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by Clifford Grobstein

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The Recombinant-DNA Debate

The four-year-old controversy over the potential biohazards presented by the gene-splicing method and the effectiveness of plans for their containment is viewed in a broader context

by Clifford Grobstein

The guidelines for research involving recombinant-DNA molecules issued a year ago by the National Institutes of Health were the culmination of an extraordinary effort at self-regulation on the part of the scientific community. Yet the policy debate over recombinant-DNA research was clearly not laid to rest by the appearance of the NIH guidelines. Instead the debate has escalated in recent months both in intensity and in the range of public involvement. A watershed of sorts was reached in March at a public forum held by the National Academy of Sciences in Washington. The forum was in part a repeat performance by scientists arguing fixed positions that were established early in the debate. There were, however, new participants on the scene, and they presented a varied and rapidly shifting agenda. They made it clear that research with recombinant DNA had become a political issue. As one speaker remarked, the Academy forum may have been the last major public discussion of recombinant DNA arranged by the scientists involved in the research. Nonscientists at the forum, by word and deed, reiterated the theme that science has become too consequential either to be left to the self-regulation of scientists or to be allowed to wear a veil of political chastity.

Science of course is crucially consequential to society, precisely because it is an intensifying source of both benefits and risks. Research with recombinant DNA may provide major new social benefits of uncertain magnitude: more effective and cheaper pharmaceutical products; better understanding of the causes of cancer; more abundant food crops; even new approaches to the energy problem. These and other possible outcomes are envisioned in "best-case scenarios" for the future application of recombinant-DNA technology. "Worst-case scenarios" can also be conceived: worldwide epidemics caused by newly created pathogens; the triggering of catastrophic ecological imbalances; new tools for militarists and terrorists; the power to dominate and control the human spirit.

Both the best-case and worst-case scenarios are largely speculative; the gap between them symbolizes the large degree of uncertainty that surrounds this major step forward in molecular genetics. The material basis of biological heredity has been broken into in the past two decades, and it seems as though each of the fragments has acquired a life of its own. In this resulting period of instability fear threatens to override wonder as the implications of the research diffuse more widely. The fear is not so much of any clear and present danger as it is of imagined future hazards. The classic response to such fears is rigid containment: the Great Wall, the Maginot Line, the cold war. All are manifestations of the effort to provide absolute security against unpredictable risks, and yet each generates its own risk. The escalation of the recombinant-DNA debate has a component of this kind of behavior, but there is a more rational component as well.

The first round of the fateful debate began in 1974, when investigators at the leading edge of work in this field declared a voluntary moratorium on several types of experiment judged to be conceivably risky. A set of techniques had been developed that made it possible to cut the long, threadlike molecules of DNA into pieces with the aid of certain enzymes, to recombine the resulting segments of DNA with the DNA of a suitable vector, or carrier, and to reinsert the recombinant into an appropriate host cell to propagate and possibly to function.

The significance of the new developments is rooted in the central biological role of DNA as the transmitter of genetic information between generations. The transmission of the encoded genetic message depends on the ability of a cell to generate exact replicas of the parental DNA and to allocate the replicas among the offspring. In addition the success of genetic transmission depends on the ability of the offspring to "express" the

GENETIC CODE of an extremely small bacterial virus, the bacteriophage designated φX174, is given by the sequence of letters on the opposite page. The letters stand for the four nucleotides cytosine, guanine, adenine and thymine, which are linked end to end to make up each strand of the normally double-strand DNA molecule. The genetic message embodied in each strand of DNA is represented by the particular sequence of nucleotides, any one of which may follow any other. In the φX174 virus the DNA molecule, which has only a single circular strand for part of its life cycle, consists of approximately 5,375 nucleotides; the nucleotides are grouped into nine known genes, which are responsible in turn for coding the amino acid sequences of nine different proteins. For example, the dark-color segment of the molecule, called gene *J*, codes for a small protein that is part of the virus; this segment also happens to be the shortest gene in the φX174 genome. The complete nucleotide sequence for the DNA in φX174 was worked out recently by Frederick Sanger and his colleagues at the British Medical Research Council Laboratory of Molecular Biology in Cambridge. About 2,000 pages of this type would be required to show the nucleotide sequence for the DNA in the chromosome of a typical single-cell bacterium; roughly a million pages would be needed to similarly display the genetic code embodied in DNA molecules that make up chromosomes of a mammalian cell.

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encoded information properly by referring to it to control essential life processes. The mechanism of genetic expression in higher organisms is at present only dimly understood, and the discovery of the new recombinant-DNA techniques seemed immediately to open a broad new avenue to increased knowledge in this field.

The detailed mechanisms of genetic replication and expression are enormously complex. The essence of the matter, however, is found in the famous "double helix" structure of DNA. Both of the two long, interwound and complementary strands of the DNA molecule are made up of four kinds of nucleotides, cytosine, guanine, adenine and thymine (abbreviated *C, G, A* and *T*), which are linked end to end like a train of boxcars. The genetic message of each strand is embodied in the particular sequence of nucleotides, any one of which may follow any other. For example, the sequence *CATTACTAG* contains five identifiable English words: *CAT, AT, TACT, ACT* and *TAG*. The genetic message, however, is "written" in triplets: *CAT, TAC* and *TAG*. In general each triplet "codon" determines, through a series of intermediate steps, the position of a specific amino acid in a protein molecule.

Proteins, like nucleic acids, can be visualized as long trains of boxcars coupled end to end; here, however, the subunits are amino acids rather than nucleotides. The sequence of nucleotides in a given DNA molecule determines the sequence of amino acids in a particular protein, with each triple-nucleotide codon placing one of 20 possible amino acids at each successive position in the protein chain. The sequence of amino acids in turn specifically establishes both the structure and the function of the protein. Thus the nucleotide sequence of DNA precisely specifies the protein-building properties of the organism. Moreover, virtually every property of the organism, from enzymatic action to eye color, depends on protein structure in one way or another.

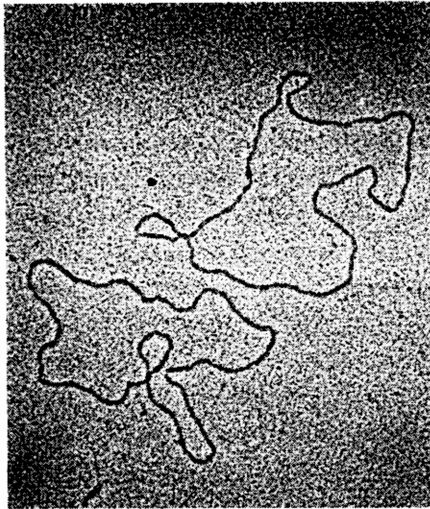
The transmission of the essential genetic information between generations depends on the precise replication of the nucleotide sequences of DNA. The mechanism for replication stems from the complementary relation between the two strands of the DNA molecule. A sequence on one strand (for example *CATTACTAG*) lies immediately opposite a complementary sequence (*GTAATGATC*) on the other strand. The strands are complementary because *C* and *G* are always opposite on the intercoiled strands, as are *A* and *T*. Complementarity depends on the special chemical affinity, or binding, between *C* and *G* on the one hand and *A* and *T* on the other. The sum of these bonds, repeating along the length of the strands, is what holds the strands together in the double helix. Under appropriate conditions affinity is reduced and the two strands can unwind and separate. The single strands can again pair and rewind when conditions for high affinity are restored.

Double-strand DNA replicates by means of an extension of these properties. The unwinding and separation of the strands begins at a localized site along the DNA molecule. In the presence of suitable enzymes and free nucleotides a new chain is formed next to the exposed portion of each unpaired older chain. Each nucleotide lines up next to its opposite number (*C* next to *G*, *A* next to *T*). The complementary sequence thus established is then linked end to end by an enzyme that closes the nucleotide couplings. When the replication process has traveled along the entire length of the original double helix, two new helixes identical with the first one have been formed. The replication of DNA is the most fundamental chemical reaction in the living world. It fully accounts for the classical first principle of heredity: like begets like.

