

COMMERCIAL BIOTECHNOLOGY

An International Analysis

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COMMERCIAL BIOTECHNOLOGY

An International Analysis

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Foreword

This report assesses the competitive position of the United States with respect to Japan and four European countries believed to be the major competitors in the commercial development of "new biotechnology." This assessment continues a series of OTA studies on the competitiveness of U.S. industries. It was requested by the House Committee on Science and Technology and the Senate Committee on Commerce, Science, and Transportation. Additionally, a letter of support for this study was received from the Senate Committee on Labor and Human Resources.

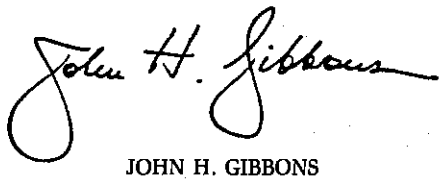
New biotechnology, as defined in this report, focuses on the industrial use of recombinant DNA, cell fusion, and novel bioprocessing techniques. These techniques will find applications across many industrial sectors including pharmaceuticals, plant and animal agriculture, specialty chemicals and food additives, environmental applications, commodity chemicals and energy production, and bioelectronics. Over 100 new firms have been started in the United States in the last several years to capitalize on the commercial potential of biotechnology. Additionally, throughout the world, many established companies in a diversity of industrial sectors have invested in this technology.

A well-developed life science base, the availability of financing for high-risk ventures, and an entrepreneurial spirit have led the United States to the forefront in the commercialization of biotechnology. But although the United States is currently the world leader in both basic science and commercial development of biotechnology, continuation of the initial preeminence of American companies in the commercialization of biotechnology is not assured. Japan is likely to be the leading competitor of the United States, followed by the Federal Republic of Germany, the United Kingdom, Switzerland, and France. In the next decade, competitive advantage in areas related to biotechnology may depend as much on developments in bioprocess engineering as on innovations in genetics, immunology, and other areas of basic science. Thus, the United States may compete very favorably with Japan and the European countries if it can direct more attention to research problems associated with the scaling-up of bioprocesses for production.

Issues and options developed for Congress include Federal funding for the basic life sciences and for generic applied research, especially in the areas of bioprocessing engineering and applied microbiology, including the training of personnel in these areas. The United States may also need to be concerned with the continued availability of finances for new biotechnology firms until they are self-supporting. Additionally, there are changes in laws and policies that could improve the U.S. competitive position. These changes include clarification and modification of particular aspects of intellectual property law; health, safety, and environmental regulation; antitrust law; and export control laws.

OTA was assisted in the preparation of this study by an advisory panel of individuals representing a wide range of backgrounds, including science, economics, financial analysis, law, labor, and new and established firms commercializing biotechnology. Additionally, over 250 reviewers from universities, the private sector, and government agencies, both domestic and foreign, provided helpful comments on draft reports.

OTA expresses sincere appreciation to each of these individuals. As with all OTA reports, however, the content is the responsibility of the Office and does not necessarily constitute the consensus or endorsement of the advisory panel or the Technology Assessment Board.



JOHN H. GIBBONS
Director

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Health and Life Sciences Division

Gretchen Schabtach Kolsrud, *Program Manager, Biological Applications Program*
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Nanette Newell, *Project Director* from September 1982
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the most potentially useful current technology obsolete in a short time. Of necessity, this assessment describes the development of biotechnology at a particular point in time, but it is important to emphasize that dynamic and progressive change has characterized biotechnology for the last decade. Figure 1 shows some prominent events that illustrate the rapid progress made in the development of biotechnology over the last decade. This pace is likely to continue into the 21st century.

The technologies

The novel techniques used in biotechnology are extremely powerful because they allow a large amount of control over biological systems. *Recombinant DNA technology,* one of the new techniques, allows direct manipulation of the genetic material of individual cells. The ability to direct which genes are used by cells permits more control over the production of biological molecules than ever before. Recombinant DNA technology can be used in a wide range of industrial sectors to develop micro-organisms that produce new products, existing products more efficiently, or large quantities of otherwise scarce products. This technology can also be used to develop organisms that themselves are useful, such as micro-organisms that degrade toxic wastes or new strains of agriculturally important plants.

Cell fusion, the artificial joining of cells, combines the desirable characteristics of different types of cells into one cell. This technique has been used recently to incorporate in one cell the traits for immortality and rapid proliferation from certain cancer cells and the ability to produce useful antibodies from specialized cells of the immune system. The cell line resulting from such

Figure 1.—Major Events in the Commercialization of Biotechnology

1973	First gene cloned.
1974	First expression of a gene cloned from a different species in bacteria. Recombinant DNA (rDNA) experiments first discussed in a public forum (Gordon Conference).
1975	U.S. guidelines for rDNA research outlined (Asilomar Conference). First hybridoma created.
1976	First firm to exploit rDNA technology founded in the United States (Genentech). Genetic Manipulation Advisory Group (U.K.) started in the United Kingdom.
1980	*Diamond* v. *Chakrabarty*—U.S. Supreme Court rules that micro-organisms can be patented under existing law. Cohen/Boyer patent issued on the technique for the construction of rDNA. United Kingdom targets biotechnology (Spinks' report). Federal Republic of Germany targets biotechnology (Leistungsplan). Initial public offering by Genentech sets Wall Street record for fastest price per share increase ($35 to $89 in 20 minutes).
1981	First monoclonal antibody diagnostic kits approved for use in the United States. First automated gene synthesizer marketed. Japan targets biotechnology (Ministry of International Trade and Technology declares 1981 "The Year of Biotechnology"). France targets biotechnology (Pelissolo report). Hoescht/Massachusetts General Hospital agreement. Initial public offering by Cetus sets Wall Street record for the largest amount of money raised in an initial public offering ($115 million). Industrial Biotechnology Association founded. DuPont commits $120 million for life sciences R&D. Over 80 NBFs had been formed by the end of the year.
1982	First rDNA animal vaccine (for colibacillosis) approved for use in Europe. First rDNA pharmaceutical product (human insulin) approved for use in the United States and the United Kingdom. First R&D limited partnership formed for the funding of clinical trials.
1983	First plant gene expressed in a plant of a different species. $500 million raised in U.S. public markets by NBFs.

SOURCE: Office of Technology Assessment.

Chapter 1

Summary

Findings

Industrial applications of biotechnology

The earliest industrial applications of biotechnology (i.e., during the next 5 to 10 years) are likely to occur in pharmaceuticals, animal agriculture, and specialty chemicals. Applications of biotechnology to **pharmaceuticals** being pursued at present are in the production of proteins such as insulin, interferon, and human serum albumin; antibiotics; MAb diagnostics; and vaccines for viral, bacterial, and parasitic diseases. As more is learned about hormone growth factors, immune regulators, and neurological peptides, their importance in the treatment of disease may increase dramatically. Eventually, the production of such regulatory proteins may turn out to be the largest application of biotechnology in the pharmaceutical industry. U.S. companies pursuing biotechnological applications in pharmaceuticals include many of the established pharmaceutical companies* and a large number of small, entrepreneurial new biotechnology firms (NBFs).** Additionally, many established companies in other sectors are using biotechnology as a way to diversify into pharmaceuticals.

In **animal agriculture,** biotechnology is being used to develop products similar to those being developed in the pharmaceutical industry. However, since animal producers cannot afford to purchase expensive products made with new technology, biotechnologically produced products may initially be limited to products for "high value" animals such as pets and breeding stock. The most important products are likely to be vaccines and growth promotants.

Unlike the production of pharmaceuticals, the production of animal health products using traditional technologies is not dominated by a few large companies. Additionally, the animal agriculture industry differs from the pharmaceutical industry in that the regulatory requirements for animal health products, especially for vaccines and diagnostics, are significantly less stringent than for human health products; markets for animal products are smaller and more accessible; and the distribution and delivery systems are different. Because of these features, many NBFs are finding animal agriculture an attractive field for the application of biotechnology.

The potential applications of biotechnology are probably more varied for **specialty chemicals** (i.e., chemicals costing more than $1/lb) and **food additives*** than for any other industrial sector at the present time. Possible applications include improvements in existing bioprocesses, such as in the production of amino acids. Other products, such as vitamins and steroid compounds, are currently made in multistep production processes involving chemical syntheses. Biotechnology could provide one or more enzymatic conversion process to increase the specificity of currently used chemical conversions. Generally, complex products, such as enzymes and some polysaccharides, can only be made economically using bioprocesses. The production of specialty chemicals represents one of the largest opportunities for the application of biotechnology because of the diversity of potential applications. Several companies in the United States are pursuing biological production of specialty chemicals, but most specialty chemicals currently produced biologically are made almost exclusively in Japan and Europe, and these countries intend to pursue new applications for specialty chemical production.

Applications of rDNA technology to **plant agriculture** are proceeding faster than anyone anticipated 3 to 4 years ago. Some important traits of plants, including stress-, herbicide-, and pest-resistances, appear to be rather simple genetically, and it may be possible to transfer these traits to important crop species in the next few years. Other traits, such as increased growth rate, increased photosynthetic ability, and the stimula-

*Established companies pursuing applications of biotechnology are generally process-oriented, multiproduct companies in traditional industrial sectors such as pharmaceuticals, energy, chemicals, and food processing.

**NBFs, as defined in this report, are entrepreneurial ventures started specifically to pursue applications of biotechnology.

*Food additives are considered together with specialty chemicals because many (though not all) food additives are also specialty chemicals, e.g., amino acids and vitamins.

Chapter 1

Summary

Introduction

In the past 10 years, dramatic new developments in the ability to select and manipulate genetic material have sparked unprecedented interest in the industrial uses of living organisms. Following the first successful directed insertion of foreign DNA in a host micro-organism in 1973, scientific researchers in the United States and other countries began to recognize the potential for directing the cellular machinery to develop new and improved products and processes in a wide diversity of industrial sectors. Potential industrial applications of those novel genetic techniques include the production of new drugs, food, and chemicals, the degradation of toxic wastes, and the improvement of agricultural products. Thus, these new techniques could have a major economic impact on industries throughout the world.

Beginning around 1976, many small entrepreneurial firms were formed in the United States specifically to build on the growing body of fundamental knowledge in molecular biology and to exploit it to a profitable end. Furthermore, large established American, Japanese, and European companies in a spectrum of industrial sectors expanded their research and development (R&D) programs to include the new genetic techniques. In the United States, private sector investments to commercialize these new techniques exceeded $1 billion in 1983.

This report assesses the competitive position of the United States with respect to Japan and four European countries—the Federal Republic of Germany, the United Kingdom, Switzerland, and France—believed to be the major competitors in the commercial development of "new biotechnology," as defined below. **Although the United States is currently the world leader in both basic science and commercial development of new biotechnology, continuation of the initial preeminence of American companies in the commercialization of new biotechnology is not assured.** Japan and other countries have identified new biotechnology as a promising area for economic growth and have therefore invested quite heavily in R&D in this field. Congressional policy options for improving U.S. competitiveness in new biotechnology are identified in this report.

Definitions

Biotechnology, broadly defined, includes any technique that uses living organisms (or parts of organisms) to make or modify products, to improve plants or animals, or to develop micro-organisms for specific uses. Biological processes and organisms have been used with great success throughout history and have become increasingly sophisticated over the years. Since the dawn of civilization, people have deliberately selected organisms that improved agriculture, animal husbandry, baking, and brewing. More recently, a better understanding of genetics has led to more effective applications of traditional genetics in such areas as antibiotic and chemical production.

This report focuses on the industrial use of recombinant DNA (rDNA), cell fusion, and novel bioprocessing techniques. To differentiate between biotechnology using these novel techniques and the more traditional forms of biotechnology, this report uses the terms "new biotechnology" and "old biotechnology," respectively. Thus, for example, traditional wine production is old biotechnology, but the use of yeast modified with rDNA techniques to produce wine with a higher alcohol content is new biotechnology. Where no specific distinction is made, the term biotechnology alone henceforth refers to new biotechnology.

Biotechnology is the most recent phase in a historical continuum of the use of biological organisms for practical purposes. Furthermore, developments arising from existing technologies are providing a base from which other technologies will emerge, and new technologies can make even

from industry, universities, and government. **The United States may compete very favorably with Japan if it can direct more attention to research problems associated with the scaling-up of bioprocesses for production.**

The European countries are not moving as rapidly toward commercialization of biotechnology as either the United States or Japan, in part because the large established pharmaceutical and chemical companies in Europe have hesitated to invest in biotechnology and in part because of cultural and legal traditions that tend not to promote venture capital formation and, consequently, risk-taking ventures. Nevertheless, several of the large pharmaceutical and chemical houses in the United Kingdom, the Federal Republic of Germany, Switzerland, and France will surely be competitors in selected product areas in the future because of their prominent position in world sales of biologically derived products. Additionally, the increased interest shown recently by the British Government in biotechnology may speed its development in the United Kingdom.

The United States could have difficulty maintaining its competitive position in the future if several issues are not addressed. If U.S. Government funding for basic life science research continues its decline, the science base, which is the source of innovation in biotechnology as well as in other fields, may be eroded. U.S. Government funding of generic applied research,* especially in the areas of bioprocess engineering and applied microbiology, is currently insufficient to support rapid commercialization. U.S. Government funding for personnel training in these areas may also be insufficient. Additionally, clarification and modification of certain aspects of U.S. health, safety, and environmental regulation and intellectual property law may be necessary for the maintenance of a strong U.S. competitive position in biotechnology.

*Generic applied research, which is nonproprietary and bridges the gap between basic research and applied research, is aimed at the solution of generic problems that are associated with the use of a technology by industry.

Analysis of international competitiveness in biotechnology

Often international competitiveness is defined as the relative ability of firms based in one country to develop, produce, and market equivalent goods or services at lower costs than firms in other countries. Competitiveness is a matter of relative prices, and these usually reflect relative costs of developing, producing, and distributing goods and services. In the case of biotechnology, two factors preclude a traditional analysis of international competitiveness. First, standard analyses of competitiveness examine the marketing of products, but as of the end of 1983, only a few products of new biotechnology had reached the marketplace—notably human insulin, some MAb diagnostic kits, and some animal vaccines. Most of these products are substitutes for already existing products, and the markets are well defined and relatively limited. Furthermore, even the markets for some new animal vaccines are quite small when compared to potential markets for applications of biotechnology in the production of some chemicals or new crop plants. Thus, the biotechnology products that have reached the market to date may be inaccurate indicators of the potential commercial success in world markets of the much larger number of biotechnology products and processes still in R&D stages. Which of the biotechnology products and processes in development are likely to be marketed and when cannot be accurately predicted. Second, even with many more products on the market, a traditional competitive analysis might not be appropriate because an economic analysis of competitiveness usually addresses a specific industrial sector. The

a fusion, known as a hybridoma, produces large quantities of *monoclonal antibodies (MAbs),* so called because they are produced by the progeny, or clones, of a single hybridoma cell. MAbs can potentially be used for many purposes, including the diagnosis and treatment of disease and the purification of proteins.

The commercial success of specific industrial applications of rDNA and cell fusion techniques will hinge on advances in bioprocess engineering. *Bioprocess technology,* though not a novel genetic technique, allows the adaptation of biological methods of production to large-scale industrial use. Most industrial biological syntheses at present are carried out in single batches, and a small amount of product is recovered from large quantities of cellular components, nutrients, wastes, and water. Recent improvements in techniques for immobilizing cells or enzymes and in bioreactor design, for example, are helping to increase production and facilitate recovery of many substances. Additionally, new genetic techniques can aid in the design of more efficient bioreactors, sensors, and recovery systems. **In the next decade, competitive advantage in areas related to biotechnology may depend as much on developments in bioprocess engineering as on innovations in genetics, immunology, and other areas of basic science.**

The same technologies that yield commercial products will also provide new research tools. The new genetic technologies described above have ignited an explosion of fundamental knowledge. The widespread use of rDNA and cell fusion techniques in the investigation of a wide variety of biological phenomena in plants, animals, microorganisms, and viruses highlights the impact of these technologies on basic science research and the advances in fundamental knowledge that they make possible. This new knowledge, in turn, may reveal new commercial opportunities.

Industrial development

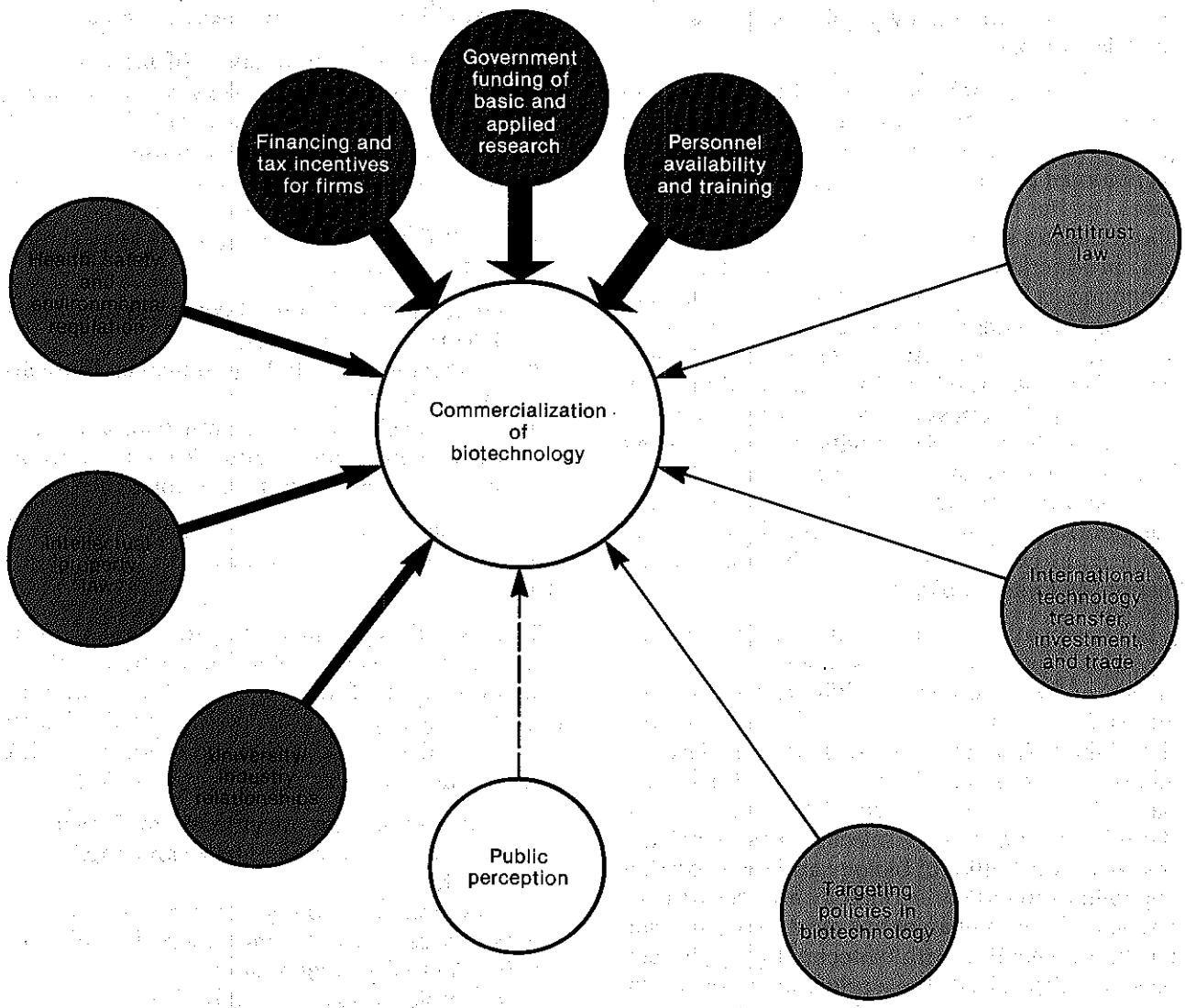
Biotechnology could potentially affect any current industrial biological process or any process in which a biological catalyst could replace a chemical one. As discussed in this report, **industrial applications of biotechnology will be found in several industrial sectors, including pharmaceuticals, animal and plant agriculture, specialty chemicals and food additives, environmental areas, commodity chemicals and energy production, and bioelectronics.**

The industrial sector in which the earliest applications of new biotechnology have occurred is the pharmaceutical sector. Reasons for the rapid diffusion of the new techniques into the pharmaceutical sector include the following:

- Recombinant DNA and MAb technologies were developed with public funds directed toward biomedical research. The first biotechnology products, such as rDNA-produced human insulin, interferon, and MAb diagnostic kits, are a direct result of the biomedical nature of the basic research that led to these new technologies.
- Pharmaceutical companies have had years of experience with biological production methods, and this experience has enabled them to take advantage of the new technologies.
- Pharmaceutical products are high value-added and can be priced to recover costs incurred during R&D, so the pharmaceutical sector is a good place to begin the costly process of developing a new technology.

Because of the rapid diffusion of the new genetic techniques into pharmaceutical R&D programs, the pharmaceutical sector is currently most active in commercializing biotechnology. For this reason, it serves as a model for the industrial development of biotechnology in much of this report. It is important to recognize, however, that the development of biotechnology in other industrial sectors will differ from its development in the pharmaceutical sector. Regulatory and trade barriers and a marketing and distribution system unique to the pharmaceutical sector limit its usefulness as a model. Furthermore, the techniques may not diffuse as rapidly into other industrial sectors, such as the chemical industry, because of difficulties companies may have in recovering investments in R&D and physical plants required to convert to biological methods of production.

Figure 2.—The Relative Importance of Factors Affecting the Commercialization of Biotechnology



SOURCE: Office of Technology Assessment.

Kingdom, Switzerland, and France. Since the importance to competitiveness of any given factor is not necessarily the same for every industrial sector in which applications are being pursued—for instance, a country's intellectual property laws may protect pharmaceuticals better than plants—the importance of each factor was evaluated for different industrial sectors.

Additional considerations taken into account in the analysis are historical patterns of industrial commercialization, the lack or abundance of particular natural resources, and the tendency toward risk taking in each country. These other considerations were used as modifiers of the results of the analysis.

OTA's principal findings with respect to the types and activities of firms commercializing biotechnology, the factors potentially important to international competitiveness in biotechnology, and the other considerations just mentioned are presented below.

tion of nitrogen fixation, are genetically complex, and it is likely to be several years before plants with these characteristics developed with rDNA technology will be ready for field testing. Micro-organisms that interact with plants offer possibilities for genetic manipulation that may be more near-term. For instance, it may be possible to manipulate micro-organisms to produce pesticides or inhibit frost formation. Companies pursuing these applications include many NBFs and established companies in agricultural chemicals and seed production.

Environmental applications of biotechnology include mineral leaching and metal concentration, pollution control and toxic waste degradation, and enhanced oil recovery. These applications may take longer to reach the market, because little is known of the genetics of the most potentially useful micro-organisms. Additionally, regulation is expected to be a major factor influencing development of this area because these applications use micro-organisms that are deliberately released into the environment. The nature and extent of this regulation remains uncertain, and this uncertainty may deter some firms from entering the field, thus slowing development.

Commodity chemicals, which are now produced from petroleum feedstocks, could be produced biologically from biomass feedstocks such as cornstarch and lignocellulose. Commodity chemical production from cornstarch will probably occur before production from lignocellulose because of the high energy inputs necessary for the solubilization of lignocellulose. Although the technology exists now for the cost-effective biological production of some commodity chemicals such as ethanol, the complex infrastructure of the commodity chemical industry will prevent the replacement of a large amount of commodity chemical production using biotechnology for at least 20 years. This distant time horizon is due more to the integrated structure of the chemical industry, its reliance on petroleum feedstocks, and its low profit margins than to technical problems in the application of the biotechnology.

In the area of **bioelectronics,** biotechnology could be used to develop improved biosensors or new conducting devices called biochips. Sensors that use enzymes for detecting specific substances are available now. However, their use is limited by the narrow range of substances they detect and by their temperature instability. Biotechnology could be instrumental in the development of more versatile sensors that use enzymes or MAbs. Better sensors would be especially useful in the control of industrial bioprocesses. Biotechnology may also make it possible to construct devices that use proteins as a framework for molecules that act as semiconductors. The anticipated advantages of these biochips are their small size, reliability, and the potential for self assembly. The production of biochips, however, is one of the most distant applications of biotechnology.

The U.S. competitive position

A well-developed life science base, the availability of financing for high-risk ventures, and an entrepreneurial spirit have led the United States to the forefront in the commercialization of biotechnology. For the most part, the laws and policies of this country have made it possible for industrialists and scientists to capitalize rapidly on the results of basic research in biotechnology conducted in the university system and government laboratories. The relative freedom of U.S. industry to pursue a variety of courses in the development of products has also given the United States a comparative advantage. The flexibility of the U.S. industrial system and the plurality of approaches taken by entrepreneurial NBFs and established companies in the development of products have facilitated the rapid development of biotechnology in the United States.

Japan is likely to be the leading competitor of the United States for two reasons. First, Japanese companies in a broad range of industrial sectors have extensive experience in bioprocess technology. Japan does not have superior bioprocess technology, but it does have relatively more industrial experience using old biotechnology, more established bioprocessing plants, and more bioprocess engineers than the United States. Second, the Japanese Government has targeted biotechnology as a key technology of the future, is funding its commercial development, and is coordinating interactions among representatives

petitive vigor in these application areas is correspondingly strong. Much of the investment in animal agriculture has been made by NBFs whereas much of the investment in plant agriculture has been made by major U.S. agrichemical companies.

In Japan, a competitive drive has been launched to enter international pharmaceutical markets. Furthermore, Japanese companies are world leaders in large-scale plant tissue culture, and MITI has identified secondary compound synthesis from plants as a major area for commercialization. Unlike the United States, Japanese companies appear to be dedicating a great deal of biotechnology R&D to specialty chemical production, an area where they are already internationally prominent.

To the extent that large companies in Europe began their commercialization efforts later than U.S. companies and may also lack the dynamism and flexibility to compete with the combined efforts of NBFs and established companies in the United States, European companies could initially be at a competitive disadvantage. The United Kingdom's major pharmaceutical companies are among the leading producers of biologically produced products, however, and their expertise in bioprocessing is impressive. Furthermore, the United Kingdom possesses some of the strongest basic research in interdisciplinary plant sciences. Whether or not the basic research will be commercialized successfully is difficult to predict.

U.S. competitive strength in biotechnology will be tested when large-scale production begins and bioprocessing problems are addressed. Pharmaceutical markets will be the first proving ground for U.S. competitive strength. The Japanese have extensive experience in bioprocess technology, and dozens of strong "old biotechnology" companies from several industrial sectors in Japan are using new biotechnology as a lever to enter profitable and expanding pharmaceutical markets. In addition to competing against Japanese companies, U.S. pharmaceutical and chemical companies will be competing against pharmaceutical and chemical companies of Western Europe, all of whom expect to recover their biotechnology investments through extensive international market penetration. There seem to be fewer European companies than Japanese companies strong in biotechnology now, but the competitive strength of European multinationals such as Hoechst (F.R.G.), Rhone Poulenc and Elf Aquitaine (France), ICI, Glaxo, and Wellcome (U.K.), and Hoffmann-La Roche (Switzerland) in the long run should not be underestimated.

Factors potentially important to international competitiveness in biotechnology

MOST IMPORTANT FACTORS

The three factors most important to the commercial development of biotechnology are financing and tax incentives for firms, government funding of basic and applied research, and personnel availability and training.

Financing and Tax Incentives for Firms.—The availability of venture capital to start new firms and tax incentives provided by the U.S. Government to encourage capital formation and stimulate R&D in the private sector are very important to development of biotechnology in the United States. Since 1976, private venture capital in the United States has funded the startup of more than 100 NBFs. Many of these firms have already obtained second- and third-round financing, while others, still seeking additional funds, are relying heavily on the currently strong stock market, R&D limited partnerships, and private placements to fund research, production scale-up, clinical trials, and early product development. Between March and July of 1983, 23 NBFs raised about $450 million. R&D limited partnerships in biotechnology are expected to total $500 million in 1983 and $1.5 billion by 1984. Corporate equity investment in NBFs, although now diminishing, has also been an important source of financing for the new firms. From 1977 to August 1983, corporate venture capital supplied over $350 million to NBFs in equity investments alone.

Current price/earnings ratios* for NBFs appear high, because most NBFs still have negative earn-

*A price/earnings ratio $\left(\frac{\text{market price per share}}{\text{company earnings per share}}\right)$ reflects the stock market's anticipation of the company's future performance based on the earnings per share.

set of techniques that constitute biotechnology, however, are potentially applicable to many industrial sectors.

Since the technologies are still emerging and most biotechnology products and processes are in early development, most of this report focuses on *potential* rather than actual products and processes. In the case of biotechnology, knowledge about market size, distribution systems, customers, production processes, and learning curve economies is lacking. Thus, traditional parameters of competitiveness are difficult or impossible to estimate. Instead of examining the classical measures of competitiveness, this analysis of international competitiveness in biotechnology examines the aggregate industrial activity in biotechnology in both domestic and foreign firms and 10 factors that *might* be influential in determining the competitive position of the United States and other countries with respect to the commercialization of biotechnology.

In investigating competitiveness in biotechnology, this report analyzes the commercialization efforts of five countries in addition to the United States: Japan, the Federal Republic of Germany, the United Kingdom, Switzerland, and France. Although companies from many countries will have biotechnology products in world markets, these five countries were selected because of their research capabilities in biology and their existing capabilities in old biotechnology and because, as a whole, their companies are most likely to reach world markets first with biotechnology-produced products. Japan leads the world both in the microbial production of amino acids and in large-scale plant cell culture, and it has a strong position in new antibiotic markets. Japan is also the world leader in traditional bioprocess engineering. Furthermore, the Ministry of International Trade and Industry (MITI) in Japan has designated biotechnology for industrial development. The European pharmaceutical houses, notably in the United Kingdom, France, the Federal Republic of Germany, and Switzerland, lead the world in pharmaceutical sales. Like Japan, three of these European countries, the Federal Republic of Germany, the United Kingdom, and France, have national plans for the promotion of biotechnology. The Federal Republic of Germany and the United Kingdom have good basic biology research and especially good bioprocess engineering research.

The first step in the analysis of international competitiveness in biotechnology was to consider the aggregate level of industrial activity and the number and kinds of firms commercializing biotechnology in the competitor countries. OTA's industrial analysis, presented in *Chapter 4: Firms Commercializing Biotechnology,* was approached from three perspectives:

- the number and kinds of companies commercializing biotechnology,
- the markets targeted by industrial biotechnology R&D, and
- the interrelationships among companies applying biotechnology and the overall organization of the commercial effort.

The analysis began with the United States and comparisons were then made with other countries.

The second step in providing an overall picture of competitiveness in biotechnology involved the evaluation of the following 10 factors identified as potentially important in determining the future position of the United States and other countries in the commercialization of biotechnology:

- financing and tax incentives for firms;
- government funding of basic and applied research;
- personnel availability and training;
- health, safety, and environmental regulation;
- intellectual property law;
- university/industry relationships;
- antitrust law;
- international technology transfer, investment, and trade;
- government targeting policies in biotechnology; and
- public perception.

The relative importance of each of the factors was first evaluated to determine their importance to competitiveness today (see fig. 2) and which ones could be important as the technology matures and more products reach the marketplace. Then, each of the factors was analyzed for each of the six competitor countries: the United States, Japan, the Federal Republic of Germany, the United

of specific products. Such research is aimed at the solution of general problems that are associated with the use of a technology by industry. Generic applied research areas in biotechnology, for instance, include development of bioreactors, screening of micro-organisms for potential products, and better understanding of the genetics and biochemistry of industrially important microorganisms. Support of basic science and of generic applied science is generally viewed as the responsibility of government, because it ultimately contributes to the public good and because it is high risk and too expensive for individual firms.

Controversy exists over the relative importance of national support of basic and applied science. Some argue that since the findings of basic research are readily accessible worldwide because they are published in journals with international distribution, strong government support for basic research is therefore not required for the maintenance of a leading position in the development of a technology. Others argue that the development of a technology within a country will progress faster if companies have access to local basic research scientists for consulting and contractual arrangements. Domestic technology transfer can help give industry a lead in innovation.

Of the competitor countries, the United States, both in absolute dollar amounts and in relative terms, has the largest commitment to basic research in biological sciences. Like the United States, the Federal Republic of Germany, the United Kingdom, and Switzerland have a strong basic science base. On the other hand, **the U.S. Government's commitment to generic applied research in biotechnology is relatively small.** The governments of Japan, the Federal Republic of Germany, and the United Kingdom fund a significant amount of generic applied science in biotechnology.

During the past few decades, the U.S. Government increased its commitment to basic biological sciences, although this commitment has decreased in the last few years. While the Government was increasing its commitment to basic science, there was a concomitant decrease in its commitment to generic applied fields such as bioprocess engineering and applied microbiology. The rationale for this policy has been that most applied science, regardless how general, is the responsibility of industry. This policy has contributed to a widening scientific gap between purely basic research funded by the U.S. Government and short-term, relatively product-specific applied research funded by private industry. **In fiscal year 1983, the Federal Government spent $511 million on basic biotechnology research* compared to $6.4 million on generic applied research in biotechnology. The relatively low level of U.S. Government funding for generic applied research in biotechnology may cause a bottleneck in this country's biotechnology commercialization efforts.**

The Japanese Government, in contrast, is devoting proportionately more public funding to the solution of generic applied science problems than to basic research. The pattern of funding in Japan may reflect a policy of placing a greater priority on generic applied research in lieu of basic research because the Japanese may rely on the United States and other countries to prove the early feasibility of new technologies for commercialization. This strategy worked well in the semiconductor industry, and Japan may very well attain a larger market share for biotechnology products than the United States because of its ability to rapidly apply results of basic research available from other countries.

Personnel Availability and Training.—Adequately trained scientific and technical personnel are vital to any country's industrial competitiveness in biotechnology. For the most part, countries with good science funding in a field also have a good supply of well-trained people in that field.

