monkey wrench in the regulatory machinery that controls the cell's dormant cancer genes.

It is possible, intentionally or unintentionally, to construct highly dangerous agents of other types, worse than anything yet envisioned in biological warfare. For example, *E. coli* or other bacteria that normally live in man and animals could be given the ability to produce deadly toxins, such as that of botulism. Protection by immunization would be out of the question. It is quite possible that an agent of this type might arise inadvertently, since in many experiments the nature of the genes implanted into a new host is unknown. The sudden appearance of new disease-causing agents is a threat not only to man directly but to the animals and plants.

There is danger even in purposely designed recombinant agents. Suppose, for instance, that drug companies are eventually successful in producing insulin [see "Good News for Diabetics!" on page 4] or other products by growing genetically engineered *E. coli* on an industrial scale. The slightest leak would constitute a major hazard. If bacteria producing insulin were to find their way into a human host, the result could be insulin shock and very likely death.

Not all the danger of recombinant work lies in cancer or in strange new diseases. There are also the hazards of success. The aim of genetic engineering is to speed up the evolution of chosen organisms. In so doing the slowly developing balance among living systems may be changed in sudden and decisive ways. Natural evolution works gradually on all species at the same time. If we undertake to control evolution ourselves, we cannot hope at once to control the myriad factors that make the world habitable.

Scientists have been slow to acknowledge all the dangers inherent to genetic manipulation. I myself must admit that, while I felt uneasy about future hazards when I carried out experiments on bacterial transformation 15 years ago, I did not ponder the full scope of the problem until recently. But today many of the hazards are immediate, and biologists can no longer shirk their social responsibility.

The question of hazards was discussed at a special meeting of the advisory committee to the Director of the National Institutes of Health called early last year by Dr. Donald S. Fredrickson. Most of the scientists present at this meeting defended recombinant DNA technology, citing all the benefits to be realized by basic biological research. Among them were David Baltimore from Massachusetts Institute of Technology, Paul Berg and David Hogness from Stanford, Donald Brown from Carnegie Institution of Washington and Charles Thomas from Harvard Medical School.

On the other side of the aisle were a few lone critics. A constant voice was that of Robert Sinsheimer. He emphasized that a major problem of recombinant DNA technology is the crossing of genetic barriers between species, an activity that opens an unfamiliar area of biology. He also has pointed out that scientists have ignored "the potential broader social or ethical implications of initiating this line of research—of its role as a possible prelude to longer range, broader scale genetic engineering of the fauna and flora of the planet, including, ultimately, man...." In a philosophic sense Sinsheimer has said, "Would we wish to claim the right of individual scientists to be free to create novel self-perpetuating organisms likely to spread about the planet in an uncontrollable manner for better or worse? I think not."

Those who have carried out the research on recombinant DNA and favor its continuation are respected scientists from large

versities. I feel, however, that there is a large silent majority of ntists who would speak out for a sane solution if given the ortunity.

The safety guidelines put forth by the National Institutes of alth have been hailed eagerly by workers in the recombinant NA field who wish to express their public concern. But in the eetings and discussions that preceded publication of the guidelines e focus remained on safety measures; scarcely a voice raised the ndamental question of whether the research should continue at l. Rather, on the implicit assumption that the work ought to oceed, they devoted painstaking effort to the formulation of afety precautions to prevent accidents. Here there was some con-oversy, the participants being divided between those who wanted ax rules, and those who wanted still laxer rules. The viewpoints aried with the participants' degrees of vested interest in recombinant esearch, and most had been involved to some degree. As Professor hargaff said, "...the incendiaries formed their own fire brigade." n pointing out that the decisions have all been made by scientists, enator Edward Kennedy, Chairman of the Senate Subcommittee on Health, objected that "the factors under consideration extend far beyond their technical competence." There has been no significant input from experts in public health, ecology, sociology, ethics or philosophy, and no effort has been made to inform the public of the dangers or to solicit popular opinions.

Briefly, the guidelines have two basic features. The first is physical containment. This means that the laboratory must be equipped to minimize the chance that experimental organisms might escape. The laboratory is kept under negative pressure so that air does not escape but is pumped out through filtered vents. For more hazardous experiments the worker must shower before entering or leaving the facility. The second feature, called biological containment, involves the use of enfeebled organisms so that if some do escape they cannot survive in the outside environment. None of the precautions is foolproof.