If DNA replication always worked without error, life would be far more homogeneous than it is. Here, however, a second classical principle of heredity intervenes: the principle of mutational variation, or the appearance in the offspring of new hereditary characteristics not present in the progenitors. Mutations arise through error, at least partly in the replication process. For example, the substitution of one nucleotide by another changes the triplet codon and puts a different amino acid in the corresponding position in the resulting protein. Single-nucleotide errors lead to single-amino-acid errors. Thus, a single-nucleotide error is responsible for the human disease sickle-cell anemia. Most mutations are not such simple, single-nucleotide exchanges; nevertheless, they correlate directly with altered, transposed or deleted nucleotide sequences in DNA. When these changes appear in a gene (that is, a segment of DNA that codes the amino acid sequence of a particular protein), a change in the protein and hence in the hereditary properties it controls is the result.

Therein lies the crux of recombinant-DNA technology. It makes possible for the first time the direct manipulation of nucleotide sequences. Changes in nucleotide sequence that are produced by "natural" errors are random, even when their overall frequency is artificially increased. In natural populations Darwinian selection "chooses" among the random errors, increasing the representation in breeding populations of those errors that lead to more offspring in particular environments. Artificial selection, practiced by human beings for millenniums, favors errors that meet human needs (agricultural breeding) or whims (exotic-pet breeding). The success of both natural and artificial selection, however, is dependent on the random occurrence of desirable mutations. There was no way to direct genetic change itself until recombinant-DNA techniques came along. The new techniques enable one to deliberately introduce known and successful nucleotide sequences from one strain or species into another, thereby conferring a desired property.

The recombinant-DNA approach involves experimental ingenuity and detailed knowledge of the DNA molecule. It begins with an attack on DNA by the proteins called restriction enzymes, which are isolated from bacteria. The enzyme attack breaks the double chain

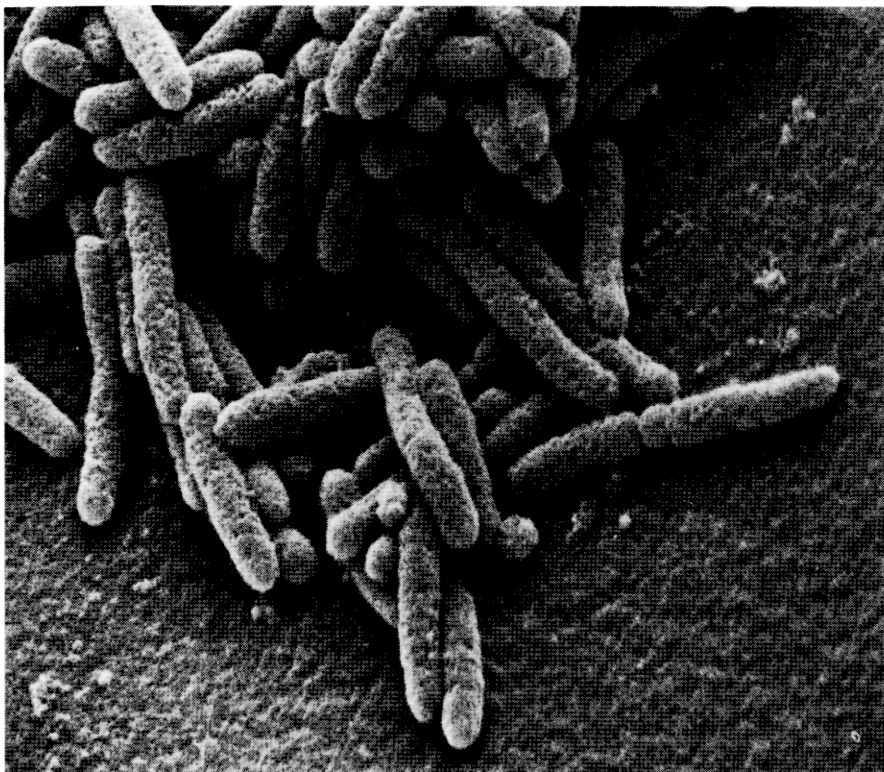


BACTERIOPHAGE ΦX174 AND ITS DNA are portrayed in this pair of electron micrographs. The virus infects the common intestinal bacterium *Escherichia coli*. In the micrograph at left, made by Jack D. Griffith of the Stanford University School of Medicine and Andrew Staehelin of the University of Colorado, two ϕX174 particles are seen attached to surface of an *E. coli* cell. In micrograph at right, made by Griffith, the DNA molecules of two ϕX174 viruses are seen in their double-strand form; each molecule is about 18,000 angstroms long.

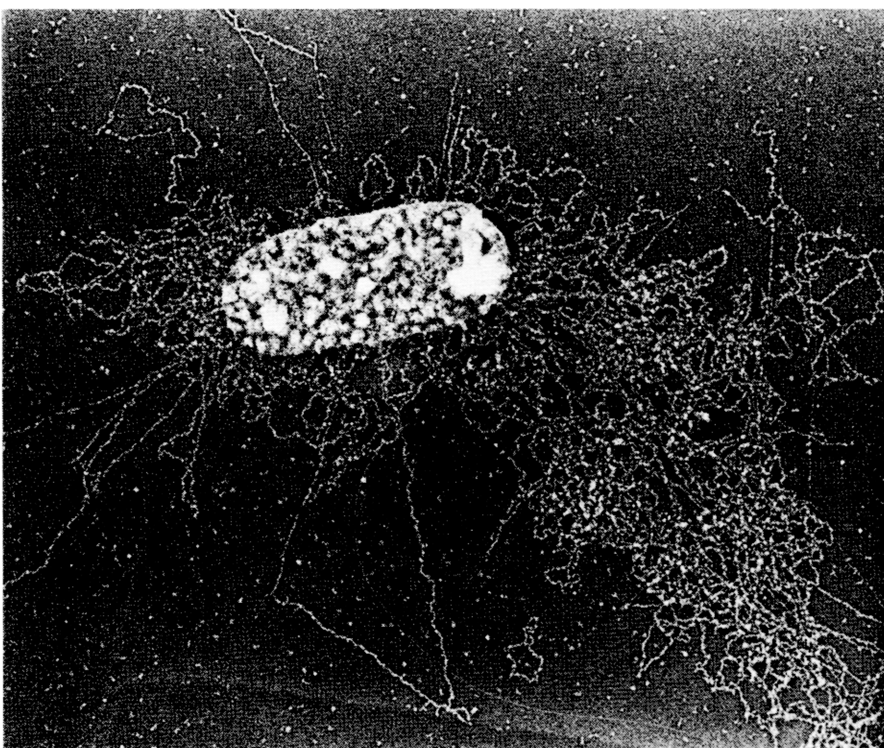
of DNA at particular sequences, say at the sequence *CATTAC*, which is opposite the complementary sequence *GTAATG*. The break does not always occur at the same point on the two strands. It may, for example, be between the two *T*'s in the first strand but just to the right of the *ATG* in the second strand. On separation one piece therefore ends in *TAC*, whereas the other ends in *ATG*. Since the single-strand ends are complementary, they will under suitable conditions stick side by side, and they can then be coupled together end to end. If the same restriction enzyme is used on the DNA from two different sources, both of which have the appropriate target sequence, then sequences with the same "sticky" ends will result. By taking advantage of this stickiness two sequences from any source can be recombined into a single DNA molecule.

The only further step necessary is to put the recombinant DNA into a suitable host organism. The recombinant must have the ability to penetrate the host and become part of its genetic system. An effective way to accomplish this has been developed for the common intestinal bacterium *Escherichia coli*. In addition to its single large circular chromosome the *E. coli* bacterium may have one or more independently replicating, smaller loops of DNA known as plasmids. The plasmids can be isolated from the bacteria, broken open by restriction enzymes and used as one component of a recombinant. After linking up the plasmid DNA with the "foreign" DNA the circular form of the plasmid can be restored and the structure returned to a whole cell. There it can resume replication, duplicating not only its own native sequence but also the foreign one. A strain of bacteria is thus obtained that will yield an indefinite number of copies of the inserted nucleotide sequence from the foreign source.

Standing alone, none of this appears to be particularly momentous or threatening; it is only a new and intriguing kind of chemistry applied to living organisms. Given the complexity of living organisms and the still more complex world of social phenomena, however, this new chemistry quickly builds into varied new potentials, both speculative and real. Suppose, for example, one were to isolate the nucleotide sequence necessary to produce a potent toxin and to transfer it to *E. coli*, usually a harmless inhabitant of every human intestinal tract. Would a dangerous new pathogen be created? Would the transformed *E. coli* release a toxin in the human gut? Might such a new pathogen escape from control and induce epidemics? Questions of this kind have answers, but they take time to find. To gain some time for reflection investigators in 1974 called for a partial and temporary moratorium on those experi-



PILE OF E. COLI CELLS appears in this scanning electron micrograph made by David Scharf. Some of the cells have been caught in the act of asexual reproduction (cell division); a few appear to be transferring their DNA by means of the threadlike connection characteristic of the process known as conjugation. *E. coli* bacteria are considered by most investigators to be most suitable host cells for recombinant-DNA experiments. Magnification is 11,000 diameters.



