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ABSTRACT

New techniques have expanded the field of biotechnology and awarded scientists an unprecedented degree of control over the genetic constitutions of living things. The knowledge of DNA science is the basis for this burgeoning industry which may be a major force in human existence. Just as it is possible to move genetic material from one organism to another, scientists intend to transfer normal genes into somatic cells in order to correct severe genetic disorders. This procedure, called gene therapy, causes some critics to fear that human gene therapy might be used to alter fundamental human traits such as intelligence, personality, or physical appearance. Although scientists counter that it is unlikely that complex human traits can ever be altered by genetic means, the anxiety continues to generate public controversy. This monograph endeavors to help gain public acceptance by providing clear and accurate information, including benefits and risks, about biotechnology by discussing the following topics: (1) definitions of biotechnology; (2) roots of the biological revolution; (3) tools and applications of biotechnology; (4) gene therapy; (5) agricultural and industrial applications; (6) biotech companies; and (7) regulating biotechnology and genetic engineering. Also included are a 14-page glossary of terms commonly used and a 68-item list of the literature cited. (RT)

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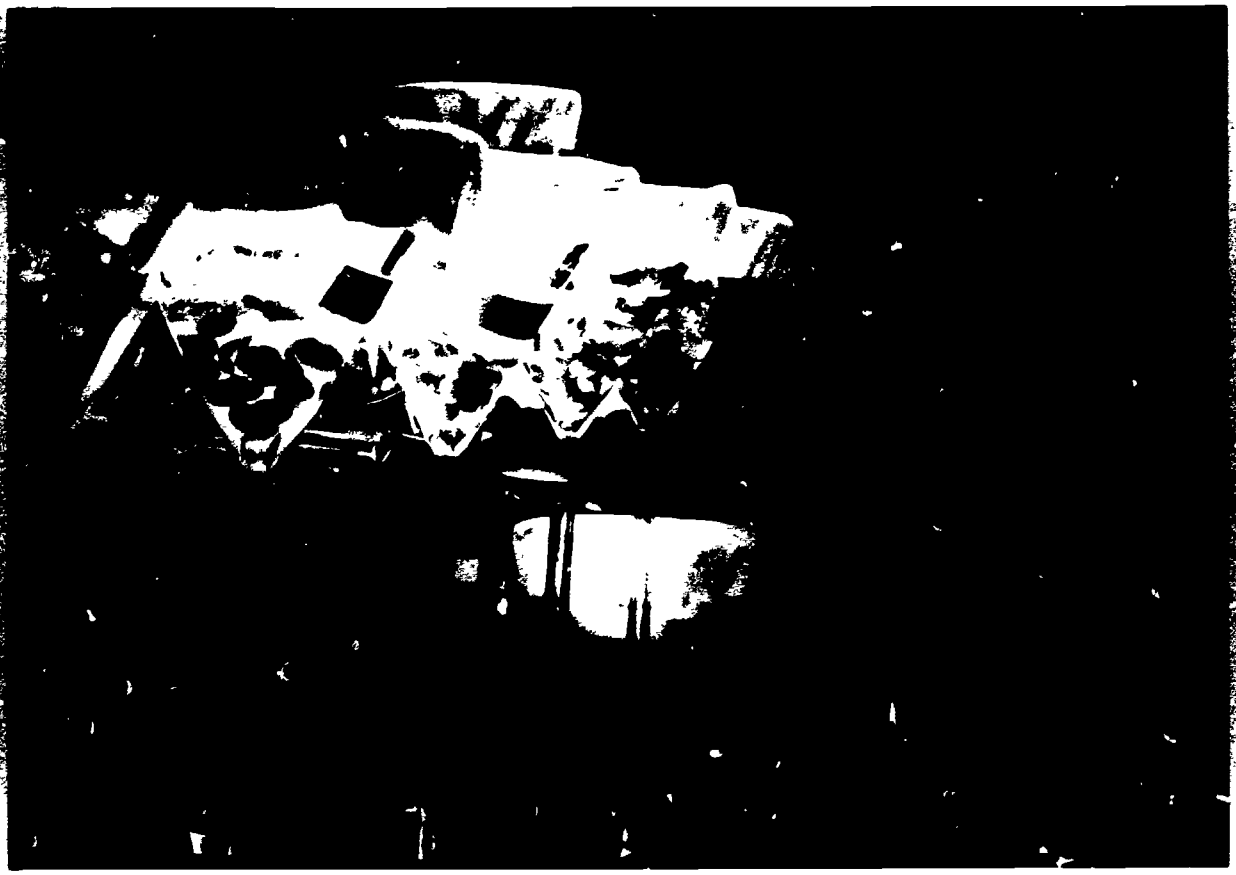

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Biotechnology, Genetic Engineering and Society

Monograph Series: III

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Contents

About the Author

George H. Kieffer, an associate professor of zoology since 1971 at the University of Illinois at Urbana-Champaign in the Department of Ecology, Ethology and Evolution in the School of Life Sciences, joined the university's staff in 1967 as an assistant professor. Previously he taught at Fresno State College, Arizona State University and the University of Colorado. Kieffer received an NIH Physiology Trainee Grant while at the University of Arizona during 1965-66. He began his teaching career at the elementary level in Michigan in 1951, later becoming an elementary school principal in Denver, Colorado.

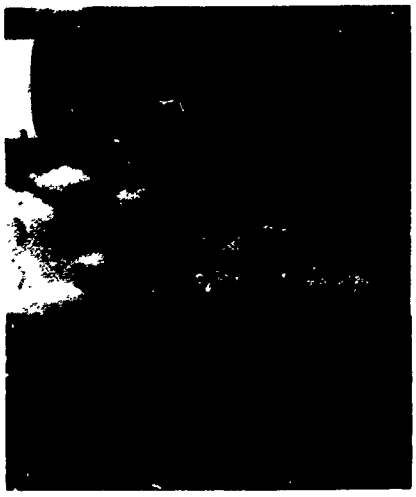
With a B.S. in Elementary Education from Concordia Teachers College in River Forest, Illinois in 1954, Kieffer went on to earn an M.S. in Alpine Biology from the University of Colorado, Boulder in 1961. In 1966, he completed his studies and earned a Ph.D. in Cell Biology from the University of Arizona, Tuscon. He has been elected a Fellow of the American Association for the Advancement of Science.

The author of several textbooks and numerous articles, Kieffer's major research interest is in biological education and ethical issues in the life sciences. His published textbooks include: *Biology 100 — Biology for Non-Majors: An Audio Tutorial Approach; Biology 101 — Biology for Non-Majors: An Audio Tutorial Approach; Ethical Issues and the Life Sciences; Biology and Human Affairs*; and *Bioethics, A Textbook of Issues*. Kieffer has published articles in such periodicals as the *Bulletin of Science, Technology, & Society, American Biology Teacher, The Science Teacher* and *AIBS Education Review*.

An Overview

When fruit juice was first changed into wine by a yeast, they called it fermentation. Now the name is biotechnology. Winemakers of an earlier age unknowingly tinkered not only with grapes but with living cells, and in doing so, they were on the edge of high tech biology.

Things have become a little more sophisticated since then. Microorganisms are used to make bread and to convert milk into cheese. Microbial factories manufacture vitamins and vinegar, and a multitude of antibiotics come from various strains of bacteria and molds.



Biotechnology will revolutionize the way we grow our food. (Rafel Olbinski © Discover Magazine 8/84, Time, Inc.)

But all of that was just a prelude; the drive to harness microorganisms to do our bidding has quickened to a blistering pace over the past 15 years. The expanding field of biotechnology is now considered not only a watershed in our understanding of nature but a commercial gold mine that has only begun to be worked.

New techniques have given scientists an unprecedented degree of control over the genetic constitutions of living things. Functioning genes now can be spliced into microorganisms and the cells of plants and animals, including humans. Genetic engineering, recombinant DNA and gene splicing are buzzwords for the DNA revolution that has brought scientists to the very brink of understanding the molecular basis of life

This knowledge of DNA science is the basis for the burgeoning biotechnology industry, and, like the explosive growth of computer technology, it portends to be a major force in human existence. The products of biotechnology — the prevention and cure of disease, the fabrication of new food sources and cheaper kinds of chemicals are seen as playing a crucial role in employment, productivity, trade and the quality of life (Markle & Robins 1985).

The biotech industry made its first big splash in the late 1970s when scientists first coaxed microorganisms to manufacture human insulin, somatostatin (a growth hormone) and interferon, and they made oil-eating microbes with voracious appetites. Now, with the marketing of new products coupled with advances in science and scientific tools, the industry is surging ahead. More than 250 companies, large and small, have been formed nationally since 1979, and enormous amounts of money have been invested in R & D to bring products on line.

Because of the inherent fits and starts of getting off the ground, not all of these

companies will survive, but those that do are expected to generate sales in the tens of billions of dollars by the year 2000.

So far, most of the impact of biotechnological findings has been in medicine. The first industrial application of recombinant DNA (rDNA) came in 1978, when the California company, Genentech, announced it had successfully engineered a bacterium to produce human insulin. Two years after, worldwide sales of this hormone amounted to $250 million (Bloomer 1986). Other products now on the market include a human growth hormone for the treatment of dwarfism and burns, the protein alpha interferon, effective against a rare form of leukemia and possibly other types of cancer, and several human and animal vaccines.

Perhaps a score or more of still others are waiting in the wings. One is the anti-blood clotting factor, t-PA (tissue plasminogen activator), a molecule that is 75 percent to 80 percent effective in dissolving blood clots. Hepatitis-B vaccine, the first genetically engineered vaccine approved by the U.S. Food and Drug Administration recently made its debut. A variety of other protein hormones and amino acids as well as vaccines now being investigated show promise. Some of the hormones under study could control pain, regulate blood pressure and lubricate the joints of arthritis sufferers.

Just as it is now possible to move genetic material from one organism to another, so it is but a technical matter to manipulate the genes of humans as well. Within the very near future, scientists intend to transfer normal genes into somatic cells in order to correct certain severe genetic disorders. The procedure called gene therapy, as startling as it appears, is considered essentially no different than other kinds of medical treatments and raises no new ethical questions (Olson 1986). Nonetheless, some critics do fear that human gene therapy might someday be used to alter fundamental human traits like intelligence, personality or physical appearance. Although scientists counter that it is extremely unlikely that complex human traits can ever be altered by genetic means, this anxiety continues to generate public controversy, sometimes leading to violent confrontation concerning the wisdom of proceeding with human gene therapy.

An area where ethical constraints already rule out genetic engineering deals with the modification of germline (reproductive) cells even though the technical and ethical problems associated with it are formidable. For example, such intentional manipulation could cause severe and lethal mutation in cells, or the insertion of new genes may alter the gene pool of the human species, raising questions about tampering with humanity's genetic heritage.

But it is in the field of agriculture that biotechnology stands to create its greatest impact — and perhaps even more headlines because of the politics of food production. Genetically engineered growth hormone injected into cows already can increase milk output 10 percent to 40 percent without an increase in feeding costs (Tangley 1986). The hormone also may be able to speed up an overall growth and reproductive capacity of livestock.

In plants, genetically induced changes could mean crops that create their own fertilizer, adapt to particular climates and soil types, and withstand herbicides and insects. These changes are coming with the real revolution being predicted for a scant 10 years from now.

However, not everyone is enthralled with these wonders of modern science. In this age of crop surpluses and overproduction, the technological changes on the horizon are viewed more as threats rather than a cornucopia. Consider the case of farm families

in the U.S. The number of family farms has declined from 3 million in 1940 to 700,000 today, and the trend is continuing. Fewer and fewer farms will produce more and more food, and more technology is perceived to be hastening the loss of the locally owned and operated farm. And this fear is not without substance. A government report in 1986 predicted that biotechnology will increase productivity dramatically over the next 14 years but it also will result in the disappearance of an estimated one million small or medium sized farms — those that have net annual sales of $250,000 or less (Tangley 1986).

But biotechnology has met no greater resistance to development than fear — from the earlier fear that engineered organisms may slip out of the laboratory to cause epidemic diseases to the more recent anxiety that certain altered microbes may inadvertently tip nature's balance against us. Some critics contend that these genetic manipulations are morally wrong. Genetic engineering's most vocal critic, Jeremy Rifkin, in his book *Who Should Play God?*, wrote, "Genetic engineers can create monstrosities beyond imagination. In a few years, they will be able to propagate a super race of beings as easily as they will be able to create a docile, subhuman breed of servants and slaves" (Rifkin & Howard 1977).

Scientists tend to dismiss most of these arguments as non-issues concocted by critics to arouse public opinion against them. Nevertheless, these matters cannot be brushed off so easily; they are deadly serious to many.

The contention is that scientists have a habit of leaping first and looking later, moving ahead too quickly without the benefit of public debate. Regardless of however much scientists may joke among themselves about fears of the unknown, public suspicion continues to be an albatross around the neck of the industry, preventing it from moving forward at a pace it would prefer. Some problems cannot even be investigated because of governmental prohibitions (e.g., human germline research).

Federal policies governing research in biotechnology were released in June of 1986. Most observers consider them somewhat benign and weighted toward promotion rather than restriction (*Bioscience* 1986). There is a lingering feeling, though, that the political climate may change. Some members of Congress believe that biotechnology may be too important to leave to biotechnologists. Any technology that deals so directly with basic life processes inevitably raises compelling questions. And legislation has been introduced into Congress from time to time that would regulate the activities of the genetic engineers, though none to date has been enacted.

This and other issues are being raised concerning the government's role. Biotechnology is growing so rapidly, and its ultimate influence is so far-reaching, that it is straining the capacity of public and private institutions to deal with it. Senator Albert Gore, Jr. exclaims, "We are running out of time in the sense that the technology is developing so rapidly that we are going to have to make some tentative decisions without the base of understanding that a democracy requires for difficult decisions. Requests for field tests of genetically engineered organisms are already beginning to be made, as companies proceed with their research programs. The first authorized human gene therapy experiments are expected to be conducted this year. Both of these facts underscore how important it is to develop a coherent set of science and ethical guidelines to help us evaluate the implications of this technology" (Olson 1986).

On one thing most everyone agrees: Biotechnology is a complicated field, so complicated that few people but the scientists and a handful of policy makers feel

competent enough to get involved. Adequate knowledge of DNA science has not yet filtered far beyond the doors of research laboratories. This state of public ignorance threatens the nation's ability to make informed policy choices about issues generated by the biotechnical enterprise (Price 1985; Crawford 1986). Public trust is essential if commercialization of this activity is to proceed. This trust can be fostered through a comprehensive and trustworthy program of public education that clearly lays out both the benefits and risks of this technology. This monograph endeavors to make a small contribution to this ambitious goal.

What is Biotechnology?

Before embarking on a detailed study of biotechnology, it is in order first to define the term. Although a definition may seem self-evident, there are in fact sharp differences of opinion over what biotechnology does include. Several countries have come up with their own official definition.

To the British, biotechnology means "the application of biological organisms, systems, or processes to manufacturing and service industries" (Markle & Robins 1986). The European Federation of Biotechnology sees it as "the integrated use of biochemistry, microbial, and engineering sciences in order to achieve technological (industrial) application of the capabilities of microorganisms, cultured tissue cells, and parts thereof." Both of these define biotechnology very broadly to include the processes of brewing and baking. According to this view, biotechnology is not very different from what was done in the past; it is simply the latest addition to 19th century industries that have slowly and steadily grown. This view tends to downplay the importance and revolutionary character of recombinant techniques, and in doing so, to place less emphasis on any related social issues.

In keeping with their national commitment to the economic aspects of biotechnology, the Japanese describe it in terms of social utility--its market potential. To them, biotechnology is "a technology using biological phenomena for copying and manufacturing various kinds of useful substances." The industrial policies of the Japanese government have implemented this interpretation by actively encouraging biotechnical development through support of applied rather than basic research, as is the case in our country.

The National Science Foundation (NSF) defines biotechnology as "the controlled use of biological agents, such as microorganisms or cellular components for beneficial use." Some take exception with this definition because it implies that everything produced by technology is "beneficial." There are critics who claim that the fruits of biotechnology are not necessarily "good", they could just as well have unintended and unanticipated negative consequences on us and our environment.

A comprehensive definition proposed by the government Office of Technology Assessment (OTA) separates biotechnology into two components, the "old" and the "new." The old refers to the broad definition preferred by the Europeans, using living organisms to produce products considered useful to humans. The new cites the "industrial uses of rDNA, cell fusion, and bioprocessing techniques." This definition for the "new" comes closest to the view held most generally in our country.

Embedded in the definition are two important points. First, biotechnology does not refer to one procedure, technique or process, but rather encompasses a diversity of means for using living organisms or their products to prepare items for the marketplace. Second, biotechnology entails the meshing of two disciplines — science and engineering.

Although it may seem that any controversy over definitions is nothing more than a quibble over words, the differences are major because they do shape subsequent

discussions about social policy. If, for example, the European view is adopted, it follows that the entire biotechnical enterprise is but the latest round in the use of organisms for industrial purposes. These applications present no unique problems other than the conventional ones normally encountered with the use of living materials — simple caution should be exercised in handling them. Engineered organisms are not sinister. Neither need we fear that deadly microbes will slip into our sewers and streets or get in our babies' cribs.

On the other hand, the "new" OTA definition confines itself to the commercial side of DNA science and it does so without implying any more than that — the products of biotechnology are not necessarily beneficial. Or is the enterprise merely a continuation of the past? It thus is left open that the new technology has an agenda of its own, in the way it uses science for making a profit and in the new social and ethical matter it raises. This definition of the "new" will be used throughout this study.

Roots of the Biological Revolution

The roots of biotechnology lay in the science of molecular biology, a branch of the life sciences that traces its start to the 1940s. At least two major events drastically changed the way biological research was done and the kinds of information that could be collected. First, a wide range of new instruments was developed, starting with the electron microscope, which greatly expanded the kinds of visual studies that could be made on living systems. The second is the host of new techniques—electrophoresis, ultracentrifugation, chromatography, radioactive tracers, and so forth, which permitted many new ways for studying life processes (Newton 1986).

These strides in the way of doing science resulted in profound additions to our knowledge. Much of the behavior and function of organisms could at last be explained in terms of known chemical and physical principles. The old belief that living matter had laws and principles of its own that governed its activities was at last put to rest.

This new science known as molecular biology united an extensive new technology and new theoretical understandings about the nature of living systems. The central problem of molecular biology since its inception has been the elucidation of the hereditary process in terms of the chemical molecule responsible for the organism's behavior.

Through the 1950s and 1960s, molecular biologists pursued theoretical questions, pursued them successfully and pursued them largely without any intent or interest in making a profit from them. The motivation driving these scientists was to gain an understanding of the molecular basis of life, especially its genetic basis.

In little more than a decade, scientists have uncovered a wealth of information about DNA and its function. They have learned a great deal about single genes and how they are arranged in cells, about oncogenes—a class of genes that causes cancer—and about the exact makeup of the genetic alphabet in a number of viruses, bacteria and human genes. Even the idea of generating a complete map of the human genome is now within reach, awaiting only the commitment of the scientific community and financial support from granting agencies.

By 1972, molecular biologists had developed the techniques by which organisms could be transformed permanently by the insertion of donor genes. The technique was made possible by the discovery of a class of enzymes — restriction enzymes — that could slice through DNA in specific locations. The segments obtained could then be isolated and recombined with DNA from another source to form recombinant DNA. A short ten years later a flourishing industry was already in place that was using transformed DNAs to manufacture commercially useful products. Biotechnology thus owes its existence to the influence, intellect and theoretical knowledge of molecular biologists.

The DNA Revolution may seem to have dawned with amazing suddenness, whereas, in fact, the astonishing breakthroughs of the past 15 years had their roots in earlier discoveries dating back over a hundred years to at least the time of Charles

Darwin. In the century and a quarter since he postulated the theory of evolution by natural selection, the quest has been to understand the nature of the hereditary process in evermore specific terms. The following questions express the high points of this search (Micklos 1986):

(1) How can the diversity of species be accounted for?
(2) How are traits passed from one generation to the next?
(3) What is the function of the gene?
(4) What molecule is the carrier of genetic information?
(5) How does the structure of DNA relate to its function?
(6) Can the DNAs of different organisms be recombined?

The chronology given in Table 1 identifies the scientists who made the pivotal discoveries to answer these questions.

Charles Darwin laid out the answer to the first question in his monumental work, *The Origin of Species*. He proposed the theory of natural selection as the mechanism whereby new species arise and change over time. However, Darwin remained puzzled as to how natural selection works since no workable theory of heredity existed in his day. This mechanism came from Gregor Mendel several years later; genes, which Mendel named factors, were able to carry hereditary information from one generation to the next. Although Mendel's genius was not recognized immediately, his so-called laws of heredity laid down the basic rules followed by most organisms. The gene, however, remained an abstract concept without any physical basis in the cell.

A contemporary of Mendel, the German chemist Friedrich Miescher, extracted a hitherto unknown molecule from the nuclei of pus cells. He named it nuclein because he thought it was a protein. Miescher, like everyone else back then, believed that proteins were responsible for the transmission of hereditary traits, an hypothesis that took a long time to die. He proposed that the newly discovered nuclein served as a reservoir of phosphorus drawn upon by the cell. Subsequent chemical analysi in the later part of the nineteenth century established that the new substance was not a protein at all but an entirely different kind of biological macromolecule given the name nucleic acid.

Mendel's laws, which were completely ignored for 40 years because they did not agree with prevailing ideas of heredity, were rediscovered about 1900 by three botanists, the Dutchman Hugo deVries, the German Carl Correns and the Austrian Erich Tschermak. The work of these three scientists demonstrated that Mendel's laws were universally applicable in the biological world. DeVries also put forth the idea of communication between the nucleus and the cytoplasm in cellular activity.

Thomas Hunt Morgan, who worked in the famous "Fly Room" at Columbia University in the early decades of this century, established *Drosophila* fruit fly as a model system for doing genetics experiments. The era of modern genetics was ushered in by this early geneticist. One of his numerous contributions was the discovery that genes are carried on chromosomes in the nucleus of the cell.

The early decades of this century were also the time when physical methods were applied to the study of large molecules and to living organisms. X-ray crystallography enabled scientists to probe the innermost structure of crystals. A few years later, Hermann Muller showed that X-rays could induce mutations in the genetic substance.

TABLE 1. LANDMARK DISCOVERIES ON THE WAY TO THE DNA REVOLUTION

1859	Charles Darwin published *The Origin of Species*. In it he proposed a mechanism to explain how hereditary changes influence the evolution of populations.
1865	Gregor Mendel presented his findings describing how genetic traits are passed from one generation to the next. From his work the concept of the gene as the basic unit of heredity was derived.
1869	Friedrich Miescher isolated DNA from the nuclei of white blood cells.
1869-1900	The basic chemistry of nucleic acids - DNA and RNA - is worked out.
1900	Mendel's work is rediscovered. It was finally realized that the laws of heredity he proposed apply almost universally in the biological world; they also provide a mechanism to drive the evolutionary process.
1910-27	Thomas Hunt Morgan proposed that genes are carried on chromosomes. His work using the *Drosophila* fruit fly as a model system marks the beginning of modern genetics.
1912	Sir Lawrence Bragg discovered that x-rays can be used to study the molecular structure of simple crystalline substances (x-ray crystallography).
1927	Hermann Muller demonstrated that mutations can be induced in the laboratory using x-rays.
1928-35	Linus Pauling elucidated the physical laws governing how atoms are arranged within molecules.
1934	Desmond Bernal showed that giant molecules, such as proteins, can be studied using x-ray crystallography.
1940	George Beadle and Edward Tatum presented the "one gene, one enzyme hypothesis" which states that each structural gene directs the synthesis of a particular protein. This brings the study of genetics to the level of biochemistry.
1944	Oswald Avery demonstrated that DNA, not protein, carries genetic information during bacterial transformation.
1950	Erwin Chargaff determined that the ratio of adenine to thymine in DNA is 1:1.
1952	Alfred Hershey established conclusively that DNA is the molecule of heredity in bacteriophages.

1952	Jean Brachet suggested that RNA, another nucleic acid, plays a part in the synthesis of proteins.
1953	James Watson and Francis Crick solved the molecular structure of DNA.
1957	Matthew Meselson and Frank Stahl demonstrated the replication mechanism of DNA.
1957	Francis Crick and George Gamov proposed the "sequence hypothesis" whereby DNA sequence specifies amino acid sequence in a protein; also, the "central dogma" that genetic information flows only from DNA, to messenger RNA, to protein.
1961	Marshall Nirenberg and Severo Ochoa "cracked" the genetic code; specific nucleotide sequences in groups of three specify each of the 20 amino acids.
1972-73	Paul Berg made the first recombinant-DNA molecule. Stanley Cohen transplanted a functioning gene between organisms.
1977	"Year One" for biotechnology. The first practical application of genetic engineering; the gene for the human hormone, somatostatin, is expressed in a bacterial cell.

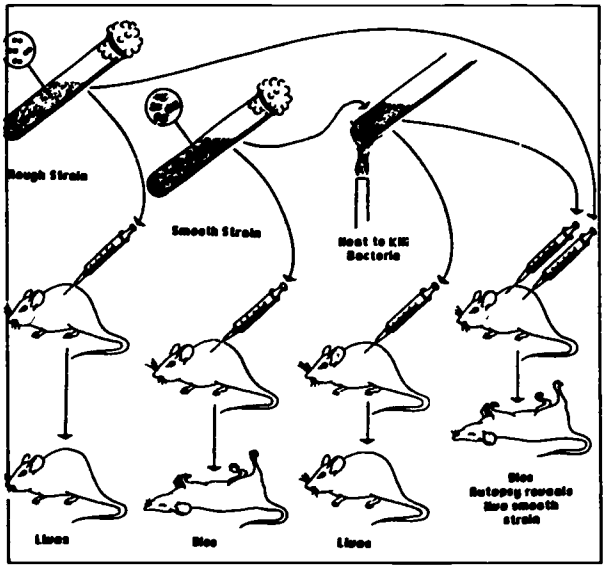
George Beadle and Edward Tatum applied Muller's X-ray techniques for inciting genetic change to the red bread mold, *Neurospora*, and were able to isolate single mutant strains from among millions. Chemical experiments with these mutants led them to conclude that genes regulate and control enzymes and make them specific. They formulated the famous "one gene, one enzyme hypothesis," which later was refined to read, "one gene, one polypeptide." Now, for the first time, genetics was reduced to the level of the molecule. At long last, the question could be investigated — "What chemical molecule is responsible for heredity?"