In the course of 25 years of Army research on biological warfare agents at Fort Dietrich, equipped with the highest level of physical containment facilities, there were 423 accidental infections and three deaths. Accidents due to human error are inevitable, all the more so because scientists under pressure become inured to taking risks. The temptation to do things the easy, quick way instead of the safe way is hard to resist. In the case of recombinant DNA, it is an all or none situation—only one accident is needed to endanger the future of mankind.

Biological containment is a new concept in laboratory safety, one that has not really been tried. The rules therefore involve guesswork, and some are arbitrary. For instance, one of the National Institutes of Health rules states that an experiment using recombinant *E. coli* is safe only if no more than one out of 100 million of the bacteria is able to survive outside the laboratory. (It should be noted that 100 million is a small number as laboratory bacteria go.) This raises some difficult questions. In testing the safety of an *E. coli* strain how much time should be allowed for all but one of the 100 million bacteria to die? Since different recombinant DNAs inserted into the bacteria may affect their survival, how can they be tested in advance, without incurring a risk during the test? Obviously it is impossible. Dr. Stanley Falkow of the University of Washington School of Medicine has said, "It is also clear from our studies that a carried plasmid [i.e. a plasmid inside a bacterium] may have a profound effect on the survival and carriage of *E. coli*...it may not be too farfetched to suggest that some DNA recombinant molecules could profoundly affect the ability of [weakened] *E. coli* to survive and multiply in the gastrointestinal tract." Moreover, how can all the possible bacterial growth conditions outside the laboratory be simulated for the test?

It is generally known that most laboratory accidents are not due to faulty equipment but to human error, and the possibility of human error increases with the eagerness of the scientist to push for faster results. Thus even if scientists swear to adhere to the guidelines, I doubt very much that they can be effective. Harvard biology professor Carroll Williams is quoted as saying, "Scientists are racing for advantage and priority in a hotly competitive field and are likely to do what they can to win the race. The competition in this field is really fantastic. One scientist even told me, 'If we don't get the containment facility we want, we'll just reclassify our experiments from a higher to a lower security requirement.'...I believe him."

The pace of recombinant DNA research is increasing daily. Students hoping to assure their future are flocking to centers of recombinant research—Stanford, Boston, Paris, Stockholm, Geneva, London—and biologists everywhere are turning to the new techniques. Reflection about ultimate values or social priorities is not part of the scene.

In the development of the atom bomb during the war there seemed to be a compelling rationale for its urgency and secrecy, even though many physicists now bemoan the actions they themselves favored at the time. There is no such compelling reason to rush into recombinant DNA research, and I believe we should do everything possible to halt its current frenzied pace. The lure of the Nobel Prize is a strong force motivating scientists in this field. I would suggest that the Nobel Committee announce that no awards will ever be given in this area. At the same time, I would urge the U.S. National Academy of Sciences to call for an immediate worldwide moratorium on recombinant research so that the issues can be examined more carefully and safety measures can be developed in a thorough manner rather than in the current crash-program atmosphere.

In my view, recombinant DNA technology is so overpowering and far reaching in its potential for harm that decisions on how to handle it must not be left to the scientists alone. At the Asilomar conference, Alexander Capron of the University of Pennsylvania Law School told the assembled recombinant researchers: "As crucial as your research seems to you to the achievement of progress, you should be prepared for the eventuality that the public may not agree."

It would make sense, certainly, for the U.S. Congress to set up a National Biohazards Commission analogous to the Atomic Energy Commission, with legal authority to evaluate, license, supervise and inspect all activities that may subject the public to biological hazards of any kind. In addition, I hope that the United States will take the lead in forming an international council on biohazards in order to establish a uniform worldwide policy.

I am aware that these suggestions may be regarded in some circles as a threat to freedom of research. Freedom to search for truth has always been a precious academic right, and every scientist jealously guards it. But this venerated 19th-century idea can no longer be entertained in the light of this new technology. A new dimension has entered the picture—the element of risk for humanity at large. We must ask, with Professor Chargaff, "Have we the right to counteract, irreversibly, the evolutionary wisdom of millions of years, in order to satisfy the ambition and the curiosity of a few scientists? This world is given to us on loan. We come and we go, and after a time we leave earth and air and water to others who come after us. My generation...has been the first to engage, under the leadership of the exact sciences, in a destructive colonial warfare against nature. The future will curse us for it."

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