E. COLI SPEWS OUT DNA through its chemically disrupted cell wall in this electron micrograph by Griffith. Most of the DNA is in the form of a single large molecule of double-strand DNA, which constitutes the chromosome of this simple prokaryotic organism. In addition the *E. coli* bacterium may have one or more of the independently replicating loops of DNA known as plasmids; one of these smaller extrachromosomal DNA molecules can be seen near the bottom. Plasmids derived from *E. coli* cells play an important role in recombinant-DNA research, since they form one class of vectors, or carriers, into which segments of "foreign" DNA can be spliced prior to their being reinserted into an appropriate host cell to propagate, thereby duplicating not only their own native nucleotide sequence but also the foreign sequence.

ments thought to be potentially the riskiest. The separation of the certainly safe experiments from the less certainly safe ones became the chief function of the guidelines released by the NIH in June, 1976. The guidelines, which replaced the temporary moratorium, were derived from worst-case analyses of various kinds of experiments; the object was to evaluate the possible range of hazards and to prescribe appropriate matching safeguards in order to minimize the unknown risks. The guidelines assigned heavy responsibility to individual investigators, and they buttressed this responsibility with special monitoring committees in the sponsoring institutions and in the funding agency.

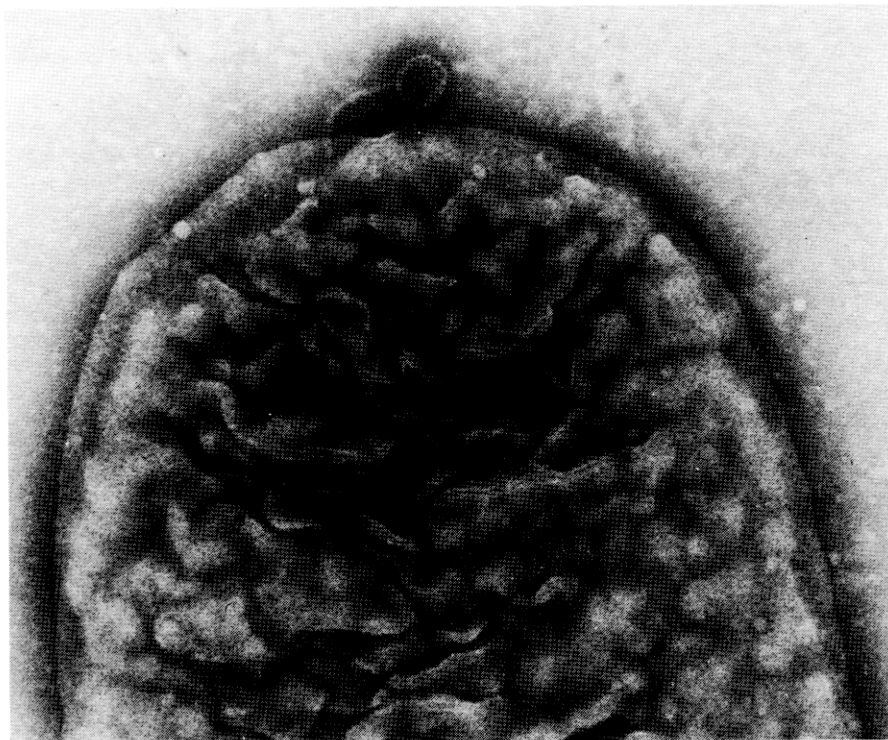
If such regulations have been adopted, why is debate continuing? Briefly, it is because the matching of estimated risk and prescribed containment adopted by the guidelines is regarded by critics as being inadequate in dealing with potential biohazards and incomplete in failing to address other important issues. The most vocal critics have presented their own worst-case analyses in the scientific and general press. These accounts have led to widespread alarm and to public-policy deliberations at the level of local communities, states and the Federal Government. The expressed concerns of the critics have generated a revised agenda for what is now emerging as a broadened second round of policymaking.

Potential biohazards and estimated degrees of risk continue to dominate the debate. The NIH guidelines balance the estimated risk of a given experiment and recommend specific measures for containing the risks. (Risk, it must be remembered, means possible danger, not demonstrated danger.) Those experiments judged to present an excessive risk are entirely proscribed. At the other end of the spectrum experiments judged to present an insignificant risk require only the safeguards of good laboratory practice. Between these extremes the guidelines establish various levels of estimated risk and prescribe combinations of suitably increasing physical and biological containment. The release into the environment of any recombinant organisms is forbidden.

Unfortunately, given the growing but still limited state of knowledge, wide disagreement is possible, both as to estimated degrees of risk and as to the efficacy of the proposed containment. Some critics project fragmentary information into the inevitable spread of dangerous, newly created organisms, threatening both the public health and the environment. Some defenders project the same fragmentary information to the conclusion that the NIH guidelines are already overly cautious. They believe the actual hazard under existing precautions will turn out to be no greater than that routinely faced in the use of automobiles, jet aircraft and other accepted technologies. The wide range of estimates is possible because of the multiplicity of conceivable experiments and because experience and critical data are inadequate for certainty on many points. One fact that is certain is that no known untoward event has yet resulted from recombinant-DNA research.

What emerges on the new policy agenda, then, is the need for effective policy-oriented research to reduce the current uncertainty as to the risk of particular kinds of experiments. For example, there is dispute over the use of *E. coli* as a host for recombinant DNA. One side argues that scientists must be mad to pick a normal human inhabitant (and a sometime human pathogen) to serve as a host for recombinant DNA. This view, in extreme form, demands the suspension of all recombinant-DNA research until an organism safer than *E. coli* can be found. The other side argues (1) that the vast amount of information available on *E. coli* makes it invaluable, (2) that the *K*-12 strain of *E. coli* actually used in laboratory research has been so modified genetically in adapting to laboratory conditions that it survives only with difficulty in the human intestine and (3) that new strains of *K*-12 have been developed with additional genetic deficiencies that will make survival outside of laboratory conditions essentially impossible. The use of such genetically deficient strains is what is meant by the term "biological containment." The concept is supported by proponents of the research as an efficacious new approach to safety and derided by critics as likely to be circumvented by natural recombination.

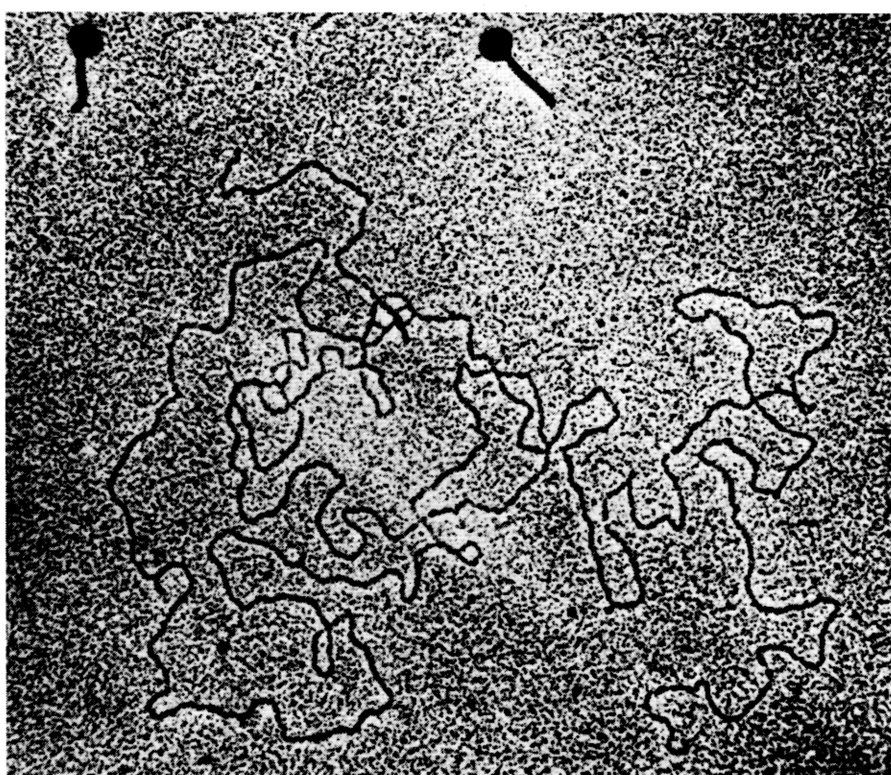
Such differences of opinion are normally reduced by scientists to experimental questions. For example, the suitability of the *K*-12 strain of *E. coli* as an experimental organism can be judged only from the effect of recombinant genes on the ecological relations of *E. coli* within the human intestine, including the degree of success of recombinant strains in competing with other strains of *E. coli* and with other organisms. Information on these matters is growing. Such questions, however, are not normally subjects of profound scientific interest. They have recently become matters of priority only because they may provide information that would be useful in arriving at a policy decision. Research on policy-oriented questions has never had a very high status among scientists engaged in basic research or even among those engaged in applied research. Therefore policy-oriented research must be encouraged through special funding mechanisms and through suitable new institutional arrangements. A regulatory agency for recombinant-DNA research and other conceivably hazardous kinds of research is urgently needed outside the NIH, and it should include a research component. The Center for Disease Control and its National Institute for Occupational Safety and Health come