An early series of experiments gave scientists their first clues. Frederick Griffiths was able to show that a harmless strain of pneumococcus bacteria could be transformed into a strain which killed mice. Griffiths concluded that something was transferred from the lethal strain to the harmless one that transformed them into killers. However, he was killed in an air raid during World World II before discovering what that something was. Fifteen years later, Oswald Avery determined that the mystery substance was none other than DNA. This was the first suggestion that the genetic substance was not a protein, but, like Mendel before him, Avery's hypothesis met with skepticism and disbelief; proponents of the opposing view continued to think of heredity in terms of protein.

The issue was finally settled in favor of DNA when the reproductive mechanism of viruses was explained. From this convincing proof came studies of virus particles that infect bacterial cells called bacteriophages (phage for short). Alfred Hershey and his coworkers showed that viruses reproduce themselves by injecting only their DNA into a bacterial host. The protein coat which encloses the virus remains attached outside of the bacterial cell, not participating at all in the reproductive process. This experiment

established beyond any doubt that the nucleic acid, DNA, was indeed the genetic molecule.

It is interesting that in the half century or so of work leading to this conclusion, the experimental organisms became progressively smaller. Mendel experimented with garden peas, Morgan and Muller with fruit flies, Beadle and Tatum with bread mold, Griffiths and Avery with bacteria, and finally Hershey with viruses. Thus, as the search became more focused, experimental organisms became smaller and smaller. And, bacteria and viruses continue to be the workhorse organisms of molecular biology and biotechnology.



Griffith's famous experiment which demonstrated that DNA functions as genetic material. Genetic information from dead bacteria that are lethal to mice is transferred to the living, nonlethal variety. The new genetic information changes the nonlethal strain into a killer.

But knowing that DNA was the molecule of heredity did not explain how it managed this wondrous task; more about its structure had to be known. Erwin Chargaff was able to show that base ratios within DNA were a constant; the number of adenines was always equal to the number of thymines; and cytosine always matched guanine. This crucial piece of structural information led James Watson and Francis Crick to proclaim their principle of "complementarity" — A bonds with T and C with G — to form the DNA double helix. The Watson-Crick structure is based on a linear sequence of nucleotides arranged in two strands; the complementary strands are held together by hydrogen bonds betwen the inner-facing nucleotides.

Today, we know that genes are pieces of DNA containing instruction to make appropriate proteins. About 1,000 base pairs make up a single gene, though the exact number is highly variable. These genes, literally thousands of them, are wound into small rods, the chromosomes. A human chromosome may contain up to 3 billion nucleotides.

Why a double molecule? Matthew Meselson and Frank Stahl demonstrated that this arrangement provides a most efficient way to reproduce duplicate copies of DNA prior to cell division. The two nucleotide strands simply unzip and a new complementary copy is formed on the exposed bases. The giant DNA molecule can therefore be replicated almost exactly over and over again as needed.

With DNA's structure in hand, scientists next turned to questioning how the genetic language of nucleotides was translated into life's essential proteins. The motivating question was: How are the four letters of nucleotide bases decoded to spell out the 20 words of amino acids? Francis Crick along with the physicist George Gamov advanced the sequence hypothesis in which they proposed that the sequence of bases in DNA corresponds with the amino acid sequence of a protein. They also suggested that bases operate in groups of three to spell out one amino acid — the triplet code: one triplet

codes for one amino acid.

Another piece of information, supplied by Jean Brachet, was that a kind of RNA serves as the messenger between DNA of the nucleus and ribosomes in the cytoplasm; ribosomes are the cellular sites of protein synthesis. Crick used this link to formulate what became known as the "central dogma" of molecular biology; the flow of genetic information is from DNA to RNA, to protein.

One last item remained unsolved, namely, which triplets code for which amino acids? Marshall Nirenberg and Servio Ochoa finally provided the solution by using laboratory-made mRNA triplets in various combinations and cell-free translation systems containing ribosomes obtained from bacteria. They showed that the 64 triplet combination could easily code for the 20 common amino acids of protein. And, indeed, that most of the amino acids had more than one code with some triplets serving as "punctuation marks" for the message.

The final question, "Can DNA be recombined to form hybrid molecules?", was answered affirmatively in the early 1970s. Paul Berg made the first recombinant DNA molecule between the viruses SV 40 and Lambda phage. The earlier discovery of restriction enzymes, a group of enzymes that selectively cuts DNA into gene length segments made Berg's success possible. Soon after Berg's work, Stanley Cohen managed to transfer a functioning gene from one organism into another. He called the composite a "chimera," for indeed it could be compared to the creature in Greek mythology which had a lion's head, a goat's body and the tail of a serpent.

With all the tools available needed to cut, glue and recombine genes, their application in technology was soon coming. A number of "firsts" were reported in 1977 — "Year One" for biotechnology (Antebi & Fishlock 1986). Foremost of these was the expression by a microorganism of the gene for somatostatin, a human hormone comprised of 14 amino acids. The gene was synthesized in the lab and spliced into a bacterium, whereupon it proceeded to make the human gene product. On December 2, 1980, a patent was awarded for this method of inserting genes into host cells but not until the argument over whether it was possible to patent living processes was settled.

With the first successes, the biotechnology enterprise was born, which within the short space of 10 years has grown into a multimillion-dollar industry. The stunning part about this phenomenal growth is not that it did happen but the astonishing speed at which it took place. Biotechnology, however one defines it, will affect almost every human activity—our health, food, fuel, wastes and virtually every biological process or interaction with our environment. But neither scientists nor the public in general have absorbed the full impact of these developments. As more is learned about DNA and how to manipulate it, more problems are certain to arise. This means that the public must be informed so that responsible public policies are formulated. To do less could stifle the DNA revolution before it really gets started.

The Tools of Biotechnology

Refinement of the basic tools for conducting biological research has permitted scientists to stretch the boundaries of current knowledge in molecular biology. The history of these developments parallels the revolution in our understanding of the hereditary process. Genes no longer were visualized as abstract units having no material basis but rather as discrete molecules that performed their functions in particular ways. The feature of life that made these remarkable discoveries possible is the near universality of the genetic code. Virtually every living organism uses the same genetic language to translate its hereditary information carried as nucleotide sequences in DNA into proteins, life's "work-a-day" molecules.* This commonality of the language makes it possible to transfer genes between entirely different species — a human gene can be spliced into a bacterium, and the hereditary machinery of the bacterial cell manufactures the human gene product.

Restriction Enzymes

DNA can be cut into recognizable pieces and the pieces arranged again in different ways. This scissoring is done by a group of enzymes called restriction enzymes, also known as endonucleases because they cleave DNA at internal positions. (Exonucleases cut nucleotides off the ends of DNA molecules.) Because endonucleases are able to recognize and cleave specific sequences of nucleotides, they are the basic tools of genetic engineering.

This class of enzymes evolved as protection against stray pieces of DNA, which may become incorporated into cells quite by random. For example, the natural enemies of bacteria are bacteriophage viruses. These infect and multiply within the bacterial cell, eventually causing it to burst, releasing viral offspring. Restriction enzymes serve as powerful weapons against the invaders; they chop up the viral DNA before it can harm the bacterial cell. Bacterial DNA is not damaged because it has been modified in such a way that the restriction enzymes do not recognize it as DNA. Bonding a methyl group ($-CH_3$) at the restriction site protects its own DNA from the degrading action of the

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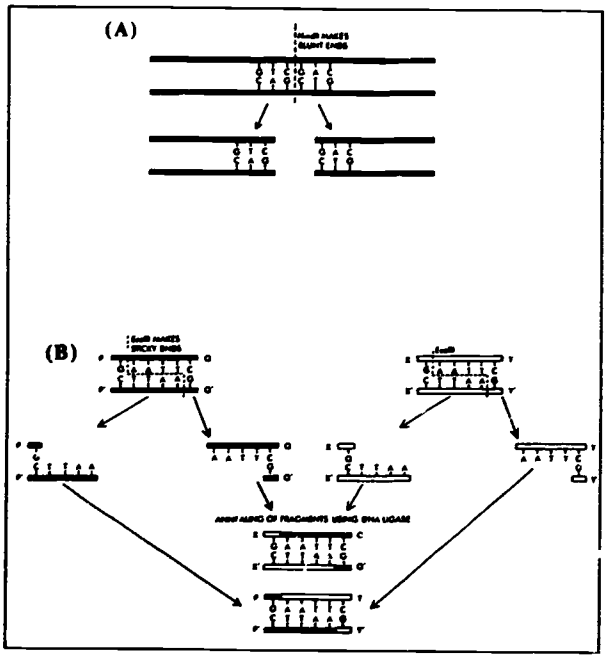
* Several exceptions to the universality of the genetic language have recently been discovered. One is found in the single celled protozoan ciliates of which *Paramecium* is a well-known member. These organisms utilize a slightly different DNA language to code for their proteins. Reading the code in three-letter combinations, codons, remains universal but the information content of a codon may be different. For instance, the *Paramecium* use TAA and TAG to signal the amino acid glutamine, whereas most other organisms use the same triplets as termination cues. Several other codon differences have also been identified. Differences like these may mean that the ciliates branched off from the main evolutionary line very early in the history of life. Mycoplasmas are another group that show variation in their amino acid codes (Antebi & Fishlock 1986).

enzyme.

There are several known kinds of restriction enzymes each of which attacks different points of the DNA molecule. Typically, the enzyme recognizes sequences that are from four to six nucleotides long and are symmetrical. The arrangement of nucleotides is known as rotational symmetry. If one nucleotide sequence of a duplex DNA is rotated through a plane, it is complementary to the opposite strand of the duplex (see figure at right). Regardless of whether the DNA is from a bacterium or a yeast cell, a plant or animal cell, various endonucleases will cleave DNA molecules wherever they encounter recognition sequences (see Table 2, page 16).



The action of restriction enzymes. A) This enzyme cuts DNA at the center of its recognition site leaving blunt ends. B) Another class of restriction enzymes cuts DNA in such a way to leave "sticky" ends. Sticky ends can be annealed to any other "sticky" end by using an annealing (joining) enzyme.

Some endonucleases cut cleanly through both complementary strands of DNA at the recognition sites to leave blunt ends. Others chop between the strands to leave exposed single-stranded ends. These so-called "sticky ends" are especially useful for splicing genes into host DNA.

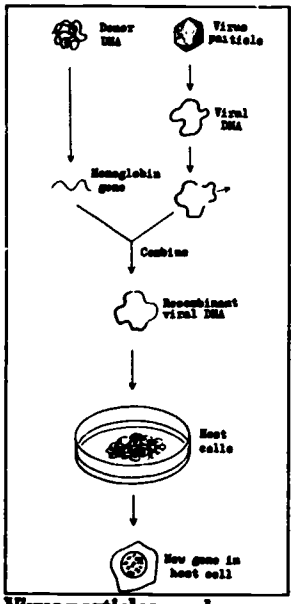
Because the two single-stranded ends produced at cleavage sites are complementary, they can pair with each other. This means that any DNA fragments created by the same endonuclease can be spliced together easily. The two strands are joined with the aid of a sealing enzymes known as a ligase. Its action is to reform the chemical bonds between complementary nucleotides.

Restriction enzymes are named according to the following plan: The first part of the name indicates the genus name of the organism from which the enzyme was taken; the second and third letters designate the species. Roman numerals denote the sequence in which the different endonucleases were isolated from a particular species (See Table 3, page 17).

Restriction enzymes are routinely used in the laboratory to form composite molecules called recombinant DNA, or rDNA. Genetic molecules from two different sources are joined together to make a novel combination of DNA.

Vectors

After a recombinant molecule is made, the next step is to insert it into a living cell to gain expression of the gene product. The simplest and most direct route is to grow cells in a medium containing the manufactured DNA. However, the rate of uptake is very low — far fewer than one percent of the cells will ever take up and incorporate the



Virus particles used as vectors to carry donor DNA into the genetic makeup of a host cell. (From Anderson & Diacumakos, "Genetic Engineering in Mammalian Cells," © 7/81 by Scientific American, Inc. All rights reserved.)

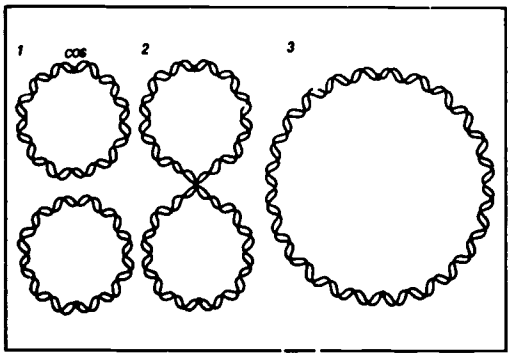
new gene. Greater success can be achieved by "piggybacking" the foreign DNA to a carrier, or vector, that transplants it directly into the target cell. Viruses and bacterial plasmids are the most common vectors used by genetic engineers.

If the host cell is to be a microorganism, the foreign DNA fragment is inserted into a bacterial virus or plasmid. Plasmids are extrachromosomal, double-stranded DNA elements occurring in the cytoplasm, displaying a ring-like structure. They are much smaller than the main bacterial chromosome, made up of a few thousand nucleotide bases compared with millions for the main chromosome. Cutting the plasmid with an endonuclease permits the insertion of a selected foreign DNA piece into the plasmid. The recombinant plasmid is then placed in a bacterial cell, whereupon the new gene may be replicated as the plasmid is reproduced or a gene product may be formed.

Some plasmids only duplicate themselves when the main chromosomes replicate and exist as single, or at most, several copies within a single bacterial cell. These plasmids are said to be under stringent replication control. Plasmids in some bacterial species are under relaxed control and duplicate themselves independently of the main chromosome. Up to several thousand copies of the same plasmid may be present in a single bacterial cell having relaxed control. Needless to say, relaxed control bacterial strains are most useful for biotechnological applications.

Colonies of bacteria that have been transformed by a recombinant plasmid under relaxed control will contain multiple copies of the introduced gene. This procedure for reproducing copies of a selected gene is known as molecular cloning, since all of the reproduced genes will be descendants and genetically identical to the original DNA piece that was spliced into the first plasmid. The cloned gene then can be harvested from the cultured cells to obtain many copies for later use.

To clone large fragments of DNA — more than 20,000 base pairs, or about 20 genes — artificial plasmids called cosmids are used. Cosmids are built up from plasmids to which "cos" sites, cohesive end sites derived from lambda phage virus DNA, are added. Between two cos fragments, one on either end of an opened up plasmid (remember plasmids are circular), large chunks of DNA can be inserted. The plasmid ring is then reformed and inserted into a bacterial cell for replication.



Cosmid formation. Each circular phage chromosome contains one so-called site where it can be cleaved and rejoined. Foreign genes can be spliced into the phage chromosome. Two or more cos-containing modified chromosomes can be joined together to form very large combinations of DNA, which can be used to transport large amounts of genetic information into a cell. (From Stahl, "Genetic Recombination" © 2/87 by *Scientific American*, Inc.)

TABLE 2. SOME RESTRICTION ENZYMES AND THEIR CLEAVING SEQUENCES

Microorganism	*Abbreviation*	*Sequence* 5'-3' 3'-5'
Bacillus amyloliquefaciens H	*Bam* HI	G G A T C C C C T A G G
Brevibacterium albidum	*Bal* I	T G G C C A A C C G G T
Escherichia coli RY13	*Eco* RI	G A A T T C C T T A A G
Haemophilus aegyptius	*Hae* II	Pu G C G C Py Py C G C G Pu
Haemophilus aegyptius	*Hae* III	G G C C C C G G
Haemophilus haemolyticus	*Hha* I	G C G C C G C G
Haemophilus influenzae Rd	*Hind* II	G T Py Pu A C C A Pu Py T G
Haemophilus influenzae Rd	*Hind* III	A A G C T T T T C G A A
Haemophilus parainfluenzae	*Hpa* I	G T T A A C C A A T T G
Haemophilus parainfluenzae	*Hpa* II	C C G G G G C C
Providencia stuartii 164	*Pst* I	C T G C A G G A C G T C
Streptomyces albus G	*Sal* I	G T C G A C C A G C T G
Xanthomonas oryzae	*Xor* II	C G A T C G G C T A G C

[From David Micklos and Jerry Freyer: *Recombinant DNA for Beginners: A Laboratory Course in Molecular Biology.* (1986). Cold Spring Harbor Curriculum Study: Cold Spring Harbor, NY. p. 15.]

TABLE 3. SYSTEM FOR NAMING RESTRICTION ENZYMES

Enzyme Name		Derivation
*Eco*RI	*E*	- *genus Escherichia*
	co	- species *coli*
	R	- strain RY 13
*Bam*HI	*B*	- *genus Bacillus*
	am	- species *amyloliquifa-ciens*
	H	- strain H
*Hind*III	*H*	- *genus Haemophilus*
	in	- species *influenzae*
	d	- strain Rd

(From Micklos 1986, p. 17)

Cosmids make it possible to build up large bands of genes wrapped up in packages ready for shipment into bacterial cells; cosmids also have been successfully inserted into animal cells. This way of getting genetic information into a host cell is known as the "cosmidic shuttle" (Antebi & Fishlock 1986).

Easier entry into a host cell can be achieved by splicing the foreign DNA fragment into the genome of a virus. Viral vectors can be used to insert genes into any type of cell — bacterial, plant or animal, including human.

An important step must be taken when using a virus vector; it first must be disarmed. The disease-causing gene or genes are removed using recombinant techniques and the selected genes put in their place. The infective portion, though, remains intact. Thus, the virus can gain admittance into a host cell but it cannot harm it. The foreign gene, now inside, may take a position somewhere on the host cell's chromosomes and begin to produce its product.

There has been considerable discussion about the potential danger of inadvertently creating an undesirable life form in the course of a recombinant experiment. What if, for example, a disease-causing gene was unintentionally incorporated into a virus that in turn was propagated within a bacterial or human cell? Or, what if a disarmed virus mutated back again to the virulent form? In either case, is there the danger that an infective epidemic could be propagated through a population?

Even though most recombinant DNA experiments are now thought to be safe, concerns like these are real and need to be taken seriously. Both scientists and government officials monitor these experiments to detect and forestall any hazards that may

arise. For its part, the scientific community has gone to considerable lengths to establish stringent safeguards. For example, the bacteria used in many recombinant experiments are unable to live outside of the laboratory; also, certain experiments thought to be dangerous are prohibited. (This and related problems will be discussed more extensively in the section, "Regulating Biotechnology and Genetic Engineering.")

The Problem of Gene Expression

The great enigma of molecular biology continues to be inducing expression of the foreign gene in the host cell. Recombinant techniques make it a relatively simple matter of laboratory fiddling to get a donor gene into a host cell, and theoretically any gene can be put into any cell. But it is quite another matter to get the donated gene to perform in its new surroundings. Unlike the genetic code, which is basically the same in all organisms, the signals that turn genes on and off vary from species to species.

Regulatory signals can take a variety of forms — genes that control other genes, proteins that function as molecular switches, and even physical factors like ultraviolet light or changes in a few temperature degrees. It is also known that regulation operates on different levels within the same cell — the choice of the DNA segment to be read, the choice of the transcribed sequences to be carried by the messenger RNA, and the rate at which protein is translated. Any of these, or a combination of them, can turn on or block gene action.

Another problem standing in the way of getting expression is that there is no known way to insert the new gene into an exact position in the host cell's chromosomes: it could just as well be one chromosome as another. Clearly, if an introduced gene is to function in a host cell, it must have the appropriate regulatory signals associated with it, and these are very often site specific. A gene must be physically related to its control elements in a certain way if it is to function. For this reason, the question of gene regulation is one of the most intensely studied problems in molecular biology today.

One promising lead is to use a genetic unit called a transposon as the vector for carrying a gene into a host cell. Transposons — sometimes called "jumping genes" — are small fragments of DNA which function as regulatory signals for genes (Morse 1984). Another property which makes them attractive to genetic engineers is that they can move from one chromosome to another within the cell, and even between cells, without losing their regulatory capacity. Replacing part of the transposon with a gene of choice using rDNA methods would accomplish two significant things: a donor gene could be inserted into the host cell and it would be functional in its new surroundings. The transposon technique therefore enables scientists to bypass a difficulty of the previous work: the unpredictable arrangement of the DNA being transferred.

But still, these artificial regulatory systems are crude compared with the exact mechanism of cells. Much work remains to be done to achieve the precision which nature demonstrates in controlling gene function.

As genetic engineers learn more and more about the switching systems of genes, the day may soon be here when they will be able to turn genes on and off as desired. This will have far-reaching consequences, not only for genetics, but in understanding and controlling other aspects of our biology as well — the differentiation of cells in embryonic growth, the events of the aging process, and maintaining our immunological de-

fenses against diseases, including cancer.

Reverse Transcriptase and cDNA

Finding and isolating a single gene from among the hundreds of thousands in a single cell nucleus would be extremely complicated if it were not for another enzyme, reverse transcriptase. It was discovered in 1970 in a special class of viruses named retroviruses. (Both cancer and AIDS have been linked to this group.)

Like other viruses, retroviruses pass from cell to cell enclosed within a protein sheath, but they are different in that their genetic information is carried as RNA rather than DNA. This characteristic explains their other name -- RNA viruses. Once inside a host cell, the RNA virus reproduces itself in an original way. Its RNA genome is first copied into DNA by an enzyme that it carries: reverse transcriptase. The process is transcription in reverse, which accounts for the name of the enzyme. The DNA copy, called complementary DNA (cDNA) because it is a complementary copy of RNA, is then stitched into the host cells' genetic material whereupon it may reproduce more of itself or become quiescent. The inactive condition may be permanent or the gene may be triggered into action by some event later in the life of the cell.

Molecular biologists have put reverse transcriptase to work as a powerful tool for fishing out a particular gene from among many. The messenger RNA (mRNA) is first extracted from a cell that is actively making a desired protein; for example, hemoglobin in a red blood cell. This step is relatively easy because there may be hundreds or even thousands of mRNA copies of a single gene in cells actively synthesizing a particular protein. The mRNA is then purified and recopied into DNA, catalyzed by reverse transcriptase. This is also known as cDNA (complementary DNA), a reverse transcriptase copy of mRNA. The double helix of DNA is made from the cDNA by exposing it to a mixture of nucleotides and the enzyme DNA polymerase. The result is a complete copy of the desired gene.

The synthetic gene can then be glued to a selected vector by recombinant methods, and inserted into a host cell where its product may be made. Alternatively, numerous copies of it can be cloned by putting it into the plasmid of a bacterial cell.

DNA Probes

The DNA probe provides a way to locate a particular gene within the complex structure of a cell. It is possible to separate the two strands of the DNA molecule by thermal or chemical treatment, and then by using a variety of methods, bring the two strands back together again. Before the strands rejoin, single strands from another source can be introduced into the medium, which, if they fit by complementary base pairing with one or the other of the original strands, will join chemically to form a double helix. This technique is called DNA hybridization, from the fact that the reformed helix is a combination, or hybrid, derived from two sources. Hybridization experiments are usually done to measure the closeness of a genetic relationship.

The DNA probe technique is nothing more than a modified hybridization experiment. Instead of hybridizing whole chromosomes together, pieces of DNA (or RNA) having an appropriate nucleotide sequence are given a radioactive or fluorescent label in the laboratory. The helical DNA of the experimental cell is then separated to

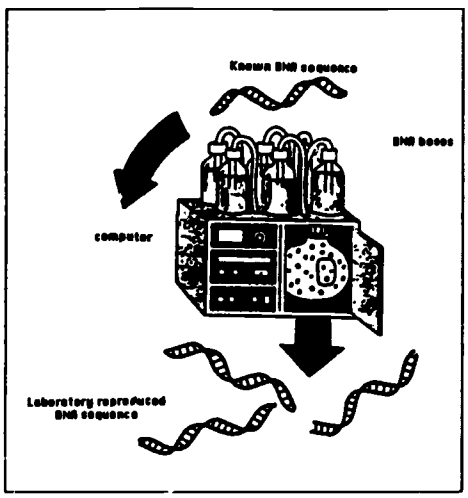
expose the bases, and the probe is introduced. When coming back together again, the labeled DNA will then attach to its complement if one is present. The location of the piece is rather easily identified by its label. This would reveal the place on the chromosomes where the gene in question is found.

These powerful tools have been used not only to investigate and isolate genes within a genome, but as a strategy for studying human diseases — medical diagnosis using genetic probes (Lewin 1983). The desired nucleotides sequences are assembled in the laboratory and used to test for the presence or absence of a particular disease or genetic defect. Cells from a patient are fragmented, the DNA exposed, and the labeled probe added. Hybridization with the patient's DNA is a positive test for the suspected condition. DNA probes are now available that diagnose hard-to-detect human diseases like the early stages of cancer and herpes, intestinal and venereal diseases, and certain hereditary defects. In the longer term it is hoped that probes will be developed to detect tendencies in adults to particular illnesses that have a genetic base. Whether people will want to know that they are carriers of latent genes for hereditary diseases is still unknown. What effect will the information have on a person's emotional well-being knowing that a heart attack or cancer awaits them if the wrong move is made? Will it influence one's decision to marry, or to have children? And equally important, will an insurance company risk insuring such persons or an employer risk hiring them? Here, too, many questions exist but few answers are known.

"Gene Machines"

The plentiful supply of genes for doing genetic engineering took a sudden and dramatic leap forward with the development of the gene synthesizer or "gene machine" (Menosky 1984). Once the nucleotide sequence of a gene is known, and there are simple ways for determining it, the information can be programmed into a computer which automatically synthesizes a workable gene segment, usually in less than a day. Before this breakthrough, the same job often took scientists and technicians four to eight months of painstaking laboratory tedium, depending on the size of the gene. With the machine, all the user need do is type the code of the desired gene onto the machine's keyboard and wait; the machine does the rest. It will deliver a quantity of synthetic gene fragments that then can be spliced together and put into the DNA of a living organism. Because the machine is so fast, its use in genetic engineering is having the same effect that the computer has had on mathematics. New problems are being tackled and solved; the once difficult has become easier, the impossible, feasible. For instance, the scientists now can readily modify naturally occurring genes, or even design new ones. They can be spliced into cells to check out their functionality.

Another machine, the protein se-



"Gene machines" tailor-make genes. Desired DNA sequences are programmed into a computer which automatically translates the information into a segment of DNA.

quencer quickly tells the amino acid makeup of any protein. Put a sample of the protein in one end, and after several hours, the machine spells out which amino acids are present and the order in which they occur. By working backwards, an experimenter can use this information to determine the nucleotide sequence of the gene that manufactured the protein. This data is then programmed into the gene synthesizer, the proper gene manufactured, spliced into a bacterial cell, and if expression is gotten, quantities of the protein may be harvested. Nucleotide sequencers, which function in much the same way, can quickly reveal the sequence of bases that are present in a segment of DNA or RNA (Lewis 1986).