The commercial development of biotechnology will require several specific types of technical personnel. Especially important categories include specialists in rDNA and MAb technology such as molecular biologists and immunologists; specialists in scale-up and downstream processing such as microbiologists, biochemists, and bioprocess engineers; and specialists for all aspects of biotechnology such as enzymologists and cell culture

*From $20 million to $30 million of the $511 million may actually be generic applied research, because definitions of biotechnology differ among agencies.

The importance of established and new firms in the commercialization of biotechnology

U.S. and foreign efforts to develop and commercialize biotechnology differ substantially in character and structure. In the United States, two distinct sets of firms are pursuing commercial applications of biotechnology—NBFs and established companies. Because NBFs were founded specifically to exploit perceived research advantages, they are providing the United States with a commercial edge in the current research-intensive phase of biotechnology's development. Through their R&D efforts, NBFs are contributing to innovation, expansion of the U.S. research base, technology diffusion, and encouragement of technical advances through the increased domestic competition they create. All of these contributions provide the United States with a competitive advantage.

Although NBFs have assumed much of the risk for biotechnology's early development in the United States, established U.S. companies are making substantial contributions to the U.S. commercialization effort. Through equity investments and licensing and contract research agreements with NBFs, established U.S. companies are providing many NBFs with the necessary financial resources to remain solvent. Through joint development agreements with NBFs, many established companies will also provide the necessary production and marketing resources to bring many NBF products to world markets. These resources could help to sustain the rapid pace of technical advance spurred by NBFs. Recently, more and more established U.S. companies have been investing in their own research and production facilities, so the role of established companies in the U.S. biotechnology effort is expanding.

U.S. efforts to commercialize biotechnology are currently the strongest in the world. The strength of U.S. efforts is in part derived from the unique complementarity and competition that exists between NBFs and established U.S. companies in developing biotechnology for wider commercial application. At present, most NBFs are still specializing in research-oriented phases of development, precisely the commercial stage where they excel. The established companies, on the other hand, have assumed a major share of the responsibility for production and marketing of, and, when necessary, obtaining regulatory approval for, many of the earliest biotechnology products— the commercial stages where their resources are strongest. **Since established companies control the later stages of commercialization for many new products being developed through production and marketing agreements with NBFs, they will also have considerable control over the pace at which these new products reach the market. Whether the dynamism arising from the competition and complementarity between NBFs and established companies will continue giving the United States a comparative advantage in the context of product introduction remains unclear.** Some established companies, for example, might have disincentives to market new products because the new products might compete with products they already have on the market.

In Japan, the Federal Republic of Germany, the United Kingdom, Switzerland, and France, biotechnology is being commercialized almost exclusively by established companies. **The Japanese consider biotechnology to be the last major technological revolution of this century, and the commercialization of biotechnology is accelerating over a broad range of industries, many of which have extensive bioprocessing experience.** The general chemical and petroleum companies especially are leaning strongly toward biotechnology, and some of them are making rapid advances in R&D through their efforts to make biotechnology a key technology for the future. In Europe, large pharmaceutical and chemical companies, many of which already have significant strength in biologically produced product markets, are the major developers of biotechnology. Their inherent financial, production, and marketing strengths will be important factors as the technology continues to emerge internationally.

The commercial objectives of biotechnology R&D vary across national boundaries. In the United States, commercial research projects appear primarily focused on pharmaceutical and plant and animal agriculture, and American com-

Drug Administration has taken the position that rDNA products whose active ingredients are identical to ones already approved or to natural substances will still need to go through the new product approval process. However, data requirements may be modified and abbreviated. This appears not to be the situation in the competitor countries, although there have not been definitive pronouncements by their regulatory agencies.

Regulation may also influence where companies locate their production facilities. A country with liberal regulation may attract production facilities and, as a consequence, gain access to technology. Alternatively, companies may set up facilities in the United States and Japan regardless of regulation because of market size and as a way to avoid certain nontariff trade barriers on imports. NBFs may not have the capital to establish foreign subsidiaries in order to avoid regulatory barriers. Thus, they may be at a competitive disadvantage with respect to larger firms for entering world markets.

Countries wishing to market their products abroad will have to abide by the regulations of the countries to which they are exporting. Thus, countries can control access to their domestic markets by the regulations they impose. This is a form of nontariff trade barrier. These barriers are considered further in the discussion of trade policy.

Intellectual Property Law.—The ability to secure property interests in or otherwise protect processes, products, and knowhow will encourage development of biotechnology, because it provides incentives for a private company to invest the time and money for R&D. Without the ability to prevent competitors from taking the results of this effort, many new and risky R&D projects would not be undertaken. Thus, a strong intellectual property law system will enhance a country's competitiveness in biotechnology.

The areas of intellectual property law most relevant to biotechnology are those dealing with patents, trade secrets, and plant breeders' rights. These areas work together as a system; an invention may be protected by one or more of them, and if one has disadvantages, a company can look to another. Thus, to the extent that a country's intellectual property law provides several alternative ways for companies to protect biotechnological inventions, it is more likely to be competitive in biotechnology.

The patent laws of the competitor countries provide fairly broad protection for biotechnological inventions, but the laws differ to some degree in the types of inventions that are protected, the effect of publication on patent rights, and the requirements regarding public disclosure of the invention, which is the quid pro quo for the grant of the patent. The United States provides the widest coverage. Patents are available for living organisms (including plants and possibly animals), their products, their components, and methods for making or using all of these. In addition, patents can be granted on therapeutic and diagnostic methods. In the United Kingdom, the Federal Republic of Germany, France, Switzerland, and Japan, patent coverage is almost as broad, but patents are not permitted on plants and animals nor on therapeutic and diagnostic methods. In addition, Switzerland does not permit patents on micro-organisms. In Japan, the relatively strict guidelines governing rDNA research also may bar patents on those genetically manipulated organisms viewed as hazardous.

With regard to the effect of publication on patent rights, the United States also has a slight advantage over the other countries analyzed here. The four European countries do not permit a patent to be granted to an inventor who has disclosed his or her invention in a publication before the patent application is filed, assuming the disclosure enables others to make it. This absolute novelty requirement is viewed as impeding the free exchange of scientific information and possibly providing a disincentive for scientists to seek patent rights. The United States, on the other hand, provides a 1-year grace period between the date that an inventor publishes an article and the date on which the patent application must be filed. Japan provides a 6-month grace period for certain activities, such as presenting scientific papers. The U.S. advantage is limited, however, because when U.S. inventors wish to secure patents in other countries, they must refrain from publication in order to protect their patent rights in those countries.

ings records. Continued reliance on the stock market and R&D limited partnerships to raise funds will place increased pressure on the new firms to begin showing profits. If NBFs do not begin showing profits within the time frame expected by investors, additional financing from public offerings and R&D limited partnerships may be difficult to obtain.

The future performance of NBFs now extensively using the stock market and R&D limited partnerships for financing may influence the availability of financing for other firms seeking capital in the future. If some of these companies do not begin to manufacture soon in order to generate product revenues, investors may lose confidence in many of the firms' ability to commercialize biotechnology.

In the United States, venture capital is generally more difficult to obtain for later rounds of financing than for initial rounds, in part because venture capitalists are more eager to invest in the earlier rounds to maximize their investment returns. The difficulty in getting subsequent financing for production scale-up may prove to be an insurmountable problem for some NBFs; the ability to self-finance may still be 5 to 10 years away.

Of all the six competitor countries, the United States has the most favorable tax environment for capital formation and financing small firms. Tax incentives, more than government funding, are used in the United States to stimulate business and encourage R&D expenditures. Thus, R&D limited partnerships, low capital gains tax rates, R&D tax credits (due to expire in 1985), and subchapter S provisions all benefit small firms.

In Japan and the European competitor countries, venture capital has played a very minor role in the commercialization of biotechnology, because these countries do not have tax provisions that promote the formation of venture capital and investment in high-risk ventures. As a consequence, few NBFs exist outside the United States. Instead, established foreign companies have initiated efforts to commercialize biotechnology because they generally can finance R&D activities through retained earnings. Established companies also have access to financing from bank loans. Additionally, the governments of Japan, the United Kingdom, the Federal Republic of Germany, and France have provided the private sector with public funds for biotechnology.

After the United States, Japan has the most financing available for companies using biotechnology. **The Japanese Government has made the commercialization of biotechnology a national priority and is financing cooperative interindustry biotechnology projects.** Most of the established companies commercializing biotechnology in Japan have at least one bank as a major shareholder that provides the company with low-interest loans for R&D. Wealthy individual investors in Japan, although few in number, have also provided some risk capital for new ventures.

Tax incentives relevant to established companies commercializing biotechnology are those which stimulate R&D investments and those which encourage capital formation. Corporate tax rates are also important. For purposes of international comparisons, the most reliable basis is the overall effective corporate tax rate. Unlike statutory rates, the effective rate takes into account different definitions of taxable income and treatments of depreciation. Available studies suggest that Switzerland, followed by Japan and the United Kingdom, have the lowest effective corporate tax rates. The effective rates in the United States, the Federal Republic of Germany, and France are higher and about equal.

Government Funding of Basic and Applied Research.—The objective of basic research is to gain a better understanding of the fundamental aspects of phenomena without goals toward the development of specific products or processes. Such research is critical to maintaining the science base on which a technology rests and to stimulating advances in a technology. Basic research is usually conducted by academic researchers who receive government funds. The objective of applied research is to gain the knowledge needed to supply a recognized and specific need, through a product or process. Such research is usually funded by industry. Generic applied science can be viewed as bridging a gap between basic science done mostly in universities and applied, proprietary science done in industry for the development

the Japanese Government is implementing new policies to encourage closer ties between basic research scientists and industry. In the Federal Republic of Germany, the Federal Ministry of Science and Technology (BMFT, Bundesministerium für Forschung und Technologie) has a history of promoting close contact between academia and industry and is cosponsoring with industry many projects important to biotechnology. Switzerland encourages communication between individuals in academia and industry, and relationships are easy to maintain. The universities in both the United Kingdom and France have had very few ties with industry in biotechnology, but the governments of both countries have recently set up programs designed to encourage university/industry relationships.

Industrial funding for research in American universities is helping to promote the transfer of technology. However, the multimillion dollar arrangements that have characterized the initial relationships in biotechnology are most likely short term and will probably become less important as the firms develop in-house expertise and their research becomes more applied. **As in other fields, consulting and contractual research agreements are likely to predominate in university/industry relationships in biotechnology in the future.**

LEAST IMPORTANT FACTORS

The least important of the 10 factors analyzed were found to be antitrust law; international technology transfer, investment, and trade; government targeting policies in biotechnology; and public perception. Any of these factors, however, could become important as the technology develops and products reach the marketplace.

Antitrust Law.—Antitrust laws are based on the general economic assumption that competition among a country's industries will result in greater productivity, innovation, and general consumer benefits than will cooperation. Recently there has been much public debate about whether U.S. antitrust laws have, in fact, accomplished these goals in all cases and whether they place U.S. companies at a competitive disadvantage in the international marketplace when foreign companies face allegedly less restrictive antitrust laws.

The antitrust laws of the United States and the other major competitors in biotechnology are generally similar in that they prohibit restraint of trade and monopolization. However, the foreign laws generally provide for exemptions and vest much discretion with the enforcement authorities, especially in Japan. Thus, in practice, they are often less restrictive than in the United States. In addition, countries differ in the consequences to firms for failure to comply with antitrust laws. In the United States, the consequences of noncompliance can be more severe than in the competitor countries because private, in addition to Government, suits can be brought against alleged antitrust violators, and treble damages are assessed if a violation is found.

U.S. companies commercializing biotechnology face no major antitrust compliance problems, because the lack of concentration and the absence of measurable markets mean that most types of joint research arrangements would not be anticompetitive. Technology licensing agreements can raise antitrust concerns, but these generally are not unique to biotechnology. However, there is some degree of uncertainty about the scope and applicability of the antitrust laws to R&D joint ventures and licensing agreements. This uncertainty, plus the expense of litigation and the threat of treble damages, could deter some activities that might lead to innovation in biotechnology, thus limiting the ability of U.S. companies commercializing biotechnology to exploit their technology.* For these reasons, the current U.S. antitrust laws may have some modest adverse effect on biotechnology.

International Technology Transfer, Investment, and Trade.—Technology transfer across national boundaries can be promoted or inhibited by export control laws and by laws governing international joint ventures and technology licensing. Most export controls are directed at overseeing technology transfer for national security reasons, and the concept of national security is fairly narrowly interpreted in all of the competitor countries except the United States. Therefore export controls may not be very

*In addition, the rigid application of certain "per se rules" in the area of licensing may actually lead to anticompetitive results.

specialists. Scale-up personnel will become more important as companies using biotechnology move into production.

The United States currently has a competitive edge in the supply of molecular biologists and immunologists able to meet corporate needs, in part because the U.S. Government has provided substantial funding since World War II for basic life sciences research in U.S. universities. The supply of Ph. D. plant molecular biologists and scale-up personnel in the United States, however, may be inadequate. Like the United States, the United Kingdom and Switzerland have funded life sciences well and have a sufficient supply of basic biological scientists. Unlike the United States, Japan, the United Kingdom, and the Federal Republic of Germany maintained a steady supply of both industrial and government funding for generic applied microbiology and bioprocess engineering in the past few decades and have adequate personnel in these fields. In Japan and the Federal Republic of Germany, slight shortages of molecular biologists and immunologists exist; Japanese companies are seeking to train personnel abroad. France appears to have shortages in all types of personnel.

The training of personnel is important to the continuing commercialization of biotechnology. The United States has, for the most part, good training programs for basic scientists. Specialists in plant molecular biology may be in short supply now, but training in this discipline can be readily achieved with interdisciplinary programs in biology departments in universities. On the other hand, the United States does not have more than a handful of training programs for personnel in the more applied aspects of biotechnology, nor does it have Government programs, such as training grants, to support training in these fields. The training of bioprocess engineers and industrial microbiologists will require greater interdisciplinary cooperation between engineering and biology departments within universities.

The United States promotes and funds the training of foreign nationals in laboratories in the United States, yet funds very little training of Americans abroad. Foreign countries have many significant research programs in biotechnology that U.S. researchers could be visiting were funding available.

FACTORS OF MODERATE IMPORTANCE

The three factors found to be of moderate importance to international competitiveness in biotechnology are health, safety, and environmental regulation; intellectual property law; and university/industry relationships.

Health, Safety, and Environmental Regulation.—The analysis of the effect of health, safety, and environmental regulation on competitiveness in biotechnology was made by determining how restrictive a country's laws would be with respect to marketing biotechnology products and whether there were any uncertainties about their application. The analysis focused on the drug laws for humans and animals and, to a lesser extent, on laws governing the production of chemicals and the deliberate release of novel organisms into the environment. **In all the competitor countries, there is some uncertainty as to the environmental regulation governing the deliberate release into the environment of genetically manipulated organisms.**

The only government controls directed specifically toward biotechnology are the rDNA guidelines adopted by the six competitor countries. They are essentially voluntary and directed primarily at research. Their containment and oversight provisions have been substantially relaxed since they were originally adopted, and this trend is expected to continue. The United States has the most liberal guidelines, whereas Japan has the most stringent.

Since companies generally approach domestic markets first, the countries with the least stringent regulation may have products on the market earlier. Japan has the most stringent health and safety regulation for pharmaceuticals and animal drugs, followed by the United States. Switzerland appears to be the most liberal. Thus, **the regulatory environment favors the European companies over those of Japan and the United States reaching their own domestic markets sooner for pharmaceuticals and animal drugs.** In the United States, the Food and

lessen corporate duplication in biotechnology R&D. A variety of policy measures are used within each country. In Japan and West Germany, the Governments carry out their policies mostly through projects that combine the resources of the Government and private companies to meet specific objectives set by the Government. The United Kingdom and France have adopted a different approach; they support startup of small firms, which are expected to commercialize the results of Government-funded basic and applied research.

At this early stage, any evaluation of the eventual success of foreign targeting programs is preliminary. History has shown that even the best thought-out targeting policies do not guarantee competitive success. Whether targeting policies of foreign governments in biotechnology are superior to the U.S. Government policy of funding basic research in the life sciences and encouraging R&D in all industries with tax credits remains to be seen. Though targeting policies are not of great importance when compared to other competitive factors, they could tip the balance of a competitive position in the future.

Public Perception.—Public perception of the risks and benefits of biotechnology is of greater importance in countries with representative, democratic forms of government than it is in countries with other forms of government, simply because of the greater attention paid to public opinion in democracies and the independence of the media. Therefore, **public perception could influence commercialization of biotechnology in all of the countries examined here.** As a factor influencing competitiveness, however, public perception is probably of greater importance in the United States than in the other competitor countries. Historically, the American public has been more involved than the public in Japan or the European countries with issues pertaining to genetic research and technology (e.g., issues regarding the safety of rDNA research).

In all countries, the importance of public perception as a factor influencing competitiveness will be greatly increased in the event of an accident or perceived negative consequence of biotechnology. Particularly in such a case, the level of scientific and technological literacy in the various competitor countries becomes important, as judgments must be made concerning complex issues. In the United States, survey data show that only a small fraction of the public is fully informed about genetics in general and therefore, probably, about biotechnology in particular. Survey data also suggest that there is public apprehension concerning applied genetics. Thus, an accident associated with biotechnology could arouse strong public reaction in the United States, a reaction that might be greater than in the competitor countries.

Given the lack of public knowledge in the United States, it is particularly important that the media play a responsible role with respect to biotechnology. The role of the media already extends beyond mere reporting of the facts, by virtue of the events and issues the media elect to cover.

At the current time, public perception is not an important factor in the commercialization of biotechnology. However, the volatility of a potential public response must be noted. Were there to be an accident due to commercial biotechnology, the public's reaction could be extremely important to the future of biotechnology.

Other influences on competitiveness in biotechnology

Three other considerations that should be noted in evaluating competitive positions in the commercialization of biotechnology are, for each country, historical patterns of industrial commercialization, the availability of natural resources, and cultural attitudes toward risk-taking.

Historically, industries in some countries have moved research results into commercialization rapidly, while industries in other countries have moved more slowly. This observation is especially important in this analysis of biotechnology. For instance, the United Kingdom has a good science base, trained personnel, and industries that could be using these new technologies; however, the United Kingdom may not be a major contender in the commercialization of biotechnology mainly because it does not have a history of rapid commercialization. On the other hand, both the United States and Japan historically commercialize scientific advances rapidly.

The patent law requirement that an invention be described in sufficient detail so that it could be replicated creates unique problems for biological inventions. Since a living organism generally cannot be described in writing with sufficient specificity to allow others to make and use it, granting of patents on such organisms and methods of using them generally is contingent on their deposit in a public depository. However, these deposits, in effect, turn over the factory for making a product to one's competitors, unlike patents in other technologies. The four European countries, and particularly the Federal Republic of Germany, place restrictions on access to such deposits that may be advantageous for their inventors.

Most aspects of biotechnology lend themselves to protection as trade secrets, and owners of such technology may rely on trade secrets when patent rights are uncertain or when they judge trade secrecy to be more advantageous. All of the competitor countries protect trade secrets relating to biotechnology, but the Federal Republic of Germany and, to a lesser extent, Switzerland, provide the greatest degree of protection. Japan appears to provide the least degree of protection.

All of the competitor countries recognize property rights in new varieties of plants, but the United States provides the greatest degree of protection. Protection in the United States is most favorable because the plant breeder has the greatest number of options among which to choose in securing property rights for a new variety of plant, including pursuing a patent under the traditional patent laws.

In the final analysis, **the U.S. intellectual property system appears to offer the best protection for biotechnology of any system in the world,** thus providing the United States with a competitive advantage with regard to this factor. This advantage results from the fact that the system provides the widest choice of options for protecting biotechnological inventions, the broadest scope of coverage, and some of the best procedural safeguards.

University/Industry Relationships.—A factor that has moderate overall importance is the relationship that exists between universities and industries. Interest in the commercial potential of biotechnology has dramatically increased university/industry interactions, especially in the United States. Established U.S. and foreign companies have invested substantial amounts of money in U.S. universities doing work in biotechnology in order to gain a "window on the technology." Many university/industry agreements in biotechnology focus on research directed toward applications of biotechnology in a specific industrial sector, whereas other university/industry agreements are directed at many applications of biotechnology. **The various agreements in the United States appear to be working well, and fears concerning conflict of interest and commingling of Government and industry funds have diminished.**

The increase of industry funding of university research in the United States in several disciplines came at a time when Federal funding of science was decreasing in constant dollars. Although the infusion of industry funds to the U.S. universities has been substantial, it accounts for only a small fraction (less than 10 percent) of the total funding of university research. In some university departments, however, such as electrical engineering, chemistry, and possibly now molecular biology, industrial funding of university research may exceed 10 percent. Even with the increase in industrial support, industrialists agree that private funding can never replace Federal funding of basic science research if past and current levels of basic research are to continue.

University/industry interactions are a very effective way of transferring technology from a research laboratory to industry. Such interactions promote communication between industrialists and academicians, a two-way interaction that benefits both sides. Industrial scientists learn the latest techniques and research results, while academicians gain increased familiarity with challenges of industrial R&D.

Neither Japan nor the European competitor countries identified in this assessment have as many or as well-funded university/industry relationships as the United States does, but varying degrees of cooperation do exist. In Japan, the ties between university applied research departments and industry have always been close. Additionally,

Issues and options

Congressional issues and options for improving the competitive position of the United States in biotechnology are presented at the end of most of the chapters in part IV. To improve the competitive position of the United States, legislation could be directed toward any of the 10 factors OTA identified as influencing competitiveness, although coordinated legislation directed toward all of the factors might be more effective in promoting U.S. biotechnology efforts. The chapters in part IV discuss only those options that are specific to the development of biotechnology. Some of the options presented in part IV are limited and straightforward, such as some options concerning health and safety regulation and R&D limited partnerships. Other options are much broader with potentially large political, ethical, and financial considerations. Some examples of the latter include establishing university/industry cooperative research centers, regulating the deliberate release of genetically manipulated organisms into the environment, and changing patterns of research funding. Thus, the adoption of some options may occur more rapidly than others.

Policy options in some areas are not specific to biotechnology but apply to high technology or industry in general. These options are to:

- improve U.S. science and engineering education and the retraining of industrial personnel,
- change U.S. antitrust law to promote more research collaboration among domestic firms,
- regulate imports into the United States to protect domestic industries,
- regulate the transfer of technology from the United States to other countries, and
- target specific industries or technologies for Federal assistance.

There are many arguments for and against these options that are beyond the scope of this report. Because of their broad applicability to industry in general, these options are not discussed in part IV. It is important to note, however, that legislation in any of these areas could affect the development of biotechnology and potentially have a large influence on the U.S. competitive position.

important for the international development of biotechnology. However, the export controls of the United States, which are the most restrictive of the competitor countries, include the control of pharmaceuticals and of many micro-organisms that potentially could be used in biotechnology product production. **These controls may have a slightly adverse affect on the competitiveness of U.S. companies commercializing biotechnology because they could cause delays that result in sales' being lost to foreign competitors.** U.S. export control laws may need clarification as biotechnology products proceed to the marketplace because there is some uncertainty as to what products or data will be restricted. In addition, the current U.S. export control law expired in October 1983. While it is virtually certain that a new law will be passed, the form that law will take is still unclear.

The U.S. Government has no laws governing international joint ventures and technology licensing among U.S. and foreign companies. As a consequence, technology can be transferred readily to other countries. The predominance of NBFs in the United States and their need for capital has led to the formation of many transnational joint ventures involving NBFs. Because of this, the United States appears to be transferring more technology outside of its national borders than are other countries at the present time. However, as biotechnology products reach the market, foreign firms will probably set up subsidiaries in the United States in order to have access to U.S. markets. If this happens, the United States could become a net importer of technology.

In contrast with the United States, France and Japan have Government programs for the review of potential transnational agreements, but it is uncertain whether such programs help or hinder the transfer of technology into those countries. As of now, laws governing the transfer of technology are not very important to the U.S. competitive position in biotechnology. However, if other countries establish themselves more favorably in world markets, the current outward flow of technology from the United States may hurt the U.S. competitive position.

Foreign exchange and investment control laws help prevent access to domestic markets and technology by foreign firms. The United States has the fewest controls, whereas Japan and France have the most control mechanisms. Japanese controls exist in the form of nontariff barriers such as ministerial review and screening of foreign investments and licensing agreements with respect to a number of criteria ranging from national security to competition with other Japanese business. Ministries also have the power to designate specific companies for special controls on foreign ownership. In France, the Government has the ability to object or order alteration of licensing agreements and foreign investments. Foreign direct investment in certain domestic industries is not encouraged. Thus, **U.S. markets are the most accessible to foreign firms and therefore the most vulnerable to foreign competition,** whereas Japanese and French markets are the least accessible and the most protected against foreign competition.

Trade policy was assessed by examining the competitor countries' abilities to protect domestic industries from imports and to control foreign investment in domestic industries. **Trade policy is not important for the commercialization of biotechnology today because of the small number of products that have reached the market and because trade in biotechnologically produced products is not likely to raise any unique trade issues.** However, trade policy will become increasingly important as more products reach the marketplace, especially in the area of pharmaceuticals, where significant nontariff barriers, such as conforming to country standards with appropriate testing data, quality control standards, and packaging requirements exist. Problems with nontariff barriers are now being negotiatied with Japan and other countries including the European Economic Community, and it apears as though some trade barriers may become less stringent.

Government Targeting Policies in Biotechnology.—The governments of four of the competitor countries—Japan, the Federal Republic of Germany, the United Kingdom, and France—have instituted comprehensive programs to help domestic companies develop certain areas of biotechnology. The targeting policies are intended to reduce economic risk and

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Another historical consideration is the quantity of sales of specific products in a country. For example, Japan's per capita consumption of pharmaceuticals is significantly higher than that of the other competitor countries; therefore, Japan may have more interest than other countries have in applying biotechnology to the production of pharmaceuticals. In other words, cultural differences will probably play a role in determining the markets each country will attempt to dominate.

The absence or presence of certain natural resources may also determine how quickly a country moves into the commercialization of biotechnology. For instance, Japan does not have domestic petroleum resources. Because biomass can potentially replace petroleum as a feedstock in the chemical industry, Japan may be more interested in applying biotechnology in the chemical industry than a country, such as the United Kingdom, which has domestic petroleum resources. The United States, a country that produces excesses of grain each year, may find commercialization of processes that can use grain as a feedstock particularly attractive. However, it is too early to predict the degree to which natural resources will determine the commercial applications of biotechnology a country may undertake.

The United States, as a general rule, is not averse to risk-taking in business. Risk-taking is a part of the American lifestyle. European countries are more risk averse. Since investment in biotechnology is considered risky, countries that are more risk averse are less likely to move rapidly to commercialize biotechnology.

Conclusion

The unique complementarities between established and new firms, the well-developed science base, the availability of finances, and an entrepreneurial spirit have been important in giving the United States its present competitive advantage in the commercialization of biotechnology. In order to maintain this advantage, increased funding of research and training of personnel in basic and generic applied sciences, especially bioprocess engineering and industrial microbiology, may be necessary. The United States may also need to be concerned with the continued availability of finances for NBFs until they are self-supporting. On most of the other factors influencing competitiveness, the United States rates very favorably, although there are changes in laws and policies that could potentially improve or help maintain the U.S. competitive position. These changes include clarification and modification of particular aspects of intellectual property law; health, safety, and environmental regulation; antitrust law; and export control law.

Japan will be the most serious competitor of the United States in the commercialization of biotechnology. Japan has a very strong bioprocess technology base on which to build, and the Japanese Government has specified biotechnology as a national priority. The demonstrated ability of the Japanese to commercialize rapidly developments in technology will surely manifest itself in biotechnology.

The Federal Republic of Germany, the United Kingdom, Switzerland, and France lag behind the United States and Japan in the commercialization of biotechnology. The European countries generally do not promote risk-taking, either industrially or in their government policies. Additionally, they have many fewer companies commercializing biotechnology. Thus, **the European countries are not expected to be as strong general competitors in biotechnology as the United States and Japan.** In markets for specific products, including some pharmaceuticals, specialty chemicals, and animal agriculture products, however, some European companies will undoubtedly be strong international competitors.

need to know both plant physiology and molecular genetics. People working in microbial enhanced oil recovery need training in microbiology as applied to a specific geological environment.

The multidisciplinary nature of biotechnology has extensive implications for educational and industrial structures. To excel in biotechnology, universities will need to draw on the resources of several departments. Diversified companies may have an inherent advantage over other companies, because technologies perfected for the production of one product (e.g., a pharmaceutical product) can be modified and used for the production of another (e.g., a food additive).

Biotechnology in developing countries

One area where biotechnology could certainly have an impact, though not considered extensively in this report, is in developing countries. Plants that have been genetically manipulated for growth in tropical and desert climates could improve agricultural production. Vaccines that do not need refrigeration could have widespread influence on the health of the people and their livestock. Small local factories that convert biomass to ethanol could help solve the problem of costly petroleum imports for energy.

The applications of biotechnology to developing countries was discussed in a workshop held by the National Academy of Sciences and the U.S. Agency for International Development (1). The proceedings of this workshop include suggested priorities for research and time frames for development of various biotechnology products important to developing countries. Additionally, the United Nations Industrial Development Organization has proposed the construction of an international center for biotechnology (2). The proposed center would have 50 staff scientists, 26 postdoctoral fellows, and 100 visiting scientists; the annual budget would be $8.6 million; and the research would concentrate on problems specific to developing countries.

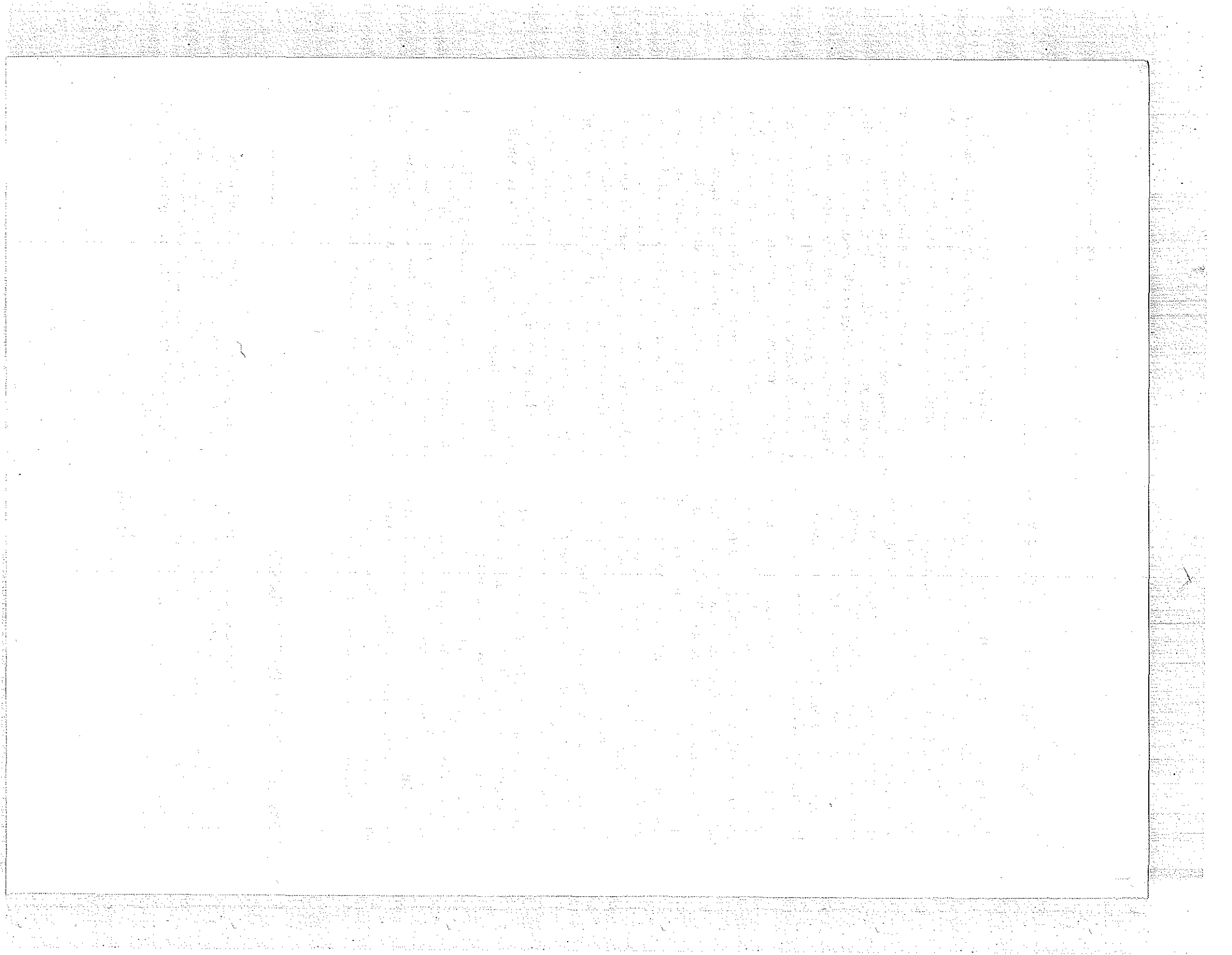
This report does not cover developing countries for two reasons. First, developing countries are not likely to compete with the United States for market shares in biotechnology in the near future. Second, all countries in a competitive position generally have equal access to markets in developing countries, allowing them equal access to international market shares. Some developing countries give preferential treatment to the first company to market a product in that country, but all countries have equal access for first introductions.