BACTERIAL VIRUS IS ATTACHED to the wall of an *E. coli* cell in this electron micrograph made by Maria Schnoss of the Stanford School of Medicine. This particular virus, named bacteriophage lambda, normally infects the bacterium by injecting its DNA into the host cell through a long taillike appendage. The magnification is approximately 140,000 diameters.

to mind as possible models for such a dual-purpose agency.

Also related to the question of biohazards is a controversy over the desirability of centralizing recombinant-DNA research facilities. Some of those who fear severe dangers from recombinant organisms have urged that the potentially more hazardous research be concentrated in remote places with extremely stringent containment procedures. Those who minimize the hazard are opposed to the concentration concept because it would tend to separate the research from the intellectual mainstream and would be unnecessarily expensive in facilities. The argument has been particularly strenuous with respect to experiments requiring *P*3 facilities, which are defined as those necessary to contain "moderate risk" experiments. *P*4 facilities for "high risk" experimentation are expected to be fewer in number because of their high cost; generally speaking they are likely also to be comparatively isolated. The current NIH guidelines provide little direction in these matters. A decision on a firmer policy belongs on the discussion agenda. Particularly urgent is careful consideration of such intermediate possibilities as the use of centralized, high-risk facilities for making particular recombinations for the first time. These activities, together with preliminary testing of new recombinants for possible hazards, might also be carried out by the proposed new regulatory agency.



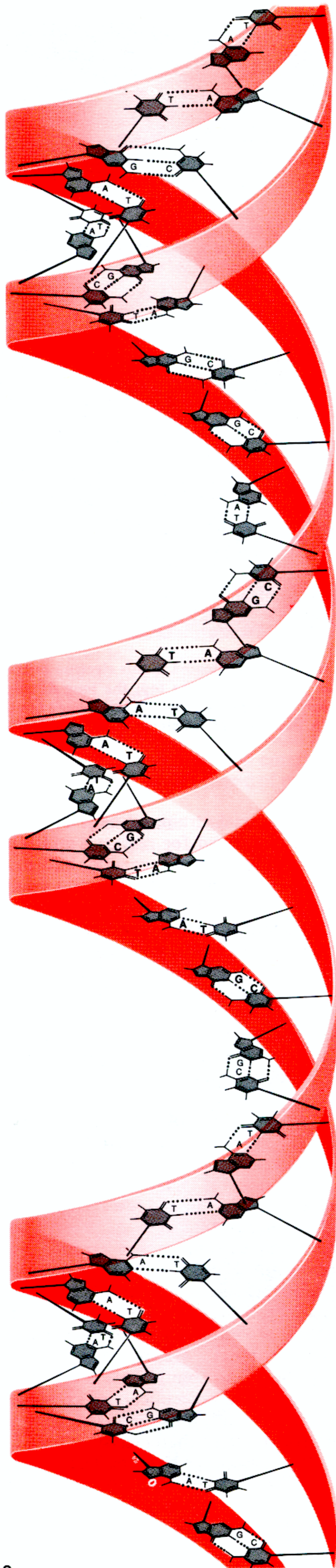
BACTERIOPHAGE LAMBDA AND ITS DNA are both represented in this electron micrograph provided by Griffith. Two complete lambda viruses are at the top; the long double-strand DNA molecule of a disrupted lambda is below them. DNA from bacteriophage lambda can also serve as a vector for recombinant-DNA experiments involving *E. coli* host cells.

A special case that emphasizes the advantages of initial testing in a central facility is provided by what are called "shotgun" experiments. These experiments, which offer special advantages to the investigator, may also present special hazards. Shotgun experiments involve exposing the total DNA of a given organism to restriction enzymes in order to obtain many DNA fragments. The fragments are then each recombined with DNA from a suitable vector and the recombinants are randomly reinserted into *E. coli* host cells. The next step is to spread the *E. coli* cells on a nutrient substrate so that each recipient cell, containing a particular inserted foreign sequence, grows into a colony. If the experiment is successful, the yield is a "library" of all the nucleotide sequences of a particular organism, each sequence growing in a separate strain and accessible to manipulation and cross-combination at will.

This experimental approach is laborious but far less so than anything else available for the exploration of the complex genetic systems of higher organisms. There is, however, a risk of unknown magnitude that portions of the DNA with unknown or repressed functions might duplicate and create unanticipated hazards. The result might be particularly unfortunate if the original DNA preparation were to contain genetic material from parasites or from viruses associated with the species under study. Under the NIH guidelines, therefore, shotgun experiments are regarded as being more dangerous than those involving purified and characterized DNA. Experiments in this category are treated as being increasingly more dangerous as the test organism under study is biologically more like the human organism. Thus experiments with primate DNA are considered to be more dangerous than experiments with mouse DNA. This approach appears to represent a reasonable precaution with respect to human health hazards, but it is less reasonable with respect to potential ecological effects. For example, shotgun recombinants involving DNA from plant sources could conceivably lead to ecologically dangerous effects if they were to escape into the environment. Shotgun procedures might therefore be best conducted first in special centralized facilities that could also act as storage and distribution centers for the recombinant products once they had been tested for safety.

These examples suggest several advantages for the creation of a Center for Genetic Resources. The center might not only carry out DNA recombinations suspected to be hazardous but also function to preserve genetic information contained in threatened natural species and in special strains of cells or organisms developed for research and other purposes. Stored genetic information can be expected to be increasingly important in the future. For example, new genetic infusions into domesticated stocks of plants and animals from their wild progenitors have long been used to strengthen the response of the domesticated stocks to changing conditions of husbandry. The sources of wild progenitors are threatened by the reduction of wild habitats all over the world.

The possibility of a biohazard need not arise only as a by-product of basic research. The practical applications of recombinant-DNA techniques, together with the applied research and development leading to them, are at least equally likely sources. For example, recombinant techniques may enormously expand the use of bacteria (and other microorganisms) for the production of certain proteins and other pharmacological products. Microorganisms have long played an essential role in the food, beverage, pharmaceutical and chemical industries, and more precise genetic control of their characteristics has already yielded large benefits. The recombinant-DNA techniques not only offer advances on current practice but also suggest a new realm of "bacterifacture" in which the rapid, controlled growth of microorganisms is coupled to the pro-



duction of specific products normally made only by higher organisms. Included among the possibilities are the production of insulin, blood-clotting factors and immunological agents. The probability of those possibilities ever being realized is no more easily assessed than the risks, but success in realizing them clearly could provide substantial economic and social benefits. Accordingly entrepreneurial interests have been aroused.

The NIH guidelines are silent on the matter of commercial applications other than stipulating that large-scale experiments (beyond production batches of 10 liters) with recombinants "known to make harmful products" be prohibited unless specially sanctioned. The guidelines also require detailed reporting of proposed recombinant-DNA experiments, a provision that runs counter to the protection of proprietary interest. There have been discussions of these matters between the NIH and representatives of industry. In addition industry spokesmen have testified at Congressional hearings. It is known that some industrial research already is under way and that representatives of industry generally endorse the precautionary approach of the NIH guidelines, but they are resistant to limitations on proprietary rights and on the size of batch production. Moreover, patent policy has come up as an issue and there has been some uncertainty in the Department of Commerce as to how it should be handled. Indeed, the possible commercial applications of recombinant-DNA techniques have yet to be publicly evaluated as a serious policy question, and they must be high on the agenda of the next round of discussions.