Electrophoresis

Individual genes, of course, are much too small to be seen even with the most powerful microscopes. But genetic engineers must know which genes they are experimenting with. One can resort to the traditional method of classical genetics by searching for the product of a gene in the phenotype of the organisms possessing the gene. But this approach may be terribly slow; at other times the gene may be present but it is inactive (e.g., it could be in the recessive condition).

The method of gel electrophoresis makes it possible to visualize gene fragments directly. Electrophoresis literally means "to carry with electricity." Pieces of DNA taken from restriction enzyme digests are carefully placed in small wells cut into a gel slab. The gel is immersed in a buffer solution that provides ions to increase the conductivity of water. An electric current is then applied to either end of the solution which sets up an electric field within the gel. Phosphate groups radiating out from each side of the DNA molecule discharge hydrogen ions to give the whole DNA molecule a net negative charge. Hence, the DNA migrates within the gel toward the positive pole of the electric field.

But molecules cannot move unimpeded through the gel. The gel is usually made of agarose, a highly branched carbohydrate which forms a net-like maze though which the molecules must pass. The speed at which they pass is proportional to their molecular size. Small fragments will move relatively faster from the point of origin compared to the larger fragments.

Gel electrophoresis thus provides a convenient method for separating DNA molecules according to size. By adjusting the concentration of the agarose gel, the ability to resolve the molecular size of the fragments is refined.

The finished gel is stained with a coloring agent which binds to the DNA. The stain (ethidium bromide) glows under the effect of ultraviolet light. The positions of the various fragments on the slab can be readily determined. Selected fragments can also be washed from the gel for further study or use.

Fermentation

As mentioned earlier, many of the commercial products of biotechnology are proteins manufactured by genetically engineered microorganisms. The human protein hormone insulin and growth hormone in cattle, enzymes for industrial purposes, interferon to combat disease, blood-clotting factors to control bleeding, and amino acids as food additives are but a few of the possibilities.

The transition from successful laboratory experimentation to full scale industrial production, however, poses significant problems of scale. Producing a commodity for market requires more than knowledge of the processes involved and the ability to manipulate them. The product must be manufactured in large quantities, and it must be offered at a cost people are willing to pay, with enough left over for a profit to be made by investors.

Commercial scale industrial synthesis of a molecular product is done in large, carefully regulated tanks called bioreactors, which utilize the biological process of fermentation. It is for this reason that the design of cost-efficient bioreactors and associated production techniques is one of the ongoing concerns of biotechnology.

Bioprocess technology using living organisms or their enzymes to manufacture commercial products is an extension of ancient techniques applied to human needs. When our early ancestors made alcoholic beverages from grains and berries, they used a bioprocess — a mixture of yeast cells and starch to form a fermentation system which made alcohol and carbon dioxide. Later it was learned that bread dough could be leavened with yeast and milk could be made into cheese by the action of various molds.

But it wasn't until 1815 that the process of fermentation was first studied in a scientific way. The French chemist, Louis Gay-Lussac, concluded that it was a physical phenomenom in which inert matter decomposed into carbon dioxide and ethyl alcohol (Antebi & Fishlock 1986). This view persisted well into the nineteenth century until Louis Pasteur showed that fermentation was really caused by living organisms.

Later studies led to the discovery that the organisms of fermentation could synthesize other products besides carbon dioxide and alcohol. Glycerol, an ingredient used in explosives, was produced when sodium bisulfite was added to the fermentation tank. Acetone was found to be another product of fermentation.

The great turning point in this technology came during World War II with the production of commercial-size quantities of penicillin, an antibiotic made by the microscopic fungus, *Penicillium notatum*. This breakthrough was made possible by the discovery of a way to prevent contamination of the culture medium by undesirable bacteria (Antebi & Fishlock 1986).

The next step was added by Japanese scientists in the 1950s. They learned how to regulate microbial metabolism systematically by applying knowledge of biochemistry and microbial genetics. The trick was to change the direction of normal metabolism and pick up desirable molecules along the way. For example, bacteria could be coaxed into producing the amino acid glutamic acid by modifying the culture medium and inhibiting certain genes.

Systematic screening, which resulted in the discovery of microorganisms having novel functions, new kinds of bacteria and molds, was also vigorously pursued in the search for "living tools" that could manufacture new products. Today, over 190 chemical products are produced by industrial fermentation and there are thousands of others that could be synthesized. Antibiotics, numbering more than 100 kinds, are the largest group, but in terms of volume, the food and energy industries produce the largest quantities. Some examples are beer, wine and vinegar.

Recombinant DNA technology will play an essential role in the development of microbial cultures for industrial fermentation. This way biosynthetic processes will be enhanced and productivity raised. Another contribution of the technique will be for engineering unique microorganisms to make new substances. All of these, coupled with

improvements in the fermenters themselves and better purification and extraction methods, have switched bioprocess technology from simple cooking to industrial production.

In the process, living cells are mixed with nutrients in a fermentation tank, the bioreactor. Nutrients may be sugars, starches, vegetable oil, or even petroleum derivatives, plus additional substances needed to stimulate cellular growth. The simplified chemical equation for the culture of cells is written:

source of carbon for energy + oxygen (for aerobic processes) + nitrogen source + growth factors—> cells + product + carbon dioxide + heat + water.

The first part of the equation represents the culture medium, the second the products obtained.

The synthesis of a product in the bioreactor is called bioconversion, a chemical reaction in which microorganisms, or possibly plant or animal cells, play the role of catalyst. Depending on the process, the cells may be alive, or enzymes by themselves may be used. Temperature, pressure and pH of the culture must be carefully regulated during the bioconversion process.

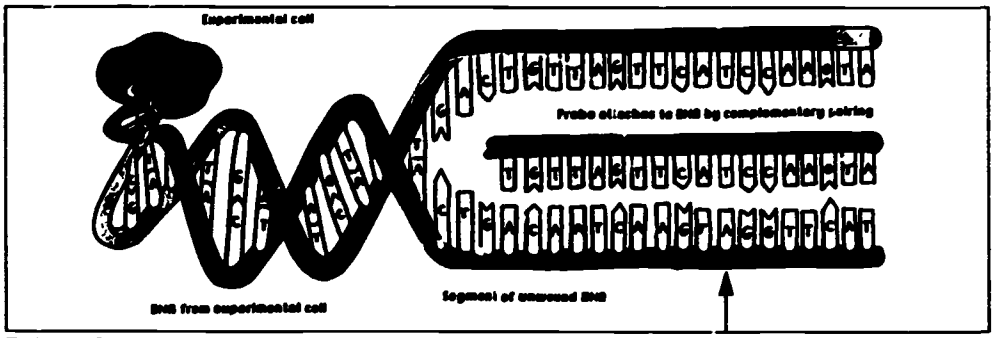
Upon completion of the reaction, the mixture is removed and the desired product separated and purified. This may not be an easy task, however, for the substance is contained in a large volume of material and results are never totally assured. Any contamination or slight variation of metabolism during fermentation might provoke the breakdown of the results sought.

Two kinds of fermentation technology dominate the industry: batch processing and continuous processing (Olson 1986). In the first, the whole mixture of nutrients and microorganisms are removed from the tank and the reaction stopped upon completion of the processing, followed by isolation of the product. The reaction is not stopped in continuous processing; nutrients enter the vessel, and spent medium containing the desired product exits continuously. Cells or enzymes doing the fermenting are usually immobilized within the reactor so they are not swept out with the outflow. This can be done by bonding cells or molecules to a solid support, trapping them in a polymer matrix, or encapsulating them within semipermeable membranous spheres.

Continuous processing is economically more advantageous because of its higher productivity and lower costs since cells or enzymes are continuously reused; separating the product from the effluent is also easier.

Despite continuing improvements in the technology, bothersome problems remain. These include maintaining a homogeneous mixture of nutrients, dissipating the large quantities of heat generated during fermentation, avoiding contamination, and separating and purifying products. Another problem is that genetically engineered organisms may also mutate or revert back to an earlier genetic state, rendering them useless.

Furthermore, there is a pressing need to find microorganisms better suited to bioprocessing technologies. *Escherichia coli* (*E. coli*) is the organism most frequently cultured, but it has its drawbacks. The bacterium produces toxic substances called endotoxins which must be removed, its growth is slow, and it is hungry for nutrients (which are expensive). Moreover, the products it manufactures remain inside the cell making



DNA probes are pieces of laboratory-made nucleotide sequences which match up with a small portion of an experimental cell's DNA. The DNA of the experimental cell is unwound and the probe will attach if a complementary region is present. The joining up indicates that a particular DNA segment is present in the experimental cell. (From *What is Biotechnology?* by the Industrial Biotechnology Association.)

it necessary to fracture the cell to get at them. Ideally, the fermenting cells should grow fast, not make toxic compounds, and secrete their products into the surrounding medium. Yeast cells have these features and are predicted to become the new "workhorse" of the genetic engineers.

Ways must also be found to grow large numbers of complex cells like plant, animal and human cells in bioreactors. It is now thought that these cells of higher organisms will be more useful for producing commercial grade substances.

Monoclonal Antibodies

There is a mistaken tendency to use the terms "genetic engineering" and "biotechnology" interchangeably. They are not synonyms. The engineering of genes, also called recombinant DNA technology, is but one technique of biotechnology. We have already encountered another, the technology involved with bioprocessing. A third important tool is monoclonal antibody technology in which considerable research is presently being conducted (see example, Milstein 1980).

Although still in its infancy, monoclonal antibody technology promises many valuable applications: in the diagnosis and treatment of many diseases; as a way for separating and purifying proteins from cellular components; for monitoring pregnancy; and for the detection of cancer or blood clots.

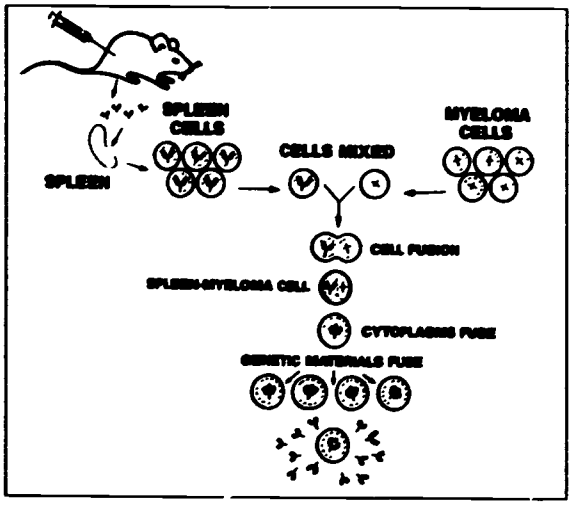
Substances foreign to the body such as disease-causing agents, have structural features on their surfaces called antigens. Antigens are recognized by the body's immune system as being foreign. The natural defense against these foreign invaders is antibodies, complex proteins that are produced and secreted by B lymphocytes, a type of white blood cell. Antibodies seek out and help destroy antigens. A lymphocyte usually produces only a single kind of antibody so that there are countless kinds of lymphocytes in the human body, one for each antigen encountered. A transformed lymphocyte (one manufacturing a particular antibody) may proliferate rapidly when it detects its corresponding antigen.

The two features of antibodies are these: (1) they are extremely specific, that is, they bind to and attack only one particular kind of antigen, and (2) some antibodies, once formed, persist indefinitely in the body to confer lasting resistance to a disease. Examples are the antibodies against childhood mumps and measles which last a lifetime.

The second characteristic makes it possible to induce resistance by vaccination. In this procedure, a vaccine of the weakened or killed infective agent is prepared. When introduced into the body it stimulates the synthesis of an antibody against the antigen. Development of a new generation of vaccines using genetic engineering will be discussed under "Human Application of Biotechnology."

The first property of antibodies, their specificity, makes monoclonal antibody technology so valuable. The traditional way for making antibodies in the laboratory is to inject an animal with a particular antigen. After the antibodies are formed, they are collected from the animal's blood serum (blood minus cells). There are at least two limitations with this method, however. The yield of usable antibody is very small, and the injected antigen usually contains impurities to which the animal also may make antibodies. Monoclonal antibody technology solves both of these; large quantities of a single antibody can be made, and it is very pure.

The recipe for making them is as follows. Two kinds of cells are isolated and purified: B lymphocytes that are producing a particular antibody from the spleen, and tumor cells which cause a type of cancer known as myeloma from bone marrow. The myeloma cells can be induced to grow continuously in cell culture. The two cell types are mixed together in a dish where they will fuse to form single hybrid cells when PEG (polyethylene glycol) is added. PEG causes cell membranes to melt into one another. The new combinations are called hybridomas and they display the combined characteristics of the two. The tumor portion bestows "immortality," stimulating the hybridoma to grow and divide indefinitely; the lymphocyte component synthesizes virtually unlimited quantities of chemically identical antibody.



The production of monoclonal antibodies. Cancer cells fuse with spleen cells synthesizing the antibody of interest. The resulting hybridoma makes large quantities of the single antibody.

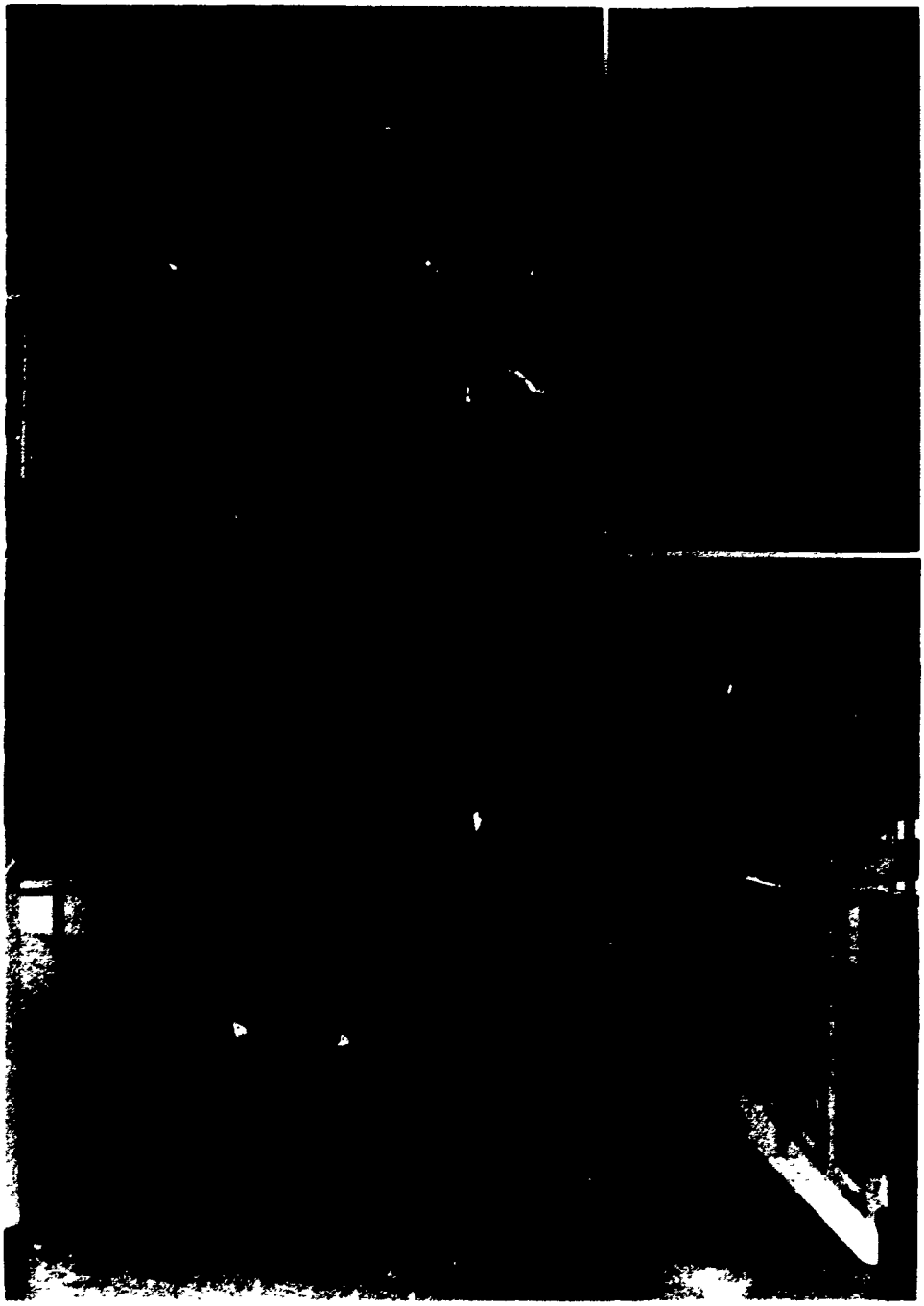
The antibodies produced by this method are named monoclonal because they come from one type of cell, the hybridoma. Antibodies synthesized by the conventional method, called polyclonal, are prepared from many kinds of lymphocytes. Because selected hybridomas make only one specific kind of antibody, the product is much more pure than polyclonal antibodies. Antibodies produced by monoclonal methods will attack only single target molecules and no other. This property, plus the ease with which large quantities of them can be made, make them especially useful for application in biotechnology.

Ordinary laboratory mice are used to make monclonal antibodies — antibody-producing spleen cells and "immortal" rodent cancer cells. But many scientists believe that it would be more desirable if the source was human because people may have negative reactions to protein antibodies from nonhuman sources. In order to produce antibodies by the same technique, though, people would have to give up their spleens.

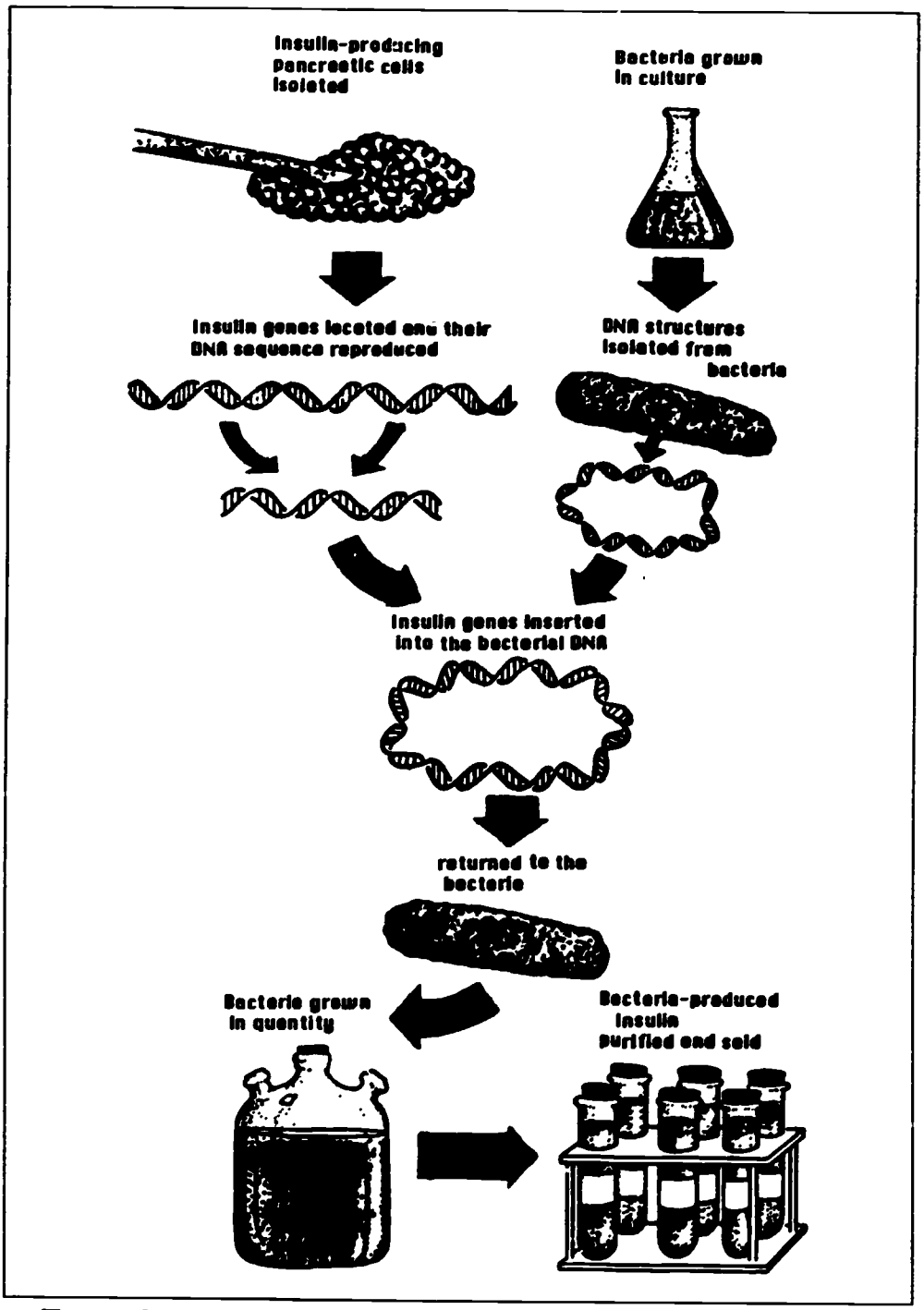
A new technique gets around this requirement by exposing human white blood cells to antigens against which they begin to produce antibody (*Science News* 1986). These cells are then "immortalized" by inserting a cancer-causing virus into them (i.e., Epstein-Barr virus). If it can be demonstrated to work against a range of antigens, the procedure will be a major advance in monoclonal antibody technology.

A Summary Thought

As we have seen, the tools of genetic engineering are not limited to recombinant DNA, gene isolation, cloning, splicing and gaining expression. They include a host of other techniques without which this technology could not exist. Monoclonal antibody technology is one of these; others not discussed here are microbial engineering, sophisticated instruments for studying the activities inside living cells and complex computer systems to analyze and simulate data. In the future, it may be possible to engineer cells much more precisely than we can now. As always, enormous powers to manipulate nature have gigantic impacts on the very foundations of our social structure, in how we view our world and ourselves. Insofar as this is true, we may very well speak of our new powers as the instigators of a real "biological revolution!"



An industrial-size fermentation installation. (Adapted from cover of *Science*, 6/13/86, Vol. 232(5756) © 1986 by the AAAS. Photos courtesy of Celltech, Ltd. and Paul H. Williams.)



The procedure used to mass produce human insulin using recombinant DNA techniques.

Applications of Biotechnology

Biotechnology is still in its infancy, but already it is clear that its impact on industry will be far-reaching. As our scientific knowledge of how living organisms grow, reproduce and change expands, the range of possible applications widens. Geneticists, microbiologists and specialists in related fields all will make a contribution. The rewards will be the production of new drugs and chemicals, the improvement of agricultural processes and food, the amelioration of some human genetic diseases, better ways to protect human and animal health, and the degradation of toxic substances and wastes from the environment.

To convey some sense of this beehive of activities presently going on in biotechnology, we will look at its application in three general areas: human; agricultural; and energy/environmental management.

Human Applications

Recombinant DNA and monoclonal antibody technology are at the center of a revolution in the improvement of human health. Over the last 10 years, these technologies have led to a veritable explosion of research information that now is beginning to yield tangible results. Applications of recombinant DNA methods are spawning a powerful generation of new drugs and ways to diagnose and treat age-old human maladies.

The first genetically engineered protein, or natural drug, to reach the marketplace was human insulin, Humulin; that was in 1983 (Olson 1986). Insulin is the hormone that regulates the metabolism of glucose, and persons who lack sufficient quantities of it suffer from the disease *Diabetes mellitus*. The synthetic hormone was made by fusing the human gene for insulin with a bacterial plasmid. Put back into bacteria, the recombinant plasmid proceeded to synthesize insulin. (Although the first successes were achieved in 1978, it took four years to test and upgrade the process for commercial production.)

The next commercial success was the manufacture of a human growth hormone by the same method. This synthetic substance, called protropin, is used to treat certain types of dwarfism in children. Without the hormone, children whose pituitary gland doesn't produce enough of it grow to only about four feet tall. If administered protropin, they attain normal height.

These first demonstrations that engineered microorganisms could be converted into living "factories" for manufacturing useful molecules were followed by an avalanche of experimentations searching for commercially valuable products. The power of recombinant DNA technology applied to human problems was that for the first time the opportunity existed to obtain virtually unlimited quantities of practically any protein.

Successes include: blood clotting elements, Factors VIII and IX, for the treatment of hemophilia; the human lung surfactant protein to prevent lung collapse in

infant respiratory distress syndrome; the brain's natural pain killer encephalin; protein tissue plasminogen activator (t-pa) effective in selectively dissolving blood clots that cause heart attacks; and key components of the body's immune system known as the lymphokines. More than 50 lymphokines are known: interferons; interleukins; macrophages; activation factor; B cell growth factor; and tumor necrosis factor, among them.

Of the lymphokines, the interferons are attracting a great deal of research interest. Natural interferons are the body's first line of defense against viral infections; they make uninfected cells more resistant to viral penetration. These lymphokines also have a growth-retarding effect on certain kinds of cancers.

Interferon is not a single molecule but a class of substances comprised of three major groups — alpha, beta and gamma interferon. Each group also has a number of subtypes as well; for example, there are 12 to 14 alpha interferons alone. The various kinds have their own chemical and physical properties, and mode of action (Godown 1985).

Within the human body, interferons are produced and secreted by different body cells, especially certain kinds of white blood cells and fibroblasts, but in extremely minute quantities. They also are very difficult to extract in pure form from body fluids. Natural interferon obtained from natural sources was, therefore, very costly, and even the best preparations contained less than one percent of the chemical mixed in with many impurities. For a long time, these problems restricted the kinds of research that could be done with this wondrous protein.

In the late 1970s, genetic engineers successfully transferred the genes for several interferons into bacterial plasmids. Their insertion into *E. coli* resulted in the production of large quantities of the pure substance. Human interferon was thus the first trace biological substance, one that is made in very small quantities in the body, to be manufactured by recombinant DNA technology. With a plentiful supply of the molecule, scientists next turned their attention to an intensive investigation of the properties of interferon as well as the clinical usefulness for the treatment of viral infections and cancer.