Local efforts to promote the development of biotechnology in the United States

Many State governments are actively promoting the establishment of local high-technology centers to stimulate the local economy, and many of these include centers for biotechnology. The oldest and best known of these is the North Carolina Biotechnology Center. This report does not analyze the development of these centers because they are analyzed in another OTA report, *Technology, Innovation, and Regional Economic Development,* due to be published in 1984. It is important to note, though, that it will take several years to recoup the costs of initiating one of these centers. Local biotechnology centers cannot be viewed as a short-term solution to economic problems.

Chapter 2

Introduction



Chapter 2

Introduction

This report assesses the international competitive position of the United States with respect to the development and commercialization of industrial applications of new biotechnology. New biotechnology is defined as the use of novel technologies—recombinant DNA (rDNA) technology, monoclonal antibody (MAb) technology, and new techniques used in bioprocess engineering—to develop commercial products and processes that use living systems.

Despite its rather narrow focus on new biotechnology, this report can be viewed as an introduction to the entire subject of biotechnology, a field that will become increasingly important in industrial production during the next few decades. Developments associated with new biotechnology could spur a renaissance in traditional biotechnology. The lure of profitability in new biotechnology, for instance, will very likely attract students to bioprocess engineering, and an increase in the number of engineers will probably improve bioprocess technologies applicable to the traditional uses of biotechnology. Another reason biotechnology may increase in importance is the movement, albeit not very rapid, toward the use of renewable resources. Diverse micro-organisms able to convert biomass into useful chemicals, some of which are a source of energy, are known, and these micro-organisms have yet to be exploited to the fullest extent. Furthermore, the industries that use traditional biotechnology are showing interest in the novel techniques mentioned above, and many of these industries will probably be using these techniques, because of their broad applicability, in some aspect of their operations in the future.

Impact of biotechnology on the research community

A point to be mentioned that does not relate directly to this report is the impact of the novel technologies, especially rDNA technology, on the biological research community. Recombinant DNA technology has already allowed a greatly increased understanding of the basis of life, and thus, of the genetic basis of disease. Research over the next 10 years may yield an increased understanding of the mechanism of carcinogenesis, genetic susceptibility to disease, the functioning of the immune system, the basis of debilitating diseases such as diabetes and arthritis, and some knowledge of brain function. Additionally, gene transplantation technology may reach a stage where some genetic diseases could be cured. It may be that the main benefit of the new biological technologies will be the advances in fundamental knowledge that accrue. Thus, even if no commercial products were to result from them, these technologies would still have a substantial impact on the quality of life.

The multidisciplinary nature of biotechnology

Biotechnology is unusual among most technologies in that it spans an array of scientific disciplines. Individuals seeking to be well versed in applications of biotechnology must have interdisciplinary training. Bioprocess engineers, for example, need some knowledge of biochemistry and microbiology as well as knowledge of engineering design so that the most efficient combination of micro-organism and bioreactor can be determined. Similarly, plant molecular biologists

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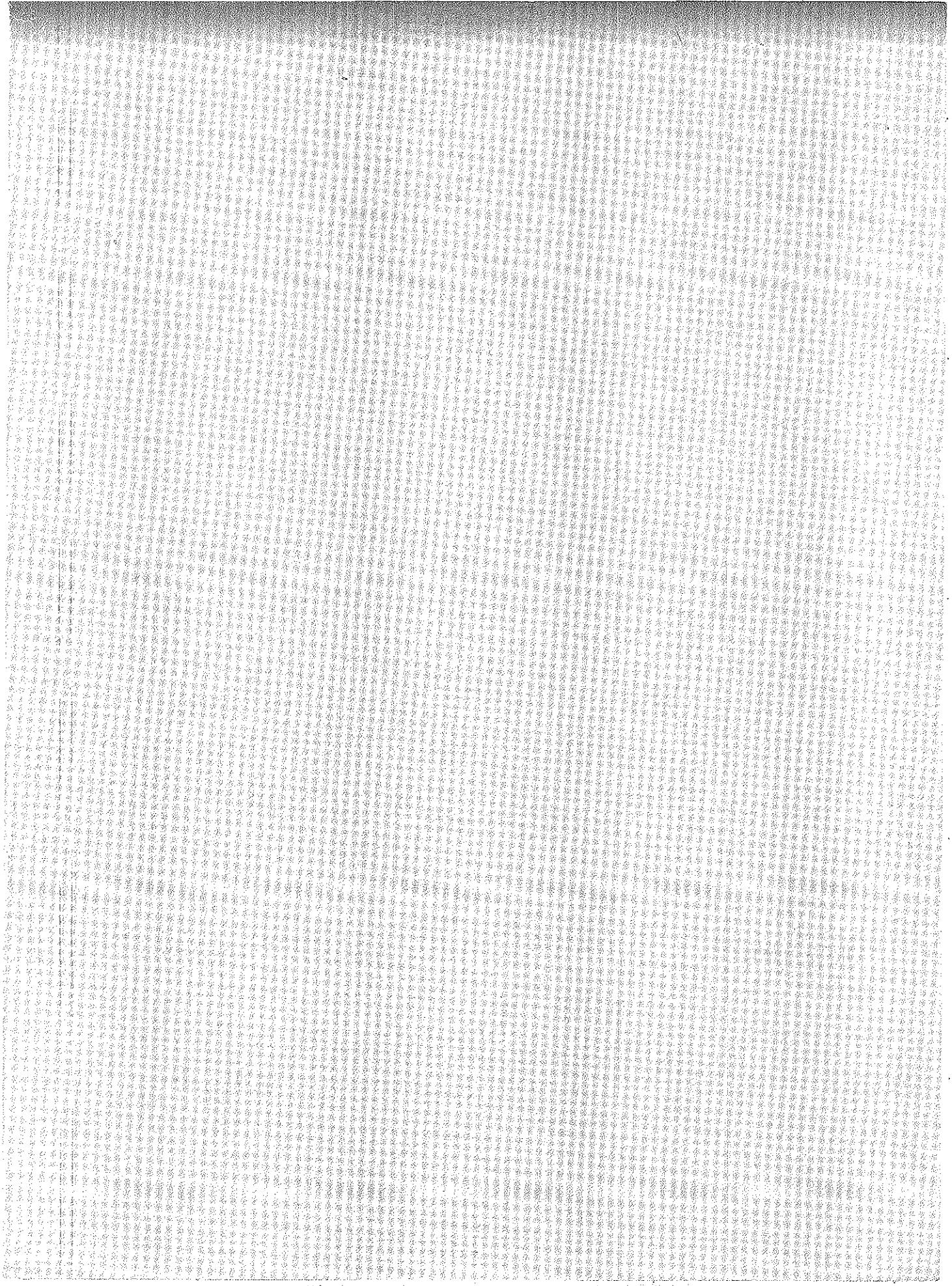

Organization of the report

This report is organized into four parts. Part I introduces the scientific background of the new technologies and forms a basis for discussion of the commercialization of new biotechnology. The three chapters consider the construction of rDNA, the formation of MAbs, and the relevant engineering principles for the large-scale growth of microorganisms and the use of immobilized enzymes to perform specific catalytic functions. Each emphasizes the industrial use of the technologies and identifies the problems yet to be solved.

Part II is an overview of the companies using biotechnology in the United States and its five major competitors in biotechnology: Japan, the Federal Republic of Germany, the United Kingdom, Switzerland, and France. The discussion considers the relative importance of and level of collaboration between established companies and new biotechnology firms in determining a competitive advantage. This part also includes a discussion of the firms producing the necessary reagents and equipment for the commercial use of biotechnology. Joint ventures among firms, both foreign and domestic, are analyzed.

How specific industrial sectors are applying biotechnology is the subject of the several chapters in Part III. The sectors discussed are pharmaceuticals, agriculture, specialty chemicals and food additives, environmental applications, commodity chemicals and energy, and bioelectronics. The order of the chapters corresponds to the approximate time frames for the development of products and processes in the various sectors—beginning with the sectors in which developments are likely to occur first. Priorities for future research to promote the development of biotechnology in each of the specific industrial sectors are outlined at the end of each chapter.

Part IV is an analysis of specific factors believed to influence a country's competitiveness in biotechnology. It considers only those factors that government policies could potentially affect. The first chapter of Part IV describes the framework used for the analysis. Subsequent chapters analyze specific factors, more or less in order of their importance: private sector financing and tax incentives, government funding of basic and applied research, personnel availability and training, health, safety, and environmental regulation, intellectual property law, university/industry relationships, antitrust law, international technology transfer and trade policy, targeting policies in biotechnology, and public perception. The analysis of the relative importance of each factor in determining a country's competitive position in biotechnology and where the United States stands is presented in *Chapter 1: Executive Summary*. Throughout Part IV, issues of congressional interest and a range of policy options are examined with respect to improving the U.S. competitive position in biotechnology.

This report is a follow-on study to OTA's April 1981 report entitled *Impacts of Applied Genetics: Micro-Organisms, Plants, and Animals* (3). Much useful information is contained in that report and is not repeated here. The reader is advised to read the earlier report for more information on the biological technologies and market forecasts.

Chapter 2 references

1. Board on Science and Technology for International Development, Office of International Affairs, National Research Council, *Priorities in Biotechnology Research for International Development: Proceedings of a Workshop* (Washington, D.C.: National Academy Press, 1982).
2. Newmark, P., "International Biotechnology: U.N. Center To Be Based in India," *Nature* 302:100, 1983.
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PART I

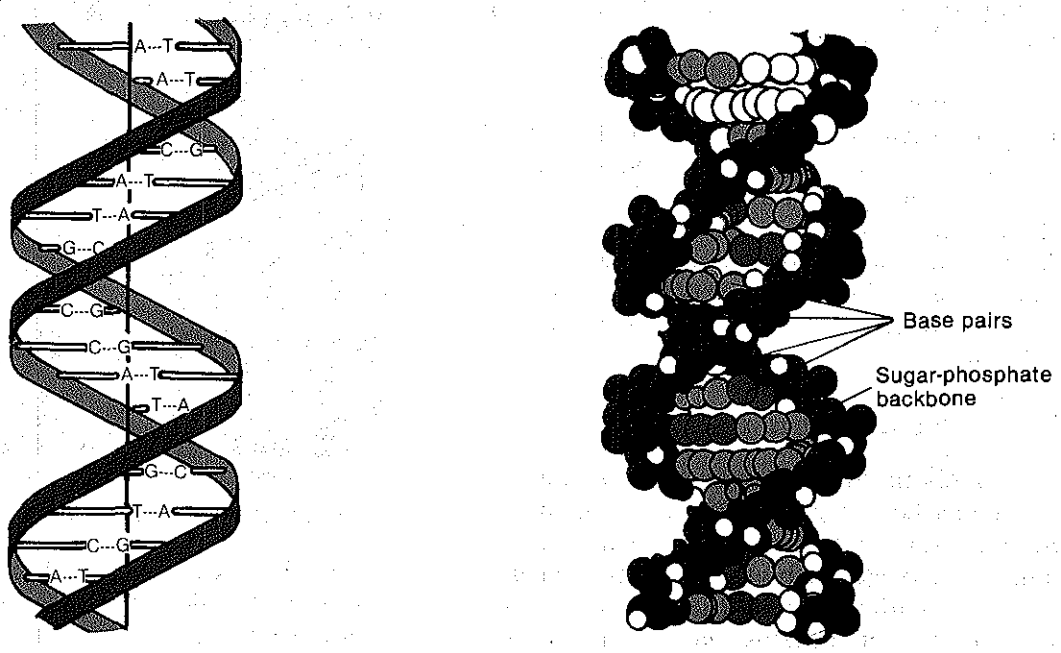
The Technologies

The mechanism by which DNA replicates is inherent in the structure of DNA itself. As can be seen from figure 3, the nucleotide bases are paired to form the rungs of the twisted DNA ladder. This pairing is absolutely specific: A always pairs with T and C always pairs with G. The pairing is accurate, but not very strong. Thus, in cell division, the DNA can "unzip" down the middle, leaving a series of unpaired bases on each chain. Each free chain can serve as a template for making a complementary chain, resulting in two identical DNA molecules, each a precise copy of the original molecule. Figure 4 illustrates the replication of DNA.

The DNA present in every cell of every living organism has the capacity to direct the functions of that cell. Gene expression, shown in figure 5, is the mechanism whereby the genetic directions in any particular cell are decoded and processed into the final functioning product, usually a protein. In the first step, called transcription, the DNA double helix is locally unzipped near the gene of interest, and an intermediate product, messenger RNA (mRNA), a single-stranded, linear sequence of nucleotide bases chemically very similar to DNA, is synthesized. The transcription process dictates the synthesis of mRNA that is complementary to the section of unzipped DNA in a manner that is somewhat similar to the replication of DNA. In the second step of gene expression, translation, the mRNA, after release from the DNA, becomes associated with the protein-synthesizing machinery of the cell, and the sequence of nucleotide bases in the mRNA is decoded and translated into a protein. The protein goes on to perform its particular function, and when the protein is no longer needed, the protein and the mRNA coding for that protein are degraded. This mechanism allows a cell to "fine tune" the quantity of its proteins while keeping its DNA in a very stable and intact form.

Proteins perform most of the necessary functions of a cell. By far the most diverse group of proteins is the enzymes, which are the proteins

Figure 3.—The Structure of DNA



A schematic diagram of the DNA double helix.

A three-dimensional representation of the DNA double helix.

The DNA molecule is a double helix composed of two chains. The sugar-phosphate backbones twist around the outside, with the paired bases on the inside serving to hold the chains together.

SOURCE: Office of Technology Assessment.

Chapter 3

The Technologies

in another will code for the same protein as it did in its native system, but its synthesis needs to be induced by the proper host signal. One of the great challenges of rDNA technology is to construct DNA molecules with signals that optimally control the expression of the gene in the new host.

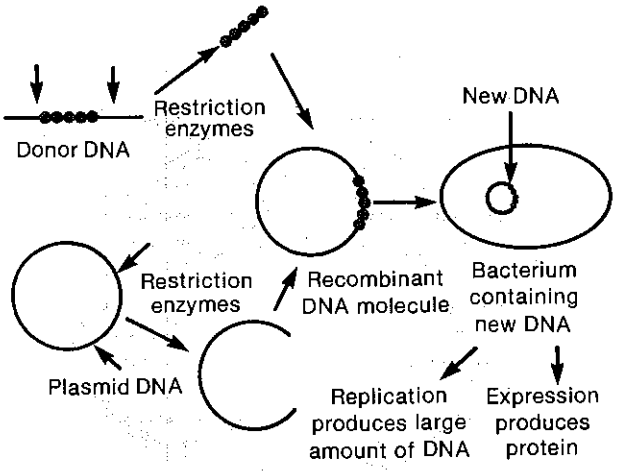
Preparing recombinant DNA

The amount of DNA present in each cell of a human (or most higher animals) is approximately 3 billion base pairs (2), and an average gene is about 1,000 base pairs, or about one millionth of the DNA. It is extremely difficult to study one gene in a million. Therefore, powerful tools have been developed to isolate genes of interest, place them in a foreign, simpler system, and replicate them many times to give a large amount of a single gene. The isolation of genes from higher organisms and their recombination in simple cells has already yielded a wealth of information, including insight into how genes determine the differences between different types of cells, how gene expression is regulated, and how genes may have evolved. For industrial uses, however, not only must the gene be cloned (reproduced), but that gene must also be expressed (the protein based on the gene be produced).

The basic technique of preparing rDNA is shown in figure 6. Preparations of restriction enzymes (enzymes that are made in certain bacteria and cut DNA at specific sites) are used to cut donor DNA (usually from a higher organism) into fragments, one of which contains the gene of interest. The resulting DNA fragments are then inserted into a DNA "vector," which is most often a plasmid.* Each plasmid vector will contain a different donor DNA fragment. These rDNA plasmids are introduced into host cells in a process called "transformation." Once inside the host cells, the rDNA plasmids replicate many times, thus providing many copies of each donor DNA fragment. Of the many bacteria transformed by plasmids containing donor DNA, only a few will contain the DNA fragment of interest. The desired

*A plasmid is a circular, double-stranded piece of DNA which replicates in cells apart from the chromosome.

Figure 6.—Recombinant DNA: The Technique of Recombining Genes From One Species With Those of Another



Restriction enzymes recognize certain sites along the DNA and can chemically cut the DNA at those sites. This makes it possible to remove selected genes from donor DNA molecules and insert them into plasmid DNA molecules to form the recombinant DNA. This recombinant DNA can then be cloned in its bacterial host and large amounts of a desired protein can be produced.

SOURCE: Office of Technology Assessment.

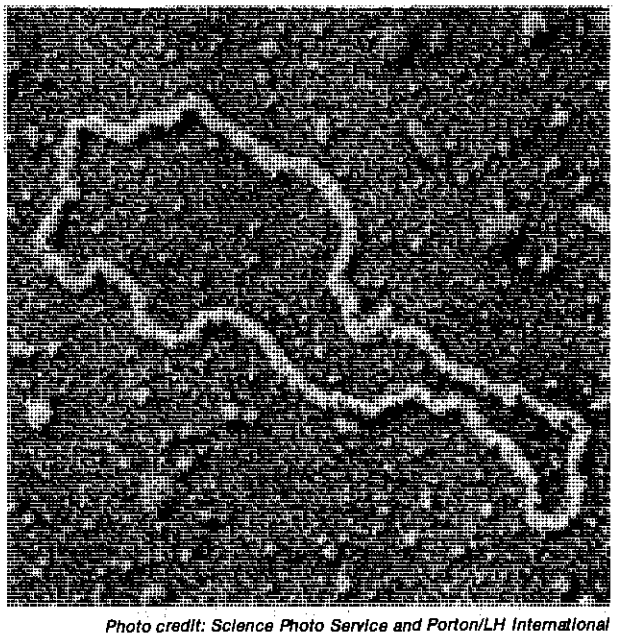


Photo credit: Science Photo Service and Porton/LH International

Bacterial plasmid

Chapter 3

The Technologies

Introduction

This chapter reviews the scientific bases for the technologies discussed in this assessment. The most publicized and broadly applicable of these technologies is recombinant DNA (rDNA) technology, which includes gene cloning, and is explained first. The second technology discussed is monoclonal antibody (MAb), or hybridoma, technology. This technology, used to prepare complex molecules known as MAbs which can be used to recognize or bind a large variety of molecules, has an expanding number of applications. The last technology discussed, bioprocess technology, allows the scaling-up of a biological production process so that large quantities of a product can be made. Bioprocess technology is, in many respects, the most difficult and least understood of the technologies, so it receives a more intensive discussion in this chapter. Because of the lack in the United States of broadly applicable knowledge in bioprocess engineering, the section on bioprocess technology also ends with priorities for future research, giving a focus to where Federal research funds might best be spent.

Recombinant DNA technology

The development of rDNA technology—the joining of DNA from different organisms for a specific purpose—has allowed a greatly increased understanding of the genetic and molecular basis of life. This technology has also led to the founding of many industrial ventures that are addressing the production of numerous compounds ranging from pharmaceuticals to commodity chemicals. This section introduces some aspects of the scientific basis of rDNA technology, discusses methods that are used to construct rDNA, and notes several additional features of the commercial use of rDNA technology.

Structure and function of DNA

Throughout the spectrum of life, the traits characteristic of a given species are maintained and passed on to future generations, preserved simply and elegantly by the information system contained within DNA. DNA can be thought of as a library that contains the complete plan for an organism. If the plan were for a human, the library would contain 3,000 volumes of 1,000 pages each. Each page would represent one gene, or a unit of heredity, and be specified by 1,000 letters. As shown in figure 3, DNA, a double-stranded, helical molecule, is composed, in part, of four nucleotide bases—adenine (A), cytosine (C), guanine (G), and thymine (T)—which are the letters of the chemical language. A gene is an ordered sequence of these letters, and each gene contains the information for the composition of a particular protein and the necessary signals for the production of that protein.

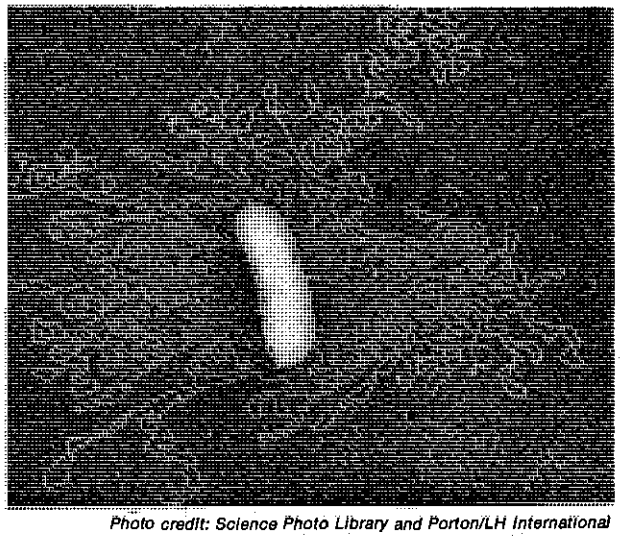


Photo credit: Science Photo Library and Porton/LH International

The DNA of the bacterium *Escherichia coli*

then be "switched on" by using host-regulated controls (1). Moreover, it is possible to alter specifically the regulatory sequences so that the gene is expressed at higher levels or so that its expression is more readily controllable in an industrial situation (3).

The purification of a protein from an industrial bioprocess is greatly simplified if the protein is secreted from the cell into the growth medium. If the protein is secreted, it does not have to be purified away from all the other cellular components. It is possible to attach additional regulatory signals to the vector DNA that direct the cell to secrete the protein and, thus, simplify its purification. The successful development of methods to enhance gene expression and product function and secretion will undoubtedly enhance the commercial applicability of rDNA technology.

The computer-aided design of proteins is another technology that will expand the use of rDNA molecules industrially. In the past, enzymes were modified by mutagenizing the host cell and then selecting or screening for mutants that contained an altered enzyme. Now, through use of the techniques of X-ray crystallography, protein sequencing, and computer modeling, the amino acid sequence and three-dimensional structure of the protein can be determined and amino acid changes that should bring about altered enzyme properties can be selected. The DNA sequence of the cloned gene for an enzyme can then be modified to incorporate the amino acid changes. Specific gene modification is made possible because of technical advances resulting in rapid and inexpensive synthesis of small DNA segments that can be used to change specific base pairs in a DNA sequence. Near-term protein modification experiments could result in enzymes with increased temperature and pH stability. Longer term experiments could define the structure of active sites of enzymes to be used for specific catalytic functions.

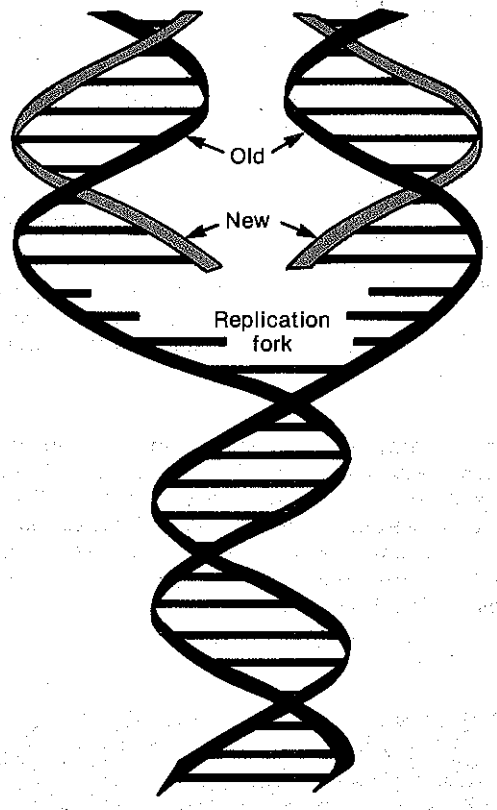
Monoclonal antibody technology

The production of antibodies in higher animals is one aspect of a complex series of events called the immune response. Specialized cells called B lymphocytes, present in the spleen, lymph nodes, and blood, recognize substances foreign to the body, or antigens, and respond by producing antibodies that specifically recognize and bind to those antigens. Any given B lymphocyte can recognize only one antigen. Thus, when a B lymphocyte meets and recognizes an antigen for the first time, the B lymphocyte is stimulated and becomes committed to producing a single type of antibody for the duration of its life. The end result of this aspect of an immune response is the antigen's removal from the body.

Antibodies bind to antigens and carry out their functions by virtue of the antibody's unique structure. All antibodies are comprised of four protein chains in a precise orientation, as shown in figure 7. One end of the antibody (the constant, or effector region) is nearly identical among antibodies. This effector region is associated with functions such as the secretion of antibodies from the B lymphocyte and "signaling" to the immune system after the antibody binds with the target antigen. The other end of the antibody, the variable region, contains the site that recognizes and binds to a particular antigen, and the structure of this end varies greatly from antibody to antibody to accommodate a wide range of antigens.

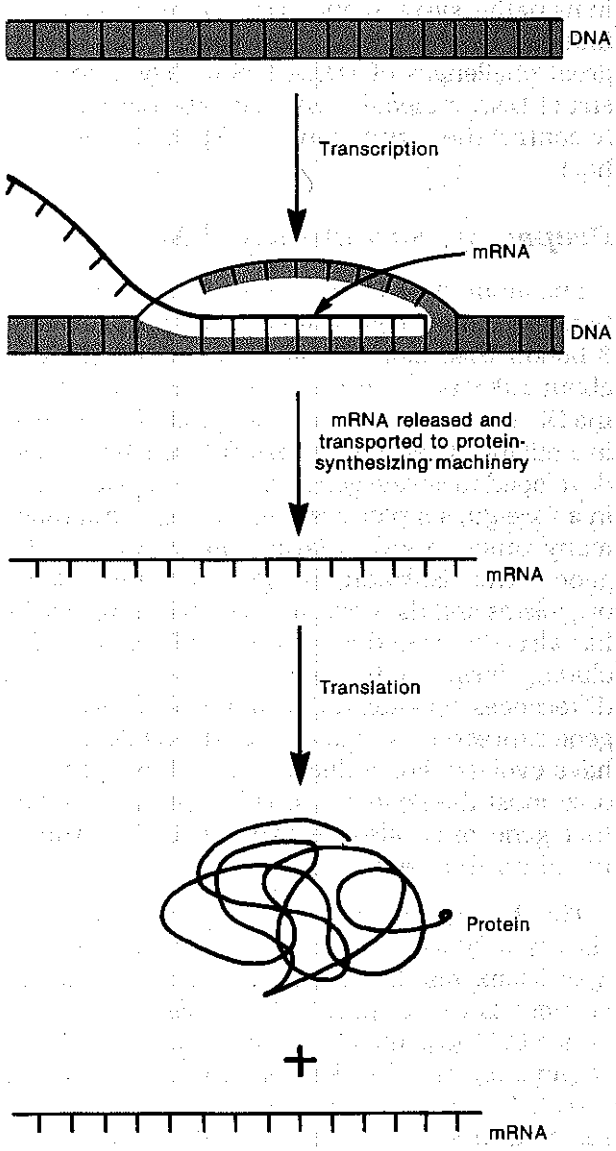
Apart from their natural functions in the protection of organisms via the immune response, antibodies have long been important tools for researchers and clinicians, who use an antibody's specificity to identify particular molecules or cells and to separate them from mixtures. Antibodies also have a major role in diagnosis of a wide variety of diseases. Antibodies that recognize known antigens are used to detect the presence and level of drugs, bacterial and viral products, hormones, and even other antibodies in sensitive assays of blood samples.

Figure 4.—The Replication of DNA



When DNA replicates, the original strands unwind and serve as templates for the building of new complementary strands. The daughter molecules are exact copies of the parent, with each having one of the parent strands

Figure 5.—Mechanism of Gene Expression



SOURCE: Office of Technology Assessment.

that catalyze biological reactions. Another group is the structural proteins, which are found, for instance, in cell membranes. Other proteins have regulatory functions; these include some hormones. Still others have highly specialized functions (hemoglobin, for example, carries oxygen from the lungs to the rest of the tissues).

The code by which genetic information is translated into proteins is the same for all organisms. Thus, because all organisms contain DNA and all organisms interpret that DNA in the same manner, all organisms, in essence, are related. It is this concept that forms the basis for the industrial use of DNA. In nearly every instance, a production process using rDNA technology depends on the expression of DNA from one species in another species. Only a universal genetic code would allow DNA to be used in this manner.

Despite the existence of a universal genetic code, regulatory signals indicating starts and stops of genes are known to vary among species. Thus, a gene removed from one organism and placed

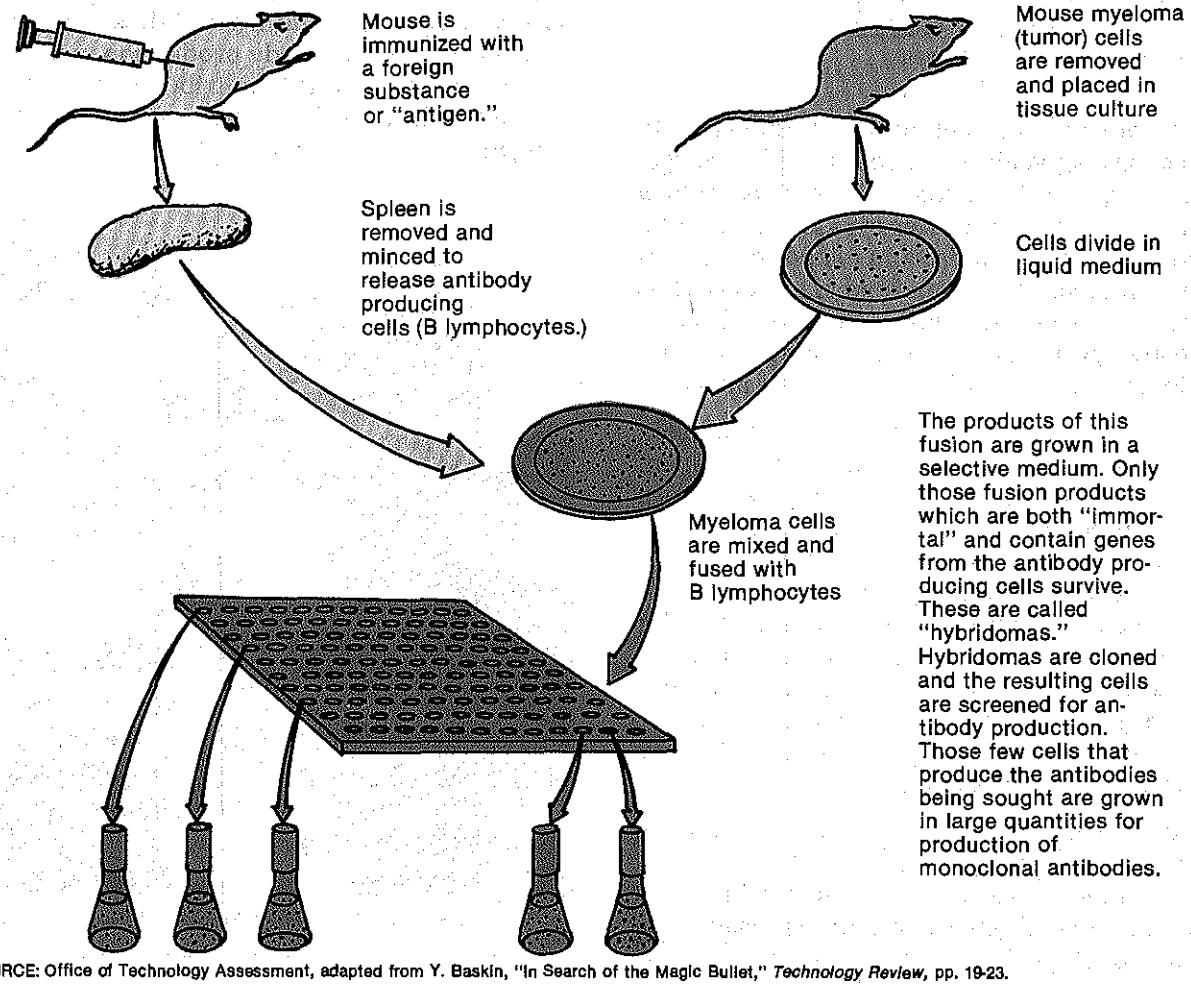
By using the method of hybridoma, or MAb, technology, it is now possible to "immortalize" individual antibody-producing cells by fusion with tissue culture-adapted myeloma tumor cells in the laboratory (4,5,8,13,22,25).

Preparing monoclonal antibodies

The method used to prepare MAbs is summarized in figure 8. The purified antigen of choice is injected into a mouse, and a few weeks later, the spleen of the mouse is removed. The B lymphocytes (antibody-producing cells) are isolated from the spleen and fused with myeloma cells. The resulting cells are placed in a cell culture medium that allows only the hybridomas to grow. The many hybridomas that result are cloned, and each clone is tested for the production of the antibody desired. A particular hybridoma clone either may be established in an in vitro culture system or may be injected into mice, where the hybridoma grows in abdominal cavity fluid (ascites) from which the antibodies are readily collected.

This method allows the preparation of large quantities of highly specific MAbs against almost any available antigen. The antibodies produced by MAb technology are homogeneous, and their production is predictable and repeatable, as compared to polyclonal antibodies produced with conventional immunological methods.

Figure 8.—Preparation of Monoclonal Antibodies



SOURCE: Office of Technology Assessment, adapted from Y. Baskin, "In Search of the Magic Bullet," *Technology Review*, pp. 19-23.