The problems of commercial applications lead from immediate issues to broader ones and to a larger time frame. Recombinant-DNA techniques have revived the debate over "genetic engineering" and have once again raised questions about the applications of fundamental biomedical research to technology, to the quality of life and to the future of society. Recombinant DNA has now joined nuclear fission, overpopulation, famine and resource shortages in the doomsday scenarios of "creative pessimism." These issues are even more difficult to deal with objectively than those related to potential biohazards, but they are plainly apparent in the general public discussion and in the public statements of respected scientists.

For example, Robert L. Sinsheimer of the California Institute of Technology has persistently raised issues that are in part practical and in part philosophical. Along with George Wald of Harvard University and Erwin H. Chargaff of the Columbia University College of Physicians and Surgeons, he suggests that the entire recombinant-DNA approach to gaining an understanding of the complexities of higher genetic systems is misbegotten. The argument is not that the approach may not work but that its alleged huge risks are unnecessary because less risky, although slower, means are available. Sinsheimer emphasizes the fundamental difference between simple prokaryotic organisms such as bacteria and complex eukaryotic organisms, including human beings. Prokaryotes, typically one-cell organisms, have a single, comparatively simple chromosome floating freely within the cell body, whereas eukaryotic cells have a nucleus that is bounded by a membrane and contains a number of far more complex chromosomes. The paleontological record suggests that prokaryotes existed on the earth for a billion or more years before the more complex eukaryotes arrived on the scene. Sinsheimer proposes that throughout the evolution of the eukaryotes there has been a genetic barrier between them and the prokaryotes, behind which eukaryotes have developed their more complex mechanisms of genetic control. To transfer these mechanisms, which are possibly the key to the evolutionary success and enormous diversity of eukaryotes, to prokaryotes may introduce, he says, incalculable evolutionary dangers. The prokaryotes may be made far more effective, both as competitors and as parasites, negating an ancient evolutionary strategy.

Sinsheimer's argument has won only a few vocal adherents among biologists, and he himself concedes that it is speculative. Nevertheless, his argument

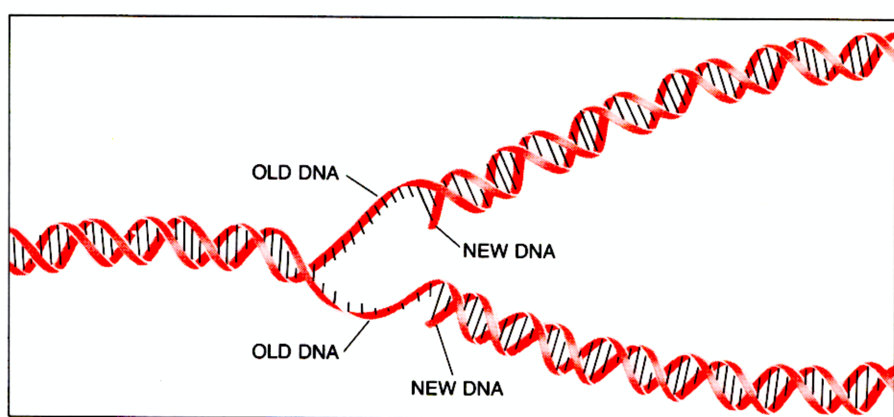
DOUBLE-HELIX STRUCTURE OF DNA is evident in this simplified diagram of a short segment of the deoxyribonucleic acid (DNA) molecule. The sugar and phosphate groups that are linked end to end to form the outer structural "backbones" of the double-strand molecule are represented schematically here by the two helical colored bands. The inner portion of each polynucleotide chain, drawn in somewhat greater detail, consists of a variable sequence of four kinds of bases: two purines (adenine and guanine, or *A* and *G*) and two pyrimidines (thymine and cytosine, or *T* and *C*). The two chains, which run in opposite directions, are held together by hydrogen bonds (*dotted black lines*) between pairs of bases. Adenine is always paired with thymine, and guanine is always paired with cytosine. The planes of the bases are perpendicular to the common axis of the two helixes. The diameter of the double helix is 20 angstroms. Adjacent bases are separated by 3.4 angstroms along the axis and are displaced successively around the axis by an angle of 36 degrees. The structure therefore repeats after 10 bases on each chain (360 degrees), or at intervals of 34 angstroms. The genetic information is stored in the sequence of bases along each chain. In this case the sequence *CATTACTAG* on one strand is identified in boldface type opposite complementary sequence *GTAATGATC* on other strand.

has attracted significant public attention, and it is widely cited to support opposition to continued recombinant-DNA research. Bernard D. Davis, a Harvard Medical School microbiologist, has provided a rebuttal, particularly with respect to the concept of a genetic barrier between prokaryotes and eukaryotes. He believes there has been an ample and continuous opportunity for the exchange of DNA between the two groups. He points out that bacteria can take up naked DNA from their immediate environment and that *E. coli* would be exposed to such DNA arising from dead human cells in the human intestine. Microorganisms might similarly take up DNA in the process of decomposing dead animals. Therefore, Davis argues, most recombinants probably have already been tried in the natural evolutionary arena and have been found wanting. Reasoning on analogy with extensive information on pathogenic bacteria, Davis concludes that under the existing NIH guidelines the probability for survival in nature of laboratory-produced prokaryote-eukaryote recombinants is vanishingly small.

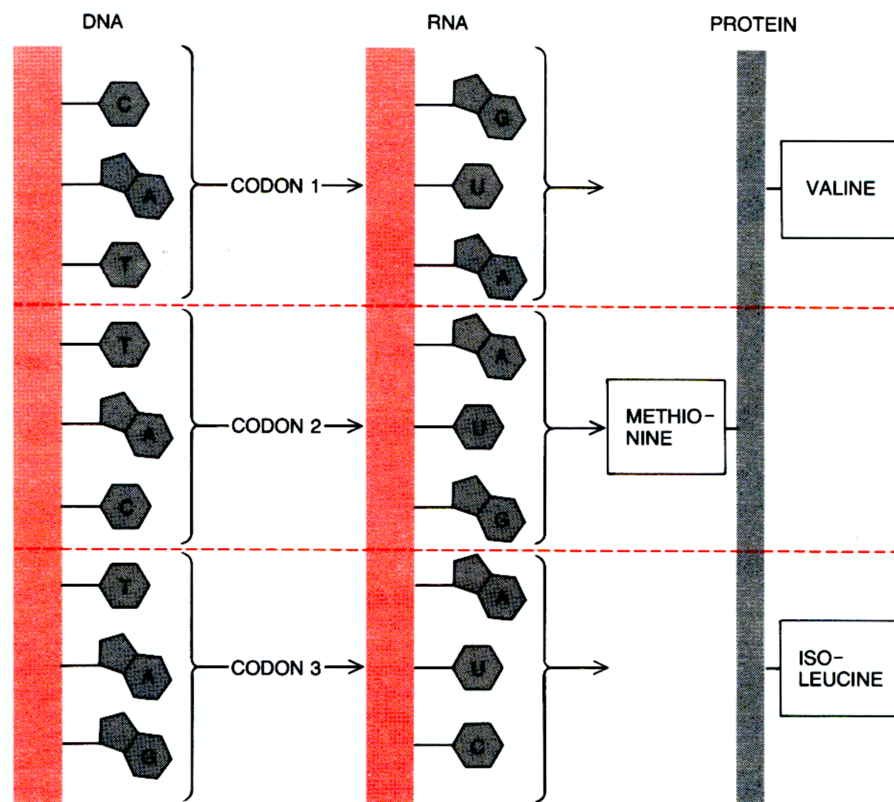
This clash of opinion on a major biological issue illustrates the difficulty of assessment of even comparatively value-free questions when critical information is fragmentary. The controversy over the risk-benefit ratio becomes even more intense when issues involve substantial value judgments as well. Here again a concern of skeptics and opponents of recombinant techniques is sharply articulated by Sinsheimer. He asks: "Do we want to assume the basic responsibility for life on this planet? To develop new living forms for our own purposes? Shall we take into our own hands our own future evolution?" Since the questions include such concepts as responsibility, purpose and control of the future, they clearly involve considerations beyond science alone.