The suppression of tumor growth was an unexpected and startling observation and one that is still only partly understood. One possibility is that interferon decreases the rate at which both normal and tumor cells multiply, but because the cancerous ones divide relatively faster than normal cells, their growth is retarded more. Multiple myeloma, Karposi's sarcoma, superficial bladder cancer, malignant melanoma and renal cell cancer are some of the cancers treated with this substance. Alpha interferon was approved for legal sale in the United States in 1986 for the treatment of hairy cell cancer by the Federal Food and Drug Administration. The common cold, herpes simplex, shingles and warts are a few of the virus-induced diseases that interferon can control.

The effectiveness of these compounds against cancer and viruses shows great promise but much remains yet to be learned. The same is the case with several of the other lymphokines, especially the interleukines (see p. 35). But recombinant technology has made quantity manufacture of these possible; we therefore can expect that medical research will provide the answers we eagerly seek. The availability of this panoply of drugs to physicians in the future holds the promise of successfully combating many of humankind's most fearful afflictions — viral diseases, cancer and the most dreaded of

all, AIDS (Acquired Immune Deficiency Syndrome).

Vaccines, too, can be made by recombinant DNA technology. Historically, vaccine was the first weapon used in the fight against viral disease. Edward Jenner, in 1797, induced immunity by introducing the less virulent cowpox virus into humans to protect against the more serious smallpox virus (Antebi & Fishlock 1986).

Jenner's method for inciting immunity relies on so-called "first generation" vaccines; the method still is widely used. Attenuated (weakened) natural disease-causing agents are injected into a person to create an immunity without causing the disease. The trend now is toward "second generation" vaccines made possible by genetic engineering. Underlying the research is the need for safer, and better, vaccinating agents to induce immunity.

The problem with the older method is that the substance used may, under certain conditions, result in undesirable side effects in the person being vaccinated. In the most extreme case, the attentuated agent may revert back to the virulent condition to cause the full-blown illness. Fortunately, this occurrence is rare and usually is associated with normally virulent pathogens that have been weakened by chemical or physical means. (The outcome, though, is more common with farm animal vaccines where up to 50 percent of those injected may die. It is for this reason that many American farmers do not vaccinate their animals. They prefer instead to use other methods to control the spread of disease, including the occasional slaughter of whole herds when a serious pathogen appears.)

Another undesirable consequence is that the vaccine, usually from a nonhuman source, contains a number of impurities in the form of other proteins that are difficult or expensive to remove. These can trigger adverse reactions in individuals receiving the vaccine, at times resulting in crippling or even deadly outcomes.

The biology of making second generation vaccines is rather straightforward. Infective agents like viruses, bacteria or parasites that live in body tissues or blood, carry proteins on their surface that the immune system recognizes as antigenic. It is against these surface proteins that the immune system naturally responds by constructing antibodies; they are not made against the whole organism or agent. The strategy then is to identify the particular component of a pathogen that induces the immunity. Being a protein, the antigen is coded for by genes. When located, the gene or genes are scissored out of the pathogen and spliced into a bacterial plasmid, or more recently, into yeast cells and the recombinant grown in a culture medium. The antigenic protein is separated from the medium, purified and made into a vaccine. When injected into the body, that protein is recognized by the immune system as foreign, and the appropriate antibody is made to neutralize or destroy it. Later, if the natural agent that normally carries the same protein is encountered, an immune response will be triggered to eliminate it. The fact that only part of the disease-causing agent is present is the reason why these are also called subunit vaccines (Olson 1986).

The first subunit vaccine was developed in 1981; it was an animal vaccine against the highly contagious hoof-and-mouth disease. In 1986, the FDA approved the first engineering human vaccine for use against hepatitis B (*BioScience* 1986). Subunit vaccines against a whole host of animal and human diseases are now actively being researched: herpes, of which there are 300,000 new cases each year in the United States alone; AIDS, which is described as a modern day plague; rabies, a potentially deadly disease that is treated with painful antirabies shots; toxic shock syndrome that incapaci-

tates or slays its victims; malaria, still the major killer in the world today; and other infectious pathogens too numerous to mention The future of these vaccines seems full of promise, and we can confidently expect that the payoff will come soon, made possible by advances in biotechnology.

The ability to diagnose health problems is another area where great strides are being made. Many diseases, including sickle cell and other forms of anemia, Tay-Sachs and Huntington's disease, and certain blood disorders, are hereditary. They arise from a defective gene that is passed from parent to child. Using the tools of genetic engineering, it is now possible to identify and characterize the specific genes that encode for many congenital disorders.

One highly sensitive approach for detecting defective genes uses a DNA probe to locate and study them. Recombinant DNA technology is used to make a labeled DNA fragment, which corresponds to the defective gene. As we saw in an earlier discussion, the synthesized probe can hybridize with the DNA of a whole cell if the particular gene is present in the genome. The hybrid DNA then can be isolated, and the gene segment of interest located and analyzed.

DNA probes are used in this way to diagnose the presence of numerous genetic disorders (Gedown 1984). For example, to test for sickle cell disease in a fetus, a sample of the mother's amniotic fluid is taken. Fetal cells sloughed off by the fetus are centrifuged out of the fluid and the DNA exposed to a radioactive probe. Hybridization of the artificial gene with the fetal DNA confirms that the gene for sickle cell disease is present (White 1986).

The first commercial tests for prenatal diagnosis of cystic fibrosis, the most common lethal inherited disease in Caucasians, became available in 1986 (Kolata 1986). Testing for individuals who might carry the gene for Huntington's disease, the gene for Duchennes muscular dystrophy, and at least one kind of cancer (retinoblastoma) soon will be ready (Motulsky 1983).

DNA probes have also opened doors to detect food contaminants and infectious microorganisms within the body. These tests can be done in a matter of hours rather than the days that the old methods required. Treatment can be started sooner, thereby increasing the chance of a rapid and successful outcome. Many other screens can and will be devised when the genes that cause a diseased condition have been identified and cloned.

The workhorse of these diagnostic procedures is the laboratory-made DNA probe, another product of gene technology. Genetic markers are bits of DNA that lie near a disease gene that has not yet been identified. If the marker consistently is inherited by victims of a disease, it signals that the defective gene must be nearby. Therefore, genetic markers serve as proxies for the hidden gene in diagnostic testing. Their use, along with DNA probes, has made it theoretically possible, at least, to detect most of the diseases caused by single gene mutations.

Both can be applied in another way, too, in "predictive medicine." Predictive medicine attempts to identify, in advance, individuals at risk for certain disorders so that they can take precautions to prevent or postpone the onset of the disease (McAuliffe & McAuliffe 1983). One of these is emphysema, a particularly debilitating lung disease that frequently kills its victims within a few years after the symptoms first appear. One out of every 2,000 caucasians of Northern European descent will be afflicted with the disease.

Affected persons have a low level of the lung-protective enzyme, alpha-1-antitrypsin (AT), an inherited abnormality. The patient's gene for the enzyme differs from the normal by only a single changed nucleotide base. The defect can be detected by using a radioactive DNA probe. By knowing this, the individual can avoid those situations that could trigger the disease by not smoking cigarettes or staying out of heavily polluted air. The onset of the illness can be postponed for up to 30 years by following these precautions (Ellis 1985).

Another probe is being researched that can pinpoint the several mutations that lead to one of the leading killers in the United States today, arteriosclerosis, or hardening of the arteries. The faulty genes, present in one out of every 500 people, interferes with the normal cellular function of absorbing and breaking down excess cholesterol. Instead, the substance builds up, eventually clogging arteries. Blocked arteries in the heart are the most frequent cause of premature death (death before age 65). The condition begins early, in the teenage years, but shows no symptoms until middle age when heart attacks begin. Again, if people having the genetic defect were aware in advance of their condition, high cholesterol foods could be avoided to postpone or prevent the deadly consequences of fat in the arteries.

Another use of probes is in paternity testing to establish a child's biological father. The DNA of sperm can be matched to that of the child. Such tests are much more accurate than more frequently used blood tests. Probes can establish guilt or innocence in certain criminal cases: rape, for example. Again, sperm from the rape victim can be compared with that of the suspected rapist to determine a verdict (Saltus 1986).

Clearly, marker technology has opened up a whole new future for medicine and many biotech companies are rushing in to develop the necessary probes.

The ability to spot health problems is being aided by another product of biotechnology, monoclonal antibodies. Since the antibodies are highly purified and bind very specifically to an antigen, they are ideal for detecting a disease already present in the body. It is now thought that the application of monoclonal antibodies in diagnosis could revolutionize the entire concept of clinical examination (Antebi & Fishlock 1986). Tests for allergies, anemia, leukemia, pregnancy, blood group determination, venereal diseases, hepatitis B, genetic defects in fetuses and the detection of hard-to-diagnose cancers are some of these. Monoclonal antibodies make it possible to test for compatibility in organ transplants, between a donor and recipient, by comparing histocompatibility proteins.

Histocompatibility proteins are cell surface antigens that participate in cell-cell recognition. They are also the antigens that trigger tissue rejection when unmatched organs are transplanted. Although no two persons, other than identical twins, have the same histocompatibility proteins, the closer they match, the better the chances of a successful graft between donor and recipient.

Monoclonal antibodies for diagnosis are not viewed as competitors of DNA probes but rather as their partners. In the future, these two techniques, along with sophisticated computers, should make rapid and accurate diagnosis of diseases possible, with one consequence being the earlier selection of a therapy to effect a cure.

Armed with genetic engineering technology, medical scientists are gaining powerful insights into the deep-rooted causes of cancer. Potential for the disease lurks within every one of us in the form of normal cellular genes which control such biological processes as development and growth. They can change into oncogenes, cancer-causing

genes, by alteration of their DNA codes. Ionizing radiation, certain chemicals, some retroviruses and other poorly understood factors can bring about the transformation. More than 40 different human oncogenes have been identified so far (Weinberg 1985; Langmore 1986).

It is presently thought that at least two distinct oncogenes must be activated if cancer is to appear somewhere in the body. One oncogene change stimulates a cell to escape normal mitotic controls to grow indefinitely; this step is named "immortalization." A second gene modification allows cell growth even though the normal growth stimulus is lacking. The locations in the genome where these changes take place can be identified and studied using recombinant DNA methods. For example, the single base change of thymine for guanine in a 350 base-long gene is now known to be responsible for the immortalization steps.

A distinguishing feature of cancer cells which sets them apart from normal cells, besides their pathology, is the presence of unique antigens on their surfaces. Thus, from the viewpoint of the body's immune system, cancer cells are foreign. Monoclonal antibodies can be made to detect these antigens; the antibodies can then be used to signal the presence of cancer in its early stages. Early detection, while the cancer is still localized and more susceptible to treatment, increases the chance that therapy will check its growth and prevent the cancer from spreading to other parts of the body.

Although the three main modalities for treating cancer continue to be surgery, radiotherapy and chemotherapy, recent advances in cancer immunology and biotechnology are making innovative treatment strategies possible, especially the use of natural chemical inhibitors. These now are being made using recombinant DNA technology.

We already mentioned the role that the interferons are playing in the battle against cancer. The interleukins are another group of lymphokines that show anti-cancer activity. These chemicals act as the key to the immune system; they turn it on when a foreign protein is detected. There are several groups of interleukins, and one of these, interleukin 2, secreted by "helper" T cells, attaches to the surface of "killer" T cells during an infection. "Killer" T cells then start to divide and to secrete substances that destroy the invader, for example, a virus or tumorous cell. This property of being able to mobilize "killer" T cells against cancer has raised the hopes of medical experimenters that someday a "magic bullet" will be found that selectively annihilates cancerous tissue.

The search for the "bullet" applies to monoclonal antibody technology in another way; to program a killer drug so it will home in only on its target cancer cells (Antebi & Fishlock 1986). The main drawback with conventional chemotherapy for neoplastic disease is not that the chemicals used are unable to kill cancer cells but that they lack specificity. Anti-cancer drugs are powerful poisons that destroy both malignant and normal cells. Because these drugs circulate randomly throughout the body, it often is necessary to administer quite large doses to ensure the cancer has been exposed. The well known consequence is that the patient is left in a weakened, frequently sickly, condition; nausea and vomiting, weight and hair loss are some unintended outcomes.

This lack of specificity could be overridden if a cytotoxic molecule, a chemical that kills cells, was able to pick out and attach only to malignant cells. The wherewithal to create such a biological missile is now on hand, the product of biotechnology.

The blueprint for its construction is simple. A monoclonal antibody to the cancer antigen is first made, and then a cytotoxic chemical is bonded to it. The resulting hybrid is called an immunotoxin, an appropriate name since it is half antibody and half poison.

A cell can become cancerous by one or a few nucleotide changes. In the diagram, T (thymine) has been substituted for G (guanine). Such changes in at least two different genes are thought necessary to produce cancer.

Diphtheria toxin and ricin, an extract from seeds of the castor oil plant, are two cellular poisons being investigated. Both kill cells by wrecking the protein synthesis machinery located in ribosomes.

The immunotoxin, when injected into a patient, searches out and attaches to the antigen on the cancer cell, whereupon it delivers its deadly cargo. Ideally, only designated target cells will be demolished. An added plus is that the patient needs much smaller doses of the cytotoxin.

Leukemia and lymphoma, two liquid tissue cancers are two forms on which the therapy is now being tried with some success. Their effect on solid tumors is not so promising since several problems must first be overcome. For one, cells comprising a tumor are not homogeneous in their surface antigens; therefore, a single immunotoxin will not wipe it out. Another is accessibility; the tumorous mass is rather tightly packed, restricting uptake of the drug.

Immunotoxins also have been used to prevent tissue rejection following an organ transplant. The period immediately following the surgery is one of the critical times when rejection can occur. This is because healing is taking place between the grafted tissue and host and is accompanied by extremely high titers of circulating antigen coming from bits broken off from the transplanted tissue. Therefore, the rejection phenomenon must be rigorously prevented during this time.

The body's immune system is completely suppressed for this period of about two weeks by the administration of very powerful immuno-suppressants, but again these are chemicals that compromise the entire body. It is much more desirable to knock out selectively that component of the immune system responsible for the rejection phenomenon, and selected immunotoxins do just that. Monoclonal antibodies are made against T cells and a cytotoxin is attached to form the hybrid. These will perform as described earlier, selectively knocking out that part of the immune system involved with tissue rejections. The patient's recovery therefore is aided, because his or her body is not swamped with chemical poisons.

In all of these areas, it is well to note that the research stage is still experimental; wide scale clinical applications are not yet available for most of them. However, if immunotoxins, like the other products of biotechnology, live up to expectations, they will add yet another weapon to the clinician's arsenal for combatting some of humankind's most fearful diseases.

Before leaving this topic, let us return for a moment to predictive medicine. There is another application outside of the medical setting that is now raising some serious ethical and social questions in the practice called susceptibility screening. For example, genetic testing by industry to screen the health of potential employees already has been done. The aim of this testing is to identify individuals who may be at risk if they are exposed to certain workplace environments, especially environments where certain chemicals will be encountered. Employers find it cheaper to exclude people

rather than pay their health care costs should they come down with a sickness due to the exposure. In a related instance, workers may be passed up for promotion if it becomes known that they carry the gene for high risk to premature heart attack (McAuliffe & McAuliffe 1983).

Predictive medicine undoubtedly will become increasingly easier, and more diseases can be screened for as genetic technology breaks new ground. Diabetes, high blood pressure, allergies of various kinds, peptic ulcers — these and many more maladies of middle life are on the drawing board. By knowing one's predispositions in advance, it will be possible to reduce the risk of a full blown expression by modifying designated aspects of one's lifestyle. But, by knowing, are there other risks that are not intended? Consider these

- Do employers have the right to bar from certain jobs, individuals who carry genes that predispose them to a particular disease?

- If so, what does this do to the egalitarian ideal we cherish in a democracy?

- Could genetic information be used by social and health planners to assign individuals their niche in society?

- Should insurance companies be permitted to refuse insurance to persons at risk?

- And, underlying all of these, does a person have the right not to know, considering that the information may have devastating consequences on one's emotional well-being?

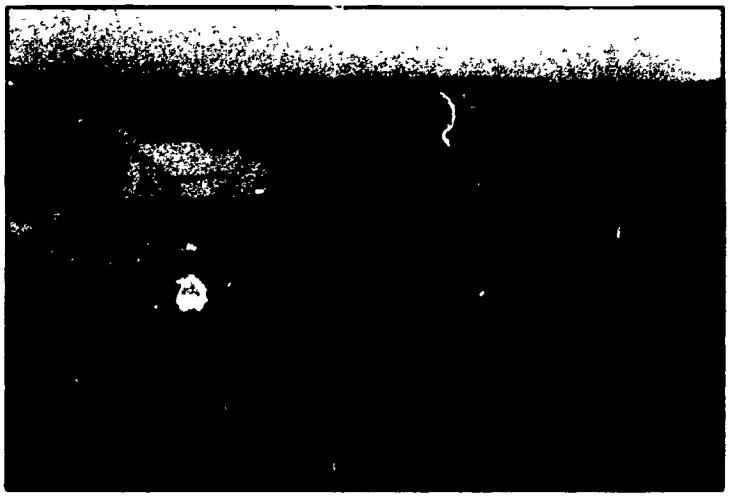
There are few clear-cut answers to any of these at present but progress, whatever its form, inevitably is accompanied by growing pains. Society surely will learn to cope.

The startling advances being made in applying biotechnology to human problems also are being used in veterinary medicine. Subunit vaccines protect newborn pigs and calves from infant diarrhea, an affliction that kills many animals early in life. The already mentioned subunit vaccine against hoof-and-mouth disease is being used. Many others are now, or soon will be, in the pipeline.

Monoclonal antibodies for diagnosing and treating infectious bronchitis virus in poultry, bovine mastitis, an inflammation of the mammary glands, and rabies soon will be available. Outbreaks of these illnesses can have serious economic consequences; hundreds of millions of dollars are lost annually by the world's farmers due to animal diseases. Clearly, the application of biotechnology to farm problems constitutes an enormously large and important area, and many biotech scientists are pursuing research that will have applications here.

One of the more controversial areas today is to use these methods to increase food production. Recombinant DNA has been used to clone the gene for growth hormone of cattle (Sun 1986). The synthetic growth hormone produced by bacterial plasmid when injected into cows, increases milk production by up to 40 percent. This has raised the sensitive political and economic question of whether the practice ought to be continued considering that the nation already produces an enormous oversupply of milk. If children go without, it is not because the industry does not produce enough.

enough. The federal government now pays out millions of dollars in subsidies annually to protect dairy farmers. (Milk shortages may exist in Third World countries, but the hormone is too expensive for them to use.)



One of the many quandaries created by biotechnology: Will the use of growth hormone in cows further weaken the economic position of the nation's dairy farmers? (USDA-Soil Conservation Service by Erwin W. Cole.)

This perplexing problem, like many others raised by new technologies, has no easy solution. Citizens, however, must be involved in discussions about them if policy choices are to be made wisely.

Although not as controversial, a synthetic hormone has been used on the farm to produce slimmed down pigs (*Science News* 1986). The gene for porcine growth hormone produced by bacteria, when injected, makes young pigs develop faster, more efficiently and with leaner meat. The treated hogs reach the market size of 220 to 230 pounds a week to 10 days sooner, thereby lowering overall feed cost by about 25 percent and a carcass with as much as 55 percent less total fat. The improvement in swine could provide the health-conscious consumer with a markedly leaner product.

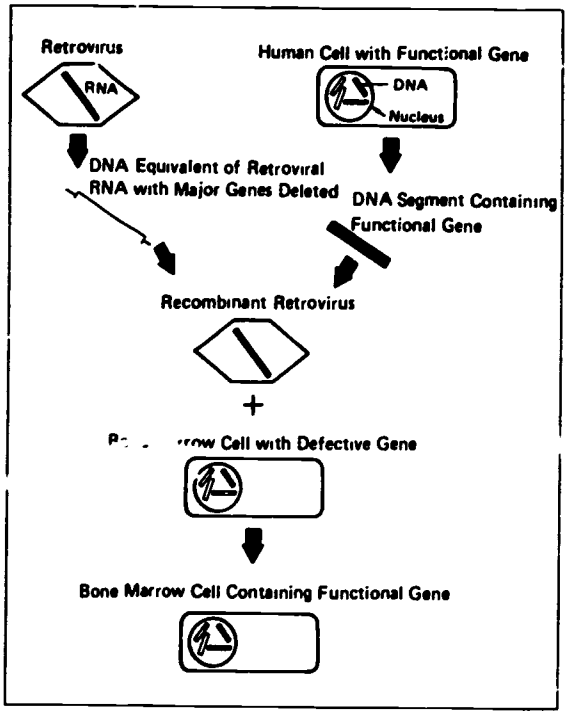
Gene Therapy

It has long been a dream, ever since it first became known that defective genes could cause disease, to replace the offender with its normal counterpart. And it remained a dream for most of this century. But in the last few years, advances in genetic engineering have brought researchers close to a method for transplanting the healthy gene into a patient to restore the missing or impaired function. This new class of treatment is known as gene therapy and it may be ready for human testing in the very near future.

Thousands of people suffer from a genetic disease of one sort or another. This is not hard to imagine for there are at least 2,000 kinds already known (Tangley 1985). None of them can be cured as of yet, and in many instances, very little can be done to alleviate the condition.

To cure a genetic disease with gene therapy, the normal gene for the deficient trait must first be located, isolated and cloned to secure copies of it. Next, a small portion of the patient's tissue is removed, usually bone marrow, because only rapidly dividing cells are suitable. Nuclei of the marrow cells will be induced to take up the normal gene probably by attaching it to a retrovirus vector. The genetically transformed cells will then be reintroduced back into the patient. Hopefully, the delivered genes will be appropriately expressed in their new surroundings producing neither too much nor too little enzyme to correct the defect. It is also hoped that the engineering cells will have an advantage over the genetically defective ones by growing faster, eventually replacing them. Another indicator of success is that the engineered cells must do no harm in the patient's body (Anderson 1984).

Since so little is known about the normal feedback controls in DNA regulation, the first gene therapy attempts will almost certainly be done using genes that have



The genetic engineering of human cells. Retroviruses will be used as vectors to insert a single normal gene into the DNA of a person having a genetic defect. The engineered retrovirus, which has had its disease-causing genes replaced with the desired human gene, will be used to infect bone marrow cells withdrawn from the patient having a defective gene. The transformed marrow cells will then be reimplanted into the patient. (From *Biotechnology: An Industry Comes of Age* 1986 by the National Academy of Science.)

the simple "always on" type of regulation (Anderson 1984). Many of the body's enzymes are produced by a mechanism like this. It is also important that the procedure select only tissues that rapidly regenerate themselves. Bone marrow as mentioned earlier, skin, certain elements of the blood and the intestinal tract lining qualify. If non-dividing tissues are used, the engineered cells will not be able to multiply within the body to supplant the defective cells with healthy ones. The brain, for example, does not grow after birth, so that the methods described here offer little hope for victims of neurological conditions like Huntington's disease or metachromatic leucodystrophy, a disease that affects the myelin sheath surrounding nerve cells of the brain.

The procedure of gene replacement is a revolutionary new approach in treatment. It will, however, not affect genes in germ line cells located in the ovaries or testes; only somatic cells will be manipulated. People whose cells have been engineered will still carry the abnormal gene in their reproductive cells, and if they are able to reproduce, will transmit it to their children. Gene therapy is, therefore, no different from other therapies used in medicine that attempt to improve the health of a sick person. The only difference is that DNA, rather than drugs or surgery is used. Gene therapy is best considered a form of euphenics rather than eugenics. The phenotype but not the reproducing genotype is altered. The point here is important because critics of genetic engineering claim that gene therapy is the first step leading to the complex genetic manufacture of humans. I will discuss this further in a later section.

Although interest in gene therapy as a new procedure for treating genetic disease currently is running high, it has been tried once before, in 1980. Dr. Martin Cline of UCLA attempted to replace the defective gene for beta thalassemia, a painful and deforming blood disorder, in two patients. The effort, though, proved to be unsuccessful; neither patient gained any relief. Dr. Cline was heavily censured for these experiments because he did not secure proper authorization to proceed. Moreover, many scientists felt that this attempt was premature in the absence of full animal experimentation (Olson 1986).

Despite the failure, the experiment demonstrated in clear and dramatic fashion that attempts to alter the human genetic constitution were not a distant prospect but an imminent reality. Another outcome interpreted by some to be at least a partial success was that the patients, although not helped, were not harmed either, suggesting that the procedure did not have untoward side effects.

Since the first attempt, progress has been made on a number of fronts including improving delivery systems for genes. Animal experiments have shown that genes can be inserted into host cells by using a retrovirus vector. The replacement of defective genes in fruit flies and mice to result in a correction also has been demonstrated.

The feat in fruit flies used transposons to ferry the wild-type red eye gene into eggs that carried the brown-eyed mutation. Roughly one-half of the treated eggs had red eyes and they in turn, had red-eyed progeny (Morse 1984).

But, perhaps more startling because they showed phenomena that come closer to humans were two reports in 1986 that normal genes were infused into mice to correct genetic defects. In one case, a globin gene was tranferred to cure the blood disease thalassemia, in the second a gene for gonadotropin releasing hormone (GnRH) was microinjected into mouse eggs to result in a correction (Constantini, Chada & Magrum 1986; Mason et al. 1986). Animals lacking the gene for the hormone are sterile because they cannot produce gametes. A few of the mice having the inserted gene did synthesize

and secrete the hormone and were fertile. Another feature of the correction was that the genes were expressed by the appropriate tissues — the globin gene in bone marrow and the GnRH gene by neurons in the hypothalamus, the location where the hormone is naturally secreted. For the first time, genetic defects in mammals were corrected.

At least six major centers in the United States are conducting full-scale gene replacement experiments. The bottom line objective, plainly stated, is someday to be able to correct human genetic illnesses. A second attempt with human patients, therefore, can be expected very soon.

The prime candidates for correction by retrovirus insertion probably will be single gene disorders in which the gene product is continuously supplied (a mild overproduction of the enzyme then should not be harmful). The following diseases fit this qualification: Lesch-Nyhan syndrome, a rare defect characterized by cerebral palsy; mental retardation and self-mutilation, caused by the absence of the enzyme HPRT (hypoxanthine guanine phosphoribosyl transferase); and two serious immunodeficiency diseases, purine nucleoside phosphorylase (PNP) and adenine deaminase deficiency (ADA) (Anderson 1984). All three are severely debilitating; the defects are associated with bone marrow cells, and the production of even a small amount of the missing enzyme is likely to be beneficial.