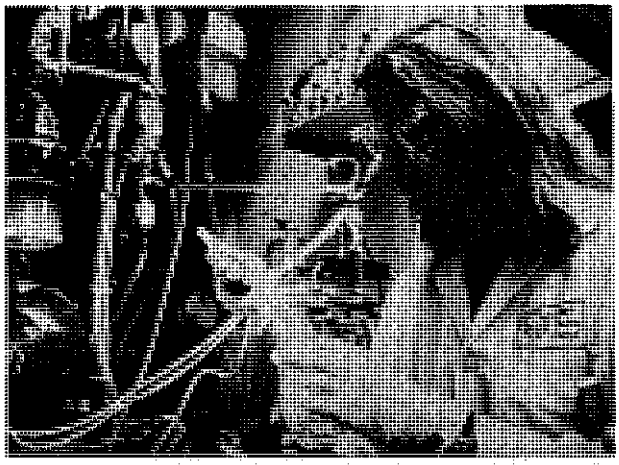


Photo credit: Science Photo Service and Porton/LH International

Molecular biologist in laboratory

gene is located among the vast number of bacteria containing plasmids with a suitable probe.* Vectors other than plasmids can be used for cloning DNA. One method uses the DNA of viruses, and another uses cosmids, artificially constructed hybrids of plasmids and viruses. Another method uses transposable elements, fragments of DNA that can insert themselves into the host cell's chromosomes.

All rDNA methods require the following:

- a suitable vector that is taken up by the host, is capable of autonomous replication, and, during the process, replicates the segment of donor DNA faithfully;
- an adequate selection system for distinguishing among cells that have, or have not, received rDNA; and
- an appropriate probe for detecting the particular DNA sequence in question.

The most difficult part of the cloning process is isolating an appropriate probe. The genes that were first cloned were those that, in certain cells, produced large quantities of relatively pure mRNA. Since the mRNA was complementary to the gene of interest, the mRNA could be used as a probe. This method severely limited the number of genes that could be cloned, however, because most genes do not produce large amounts of mRNA. More recently, a different technique has been used that allows a much greater diversity of genes to be cloned. If the amino acid sequence of a protein is known, then, working backwards through the gene expression scheme, the nucleotide base sequence can be determined. Because of the advent of automated DNA synthesizers, a portion of DNA can be synthesized that is complementary to the gene. This piece of DNA can then be used as a probe. Thus, if enough of a particular protein can be isolated and sequenced, its corresponding gene can be cloned.

At present, rDNA is grown principally in simple micro-organisms such as bacteria and yeast. Yeasts, in addition to bacteria, are being used as hosts for rDNA cloning because they more closely resemble cells of higher organisms. Yeasts perform functions similar to those of higher eukaryotic cells. These functions include adding sugar groups to some proteins. For the function of many proteins, these sugar groups are essential. Recently, scientists have learned how to introduce novel genetic material into higher plants and animals. The special techniques that pertain to cloning DNA in plants are discussed in *Chapter 6: Agriculture*.

Recombinant DNA technology in industrial processes

The commercial use of rDNA technology has several features in addition to those just discussed. In order to produce a product or improve a process, the cloned gene must be expressed to give a functional product. Since the signals that regulate gene expression vary from species to species, achieving the expression of a gene in a foreign cell may be difficult. The commercial development of biotechnology is highly dependent on the ability to achieve gene expression, for it is proteins (or their metabolites) that either are the marketable products themselves or establish the cellular environment necessary for performing such practical tasks as degrading toxic wastes or increasing the efficiency of photosynthesis. To a large extent, the problem of gene expression has been addressed through the manipulation of the adjacent vector DNA so that it contains the host's regulatory sequences. The cloned gene can

*A probe is a sequence of DNA that has the same sequence as the desired gene and has been prepared in such a way that it can be identified after it base pairs with that gene.

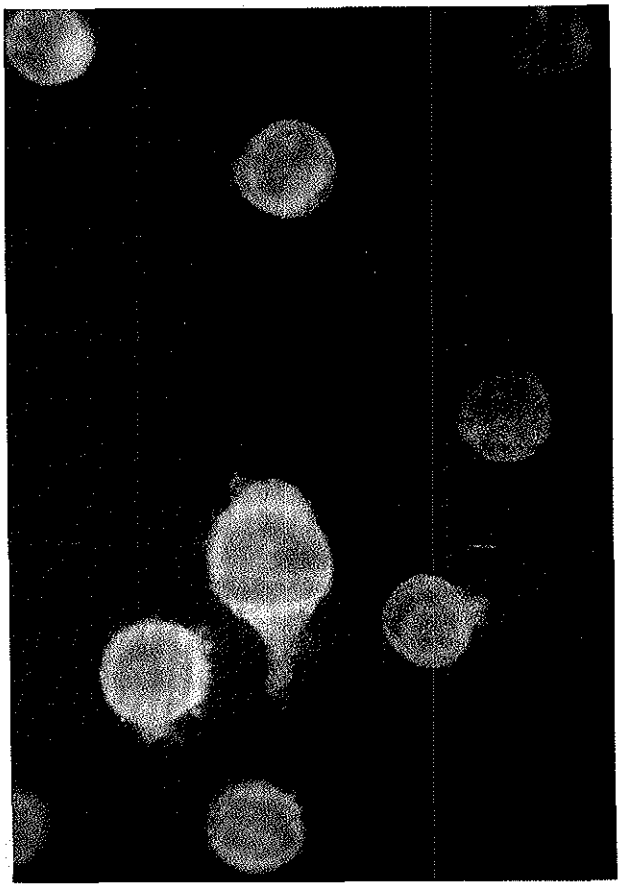


Photo credit: Science Photo Library and Porton/LH International

In vitro identification of specific cells using fluorescently labeled monoclonal antibodies

hybridoma preparation (9,17,23,27). Successful fusions apparently result from using these cell lines (6).

Monoclonal antibodies and recombinant DNA technology

The combination of MAb technology and rDNA technology offers intriguing possibilities for further technological exploration. Recombinant DNA techniques could be used to produce portions of antibody molecules in bacteria to circumvent some of the problems (e.g., hybridoma instability) associated with MAb production in mice or tissue culture. Additionally, these MAbs would be free of impurities, such as viruses, found in animal cells and possibly could be produced in large amounts with a concurrent savings in cost.

The first cloning and expression of a complete antibody molecule in a bacterial system was announced recently by the U.S. firm Genentech and the City of Hope Medical Center and Research Institute (19). The protein chains were expressed separately in bacteria and reconstituted by the researchers. The pharmaceutical applications of bacterially produced antibody genes will be limited. Antibody molecules must be modified by the cell to function in most diagnostic and therapeutic applications. Bacteria do not perform the modifications necessary for proper function. However, it may be possible to clone the antibody genes in a cellular system such as yeast where the proper modifications can be made.

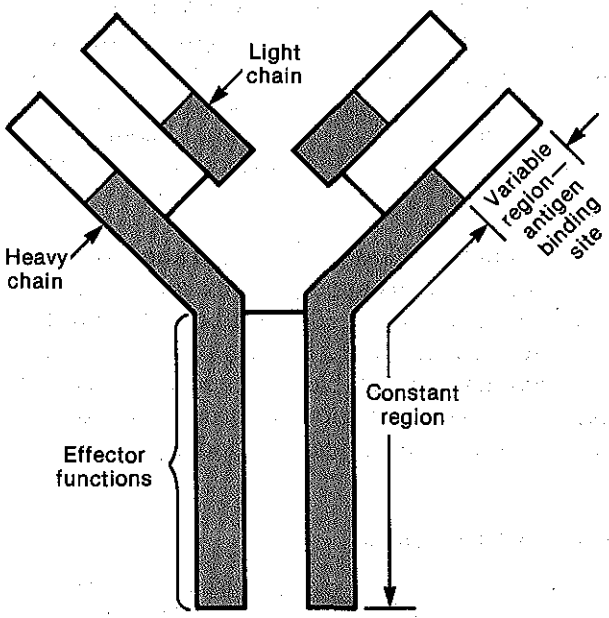
The production of MAbs in rDNA systems may prove useful for making reagents used in industrial applications where only the antigen-binding function may be necessary. With genes cloned for the antigen-binding regions of the antibody, portions of MAbs may be produced more economically in bacterial rDNA systems than in a large-scale mouse ascites or cell culture protocols.

Large-scale production of monoclonal antibodies

Although MAbs can be produced by several methods, manufacturers primarily use mouse ascites to produce the modest amounts of MAbs needed to service current diagnostic and research markets. As applications for MAbs to human therapy are developed, the need for larger quantities of MAbs (free from mouse-derived contaminants that might cause allergic reactions) may encourage a switch to the use of large-scale cell culture to produce MAbs. If MAbs are to be used in industrial applications (e.g., in the purification of proteins), production methods will be needed to produce even larger quantities of antibodies. In these cases, efficient cell culture or microbial bioprocess techniques will probably be necessary to provide enough antibodies to fill these needs.

Improved, more controllable cell culture systems will be needed for the production of MAbs in the future. A crucial need for large-scale cell culture is either the isolation of hybridoma cell lines that attach to surfaces or the use of techniques for immobilizing cells on a solid matrix.

Figure 7.—Structure of an Antibody Molecule



SOURCE: Office of Technology Assessment.

The conventional method of producing antibodies for diagnostic, therapeutic, and investigational purposes is to inject an antigen into a laboratory animal and, after evoking an immune response, to collect antiserum (blood serum containing antibodies) from the animal. Although this method has been and continues to be widely used, there are several problems associated with conventional antibody technology. These include:

- minor contamination of the injected antigen with other molecules, so that the antiserum collected from the animal contains a mixture of antibodies against both the target antigen and the contaminating molecules;
- heterogeneous populations of antibodies with concomitant differences in activity, affinity for the antigen, and biological functions, especially when a number of different animals are used to prepare the antiserum; and
- the limited supply of quality antisera for any given purpose (10,28,32).

Since these difficulties are almost unavoidable in standard antibody preparations, the standardization of immunoassays and the accumulation of large amounts of reference antisera have been difficult. Such problems, although time-consuming and expensive, have not prevented the effective use of antibodies as diagnostic, therapeutic, and investigational tools for both research scientists and clinicians, but the search for new methods for continual production of large amounts of pure antibodies has continued.

By what Cesar Milstein calls a "lucky circumstance," he and Georges Köhler began experimenting with the well-established technique of cell fusion in myeloma (antibody-producing tumor) cells adapted for cell culture. Milstein and Köhler fused myeloma cells with antibody-producing spleen B lymphocytes from mice that had been immunized with sheep red blood cells (SRBCs), and they found that some of the resulting hybrid cells, called hybridomas, secreted large amounts of homogeneous (monoclonal) antibodies directed against SRBCs (20,21). The myeloma parent cell conferred on the hybridoma the ability to grow permanently in cell culture and thus to support almost unlimited antibody production, while the B lymphocyte parent contributed the genes coding for the specific antibody against an SRBC antigen.

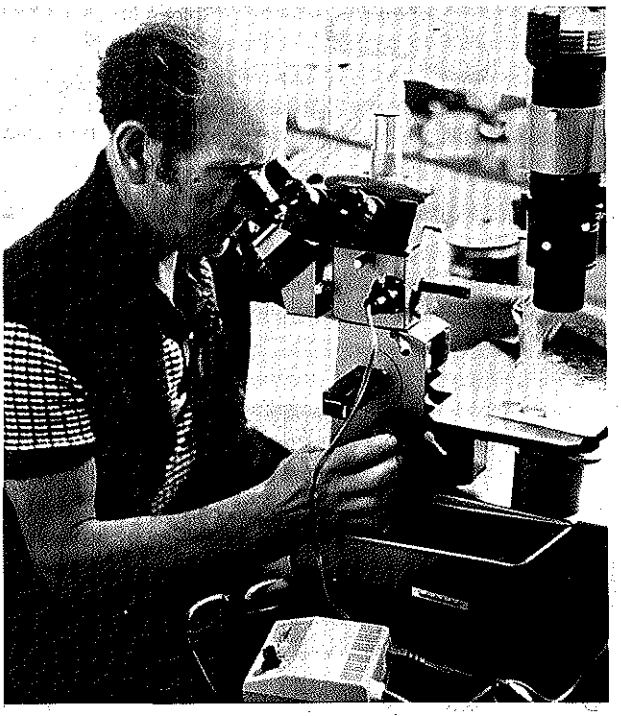


Photo credit: Science Photo Service and Porton/LH International

Dr. Cesar Milstein, discoverer of monoclonal antibodies

Bioprocess technology*

Bioprocesses are systems in which complete living cells or their components (e.g., enzymes, chloroplasts, etc.) are used to effect desired physical or chemical changes.** Since the dawn of civilization, bioprocesses have been used to produce alcoholic beverages and fermented foods. Until the 19th century, alcoholic fermentation and baker's yeast production were carried out in the home or as local cottage industries. As industrialization occurred, both these bioprocesses moved into factories.

Although other minor products made with bioprocesses were added over the years, bioprocesses did not become significant in the overall spectrum of chemical technology in the United States until the introduction of commercial acetone and butanol production during and after World War I. Somewhat later, large-scale microbial production of citric acid was introduced, and by the beginning of World War II, the U.S. bioprocess industry was thriving, with solvent alcohols and related low molecular weight compounds comprising the bulk of bioprocess production. The rapid growth of the petrochemical industry during World War II caused the displacement of microbial production of industrial solvents, however, and by 1950, microbial production of such solvents (including nonbeverage alcohol) had virtually disappeared in the United States.

This contraction of bioprocess manufacturing might have been the death-knell for old biotechnology had it not been for the introduction of, and the proliferation of markets for, antibiotics during the 1940's. The unique qualifications of biological processes for the synthesis of complex molecules such as antibiotics rapidly became apparent. Microbial production of a number of vitamins and enzymes was initiated at about this time, although only on a small scale. Thus, in the decade from 1940 to 1950, there occurred a complete transformation of industrial bioprocesses. Production of high-volume, low-value-added industrial chemicals (e.g., acetone, butanol) by anaerobic processes employing primarily yeasts and bacteria was largely replaced by more modest-scale production of high-value-added products (e.g., pharmaceuticals, vitamins, enzymes) made by highly aerobic processes in a variety of less familiar bacteria (e.g., the actinomycetes) and some fungi (see table 1). These aerobic processes are generally quite vulnerable to contamination by other micro-organisms and require much closer control of process conditions. Such aerobic processes continue to be used in industry today.

The advent of new biotechnology has sparked renewed interest in the industrial use of bioprocesses. The discussion that follows examines the dependence of new biotechnology, including rDNA and MAb technology, upon bioprocess technologies. Two aspects of the interrelationship between new genetic technologies and bioprocess technologies are emphasized:

- the engineering problems unique to genetically modified organisms, and
- the ways in which genetically modified organisms or parts of organisms may be used to enhance the efficiency and usefulness of bioprocesses.

In order to be viable in any specific industrial context, bioprocesses must offer advantages over

Table 1.—Volume and Value of Biotechnology Products

Category	Examples
High volume, low value ...	Methane, ethanol, animal feed, waste treatment
High volume, intermediate value	Amino and organic acids, food products, polymers
Low volume, high value ...	Pharmaceuticals, enzymes, vitamins

SOURCE: Office of Technology Assessment, adapted from A. T. Bull, G. Holt, and M. D. Lilly, *Biotechnology: International Trends and Perspectives* (Paris: Organisation for Economic Co-Operation and Development, 1982).

*This section is based largely on a contract report prepared for the Office of Technology Assessment by Elmer Gaden, University of Virginia. The information in that report was extensively reviewed and added to by James Bailey, California Institute of Technology; Harvey Blanch, University of California, Berkeley; and Charles Cooney, Massachusetts Institute of Technology.

**The term bioprocess is used here in preference to the more familiar term "fermentation" because it more correctly identifies the broad range of techniques discussed. A fermentation process, though often used to denote any bioprocess, strictly speaking refers only to an anaerobic bioprocess.

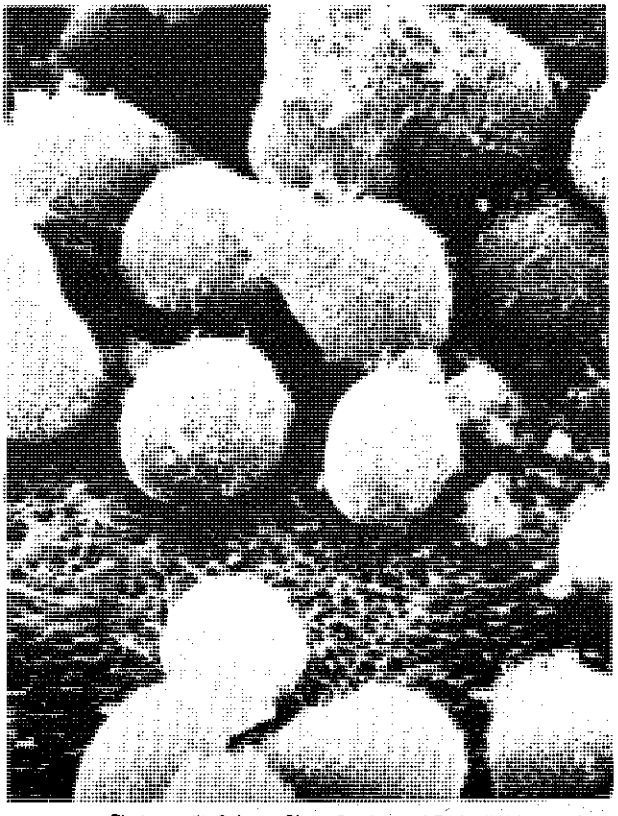


Photo credit: Science Photo Service and Porton/LH International

Scanning electron micrograph of human hybridoma cells

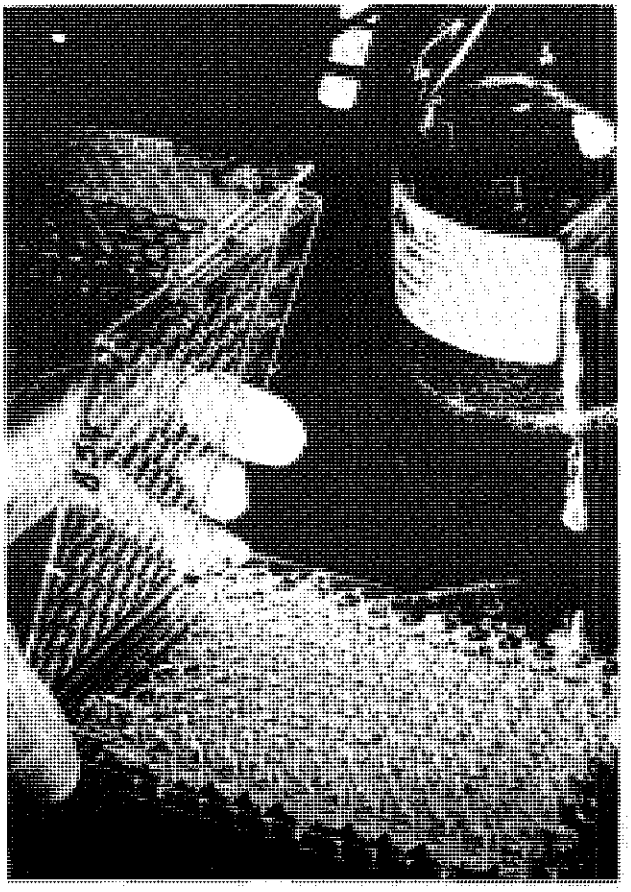


Photo credit: Science Photo Library and Porton/LH International

One step in the isolation of hybridomas

Despite the great promise of MAbs, there are several persistent technical problems to be considered:

- obtaining MAbs against certain weak antigens (antigens that do not produce a large immune response) remains difficult (11,24);
- homogeneous antibodies cannot perform some functions such as forming a precipitate with other antigen-antibody complexes, a necessary function for some diagnostic assays;
- low frequency of fusion is a continuing problem in the preparation of hybridomas, as is the stability of the hybridomas and antibodies (14); and
- some MAbs are sensitive to small changes in pH, temperature, freezing and thawing, and can be inactivated during purification.

Many of these problems are being alleviated or solved as research with MAbs progresses.

Another problem being addressed is the development of hybridomas for specific species. Some suitable myeloma cell lines exist for mice, rats, and humans (12,20,27), but a wider variety of human cell lines and cell lines for other species are needed if wider applications of MAb technology are to be made. Hybridomas are often made with cells from two different species, but these fusions regularly result in the preferential loss of the spleen B lymphocyte chromosomes, resulting in an absence of antibody production (24). For therapeutic applications, it is desirable to treat people with human antibodies to avoid allergic reactions and other problems of antibody cross-reactivity. Thus, MAbs from a human myeloma/human spleen cell fusion are needed. Several investigators have reported the development of human myelomas that are suitable for

not been economical. Now, however, a combination of improved engineering design and procedures and rDNA technology may yield bioprocesses that are more efficient than they have been in the past and therefore more competitive.

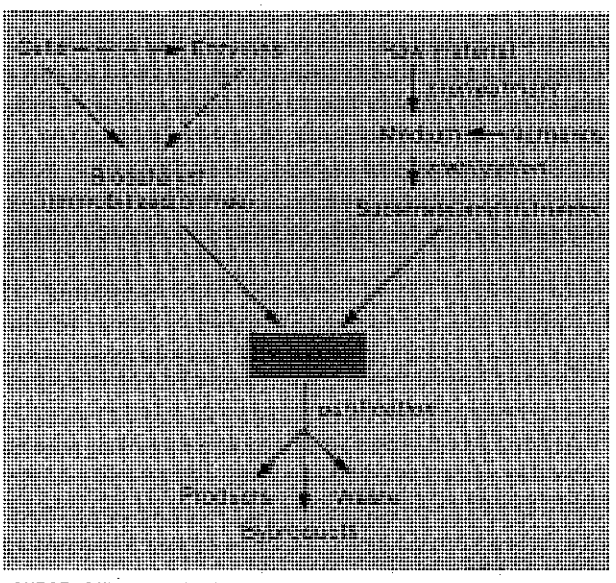
*Bioprocess essentials**

The steps in bioprocessing are presented schematically in figure 9. The substrate and nutrients are prepared in a sterile medium and are put into the process system with some form of biocatalyst—free or immobilized cells or enzymes. Under controlled conditions, the substrate is converted to the product and, when the desired degree of conversion has been achieved, byproducts and wastes are separated.

Water is the dominant component of the medium for virtually all current bioprocesses. Even when micro-organisms are grown on solid materials, an unusual processing mode, the substrate must be dampened in order to permit microbial growth and enzyme action. Products must usually be purified from dilute, aqueous solutions.

*The bioprocesses discussed here exclude uncontrolled environmental applications.

Figure 9.—Steps in Bioprocessing



SOURCE: Office of Technology Assessment.

Bioprocesses require a closely controlled environment, and this necessity markedly influences their design. Biocatalysts generally exhibit great sensitivity to changes in temperature, pH, and even concentrations of certain nutrients or metal ions. The success of a bioprocess depends on the extent to which these factors are controlled in the medium where interaction between biocatalyst and substrate takes place.

SUPPLY OF NUTRIENTS

In addition to establishing a suitable environment, the medium must provide for the nutritional needs of living cells. A primary requirement is a source of carbon. In addition to supplying the energy needed for metabolism and protein synthesis, carbon sources contribute structural elements required for the formation of complex compounds. Often, the carbon source may itself be the substrate for the catalyzed reaction, as in the fermentation of sugar to ethanol. Sugars, starches, and triglycerides, and, to a lesser extent, petroleum fractions, serve as carbon sources.

Other important nutrients required by living cells are nitrogen, phosphorus, and sometimes oxygen. Nitrogen and phosphorus are incorporated into structural and functional molecules of a cell and may also become part of product molecules. Most of the micro-organisms currently used by industry are highly aerobic and require an adequate supply of oxygen, but others are strictly anaerobic and must be protected from oxygen. A number of other nutrients, such as vitamins and metal ions, though required only in very small amounts, are nevertheless essential. Some of these nutrients, especially metals, may appear in the product.

In order to make the substrate and nutrients accessible to the biocatalyst, the medium must be thoroughly mixed. Most bacteria and some yeasts used in bioprocesses commonly grow as individual cells or as aggregates of a few cells suspended in the medium, whereas fungi and actinomycetes grow in long strands. As they grow, all these types of cells increase the viscosity of the fluid in which they are growing in a batch process, making the fluid more difficult to mix, and thus more difficult for nutrients to reach them.

Immobilized cells could be grown in large quantities in culture; the MAbs secreted from the cells could then be routinely collected from the medium. Immobilized cell methods may prove valuable for large-scale MAb production. Such methods are already used industrially, for example, in growing cells that produce polio virus for subsequent vaccine production (26,31).

Damon Biotech Corp. (U.S.) has recently introduced the technique of microencapsulation to MAb technology (7). This method uses a porous carbohydrate capsule to surround the hybridoma cells and to retain the antibodies while allowing the circulation of nutrients and metabolic wastes. After several days in culture, the encapsulated colonies are harvested and washed to remove the growth medium, the capsules are opened, and the antibodies are separated from the cells. According to Damon Biotech, 40 to 50 percent (by weight) of the harvested medium is made up of MAbs. The company claims the microencapsulation method for producing MAbs is significantly less expensive than the ascites method, provides a high concentration of antibodies, and does not require the maintenance of animals (18).

Industrial uses for monoclonal antibodies

Because of their unique properties of homogeneity, specificity, and affinity, MAbs can be used effectively in downstream purification systems for molecules, especially proteins. A MAb purification system relies on the binding of a target molecule to a MAb immobilized on a solid support such as a bead. The beads are packed in a column, and a mixture containing the target molecule is passed through the column. The MAb binds the molecule while the impurities wash through the column. Then the binding is reversed, and the target molecule is released and collected from the column.

Before MAb-based purification systems can be used in large-scale, several practical and technical factors must be optimized. These include cost, purification of the antibody itself, and elution of the desired product after purification by the antibody. Elution requires the use of an antibody of somewhat lower affinity than one would use for diagnostic or therapeutic applications so that the binding can be reversed easily.

Various important proteins, including alpha-fetoprotein and leukocyte interferon, are now purified using MAbs (29,30). MAb purification systems may be used in the future to purify a vast number of compounds, particularly substances present in small amounts.

A simple extension of the procedure just described involves using MAbs to bind unique surface proteins and, with them, the cells to which they are attached. This permits separation of cells with surface proteins of interest and is carried out by passing the cells over a suitable matrix to which the antibodies have been bound. In another procedure, fluorescence-activated cell sorting, cells are mixed with fluorescently labeled MAbs, and the mixture is passed through a special instrument called a flow cytometer, which responds to the fluorescent marker and sorts the cells into labeled populations at rates of 50,000 cells per minute (15,16). So far, fluorescence-activated cell sorting has been used mostly for research purposes, but as the method is improved, it may be employed in a range of clinical applications.

Conclusion

Many fields of biological research are being affected by MAb technology. Researchers now use MAbs to study problems in endocrinology, biochemistry, cell biology, physiology, parasitology, and many other fields, because the products of MAb technology are easily standardized and reproduced. Furthermore, many diagnostic, therapeutic, and industrial uses for MAbs are becoming apparent, and, as outlined in subsequent chapters of this report, several U.S. and foreign firms are developing these applications. Industrial purification applications of MAbs and the widespread advantages of MAb technology in preparing pure and easily standardized antibodies offer substantial benefits in industrial, research, and clinical laboratories. Recombinant DNA and MAb technologies can complement each other, because rDNA technology can lead to the production of new compounds, and MAbs can aid in their identification and purification.

Box A.—Continuous Bioprocessing

Biocatalyst Immobilization Techniques

In recent years, several techniques for immobilizing both enzymes and whole cells have been devised (see fig. BXA-1 and table BXA-1). The oldest technique involves simple *adsorption.* Enzymes or whole cells adhere loosely (without chemical bonding) to the surface of a support material such as alumina, charcoal, clay, or cellulose. Eventually such adsorbed agents will wash away, but some last a surprisingly long time.

For isolated enzymes, *chemical bonding* between the enzyme molecule and the surface of the support material achieves a much firmer hold and results in a stable biocatalyst preparation capable of extended service. Binding supports include cellulose, glass, and various synthetic polymers. In some cases, fixation of the enzyme seems to interfere somewhat with its catalytic activity. Other reports claim increased activity after immobilization.

Entrapment in a polymer matrix represents a third method of immobilization, applicable to both enzymes and cells. Starch, silica gel, collagen, and a variety of synthetic polymers readily form gels containing microscopic cages through which water can pass, but which trap enzyme molecules or microbial cells. This technique reduces the rate of reaction, because substrate, nutrient, and product molecules must all diffuse through the solid matrix structure. A compensating advantage, however, is that living cells can be firmly retained without damage. At least one Japanese pharmaceutical and fine chemical manufacturer (Tanabe Seiyaku) has successfully employed this method for the commercial production of a number of specialty chemical products.

Finally, in the technique called *microencapsulation,* enzymes or cells are enclosed in a spherical polymer membrane. The resulting capsules range in diameter from 5 to 300 micrometers and look much like enlarged cells. The membrane is semipermeable; the relatively small substrate and product molecules pass readily through the encapsulating membrane, but the larger enzyme molecules and the still larger structure of a complete cell cannot escape.

Continuous Bioprocessing Modes

Both packed- and fluidized-bed reactors, similar to those widely employed in conventional chemical technology, can be employed in continuous bioprocesses using immobilized catalysts (see fig. BXA-2). Continuous packed-bed bioreactors have, in fact, long been used for making vinegar. Diluted wine or fermented cider percolates through a bed containing wood shavings or some similar material. The microorganisms that convert ethanol to acetic acid form a slimy film on the surface of the solid support and so remain in the reactor. The treatment of sewage and other wastes is similar. The waste stream trickles through a bed of stone, ceramic, or plastic on which a microbial film has grown. Waste particles are adsorbed and converted to nonpolluting compounds. More sophisticated packed-bed reactors are beginning to be employed to perform a variety of conversions.

The development of better methods of immobilization has allowed the introduction of fluidized-bed reactors. Biocatalyst particles are suspended in an upward flowing stream of medium, with the flow rate adjusted to maintain suspension while preventing the loss of excessive amounts of catalyst from the reactor. Though a variety of designs have been proposed and several have been carried to the pilot scale, no industrial application of this approach to bioprocesses has been realized as yet.

Technical Limitations on Continuous Bioprocessing

The technical factors that limit the adoption of continuous as opposed to batch processing fall into the following categories:

- *Kinetic.* In principle, it is seldom possible to establish optimal conditions for economic secondary metabolite production (e.g., antibiotics) in a single-stage process. Separate growth and product formation stages require different process conditions.

competing methods of production. In most cases, bioprocesses will be used industrially because they are the only practical way in which a desired product can be formed. Biological processes may be desirable:

- in the formation of complex molecular structures such as antibiotics and proteins where there is no practical alternative,
- in the exclusive production of one specific form of an isomeric compound,
- because micro-organisms may efficiently execute many sequential reactions, and
- because bioconversions may give high yields.

Examples of the categories of current uses of bioprocesses are the following:

- production of cell matter ("biomass" itself) (e.g., baker's yeast, single-cell protein);
- production of cell components (e.g., enzymes, nucleic acids);
- production of metabolites (chemical products of metabolic activity), including both primary metabolites (e.g., ethanol, lactic acid) and secondary metabolites (e.g., antibiotics);
- catalysis of specific, single-substrate conversions (e.g., glucose to fructose, penicillin to 6-aminopenicillanic acid); and
- catalysis of multiple-substrate conversions (e.g., biological waste treatment).

Bioprocesses may offer the following advantages over conventional chemical processes:

- milder reaction conditions (temperature, pressure, and pH);
- use of renewable (biomass) resources as raw materials for organic chemical manufacture, providing both the carbon skeletons and the energy required for synthesis;
- less hazardous operation and reduced environmental impact;
- greater specificity of catalytic reaction;
- less expensive or more readily available raw materials;
- less complex manufacturing facilities, requiring smaller capital investments;
- improved process efficiencies (e.g., higher yields, reduced energy consumption); and
- the use of rDNA technology to develop new processes.

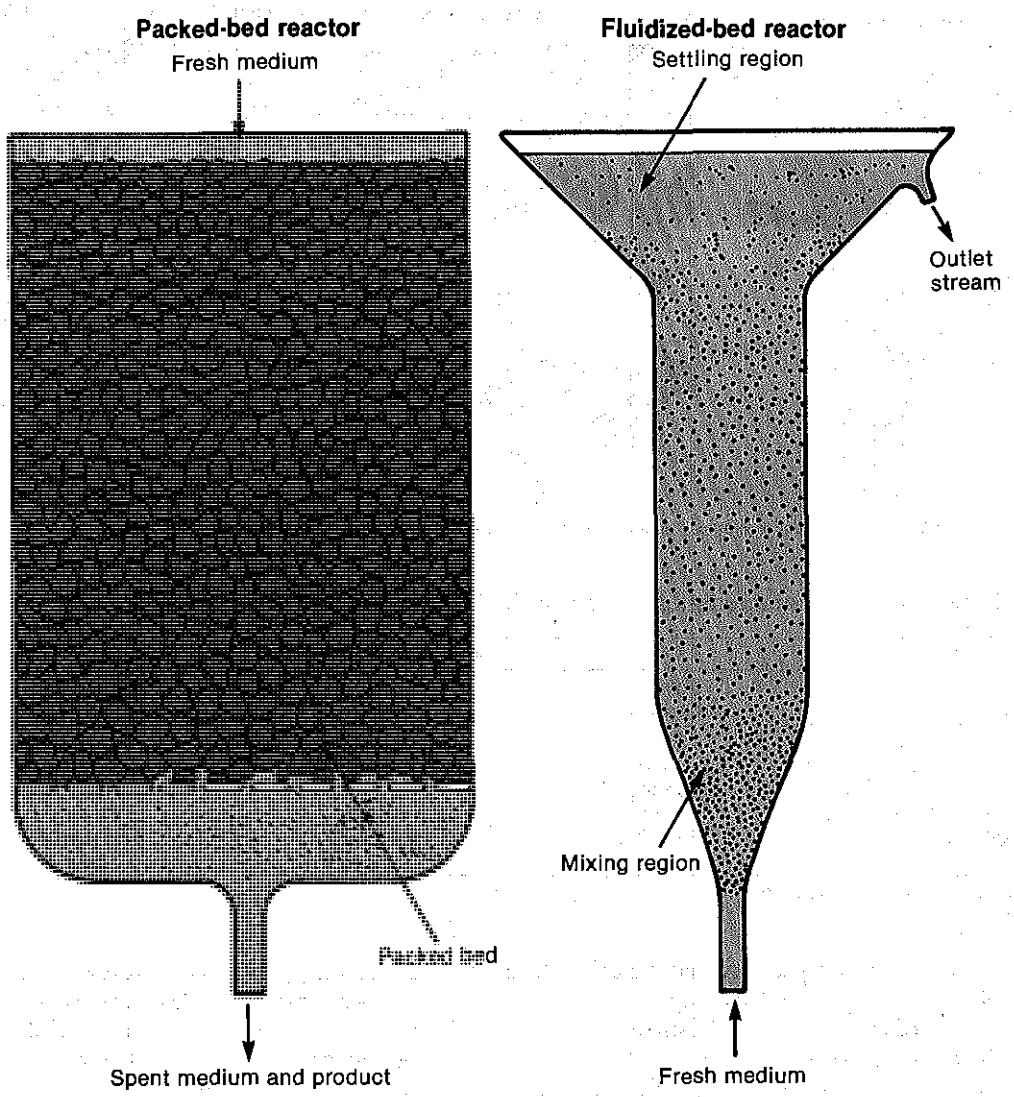
Some of the conceivable disadvantages of bioprocesses, on the other hand, are the following:

- the generation of complex product mixtures requiring extensive separation and purification, especially when using complex substrates as raw materials (e.g., lignocellulose);
- problems arising from the relatively dilute aqueous environments in which bioprocesses function (e.g., the problem of low reactant concentrations and, hence, low reaction rates;* the need to provide and handle large volumes of process water and to dispose of equivalent volumes of high biological oxygen demand wastes; complex and frequently energy intensive recovery methods for removing small amounts of products from large volumes of water);
- the susceptibility of most bioprocess systems to contamination by foreign organisms, and, in some cases, the need to contain the primary organism so as not to contaminate the surroundings;
- an inherent variability of biological processes due to such factors as genetic instability and raw material variability; and
- for rDNA systems, the need to contain the organisms and sterilize the waste streams, an energy-intensive process.