The human species has, of course, been altering life on this planet from the beginnings of human culture. When hunting and gathering gave rise to animal husbandry and agriculture, human choice and purpose began to influence the evolution of selected species. Unconscious human selection was replaced by deliberate plant and animal breeding, and the further development of human culture is now clearly altering the entire ecosystem. Moreover, the biocultural progression of the human species, based partly on human purpose, is undoubtedly altering the human gene pool and will slowly modify the species in unpredictable ways. Nevertheless, the advent of recombinant-DNA techniques has obviously enhanced the prospects for genetic engineering and has restressed the need to assess its implications. Can it be assumed that success in introducing recombinant DNA's into *E. coli* means that there will be similar success in introducing them into the human species? If it can, what is the probable time frame for applying the technique to the human species? Is it accurate and responsible to suggest that we have almost in hand control of "our own future evolution"? These certainly are questions for scientific assessment, and they should have a prominent place on the new policy agenda.

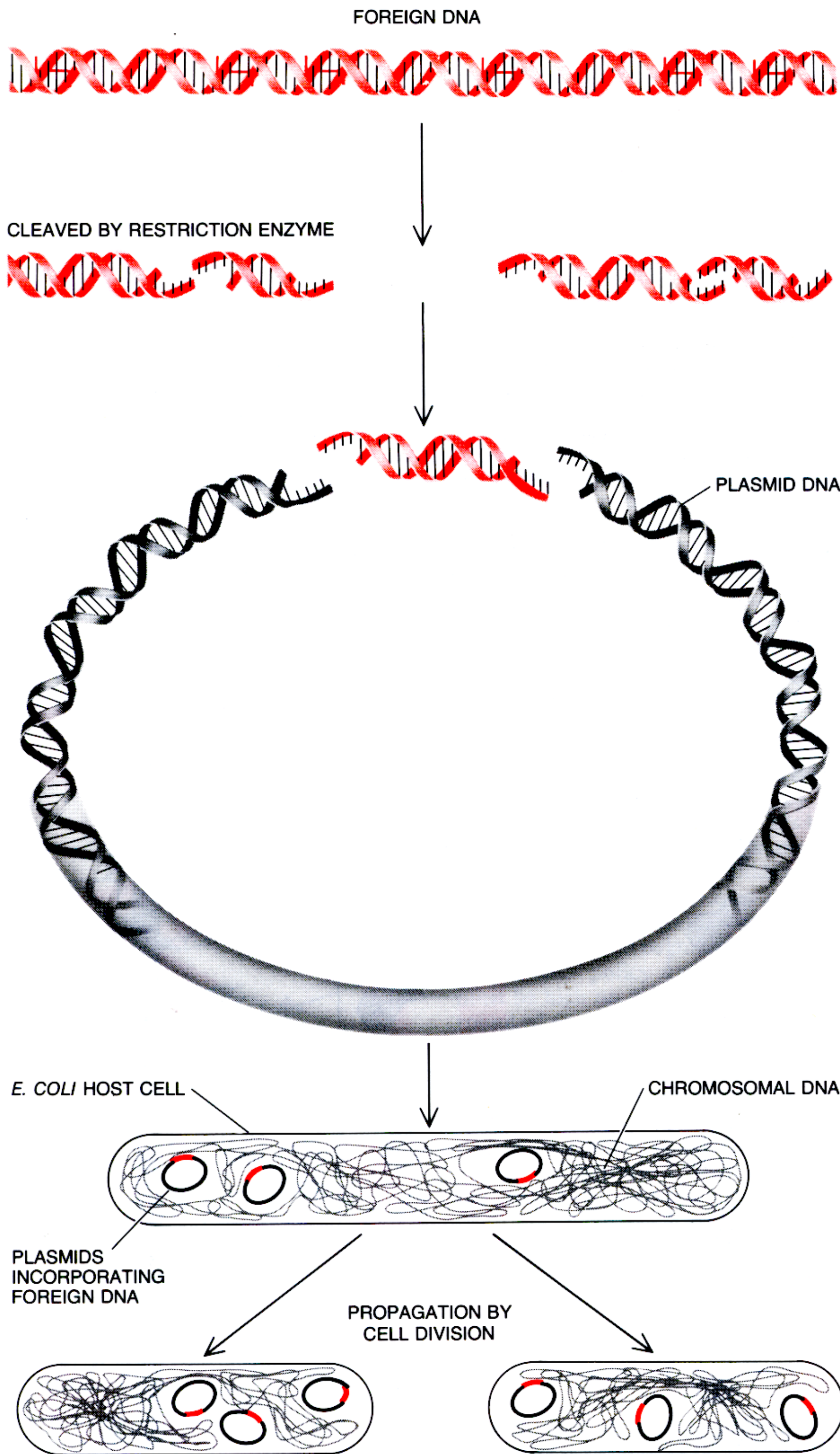
Sinsheimer has gone into still another controversial area, not only for the scientific community but also for the entire



REPLICATION OF DNA depends on the complementary relation between the nucleotide sequences on the two strands of the DNA molecule. Under appropriate chemical conditions the hydrogen bonds between the bases are weakened and the two strands can unwind and separate. In the presence of suitable enzymes and free nucleotides a new chain can be formed next to the exposed portion of each unpaired older chain. The complementary sequence that is formed by each nucleotide lining up next to its opposite is then linked end to end by an enzyme that "zips up" the nucleotide couplings. In this way two new helixes identical with the first can be formed.



ROLE OF DNA IN PROTEIN SYNTHESIS is suggested by this highly schematic diagram. The genetic message contained in the nucleotide sequence *CATTACTAG*, for example, is "written" in the form of the triplet "codons" *CAT*, *TAC* and *TAG*. Each codon determines, through a series of intermediate steps involving a molecule of ribonucleic acid (RNA), the position of a specific amino acid in a protein molecule. Thus the sequence of nucleotides in a given DNA molecule specifies the corresponding sequence of amino acids in a particular protein, with each triple-nucleotide codon placing one of 20 possible amino acids at each successive position in the protein chain. Since the sequence of amino acids in turn establishes both the structure and the function of the protein, the nucleotide sequence of DNA determines virtually every property of organism. Letter *U* stands for the pyrimidine uracil, a constituent of RNA.



RECOMBINANT-DNA TECHNIQUE makes it possible for the first time to deliberately introduce nucleotide sequences from the DNA of one strain or species of organism into the DNA of another. The DNA of the "foreign" organism is first treated with restriction enzymes, which cleave the double-strand molecule at particular nucleotide sequences (typically thousands of base pairs apart) on a random basis. The same enzyme is then used to cleave the DNA of a suitable vector, in this case a plasmid isolated from *E. coli* bacteria. Since the break caused by the enzyme does not occur at the same point on both strands, the chemical treatment results in a mixture of DNA segments that have complementary single-strand ends. Under suitable conditions the "sticky" ends of two different sequences can be coupled to form a single DNA molecule. For example, after recombining the foreign DNA with the plasmid DNA the circular form of the plasmid can be restored and the structure can be inserted into a suitable host cell (in this case *E. coli*), where the plasmid can resume replication, thereby propagating an indefinite number of "cloned" copies of the inserted nucleotide sequence from the foreign source.

society. Arguing that time may be needed to "pace" new genetic knowledge to human capacities for putting nature to intelligent use, he wonders whether "there are certain matters best left unknown, at least for a time." This is high heresy in the scientific community, whose fundamental premise is that the growth of knowledge is the driver and not the captive of other values. The rejection of the concept of "forbidden knowledge" was part of the heroic period at the beginning of modern science, when it included willingness to face the Inquisition and the stake. Having been seared by the nuclear flame and now confronting the more subtle implications of the innermost language of life, 20th-century scientists fear not the stake but the judgment of history. Chargaff, a pioneer in the investigations that led to the decipherment of the genetic language, says: "My generation, or perhaps the one preceding mine, 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."

Sinsheimer and Chargaff, along with a number of philosophers, historians and sociologists of science, are clearly suggesting that the possible consequences of knowing must be consciously included in decisions about the directions of the search for knowledge itself. No issue cuts more deeply to the core of modern science. The self-doubt expressed by some scientists reflects a general questioning in the U.S. of the net benefits of science and technology. Cost-benefit analysis is a current preoccupation, and it is being increasingly applied to the generation of knowledge itself. It is hard enough to assess what we may gain or lose from particular new knowledge; it is even harder to assess the costs of not having it. This problem is epitomized by the recombinant-DNA controversy. The rise of molecular genetics in the U.S. is the direct product of a series of decisions made after World War II that provided funds for biomedical research. The objective was the conquest of the "killer" diseases: cancer, heart disease and stroke. Those diseases are still much with us, although they are better understood and cared for. Meanwhile, out of Federally supported research also came the impetus that led to the discovery of the double helix, the genetic code, the structure of proteins and recombinant DNA. In a classic "double take" the public is now asking whether it has been buying health and well-being or chimeric monsters. Is molecular genetics and all biomedical technology a sorcerer's apprentice? Are we increasing rather than lessening our burden of pain and anxiety?