It was initially thought that the first tries at correction would be for blood disorders, such as thalassemia and sickle cell disease. These are now regarded as more distant possibilities because the crucial gene product, hemoglobin, demands precise regulation of the gene, something that current knowledge does not allow. (The earlier mentioned correction of thalassemia in mice was achieved by microinjection of the gene into a fertilized egg, not through bone marrow transplant.)

W. French Anderson, a leading expert in the field of gene therapy, cautions that it is "unrealistic to expect a complete cure from the initial attempts at gene therapy" (Olson 1986). The likelihood is low that these first experiments will even alleviate many of the symptoms. The uncertainties are many and so much more remains to be learned.

Children probably will be selected as the first patients because this age group is likely to benefit more in the long run; children also make better (healthier) patients. Furthermore, it will be necessary to weigh the potential risks to the patient against the anticipated benefits to be gained. Producing a pathogenic virus from the disarmed virus vector or malignancy are among the consequences that could leave the patient worse off than before the gene transplant.

In the opinion of some, including Dr. Anderson, it is unethical to further delay human trials, providing safety of the procedure has been established. The argument is that patients with serious genetic disease have little or no hope at the present for alleviating their medical condition.

This view is opposed by loud critics who protest against all forms of gene transfer experiments, in humans as well as animals. The most vociferous of this group is Jeremy Rifkin, the social and environmental crusader referred to earlier. Uneasiness over the technology focuses on the potential role somatic cell gene therapy could play in developing techniques that are clearly socially unacceptable. These are gene transplants to enhance desirable characteristics rather than to cure disease and the fear is that the techniques could lead to making intentional changes in germ line cells for social reasons. Germ line changes could alter the gene pool of the human species. and future generations would have to live with that change, for better or worse.

But most scientists agree genetic surgery for these purposes remains a very distant prospect. As for remodeling humans by inserting genes, most of the characters targeted — intelligence, longevity, physical appearance and health — are not controlled by single genes at all. They result from the cumulative effect of dozens, hundreds or even thousands of genes, interacting with the outside environment. Manipulation of such complex and poorly understood systems has barely begun in animal experiments. Its unraveling, if ever, is many years away.

Germ line changes may be much more of a possibility. The landmark experiment, done in 1984, showed genes could be introduced into fertilized eggs followed by expression. The gene for growth hormone was successfully inserted into fertilized mouse eggs. The transgenic mice — those that had incorporated a foreign gene into their genome — grew to more than twice their normal siblings' size. The experiment's success has generated great interest in the possibility of engineering farm animals in the same way; presumably they would be more productive as food producers, or more resistant to disease. The recent achievement of the genetic transfer of the GnRF gene, also in mice, to correct a genetic defect, makes this genetic modification method a realistic possibility.

Transgenic mice. 10-week-old littermates, the one on the left contains a new gene composed of the mouse metallothionein promoter fused to the rat growth hormone structural gene. The male with the new gene weighs 44 gms. and his sibling without the gene weighs 29 gms. The gene is passed on to offspring which also grow larger than controls. In general, mice that express the gene grow 2-3 times as fast as controls and reach a size up to twice normal. Organisms or cells that have been transformed by the transplantation of a new gene or genes are called transgenic. (Photo by Brinster. From Palmiter, Brinster, Hammer, Trumbauer, Rosenfeld, Birnberg & Evans, "Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes," 1982, *Nature, 300*, 611-615.)

But the procedure is not as simple as it may sound, particularly if humans are the subject. Current techniques are far too risky to even consider trying them on human eggs. Gene implants are successful in only a small fraction of the eggs treated — at best, one in 20, but usually far fewer. The gene is introduced by microinjection; a thin, hollow glass needle is used to penetrate through the egg membrane into the interior and the foreign DNA infused. This puncturing traumatizes the egg, frequently killing it. The success rate with human eggs would probably be much lower because our genetic makeup is much more heterogeneous, making placement and expression of the inserted gene much more problematical.

Concerning the matter of expression, recall that there is no known way to control where a gene implants into a chromosome. Yet, this position can affect the operation of the gene, whether or not it will be expressed. The inserted gene also can change the normal function of the DNA that is already present. It can, for instance, separate a functional gene into two sections and block its action, the result would be a new genetic defect induced by the experimental procedure. Then, too, the inserted gene can turn on other genes within the genome, causing a cancer if the activated gene is an oncogene

(Lepkowski 1982).

The transplanted growth hormone gene in mice was actually expressed by liver cells rather than the pituitary gland, its natural location, and at a very high level. Although the mice grew, the gene was not properly controlled and gigantism resulted. The GnRF gene did express itself in the correct body tissue, but there was no way to predict this in advance. Until more is known about regulation, this method for altering the genome remains one of chance rather than of science.

Because of these and other technical difficulties, the prospects are dim that germ line modification will soon be tried with human eggs. To make matters all the more secure, genetic modification experiments with human germ line cells now are explicitly prohibited by government regulation.

Will we ever reach the level of technical expertise at which safe genetic manipulation of human eggs will become possible? And if so, will the technology be applied? Answers to these are difficult to see. Concerning the first, the response is probably yes — in time. The knowledge, however, will in all likelihood be learned from animal experiments. The second is more uncertain. But, if history teaches anything, it is that morality does change over time. What is deemed unacceptable today may very well be embraced by future generations.

Agricultural Applications

The economic and environmental benefits expected from agricultural uses of recombinant organisms are enormous; some liken it to a revolution (Kenny 1986). By splicing foreign genes into plant chromosomes, it may be possible to create plants resistant to a wide array of pests. The hope and expectation is that such plants will lead to decreased uses of chemical pesticides, many of which are toxic to humans and the environment. Recombinant DNA technology may be used to generate plants that utilize fertilizer more efficiently, or even to create varieties that make their own fertilizer, thereby minimizing fertilizer runoff into streams and lakes. Genes could be spliced that enhance protein synthesis and nutrient storage in plants, resulting in improved nutritional quality and larger yields. This would assure adequate food to feed the world's hungry 10 to 20 years from now. Genetic engineering could be used to stem the decline in genetic diversity of crop plants. Genetic diversity provides a reservoir of genes from which to breed plants that are resistant to disease and to increase yield.

Thus, there is little doubt that world agriculture will be transformed and our food will be produced using the technologies, possibly as early as the year 2000. Animal production will change first (Kenny 1986). The life cycle of the cow will be controlled more rigorously. New reproductive technologies have made it possible already to induce a champion cow to produce an average of eight and up to 20 ova at a single ovulation (superovulation) which then are fertilized outside of the body (in vitro). Ova can be artificially inseminated and the resulting embryos flushed out of the cow after six days. Embryos can be twinned by splitting to obtain identical copies, or quadruplets, octuplets, or whatever number desired. The embryos secured by any of these methods can then be implanted into surrogate cows. The sexing of embryos makes it possible for dairy farmers to transplant only females, cattle raisers selecting only males. (The newborn then are surgically castrated to result in better beef.) The estrus cycles of the surrogates are synchronized to match the donor cow using injected hormones, some of which can be made by biotechnology. Hence, bovine reproduction will soon be a completely managed process and even the calf will be a genetically selected individual.

New vaccines that prevent infectious diseases such as scours will assure healthy growth of the calf. Scours is a common, often fatal, diarrheal infection. (The vaccine is now on the market.) Bovine interferon is being tested for use against shipping fever, which costs the cattle industry more than $250 million annually. Bovine growth hormone will increase milk supply in cows without increasing feeding costs: it also will speed growth in feeder cattle.

The cumulative effect of these chemicals of bioengineering will be to shorten the time for cattle to reach market size, and to allow cattle-raising to be carried out under less sanitary conditions and at increased densities. The coming on-line of farmer-administered diagnostics using monoclonal antibodies will also lessen the need for expensive veterinary services.

Other meat producing industries will profit as well. Growth hormone, interferon and various vaccines already are available for hogs. Success in breeding faster

growing pigs containing less body fat has been reported. The gene to produce chicken growth hormone has been cloned, which could decrease the time it takes to grow a broiler from a chick by 15 percent (from eight weeks to seven weeks) with a corresponding decrease in feeding costs. Molting hormone administered to laying hens has been shown to result in an increase in the production of eggs.

Feed, the major cost item in raising livestock, also will be affected by biotechnology. Presently in the U.S., soybeans supply the two major constituents of animal feed, carbohydrates and proteins. Biotechnology may make it possible to meet these needs with a unique source, single cell protein. Although the method is not yet competitive with soybeans, single cell protein could be the food of the future, especially if certain requirements are met (e.g., a cheap and efficient feedstock can be found for growing the bacteria and yeast). Microorganisms also could be engineered to superproduce the amino acids lacking in corn such as lysine and tryptophan. These now are added as a supplement to feed.

But, even though animal biotechnology is more advanced today, the greatest long-term potential for agricultural applications lies in plant bioengineering. The most important opportunities will be in manipulating the genetic information of seeds. Important food plants can be tailored to produce high-yielding varieties or having enhanced nutritional value (e.g., certain amino acids). Incorporating the drought resistant trait into plants will permit the cultivation of land that is too dry using present methods. Faster growth and quicker maturation would allow the production of more than one crop in a single growing season, and herbicide resistance would make it possible to kill weeds selectively without harming crop plants.

Farther into the future, the discoveries in plant molecular biology will make it possible to understand how herbicides and plant growth regulators work. This knowledge could make it possible either to design better chemicals or to design plants that would respond to crop chemicals in desirable ways.

Even the chemical and pharmaceutical industries may find agricultural applications helpful. New chemicals could be grown in large quantities at competitive prices (methane and alcohol are already produced in this way). The human gene that encodes for human chorionic gonadotrophic hormone (HCG) has been delivered into petunia plant cells which then proceed to make minute quantities of the hormone (Seeber 1985).

These are still distinct possibilities which will change the very practice of farming and affect productivity. The practical returns from these applications will be unparalleled to anything since the dawn of agriculture 15,000 to 20,000 years ago.

While there is little doubt that the new technologies will transform food production, it is prudent to point out that this cornucopia, although coming, is not yet here. Many of the techniques will not become available for widespread use for at least another five years. Furthermore, certain social and political issues must be dealt with first.

Agricultural applications of biotechnology will take longer to emerge than in the other areas for a number of reasons. First is that the opportunities in agriculture were discovered a few years after they were in medicine. Molecular biology in its earlier stages was largely confined to medical school laboratories and those of non-land grant universities, institutions which traditionally are not interested in agricultural problems. Early interest dealt mainly with health care products — insulin, interferon and monoclonal antibodies. The next step came with the application of medical knowledge to

problems of animal health with vaccines being the first developed. Only most recently has biotechnology looked at applications in plant biology. The new movement in the agricultural industry is to use the advanced technologies to produce patentable materials — varieties of plants that are different from those presently available.

But another part of biotechnology's lateness in this area can be traced to the complex genetics of plants. Plant systems are far more complicated than the genetics of bacteria and even mammals. Some plants, string beans for example, have ten times as much DNA as humans. Other plants have multiple sets of identical chromosomes (polyploidy). In both cases, scientists are unsure about the role of the extra genetic material.

Unscrambling the genetic message of plants so that they can be thus altered will not come easily. Unlike bacteria in which single genes usually control a trait, plants often have many more involved in this determination. The genetic system which regulates nitrogen fixation, for example, contains at least 17 individual genes, and scientists suspect that the genetics of other economically important traits may be just as complicated (Antebi & Fishlock 1986).

And then there is the problem connected with the number of scientists specializing in the molecular biology of plants. Various estimates put the number at no more than a thousand in the entire world. This is about 50 times fewer than the number of researchers in general molecular biology (Hansen, Busch, Burkhardt, Lacy & Lacy 1986). Plant research, for whatever reason, does not attract scientists in the way that experimentation with humans, animals and microbes attracts them. This also means that much less is known about overall plant functions, knowledge that is proving essential if plant behavior and biochemistry are to be manipulated.

And last, fewer investment dollars are channeled into agricultural research. Simply put, people are more ready to invest their money in medical research than they are in the study of plants. The ratio now is about 2:1; for every two dollars spent in other areas, one dollar goes for plant research. [In 1984, the estimated total was $3 billion (Antebi & Fishlock 1986).] This lower investment factor, too, serves as a further impediment to progress in plant engineering since research and development in biotechnology of any kind is very costly.

Yet, in the medium term, agricultural applications continue to represent a truly enormous economic opportunity for biotechnology, a proposition not hard to understand; food, after all, is one of the most basic human resources along with shelter and health.

Methods

Plant breeders have been introducing new genes into plants for centuries using techniques of cross-breeding and selective breeding. Nearly all of our high-yielding crops, productive forest trees, popular ornamental shrubs and garden plants have been derived through breeding programs. But this approach is labor-intensive, tedious and slow, especially for the higher plants. Hundreds of crosses taking many years may be needed to introduce a single trait into a plant. Moreover, the method is restricted to species that are sexually compatible, a requirement that limits the size of the gene pool that can be used.

Genetic engineering provides a way to shortcut these. The new methods

eventually could allow the insertion of a wide variety of beneficial genes into plants in a matter of days. It would also take the present "hit-or-miss" chance of success due to random genetic recombinations out of plant breeding.

Much of the present progress in the genetic manipulation of plants is attributable to the solution of a problem that has long frustrated plant scientists — how to slip foreign genes into the DNA of plant cells. Three different techniques have been devised for achieving this — micropropagation, protoplast fusion and a plasmid vector that normally infects plant cells.

The technology called micropropagation is the oldest of these methods dating from the early 1960s (Miller 1985). It involves removing the growing points of plants normally found at the tips of stems, branches or roots. This tissue, the meristem, is still undifferentiated in that it has not yet grown into any adult structure. It still retains the entire genetic potential to become any adult tissue. This characteristic is known as totiopotency.

The meristem tissue is enzymatically digested into isolated, individual cells, each of which can be used to regenerate an entire plant when prompted to grow in special growth media. The resulting plants are true clones of the parent meristem (the method is sometimes called somatacloning).

A single gram of meristem can give rise to a thousand progeny plants in a matter of weeks. In the course of only nine months, 1,000 rose bushes can produce a million new plants. The micropropagation process expressed in human terms is equivalent to growing near carbon copies of people from a piece of skin tissue.

Nurseries purchase tissue-culture produced plants because they far surpass seeds, not only in the reduced time it takes plants to grow to market size, but also in the genetic uniformity of traits like size and shape. Plant growers already culture everything from ferns to strawberries with assembly line efficiency.

Propagating cells in this way also makes it possible to identify interesting traits, isolate them and regenerate whole plants having the desired qualities. These so-called variants (each may vary genetically in a slight way) have demonstrated differences in plant size, flowering, pigmentation, growth habits, disease resistance and other characteristics.

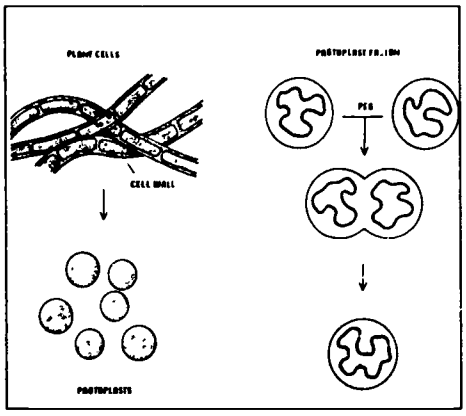
The cell culture technique permits the simultaneous screening of large populations of cells from which a particular trait, or traits, may be selected for propagation. For example, to breed for herbicide resistance, one need only introduce a chemical into the culture medium to see which cells are most resistant and then regenerate them into whole plants.

This method has been used to cure diseases in some plants (strawberries, carnations and chrysanthemums), to grow disease-free plants including potatoes (which periodically are ravaged by outbreaks of rusts), and to increase the yields and quality of some plants (tomatoes and coffee).

A key requirement needed for success of the method unfortunately functions as one of its limitations — selected cells must be capable of regeneration into whole plants (Marx 1985). Dicotyledonous plants — those having two seed leaves — are rather easily cloned from single cells. Examples here are tomatoes, alfalfa and petunias. But the crop plants whose regeneration would be most valuable from a food standpoint are the monocotyledons, plants having only a single seed leaf. These are the cereal grains — wheat, barley, rice and so forth. For a long time, scientists were unable to clone these,

hence, micropropagation could not be used with these important crop plants. But early in 1987, the first successes were reported; entire rice plants were regenerated from single, isolated cells. The achievement opens the way to introducing desirable new traits into monocotyledonous plants (Marx 1987).

One of these ways is protoplast fusion (Hansen et al. 1986). Protoplasts are plant cells whose cell walls have been digested away by enzymes. Without their rigid cell wall, the naked cells can be caused to fuse together when grown in a medium containing PEG — polyethylene glycol (see p. 25). Between 10 percent and 20 percent of the cells that come in contact with each other in the culture will fuse. The hybrid cells then are stimulated to regrow a cell wall and regenerated into a complete adult plant.



Protoplast fusion. Plant cell walls are digested away to result in 'naked' cells called protoplasts. Two protoplasts, each carrying different genetic information, are induced to fuse when exposed to PEG (polyethylene glycol). The heredity information of each also combines to form a single, large genome. (From Hopwood, "The Genetic Programming of Industrial Microorganisms," Copyright © 9/81 by Scientific American, Inc. All rights reserved.)

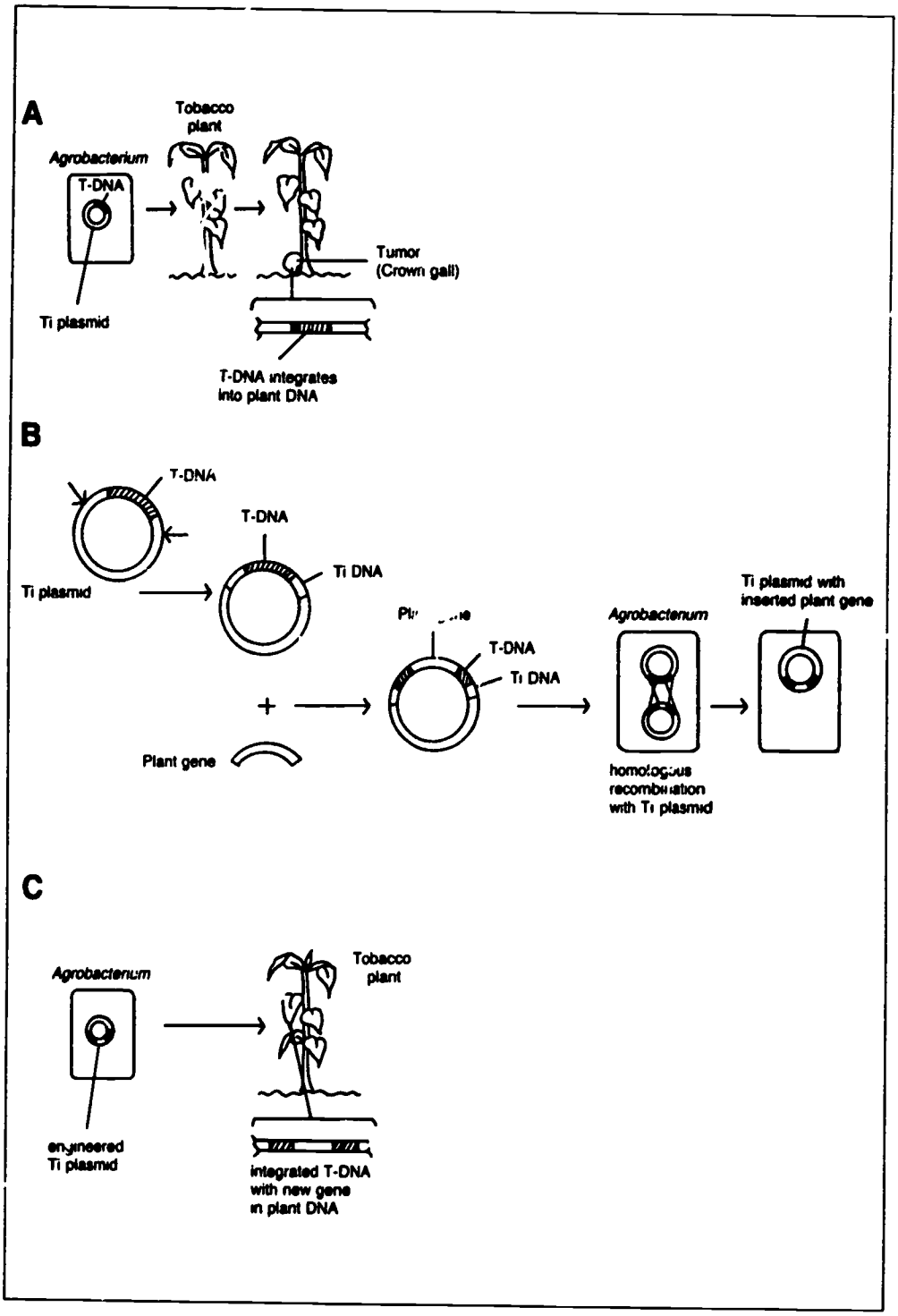
Cells from any source can be fused — different tissues from the same plant, different plants of the same species, plants of different species, and even plant-animal combinations. The first plant propagated from protoplast fusion was a pomato — a potato, tomato mixture. Unfortunately, this handsome vegetable was sterile; it was also quite bitter to the taste.

Not only can fusion produce hybrids that would otherwise be impossible, but additional genetic information from another source can be implanted into isolated protoplasts. Genetic information which confers certain traits like disease resistance may be incorporated into the makeup of the regenerated plant.

But while the technology is an exciting one, it has yet to contribute a significant advance in the formation of hybrid plant materials. A stumbling block is that many protoplasts resulting from the fusion do not produce viable cells. Furthermore, in those few cases where the hybrid does regenerate, the selected trait is poorly expressed, or not expressed at all in the resulting plant, or the new plant cannot reproduce the change. Perhaps these problems can one day be solved, and protoplast fusion will take its place in the plant scientist's tool kit for manufacturing new and unique varieties of plants.

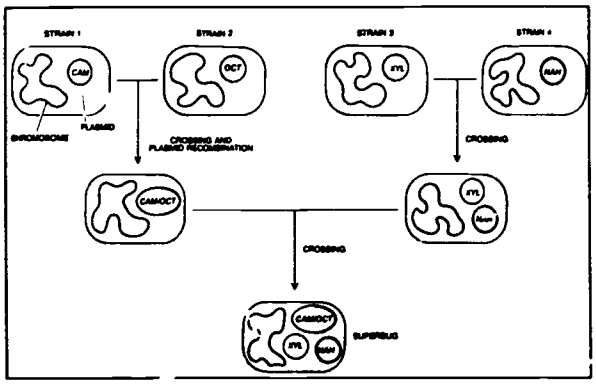
The most revolutionary method for implanting new genetic information into plant cells uses the now familar method of genetic engineering. While the genetic modification of plant cells has not yet reached the microbial or animal stage of sophistication, plant engineering is likely to have as important an impact on all phases of agriculture as the other two have had on improving human health.

Securing an effective recombinant vector that could carry genes into cells was an obstacle to applying recombinant methods to plants for a long time. The solution finally came from the bacterium, *Agrobacterium tumefaciens* (Moses 1987). This microbe causes small brown tumors called crown galls on such economically important plants as grapes and tobacco, which usually weakens them and stunts their growth. The parasitic bacterium reprograms plant cells to manufacture a specialized food for its own



Gene transfer in plants using the Ti plasmid of *Agrobacterium*. (A) A natural infection resulting in a tumorous growth called a crown gall. (B) The Ti plasmid is modified by removing the tumor-causing genes and replacing them with a desired plant gene. The plasmid is returned to the bacterium, which transfers the selected gene into a plant cell. (C) The new gene integrates into the plant cell's DNA and may become functional in its new surroundings. (Adapted from Watson, Tooze & Kurtz, *"Recombinant DNA, A Short Course,"* 1983. W.H. Freeman, New York in *BioScience*, 37(1), 8.)

exclusive use. Like all bacteria, *Agrobacterium* has a plasmid in its cytoplasm. The plasmid is designated Ti, for tumor-inciting. The genes essential for tumorigenesis are contained in it. This disease-inducing bacterium can be cultured in the laboratory and its plasmid separated out for genetic modification.



The manufacture of 'superbugs' to eat oil spilled into the environment. Each of four different bacterial strains has the capacity to metabolize a particular component of oil, a very complex hydrocarbon. The plasmids of each strain are combined into a single bacterial cell which then has the appetite to eat all four components. (CAM=camphor; OCT=octane; XYL=xylene; NAH=napthalene.) (From Hopwood, "The Genetic Programming of Industrial Microorganisms." Copyright © 9/81 by Scientific American, Inc. All rights reserved.)

An important first step is to construct a benign version of the plasmid by scissoring out the tumor-causing genes while preserving the all-important control genes. Foreign genes can then be spliced into the disarmed plasmid, and the plasmid returned to the bacterium. The recombinant organism is then used to infect plant cells grown in culture — a procedure called agroinfection — and if successful, the new gene will insert into the host's genome. Regeneration of the transformed cell into whole plants should result in a plant expressing the new trait.

A number of research teams throughout the world have shown that this method is effective for implanting new genes into cells of dicot plants which are later expressed in the propagated plants. Some examples are the bean storage protein gene in tobacco, a bacterial herbicide resistant gene in tobacco, and a bacterial gene that degrades an antibiotic in petunias. An experimental team has even put the gene for light-generating luciferase enzyme into tobacco plants, thereby prodding plants that light up like fireflies when they are watered with a solution containing the substrate for luciferase (Ow et al. 1986).

None of these have economic importance; the genes were selected because they provide an easy way to determine whether a successful gene transplant has taken place. The result that the genes were spliced into and expressed by host plants via this method is the real significance of these experiments.

As with animals, scientists still cannot predict where inside a plant's extensive library of genes the new DNA will be deposited; it seems to do it randomly. And only very recently have they succeeded in regenerating monocots from single protoplasts. This important breakthrough removes the last barrier to the genetic engineering of monocots. The procedure for introducing genes into dicots is now at last applicable to the cereal crop plants with the initial successes first reported in early 1987 (Edwards 1987). The *Agrobacterium* vector was used to splice a virus gene into corn. (A viral gene was used because its presence in host cells is easy to detect.)

Various plant viruses continue to be studied as potential gene carriers. Some that show special promise are the Gemini viruses because they infect a wide variety of plant species.

Finally, the use of microinjection is also being investigated. Rather than

introducing genetic material into a fertilized egg as in animals, fine glass needles inject DNA directly into protoplasts. The approach is especially promising because it bypasses some of the restrictions mentioned earlier. Single whole chromosomes have already been injected into petunia protoplasts. Plants regenerated from these protoplasts produced some of the proteins coded by genes of the chromosome (Bennett & Miller 1985).