Solutions to some of these problems through the use of biotechnology may make bioprocesses more competitive with conventional chemical syntheses. Genetic intervention may be used in some instances to modify micro-organisms so that they produce larger amounts of a product, grow in more concentrated media, have enzymes with increased specific activity, or grow at higher temperatures to help prevent contamination. Recombinant DNA technology may lead to the development of completely new products or modification of important existing ones. In the past, some potentially useful bioprocesses have

*It is often said that biochemical catalysis is many times more effective than conventional chemical catalysis. This contention is based on the very high specific activities observed for individual enzymes in vitro. Such rates are seldom encountered under large-scale conditions. In general, bioprocesses are extremely slow in comparison with conventional chemical processes.

Figure BXA-2.—Packed-Bed and Fluidized-Bed Reactors



SOURCE: Adapted from E. Gaden, "Production Methods in Industrial Microbiology," *Scientific American*, September 1981, p. 196.

Batch operation currently dominates specialty chemical and pharmaceutical bioprocesses and is likely to continue to do so in the near future. In addition to technical limitations on continuous processing (see box A), other considerations have led manufacturers to choose the batch mode. Batch processing is often used, for example, because it offers the operational flexibility needed when a large number of products are manufactured, each at fairly low production levels; each process unit, more or less standard in design, can easily and rapidly be switched from one product line to another. Furthermore, a switch from batch to continuous processing is expensive, and, if a company has unused batch equipment, it may find that a switch to continuous processing is not economically warranted in the near term.

Increased use of genetically manipulated biocatalysts could affect the design and operation of bioconversion units. Harvey Blanch points out (33):

> . . . one of the difficulties which arises from the insertion of foreign DNA into the organism is re-

Since most of the micro-organisms currently used by industry perform their conversions aerobically, they demand a constant supply of oxygen. Oxygen's low solubility in water represents a significant stumbling block to efficient bioprocessing. Since oxygen is depleted during conversion, the medium must be constantly aerated; the more viscous the medium, however, the more difficult it becomes to supply oxygen. Approaches to maintaining an adequate oxygen supply include:

- increasing reactor pressure to increase oxygen solubility,
- the use of oxygen-rich gas for aeration, and
- changes in process design and operation.

PURE CULTURES AND STERILIZATION

Most of the products of bioprocesses are formed through the action of a single biocatalyst, either a micro-organism or an enzyme.* If foreign organisms contaminate the process system, they may disrupt its operation in a variety of ways. They can directly inhibit or interfere with the biocatalyst, whether it is a single enzyme or a complete cell, and they may even destroy the biocatalyst completely. Alternatively, contaminating organisms may leave the catalyst unaffected, but modify or destroy the product. Foreign organisms can also generate undesirable substances that are difficult to separate from the primary product. In the manufacture of pharmaceutical products, the risk of toxic impurities is of particular concern.

To avoid or minimize contamination, most current bioprocess technologies employ pure culture techniques. The medium and its container are sterilized, and a pure culture consisting of a population of a particular species is introduced. In order to avoid subsequent contamination, all materials entering the system, including the large amounts of air required for aerobic processes, are sterilized. The apparatus must be designed and operated so that opportunities for invasion by unwanted organisms are minimized.

*A significant exception to this generalization is the broad group of biological waste treatment processes. These processes use mixed and varied populations of micro-organisms developed naturally and adapted to the waste stream being treated.

Processing modes

Bioprocesses may, in principle, use any of the operating modes employed by conventional chemical technology. These modes range from batch processing to continuous steady-state processing.

In batch processing, the reaction vessel is filled with the medium containing the substrate and nutrients, the medium is sterilized, the biocatalyst is added, and conversion takes place over a period ranging from a few hours to several days. During this period, nutrients, substrates, agents for pH control, and air are supplied to, and product gases are removed from, the reaction vessel. When conversion is complete, the reaction vessel is emptied, and the purification process begins. Turnover time between batches can account for a significant portion of total processing time.

In continuous steady-state processing, which lies at the other end of the operational spectrum from batch processing, raw materials are supplied to, and spent medium and product are withdrawn from, the reaction vessel continuously and at volumetrically equal rates. Potential advantages offered by continuous processing over batch processing include significantly higher productivity, greater ease of product recovery due to the lack of contaminating biocatalyst, and lower cost due to reuse of biocatalyst.

The simplest approach to the implementation of a continuous processing system is to modify a batch reactor so that fresh substrate and nutrients can continually be added while a product stream is removed. This simple arrangement has one serious drawback: the biocatalyst leaves the reactor continuously with the outlet stream and must be separated from the product. Several techniques, all of which involve fixing the biocatalyst in some manner, have been developed to avoid the biocatalyst's escape with the reaction mixture and allow its repeated use. The development of techniques for the immobilization of biocatalysts has greatly expanded the possibilities for continuous bioprocesses. Although still not widely employed for large-scale bioprocesses, the biocatalyst immobilization techniques now available offer a diversity of new opportunities for more effective bioprocessing (see *Box A.—Continuous Bioprocessing*).

organisms can be transferred to cells whose large-scale growth is well understood.

Wider availability of thermotolerant biocatalysts is important for all industries using bioprocesses. Recent research on the development of thermotolerant biocatalytic agents has advanced the potential efficiency of bioprocesses. The advantages of thermotolerance include:

- reduced susceptibility to contamination;
- easier removal of metabolic heat;
- more complete and rapid conversions when volatile inhibitors are present (but oxygen solubility is reduced); and
- easier recovery of volatile products (e.g., ethanol).

Biocatalysts that can withstand high pressure may also be useful industrially. For instance, higher pressures will increase the solubility of oxygen.

Finally, research investigating the relationship between the structure and the function of enzymes is proceeding. Ultimately, the aim is to be able to design, with the help of computers, an enzyme to perform any specific catalytic activity under given conditions. Although this procedure will not be done routinely for many years, it will soon be possible, using rDNA technology, to modify the structure of an enzyme to improve its function in a given condition, such as at a particular pH or temperature. Thus, biotechnology could greatly affect the efficiency of bioprocesses.

Bioprocess monitoring and associated instrumentation

Despite the need for close control of process variables during a bioprocess operation, the techniques available for making measurements on-line are extremely limited. Existing equipment can readily monitor only temperature, pH, dissolved oxygen concentration, and evolution of gases. Although many other sensors have been developed to measure other variables (e.g., glucose levels), all are sensitive to steam sterilization. Thus, their usefulness in monitoring most bioprocesses is limited. Many critical variables are able to be monitored only by withdrawing samples from the reaction vessel and analyzing them off-line, and, even then, it is difficult to determine key characteristics accurately. When measuring cell mass (an indicator of growth), for example, most process operators simply note such crude indicators as packed cell volume, turbidity, or, at best, dry weight.

It is possible to measure the compositions and flow rates of gaseous streams entering and leaving the reactor and to use the values obtained from such measurements to help estimate key process conditions indirectly. Such procedures have been greatly facilitated by the use of computers. The real potential of computer control, however, will not be realized until a greater range of reliable on-line sensors becomes available.*

A number of European, Japanese, and American groups have developed improved sensors for bioprocess control, but, so far, most devices require removal of samples for off-line analysis because the sensors cannot withstand sterilization. Continuous sampling combined with various types of rapid instrumental analyzers offers a reasonable compromise, but, with this approach, there is a time lag between the actual sample time and the time at which the assay information becomes available.

Sophisticated instrumentation will have increasing use in bioprocess monitoring. High performance liquid chromatography, for example, is used to identify particular compounds in a mix of compounds and is one of the fastest growing instrumentation fields. Flow cytometry has potential use in measuring process variables such as cell size (an indicator for adjusting nutrient flows) and cell viability. Other instrumentation will surely be used as bioprocess monitoring becomes more widely investigated.

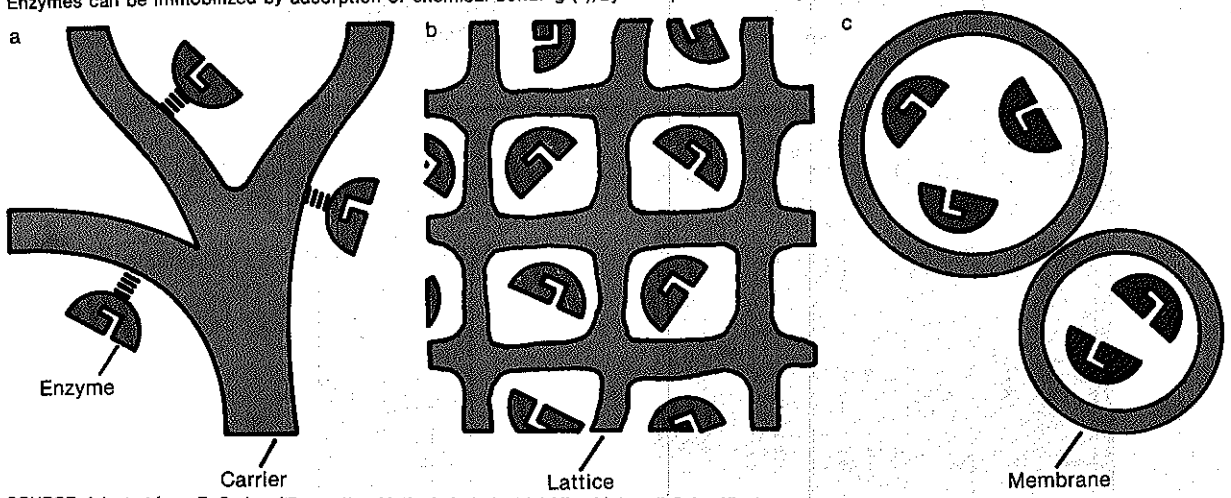
Computer-coupled bioprocesses can greatly improve monitoring and controlling the growth conditions during a bioprocess run. Computers can be used to analyze the data from sensors and other monitoring instrumentation and respond to these data by adjusting process variables, such as nutrient flow. Additionally, computer interfaces can be used:

- to schedule efficiently the use of equipment;
- to alarm operators when necessary;

*For a discussion of biosensors, see *Chapter 10: Bioelectronics.*

Figure BXA-1.—Techniques for Immobilizing Enzymes and Whole Cells

Enzymes can be immobilized by adsorption or chemical bonding (a), by entrapment in a polymer matrix (b), or by microencapsulation (c).



SOURCE: Adapted from E. Gaden, "Production Methods in Industrial Microbiology," *Scientific American*, September 1981, p. 182.

Table BXA-1.—Characteristics of Immobilization Methods for Enzymes and Cells

Characteristic	Immobilization method		
	Physical adsorption	Chemical bonding	Entrapment; encapsulation
Preparation	Easy	Difficult	Moderate
Activity	Low	High	High
Specificity	Unchanged	Changeable	Unchanged
Binding force (retention)	Weak	Strong	Strong
Regeneration	Possible	Impossible	Impossible
Cost	Low	High	Low

SOURCE: Gaden, personal communication, 1983.

- *Biological.* Biocatalyst stability may be difficult to maintain for long periods of continuous operation. The phenomenon of "culture degeneration," reported in many instances, deserves careful study. The results of such studies will surely be case-specific and may simply reflect inadequacies in the knowledge of nutrient requirements necessary to sustain long-term productivity. As the use of rDNA organisms grows, this matter will require close attention because of concerns over the stability of these types of organisms.
- *Operational.* The primary technical factors acting to limit continuous bioprocessing in the past have been difficulties in maintaining sterile conditions and in handling biocatalytic suspensions, especially those of filamentous fungi or large cell clumps. The perplexing contamination problem has focused improvement efforts on the deficiences of equipment (mainly pumps) for moving liquids and slurries and on valves and transfer lines. Many specific difficulties have already been overcome in connection with batch operations, and improved equipment design and more rigorous operating procedures may result in successful continuous processes.

As automation reduces the labor intensity of laboratory tasks, the pace of competition will quicken, and countries with sophisticated software to direct the automation will possess an advantage in the commercialization of biotechnology.

Separation and purification of products

Separation and purification techniques used in bioprocesses are the aspect of bioprocess engineering most in need of attention, especially for the production of novel products such as proteins. Research is needed to find highly selective recovery techniques that leave as little residual product as possible in the medium and thus lessen the labor intensity associated with downstream processing. An example of the effort expended in downstream processing is provided by the new plant Eli Lilly built to produce human insulin (Humulin®). The plant employs 220 people, 90 percent of whom are involved in recovery processes.

Some of the possibilities for improving recovery techniques now under consideration include the following:

- *Ultrafiltration.* Membranes and other filtration systems, such as porous metals, offer many advantages, and considerable experience in other areas of chemical technology is already available. Some U.S. companies, such as Millipore, Amicon, and Nucleopore are making advances in this area.
- *Continuous chromatography and high performance liquid chromatography.* If these approaches, already available on the laboratory scale, could be scaled-up, it would be possible, in principle, to collect a crude product from the medium and then, by selective elution, recover product, reusable nutrients, and inhibitory substances separately. One American manufacturer (Waters, a Millipore subsidiary) claims to have developed a pilot-scale chromatographic unit.
- *Electrophoresis.* Electrophoretic methods, especially continuous flow, can separate proteins, peptides, and nucleic acids on the basis of their electrical charge. The advantage of this separation method over some others is that it can run continuously and can effectively separate molecules in large sample volumes. The potential of continuous-flow electrophoresis for producing commercial quantities of high purity substances such as pharmaceuticals was demonstrated on a recent space shuttle mission. The electrophoresis experiment, cosponsored by McDonnell Douglas and the National Aeronautics and Space Administration, demonstrated that under weightless conditions an electrophoresis system, identical to one tested on Earth, separated about 700 times more material in a given period of time and also achieved four times the purity while processing 250 times more material.
- *Monoclonal antibodies.* Immobilized MAbs are being used as purification agents for protein products (see "Monoclonal Antibody Technology" section above). This technique best suits large molecular weight and high-value-added products such as proteins.

Genetic modifications of micro-organisms used in bioprocessing could also aid in recovery processes. Two changes in particular would greatly improve the yield and ease recovery of proteins. First, micro-organisms could be developed that have minimal intracellular protein-degrading enzymes. The presence of these enzymes will decrease the yield of protein product. Second, a protein is much more easily purified if it is secreted from the cell into the surrounding medium. The genetic incorporation of protein secretion mechanisms will lower production costs dramatically.

Although purification and separation protocols have been developed for existing bioprocesses, new bioprocesses will present new challenges. For example, rDNA technology has led to a new set of bioprocesses that synthesize protein products, and substantial work is needed to improve recovery strategies for large-scale protein purifications. In addition, one of the factors that restricts the use of bioprocesses for producing commodity chemicals is the expense of recovering these low-value-added chemicals from dilute aqueous solutions.

version. This can be minimized by placing the cell in an environment in which cellular replication is minimized, while cellular activity, such as the production of enzymes and products, is maintained at high levels.

Achieving the dual objective of minimal growth and maximum conversion activity requires restrictive nutrient supplies and high cell densities. Immobilized biocatalysts could be used to achieve these objectives.

Bioprocesses, unlike petroleum refining or petrochemical operations which completely convert raw materials to products or consume them as process fuels, regularly produce large amounts of waste, mainly cell matter and residual nutrients. Bioprocesses also require large volumes of clean water, discharge equivalent amounts of dilute, high biological oxygen demand wastes, and produce products in low concentrations. One solution to problems associated with bioprocessing might be the use of cleaner, more defined media, which produce fewer byproducts. Another solution might be the use of more concentrated media. The latter option is normally considered in bioprocess development, but the microorganisms now in use are limited in their tolerance for high nutrient concentrations. Genetic manipulation may provide micro-organisms that are less sensitive to increased product concentration.

Raw materials

Current bioprocess technology uses an extremely limited range of raw materials. Just a few agricultural commodities—starch, molasses, and vegetable oil—are employed as raw materials in many of the existing industrial bioprocesses. Industry chooses these feedstocks for several reasons. There are established markets for these materials and, for primary products like starch, reasonably defined quality standards and assay procedures. Several competing suppliers guarantee uniform quality and fairly stable prices. Bioprocess applications constitute only a relatively small fraction of the market for agricultural commodities. The need for raw materials for bioprocesses, however, could become a major factor in commodity grain markets if bioprocesses find a place in large-scale fuel or chemical production.

Less important raw materials are some byproducts of agricultural and food processing, such as "corn steep liquor" and "distillers solubles." Petroleum hydrocarbons are little used because of their high cost. The potential for relatively pure cellulose (e.g., delignified wood) remains unrealized.* For various carbohydrate wastes—agricultural, food, industrial, or municipal—in spite of frequent claims of their availability and low cost, no economical bioprocess applications have yet been found.

Biocatalysts

The substances that actually cause chemical change in bioprocesses are the enzymes produced by a living cell. For simple enzymatic conversions, isolated enzymes can be used as biocatalysts. When biological transformation of the substrate involves several sequential and interrelated chemical reactions, each catalyzed by a separate enzyme, however, whole cells (most commonly, but not exclusively, micro-organisms—bacteria, yeast, or fungi) are used as biocatalysts. Bioprocesses used for the synthesis of complex molecular structures (e.g., antibiotics or proteins such as insulin), for example, require entire systems of enzymes. Such systems do not yet function in concert outside a living cell. Indeed, when the desired product is the cell itself (e.g., baker's yeast or single-cell protein), all the enzymes comprising the cell's growth machinery are components of the catalytic system.

An inspection of the immense spectrum of organisms whose biochemical capabilities have been reasonably well defined reveals that bioprocesses employ only a small, select group of biocatalysts. If one eliminates those organisms considered "natural populations" in food fermentation or biological waste treatment, the range of biocatalysts employed in bioprocesses is even more limited. Some animal cells and tissues are employed for vaccine production and related activities, but the catalytic capabilities of plant cells, except for some algae, have not yet been employed commercially. It is possible that biotechnology will provide a means whereby important catalytic activities from poorly understood

*See *Chapter 9: Commodity Chemicals and Energy Production.*

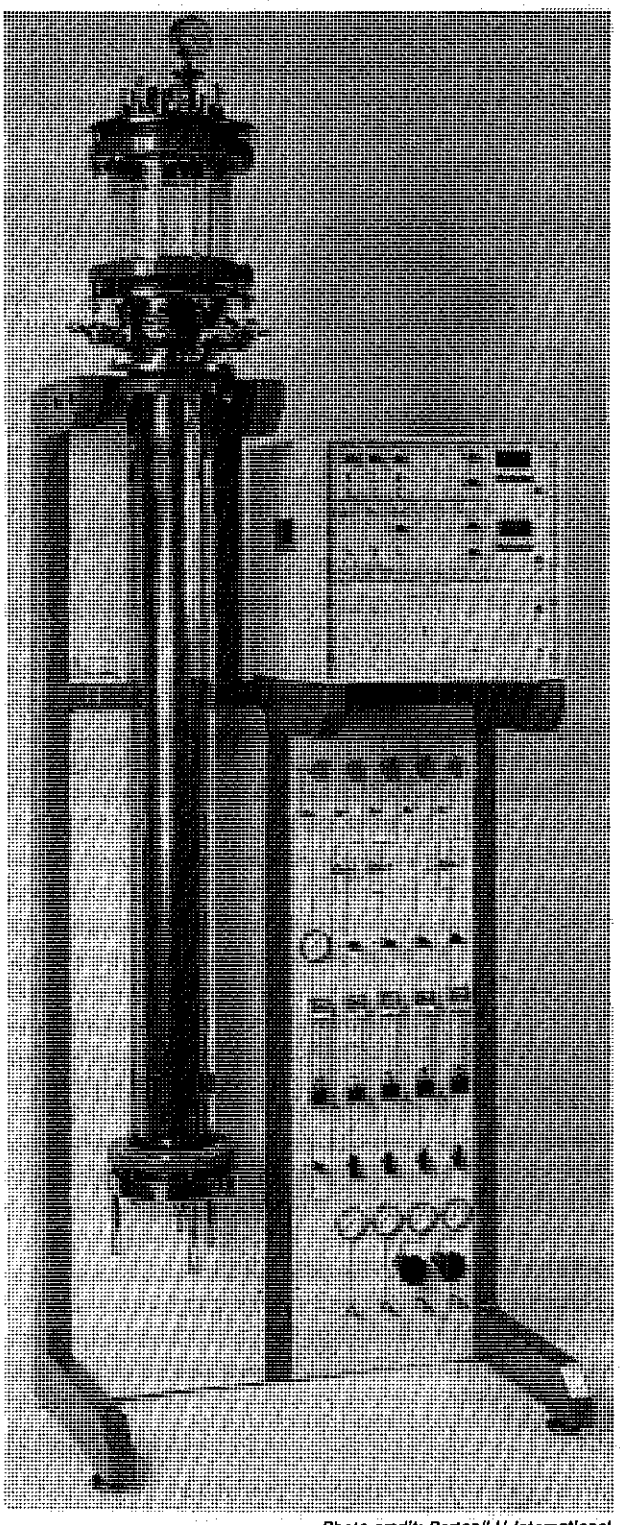


Photo credit: Porton/LH International

Bioreactor specially designed for the growth of plant or animal cells

Furthermore, mammalian cells have very complex nutritional requirements, which have not been completely defined. They require serum from blood for growth, and the essential composition of serum is not well characterized. In contrast to microbial cells, mammalian cells are not normally exposed to the environment, but are constantly surrounded by a circulatory system that supplies nutrients and removes wastes. When these cells are grown in culture, the medium is initially clean and nutritionally balanced; as the cells take up the nutrients and excrete waste products, however, the medium becomes much less like the cells' normal environment. This problem, along with the problem of fragility, requires modified reactor design (39).

Some mammalian cells grow in suspension like microbial cells, but most higher cells must attach to a solid surface. A major problem with large-scale cell growth of mammalian cells has been the availability of large, accessible surfaces for cell growth. The attachment of cells to microcarriers, or very small beads, has begun to solve many of the problems associated with large-scale mammalian cell culture. The beads provide a large amount of surface area and can be placed in a column where either a continuous-flow or fluidized-bed bioreactor can be used for cell growth and product formation (see box A). Either of these bioreactors is gentler than a stirred tank reactor. Additionally, because of the continuous nature of these bioreactors, fresh nutrients are added and wastes are removed continuously.

The instrumentation requirements for mammalian cell growth are different than those for microbial growth. The lower rates of metabolism and lower density of mammalian cells require more sensitive sensor systems than for microbial cell growth. Additionally, because the nutritional requirements are so much more complex, different strategies are needed to monitor and control cell growth. These problems are just beginning to be addressed (40).

Priorities for future research

Priorities for future generic research in bioprocess engineering that would be applicable

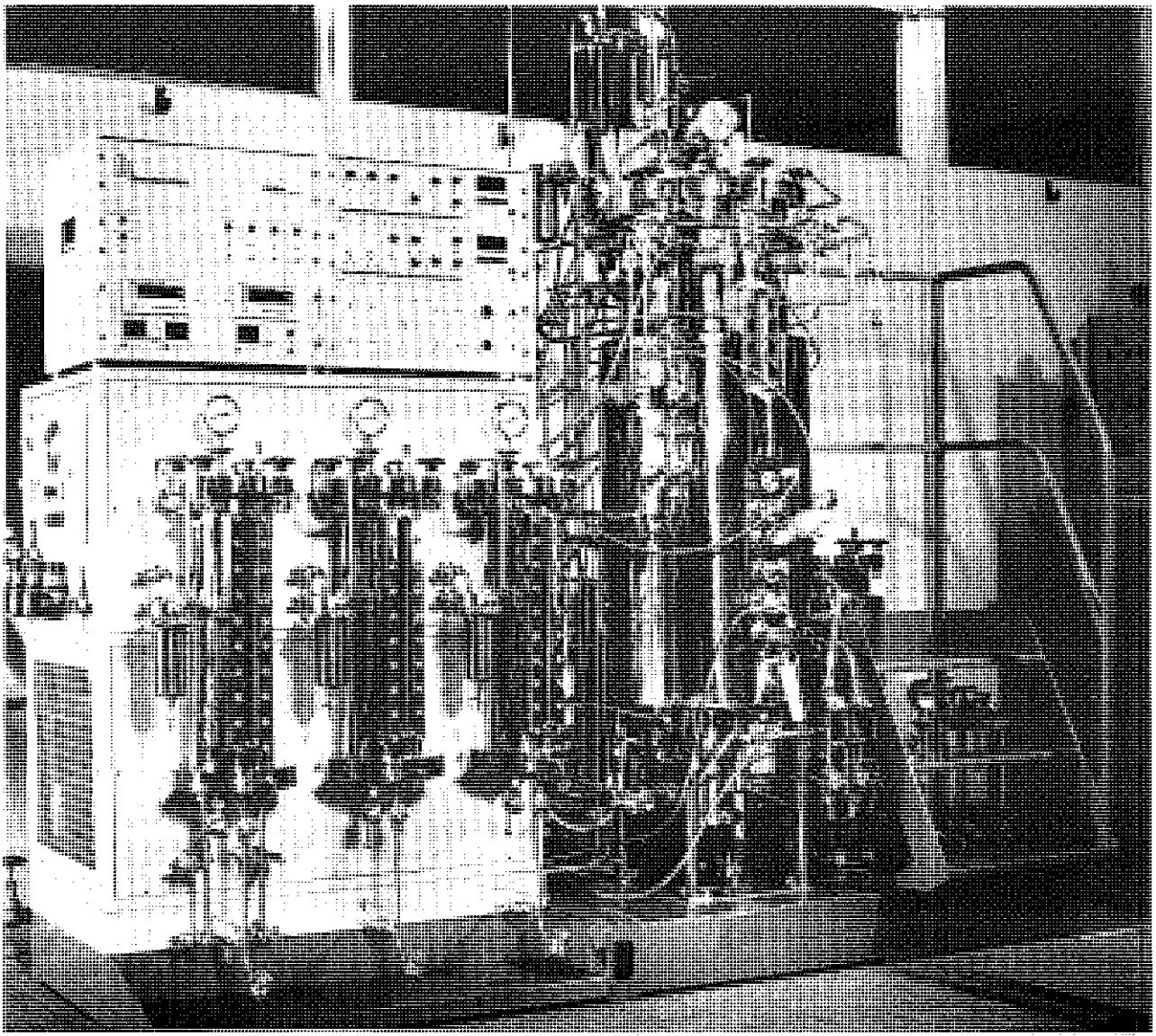


Photo credit: Porton/LH International

100-liter pilot plant bioreactor with computer controls

- to log, store, and analyze data; and
- to inventory raw material depletion and product synthesis.

These functions optimize the methodology and organization of bioprocessing within a plant. Companies are only now starting to use computer-controlled bioprocesses because of cost, lack of good sensors, and interfacing problems. Yet advances in this field are sure to occur soon because of increased interest in bioprocesses by electronics experts, as evidenced by the recent joint venture between Genentech and Hewlett-Packard.*

The automation of bioprocessing will be of critical importance in the future as companies compete for shares in biotechnology product markets.

*See *Chapter 4: Firms Commercializing Biotechnology.*

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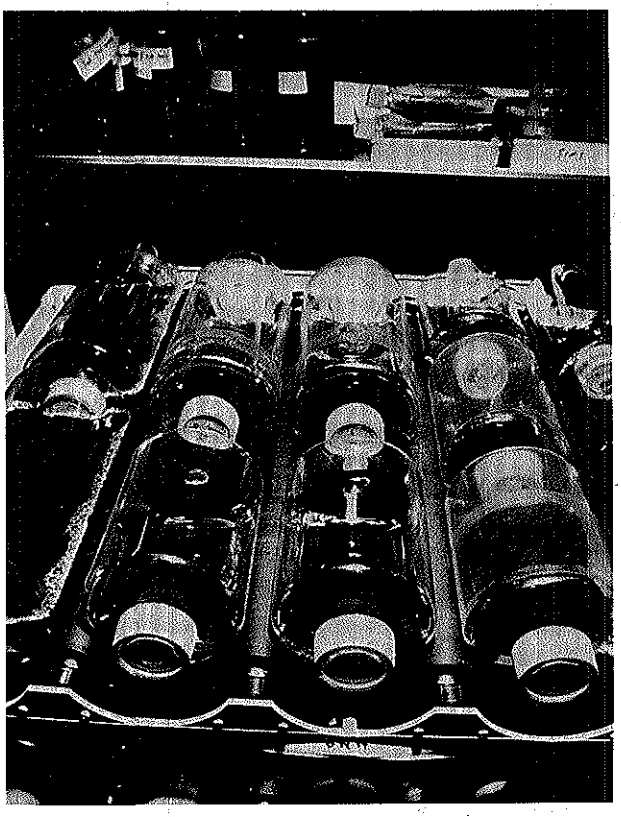
**Most of these references are of general interest for those wishing to pursue the subject of bioprocess technology further.

Culture of higher eukaryotic cells

The organisms used most extensively in large-scale bioprocesses are prokaryotes (e.g., bacteria) or simple eukaryotes (e.g., yeast). These are hardy organisms which grow to high cell densities and consequently give high product yields.

Certain products can be obtained in some situations only from the large-scale cultivation of higher eukaryotic cells. As noted in table 2, for instance, many proteins that are potentially useful (e.g., in medicine) have not been isolated in large enough quantities to study adequately. If eukaryotic cell culture made these proteins available in larger quantities, their amino acid sequence could be determined, their genes cloned, and even more of the proteins could be produced. Furthermore, some proteins probably need "post-translational modifications" (changes in protein structure after the protein is made from mRNA) that only higher eukaryotes can perform. These modified proteins may only be made in eukaryotic cells. Also, in many cases, the production of secondary metabolites in plant cells is a function of several enzymatic functions, most of which are not known. Therefore, the growth of plant cells in culture might be the easiest way to produce useful plant compounds. Finally, many individuals think that the growth of hybridomas would be easier and more economic in culture if the culture technology were better developed (see "Monoclonal Antibody Technology" section above). As biotechnology becomes more integrated into the industrial structure, the development of more efficient and economic bioprocess technologies for higher eukaryotic cells will increase in importance.



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Laboratory tissue culture production

The technologies developed for the growth of micro-organisms have limited applicability to the growth of higher eukaryotic cells because of differences between microbial and mammalian cells (see table 3). Mammalian cells are larger, more fragile, and more complex than microbial cells.*

*Most cell culture research has been done with mammalian cells, so the work reported here focuses on those cells. Problems with plant cell culture are similar to those of mammalian cell culture.

Table 2.—Situations Potentially Requiring Large-Scale Eukaryotic Cell Culture

Cell culture system	Reason for large-scale eukaryotic cell culture
Cells producing useful proteins	Not large enough quantity to determine amino acid sequence; therefore cannot use rDNA technology
Cells producing modified proteins	Modification systems present only in higher cells
Plant cells producing useful secondary metabolites	Enzymatic pathways for metabolic production not well understood; therefore cannot use rDNA technology
Hybridomas	Mouse acites system has limited capacity and applicability

SOURCE: Office of Technology Assessment.

Table 3.—Comparison of Microbial and Mammalian Cells

Characteristic	Microbial cells	Mammalian cells
Size (diameter)	1-10 microns	10-100 microns
Metabolic regulation	Internal	Internal and hormonal
Nutritional spectrum	Wide range of substrates	Very fastidious nature
Doubling time	Typically 0.5-2.0 hours	Typically 12-60 hours
Environment	Wide range of tolerance	Narrow range of tolerance
Other characteristics		Limited life span of normal cells
		Lack of protective cell wall

SOURCE: Office of Technology Assessment, adapted from R. J. Fleischaker, Jr., "An Experimental Study In the Use of Instrumentation To Analyze Metabolism and Product Fermentation in Cell Culture," thesis, Massachusetts Institute of Technology, Cambridge, Mass., June 1982.

to all industries using biotechnology include research in the following areas:

- continued work on the practical use of and design of bioreactors for immobilized cell and enzyme systems;
- development of a wider range of sterilizable sensors for process monitoring and control;
- improved product recovery techniques, especially for proteins;
- general reactor design and practical approaches to better oxygen transfer;
- inhibition of intracellular protein-degrading enzymes;
- improving the genetic stability of rDNA organisms;
- protein secretion mechanisms;
- improved methods for heat dissipation during bioprocessing; and
- biochemical and physiological mechanisms for temperature and pressure tolerance.