The last question leads to yet another issue. Biohazard and ecohazard may arise inadvertently, but "sociohazard" may be the product of deliberate malev-

olence. The U.S. is a signatory to an international legal convention that has renounced biological warfare, including research to produce the necessary agents. Not all countries have taken this step, and public renunciation without adequate inspection cannot ensure that covert activities do not exist. Opponents of recombinant-DNA research see its techniques as being ideally suited to serve malevolent purposes, either as agents of organized warfare or of sabotage and terrorism. The techniques do not require large installations or highly sophisticated instrumentation. Contrary views have not denied this but have noted that recombinant-DNA techniques would not be the first technology to have potential malevolent applications. Explosives have such applications, but society does not completely ban them; it takes prudent precautions against their misuse.

Nevertheless, the issue of the possible misuse of recombinant-DNA technology deserves a place on the policy agenda, because it emphasizes the need for international discussion of the implications and management of recombinant-DNA research and recombinant-DNA applications. It can be argued that the U.S. is not ready for such discussion until its own policies are in better order. It is not too early, however, to begin the internal consideration of how best to approach the international arena.

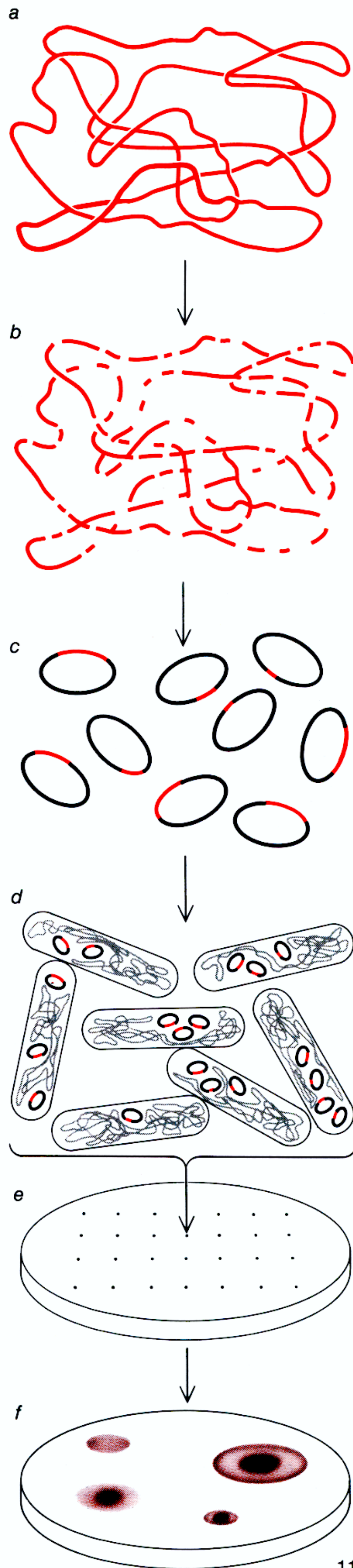
These are the chief issues that have emerged from the policy debate so far. It is a not inconsiderable list. The debate has not been raging on every street corner, but it became strenuous enough in Cambridge last summer to have repercussions across the continent. For example, an evaluation presented by a panel of nonscientists to the Cambridge City Council was not too different in content from one produced by a task force of the Quality of Life Board of the City of San Diego, where I live. Both groups accepted within their community the continuance of recombinant-DNA research requiring *P*3 facilities but sought somewhat greater assurances of safety than those provided by the NIH guidelines. Meanwhile the Attorney General of the State of New York held a public hearing, and a joint hearing was conducted by two committees of the Assembly of the State of California Legislature. Legislation regulating DNA research was later introduced in the California Assembly, and it is still under consideration. Congress has also held several hearings and various items of regulatory legislation have been introduced in both the Senate and the House of Representatives. These local, state and Federal initiatives emphasize the necessity to get on with the policy agenda.

The agenda should be viewed in at least two time frames: immediate and longer range. A consensus has been growing that there is an immediate need to give the quasi regulation represented by the NIH guidelines a statutory base. In particular, regulation must be extended to activities not supported by Federal agencies, especially in the industrial sector. However this is to be done, it is important to maintain flexibility, since the problems to be dealt with will change as greater knowledge and experience are acquired.

Moreover, given the complexity of the longer-term issues, immediate legislation probably should be provisional and limited. A mechanism should be included, however, that actively leads toward a more definitive future policy. This requires provision for a new, comprehensive assessment of all the issues raised by recombinant-DNA research, including the probable effectiveness of the regulatory devices put in place under the NIH guidelines.

The need for such a new national assessment is demonstrated by the nature of the critical challenge to the product of the earlier assessment. First, it has been alleged that the 1975 Asilomar conference establishing the pattern for the NIH guidelines was dominated by scientists involved in the research, and therefore it could not yield a broad enough perspective. Second, it is argued that the earlier assessment was devoted primarily to the question of potential biohazards and did not address in any depth other gravely important questions. The passage of time has added several more points: that circumstances already have changed as research has progressed, that experience has grown and that a wider range of opinion has come to bear on the issue. Whatever format is adopted for the reappraisal of the recombinant-DNA issue, the public must be assured that the process is a comprehensive and objective one.

Whoever undertakes this new national review should first carefully examine the current situation, including the actual effectiveness of the regulatory mecha-



"SHOTGUN" EXPERIMENT is a type of recombinant-DNA experiment in which the total DNA of an organism (*a*) is exposed to restriction enzymes in order to yield many fragments (*b*), which are then recombined with the DNA from a suitable vector (*c*) and randomly reinserted with the vector into the host cells (*d*). The *E. coli* hosts are next spread on a nutrient substrate (*e*) so that each recipient cell, containing a particular inserted foreign nucleotide sequence, can grow into a colony (*f*). The result, if the experiment is successful, is a "library" of all the nucleotide sequences of the organism. Under the guidelines issued by the National Institutes of Health last year shotgun experiments are regarded as being potentially more hazardous than those involving purified and characterized DNA, since it is not known whether portions of the DNA with unknown or repressed functions might cause unexpected problems.

nisms provided by the NIH guidelines. Particular attention needs to be paid to the local institutional biohazards committees mandated by the NIH guidelines. Beyond the responsibility assigned to the principal investigator these committees are the only source of local surveillance and standard-setting. Their composition and charge are unique, yet their authority and procedures are stipulated only generally in the NIH guidelines. They may well need the stimulus and support of external interests to carry out their important task. Moreover, no provision has been made for budgeting what may turn out to be their considerable cost for technical surveillance, personnel training and medical monitoring. Like all insurance, security against biohazard must be bought. The cost should be borne as an additional expense of the research, not as a competitor for existing funds.

Similarly, the actual performance of the NIH study sections, which are mandated by the guidelines to be independent evaluators of biohazards and containment, needs to be examined. Study sections are already heavily overloaded with the job of evaluating scientific quality. Yet these part-time peer groups are asked to assume another difficult function. If the responsibility is to be taken seriously, it too will entail additional costs.

Of special importance for early attention is an effective monitoring system for following the actual directions of recombinant-DNA research. The techniques involved are so rich in possibilities, whether for fundamental research or applications, for benefit or risk, that "early warning" is essential. Systematic following of the directions of investigators' interests, from applications for support through informal communication to formal publication, is essential to the early detection and assessment of either risks or opportunities. Needless to say, monitoring is particularly difficult in industrial research. It might therefore be desirable to limit or postpone certain development efforts pending closer study and greater knowledge of the underlying problems.

Equally urgent is a determined effort toward a more effective assessment of risks and their limitation. The specific assignment of responsibility for this kind of policy-oriented research should be an early recommendation of the body undertaking the reassessment. Given the differing perspectives required by regulation and the NIH mission to promote health-related research, the regulatory function probably belongs elsewhere in the long run. On the other hand, given the need for careful study of the implications of relying on existing agencies or of establishing a new one, the temporary continued assignment of this responsibility to the NIH may be desirable. This interim solution, if it is adopted, must be accompanied by additional funding to carry it out effectively.