The Problem of Field Testing

Many who view biotechonology as a revolution also fear that it may be used in ways that will make matters worse than they are today. For example, a coalition of farmers and environmental activists is trying to block marketing of the genetically engineered bovine growth hormone; the concern is that up to half of today's dairy farmers will be forced into bankruptcy if milk production is raised in this way.

Others who are hesitant fear that biotechnology is galvanizing a trend toward a consolidation of the industries that supply farmers with their seeds, pesticides and fertilizers. The day soon may come when the same multinational conglomerate will control all the supplies farmers buy, the prices farmers will be paid for their products and the research agenda for biotechnology itself. All of these issues will have to do with the way farming in the future will be done (Tangley 1986).

A problem having more immediacy concerns the matter of field testing engineered organisms. The first field tests of genetically engineering organisms designed to improve crop production are now ready. Approval to proceed is all that is needed — but it is slow in coming. Already, two release experiments have been done outside of the country, one in Argentina, the other in New Zealand, because of the uncertainty surrounding testing in the U. S.

A number of projects are now ready for field testing. An engineered bacterium has been designed to make plants more resistant to frost. Another uses a transformed microbe as a pesticide to kill root-eating insects. The bacterium has been given a gene for the production of a toxin that is lethal to insects; the bacterium lives in the soil on plant roots. An insect lunching on roots would at the same time ingest the engineered vector with lethal consequences.

The only genetically modified plant proposed for field testing so far is a tobacco plant having a new gene that makes it tolerant to the herbicide glyphosphate. Because herbicides frequently harm crops as well as associated pest plants, researchers are anxious to find ways to alter the desirable plants genetically to better withstand herbicides. The altered plant should have this trait.

These are only three of the many possibilities that someday must be tested in nature. Others are plants that convert carbon dioxide from the atmosphere into carbon-containing compounds more efficiently, plants that can withstand the environmental conditions of high acidity, alkalinity and salinity and plants that degrade toxic substances used as pesticides such as 2,4-D.

But, before any of these can be brought on-line, a number of issues first must be resolved. Some of these will require more science; others are not scientific at all but are social and political. Let's examine one example as a type case to illustrate the nonscientific considerations of this work. The case is the use of a modified microorganism to protect plants against frost damage (Maranto 1984, 1986).

Two California research groups have requested permission to field test this organism. One team, from the University of California at Berkeley intends to spray the bacteria on several rows of potatoes; the second group from a biotechnology company, Advanced Genetic Systems in Oakland, wants to apply these microorganisms to 2,400 strawberry plants.

The proposed experiment involves a modest bit of genetic engineering. *Pseudomonas syringae*, the organism, lives as a parasite on the leaves of many plants. When temperatures drop below 32 degrees Fahrenheit, the bacterium produces and secretes a protein that functions as a nucleus for the formation of ice crystals. The frost-damaged tissues are then fed on by the bacterium. Plants not having *Pseudomonas* on their leaves withstand temperatures briefly down to 23 degrees F. before being harmed. Without the ice-producing seed, dew can be cooled to that point before it freezes.

The engineering strategy was to produce *Pseudomonas* without the gene that codes for the offending protein, and spray them onto crops in sufficient quantities to displace the natural variety. Bacteria without the gene have been dubbed "ice minus" and those with it "ice plus." If the ice minus strain could establish itself successfully in the environment, it would protect agricultural plants from unseasonable frosts — which cost American farmers $1.5 billion annually — and also extend the growing season to permit the planting of more than one crop.

Greenhouse experiments under controlled conditions have already shown that the engineered organisms do increase frost tolerance in plants; now field tests under natural conditions must be done. This is necessary because different soils, soil treatments and weather conditions can change the outcome significantly; greenhouse or growth chamber experiments have little relevance to field results. The proposed tests were to be the first in which anyone had intentionally released an organism altered by genetic engineering into the environment; up to then, rigorous laboratory procedures were followed to keep such organisms contained so that they could not enter the environment.

(An interesting footnote to the proposed experiments is that the engineering involves removing a gene rather than adding one to an organism).

Permission for the experiments was granted by two federal agencies responsible for such requests — the Environmental Protection Agency and the United States Department of Agriculture — after a long period of study, but neither was done. The potato experiment was blocked by a court injunction secured by Jeremy Rifkin. He successfully argued that the EPA had violated its own rules by failing to assess adequately the environmental impact of the experiment.

The strawberry experiment was not done because the EPA recalled its initial approval when it became known that there were certain irregularities in the proposal requesting testing (see page 52). Rifkin also discovered these and made them public. In addition, AGS was assessed a $20,000 penalty for the presumed wrongdoing. The county board of supervisors of Monterey County, California, the location where the experiment was to be done, voted twice to block it, responding to pressure brought by local citizens.

The court ruling that prevented the first experiment raised two important issues, one legal, the other having to do with how science is done (Norman 1984). The legal point was that the EPA had not assessed the risks in a manner prescribed by law. The second and more important issue is whether society, rather than the scientific commu-

nity, should regulate the growing field of biotechnology. This issue is still not settled, because it raises some rather fundamental questions all of which ultimately hinge on yet another question, "How safe are the engineering organisms?"*

In June of 1985 a conference was held to examine the scientific dimensions of the question (Kolata 1985; Tangley 1985). This was the first time that molecular biologists and ecologists sat down together to discuss the problem. The meeting brought out the sharp differences between the two and made clear that it was still too early to agree upon a general set of procedures to be followed for using genetically engineered organisms in agriculture.

Basically, ecologists think that molecular biologists know precious little about the ecosystems they want to invade with their organisms and do not appreciate the possible adverse consequences. Molecular biologists contend that ecologists are alarmists, conjuring up terrible and mostly implausible scenarios of engineered organisms overturning the environment and threatening human health and life.

It is, of course, true that neither side can be sure about its position since the hazards of recombinant DNA modified organisms remain largely speculative. Never once has it been conclusively demonstrated that such organisms do what they are purportedly capable of doing either to humans or to the environment for the simple reason that there is no record that any have been put out there, intentionally or otherwise.

The argument for less stringent regulation is that genetic engineering is actually nothing new. Genes are exchanged among organisms all the time, only nature is better at it than molecular biologists. And no catastrophes are known from nature's experiments. Moreover, the manufactured organisms are feeble compared to the wild strains.

Ecologists counter that such comparisons are misleading. For instance, one can't automatically assume that engineered cells will be less fit than extant species. Furthermore, sporadic genetic transfers in nature are quite different from engineering experiments in which millions of copies of a new microbe are intentionally made in ways that ensure their survival. In nature, a new combination must pass the test of survival on its own by outcompeting the existing forms, and many fail this hurdle; they die. The key principal is that natural organisms have been selected over millions of years for adaptation to their particular environment. Any new genetic combination therefore has a difficult time in establishing itself by outcompeting the already established forms. Engineered organisms, in effect, are given a head start by their creators in the competition for survival and could very well use this to their advantage by driving out the natural forms.

Ecologists stress that they are not concocting stories to block progress; instead they have something positive to offer biotechnology. They have learned from experience to ask questions that may not occur to molecular biologists. Some of these

*Advanced Genetic Systems scientists were accused of having tested the "ice minus" bacteria outdoors before gaining EPA approval. They injected the modified bacteria into some trees located on the rooftop of their laboratory. AGS contended that this was not a release since the bacteria were injected, not sprayed, and the trees were on their property. EPA argued that the procedure was a potential release because birds could have picked some of the bacteria and carried them away. AGS was again granted approval to proceed with field testing in March, 1987 by EPA. The company, though, did not conduct the experiments in Monterey County but in a more rural location (Crawford 1987).

questions ask what other organisms will be affected by the released organisms before they are let loose in the environment, how likely it is that the new genes move and spread in the environment, and what organisms will spread them. After these questions are satisfactorily answered in laboratory and greenhouse experiments, then outdoor testing is in order.

By the end of the meeting, each side began to understand the other's position more clearly. A two-way educational process thus is in order between the two scientific communities. There seems to be no reason to think that effective policies regarding the issue of recombinant organisms in agriculture cannot be worked out so that the enormous potential of this technology can be put to good use. Most ecologists just want to see the industry properly monitored to preclude the possibility of unintended environmental harm.

A step in this direction was taken when the U.S. Patent and Trademark Office ruled in 1985 that engineering plants, seeds and tissues can now be protected by patent (Sun 1985). Previously, new types of plants were only narrowly covered by federal rules. Competitors could copy the discovery easily and offer their own version of it for sale. This prevented some companies from committing the large outlays of money, time and scientific talent required to breed new and better plants. Now, developers have complete control over their discoveries, an incentive that will encourage more biotech companies to enter the field.

Note: The first outdoor testing of whole plants altered by rDNA methods began in May of 1986 in Wisconsin (Sun 1986). Two hundred tobacco seedlings that were modified to resist crown disease were planted. The purpose of the test was to analyze whether the genetic changes affect plant yield in an outdoor environment. More recently, often-delayed field testing of "ice-minus" bacteria was begun in April 1987, following a court order which rescinded the previous prohibition (Marx 1987). Additional requests for approval of outdoor studies are in the regulatory pipeline. Thus, the technology that was once confined to the laboratory is taking its first steps to what will almost certainly become a large-scale commercial enterprise.

Industrial Applications

Waste Management and Energy

No one doubts that biotechnology will contribute immensely to human welfare in many areas. Two of these we have examined in considerable detail — pharmaceutical and agricultural applications; a third is industrial. The potentials here, though, are still mostly at the drawing board stage. None will be more affected by the changes than the chemical industry. It already makes much of its profit from pharmaceuticals, agrochemicals and materials made for biological use (e.g., enzymes for food processing). A number of these already are manufactured by biological processes. The industry is, therefore, anxious to adopt more bioprocessing into its production practices both to improve the efficiency of what it now makes, and to diminish the environmental effects caused by the more traditional chemical methods of synthesis it presently uses. Monsanto, DuPont, BASF and Shell are some of the companies who have invested heavily in biotechnology.

But chemicals are not the only industries serious about this potential. Mining and waste management are two that are experimenting with bioengineering. And for good reason: both employ living microorganisms to aid them in their work.

For centuries, microbes have been used to recover copper from solutions draining from underground mines. Today, 10 percent of the copper produced in the U.S. is obtained by using bacteria (Antebi & Fishlock 1986). The organism, *Thiobacillus ferroxidans*, lives in metallic ore deposits, an environment that will kill most other microbes. The enzymes of this bacterium are able to change the electron configuration of metal atoms to form compounds that are highly soluble in the acid solutions of mine drainage. The metals thus liquified can be extracted.

Thiobacillus displays another unusual property: its cells can concentrate many kinds of metals from the environment. Copper, nickel, cadmium, lead, zinc, cobalt, strontium, rubidium, arsenic and antimony are examples. At least one mining firm, Advanced Mining Technologies, in New Mexico, is conducting research with microorganisms that can extract metals from low grade ores. They estimate the potential market using this technology to be about $5 billion by the year 2000 (Antebi & Fishlock 1986).

One method for using "bug" technology is now being evaluated in Ireland. A solution containing *Thiobacillus* is sprayed directly into mines to dissolve the metal. The water is pumped out and the metal (copper) is recovered. Yields are low still but the method is promising (Antebi & Fishlock 1986). It will be years, though, before the process becomes commercially feasible and, so far, not many investors have been attracted to this technology. (An unrelated reason may be that the metals market is weak right now.)

The precious metals people, on the other hand, have a far greater interest in utilizing microbes for the recovery of metal. At Kodak Films, a bacterium that extracts silver from waste silver sulfide solutions has been isolated. Silver compounds are important ingredients in photographic film emulsions. In South Africa, researchers have

discovered several genes in bacterial plasmids that bestow a tolerance for arsenic on the microorganisms that carry them. The motivating interest here is that many veins of gold are rich in arsenic and South Africa is the world leader in gold production. Arsenic has the effect of slowing down the metabolism and growth of bacteria. If these plasmids, or their genes, can be placed into "gold-eating" bacteria like *Thiobacillus*, they could serve as very effective gold miners. Bacterial mining has the advantage of being more efficient than the traditional pick and shovel method; 86 percent to 90 percent yields are possible. It is also less costly and not nearly as polluting. Getting rid of the contaminating arsenic and controlling the sulfurous gases from the refining process pose serious environmental problems (Antebi & Fishlock 1986).

Despite its promise, mining with microorganisms remains mostly a futuristic potential; many problems still exist. Among these is cultivating bacteria on solid (rock) substrates. Also, many of the useful forms cannot tolerate very low acid conditions, pH 2 and less, environments that are quite typical in mines.

But the capacity to extract metals from solutions makes these microbes useful in other ways. They can be put to work extracting toxic substances from industrial wastes. For example, a mixture of bacteria that includes *Thiobacillus* and *Pseudomonas*, is able to remove the heavy metals, lead, zinc, nickel and cadmium, from industrial effluents with up to 90 percent efficiency. Some of these bacteria can even thrive under near-boiling temperatures. Bacteria like *Sulfobolus* naturally inhabit volcanic cracks and sulfurous waste piles (Antebi & Fishlock 1986). These microbial cocktails thus can be made to grow even in heated industrial waste water, cleansing it of toxic metals before being recycled back into the environment. This probably will be the first use of the engineered "metal eaters."

Biotechnology may very well play a crucial role in the more conventional forms of water treatment, too. A large portion of the world's water management problems has always been solved by microorganisms that break down matter for their own use, but as the human population continues to concentrate in cities, the natural degrading capacity of these decomposers is being overtaxed (Godown 1984). The opportunities for applying biotechnical solutions to protect the environment, therefore, are immense, not only for the treatment of water but in the degradation of solid materials as well. But again, the application of this technology awaits the solution of many problems.

Waste water treatment deals with pollution in its most complex and variable state. Industrial effluents, street and parking lot runoffs, solid chunks of sewage, slime, grease and assorted odds and ends are churned in a heterogeneous combination that is continuously changing its pH and consistency. Improving on the microbes that digest this mess will not be an easy task.

However, it is quite possible to envisage cultivating selected bacterial strains that are highly effective in degrading this or that waste, and seeding them into huge bioreactors as needed. These microbes would have been genetically modified to enhance their degrading properties. American and Japanese scientists already have patented strains that attack industrial substrates. The Japanese have launched a program called BIOFOCUS designed to develop by cell fusion, microorganisms that eliminate organic pollutants, nitrogen and phosphorus from waste water (Antebi & Fishlock 1986).

A bacterial strain that breaks down petroleum in the environment was the first engineering microbe to receive a U.S. patent, in 1980 (Wade 1980). Anada Chakrabarty

discovered plasmid genes for the enzymes that do this. He then transplanted three of these genes into a single plasmid to create a "super bug," one having a voracious appetite for oil. Its intended use to clean up accidental oil spills in water and on beaches, however, has never been tried outside of the laboratory. The same reservations that opposed the antifrost microbes concerning intentional environmental release have been voiced against this process.

These ravenous eaters might also be capable of drawing raw petroleum out of oil shale and tar sands. If feasible, a considerable market would be opened up considering that a large fraction of the world's known oil reserves are in this form, and extracting it now requires a complex and polluting technology.

Dr. Chakrabarty is presently working on a microorganism that can decompose Agent Orange, a devasting herbicide used extensively in Vietnam and which many veterans claim poisoned them. The components of this plant killer, 2,4-D and dioxin, are still being used in plant sprays and have been linked to stillbirth in women exposed to them during their pregnancy. Exposure to the herbicide also increases the risk of one type of cancer, non-Hodgkin's lymphoma (Silberner 1986).

Finally, biotechnology has a great potential to add to our energy resources, and major efforts are underway to improve the ways in which microorganisms convert biomass into useful fuels. An important advantage of these processes is that biomass derived from plant materials and animal wastes are a renewable resource in contrast to fossil fuels that when once used are gone forever.

The world's biomass represents an enormous amount of renewable energy and already some of it is being used for energy production. Biogas, which contains 50 percent to 80 percent natural gas (methane) is made by fermentation in methane digesters in many parts of the world — in India and China where it is an important supplement to conventional sources, and in Great Britain and the United States as experimental projects. The pile of garbage produced by Americans in a single year has the potential to be changed into 5.6 billion cubic meters of methane, which, in petroleum equivalents, equals about five million tons. But many technical problems again stand in the way of producing biogas in industrial quantities.

The bacteria involved have a low growth rate and are sensitive to heat and low pH. And then, too, the organic matter fermented is extremely varied requiring a veritable army of microorganisms to break it down. Problems like these put serious limitations on the technology, at least for large-scale production; home-type methane digesters, though, are not nearly as sensitive.

Microbe-produced alcohols -- methanol, ethanol and acetone-butanol — seem to have a much brighter future than biogas. Today, nine-tenths of the cars in Brazil run on alcohol, and in the U.S., a gasoline/alcohol mixture of 9:1 is sold as gasahol, a premium grade, lead free fuel. Since 1979, the use of ethanol as a gasoline substitute has taken over 5 percent of the American oil market (Antebi & Fishlock 1986). Other countries around the world are producing varying amount of these alcohol fuels.

The microorganisms for ethanol are mainly yeasts, and for others they are bacteria. Experimentation with these cells continues as scientists search for more efficient strains. The additional property of heat tolerance, for instance, would increase alcohol production inasmuch as heat added to the fermentation mixture increases speed of the reaction.

The ideal organism would simultaneously be able to tolerate high concentra-

tions of alcohol and high temperature, and synthesize the enzyme amylase to break starch into sugar. The genes for some of these have been identified and in all probability could be transferred into yeast by genetic engineering. The result would be a super agent for fermentation.

One of these steps is already history. A bacterial gene that codes for a starch-digesting enzyme has been spliced into brewer's yeast (*Scientific American* 1987). The altered yeast can turn starch into sugar on its own. Its developers claim that the yeast achieves the same conversion efficiency as enzyme additives presently mixed in with the fermentation medium to convert starch to sugar.

Raw materials now used in commercial fermenters are principally sugars — sugar cane in Brazil, corn in the U.S., sweet potatoes in Japan, casava or grass in other parts of the world. Enzymes in yeast break the glucose-fructose bond of sucrose to secure the simple sugars. The engineered strain may eventually permit the use of a wider variety of feed stocks, such as the white potato which is almost entirely starch.

Another great potential is presently being lost in cellulose, a complex carbohydrate which itself comprises the most widely distributed organic molecule on the planet. Wood, straw, corn stalks, husks, cobs, beet pulp, and so forth are almost pure cellulose or its derivatives. The gene for breaking the glucose-glucose bonds of cellulose is known but it is not present in yeast. If it could be spliced in, this enormous resource would become available for fermentation. Price of production would drop and large quantities would become available. (Actually, most of the alcohol produced in our country today is synthesized from ethylene, a petroleum derivative, because it is cheaper and easier.)

The use of hydrogen as a fuel derived from photosynthesis is a more distant possibility. Radiant energy from the sun is captured by chlorophyll and is transferred through a series of chemical steps to the chemical bonds of organic molecules. One of the reaction steps extracts hydrogen ions from water; these ions eventually become incorporated into the photosynthetic product molecule.

It may be possible to short circuit the process and seize the hydrogen ions before they are incorporated. Electrons could be attached to them to form hydrogen molecules. The hydrogen then would be combined with oxygen in a fuel cell to form water; electrical energy is also created by the process. Although the proposal has a science fiction ring to it, scientists are investigating this possibility seriously as a way to help satisfy our future energy needs.

In all of these proposed technologies, everything depends upon costs. Few of the bioprocesses for securing energy described here are competitive as yet at the industrial level. For example, the use of alcohol as an automobile fuel in our country is made possible only by federal subsidy of the industry. Some, though, have potential at a local level. With these technologies, we are looking into the future, the expectation being that when whatever limits them is eventually overcome, biotechnology will be at the forefront leading the change.

The Biotech Companies

The number of biotechnology firms has snowballed since recombinant DNA techniques were first applied to commercial production. In 1978, there were only four (Miller 1981); today more than 200 are operational in our country alone (Antebi & Fishlock 1986). By all accounts, the benefits of genetic engineering are expected to be substantial and even if only some of today's hopes become realities, enormous profits will be made by successful companies. Current sales of genetically engineered products are modest, less than $500 million in 1985, but are expected to climb to more than $100 billion by the year 2025 (Hardy & Glass 1985). Biotechnology thus offers a new source of industrial strength for the United States.

Two distinct kinds of companies are pursuing commerical applications in the U.S.; small, start-up firms, and large, multiproduct companies (Olson 1986). Start-up companies often attract the best scientific talent by offering participants a share of the company, hence, the potential to get rich, a favorable research climate, and the opportunity to be part of a new venture. With their talented technical staff, start-up companies are the basis of present U.S. leadership in biotechnology. Their most popular applications to date have been in use of monoclonal antibodies, for diagnostic medical tests and the treatment of diseases. Some pharmaceuticals now on the market are: human insulin; alpha interferon; interleukin 2; diagnostics for blood viruses, hepatitis and Chlamydia (a sexually transmitted disease); a number of monoclonal antibodies; a pregnancy test; and several animal vaccines.

Agrochemicals and animal agriculture is the second area attracting th small companies. Animal vaccines and growth hormone are already commercial' ilable and not far behind are plants tolerant to pesticides, herbicides and frost ny new biotech firms find agriculture an attractive field because markets are more a sible and the industry must comply with fewer government regulations. Feder proval of medicines for animals, for instance, does not require the same stringent he and safety testing as products applied to humans.

Production of special chemicals — ethanol, organic acids and food additives like vitamins and amino acids — wholly or partly made by fermentation, is als undergoing considerable development. But most of this is by large companies that have the facilities and equipment for doing so. Relatively few firms are working on such applications as waste management and mining because much more research is needed to demonstrate their commercial feasibility.

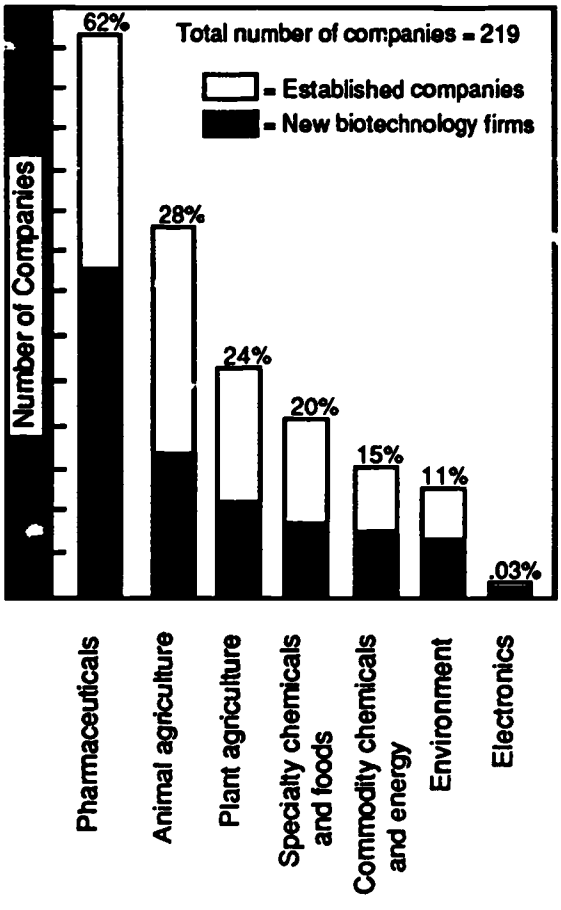
Figure 2 compares the percentage of firms in the United States pursuing applications of biotechnology in specific industrial sectors. Of the 219 companies engaged in these activities, the health care industry (pharmaceuticals) is far and away the leader, with agriculture in second place (Hardy & Glass 1985).

Established companies have been slower in moving into genetic engineering, but since 1981 their involvement has increased. The recognition that the technology will affect many industries substantially was the motivator. Their involvement has taken many forms. Initially, numerous chemical, pharmaceutical, food, energy and other

companies established partnerships with start-up companies, perhaps as a way for keeping informed. In the usual case, the small firm did the initial research and the large firm handled getting the approval, manufacturing and marketing the product. Both would share proportionally in whatever profits were made. This merging of expertise continues today but more and more large companies are establishing their own major R&D laboratories.

This has raised the significant issue of whether the small companies have the necessary financial power to compete with their much wealthier counterparts. Already, a number of these have been bought up by the larger companies, some continue with working partnerships, and others have remained profitable by selling products that don't interest the big companies because of their smaller markets.

Although overall the industry continues to sparkle, biotechnology has been oversold as a way to make lots of money (Antebi & Fishlock 1986). The ultimate test of any business is profitability, and investors are becoming less willing to put money into companies that do not have products already in hand. The balance between what is possible and what it costs easily can lead a potential application to oblivion. It is a long and costly path from idea, to research, to commercial payoff. Until 1983, no new firms registered even a nickel of profit.



Percentage of U.S. firms pursuing applications of biotechnology in various industrial sectors. The proportion of large established companies to small start-up companies is also shown. (OTA Assessment as reported in *BioScience*, May 1985, p. 273.)

In the pharmaceutical game, the new biotech firms are up against fierce competition in marketing. And then, added to this is the rise in public concern and suspicion of these new products and their manufacturers. These have provoked policy makers into devising ever-longer and more elaborate tests for the new products. Safety testing now accounts for a huge and still growing proportion of the total research and development costs for any new bioproduct.

The main element in the future development of small biotech firms lies in their ability to attract capital to finance their R & D of new products, and to bring the products to market within a reasonable time frame. It would appear, though, that the number of biotech companies will shrink over the next few years. Small companies will merge with

A Partial List of Biotechnology Firms

Small, Start-up Companies	*Large, Well-Established Companies*
Agracetus	Abbott Laboratories
Agricultural Genetics	American Cyanimid
Agrigenetics	Archer, Daniels, Midland
Biogen	Arco Petroleum
Biogenex	BASF
Bionova	Bayer
Biotech Research Labs	Carlsberg Laboratories
Calgene	Ciba-Geigy Pharmaceuticals
California Biotechnology	Dow Chemicals
Centocor	Eli Lilly
Cetus	Getty Synthetic Fuels
Cytotech	Hoechst
Ecogen	Hoffman-LaRoche
Genencor	Lederle Labs
Genentech	Merck
Genetic Systems	Miles Laboratories
Genex	Mitsubishi Chemical Industries
Gentronix	Monsanto
Gen Probe	Sandoz
Hybritech	Searle
Innoven	Shell Oil
Integrated Genetics	Texaco
Molecular Genetics	SmithKline Beckman
Molecular Antibodies	Upjohn
Sungene	
Transgene	
Wilson Hybrids	

larger ones, others will continue to be nourished by funds from well-established firms. Whatever form the new industry eventually takes, we can be assured that biotechnology will continue to grow and to have an impact on many commercial enterprises, changing some beyond recognition.