The large-scale culture of eukaryotic cells is beginning to receive some research attention. Because of the complex nutritional requirements of eukaryotic cells, the cost of the medium is high. If industry is going to adopt eukaryotic cell culture technology, the development of economic artificial media is important. Also important is the development of new bioreactor design and instrumentation for the control of cell growth.

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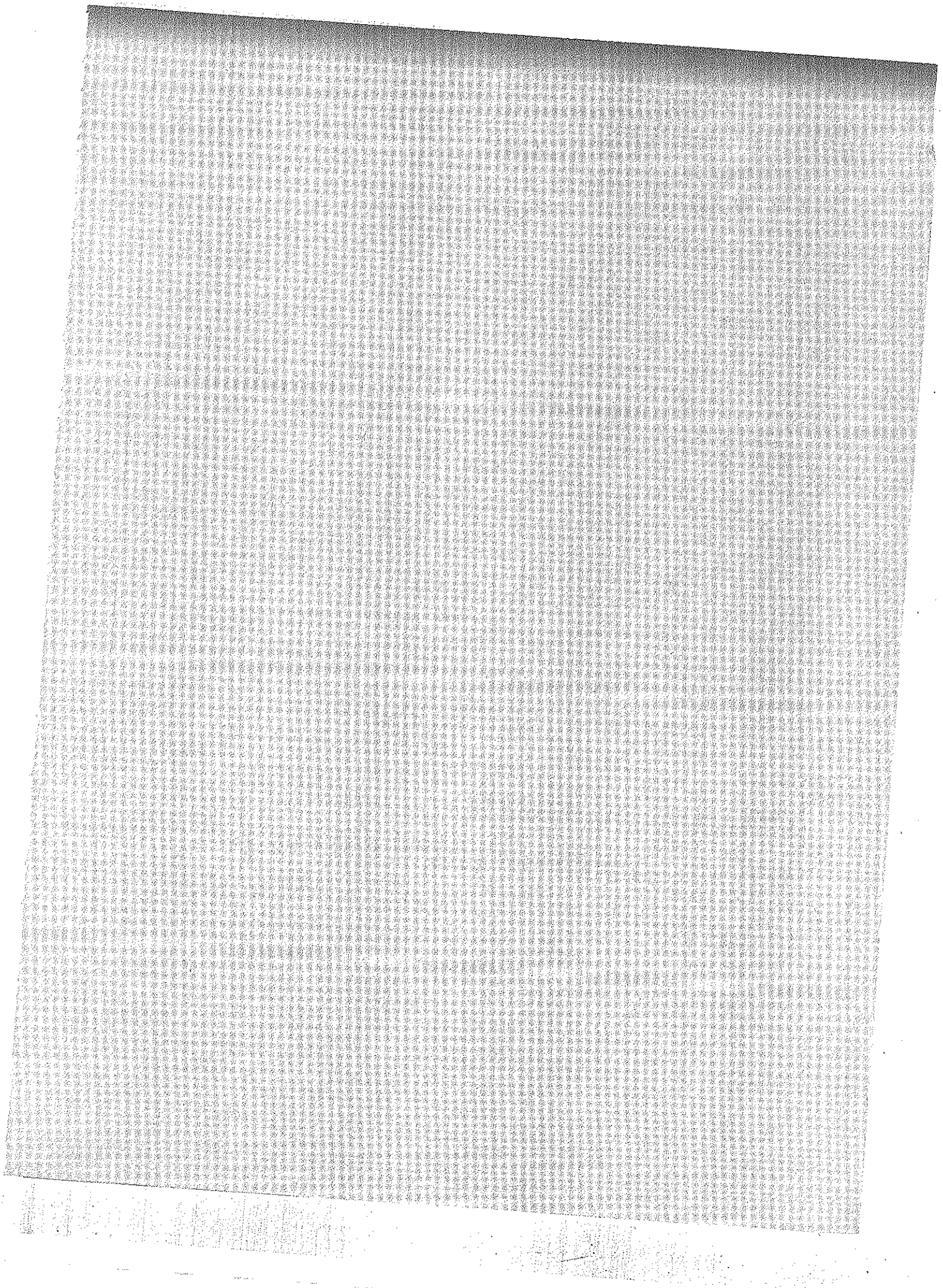

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PART II
Firms Commercializing Biotechnology

Overview of U.S. and foreign companies commercializing biotechnology

U.S. and foreign efforts to develop and commercialize biotechnology differ substantially in character and structure. The manner in which the United States and other countries organize their development efforts is important for two reasons: it can influence their respective commercial capabilities; and it will ultimately shape the character of international competition.

In the United States, two distinct sets of firms are pursuing commercial applications of biotechnology—NBFs and established companies. NBFs, as defined by this report, are entrepreneurial ventures started specifically to commercialize innovations in biotechnology. For the most part, they have been founded since 1976—the same year the U.S. firm Genentech was founded to exploit the recombinant DNA (rDNA) technology patented in the United States by Cohen and Boyer.* Typically, NBFs are structurally organized specifically to apply biotechnology to commercial product development. The established companies pursuing applications of biotechnology are generally process-oriented, multiproduct companies in traditional industrial sectors such as pharmaceuticals, energy, chemicals, and food processing. These companies have undertaken in-house biotechnology R&D in an effort to determine how and where best to apply biotechnology to existing or new products and processes. Table 4 provides a list of NBFs and established companies currently applying biotechnology in the United States and the targeted commercial areas of their research. Figure 10 illustrates the percentage of U.S. firms pursuing biotechnology R&D in specific application areas.

Sixty-two percent (135) of the 219 U.S. companies for whom commercial application areas are known* are pursuing applications of biotechnology in the area of pharmaceuticals; 28 percent are pursuing applications in animal agriculture, and 24 percent in plant agriculture.** In the area of specialty chemicals and food additives, commodity chemicals and energy, the environment, and electronics, respectively, relatively fewer U.S. firms are pursuing commercial applications of biotechnology. In some of these sectors, conventional technologies are working well or existing investments in capital equipment are very substantial. In others, much uncertainty still surrounds the potential of biotechnology or the research needed to develop applications of biotechnology is long term.

In Japan, the Federal Republic of Germany, Switzerland, France, and the United Kingdom,*** biotechnology is being commercialized almost exclusively by established companies. Most European nations and Japan, unlike the United States, tend, for different reasons, to emphasize the importance of large companies instead of small ones. Thus, the development of biotechnology in these countries is biased considerably toward the large pharmaceutical and chemical companies.

It should not be assumed that the small number of NBFs in the European countries or the lack of

*Two U.S. firms, Cetus and Agrigenetics, though established before 1976, are considered to be NBFs. Cetus was founded to capitalize on classical genetic techniques for product development, but showed early interest in biotechnology and began aggressively pursuing product development with the new techniques. Agrigenetics was formed in 1975 to link new genetic research with the seed business. Thus, the behavior and research focus of both Cetus and Agrigenetics place them in the new firm category despite their early founding dates.

*This figure does not include the companies listed that are specializing in bioprocessing, because the bioprocessing R&D may not be associated with specific products. See *Appendix D: Firms Commercializing Biotechnology in the United States* for an explanation of how the list was obtained.

**These percentages add up to more than 100 percent because many of the firms are engaged in more than one area of commercial application.

***In the United Kingdom, some NBFs, not including Celltech and Agricultural Genetics, are beginning to form on the periphery of universities. Plant Science, Ltd., for example, is linked to the University of Sheffield; Imperial Biotechnology, Ltd., is linked to the Imperial College in London; IQ (Bio) was formed by some Cambridge University biochemists; Boscot, Ltd., a joint venture between two Scottish institutions, was established by the University of Edinburgh and Heriot-Watt University, and Cambridge Life Sciences pursues biosensors based on work at Southampton University. As an indication of the increased number of NBFs forming in Britain, Biotechnology Investments, Ltd., the venture fund managed by N.M. Rothschild (the bank) now has for the first time since the fund was established more proposals from British firms than from companies in the United States (56).

Chapter 4

Firms Commercializing Biotechnology

Table 4.—Companies Commercializing Biotechnology in the United States and Their Product Markets[a,b] (Continued)

Company (date founded)	Commercial application of R&D[c]	Ph.D.s[d]
Crop Genetics International (1981)	PA	
Cutter Laboratories, Inc.	Ph	
Cytogen Corp. (1981)	Ph	7
Cytox Corp. (1975)	Env	
Damon Biotech, Inc. (1981)	Ph	10
Dairyland Foods Corp.	SCF	
Dart and Kraft, Inc.	SCF	
Davy McKee Corp.	Bioprocessing	
DeKalb Pfizer Genetics (1982)	AA	
Diagnon Corp. (1981)	Ph	
Diagnostic Technology, Inc. (1980)	Ph	
Diamond Laboratories	AA	
Diamond Shamrock Corp.	AA,CCE	
DNA Plant Technology (1981)	PA	10
DNAX Corp.	Ph	
Dow Chemical Co.	Ph,PA,CCE,SCF, AA,Env	
Ean-tech, Inc. (1982)	El,Env,Ph	3
Eastman Kodak Co.	Ph,Env	
Ecogen (1983)	PA	
E. I. du Pont de Nemours & Co., Inc.	Ph,PA,CCE,SCF	
Electro Nucleonics Laboratories, Inc.	Ph	
Eli Lilly & Co.	Ph,PA	
EnBio, Inc. (1975)	Bioprocessing	
Endorphin, Inc. (1982)	Ph	
Engenics, Inc. (1981)	Bioprocessing	25
Enzo Biochem, Inc. (1976)	Ph,AA,CCE,SCF,PA	
Enzyme Bio-systems, Ltd.	SCF	
Enzyme Center, Inc.	SCF	
Enzyme Technology Corp.	SCF	
Ethyl Corp.	CCE,SCF,Env	
Exxon Research & Engineering Co.	CCE,Env,SCF	
Fermentec Corp. (1978)	Bioprocessing	
FMC Corp.	Ph	
Frito-Lay, Inc.	PA	
Fungal Genetics, Inc. (1982)	Ph,SCF	
Genencor (1982)	SCF,CCE	
Genentech, Inc. (1976)	Ph,AA,CCE,El	75
General Electric Co.	El,Env,Ph,SCF	
General Foods Corp.	PA	
General Genetics (1982)	Ph	
General Molecular Applications (1981)	Ph	
Genetic Diagnostics Corp. (1981)	Ph	3
Genetic Replication Technologies, Inc. (1980)	Ph,AA	
Genetic Systems Corp. (1980)	Ph	14
Genetics Institute (1980)	Ph,PA,SCF,Env	24
Genetics International, Inc. (1980)	AA,Ph,SCF,CCE, Env,El	17
Genex Corp. (1977)	Ph,AA,SCF,Env	48
Gentronix Laboratories, Inc. (1972)	El	
Genzyme (1981)	SCF	6
W. R. Grace & Co.	AA,SCF,Env,PA,Ph	
Hana Biologics, Inc. (1978)	Ph	
Hem Research (1966)	Ph,AA	
Hoffmann-La Roche, Inc.	Ph	
Hybridoma Sciences, Inc. (1981)	Ph	
Hybritech, Inc. (1978)	Ph	13
Hytech Biomedical, Inc. (1981)	El,Ph	10
IBM Corp.	El	
IGI Biotechnology, Inc. (1975)	Ph	
Immulok, Inc. (1980)	Ph	

Chapter 4

Firms Commercializing Biotechnology

Introduction

Biotechnology has the technical breadth and depth to change the industrial community of the 21st century because of its potential to produce substantially unlimited quantities of:

- products never before available,
- products that are currently in short supply,
- products that cost substantially less than products made by existing methods of production,
- products that are safer than those now available, and
- products made with raw materials that may be more plentiful and less expensive than those now used.

By virtue of its wide-reaching potential applications, biotechnology lies close to the center of many of the world's major problems—malnutrition, disease, energy availability and cost, and pollution. It is because of biotechnology's promise that the developed countries of the world have commenced a competitive battle to commercialize its applications.

Nowhere in the world are efforts to commercialize biotechnology stronger than in the United States.* Large established U.S. companies in industries ranging from pharmaceuticals to petroleum have followed the lead in developing biotechnology that was set by entrepreneurial new biotechnology firms (NBFs) in the United States whose dedication to biotechnology is unmatched anywhere. Major competitive challenges to the United States in current product markets, as well as in new biotechnology markets yet to be defined, will be mounted by established companies in the Federal Republic of Germany, United Kingdom, Switzerland, and France—but the most formidable challenge will come from established companies in Japan. The Japanese consider biotechnology to be the last major technological revolution of this century (58). More immediate than its promise of helping to alleviate some world problems, biotechnology offers Japan an important opportunity to revitalize its structurally depressed basic industries whose production processes are reliant on imported petroleum.

This chapter provides an overview of U.S. and foreign private sector research and development (R&D) and commercialization efforts in biotechnology to help answer the broader question being addressed by this report: Will the United States be able to translate its present technological lead into worldwide commercial success by securing competitive shares of biotechnology-related product markets? The first section of the chapter provides an overview of the types of companies that are commercializing biotechnology in the United States and the five foreign countries expected to be the major competitors in the area of biotechnology. This section briefly examines the four fields where biotechnology is being applied most vigorously—pharmaceuticals, animal health, plant agriculture, and specialty chemicals. The second section analyzes and compares the strength of the U.S. support base with that of the competitor countries, using three important product areas for comparison: biochemical reagents, instrumentation, and software. The third section analyzes the respective roles of the firms applying biotechnology in the United States—NBFs and established companies—in the domestic and international development of biotechnology. It also describes collaborative ventures between NBFs in the United States and established U.S. and foreign companies that are seeking to commercialize biotechnology. The chapter concludes by summarizing major findings with respect to the role of NBFs and established companies in the U.S. commercialization effort.

*For a summary of activities in biotechnology in countries other than the United States, see *Appendix B: Country Summaries.*

Table 4.—Companies Commercializing Biotechnology in the United States and Their Product Markets[a,b] (Continued)

Company (date founded)	Commercial application of R&D[c]	Ph.D.s[d]
Replicon (1982)	Ph,SCF	
Repligen Corp. (1981)	Ph,AA,CCE,SCF	
Ribi Immunochem Research, Inc. (1981)	AA,Ph	3
Rohm & Haas	PA	
Salk Institute Biotechnology/ Industrial Associates, Inc. (1981)	Ph,AA,CCE	9
Sandoz, Inc.	Ph,PA,AA	
Schering-Plough Corp.	Ph,AA	
SDS Biotech Corp. (1983)	AA	
G. D. Searle & Co.	Ph,SCF	
Serono Laboratories, Inc.	Ph	
SmithKline Beckman	Ph,AA	
E. R. Squibb & Sons, Inc.	Ph	
A. E. Staley Manufacturing Co.	AA,PA,SCF	
Standard Oil of California	Env	
Standard Oil of Indiana	Ph,PA	
Standard Oil of Ohio	PA	
Stauffer Chemical Co.	PA	
Summa Medical Corp.	Ph	
Sungene Technololgies Corp. (1981)	PA	4
Sybron Biochemical	Env	13
Synbiotex Corp. (1982)	Ph,AA	
Syncor International	Ph	
Synergen (1981)	AA,SCF,CCE,Env	21
Syngene Products and Research, Inc.	AA	8
Syntex Corp.	Ph,AA	
Syntro Corp. (1982)	AA,CCE	5
Syva Co. (1966)	Ph	
Technicione International Corp. (1982)	Ph	6
Unigene Laboratories, Inc. (1980)	Ph,AA	12
Universal Foods Corp.	SCF,PA	
University Genetics Co. (1980)		
Genetic Clinics	Ph	
U.O.P., Inc.	SCF,CCE	
The Upjohn Co.	Ph,AA,PA	
Viral Genetics (1981)	Ph	
Wellcome Research Laboratories	Ph	
Worne Biotechnology, Inc. (1982)	PA,CCE,Ph,AA, Env,SCF	10
Xenogen, Inc. (1981)	Ph,PA	
Xoma Corp. (1981)	Ph	
Zoecon Corp. (1968)	PA,AA	
Zymed Laboratories	SCF,CCE	5
Zymos Corp. (1982)	Ph,SCF	

[a]Does not include support firms.
[b]See *Appendix D: Index of Firms in the United States Commercializing Biotechnology* for a description of how the data were collected.
[c]Ph: Pharmaceuticals, PA: Plant Agriculture, AA: Animal Agriculture, SCF: Specialty Chemicals and Food, CCE: Commodity Chemicals and Energy, Env: Environmental (Microbial Enhanced Oil Recovery, Microbial Mining, Pollution Control, and Toxic Waste Treatement), El: Electronics.
[d]As of March 1983.
[e]M.D.s and Ph.D.s.

SOURCE: Office of Technology Assessment.

Table 4.—Companies Commercializing Biotechnology in the United States and Their Product Markets[a,b]

Company (date founded)	Commercial application of R&D[c]	Ph.D.s[d]
Abbott Laboratories	Ph	
Actagen (1982)	Ph	5
Advanced Biotechnology Associates, Inc. (1981)	Ph	
Advanced Genetic Sciences, Inc. (1979)	PA	27
Advanced Genetics Research Institute (1981)	AA	8
Advanced Mineral Technologies, Inc. (1982)	Env	
Agrigenetics Corp. (1975)	PA,SCF	46
Allied Chemical Corp.	PA	
Alpha Therapeutic Corp.	Ph	
Ambico, Inc. (1974)	AA	
American Cyanamid Co.	Ph,PA,AA	
American Diagnostics Corp. (1979)	Ph	
American Qualex (1981)	Ph,AA	
Amgen (1980)	Ph,PA,AA,SCF	45
Angenics (1980)	Ph	5
Animal Vaccine Research Corp. (1982)	AA	
Antibodies, Inc. (1960)	Ph,AA	
Applied DNA Systems, Inc. (1982)	Ph,SCF,CCE,Env	
Applied Genetics, Inc. (1981)	AA	
ARCO Plant Cell Research Institute	PA	18
Atlantic Antibodies (1973)	AA	2
Axonics	Ph	
Baxter-Travenol Laboratories, Inc.	Ph	
Becton Dickinson & Co.	Ph	
Bethesda Research Laboratories, Inc. (1976)	Ph,AA	
Biocell Technology Corp. (1980)	Ph	
Biochem Technology, Inc. (1977)	Bioprocessing	
Bio-con, Inc. (1971)	AA	
BioGenex Laboratories (1981)	Ph	
Biogen, Inc. (1980)	Ph,AA,CCE,Env	79
Biological Energy Corp. (1981)	CCE,SCF	3
Bio Response, Inc. (1972)	Mass cell culture	6
Biotech Research Laboratories, Inc. (1973)	Ph,CCE	11
Biotechnica International, Inc. (1981)	PA,CCE,SCF,Env, AA,Ph	12
Bio-Technology General Corp. (1980)	PA,AA,Ph	5
Brain Research (1968)	Ph	
Bristol-Myers Co.	Ph	
BTC Diagnostic, Inc. (1980)	Ph	3
Calgene, Inc. (1980)	PA	21
California Biotechnology, Inc. (1982)	Ph,AA	21
Cambridge Bioscience Corp. (1982)	Ph,AA	
Campbell Institute for Research & Technology	PA	
Celanese Corp.	CCE	
Cellorgan International, Inc. (1972)	Ph	
Celtek, Inc. (1980)	Ph	5
Centaur Genetics Corp. (1981)	Ph,PA,AA	4
Centocor (1979)	Ph	14
Cetus Corp. (1971)	Ph,AA,CCE	45
Madison (1981)	PA	25
Palo Alto (1980)	Ph	2
Immune (1980)	Ph	
Chiron Corp. (1981)	Ph,AA	26[e]
Ciba-Geigy	Ph	
Clonal Research (1970)	Ph	3
Codon (1980)	CCE	15
Collaborative Genetics, Inc. (1979)	Ph,SCF,CCE	12
Collagen, Inc. (1977)	Ph	
Cooper Diagnostics, Inc.	Ph	
Cooper-Lipotech, Inc. (1981)	Ph	
Corning Glass Works	SCF	

cals. Discussion of U.S. private sector activities in specialty chemicals, commodity chemicals, and the environmental and electronics fields is reserved for the chapters in part III. It is important to recognize that there is no "biotechnology industry." Biotechnology is a set of technologies* that can potentially benefit or be applied to several industries.

The industrial sector in which the earliest applications of biotechnology have occurred is the pharmaceutical sector. Because of the rapid diffusion of the new genetic techniques into pharmaceutical R&D programs, the pharmaceutical sector is currently the most active in commercializing biotechnology. For this reason, the pharmaceutical sector serves as a model for the development of biotechnology in this chapter and in much of this report. It is important to recognize however, that the development of biotechnology in other industrial sectors will differ from its development in the pharmaceutical sector. Regulatory and trade barriers and a marketing and distribution system unique to the pharmaceutical sector limit the applicability of the model to other industrial sectors.

*Pharmaceutical industry***

The pharmaceutical industry is one of the most successful high-technology sectors of the world economy (80). Because research is the foundation of competitive strength for modern pharmaceutical companies (55), and because pharmaceuticals are the first products to which biotechnology has been applied, the first and perhaps most intense proving ground for U.S. competitive strength in biotechnology will be in the area of pharmaceuticals.

U.S. COMPANIES

The first applications of biotechnology have emerged in the area of pharmaceuticals for several reasons. First, rDNA and MAb technologies were developed with public funds directed toward biomedical research. The first biotechnology products—MAb in vitro diagnostic kits, rDNA-produced human insulin, and interferon—are a direct result of the biomedical nature of the basic research that led to these new technologies. Second, pharmaceutical companies have had years of experience with biological production methods, and this experience has enabled them to take advantage of the new technologies. Finally, since some pharmaceutical products, such as large polypeptides and antibiotics, can only be produced by biological methods, there are no competing production methods that might inhibit the application of biotechnology to their production.

Pharmaceuticals are profitable products because they are low volume, high-value-added products.* This and other financial considerations such as the following have led many U.S. companies to apply biotechnology to the pharmaceutical field.

- The time required to develop some pharmaceutical applications of biotechnology, in particular MAb or DNA probe in vitro diagnostic products for humans, is much less than that required to develop other industrial applications (except possibly some animal health applications).
- Many of the pharmaceutical products being developed with biotechnology are replacements for or improvements in pharmaceutical products already on the market, and they can quickly generate income to finance the development of additional products.
- The pharmaceutical industry offers high rates of return on both sales and equity and is thus an attractive and profitable industrial sector into which firms might diversify.
- Many of the biotechnology pharmaceutical markets may be relatively small. Small firms with limited production and financial resources are able to compete more equally with large firms in small product markets rather than in large markets, because economies of scale and costs of marketing in small product markets are small.

*See *Chapter 3: The Technologies*.

**Applications of biotechnology to the area of pharmaceuticals are discussed further in *Chapter 5: Pharmaceuticals*.

*Value added is the value that a company adds to goods and services that it purchases from other companies. It is the difference between the sales revenues and the cost of resources that it has purchased from other companies. For a "high-value-added" product, therefore, the difference between the resources expended to produce the product and the sales revenues generated by the product is greater than average.

Table 4.—Companies Commercializing Biotechnology in the United States and Their Product Markets[a,b] (Continued)

Company (date founded)	Commercial application of R&D[c]	Ph.D.s[d]
Immunetech, Inc. (1981)	Ph	
Immunex Corp. (1981)	Ph	18
Immuno Modulators Laboratories, Inc. (1982)	Ph	
Immunogen (1981)	Ph	
Immunotech Corp. (1980)	Ph	
Imreg, Inc.	Ph	
Indiana BioLab (1972)	PA,AA,SCF,CCE	
Integrated Genetics, Inc. (1981)	Ph	17
Interferon Sciences, Inc. (1980)	Ph	7
International Genetic Engineering, Inc. (Ingene) (1980)	Ph,PA,CCE	16
International Genetic Sciences Partnership (1981)	PA,AA	
International Minerals & Chemical Corp.	AA,PA,Env,CCE	
International Plant Research Institute (IPRI) (1978)	PA	35
Kallestad Laboratories, Inc.	Ph	
Kennecott Copper Corp.	Env	
Lederle Laboratories	Ph,AA	
The Liposome Co., Inc. (1981)	Ph,AA	
Liposome Technology, Inc. (1981)	Ph,AA	
Litton Bionetics	Ph	
3M Co.	Ph	
Mallinckrodt, Inc.	Ph	
Martin Marietta	SCF,PA	
Meloy Laboratories, Inc. (1975)	Ph	
Merck & Company, Inc.	Ph,AA	
Microlife Genetics (1981)	SCF,Env	
Miles Laboratories, Inc.	Ph,SCF,CCE,AA	
Miller Brewing Co.	PA	
Molecular Biosystems, Inc. (1980)	Ph	7
Molecular Diagnostics (1981)	Ph	
Molecular Genetics, Inc. (1979)	Ph,PA,AA	20
Monoclonal Antibodies, Inc. (1979)	Ph,AA	7
Monsanto Co.	PA,AA	
Multivac, Inc.	Ph,PA,AA,SCF	
Nabisco, Inc.	PA	
National Distillers & Chemical Co.	CCE	
NPI (1973)	PA,CCE,SCF	25
Neogen Corp. (1981)	PA,AA	
New England Biolabs	Ph	
New England Monoclonal Resources (1982)	Ph	
New England Nuclear Corp.	Ph	
Norden Laboratories	AA	
Novo Laboratories, Inc.	Ph,SCF	
Nuclear & Genetic Technology, Inc. (1980)	Ph	
Ocean Genetics (1981)	SCF	
Oncogen (1982)	Ph	
Oncogene Science Inc. (1983)	Ph	
Organon, Inc.	Ph	
Ortho Pharmaceutical Corp.	Ph	
Petrogen, Inc. (1980)	Env	7
Pfizer, Inc.	Ph,PA,CCE,AA, SCF,Env	
Phillips Petroleum Co.	Env,SCF,CCE	
Phytogen (1980)	PA	5
Phyto-Tech Lab	PA	
Pioneer Hybrid International Corp.	PA	
Plant Genetics, Inc. (1981)	PA	11
Polybac Corp.	Ph,SCF,Env	
PPG Industries	SCF	
Purification Engineering, Inc.	Bioprocessing	
Quidel Home (1982)	Ph	

number of foreign firms (80). At least one study has suggested that substantially fewer U.S.-originated new chemical entities will appear on the market in the mid to late 1980's than are appearing today because of a decline in self-originated investigational new chemical entities since the mid-1970's (83). Table 6 indicates the number of new pharmaceutical products introduced by the United States, four European countries, and Japan in the period 1961-80 and each year since. As the figures in that table show, the United States and France were the leaders in 1961-80, with 23.6 and 18.1 percent of new product introductions, respectively. They were followed by West Germany, Japan, Switzerland, and the United Kingdom. The world leader for the years 1981-83 is Japan, with an average of 27 percent of new product introductions. Although the United States had an average of only 16 percent of new product introductions for the years 1981-83, the drive by NBFs and established U.S. companies to apply biotechnology to the development and production of pharmaceuticals could help reverse the downward trend in U.S. innovation and thereby contribute to the competitive strength of U.S. companies in world pharmaceutical markets.

FOREIGN COMPANIES

Established European and Japanese companies, following the lead of NBFs and established companies in the United States, are now vigorously pursuing pharmaceutical applications of biotechnology.* On average, European companies' biotechnology R&D budgets lag somewhat behind the budgets of established U.S. companies and some U.S NBFs as well (see table 7). As biotechnology processes gain wider acceptance in the pharmaceutical industry, however, European manufacturers—e.g., the West German companies Bayer AG and Hoechst, the Swiss companies Hoffmann-La Roche, Ciba Geigy, Sandoz, and the French company Rhone Poulenc—are expected to challenge U.S. companies, if for no other reasons than their prevailing strength in bioprocessing, their strength in international pharmaceutical markets (see table 5)* and their intentions to maintain this strength,

*Japanese companies are though to have begun making a serious commitment to biotechnology as early as late 1981 (70). West German companies were among the last European companies to begin commercializing biotechnology and did not intensify their R&D efforts in biotechnology until late 1982. Other European countries have paralleled the Japanese in their date of entry into biotechnology.

*Although no British pharmaceutical companies appear in table 5, British companies such as Beecham, Wellcome, Glaxo, and ICI are important international manufacturers of biologically produced products and are applying biotechnology to product development. Additionally, Beecham and Glaxo are among the world's largest producers of biologically made products (48).

Table 7.—Biotechnology R&D Budgets for Leading U.S. and Foreign Companies, 1982[a]

Company[b]	Biotechnology R&D budget (millions of dollars)
Hoechst (F.R.G.)	$4.2[c]
Schering A.G. (F.R.G.)	4.2
Hoffmann-La Roche (Switz.)	59
Schering-Plough (U.S.)	60
Eli Lilly (U.S.)	60
Monsanto (U.S.)	62
DuPont (U.S.)	120
Genentech (U.S.)*	32
Cetus (U.S.)*	26
Genex (U.S.)*	8.3
Biogen (U.S.)*	8.7
Hybritech (U.S.)*	5
Sumitomo (Japan)	6+
Ajinomoto (Japan)	6+
Suntory (Japan)	6+
Takeda (Japan)	6+
Elf-Aquitaine (France)	4+

[a]Biotechnology R&D figures for British companies not available.
[b]Companies with asterisks are NBFs.
[c]1983 figure.

SOURCE: Office of Technology Assessment.

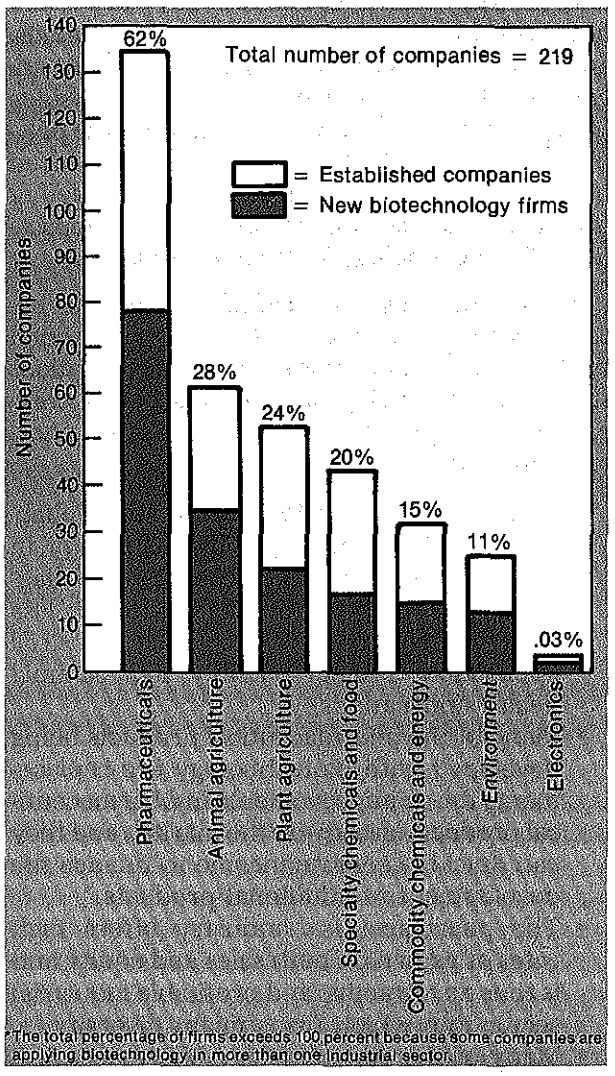
Table 6.—Introduction of New Pharmaceutical Products by Country of Origin Between 1961 and 1983

Country	Number of new products introduced by year[a]			
	1961-80	1981	1982	1983 (est.)
Japan	155 (10.3%)	15 (23.1%)	9 (23.1%)	17 (35.4%)
West Germany	201 (13.4%)	8 (12.3%)	1 (2.6%)	7 (14.6%)
United States	353 (23.6%)	9 (13.9%)	9 (23.1%)	6 (12.5%)
France	271 (18.1%)	3 (4.6%)	5 (12.8%)	5 (10.4%)
United Kingdom	— (—)	3 (4.6%)	— (—)	3 (6.2%)
Switzerland	109 (7.3%)	6 (9.2%)	4 (10.2%)	— (—)

[a]Numbers in parentheses indicate share of total number of new pharmaceutical products introduced for the years indicated.

SOURCE: Nomura Research Institute, "Trends of Biotechnology in Japan," Tokyo, July 1983.

Figure 10.—Percentage of Firms in the United States Pursuing Applications of Biotechnology in Specific Industrial Sectors*



*The total percentage of firms exceeds 100 percent because some companies are applying biotechnology in more than one industrial sector.

SOURCE: Office of Technology Assessment.

NBFs in Japan will retard those countries' development of biotechnology. Varying strategies, organizational differences, and cultural factors all contribute to the competitive strengths of foreign countries' established companies. It is important to note, however, that the complementary efforts of NBFs and established companies in the United States have been a major factor in providing the United States with an early competitive advantage in the commercialization of biotechnology.

Although there are few NBFs outside the United States at present, some European countries are beginning to sense that small firms can make important contributions to innovation, particularly in high-technology fields such as biotechnology. Thus, in contrast to the West German Government, which believes that the development of biotechnology in West Germany is the province of the large chemical companies for which the country is noted and that NBFs are "not in line with the German mentality" (5), the British and French Governments have aided in the establishment of small firms such as Celltech, (U.K.), Agricultural Genetics (U.K.), and Transgene (France's leading biotechnology venture company).