Considerations of biohazards and physical and biological containment have necessarily had a high priority in this early phase of recombinant-DNA research. Many informed observers believe, however, that these concerns will decline in importance as research continues and experience grows. Therefore although the current furor makes a rational approach to the biohazards question an essential part of any successful recombinant-DNA policy, this approach does not exhaust the longer-term requirements and may even distort them. More crucial in the long run may be several other issues that have been raised directly or indirectly.

For example, in investing in fundamental genetic research that can profit from recombinant-DNA techniques, what relative priorities should be assigned to potential applications? In the past the national strategy in biomedical research has been to invest directly in basic research, without declared objectives, while also investing in specific objectives, allowing some of the latter support to "trickle down" to basic research. Thus an investigator of the interaction of viruses and cells, say, might be alternatively or simultaneously supported by funds for fundamental investigation and by funds intended for promoting the development of an effective therapy for cancer. What should be the priorities among possible practical applications of molecular genetics? Competing lines of inquiry include the microbiological synthesis of drugs, specific human gene therapies, the improved efficiency of photosynthesis, nitrogen fixation by food crops, enhanced agricultural production and so on. There are quite different potential risks and benefits in each of these directions, and all are unlikely to be maximally supported at once. In the new areas that are opening up is a new research strategy called for? If it is, by what procedures should it be formulated and how should it be implemented?

It is widely recognized that there is a logical continuum running from basic research through applied research and development to technological application. It is also recognized that movement along this continuum is neither smooth nor fully predictable and that varying motivations and institutional arrangements operate along its length. Recombinant-DNA techniques are the product of fundamental investigation, supported almost entirely by the partnership of the Federal Government and the universities. For the moment, at least, the techniques are likely to remain useful primarily in that area. The techniques may also be useful for various industrial purposes, however. Given the nature of the original investment as well as the complex issues raised, should technological uses, at least for a time, be kept under Federal control? Should some of the return from successful applications be employed to recycle the original investment of Federal resources? Should this promising new technology be a prototype for establishing a revolving capital fund to support a more stably financed basic-research effort?

The possibilities of genetic engineering and evolutionary control illustrate the fundamental dilemmas raised by the new capabilities conferred by scientific knowledge. Society has entered an age of intervention, in which the automatic operation of natural processes is increasingly, through informed intervention, brought consciously into the orbit of human purpose. Many events that humanity formerly could regard only as a boon or a scourge—an act of God or of nature—are now the partial product of human decision and intervention. If human beings do not have the capability today to invent new organisms or to initiate life itself, they may soon have that capability. If they cannot today consciously and fully control the behavior of large ecosystems, that power is not far beyond what has already been achieved. The humility of individuals understandably shrinks from awesome powers that were earlier assigned to divine will. It was not, however, the humility of individuals that conferred these emerging capabilities or is called on to control them today. It was the social interaction of individuals, operating through social institutions, that brought us to the present fateful decision making. Imperfect though they are, our social institutions built the platform for the age of intervention.

The policy challenge we face, refracted in the exquisite structure and potential of the double helix, is whether we can create institutions able to transform the fruits of an age of reason into the achievements of an age of intervention. There are voices today urging us not only to eschew conscious intervention but also to distrust and limit the uses and consequences of reason itself. Perhaps it needs to be restated that it was, after all, natural selection that evoked the double helix and all it conveys. Included among the products are human knowledge and judgment, to which has now passed the duty of designing social processes and structures that can cope with the manipulability of the double helix itself.

The concept and control of the double helix signal a new frontier of biocultural progression. A stereoscopic vision that includes both "creative pessimism" and "creative optimism" is now required. Neither alone can do justice to the profound revelations human beings have recently experienced. A single eye is particularly limited in yielding depth and perspective. For the age of intervention at least two are needed.

PHYSICAL CONTAINMENT	BIOLOGICAL CONTAINMENT (FOR *E. COLI* HOST SYSTEMS ONLY)		
	*EK*1	*EK*2	*EK*3
*P*1	DNA from nonpathogenic prokaryotes that naturally exchange genes with *E. coli* Plasmid or bacteriophage DNA from host cells that naturally exchange genes with *E. coli*. (If plasmid or bacteriophage genome contains harmful genes or if DNA segment is less than 99 percent pure and characterized, higher levels of containment are required.)		
*P*2	DNA from embryonic or germ-line cells of cold-blooded vertebrates DNA from other cold-blooded animals and lower eukaryotes (except insects maintained in the laboratory for fewer than 10 generations) DNA from plants (except plants containing known pathogens or producing known toxins) DNA from low-risk pathogenic prokaryotes that naturally exchange genes with *E. coli* Organelle DNA from nonprimate eukaryotes. (For organelle DNA that is less than 99 percent pure higher levels of containment are required.)	DNA from nonembryonic cold-blooded vertebrates DNA from moderate-risk pathogenic prokaryotes that naturally exchange genes with *E. coli* DNA from nonpathogenic prokaryotes that do not naturally exchange genes with *E. coli* DNA from plant viruses Organelle DNA from primates. (For organelle DNA that is less than 99 percent pure higher levels of containment are required.) Plasmid or bacteriophage DNA from host cells that do not naturally exchange genes with *E. coli*. (If there is a risk that recombinant will increase pathogenicity or ecological potential of host, higher levels of containment are required.)	
*P*3	DNA from nonpathogenic prokaryotes that do not naturally exchange genes with *E. coli* DNA from plant viruses Plasmid or bacteriophage DNA from host cells that do not naturally exchange genes with *E. coli*. (If there is a risk that recombinant will increase pathogenicity or ecological potential of host, higher levels of containment are required.).	DNA from embryonic primate-tissue or germ-line cells DNA from other mammalian cells DNA from birds DNA from embryonic, nonembryonic or germ-line vertebrate cells (if vertebrate produces a toxin) DNA from moderate-risk pathogenic prokaryotes that do not naturally exchange genes with *E. coli* DNA from animal viruses (if cloned DNA does not contain harmful genes)	DNA from nonembryonic primate tissue DNA from animal viruses (if cloned DNA contains harmful genes)
*P*4		DNA from nonembryonic primate tissue DNA from animal viruses (if cloned DNA contains harmful genes)	

"SHOTGUN" EXPERIMENTS USING *E. COLI K*-12 OR ITS DERIVATIVES AS THE HOST CELL AND PLASMIDS, BACTERIOPHAGES OR OTHER VIRUSES AS THE CLONING VECTORS

EXPERIMENTS IN WHICH PURE, CHARACTERIZED "FOREIGN" GENES CARRIED BY PLASMIDS, BACTERIOPHAGES OR OTHER VIRUSES ARE CLONED IN *E. COLI K*-12 OR ITS DERIVATIVES

SOME EXAMPLES of the physical and biological containment requirements set forth in the NIH guidelines for research involving recombinant-DNA molecules, issued in June, 1976, are given in this table. The guidelines, which replaced the partial moratorium that limited such research for the preceding two years, are based on "worst case" estimates of the potential risks associated with various classes of recombinant-DNA experiments. Certain experiments are banned, such as those involving DNA from known high-risk pathogens; other experiments, such as those involving DNA from organisms that are known to exchange genes with *E. coli* in nature, require only the safeguards of good laboratory practice (physical-containment level *P*1) and the use of the standard *K*-12 laboratory strain of *E. coli* (biological-containment level *EK*1). Between these extremes the NIH guidelines prescribe appropriate combinations of increasing physical and biological containment for increasing levels of estimated risk. (In this table containment increases from upper left to lower right.) Thus physical-containment levels *P*2, *P*3 and *P*4 correspond respectively to minimum isolation, moderate isolation and maximum isolation. Biological-containment level *EK*2 refers to the use of new "crippled" strains of *K*-12 incorporating various genetic defects designed to make the cells' survival outside of laboratory conditions essentially impossible. Level *EK*3 is reserved for an *EK*2-level host-vector system that has successfully passed additional field-testing. Because of the very limited availability of *P*4 facilities and because no bacterial host-vector system has yet been certified by the NIH as satisfying the *EK*3 criteria, the recombinant-DNA experiments now in progress in the U.S. with *E. coli* host systems are with a few exceptions limited to those in the unshaded boxes. Experiments with animal-virus host systems (currently only the polyoma and SV40 viruses) require either the *P*3 or the *P*4 level of physical containment. Experiments with plant-virus host systems have special physical-containment requirements that are analogous to the *P*1-to-*P*4 system.

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