Turning our attention to the international scene, the United States continues to hold a tenuous world leadership position in biotechnology but other countries are trying hard to overtake us (Walton 1984). Japan is our nearest competitor, with West Germany, France, the United Kingdom and Switzerland rapidly closing the gap. The U.S. position is due to three factors: (1) a well-developed life science base, (2) the availability of financing for high-risk ventures, and (3) an entrepreneurial spirit (Antebi & Fishlock 1986). It is the entrepreneurial spirit to be part of a new venture that has made start-up companies possible. Their major source of funding has been the stock market, where stocks in biotechnology firms set records on Wall Street when they were first offered. More recently the glow has faded somewhat but the stock market remains a promising source of capital for small biotech companies.

The American approach contrasts with other countries where commercialization of the technology is done almost exclusively by established companies with strong government support. The Japanese government has declared commercialization to be a national priority. That country is creating a climate which fosters a strong competitive industry. Whereas the U.S. has the strategic advantage in basic biological research, the Japanese have the greater experience with production methods to manufacture products efficiently and at lower costs. (The clear examples here are the Japanese auto and electronics industries, whose products have swept world markets.)

The United States, if it is to retain the competitive edge, must refine its mastery of biotechnological processing while continuing to innovate in genetics, immunology and the culture of organisms. Furthermore, the current public demand for a risk-free society must somehow be changed. Such attitudes have the effect of increasing the costs of production in many areas, rendering them less competitive.

The most ideal solution might be to work cooperatively with our competitors, each contributing our respective expertise. The first signs of this complementary relationship are beginning to emerge — international partnerships have been formed between American and overseas companies. Whether this arrangement can work out for the good of all remains to be seen.

Regulating Biotechnology and Genetic Engineering

Genetic manipulation of microorganisms, plants and animals will undoubtedly result in enormous benefits for human health, food production, veterinary medicine, new chemicals and energy sources and pollution control. The benefits will be real and often economically profitable to the companies involved. But, as happens with every emerging technology, plausible arguments have been made which both condone and condemn its activities. Many proponents of the new technology come from the private sector, having a vested interest, and therefore are motivated by the prospect of financial gain. Some proponents are potential users of the new products — for example, the parents of a child born with a serious genetic defect. Others are scientists who are anxious to do certain experiments.

The other side is often espoused by environmental organizations, public interest groups, concerned individuals and highly capable scientists who view the new field with some misgiving. The common anxiety of this diverse group is that that the new technologies constitute a modest or a major threat to human health, human and animal populations, or the natural environment (Alexander 1985). Moreover, the genetic engineering of humans, no matter how humanitarian its present goals may in the long term lead to a dehumanization rather than an uplifting of our species.

Underlying all technological applications of the past is the conclusion that e were without certain risks, and a risk-free technology still does not exist. Tradeoffs are a part of bringing any new technology to fruition. Another lesson from history is that when it came to a new technology, spokespersons on both sides of an issue were subsequently proven wrong — those who claimed excessive optimism for allegedly harmless endeavors and the prophets of imminent disaster.

The debates over technology and genetic engineering are no different; they are not zero risk enterprises nor are they likely to precipitate catastrophe. Biology, ecology, agriculture and medicine are not sufficiently exact to allow predictions to be made with certainty (Alexander 1985). Hence, it continues to be extremely difficult to assess in advance the human and environmental risks posed by industrial level applications of the biotechnologies. We do not know that the introduction of exotic species such as "ice-minus" bacteria into an ecosystem can have an unexpected and detrimental consequence. We do not know that a normal gene transplanted into a patient to ameliorate an inherited condition may not run awry in the body to cause a new disease or cancer. And, we cannot know that the characteristics of a new technology that make it beneficial to one group of people may make it harmful to another. An instance might be an application that lengthens the growing season resulting in a glut of crops, which worsens the economic position of small farmers forcing them out of business. The use of bovine growth hormone to stimulate milk production is a real case in point.

Another issue concerns the international threat of our country's present position of world leadership in biotechnology. A major factor in determining the continued strength of the U.S. position will be the kinds of regulation that will be applied to the infant industry. According to some, unrealistic rules could threaten our current lead

(McGarity 1985). New products will take longer to reach the marketplace allowing foreign competitors to secure patents and the market for certain products, which thereafter would be denied to American firms. In the words of one biotechnician, "There is a possibility that (with) regulatory delays (which) prevent the timely development of these products in the U.S., we will lose our lead. Americans have pioneered a truly great technology, and we deserve some of the economic benefits that will flow from it" (Olson 1986). Proponents of this view envision the loss of a rare economic opportunity if overly stringent regulations are imposed.

Scientific research and economic studies can reduce some of the uncertainties, but they cannot eliminate them altogether. What these studies can do is help in formulating policies and regulatory procedures by laying out alternatives in terms of certain trade-offs between protecting the public from unknown risks of a new technology while allowing society and the industry to reap the benefits of the technology.

Genetic engineering technology has been controversial from the start, and anyone who applies it for industrial ends will have to win public acceptance if he is to prosper (Hardy & Glass 1985). Ethical, religious and moral matters still trouble a segment of the population, as does the issue of safety. The public is not willing to accept the new technologies without some assurance that they do not pose undue risks to human health and to the environment, as well as to long-cherished values.

The biotechnology industry recognizes this, too, acknowledging that a reasonable regulatory system administered by the government is essential if many of the promised benefits are to be realized. A wise regulatory policy could help build the public trust and allay fears.

By all indicators, biotechnology in the United States is at a turning point; we now seem poised for a full-scale debate on the risks and benefits of the new industry. The outcome of the debate will profoundly affect the future of this budding enterprise. Lack of public support could mean a steady stream of litigation for years to come, tying up future development. Already, several planned field tests have been held up by court action, one for several years – the spraying of a potato patch with "ice-minus" bacteria.

William Ruckelshaus, former head of the federal EPA, asserts that the critical element is educating the public. It must be informed, "fairly, honestly, and straightforwardly" concerning the risks and benefits. "This may be the last chance to do it right," he concludes (Quoted in Olson 1986).

In the final analysis, the federal government has the responsibility to protect human health and the environment from any risks posed by biotechnology, even though the extent of these risks cannot be fixed with certainty. The problem is made all the more perplexing by the demands of a burgeoning industry that requires a climate that will encourage rather than discourage its growth. The insistance is that biotechnology is a new source of industrial strength for the U.S. and ought to be supported.

What then should be the safeguards that will serve the interests of both the public and industry? And who should decide? How should they be enforced? And what penalties should there be for those who do not follow the rules?

So far in our country, authority is in the hands of existing regulatory agencies; no new legislation has passed through Congress although a number of bills have been introduced. The area of regulation, however, is still so new that it is yet unclear what form the rules will take eventually and how successful they will be. What follows is a general overview of the present federal regulatory mechanism. While not meant to be

all-inclusive, the summary will provide a sense of the thinking involved in this important and timely matter.

NIH Guidelines

Public concern over the uses of recombinant DNA methods has been strong almost since the very first successful experiments. In the early 1970s a group of molecular biologists whose work put them at the leading edge of genetics, expressed some hesitance about their discoveries; the techniques could be used for unlimited good if applied wisely but unlimited bad was also a possibility if untoward consequences resulted. A new term, biohazard, was introduced into the scientific vocabulary to refer to engineered organisms or their products which pose a possible risk to human health or to the environment. Their hesitation resulted in a voluntary moratorium on certain types of gene splicing experiments and a call for an international conference on recombinant DNA molecules (Tangley 1985; Miller 1985). The meeting was held at Asilomar in California in February, 1975. The conference agenda dealt entirely with a discussion of the scientific risks associated with gene transfer experiments and whether certain safety regulations ought to be put in place. These were strictly scientific issues; ethical and social questions associated with altering the genetic makeup of organisms were not considered. (Some critics fault the scientists for this omission.) In the end, it was concluded that a framework for conducting this kind of research should be developed, that it be written by scientists, and that it be enforced by a research granting agency of the federal government. This was judged to be the most acceptable way to proceed rather than to risk the imposition of rules from outside the scientific community, which could be more stringent and stifling.

A committee of scientists was then appointed by the National Institutes of Health (NIH), since this agency was and still is a major supplier of research funds for recombinant research. The committee became known as the Recombinant DNA Advisory Committee (RAC) to NIH. The RAC continues to this day as a major regulator of gene splicing experiments; its membership, though, has been expanded to include nonscientists (e.g., ethicists and lawyers).

The RAC developed a set of procedures which became know as the "Guidelines for Research Involving Recombinant DNA," or "The Guidelines," for short; they were made public in 1976. These guidelines assigned different categories of risks to different kinds of experiments. Each kind of risk required that a specified protocol be followed. The main objective of the Guidelines was containment, that is, the restriction of engineered organisms to the laboratory setting only. Altered organisms must not be permitted to gain entrance either into the environment or into humans.

Two kinds of containment, biological and physical, specified the laboratory equipment to be used, the procedures to be followed and organisms that could be used. Certain experiments perceived to be inherently dangerous were prohibited altogether; for example, transplanting a gene known to produce a disease or toxin into an otherwise benign cell was banned.

The microorganism of choice was the bacterium *Escherichia coli* (*E. coli*) strain K-12. This strain of the organism was selected because it cannot survive outside the very precise controlled conditions found only in the laboratory. Any accident, therefore, in which a modified organism may escape from the lab would pose little or no threat, either

to humans or to the environment.

With the publication of the Guidelines, the RAC established itself as the lead federal agency in regulating rDNA research. All experiments supported by federal grants came under the jurisdiction of the guidelines. Conformity was enforced by a threatened loss of research funding if the rules were broken.

On the whole, most reasonable people judge that the Guidelines have accomplished their intended purpose: human health and the environment have been protected while permitting scientists to continue with their research. To the credit of researchers, violations have been extremely few.

As experience with recombinant research was gained, it was determined that many of the earlier fears were unwarranted or overestimated. For example, the K-12 strain of *E. coli* was so enfeebled that it could not live at all outside of the laboratory; simple precautions, long followed by microbiologists, like disinfecting experimental cultures before disposing of them, would keep the altered microorganisms confined. Several revisions of the Guidelines thus were made to take into account the new understandings. The effect was a general loosening of the rules so that today, nearly 90 percent of the experiments involving rDNA are exempt from the Guidelines (Olson 1986).

Public concern with recombinant research also reached its high water mark during the middle to late 1970s. Editorial pages began to fill with discussion of the new science, focusing principally on biohazards; at issue were the potential consequences following an accidential release of an engineered bacterium. If a microorganism carrying a gene for cholera or a cancer-causing virus got into the air or water, would an epidemic sweep the land?

Activists, borrowing on their anti-war experience of the Vietnam era, took to the streets in many parts of the country — in Massachusetts, Michigan, Wisconsin and California. Environmental groups petitioned federal and state legislators and city councils, to ban or restrict experiments dealing with gene splicing. The issue always was the same: the potential immediate effects of an escaped K-12 variant.

The issue was rather quickly resolved; the danger had been exaggerated. By the late 1970s, insistent demands for restrictive legislation all but disappeared and molecular biologists continued with their research largely unimpeded. The relaxed Guidelines permitted most kinds of experiments and the public was generally satisfied that these posed no great danger.

But, the hiatus was not to last. Commercial applications of genetic engineering revived concerns about potential risks to human beings and to the environment. The Guidelines were not adequate for the new applications because their purpose was to keep engineered organisms out of humans and away from the environment; the new uses require the direct opposite — the intentional release of engineered micoorganisms into the environment or modified cells transplanted into human patients.

The Guidelines, though, are not entirely silent in these matters. Experiments involving either of these procedures must pass through a review process and be approved by both the RAC and the director of NIH. The field testing of "ice-minus" bacteria was approved according to this procedure.

However, the new uses of engineered organisms have engendered some major concerns for several reasons (McGarity 1985). First, the large-scale release of modified microorganisms into the environment greatly reduces the amount of human control that

NIH GUIDELINES*

The Guidelines specify that physical containment be used (*Genetic Technology: OTA Assessment* 1982). Physical containment requires methods and equipment that lessen the chances that a modified organism can escape. Four levels are designated P 1 through P 4, from least to most restrictive.

Physical Containment

P 1 - Standard laboratory procedures used in microbiological research; trained personnel; waste decontaminated.

P 2 - Biohazard sign prominently displayed; no public access; autoclaves in building; hand washing facility in laboratory.

P 3 - Negative air pressure, air filters, filters in vacuum lines, class 2 safety cabinets (enclosed boxes).

P 4 - Isolated and completely separate construction, air locks, all air decontaminated, autoclave in laboratory; all experiments in class 3 cabinets (glove boxes); shower room attached.

*Note: Originally, the Guidelines pertained to biological, as well as physical containment. However, the biological regulations are no longer in force.

can be exercised. These organisms are intentionally designed to survive in the real world, so that once let free they cannot be recalled if something unforeseen should happen. The purpose of their release is thwarted if they cannot survive. Moreover, the severity of any bad consequences would be magnified because these are living, reproducing organisms.

The ecologist sees the problem as exotic organisms being put into places where natural controls are missing; the results could be environmentally unsettling. Some oft-cited real cases are the kudzu vine brought to America from Japan to control soil erosion; instead the plant has become a major weed pest in portions of the nations's farm belt. Starlings were introduced from Europe to combat insects but stayed to become major nuisances. Gypsy moths were imported in 1869 to breed disease-resistant worms to produce silk; now they cyclically defoliate whole forests in the Northeast. The brown snail which was supposed to be a connoisseur's delight destroys citrus and vegetable crops in California. And the list goes on. Large-scale-release technologies have the potential for creating similar kinds of ecological havoc and the Guidelines simply do not make provision for monitoring such a possibility effectively.

Another deficiency is that they are not binding on privately funded rDNA activities of the sort typical of the industry (Olson 1986). The Guidelines apply only to federally funded research. Biotech companies need not comply, although most do voluntarily as an act of good faith. Even foreign countries have adopted versions of the Guidelines. But still, there is nothing which prevents them from carrying out research that they deem propitious. This is perhaps the most serious limitation of the Guidelines. Attempts have been made to write the Guidelines into law by an act of Congress. All of these, though, have failed.

A related issue is that the Guidelines focus mainly on biomedical research rather than commercial applications. The RAC has only limited expertise to judge commercial level applications; for example, there are no ecologists or industrial representatives on the committee.

The Guidelines also are limited in the kind of research they regulate, rDNA only. Other techniques such as cell fusion are not covered but these methods permit the genetic alteration of cells too.

Finally, the Guidelines deal only with the scientific issues of gene splicing. The ethical, moral, legal and social questions are not part of the RAC's charge. Jeremy Rifkin claims that this deficiency is the reason for his anti-genetic engineering campaign. The goal of his crusades, he says, is "to raise fundamental questions about the social, economic, and environmental consequences before the technology becomes entrenched" (Quoted in Maranto 1986). Others, including some ecologists, concur with his view.

Federal Regulative Agencies

So then, what are the alternatives to the RAC? The prevailing attitude in Washington is opposed to drafting new laws to regulate biotechnology, placing responsibility instead on existing agencies and laws. But before turning to these, let us first review the procedure for regulating gene therapy since it does not fall under the purview of any of the existing regulatory agencies other than the RAC.

Policy makers, Congress and the RAC have been deliberating the technical,

social and ethical issues of human gene therapy for several years already, the result being the formulation of a rather rigorous review process. Intensive review of any proposal must gain the approval of the "Gene Therapy Subcommittee" of the RAC. The membership of this subcommittee includes a variety of interests, including the public interest. It has developed a document entitled "Points to Consider," which spells out technical and ethical considerations to be followed. It also stipulates that the proposed gene therapy must be aimed solely at relieving a life-threatening or severely debilitating condition; gene transplantations that could alter the germline (reproductive) cells are expressly prohibited.

Besides gaining the endorsement of the subcommittee, experiments involving human gene therapy require approval at several different levels (Culliton 1986). The first step is that the experiment will have to be cleared by the research center's Institutional Biosafety Committee; in-house committees oversee procedures for the safe handling of recombinant organisms or cells. A second local review by the Institutional Review Board (IRB) enforces such matters as the protection of the patient and securing informed consent; regulations governing these must be obeyed. At the national level, an open review conducted by the Gene Therapy Subcommittee will ensure that the proposed experiment conforms to "Points to Consider". The experimental procedure will then be described in lay language in the Federal Register so any member of the public can comment. Next, the full RAC must approve the experimental procedure and pass on their approval to the director of NIH, who must also concur.

As more is learned about gene therapy, no doubt new concerns will arise, different from the ones contemplated, and the protocols will have to change to fit the new circumstances. These revisions will draw on the expertise not only of scientists and physicians, but philosophers, sociologists, legal scholars and theologians, as well. Ideally, the public, too, will make its contribution.

Returning to regulating nonhuman applications of biotechnology, it now appears that existing federal agencies and statutes will administer in this area. After more than two years of preparation, federal biotechnology guidelines were released in the fall of 1986 (Sun 1986; Wo. man 1985). Named the "Coordinated Framework for the Regulation of Biotechnology," the document has two principal subdivisions. The first designates a new group, the Biotechnology Science Coordinating Committee (BSCC) under the President's Office of Science and Technology (OST), to foster consistency and cooperation among the various agencies that will do the actual regulating. BSCC membership is made up of top officials from the relevant regulatory and research agencies.

The framework's second part details the regulatory agencies and the areas they will oversee; the principal ones are the Environmental Protection Agency (EPA), Federal Food and Drug Administration (FDA), the United States Department of Agriculture (USDA), the Occupational Safety and Health Administration (OSHA) and NIH.

The framework focuses especially on regulating the release of genetically altered organisms into the environment. Although all proposed experiments are to be reviewed in some way, certain classes of nonpathogens, such as those containing gene deletions, additions of well-characterized noncoding sequences, or genes transferred from organisms within the same genus, are subject to a much shorter, abbreviated review.

Operating policies of the various agencies are in the process of being formulated and will continue to evolve as the technology and the climate change. The various charges have, however, been specified and are outlined briefly below.

The FDA will regulate in the area of human and animal drugs, biologic substances, food and color additives, and medical devices (McGarity 1985; Olson 1986). The Food, Drug and Cosmetic Act administered by the FDA already has a permit process for approval of the items listed. Before any can be marketed, a manufacturer must demonstrate that the product is "safe and effective." The FDA also can insist that the substance is made using "good manufacturing practices," to ensure purity.

All drugs and biologics derived from methods using rDNA must pass the approval process currently required for all others of the same type. Criteria that must be met include standards of safety, purity, potency and efficacy.

As might be expected, securing FDA approval is a long and expensive process. Typically, it takes from six to eight years and costs tens of millions of dollars. This continues to be one of the major impediments in commercial biotechnology. Of course, the industry would like to see the process simplified and shortened. The request is not without precedent; only four years passed between the time that the human insulin gene was successfully spliced into a bacterium and the product was okayed for marketing.

The EPA will regulate applications of genetic engineering under several acts: the Federal Insecticide, Fungicide and Rodenticide Act; the Toxic Substances Control Act; and the National Environmental Protection Act. The first requires that before a license is issued to sell or distribute a pesticide in the U.S., it must be shown that the substance will not cause unreasonable "adverse effect" on humans or the environment.

This law can be applied to the large-scale release of microorganisms which are intended to kill or control pests that infect crop plants. The "ice-minus" bacteria field test fits this qualification if the modified bacteria are used to eliminate "ice-plus" strains that would otherwise harm crops by frost formation.

The Toxic Substances Control Act regulates the production, distribution, use and disposal of chemicals that the EPA believes pose an "unreasonable risk" to human health or the environment. By defining genetically engineered organisms as "chemical substances" (courts have made this judgment), the EPA will have considerable authority over a large number of products manufactured by the biotech industry.

The National Environmental Protection Act (NEPA) requires that federal agencies prepare an environmental impact statement if a contemplated activity would affect the environment in significant ways. Many lawsuits have been filed by environmental groups and private citizens to block projects they believed were damaging to the environment. Jeremy Rifkin successfully used the provision of this act to block the "ice minus" field test on potatoes several years ago in California. The possibility thus exists that the statute will encourage those opposed to genetic engineering to bring lawsuits which could tie up the industry for years; on the other hand, if used wisely, the ootion could engender public confidence in the regulatory process.

The USDA will have authority over plants, animals and microorganisms of agricultural interest. It also will regulate animal biologics (e.g., animal vaccines). Under the Federal Plant Pest Act the agency controls the importing and intrastate shipment of microorganisms that can harm plants. Under the Noxious Weed Act, it can regulate plants which may injure crops, fish and wildlife, or public health. The department has, therefore, important monitoring functions over novel plants, animals and microorgan-

isms, such as the recent approval to conduct the first open air field tests of a genetically engineered plant containing a gene for a pesticide. The gene, transferred from a bacterium (*Bacillus thuringiensis*) produces a protein that is toxic to a broad spectrum of caterpillars which feed on plant leaves.

Several other federal agencies also will exercise some regulatory function on biotechnology. OSHA bears the responsibility for worker safety; it can prescribe standards for the workplace to protect employees from significant risks to their health and safety. This covers the use of microrganisms used in fermentation technology.

The Agency for International Development (AID) may sponsor research on using genetic engineering to help solve problems in less developed countries. The Department of Commerce controls the exporting of biological materials. The Patent Office issues patents for modified organisms and cells and the processes involved. The Departments of Energy and Interior may also exercise regulation in cases that relate to their respective areas.

Finally, some states and local communities have various legal provisions which may be applied to certain rDNA activities.

The regulatory maze just outlined may seem to be a bureaucratic hodgepodge without clear lines of authority. You now can appreciate why the earlier mentioned BSCC (Biotechnology Science Coordinating Committee) was put into place — to promote consistency and cooperation among the various agencies.

Undoubtedly, as experience is gained, various regulatory procedures within the respective agencies will evolve to accommodate the new issues. The continuing concern is that a top-heavy and diverse bureaucracy may put unnecessary restrictions on the research and development of new biotechnical products. If that is the result, the U.S. firms may lose their incentive to innovate new products and our leadership in the world will slip away. Whether this should be the principal concern, or even a concern at all, remains an open question.

Closing Comments

Genetic engineering and biotechnology are now an important part of the public's agenda. It is an enigma in this era of science and technology that practitioners of the two are both admired and mistrusted. If the commercialization of biotechnology is to succeed, it must have public acceptance, and for people to make wise and informed decisions they must have some knowledge of biology, including genetics. Uninformed decision making can lead to prohibitions which can deny to society the benefits the new technology is poised to offer. This not to say that public policy will be based on scientific information alone; a range of other issues — political, economic and ethical — must rightly be considered, too.

Public education about biotechnology at all levels needs to be strengthened in schools and universities and within community groups (Price 1985). Clear and accurate information dispensed by the public media can provide enormous help. Hard questions must be answered. The assurance is that well informed citizens can foster the use of the new technologies in a responsible manner that will lead to better health and welfare for all. We should err seriously if this education is left to chance or omitted completely.

GLOSSARY OF TERMS COMMONLY USED IN BIOTECHNOLOGY AND GENETIC ENGINEERING

A

ACTIVE SITE - specific location on an enzyme that attaches to a substrate and the place where a chemical reaction occurs.

AEROBIC - needing oxygen for growth.

AFFINITY CHROMATOGRAPHY - a process used to separate and purify biological molecules on the basis of their structure or function. The molecule to be separated is selectively bonded to a solid matrix and recovered from it by changing chemical conditions.

ALLELE - alternative forms of a gene.

ALLOGENIC - organisms of the same species but having different genotypes.

AMINO ACIDS - organic molecules having both carboxyl and amino groups which serve as building blocks of proteins.

ANTIBIOTICS - substances secreted by fungi or some bacteria which are capable of inhibiting the growth or killing various kinds of bacteria. They can be made naturally using appropriate microorganisms, or synthetically in the laboratory.

ANTIBODY - a protein produced by lymphocytes (wbc) in response to the presence of a foreign substance (an antigen) and released into the bloodstream.

ANTICODON - the three nucleotides by which a transfer RNA (tRNA) recognizes (is complementary to) a messenger RNA (mRNA) codon.

ANTIGEN - foreign molecule which stimulates the production of an antibody by the immune system.

ANTIHEMOPHILIC FACTOR - a group of blood proteins which trigger blood clotting. One of these, Factor VIII, is used to treat hemophilia, an inherited blood disease in which blood clotting is impaired.

ASSAY - technique for measuring a biological process.

ATTENUATED - weakened. Applied to vaccine formation, a pathogen used to induce antibody formation has been treated to render it incapable of causing a disease.

AUTOIMMUNE DISEASE - an immunological disorder in which the body produces antibodies against its own tissues.

AUTOSOME - the chromosomes of animal cells that are not sex chromosomes.

B

BACILLUS SUBTILIS - a bacterium used in genetic engineering that is important because of its ability to secrete proteins it manufactures.

BACTERIOPHAGE - general name for viruses that parasitize bacteria; also, "phage."

BACTERIA - a major class of procaryotes, that is, microorganisms having a simple cell structure.

BASES - (1 ostances which react with acids to form salts; (2) the "letters" of the genetic cod adenine, guanine, thymine, cytosine, and uracil are the common bases.

BASE PAIR - two nucleotide bases on different strands of nucleic acid molecules that bond together.

BATCH PROCESSING - an industrial fermentation technology in which defined amounts of inorganic and living material are joined in a bioreactor. The desired product is selectively removed upon completion.

BIOCATALYST - an enzyme that accelerates a biochemical re ction.

BIOCHEMISTRY - the branch of chemistry that studies chemical processes in living organisms and cells.

BIOCHIPS - biological molecules that can replace semiconductors in electronic circuits.

BIOCONVERSION - using biocatalysts (enzymes) for chemical processes.

BIODEGRADABLE - capable of being broken down into simpler molecular components by living organisms such as bacteria or fungi.

BIOHAZARD - used by scientists to designate that genetically engineered organisms are being experimented on or worked with; intended as a warning to be cautious.

BIOMASS - the total mass of living matter in a given area. In biotechnology, the term refers to cellulose used for the production of chemicals that can be used for energy.