Efforts in support of small company formation are also being undertaken by organizations elsewhere in Europe. The Organisation for Economic Co-Operation and Development, for example, in an effort to spur technological innovations, has made several proposals designed to support small firm development (65). These proposals encompass the promotion of new sources of venture capital, assistance to new startups in developing high quality feasibility studies, and diverse measures to encourage high-technology startups.

Venture capitalization is almost exclusively an American phenomenon (5,69). Many would agree that the formation of venture capital and entrepreneurial drive necessary to start small high-technology firms and vigorously commercialize inventions has been inhibited in much of Europe by a historical labor attitude that gives priority to job security and a predictable business environment rather than to aggressive risk-taking. In Japan, individualism and the creation of small, entrepreneurial and independent high-technology firms appears to be discouraged by cultural traits emphasizing group identity and acceptance. Large, very successful firms typical of Japan provide workers with a group identity and a sense of security, and it is these firms that are commercializing biotechnology in that country.

The biotechnology-related activities of U.S. and foreign companies in the pharmaceutical and animal and plant agriculture sectors are introduced below. Also discussed are foreign companies' biotechnology-related activities in specialty chemi-

maceutical company that is applying biotechnology to human and animal health in areas including diagnostics, neuropeptides, serums, vaccines, and antibiotics, and has established Elf-Bioindustries and Elf-Bioresearch to develop biotechnology in the foodstuffs and agriculture sectors. To support some of its new biotechnology R&D, Elf is currently building a $10 million "genetic engineering" plant (5). Rhone Poulenc is the world's second largest producer of animal health products (84) and is considered to be the second most committed of the three French companies actively commercializing biotechnology (50). To support its biotechnology effort, in 1980, Rhone Poulenc established a small specialty biotechnology subsidiary named Genetica.

Despite the efforts of companies such as Elf and Rhone Poulenc, the initial hesitation France expressed in the early stages of biotechnology development has put French companies at a distinct disadvantage internationally, particularly vis-a-vis U.S. companies. The French Government has a formal policy designed to promote biotechnology, but it is not clear that whatever impetus this policy provides will be great enough to compensate for France's slow entry into biotechnology. Historically, the French Government's plans to promote national champions (e.g., the Plan Calcul, the Concord) have failed. As the pace of biotechnology commercialization quickens, a strong private sector effort may be necessary in order to launch France into a more competitive position.

Overall, Europe is considered to be farther behind the United States in the application of biotechnology to product-related research areas than in fundamental research (23). Strong commercialization efforts by the major chemical companies of West Germany or by the pharmaceutical companies of Switzerland or the United Kingdom, however, could significantly improve West Germany's, Switzerland's, or the United Kingdom's current competitive positions in the commercialization of pharmaceutical applications of biotechnology.

Some would argue that large companies have an inertia that is difficult or impossible to change, making rapid changes in research policy and direction impracticable (5). To the extent that large companies pursuing pharmaceutical applications of biotechnology in Europe lack the dynamism and flexibility to compete with the combined efforts of NBFs and established companies in the United States, Europe could initially be at a competitive disadvantage. If the timing of market entry for therapeutic and diagnostic products becomes the most important factor in competition for market share and market acceptance, however, the marketing strength of the European multinationals could help balance competition in pharmaceuticals between the United States and Europe.

The potential competitive challenge that will be mounted by Japan in the area of pharmaceuticals is more difficult to estimate than the challenge from the European countries for two reasons: 1) Japanese pharmaceutical companies such as Takeda, Sumitomo Chemical, Mitsubishi Chemicals traditionally have not had a significant presence in world pharmaceutical markets (55); and 2) present Japanese commercialization efforts, most being proprietary, are difficult to assess either quantitatively or qualitatively. One set of factors characterizing Japanese efforts to apply biotechnology to pharmaceutical development suggests a rather formidable challenge facing U.S. companies in future biotechnology-related pharmaceutical markets, while a different set of factors suggests less of a future challenge. Each set of factors is discussed in turn below.

Factors that suggest that Japan will have international competitive advantages in the application of biotechnology to pharmaceutical development include the following:

- The application of biotechnology to pharmaceuticals in Japan has stimulated the involvement in pharmaceuticals of many Japanese companies from a broad variety of bioprocess-based industries. Table 9 shows the diversification of Japanese chemical, food processing, and textile and pulp processing companies into pharmaceuticals.

A 1982 Keidanren* survey of 132 Japanese companies using biotechnology found that 83 percent

*Keidanren, the Japan Federation of Economic Organizations, is a national organization composed of about 700 of the largest

U.S. pharmaceutical companies are quite active internationally. Table 5 illustrates the distribution of sales by the top 20 U.S. and foreign pharmaceutical companies in 1981. Sales by the U.S. companies listed represented almost 60 percent of the total pharmaceutical sales for the top 20 pharmaceutical companies in the world. On the average, almost 42 percent of the sales by these U.S. companies were foreign sales. According to the Institute for Alternative Futures, foreign sales accounted for roughly 43 percent of total U.S. prescription drug sales in 1980 (45), and U.S. pharmaceutical subsidiary sales in foreign countries exceeded $10 billion in 1980.* Given established U.S. pharmaceutical companies' strong export performance in the past, the U.S. posture in world pharmaceuticals markets will be a subject of great interest as biotechnology develops.

Up until about 1976, the average participant in the U.S. pharmaceutical industry could be described as a research-based, integrated, multinational company that spent (and still does) approximately 11.5 percent of its annual pharmaceutical sales on R&D (67). Since about 1976, the profile of the participants has changed considerably. Approximately 70 new U.S. companies have entered the pharmaceutical field just to apply biotechnology. Many of these NBFs are wagering their existence on the success of commercial pursuits of biotechnology in nascent pharmaceutical product markets. In total, about 135 U.S. companies—78 NBFs and 57 established companies—are known to be pursuing pharmaceutical product and process development using biotechnology.*

Since the early 1960's, the U.S. share of world pharmaceutical research, innovation, production, sales, and exports has declined, as has the number of U.S. companies actively participating in the various ethical drug markets compared to the

*This figure is from a survey of Pharmaceutical Manufacturers Association member companies that had not been published as this report went to press.

*The high level of U.S. firms' interest in pharmaceutical applications of biotechnology is in part a reflection of the large number of old and new firms producing MAbs. Many companies included in table 4 are using hybridoma technology to produce MAbs for the markets traditionally addressed by the pharmaceutical industry. In some cases, OTA did not have sufficient information to determine the specific application for MAbs. For example, some companies indicated that they were engaged in the production of MAbs, but would not specify their intended use (i.e., research, separation and purification, diagnostic or therapeutic products for humans, animals, or plants). Because a majority of firms producing MAbs are manufacturing MAbs for pharmaceutical use, OTA placed firms for whom data were incomplete in the pharmaceutical sector, even though hybridoma technology is also essential to fundamental molecular research on plants, animals, and bacterial systems.

Table 5.—Distribution of Sales by the Top 20 U.S. and Foreign Pharmaceutical Companies, 1981

Company	Home country	Percent of sales in home country	Percent of sales in other countries	1981 total pharmaceutical sales (millions of dollars)	Share of pharmaceutical sales
American Home Products	U.S.	66%	44%	$2,303	
Merck	U.S.	53	47	2,266	
Bristol-Myers	U.S.	71	29	2,190	
Warner Lambert	U.S.	55	45	2,045	
SmithKline Beckman	U.S.	59	41	1,782	
Pfizer	U.S.	43	57	1,777	
Eli Lilly	U.S.	62	38	1,664	58%
Johnson & Johnson	U.S.	56	44	1,308	
Upjohn	U.S.	62	38	1,242	
Abbott	U.S.	65	35	1,182	
Schering-Plough	U.S.	51	49	924	
Hoechst	F.R.G.	28	72	2,555	
Bayer	F.R.G.	24	76	2,400	19%
Boehringer-Ingleheim	F.R.G.	37	63	1,197	
Ciba-Geigy	Switz.	2	98	1,891	
Sandoz	Switz.	5	95	1,515	16%
Hoffmann-La Roche	Switz.	3	97	1,629	
Takeda	Japan	94	6	1,195	4%
Rhone-Poulenc	France	41	59	1,008	3%

SOURCE: Adapted from Arthur D. Little, estimates based on publicly available company data.

Table 10.—Japanese Joint Ventures in Pharmaceutical Applications of Biotechnology

Companies	Product area
Otsuka Pharmaceutical/Hayashibara/Mochida Pharmaceutical	Production of alpha, beta, and gamma interferon
Yamanouchi Pharmaceutical/Ajinomoto	Large-scale production of thrombolytic agent
Yoshitomo Pharmaceutical/Takeda Chemical	Large-scale production of thrombolytic agent
Ajinomoto/Morishita Pharmaceutical	R&D on pharmaceuticals
Yoshitomi Pharmaceutical Industries, Ltd./ Yuki Gosei Kogyo Co., Ltd.	Developing rDNA products for circulatory system
Takara Shuzo/Taiho Pharmaceutical	Development of heart drugs using rDNA
Toray Industries/Kyowa Hakko Kogyo/Gan Kenkyu Kai (Cancer Research Association)	Development of beta and gamma interferon by rDNA
Asahi Chemical Industry/Dainippon Pharmaceutical/Tokyo University	R&D on alpha and gamma interferon
Toray Industries/Daiichi Seiyaku Co., Ltd.	Using rDNA to produce gamma interferon
Ajinomoto/Takeda Chemical Industries, Ltd.	Development of interleukin-2
Asahi Chemical Industries Co., Ltd./ Dainippon Pharmaceutical Co.	Development of tissue necrotic factor

SOURCE: Office of Technology Assessment.

that the separate approach being taken was costly both in terms of funds expended and time taken (73). Many other examples of technical collaboration in biotechnology in Japan can be cited, and many more Japanese companies have intentions to cooperate with one another in research or development and/or in commercialization in the future. In 1981, a scientist from the Fermentation Research Institute of Japan's Ministry of International Trade and Technology acknowledged that almost half of the companies who work or intend to work in "genetic engineering" will cooperate or have already cooperated in some biotechnology activities (79). Joint ventures such as those listed in table 10 might provide Japanese companies with commercial advantages for two reasons: 1) each firm participating in the venture brings different resources and expertise to the project, thereby making the group effort more efficient; and 2) the intention of some of the joint ventures is to secure patents in fields not yet pre-empted by foreign competition (e.g., new host-vector systems and sophisticated sensors for bioprocessing) or to undertake joint clinical testing (70).

- Japan's share of world pharmaceutical R&D expenditures has been increasing steadily since 1964 (see table 8) as has its share of the worldwide total of newly introduced pharmaceuticals (see table 6).

In 1981, Japanese companies ranked first in terms of the largest number of major new drugs introduced into world markets, being responsible for 15 (23 percent) of the 65 newly introduced pharmaceuticals (see table 6). In 1982, Japanese companies again accounted for roughly 23 percent of the new pharmaceuctical products introduced. They also accounted for over 16 percent of all U.S. patents issued for pharmaceutical and medicinal products and for 38 percent of all U.S. pharmaceutical and medicinal patents granted to foreign firms (14).

- Japanese companies applying biotechnology to pharmaceutical development (in contrast to U.S. companies) appear to be dedicating relatively more research effort to the later stages of commercialization (i.e., bioprocessing) and cancer treatment. Seventy-five percent of all Japanese medical and drug companies are engaged in MAb research, and a large proportion of the MAb R&D is targeted toward developing a "magic bullet" for cancer treatment, monitoring bioprocesses, and recovering proteins (70).

Factors that suggest that the Japanese may not have significant advantages in future biotechnology-related pharmaceutical markets include the following:

- Barriers to entering foreign pharmaceutical markets are high, and Japanese companies at present have neither distribution channels in place nor a sufficient sales force to permit aggressive marketing of pharmaceutical products in Western markets.

Japanese companies' lack of distribution channels in Western pharmaceutical markets is one fac-

and their increasing shares of worldwide pharmaceutical R&D expenditures as compared to U.S. companies. (Pharmaceutical R&D expenditures by country for the years 1964, 1973, and 1978 are shown in table 8).

The average European company's involvement in biotechnology is largely characterized by research contracts with universities and research institutes rather than by investments in new in-house biotechnology facilities.* Some of the large pharmaceutical companies of Switzerland have, however, begun to make substantial investments in biotechnology facilities. Hoffmann-La Roche, for example, spent $59 million on biotechnology R&D in 1981 (26) and ranks eleventh in worldwide pharmaceutical sales (28). Ciba-Geigy, which commands 3.1 percent of the global drug market, is building a $19.5 million biotechnology center in Switzerland and a $7 million agricultural biotechnology laboratory in North Carolina (11,12).

West German chemical and pharmaceutical companies have been among the last foreign companies to move into biotechnology. Many of the companies have signed contracts with universities instead of investing in facilities to support their research (10). Some West German companies, including Schering AG and Boehringer Ingleheim, however, are making significant contributions to the German biotechnology effort. Schering AG, for example, in a joint agreement with the State of Berlin is establishing a $10.7 million institute of "genetic engineering," which is regarded as an important step for biotechnology research in Germany (29).

In terms of total sales, pharmaceutical companies in the United Kingdom are not among the world's top 20, and historically, the United Kingdom has been slow to commercialize the results of much of its basic research. It is important to note, however, that some British pharmaceutical companies (e.g., Glaxo and Beecham) possess substantial bioprocessing knowledge, a capability that may provide them with a competitive advantage as biotechnology develops. Furthermore, some British pharmaceutical companies have made in-house investments in biotechnology. ICI and Wellcome appear to be among the most strongly committed of the British pharmaceutical companies commercializing biotechnology. ICI, for example, has the world's largest continuous bioprocessing plant and is considered an international leader in bioprocessing technology. This company recently developed a new biodegradable thermoplastic polyester, Biopol®, formed by a genetically manipulated micro-organism. Although Biopol® is not a pharmaceutical, it does give some indication of ICI's innovative capacity in the biotechnology field.

The pharmaceutical and chemical companies of France appear less aggressive than British companies in developing biotechnology expertise. Three major French companies have R&D programs in biotechnology—Elf Aquitaine (67-percent Government-owned), Rhone Poulenc (100-percent Government-owned), and Roussel Eclaf (40-percent Government-owned and a Hoechst subsidiary). Of these three, Elf Aquitaine has committed the most to biotechnology. It owns Sanofi, a phar-

*Many of the established U.S. companies have made substantial investments in new in-house facilities. See section below on "Established Companies."

Table 8.—Pharmaceutical R&D Expenditures by Country: 1964, 1973, and 1978

	1964		1973		1978	
	Level (millions of dollars)	World share (percentage)	Level (millions of dollars)	World share (percentage)	Level (millions of dollars)	World share (percentage)
United States	$282	60%	$640	34%	$1,159	28%
Federal Republic of Germany	40	8	310	16	750	18
Switzerland	38	8	244	13	700[a]	17
Japan	27	6	236	13	641	15
France	28	6	166	9	328	8
United Kingdom	29	6	105	6	332	8

[a] Estimated.
Note: Data are in current dollars and represent expenditures for both human and veterinary research.
SOURCE: National Academy of Sciences, *The Competitive Status of the U.S. Pharmaceutical Industry*, Washington, D.C., 1983.

foreign pharmaceutical and chemical companies.* Most of these companies have global marketing and distribution networks and undertake animal drug production as a diversification of their principal activities. In recent years, the advent of biotechnology, the rising industrialization of animal agriculture, and changing dietary habits in foreign countries have increased the demands for improvements in old products and for completely new products. NBFs may have a major role to play in expanding animal health markets.

Sixty-one companies in the United States are known to be pursuing animal health related applications of biotechnology, as shown in table 4. Thirty-four (56 percent) of these companies are NBFs. Of special note is the role new firms appear to be playing in three major segments of the industry—diagnostic products, growth promotants, and vaccines. Possible explanations for why some NBFs might be interested in these three animal health markets include the following:

- Recombinant DNA methods used to make human vaccines are suited to making safe and effective animal vaccines against both viral and bacterial infections, just as the MAb or DNA probe technology used to produce human products is suited to making passive vaccines or diagnostic products for animals.**
- The markets for many animal health products (e.g., vaccines or diagnostic products) are relatively small and therefore allow NBFs to compete equally with larger companies without suffering from scale disadvantages.
- The commercial introduction of veterinary vaccines can generally be achieved more quickly than can that of human therapeutic products. The regulatory process allowing veterinary vaccines to enter the market typically can be completed in about 1 year (17). Thus, the lower costs of commercialization for veterinary vaccines in comparison to human pharmaceuticals and the potential for short-term product revenues may reduce NBFs' financial need to collaborate extensively with established companies.*
- Some veterinary vaccine research (e.g., on feline leukemia vaccines) could serve as a model for developing human vaccines for similar viruses that could launch some NBFs into the more profitable human pharmaceutical markets.

The fact that 34 of the 61 U.S. companies pursuing applications of biotechnology in animal agriculture are NBFs suggests the evolution of an expanding animal health market in which NBFs such as Molecular Genetics, Inc. (MGI), Amgen, Chiron, Bio-Technology General and Cetus perceive opportunities. In contrast to human pharmaceutical products, animal vaccines and diagnostic products are in many cases being developed by NBFs independently of established U.S. or foreign companies.

In the development of animal growth promotants, however, established U.S. companies are more involved. The market for animal growth promotants is the second fastest growing market in the animal health field, and because it may be the most significant commercial development area (26), it is also one of the most competitive. Global sales for growth promotants are expected to reach $515 million by 1985 (84). Several established U.S. companies, including American Cyanamid, Eli Lilly, Monsanto, and Norden (a subsidiary of SmithKline Beckman), have displayed interest in the field by sponsoring research contracts with NBFs such as MGI, Biotechnica International, Genentech, and Genex. Other established U.S. companies have shown interest by conducting initial evaluations of growth promotants developed by NBFs, as Eli Lilly did in the case of a product developed by the NBF BioTechnology General.

In an effort to expand their own technical capabilities and reach new product markets, some es-

*Major U.S. producers of animal health products include Syntex, Pfizer, Eli Lilly, Upjohn, SmithKline Beckman, American Cyanamid, Merck, Johnson & Johnson, Tech America, and Schering-Plough. Major foreign producers include Burroughs-Wellcome (U.K.), Rhone-Merieux (France), Hoechst AG (F.R.G.), Bayer AG (F.R.G.), Connaught (Canada), Beecham (U.K.), Solvay (Belgium), Boehringer Ingleheim (F.R.G.), Intervet (Netherlands), and Elf Aquitaine (France).

**The NBFs Chiron and Cetus both became involved in the veterinary products business as extensions of their research in the field of human health care (17,20). The NBF Monoclonal Antibodies, Inc., as a spinoff from research on detection kits for human pregnancy and ovulation, is developing an ovulation detection kit for large animals which will be useful in animal breeding management.

*Collaborative ventures between NBFs and established U.S. and foreign companies are discussed further below.

Table 9.—Diversification of Japanese Chemical, Food Processing, Textile, and Pulp Processing Companies Into Pharmaceuticals

Company	Pharmaceutical field of entry
Chemical companies:	
Sunstar	Antibiotics, interferon
Hitachi Chemical	Antibiotics, vaccines
Hokko Chemical Industry	Antibiotics
Mitsubishi Chemical Industries	Physiologically active agents, anticancer drugs, diagnostic reagents, monoclonal antibodies
Denki Kagaku Kogyo	Physiologically active agents
Sumitomo Chemical	Monoclonal antibodies, interferon, growth hormone
Daicel	Anticancer drugs
Mitsubishi Petrochemical Industries	Diagnostic reagents
Chisso	Diagnostic reagents
Mitsui Toatsu Chemical	Urokinase
Food processing companies:	
Ajinomoto	Antibiotics
Suntory	Antibiotics, interferon, anticancer drugs, drugs for treatment of high blood pressure
Meiji Seika Kaisha	Antibiotics, interferon
Sanraku-Ocean	Antibiotics
Kikkoman Shoyu	Physiologically active agents, antibiotics, immune suppressors
Takara Shuzo	Physiologically active agents
Meiji Milk Products	Physiologically active agents, interferon
Yakult Honsha	Physiologically active agents, anticancer drugs, diagnostic reagents for liver cancer
Kyowa Hakko Kogyo	Physiologically active agents, interferon
Kirin-Seagrams	Interferon
Kirin Brewery	Anticancer drugs
Sapporo Breweries	Anticancer drugs
Toyo Jozo	Immune suppressors
Morinaga & Co.	Diagnostic reagents for liver cancer, drugs for treatment of high blood pressure
Snow Brand Milk Co.	Interferon
Textile and pulp companies:	
Asahi Chemical Industry	Interferon
Toray Industries	Interferon
Teiji Limited	Interferon
Kirin-Seagrams	Interferon

SOURCE: Office of Technology Assessment.

Japanese companies. It enjoys the regular and active participation of the top business leaders working closely with a large professional staff to forge agreements on behalf of business as a whole. It often surveys its members on issues of economic importance.

of the 60 companies that responded were pursuing applications in the area of pharmaceuticals (70), compared to only 62 percent of U.S. companies (see table 4). Intensified competition is expected to push technical advances in the area of pharmaceuticals along in Japan at a rate that is comparable to or greater than the rate in the United States. Among the companies using biotechnology in Japan, it is already a widely accepted view that Japan can catch up with the United States within 5 years. This point is very well illustrated by the *Nikkei Sangyo Shimbun* (Japan Industrial Daily) survey undertaken in June 1981. According to the survey, 48 percent of the 128 responding firms thought Japan could catch up to the United States in the commercial development of biotechnology in 5 years, and 24 percent estimated that catching up would take only 2 to 3 years (57).

- The Government of Japan, which has targeted the pharmaceutical industry for international expansion, has improved the environment for pharmaceutical innovation, and thus, for the application of biotechnology.

The Japanese Government through targeting of the pharmaceutical industry, changes in patent laws to prevent imitation, and pricing policies in the Government-administered national health insurance system has begun an effort to coordinate trade, pricing, and health care policies to promote pharmaceutical innovation and overseas expansion (74). These Government efforts are expected to facilitate the application of biotechnology in the Japanese pharmaceutical industry.

- Joint pharmaceutical research projects and collaborative arrangements among companies, sometimes in conjunction with Government research institutions, promote biotechnology transfer throughout Japanese industry and accelerate the pace of technical advances. Table 10 provides a list of some Japanese joint ventures in pharmaceuticals derived from the Keidanren survey of 1982.

As early as 1979, the Japanese Ministry of Health set up a study group between Green Cross and Toray Industries to speed the development of interferon, because the Ministry had concluded

Table 11.—Applications of Biotechnology to Plant Agriculture for Seven New Biotechnology Firms

Advanced Genetic Sciences, Inc.:
—Development of plant varieties with increased resistance to disease, stress, herbicides and pests, and tolerance to extreme weather conditions
—Development of antagonistic bacteria that do not contain ice nucleation properties to optimize frost protection
Biotechnica International:
—Improvement of nitrogen-fixing capability of *Rhizobium*
—Introduction of nitrogen-fixing capability in plants that rely on fertilizer
—Herbicide resistance in selected plants
—Improved protein content in alfalfa
Bio-Technology General Corp:
—Development of a biofertilizer, *Azospirillum*
—Development of several strains of trichoderm, a micro-organism that controls soil-borne fungi that cause damage to many plants.
International Genetic Engineering (Ingene):
—Modification and production of bacteria that are lethal to four specific weeds and three groups of insects
—Production in micro-organisms of plant growth regulators—hormones that affect many biological functions including flowering, fruit ripening, and water loss.
—Modification of organisms that are responsible for ice nucleation in an effort to interfere with the organisms' ability to adhere to plants
Cetus Madison:
—Development of soybean and corn hybrids to increase vigor
—Development of microbial inocculant for corn, soybean, cotton, wheat, and rice to protect plants against fungal and insect diseases and to increase plant growth through nitrogen fixation and other biological processes
—Exploration of ways to add genes to make plants unpalatable to insect pests and to make plants resistant to diseases
Ecogen, Inc.:
—Development of microbial and viral pesticides
Molecular Genetics, Inc.:
—Development of herbicide-resistant corn and nutritionally enhanced field corn

SOURCE: Company prospectuses and annual reports.

to develop markets for broad-spectrum herbicides that might not otherwise be used. Some U.S. chemical companies may be investing in plant-related biotechnology to compensate for possible reductions in future sales due to the development of plants that do not require chemicals (e.g., plants that fix nitrogen, plants that produce pesticides) or due to competition from microbial insecticides or nonchemical treatments (30). Some pharmaceutical companies may invest in plant-related biotechnology, for example, to seek new sources of therapeutically active substances or to develop a commercial process for producing secondary products from plants (e.g., morphine and codeine).

One route by which some established U.S. companies have entered the plant agriculture field is through the acquisition of seed companies. Seed companies provide both an in-place marketing system and high-quality, commercially successful gene pools, often representing as much as 10 to 20 years of R&D. Through their ownership of seed companies and investments made both in-house and through sponsored research with NBFs, some established companies are assuming active roles in the modern research impetus for seed improvement. By assuming stronger roles in basic plant science research, U.S. companies like ARCO, Shell, Allied, Monsanto, and DuPont hope to play a leading role in the development of future agriculture markets.

FOREIGN COMPANIES

The commercialization of plant-related biotechnology is occurring more slowly in the European competitor countries than in the United States. For example, most West German plant tissue culture research is going on in universities (6). Some of the large European pharmaceutical companies are reportedly interested in plant tissue culture, but only Boehringer Mannheim (F.R.G.) and the Society for Biotechnology Research (GBF, Gesellschaft für Biotechnologische Forshung) have made their interests public. Boehringer Mannheim is also engaged in research to produce digitalis using immobilized plant cells (10). Although excellent basic research is conducted in centers such as the Max Planck Institute for Plant Research in Cologne,* few commercial pursuits are known.

Great Britain possesses some of the strongest basic research in interdisciplinary plant sciences and recently a new firm launched by the British Technology Group, Agricultural Genetics, was established to exploit discoveries made at the Agricultural Research Council. Whether or not the basic research will be commercialized successfully is difficult to predict.

*Bayer signed a 3-year agreement with the Max Planck Institute for research in plant cultivation with special attention to rDNA to improve plant resistance to phytotoxins.

tor that has limited Japanese companies' ability to penetrate these markets. It is expected that the mode by which Japanese companies will penetrate these markets in the future will be through joint ventures with U.S. or European companies that allow Japanese companies to take advantage of existing distribution channels.* Although Japanese companies tend to seek opportunities to penetrate foreign markets directly through manufacturing subsidiaries rather than through licensing contracts, only two Japanese companies have established equity joint ventures with U.S. firms** and only three have established U.S. subsidiaries.*** However, the international expansion of Japan's pharmaceutical industry has only just begun.

- Almost half of the Japanese companies now using biotechnology expect to "catch up" technologically to the United States in 5 years. These companies therefore intend to set their own R&D and commercialization targets beyond the 5-year catch-up period at considerable commercial risk.

The intention of Japanese companies to catch up to U.S. companies and to set their own R&D targets is a unique phenomenon. In the past, even in high- technology fields such as computers and electronics, the R&D and commercialization targets have been demonstrated in advance by U.S. and Western European companies, so Japanese companies have not had to worry about the marketability of their R&D and commercialization efforts. By selecting the best technology available and refining it, Japanese companies have been able to minimize the time required to catch up with the front runners and sometimes surpass them at the product marketing stage (70). Given the lack of established commercial targets in biotechnology and considering the barriers to entering foreign pharmaceutical markets mentioned above, it cannot be assumed that the Japanese will be major competitors in biotechnology-related pharmaceutical markets.

- Japan's traditional bioprocess-based industries, including pharmaceuticals, rely largely on conventional microbiology, genetics, and bioprocess feedstocks. These traditional approaches in bioprocessing could be challenged by new biotechnology (41).

Japan is considered to be behind the United States in fundamental biology. This weakness in fundamental biology could reduce the potential competitive threat of Japanese companies applying biotechnology to pharmaceutical development.

- Biotechnology R&D investments by Japanese companies are still low in comparison to the investments by U.S. companies.

Although Japan's aggregate investment in pharmaceutical R&D has increased steadily since 1964, investments by individual Japanese companies in biotechnology R&D are still low compared to investments by NBFs and established companies in the United States (see table 7). According to the *Nikkei Sangyo Shimbun* survey (June 1981) and the Keidanren survey (1982), only 5 Japanese companies spent more than $6 million per year on biotechnology R&D. The average R&D expenditure of 49 of the 60 Japanese companies that responded to the Keidanren survey was under $1 million. Although it is difficult to translate R&D investment into commercial success, on a quantitative basis, Japan falls far behind the United States in terms of industrial expenditures on biotechnology research.

*Animal agriculture industry**

U.S. COMPANIES

The animal agriculture industry encompasses companies engaged in the manufacture of products, the prevention and control of animal diseases, animal husbandry, growth promotion, and genetic improvement of animal breeds. The companies that dominate the production of most animal health products are established U.S. and

*In support of this expectation is a study by the Japanese Productivity Center in 1982 of the potential for Japanese drug firms in the United States. The study estimated that the establishment of a U.S. subsidiary by a Japanese company would require an investment of about $80 million over a 4-year period. The study recommended that Japanese companies form joint ventures with U.S. companies rather than establish a Japanese company or purchase a U.S. company (75).

**Takeda with Abbott (U.S.) and Fujisawa with SmithKline (U.S.).

***The three U.S. subsidiaries are Daiichi Pharmaceutical Corp., Otuska Pharmaceutical, and Alpha Therapeutics (subsidiary of Green Cross).

*Applications of biotechnology to animal agriculture are discussed further in *Chapter 6: Agriculture*.

of Bayer's specialty chemical research is taking place in the United States through these two subsidiaries. Bayer has opted for specialty chemicals as its main R&D focus; Miles is important in the enzyme and organic acid field using bioprocessing, and Cutter is expanding its R&D activity in purifying enzymes and proteins on a large scale (10). Two other German companies, Schering AG and BASF AG, are also actively applying biotechnology to the production of specialty chemicals. Schering's main research focus is on the genetic manipulation of micro-organisms to produce amino acids such as lysine (10), and BASF is building a $24 million "Biotechnicum," a combination of research laboratory and pilot plant with a product focus on optically active intermediate chemicals and vitamins. Schering has also signed two research agreements with Genex, one of which involves the development of a genetically manipulated microbe to produce an amino acid.

U.S. and foreign support firms

Companies engaged in biotechnology research have increased and expanded the demands placed on the infrastructure that has traditionally supplied biochemical reagents, instrumentation, and software for biological research and production. As "scaled-up" production of biotechnology products comes on line, the demand for these supplies as well as for new production instrumentation is likely to increase further.

The United States, with an assortment of companies supplying biochemical reagents, instrumentation, and software, has the strongest biotechnology support sector in the world. The U.S. biotechnology support sector is characterized by a large number of small specialty firms that compete in small specialty product markets such as biochemical reagents used in rDNA research (e.g., BioSearch, Vega, P-L Biochemicals (a subsidiary of the Swedish company Pharmacia), Bethesda Research Laboratories,* Collaborative Research, New England BioLabs, Applied Biosystems, Creative Biomolecules, and Intelligenetics) and several medium-sized to large firms that produce analytical and preparative instrumentation as well as bioprocess equipment** for larger, more diverse product markets (e.g., Beckman, Perkin Elmer, Varian, Hewlett Packard, Waters, New Brunswick).

In most support areas, European and Japanese support sectors are underdeveloped compared to that of the United States, although both are expanding quickly. Two factors might account for weak support sectors in Japan and Europe as compared to that of the United States:

- The United States is a recognized leader in basic biomedical research, and over the years, public funds, notably from the National Institutes of Health, have created a large well-defined market for specialty products used in biological research (1).
- Because so many large and small U.S. companies are currently applying biotechnology, the specialty research product needs are greater in the United States than in any other country, and opportunities exist for many small manufacturers. In fact, the U.S. market for custom oligonucleotides (DNA fragments) and biochemical reagents for synthesis of DNA is equal to that of the rest of the world (51).

In Europe and Japan, there are few biotechnology support firms supplying biochemicals. Thus, European and Japanese companies developing biotechnology generally have to manufacture oligonucleotides and other biochemical reagents in-house. Consequently, the expense for biochemicals in European countries and Japan is often greater than in the United States, where many support firms have achieved significant economies of scale (51). The alternative to in-house production of support materials in Europe and

*Bethesda Research Laboratories was recently purchased by Dexter Corp.'s GIBCO division. The new name for the merged company will be Life Technologies, Inc.

**See *Chapter 3: The Technologies* for a discussion of bioprocess equipment.

tablished pharmaceutical and chemical companies have contracted with NBFs for animal health projects including the development of animal growth promotants and vaccines for foot-and-mouth disease, rabies and colibacillosis (a diarrheal disease that kills millions of newborn pigs and calves each year). Norden, for example, funded research by the NBF Cetus to develop a vaccine to prevent colibacillosis in hogs. This vaccine received the U.S. Food and Drug Administration's (FDA's) approval in 1982. As other examples, American Cyanamid and Merck have both contracted with NBFs for projects involving bovine growth hormone and a vaccine for foot-and-mouth disease. Many of the products under joint development are already undergoing testing.

Several NBFs are in a strong competitive position vis-a-vis established U.S. and foreign companies in animal-related biotechnology. Most of the established U.S. companies have made relatively small investments in this area—equal to or less than investments in animal health by most of the leading NBFs (54). As established U.S. companies in the animal health field increase their biotechnology investments, the U.S. competitive position in domestic as well as foreign animal health markets should strengthen.