BIOPROCESS - the use of cells or cellular components to produce a desired end product.

BIOREACTION - apparatus used for bioprocessing.

BIOSYNTHESIS - formation of complex biological molecules from simpler ones by living organisms.

BIOTECHNOLOGY - production of commercial products by using a biological process.

B LYMPHOCYTES (B CELLS) - lymphocytes (white blood cells) developed in bone marrow involved in the production of antibody.

C

CALLUS - cluster of undifferentiated cells that can be induced to form whole plants by appropriate treatment.

CARCINOGEN - any agent capable of inducing cancer.

CATALYST - a substance that accelerates the rate of a chemical reaction by lowering the activation energy.

CELL CULTURE - a technique for growing cells outside the body of an organism.

CELL FUSION - the formation of a hybrid cell by fusing two different cells together.

CELL LINE - cells grown by culture methods having the same genetic makeup.

CHEMOSTAT - a growth chamber that maintains a constant volume of cells or microorganisms by adding fresh nutrient and removing spent culture.

CHIMERA - name for a new organism produced by any method of genetic engineering.

CHROMOSOMES - threadlike structures in cells, which serve as the physical carriers of genes. In bacteria, the chromosome consists of a single naked circle of DNA; in eucaryotes, they consist of a single linear DNA molecule and associated proteins.

CISTRON - a sequential series of nucleotide bases in DNA that code for a single polypeptide (protein) molecule.

CLONE - a group of individual organisms or cells all derived from a single progenitor by asexual reproduction and genetically identical to it.

CODON - a group of three nucleotide bases in DNA or mRNA that specify an amino acid or serve as a signal to start or stop a function.

COENZYME - a nonprotein substance needed to activate an enzyme.

COMPLEMENTARITY - the relationship between nucleotide bases on two different strands of DNA or RNA which pair together by a weak chemical bond. Adenine pairs

with thymine (DNA) or uracil (RNA) and cytosine with guanine.

COMPLEMENTARY DNA (cDNA) - DNA synthesized from a messenger RNA rather than from a DNA template. This type of DNA is used for cloning a gene or as a DNA probe to locate specific genes.

CONTINUOUS PROCESSING - a bioprocessing method in which new material is added and products removed continuously at a rate that maintains the volume at a constant level.

COSMID - vector for carrying large DNA segments into host cells, made in the laboratory; formed from a plasmid by introducing "cos" (insertion) sites from lambda phage DNA at two exposed ends of the plasmid.

CULTURE MEDIUM - a mixture of organic and inorganic substances used to grow bacteria or other cells.

CYTO - referring to cells.

CYTOGENETICS - study of the genetics of cells especially as it relates to the function of chromosomes.

CYTOTOXIC - capable of causing cellular death.

D

DNA (DEOXYRIBOSE NUCLEIC ACID) - the genetic material of all organisms; composed of two complementary chains of nucleotides wound in a double helix.

DIFFERENTIATION - production of different cell and tissue types during development.

DIPLOID - a cell or organism having two chromosome sets.

DNA LIBRARY - see genomic library.

DNA PROBE - a molecule (usually a nucleic acid) that has been labeled in some way (e.g., radioactive isotope, fluorescent dye, etc.) used to locate a particular nucleotide sequence or gene on a DNA molecule.

DNA SEQUENCE - the order of nucleotide bases in the DNA molecule.

DOWNSTREAM PROCESSING - the stages in industrial processing following the bioconversion step (e.g., fermentation); includes separation, purification and packaging of the product.

E

ELECTROPHORESIS - separation of substances based on their different electrical charge.

ENDONUCLEASE - an enzyme that breaks nucleic acids at specific interior sites thus producing nucleic acids of various lengths.

ENZYME - a protein having catalytic properties.

ENZYMATIC ENGINEERING - techniques used to increase the catalyzing power of enzymes.

ESCHERICHIA COLI (E. COLI) - a bacterium that normally inhabits the intestine of most vertebrates. A commonly used microorganism in genetic engineering because it is genetically well characterized.

EUCARYOTE - a cell or organism having cells with well-defined nuclei.

EXON - a segment of DNA that is transcribed into mRNA and translated into a protein.

EXONUCLEASE - an enzyme that breaks bonds only at the ends of nucleotide chains thus releasing one nucleotide at a time.

EXPRESSION - in genetics the appearance of a trait that is specified by a gene. In genetic engineering, the term is used to mean that a protein has been produced by a gene which was inserted into a host organism or cell.

F

FACTOR VIII - a protein that aids in the clotting of blood and is used to treat hemophiliacs.

FEEDSTOCK - the raw material used in chemical or biological processing.

FINGERPRINTING - identification of proteins from fragments they generate upon digestion with an enzyme.

FERMENTATION - an anaerobic process that releases energy from a sugar or other fermentable substance. In biotechnology it is used to synthesize various chemicals or pharmaceuticals.

FRAMESHIFT - insertion or deletion of nucleotides that are not multiples of three resulting in improper grouping into codons.

FUSION - see cell fusion.

G

GENE - a sequence of DNA nucleotides on a chromosome that functions as the basic unit of heredity. (See also, transcribable unit.)

GENE AMPLIFICATION - production of extra copies of a gene within a cell; may be spontaneous or induced.

GENE MACHINE - a computerized device used for synthesizing nucleotide sequences in the laboratory.

GENE MAPPING - determining the relative position of genes on chromosomes.

GENE SEQUENCING - determining the sequence of nucleotides in a DNA segment.

GENETIC CODE - the set of nucleotide triplets (codons) that defines the corresponding nucleotide triplets of RNA and amino acids in protein.

GENETIC ENGINEERING - altering the genetic makeup of cells or organisms so that they are capable of producing new substances or performing new functions.

GENOME - the total set of hereditary elements in a cell or organism.

GENOMIC LIBRARY - fragments of cloned DNA from a single species of organism obtained by restriction enzyme digests; fragments are used to locate specific genes using the hybridization technique.

GENOTYPE - the genetic makeup of an organism or cell.

GERM CELLS - reproductive cells (i.e., eggs and sperm); gametes.

GERMPLASM - the sum total of genetic variability available to a particular population of organisms.

GLYCOSYLATION - attachment of groups of sugars to molecules such as proteins.

H

HAPLOID - a cell or organism with a single chromosomal set.

HETEROZYGOUS - having two different alleles of a given gene or chromosome.

HISTOCOMPATIBILITY - similarity in tissue types such that grafting can be done without tissue rejection.

HISTONES - proteins associated with DNA in eucaryotic chromosomes; may play a regulatory function.

HOMOLOGOUS - alike in structure, position or function (e.g., homologous chromosomes are paired chromosomes of the diploid cell that carry equivalent genes).

HOMOZYGOUS - having two identical alleles of a given gene or chromosome.

HORMONES - chemical "messengers" produced in one part of a multicellular organism and transported to another part where they influence the metabolic activities of specific cells.

HOST - a cell or organism used for growth of a virus, plasmid or other form of foreign DNA, or the production of a cloned substance.

HOST-VECTOR SYSTEM - combination of DNA-transporting unit (vector) and DNA-receiving cell (host); used for introducing foreign DNA into a cell or organism.

HUMORAL RESPONSE - an immune response involving the production of antibodies by B lymphocytes.

HYBRID - offspring or cell of two genetically dissimilar parents.

HYBRIDIZATION - production of hybrids; in genetic engineering term refers to the binding of complementary strands of DNA or RNA.

HYBRIDOMA - a hybrid cell that produces monoclonal antibodies in culture; formed by the fusion of a myeloma (cancer) cell with a normal antibody-producing lymphocyte.

I

IMMUNE SYSTEM - in vertebrates, the surveillance mechanism that recognizes and takes action against foreign invaders and alien cells.

IMMUNITY - resistance to a disease or to the toxic effects of an antigenic material.

IMMUNOASSAY - technique for identifying substances by using antibodies that combine with them.

IMMUNOFLUORESCENCE - technique for identifying substances by using antibodies labeled with fluorescent materals; antibodies are caused to fluoresce by exposing them to ultraviolet light.

IMMUNOGLOBULINS - the class of circulating proteins that comprise the antibodies.

IMMUNOTOXIN - a molecule that kills cells attached to an antibody.

INDUCER - a substance that increases the rate of enzyme synthesis.

INSERTION SEQUENCES - short segments of DNA that can move from one chromo-

somal location to another in the same chromosome or into other chromosomes; often function as the ends of such larger transposable elements as transposons and retroviruses.

INTERFERON - a chemical messenger of the immune system that inhibits viral replication and may have anticancer properties; three major types are known: alpha, beta and gamma.

INTERLEUKIN - a class of lymphokines important in the function of the immune system (e.g., regulates the maturation and replication of T lymphocytes). Two types have been identified, interleukin 1 (IL-1) and interleukin 2 (IL-2).

INTRON - a part of the eucaryotic gene that does not encode for protein.

IN VITRO - biochemical reactions taking place in the laboratory, outside of a living organism or cell; literally meaning, "in glass."

IN VIVO - biochemical reactions taking place within a living organism or cell.

ISOZYMES (ISOENZYMES) - enzymes that perform the same biological function but have different physical characteristics.

ISOGENIC - of the same genotype.

K

KARYOTYPE - the ordered arrangement of the chromosomes of an organism.

L

LIBRARY - see genomic library.

LIGASE - enzyme used to join DNA or RNA fragments together.

LINKED GENES - genes located on the same chromosome that are inherited as a unit, but each retains its individual identity.

LOCUS - the location of a gene (i.e., a transcribable unit) on a chromosome.

LYMPHOCYTE - type of white blood cell involved in the immune response of which there are two principal classes, B cells and T cells.

LYMPHOKINE - a class of soluble proteins that play a role in the immune response not yet understood.

LYMPHOMA - cancer of the lymph tissue.

LYSIS - disruption of a cell by breaking of its membrane.

M

MACROMOLECULES - large biological molecules, e.g., proteins and nucleic acids.

MACROPHAGE - white blood cells that ingest dead cells and other debris in tissues and are involved in the production of interleukin-1; may also kill tumor cells when exposed to lymphokine "macrophage activating factor."

MEDIUM - mixture of nutrients used for tissue (cell) culture.

MESSENGER RNA (mRNA) - the type of RNA molecules that direct the incorporation of amino acids into proteins in protein synthesis.

METABOLISM - the sum total of all biochemical reactions occurring within a cell or organism.

MICROBIOLOGY - study of living organisms that can be seen only with the aid of a microscope.

MICROORGANISM - any organism that can be seen only with the aid of the microscope.

MOLECULAR BIOLOGY - the study of genetic and biochemical processes in living cells.

MOLECULAR WEIGHT - the sum of the weights in daltons of all of the atoms in a molecule; the standard reference is the hydrogen atom at one dalton.

MONOCLONAL ANTIBODY - a highly pure antibody of a single type that is produced by a laboratory-made hybridoma.

MONOCOTYLEDONS (MONOCOTS) - plants having a single seed leaf (cotyledon); cereals such as rice, wheat and corn are monocots.

MULTIGENIC - a single inherited characteristic expressed by several genes.

MUTAGEN - a mutation-causing substance.

MUTANT - a cell or organism that manifests new characteristics due to a change in its DNA.

MUTATION - a change in the hereditary material.

MYELOMA - a type of cancer cell used in the monoclonal antibody technique to form hybridomas.

N

NITROGEN FIXATION - incorporation of atmospheric nitrogen in nitrogen-containing compounds, a process that can be carried out only by certain microorganisms.

NITROGENOUS BASE - a nitrogen-containing molecule having basic chemical properties; the building blocks of nucleic acids are the principal nitrogenous bases. Also called nucleotide bases.

NUCLEASE - an enzyme that cleaves of the chemical bonds of nucleic acid to result in its constituent nucleotides.

NUCLEIC ACID - one of the major classes of cellular macromolecules; includes DNA and RNA.

NUCLEOTIDES - the building blocks of nucleic acid; a single nucelotide is composed of a sugar, a phosphate group and one of five nitrogenous bases.

NUCLEUS - the part of the eucaryotic cell which contains the genetic material.

O

OLIGONUCLEOTIDE - short segments (2 to 10 nucleotides) of DNA or RNA.

ONCOGENE - a cancer-causing gene; a mutant form of "normal" growth-regulating gene that is inappropriately turned on.

ONCOGENIC - cancer-causing.

ONCOLOGY - the study of cancer.

ORGANIC COMPOUND - molecules containing carbon other than carbon dioxide and the carbonates (CO_3 containing compounds).

OPERATOR - site on DNA at which a repressor or activator protein binds.

OPERON - group of adjacent genes regulated and transcribed as a single unit into an mRNA molecule.

P

PATHOGEN - a disease-causing organism.

PEPTIDE - two or more amino acids linked by a peptide bond.

PEPTIDE BOND - formed between two amino acids by removing an OH group from the carboxyl (-COOH) group of one amino acid and an H from the amino end ($-NH_2$).

pH - measure of the relative concentration of hydrogen ions in a solution; pH values range from 0 (highly acidic) to 14 (highly basic).

PHAGE - see bacteriophage.

PHAGOCYTE - any cell that engulfs and devours microorganisms or other particles.

PHENOTYPE - observed characteristics of an organism resulting from the interaction of genotype and environment.

PLASMID - small ring of DNA that carries accessory genes which are separate from the chromosome in bacteria.

POLYCLONAL - derived from different types of cells.

POLYMER - a molecule consisting of a sequence of similar units called monomers.

POLYMERASE - enzyme that catalyzes the formation of polymers.

POLYNUCLEOTIDE - a chain of nucleotides as in nucleic acid.

POLYPEPTIDE - a small chain of amino acids.

PROBE - see DNA probe.

PROCARYOTE - organisms composed of one or more cells without well-defined nuclei.

PROMOTER - a DNA site where RNA polymerase initiates transcription.

PROPHAGE - noninfectious bacteriophage DNA linked with the growing and dividing bacterial chromosome but does not bring about lysis of the bacterial membrane.

PROTEIN - a chain of amino acids joined together by peptide bonds.

PROTOPLAST - a cell with its cell wall removed, (e.g., plant cell or bacterium).

PSEUDOGENE - a silent gene; a copy of a gene that is not transcribed.

PURE CULTURE - in vitro growth of only a single kind of microorganism.

R

RADIOIMMUNOASSAY - a technique that uses a radioactive labeled antibody to identify a molecule or measure a process.

RECOMBINANT DNA (rDNA) - segments of DNA from two different organisms

spliced together in the laboratory into a single molecule.

RECOMBINANT DNA TECHNOLOGY - methods for transferring genes or groups of genes from one organism to another.

RECOMBINANT - the new molecule, organism or cell that is the product of rDNA methods.

REGULATORY GENE - a gene that acts to control the protein-synthesizing activity of other genes.

REPLICATION - the reproduction of a second copy of DNA that is exactly like the first, or parent, DNA.

REPLICON - any genetic element with the ability to reproduce independently (e.g., a chromosome or a plasmid).

REPRESSOR - a protein that regulates DNA transcription by preventing RNA polymerase from attaching to a DNA promoter site.

RESTRICTION ENZYMES - enzymes that cleave DNA at specific sites.

RESTRICTION ENDONUCLEASE - enzyme that cleaves DNA at internal locations.

RESTRICTION MAP - a diagram of a segment of DNA showing the relative locations of sites ("restriction sites") cleaved by particular restriction endonucleases.

RETROVIRUS a virus whose genetic information is RNA rather than DNA.

REVERSE TRANSCRIPTASE - an enzyme that transcribes RNA into DNA; found only in retroviruses.

RIBONUCLEIC ACID (RNA) - a kind of nucleic acid that includes messenger RNA, ribosomal RNA and transfer RNA. It is characterized by having a ribose sugar as part of its structure.

RIBOSOME - the cellular organelle on which protein synthesis takes place.

RNA POLYMERASE - an enzyme that synthesizes RNA molecules under the direction of a DNA template.

S

SATELLITE DNA - a nontranscribable region of a chromosome with a distinctive base composition.

SINGLE CELL PROTEIN cells or protein extracts from microorganisms grown in

large quantities for use as protein supplements.

SPLICING - a stage in RNA processing in which introns are removed and exons are joined to form a continuous coding sequence of RNA.

STRUCTURAL GENE - any sequence of nucleotides that encode a protein, tRNA, or rRNA; distinct from regulator genes which are not transcribed.

SUBSTRATE - a substance that is acted upon by an enzyme.

SUPPRESSOR GENE - a gene which can reverse the effect of a mutation in other genes.

T

T CELLS (T LYMPHOCYTES) - white blood cells which recognize, engulf and destroy specific foreign cells.

TEMPLATE - a pattern used for the formation of a complementary molecule; in DNA replication, each strand is used as a template on which a complementary strand is assembled. They are produced in bone marrow but mature in the thymus gland.

TISSUE CULTURE - in vitro culture of cells obtained from tissues.

TOXINS - poisonous substances produced by microorganisms.

TRANSCRIBABLE UNIT - the modern definition for a gene; the portion of DNA that is transcribed into RNA.

TRANSCRIPTION - the synthesis of RNA from a DNA template.

TRANSCRIPTION RNA - the transcribed copy of RNA from DNA containing both introns and exons; subsequent processing will remove the introns to result in messenger RNA.

TRANSDUCTION - transfer of genetic material by a bacteriophage.

TRANSFER RNA (tRNA) - the class of molecules that bring the amino acids to the site where they are incorporated into protein on ribosomes.

TRANSFORMATION - the transfer of DNA from one organism to another, also called gene transfer.

TRANSLATION - the process whereby mRNA is used as a template for the synthesis of a polypeptide on a ribosome.

TRANSPOSON - a segment of DNA carrying one or more genes that can move from one DNA molecule to another to result in a change of the altered DNA.

V

VACCINE - a preparation containing an antigen made up of whole disease-causing organisms (attenuated) or parts of such organisms; used to confer an immunity against the antigen.

VECTOR - the agent used to transfer DNA into a host cell, e.g., plasmid or bacteriophag.

VIRION - a viral particle consisting of genetic material and a protein coat.

VIRUS - an entity whose own genetic material can only function within the cell of another organism; classified according to its structure or properties (e.g., adenovirus - with DNA, retrovirus - with RNA).

W

WHITE BLOOD CELL - a blood cell that does not contain hemoglobin; also called a leucocyte.

WILD-TYPE - the form that is found as the most common one in nature.

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DEPARTMENT OF DEFENSE DEPENDENT SCHOOLS PACIFIC

MIDDLE AND HIGH SCHOOL SCIENCE EDUCATION PROGRAM EVALUATION GUIDE

SY88-89

EDITION ONE

MAY 1988

Revision Dates

Distribution: All Pacific Middle and High Schools

CONTENTS

(01) INTRODUCTION

The Checklist is intended for use by the DoDDS-Pacific science coordinator, school principals, science department chairs and teachers in identifying strengths and weaknesses of thei~ science programs. The guide is also used as a notebook by the science co. dinator during school visits. Wherever possible, references have been cited in context so that users may, if necessary, consult the supporting documents. A list of those references is provided in category (02) below and the relevant documents are included sequentially in the Appendix.

(02) LIST OF SUPPORTING DOCUMENTS

01. ETG/635-3001/303-5 Memorandum Quality Program Indicators, of 87MAR23.

02. DS Manual 2005.1, Administrators' Guide, section 402, of 88FEB.

03. DS Manual 2200.1, Science Objectives for 1985-1992.

04. DoDDS-P/Director Memorandum, Definition of Laboratory Science Courses and Science Laboratory Sessions, of 87OCT07.

05. 7-12 Sequential Learning Guide DSPA Manual 2000.9.

06. ERC/635-2151/308 Memorandum, Course Titles and Student Information System (SIMS) Computer Codes. of 87APR17.

07. ERH/635-2267/303-11 Memorandum Approved Textbook Listing. of 87AUG11.

08. NCA Standards For Secondary Schools (staffing).

09. The DoDDS Educator Applicant Evaluation Guide School Year 1988-1989.

10. DS Regulation 2000.1, Department of Defense Dependent Schools High School Graduation Requirements, of September 7, 1984.

11. NCA Standards For Secondary Schools (staffing).

(03) SCHOOL AND COUNTRY

(04) VISITATION DATE/S AND NUMBER

(05) PURPOSE/S OF THE VISIT

1.

2.

3.

(06) IN BRIEFING

1. Name/c of individual/s with whom the briefing was held:

2. Quality Program Indicators (ETG/635-3001/303-5 Memorandum of 87MAR23) identified by the school administrator as those upon which he or she would like the evaluation to focus:

   a.

   b.

   c.

   d.

3. **Previous Visit:**

a. **Date:** _____

b. **Recommendations for improvement made as a result of the previous visit:**

(01) _____

(02) _____

(03) _____

(04) _____

(05) _____

(06) _____

c. **Actions taken on the recommendations for improvement:**

(01) _____

(02) _____

(03) _____

(04) _____

(05)_____

_____

(06)_____

_____

d. Notes:

(01)_____

_____

(02)_____

_____

(07) TEACHERS, SPECIALISTS AND ADMINISTRATORS VISITED

1. NAMES/RESPONSIBILITIES NAMES/RESPONSIBILITIES

a._____ n._____

b._____ o._____

c._____ p._____

d._____ q._____

e._____ r._____

f._____ s._____

g._____ t._____

h._____ u._____

i._____ v._____

j._____ w._____

k._____ x._____

l._____ y._____

m._____ z._____

2. **Notes:**

a. _____

_____

b. _____

_____

c. _____

_____

d. _____

_____

e. _____

_____

f. _____

_____

3. **Observations/Recommendations for Improvement:**

a. _____

_____

_____

b. _____

_____

_____

c. _____

_____

_____

d. _____

_____

_____

e. _____

_____

_____

(08) SCIENCE DEPARTMENT

1. General Observations:	Yes	No
a. Program Administration.		
(01) A science supervisor coordinates the science program.	_____	_____
(02) A science supervisor has full administrative responsibility for the science program except teacher evaluation.	_____	_____
(03) Supervision of the science program is done by regular school administrators.	_____	_____
(04) Supervision of the science program is judged to be adequate.	_____	_____
(05) Administrative support of the science program is adequate.	_____	_____
b. Curriculum Coordination:		
(01) There is vertical coordination in the program from grade to grade.	_____	_____
(02) There is horizontal coordination among course sections at the same grade/course level.	_____	_____
(03) Repetition in course content is limited from course-to-course except where it is planned.	_____	_____
(04) Teachers have an opportunity to plan with other teachers;		
(a) in the same course.	_____	_____
(b) teaching different courses.	_____	_____
c. Decision-making Process in the Science Program:		
(01) Teachers have frequent opportunities for staff input on the science program.	_____	_____

(02) Teachers have great independence in developing their science courses. _____:_____

(03) Teachers have few opportunities to influence the science program. _____:_____

2. Name of Department Chair: _____

3. Size of Department: _____

4. Frequency of Meetings: _____

5. Minutes of Meetings: _____

6. Notes:

a. _____

_____

b. _____

_____

7. Observations/Recommendations for Improvement:

a. _____

_____

_____

b. _____

_____

_____

(09) SCIENCE BUDGET

(DS Manual 2005.1, Administrators' Guide, section 402):

1. Dollar Amount: _____

a. Consumable Materials: _____

b. Equipment: _____

(01) Replacement: _____

(02) New:_____

(03) Repair:_____

c. Library Materials:_____

d. Science Kits (Grades 7, 8 and 9)_____

e. Textbooks:_____

2. Name of Person Who Drafts the Budget:_____

3. Process Used When Drafting the Budget:_____

_____

_____

_____

_____

_____

4. Yearly Budget Deadline as Set by the Administration:_____

5. Notes.

a._____

_____

b._____

_____

c._____

_____

6. Observations/Recommendations for Improvement:

a._____

_____

_____

b._____

_____

c.

d.

(10) LIBRARY AND MEDIA CENTER

1. General Adequacy: The presence of sufficient and appropiate science books, student periodicals, professional science teaching periodicals and science media programs to carry out the conditions of the curriculum are essential to a good science education program. All of these items should be matched as closely as possible with the science program objectives and teaching methods required by the curriculum. Versatility, intended use, the user, and application to student investigations must be considered in assessing the appropiateness of existing library and media center inventories to adequately support the science education program as well as new purchases in the area of science.

To assess the general adequacy of the science library and media center portion of the science program, all components that have been met in the list below should be checked.

FUNDAMENTAL	SUBSTANTIAL	EXEMPLARY
{_} Sufficient library books and media programs are available to support all activities and topics in the courses offered.	{_} All necessary instructional resorces including audio visual resources related to the science curriculum are available in the media center.	{_} Full use is made of instructional media to supplement science learning in the classroom.
{_} An annual budget provides for the purchase of science books and media programs.	{_} Equipment and library materials provided for in the curriculum plan are available to individuals or small groups for use when conducting	{_} Lists of science media programs held by the media center are available for teacher use.
		{_} There is an on going program conducted by media specialist and science department to

	investigations.	evaluate the currency of science books and media programs.

2. Books:

   a. Approximate number of science books held:_____

   b. Are the science books well distributed across all science areas?_____

3. Reference documents_____

   a. Professional periodicals in science areas:

      (01) Number:_____

      (02) Names:

      (a)_____

      (b)_____

      (c)_____

      (d)_____

      (e)_____

      (f)_____

   b. Student periodicals in science areas:

      (01) Number:_____

      (02) Names:

      (a)_____

      (b)_____

      (c)_____

      (d)_____

      (e)_____

      (f)_____

4. Audio/Visual/Media Materials:

   a. Number of Programs: _____

   b. Distribution Across the Science Areas: _____

   _____

5. Notes:

   a. _____

   _____

   b. _____

   _____

   c. _____

   _____

6. Observations/Recommendations for Improvement:

   a. _____

   _____

   _____

   b. _____

   _____

   _____

   c. _____

   _____

   _____

   d. _____

   _____

   _____

(11) COMPUTER PROGRAM IN SCIENCE

1. Software:

   a. Number of science programs held by the school:_____

   b. Is the software compatible with the computers?_____

   _____

   c. Is the software well distributed across the science areas?_____

2. Apple IIGS Program:

   a. Are Apple IIGS computers part of the science program?_____

   b. How many computers are used in the program?_____

   c. Subjects in which the computers are used:_____

   _____

   d. Ways in which the computer/s is/are used:

      (01)_____

      (02)_____

      (03)_____

      (04)_____

3. Notes:

   a._____

   _____

   b._____

   _____

   c._____

   _____

4. Observations/Recommendations for Improvement:

   a._____

   _____

   _____