FOREIGN COMPANIES

Established U.S. and European companies control world animal health product markets, but collectively, European companies' efforts to produce new or replacement animal vaccines or growth promotants using biotechnology do not appear to be as strong as the collective efforts underway in the United States. European companies appear on the basis of reported research projects almost exclusively dedicated to the development of products for the world's two largest animal vaccine markets, rabies and foot-and-mouth disease. U.S. companies dominate the world market for animal growth promotants, and few European animal health companies have indicated an interest in entering the growth promotants product market. Furthermore, few European companies have established R&D joint ventures with the leading U.S. NBFs engaged in growth promotant R&D.

Japanese companies have exhibited relatively little commercial interest in the area of animal health, probably because meat does not constitute as large a portion of the Japanese diet as it does of the diets in Western European countries and the United States. Recently, however, the Japanese chemical company Showa Denko and the U.S. company Diamond Shamrock set up a biotechnology joint venture, SDS Biotech Corp., in Ohio exclusively for animal health research (13).

*Plant agriculture industry**

U.S. COMPANIES

The plant agriculture industry encompasses companies engaged in R&D activities to modify specific plant characteristics (e.g., tolerance to stress, nutritional content, yield, and growth rate) or to modify traits of micro-organisms that could be important to plant agriculture (e.g., nitrogen fixation, disease suppression, and insecticide production). The importance of plants as a food source and renewable resource and the potential of biotechnology to alter plant characteristics has attracted a diverse set of firms to the plant agriculture industry. Fifty-two U.S. firms listed in table 4, 30 established companies and 22 NBFs, are applying biotechnology to plants. Table 11 provides some examples of the diverse application areas that NBFs are pursuing.

Established U.S. companies from industries ranging from oil and chemicals to food and pharmaceuticals appear to be dominating the U.S. investment in biotechnology R&D in plant agriculture (25). U.S. chemical companies that have made considerable in-house investments in plant-related biotechnology research include American Cyanamid, Dow, Allied, DuPont, and Monsanto. These companies already produce chemical pesticides and herbicides and already have research using plant cell and molecular biology techniques directed toward increasing the resistance of crop plants to these chemicals (15). American Cyanamid, which has the expertise to synthesize herbicides, and the NBF MGI, which has the expertise to develop novel corn strains tolerant to new herbicides, have a joint program to develop herbicide-resistant corn. New corn strains developed for herbicide resistance might make it possible

*Applications of biotechnology to plant agriculture are discussed further in *Chapter 6: Agriculture*.

The biochemical supply situation is somewhat different in the United Kingdom, a nation strong in basic research but weak in commercial applications (51,69). As early as 1980, a well-known British Government biotechnology report, the Spinks' report, recognized that the United Kingdom had a shortage of suppliers of suitable equipment and reagents for biochemical laboratories (2). The number of new small British suppliers of biochemical reagents and restriction enzymes is increasing, but British firms using these products as well as instrumentation still purchase much of them overseas.* British firms' reliance on foreign biochemical suppliers could be reduced as an increasing number of small supply companies are beginning to form in the United Kingdom.

The demand for support materials in Japan has increased significantly since MITI designated biotechnology a priority area for the 1980's. In anticipating the increased demand for research supplies, the Science and Technology Agency (STA) sponsored an industrial research team** whose objective is "DNA extraction, analysis, and synthesis technology development" (70).

Until recently, oligonucleotides were produced in Japan only on an experimental basis and foreign products were used for domestic consumption. Now, three Japanese companies and their affiliated trading firms produce and market synthetic DNA in Japan,*** and two of them are members of the MITI research team. Only two Japanese companies, Nippon Zeon Co. and Takara Shuzo, produce restriction enzymes for the estimated $4.5 million Japanese market (35). Nippon Zeon Co., a subsidiary of Kongo Pharmaceutical Co., is manufacturing 35 kinds of restriction enzymes and 87 different synthetic DNA fragments mostly for research institutes in Japan (37). Takara Shuzo, in addition to supplying enzymes to the Japanese market, is exporting them to the United Kingdom. Because of the increasing rate at which biotechnology research is being carried out in Japan, and because of the underdeveloped support industry there, the current supply of oligonucleotides and restriction enzymes for biotechnology research in Japan is inadequate. In fact, Japanese distributors are still looking for U.S. suppliers (40).

The biotechnology support structure in Japan is expected to develop differently from that of the United States, because most companies commercializing biotechnology in Japan will continue to manufacture or import their own specialty biochemical supplies. In order to meet their own needs, Japanese companies have integrated vertically and are increasing their efforts to develop products such as reverse transcriptase and other enzymes that will reduce the cost and speed up the rate of biotechnology R&D. This pattern of vertical integration and in-house manufacture is not likely to change in the short term. The Japanese supply structure could retard research and create an early commercial disadvantage for Japanese companies in the short run.

INSTRUMENTATION

The instrumentation field includes all the instrumentation used in biotechnology from the analysis and synthesis of DNA molecules to the monitoring and control of large-scale separation and purification of commercially important biological compounds. Of particular importance to the pace of biotechnical development is the newly designed or recently modified instrumentation that is meeting the special needs of biotechnology research and production. Two of the most important instrument areas are DNA and peptide synthesizers and bioprocessing separation and purification instruments such as HPLCs.

Automated DNA and Peptide Synthesizers.—Automated DNA and peptide synthesizers significantly reduce the number of personnel and the amount of time required for synthesis. Such synthesizers will have significant impacts on the timing of research outputs and technical developments in biotechnology in the United States (61). An increased availability of specifically synthesized gene fragments arising from automated

*The British firm Amersham recently launched new product lines to meet the growing need for restriction enzymes in the United Kingdom, but rather than manufacturing the enzyme itself, Amersham will be supplied with 22 restriction enzymes by the Japanese firm Takara Shuzo Co. (9). Typically, Japanese companies do not pursue small foreign markets; in this case, however, Amersham's distribution network provided easy access to the European enzyme market.

**Ajinomoto, Wakinaga Yukuhin, Yamasu Shoyu, Yuki Gosei Yakuhin Kogyo, Toyo Soda Manufacturing Co., Ltd.

***Nippon Zeon Co.-Mitsui Trading Co., Yamasa Shoyu-Sumitomo Shoji, Yoshitomi-Yuki Gosei.

The Japanese are very interested in the development of amino acids and high-value compounds by selecting and engineering plant cells to produce secondary metabolites in vat culture. MITI has identified secondary compound synthesis as a major area for commercialization, and this area of plant-related biotechnology research will receive approximately $150 million from MITI during the next 10 years (15). With their experience in large-scale bioprocessing, the Japanese are well ahead of the United States in this aspect of plant biotechnology. Japanese companies have already reported repeated success in growing plant cells in 15,000 liter batches (68). The upper limit in the United States is only 300 liters (68).

Although biotechnology is not expected to provide foreign countries with an ability to reduce U.S. dominance in world grain markets, it may provide foreign countries with opportunities to seize specific agricultural markets. In both France and Italy, for example, there are major commercial activities in plant tissue culture techniques for eliminating viruses and propagating fruit and nut trees (15).

*Specialty chemicals industry**

The specialty chemicals industry promises to be a particularly competitive industry as biotechnology develops, because large chemical companies from both Japan and the Federal Republic of Germany as well as the United States are hoping to switch from the stagnant commodity chemicals industry into the more profitable specialty chemicals industry.

The general chemical and petrochemical firms of Japan are leaning strongly to biotechnology, and some of them are making rapid advances in R&D through their efforts to make biotechnology a key technology for the future. Japanese companies are expected to be especially strong competitors in future specialty chemical markets for reasons including the following:

- Japanese bioprocess-based companies are known to possess highly developed enzyme technology, a prerequisite for efficient biological production.
- Japanese chemical companies view specialty chemicals as a profitable area in which to diversify. Showa Denko, a leading chemical company in Japan, is expecting to become a major world producer of the amino acid tryptophan, first by using a new low-cost semisynthetic production method, and second by rDNA production.
- Two Japanese companies, Kyowa Hakko and Ajinomoto, are currently the world's major producers of amino acids. Both companies have operating production plants in the United States, and both have strong biotechnology R&D programs in Japan. Ajinomoto, for example, has succeeded in improving the production of the amino acid threonine by rDNA technology using *E. coli*, and Showa Denko has cut in half the production cost for tryptophan through a semisynthetic production process.

The commercialization of biotechnology will require many small, incremental improvements in bioprocess technology, superb quality control, and mass production to progress along the learning curve. As biotechnology development reaches large-scale production stages, well-developed bioprocessing skills will be necessary to compete in world product markets. Nowhere is the art of bioprocessing better refined than in Japan. Certainly Japan's expertise in this area will provide competitive strengths in many future biotechnology product markets.

Two West German companies that have experienced declining profits for the last 10 years because of poor chemical sales are Hoechst and Bayer, the world's largest chemical exporters and the world's two largest pharmaceutical companies (see table 5). These two companies spend more on R&D than any other pharmaceutical companies. Both these companies have targeted specialty chemicals as an area where biotechnology might increase corporate sales and profits (10). Bayer has a longstanding collaboration with its two U.S. subsidiaries, Miles and Cutter, and these two subsidiaries help keep Bayer informed of biotechnology developments in the United States. Much

*Applications of biotechnology to specialty chemicals are discussed further in *Chapter 7: Specialty Chemicals and Food Additives.*

Bioprocessing Separation and Purification Instrumentation.—Technical advances in separation and purification as well as monitoring will affect both laboratory research and commercial production and ultimately the U.S. competitive position in biotechnology (61).* The use of rDNA technology to produce low-volume, high-value-added products as well as high-volume products has greatly increased the need to develop more economic bioprocesses. As large-scale production draws closer, the ability to isolate and purify large quantities of desired products will be a determinant in how fast companies can reach international product markets. Those countries that possess the most advanced technology to separate and purify commercially important compounds might gain some commercial advantages in the early stages of production. Without more economic production, financial and commercial success in biotechnology may be difficult to achieve.

In the United States, Europe, and Japan, there is intense competition in R&D to develop improved large-scale separation and purification methods for biological compounds as well as methods for monitoring and controlling a bioprocess itself.** There is widespread effort to apply HPLC, continuous-flow electrophoresis, and flow cytometry to bioprocesses to decrease the manufacturing costs of compounds such as proteins. Increasingly, R&D efforts are being undertaken to scale-up analytical instruments, particularly HPLCs, for use in larger volume production processes. The United States is a recognized leader in analytical instrumentation used in biological research and thus stands at the forefront of many of the technical innovations being made in the bioprocess field. As automation and the use of sophisticated instrumentation to monitor and control the production process begins to transform bioprocessing from an art to a science, thereby making production more economic, U.S. companies will be in a strong competitive position.

HPLC is one of the most commonly used separative techniques and also one of the fastest growing instrumentation fields in the world (76). The growing sales are due in part to its expanded use in both analytical and preparative areas. HPLCs are considered standard analytical tools in the laboratory to accurately isolate and purify organic molecules, drugs, and some peptide hormones. More recently, HPLCs have been scaled-up successfully to monitor bioprocesses and purify large quantities of proteins such as leukocyte interferon.

Half of the $300 million worldwide HPLC market belongs to U.S. producers, and the European HPLC market is dominated by three U.S. companies, Varian, Beckman Instruments, and Waters. Japanese and European companies have tried with little success to penetrate segments of the U.S. instrument market. Pharmacia, a Swedish company, is the only exception. Large American companies such as Hewlett Packard, Perkin Elmer, and Beckman are so firmly entrenched by virtue of their service and applications networks that foreign firms (e.g., Shimadzu, a Japanese company) are having a difficult time making inroads. An absence of major foreign companies in the U.S. market and the dominance of American companies abroad highlights the prominent U.S. position in instrumentation markets.

Although U.S. companies dominate world HPLC markets, the Swedish company Pharmacia is a major competitor in separation and purification technology, especially chromatography (52). In fact, it is the only company in the world doing large-scale industrial chromotography. Waters and Beckman are thought to be catching up (52). According to John McTaggart of Tag Marketing, U.S. companies are catching up to Pharmacia in procedures for reducing the bulk of material at initial stages of isolation and purification (52). The gap is narrowing, because U.S. companies strong in hardware support (i.e., advanced solid matrix, membrane, and hollow fiber design) such as Millipore, Amicon, and Nuclepore are making advances in product recovery through ultrafiltration. The United States is considered the technological leader in hollow fiber and membrane technology.

*The reader is directed to *Chapter 10: Bioelectronics* for a discussion of sensor technology.

**See the discussion of bioprocess technology in *Chapter 3: The Technologies*.

Japan is reliance on a foreign supplier. Such reliance could impede technical advances (21) and retard commercialization in the short run. Although there are Japanese and European instrumentation manufacturers, U.S. instrumentation is considered superior to both Japanese and European instrumentation and dominates the European market (51). The Japanese instrumentation market is supplied by Japanese manufacturers, which have not made significant inroads in foreign markets (52).

Important product areas

For purposes of analysis, OTA examined three product areas thought to have significant short-term implications for research developments and technical developments in the biotechnology field:

- biochemical reagents used specifically in rDNA research (e.g., oligonucleotides and restriction enzymes);
- instrumentation used in product R&D (e.g., DNA and peptide synthesizers) and separation and purification instruments such as high-performance liquid chromatography (HPLC); and
- software designed to drive the microprocessors that automate instruments as well as software designed to analyze DNA and protein sequence data in data banks.

The United States is a world leader in all three product areas. If adequate supplies of the above products and services can sustain the present rate of growth of biotechnical advancement, the United States could possess a short-term advantage in bringing biotechnology products to international markets.

BIOCHEMICAL REAGENTS

The availability of quality biochemical reagents such as oligonucleotides (DNA fragments) and restriction enzymes (enzymes used to cut DNA) is crucial to sustaining the rapid development of the new biotechnology field and making it viable on a large scale. Between 1980 and 1990, sales of biochemicals for DNA and peptide synthesis in the United States are expected to increase at an annual rate of 20 percent (81). As more research is undertaken in plant agriculture, sales are expected to rise further. The total synthetic DNA market for 1983 to 1984 is estimated at $3 million to $4 million, and demand is expected to increase 25 to 30 percent a year (36).

Until rather recently, most oligonucleotides were made in-house in the United States; however, as demand for these materials has increased, small specialty support firms have been started to exploit these small markets. One source believes that the evolution of small support firms in the United States is gradually shifting many skilled biochemists in U.S. companies commercializing biotechnology from routine laboratory duties to basic research and that the net result has been an increase in the progress of biotechnology research in the United States (51).

Small U.S. support firms are estimated to supply about 25 percent of the total reagents used in biotechnology research in the United States at present (51). Some expect this figure to increase to about 50 percent as small firms achieve economies of scale, and their prices become lower than those of in-house manufacture. Others believe an estimate of 50 percent might be somewhat high, because some of the major users of reagents, in order to control availability, quality, and cost, are opting for in-house manufacture rather than purchase (40). In-house manufacture may in fact limit the growth of the reagent market. The Canadian firm Bio Logicals no longer manufactures oligonucleotides at all, because the market is smaller than was originally estimated, and the business is becoming one of low profit margin (4).

The unavailability of specific DNA sequences will clearly slow any research development on those sequences. Research at the U.S. firm Genentech was slowed, for example, when the company had to wait weeks for a reagent that is only available from Sweden (43). In the United States, the existence of many small custom reagent suppliers makes delays of this kind rare. In Europe, however, delays of 1 to 2 months occur more often. Nonetheless, there is little competition in Europe among firms making custom synthesized fragments, because European researchers are willing to wait a couple of months for special reagents (51). DNA probes (small pieces of DNA that recognize specific genes) are not even manufactured there (21).

ages to assist researchers with molecular genetics analysis. Some of the subscribers include SmithKline Beckman, DNAX, Hoffmann-La Roche, Biogen, and Pfizer.

Conclusion

The U.S. support sector provides competitive as well as commercial advantages to U.S. companies developing biotechnology through: 1) the timely and sufficient supply of biochemicals such as oligonucleotides and restriction enzymes for rDNA R&D, 2) new or modified instrumentation such as DNA and peptide synthesizers as well as large-scale purification instruments such as HPLCs, 3) the design of new software for research and production, and 4) a continuous exchange of information between suppliers and companies using biotechnology that results in the creation of new products and in constant improvements in existing instrumentation, equipment, and software used in biotechnology R&D.

The first advantage, timely and sufficient supply of biochemical reagents for rDNA R&D, can affect the rate at which some biotechnology research is carried out. An increasing number of small U.S. companies specializing in custom DNA synthesis has made available sufficient supplies of reagents in the United States that are priced lower than European or Japanese supplies. In Europe, although the number of companies supplying custom reagents has increased, supplies still are not adequate and delivery is slow, especially when reagents are imported (43).

The second and third advantages, new or modified instrumentation and new software design, may provide U.S. companies with a short-term advantage through more efficient research methods and production processes. DNA and peptide synthesizers, for example, are beginning to automate the long and tedious manual task of assembling DNA and peptides, thereby creating greater efficiency in the early stages of research. The scale-up of HPLCs for use in purification of commercially important compounds may also provide greater production efficiency. Software used to drive the microprocessors used in synthesizers or bioprocessing equipment, or to manipulate sequence data in data banks, or to direct computer modeling of proteins may also give U.S. companies short-term advantages in the earlier stages of commercialization. It should be noted, however, that these materials can be exported without difficulty, and that any U.S. advantage derived from their manufacture in the United States is short term.

The fourth advantage, information exchange between support firms and the companies developing biotechnology, promotes technology transfer within the United States and stimulates improvements in instrumentation and software design for biotechnology application. Not only do support companies constantly improve on the products that they themselves manufacture, but the companies that they are supplying in turn strengthen the U.S. support base by developing customized and automated instrumentation and equipment for in-house use, which they may then make available to other companies once their proprietary position has been secured. Examples of companies in the latter category include Genentech, Cetus, and Bio Logicals (Canada). Bio Logicals' DNA synthesizer grew out of in-house technology to produce oligonucleotides for itself. Cetus recently established a new subsidiary, Cetus Instrument Systems, to capitalize on the commercial value of novel instrumentation and computer systems developed for its own in-house R&D. Genentech and Hewlett Packard started a joint venture company, HP Genenchem, to develop for themselves and other companies automated instrumentation for use in biotechnology R&D. Genentech will provide the joint venture with instrumentation already developed and add early insights for research and commercial instrument opportunities (37). Possible areas of automation include DNA and protein sequencers and synthesizers and industrial-scale HPLC and flow cytometers for bioprocess monitoring and control.

In the current stage of biotechnology development, there is considerable interaction between suppliers and potential users, particularly in the area of sophisticated instrumentation. Ideas for new products are developed through in-depth conferences with customers and potential customers to determine or anticipate what kinds of R&D problems they might have. Also, in response to customers' needs, U.S. support firms are constantly upgrading and modifying instrumentation to maximize its utility. These interactions and

synthesis may give researchers more flexibility in the manipulation of genetic information. Automated synthesizers can, among other things, expand the availability and variety of linkers and adapters* for cloning DNA, provide probes for finding messenger RNA and DNA gene sequences, or manufacture whole genes themselves.

The United States leads the world in synthesizer technology. The support companies that manufacture DNA and/or peptide synthesizers in the United States include Vega Biotechnologies, BioSearch, Beckman Instruments, Sys-Tec, Applied BioSystems, P-L Biochemicals, Syncor, Genetic Design, and Sequemat. Generally, these companies have very good communication with the U.S. companies and laboratories they supply. BioSearch customers, for example, keep BioSearch continually informed of their needs so that automation can be designed based on these needs. Communication networks between European instrument suppliers and their European customers are not so well developed.** U.S. companies might, therefore, gain some competitive lead time in biotechnology, because they will be among the first to benefit from automation developments in the United States.

There are no Japanese companies actually manufacturing DNA or peptide synthesizers for commercial use (21,81), but some U.S. manufacturers of DNA and peptide synthesizers have established distribution agreements in Japan.*** The reasons given most often for the dearth of Japanese manufacturers are the high risks of bringing synthesizers to market and the small size of the Japanese synthesizer market. A 1982 market survey by American Commercial Co. (Vega Biotechnoloy's Japanese trading company) found the Japanese market at that time to be approximately 150 machines (81). Without automation to synthesize the genes or fragments necessary for research, the Japanese may find it difficult in the short run to keep pace with American research advances. Additionally, if future markets develop for total gene synthesis, Japanese research could be slowed because Japanese companies have not developed their own automation.

The only two DNA synthesizer manufacturers in Europe are Celltech and Cruachan Chemicals Co., Ltd. (U.K.). However, companies in France, the Federal Republic of Germany, Switzerland, and the United Kingdom have introduced peptide synthesizers to the market or plan to soon. Sempa (France) is not aggressively marketing its machine in the United States. The relatively small size of the European market discourages many potential large European manufacturers from entering the market. The inherent risks of introducing a new product might also discourage small European companies from entering the market as well.

Over the next 5 years, the U.S. market for automated DNA synthesizers is expected to grow to between approximately 500 (81) and 1,000 units (21). Since March 1983, Applied BioSystems (U.S.) has shipped 30 synthesizers, and in just over a year, BioSearch (U.S.) has shipped about 50 (37). Some observers expect that the largest biotechnology support markets in the near term will be those for synthesized whole genes and purification systems (21). Though some firms doubt that a market for whole genes is developing, other firms, including Creative BioMolecules (U.S.), have already begun to market whole genes. Creative BioMolecules' synthetic gene for human pancreatic growth hormone releasing factor.

New developments in continuous-flow peptide synthesizers have led to an upsurge in interest in this different type of instrument technology. The U.S. market for peptide synthesizers 5 years from now is expected to be 500 units—the same size market that is forecast for DNA synthesizers (81).

In a situation of rapidly changing technology, the United States is at a clear advantage in the short run because of the supply of automated instrumentation, an automated synthesis instrument supply standpoint, because many small U.S. companies are willing to address these small, high-risk markets. In Europe, few small or large firms are willing to do the same.

*Short nucleotide sequences that encode restriction enzyme sites.
**See the Spinks' report recommendations.
***A U.S. synthesizer manufacturer contacted by OTA was not aware of any Japanese companies that manufacture synthesizes (40).

duced 24 times as many major innovations per R&D dollar as did large firms and 4 times as many as did medium-sized firms (44). Finally, an Office of Management and Budget study concluded that small firms (i.e., firms with fewer than 1,000 employees) had a ratio of innovations to employment in R&D 4 times as great as that of larger firms (19). In combination, the results of these studies suggest that small firms appear to be more efficient than large companies in the way they use the R&D funds available to them (32).

THE EMERGENCE AND FINANCING* OF NBFs

Since 1976, more than 100 NBFs have been formed in the United States. The founders of many NBFs recognized early that most developments in biotechnology would flow from basic research carried out in academic institutions. For this reason, they formed their companies around a nucleus of talented university scientists, frequently using nonproprietary technology. Several NBFs (e.g., Genentech, Centocor, Genetic Systems) got started by placing R&D contracts with academic researchers for the commercial development of a laboratory discovery.

The character and record of the chief scientists in a new firm is important for several reasons: the amount of venture capital made available to the firm might be determined by the scientist's reputation in the scientific community; the scientist may have some influence over the flow of other well-respected scientists and skilled technicians to the company; and his or her reputation might attract the endorsement of established companies which provides valuable reinforcement to the NBF (e.g., Genentech's early relationships with the U.S. company Eli Lilly and the Swiss company Hoffmann-La Roche).

NBFs must be able to attract and retain qualified personnel if they wish to attract venture capital,** develop marketable products, and maintain their domestic competitive position. Competition in the United States for skilled personnel is intense.*** According to the First Annual Technical Staffing Survey conducted by Scherago Associates in New York, the average biotechnology firm* in the United States more than doubled its staff of scientists between 1980 and 1982 from 3.1 to 7.3 (72). Scherago expects the number of Ph.D.s to almost double again by 1984. The results from the OTA/NAS survey of firms' personnel needs** substantiate the Scherago survey findings, but they also show that the average number of scientists per firm might be growing at a faster rate than originally estimated. The average number of Ph. D.s for the NBFs listed in table 4 as of March 1983 was already 15.7.***

NBFs, by virtue of their size, incentive plans, and innovative and academic-like environment have been able to attract many talented scientists. It is expected that NBFs will continue forming, in part because new firms will continue to be able to attract good scientists.

The formation of the loosely organized and highly competitive structure within which biotechnology is developing in the United States has been shaped largely, but not exclusively, by the availability of venture capital and the willingness of scientists to pursue commercial gain through small, newly formed entrepreneurial companies. The emergence and growth of venture-capital-backed NBFs in the United States began around 1976. As shown in figure 11, not until late 1982, when venture capitalists had satisfied much of their portfolio requirements for biotechnology stock (42) and over 100 new companies had been formed, did startup activity begin to taper off.†

Many of the first NBFs (e.g., Genentech, Genex, Cetus) financed their own proprietary research by providing large established U.S. and foreign companies with research services for initial product development or by entering into licensing agreements with such companies that would re-

*The financing of NBFs is discussed in detail in *Chapter 12: Financing and Tax Incentives for Firms.*

**Because most NBFs are unable to meet many of the standard investor requirements for such things as earnings, sales, rate of growth, etc., sometimes potential investors use the number of Ph. D.s per firm as a measure of future earning power.

***See *Chapter 14: Personnel Availability and Training* for a more detailed discussion of personnel needs and availability in the United States.

*Scherago defines a biotechnology firm as a gene manipulation company.

**See *Appendix E: OTA/NAS Survey of Personnel Needs of Firms in the United States.*

***This average is based on the firms in table 4 for whom Ph. D. figures are given.

†The pace of new biotechnology startups may also have been slowed because many of the top university scientists who wanted to join new firms probably had already done so. A year or two ago a survey done by an investment company looking for an unaffiliated molecular biologist reportedly approached 20 researchers before it found one without a commercial tie (16).

SOFTWARE

The United States holds a commanding position in software designed for molecular biology and bioprocessing. With a superior capability to analyze and manipulate sequence data or to purify large quantities of valuable products, for example, the United States might gain some commercial lead by hastening research in some product development areas.

Automation will be necessary to develop more efficient bioprocesses and to lower the costs of biological production. U.S. instrumentation and software manufacturers such as Perkin-Elmer and Fisher Scientific are designing a wide range of software for use in biological research and production processes. The United States is the recognized leader in software design in general and in sophisticated computer applications to biological research specifically. Because of the dominant role U.S. companies play in instrumentation markets, and because of the increasing importance microprocessors and automation are having in biological research and production, the United States is expected to gain some short-term advantages in the commercialization of biotechnology.

Software controls all processes automated by microprocessors. Current software applications in biotechnology are wide ranging and include the manipulation of DNA sequence data contained in data banks, the automatic ordering of nucleotide bases to synthesize pieces of DNA, the modeling of protein structures, and the monitoring and control of large-scale bioprocessing. On the analytical level, purification of peptides and DNA fragments, for example, is expected to become more sophisticated through technical advances in automation (40). On a preparative level, the utility of HPLCs, for example, is being increased by interfacing HPLCs with other instruments (e.g., infrared and mass spectrometers) and computers.

The availability in the United States of software designed to analyze the data in the private and public DNA and protein data banks that have been created worldwide may give U.S. companies commercializing biotechnology some competitive advantages. Both public and private DNA sequence banks exist in the United States. The two largest private and public banks respectively are: the Nucleic Acid Sequence Database (1,200,000 nucleotide bases), operated by the National Biomedical Research Foundation, Georgetown University Medical Center; and the Genetic Sequence Data Bank (GENBANK) (1,800 DNA sequences totaling 2 million nucleotide bases) founded on data collected, organized, and annotated by the Los Alamos National Laboratory and developed through funding from the U.S. National Institutes of Health. The latter data bank will be a repository for all published nucleic acid sequences of more than 50 nucleotide base pairs in length. Georgetown also operates the world's largest protein sequence data base, which currently contains 2,100 sequences and about 360,000 amino acids.

The United States is not unique in its creation of such data bases; however, in terms of size, there are no foreign equivalents. The Europeans have their own nucleic acid data base, the Nucleotide Sequence Data Library (operated by the European Molecular Biology Laboratory, EMBL), and the Japanese will have their own equivalent soon. In addition to these foreign DNA data bases, small private foreign protein data banks exist for the exclusive use of the institutions with which they are affiliated.

A research advantage for the United States is expected to arise not only from the availability of data bases, but also from the software being designed by academic institutions, nonprofit research foundations, and private companies to analyze the data in the banks. Since GENBANK's development was made possible through public money, the data are available to the public, domestically as well as internationally. Additionally, subscribers to Georgetown's Nucleic Acid Database can use the accompanying programs to access both the GENBANK and EMBL's bank. With equal international accessibility to the data bases, competitive advantage will flow to the country that has the ability to perform sophisticated sequence manipulation through specially developed software. In fact, the utility of the data bases will be defined by the available software.

The U.S. company Intelligenetics is specializing in the application of data processing and artificial intelligence techniques to biological problems, and this company has created specific software pack-

- Relatively short development times and modest capital requirements for MAb in vitro diagnostic products afford NBFs opportunities to generate short-term cash flow from these products with which to fund the more time-consuming and costly R&D on pharmaceutical products intended for internal use.*
- Entering the MAb in vitro diagnostic products market is relatively easy for NBFs, because the diagnostic market is highly fragmented and the individual diagnostic markets relatively small. Thus, NBFs are likely to encounter few scale disadvantages in competition with large established companies.
- The markets for in vitro MAb diagnostic products are growing, thus providing expanding opportunities for entry by NBFs. The clinical immunodiagnostic market has grown at an annual rate of approximately 20 percent for the past few years, and this rate of growth is expected to continue or increase in the future (63). The 1982 market was valued at $5 million to $6 million (77). Table 12 provides 1982 and 1990 estimates for the size of various MAb markets in the United States.

Oppenheimer & Co. expects the clinical immunodiagnostics market to be the most important source of revenue to NBFs in 1983 (63). Many of the in vitro MAb diagnostic products now being developed or sold are replacement products that offer improved (more accurate) detection, shorter test times, and lower production costs (63)—and as might be expected, competition for market shares and scientific and financial resources is intense. Since 1980, more than 12 new U.S. companies (e.g., Xoma, Quidel, Techniclone, New England Monoclonal Resources) have formed specifically to exploit hybridoma technology, and most of them either already have MAb diagnostic kits on the market or are seeking FDA's approval. In 1982 alone, FDA approved some 30 in vitro MAb diagnostic kits (26).

To increase their chances for commercial success, NBFs solely dependent on MAb-based diagnostic products must find market niches. Although, a focused strategy such as MAb production could bring NBFs financial success with a smaller investment of dollars and scientific expertise in a shorter time frame than a more diverse strategy typical of some of the more heralded, multipurpose companies, such a strategy could also limit their growth potential (26). The worldwide diagnostic market represents only $2 billion out of the $80 billion annual human drug market (24). Until NBFs are capable of entering the larger drug markets, however, diagnostic products may prove crucial in supporting the high costs of pharmaceutical development.

Some NBFs are developing MAb therapeutic and in vivo diagnostic products, although the number of NBFs developing these products is less than the number developing in vitro MAb diagnostic products.* In addition to MAb therapeutics to treat cancer, MAb therapeutic products are being developed to treat bacterial infections that are sometimes difficult to treat with antibiotics and viral infections for which no antibiotics exist. As will be discussed in the section below entitled "Collaborative Ventures Between NBFs and Established U.S. Companies," the regulatory environment for pharmaceuticals imposes heavy long-term financial burdens, which many NBFs may be unable to bear alone. Since many of the new firms aspire toward short-term earnings and independent production and marketing, it is not surprising that in vitro MAb diagnostic products are the area of application most widely chosen by NBFs.

Many small markets exist for NBFs in animal agriculture, and for replacement as well as new products, the barriers to market entry are low. Furthermore, the costs of obtaining regulatory approval for most animal health products are lower than those for human pharmaceuticals. However, in order to market some animal health products, including vaccines, a large and highly

*Cetus Corp. (U.S.), for instance, is developing diagnostic products for detecting blood-borne pathogens such as hepatitis B virus with funding from Green Cross of Japan and for detecting cytomegalovirus. Cetus is also developing readily marketable biotechnology products for animal agriculture until its more profitable products, particularly anticancer drugs, are developed. Likewise Hybritech (U.S.) and Genetic Systems (U.S.) are producing MAb diagnostic products to support other longer range R&D activities such as MAb therapeutics.

*An even smaller number are developing MAbs for use in separation and purification.

tailoring of instrumentation and equipment to meet industrial needs will be critical to surmounting the numerous problems anticipated in the design, scale-up, control, and optimization of industrial biotechnological processes (22).

The U.S. biotechnology support sector currently provides a sufficient and *timely* supply of biochemicals, instrumentation, and software to U.S. firms using biotechnology. By virtue of its support strength, the United States holds research advantages over other countries—advantages that may or may not be translated into commercial products. For the United States to retain these advantages in the future, U.S. support firms must remain poised to meet the immediate and expanding supply needs of the U.S. firms commercializing biotechnology.

U.S. firms commercializing biotechnology and their role in competition

As noted at the beginning of this chapter, the commercial development of biotechnology in the United States is being advanced by two types of firms: NBFs and large established U.S. companies. It is important to keep in mind throughout this report the organizational nature of the U.S. biotechnology development and commercialization effort and the strength that the present NBF-established firm competition and complementarity lends to this effort. NBFs and established U.S. companies both have important roles to play in the present phase of biotechnology development. Not until the technology is more fully developed will the parameters of responsibility for each group of firms be more clearly defined.

New biotechnology firms

The development of biotechnology is still at an early stage, and competition at present, both in the United States and abroad, is largely in research and early product development (e.g., vector selection and gene expression). Development and commercialization have not yet progressed to a point where competition for market shares is of immediate concern. In the present research-intensive stage of biotechnology's development, NBFs are providing the United States with competitive advantages in biotechnology through contributions to innovation. In the early stages of a new technology, small firms in the United States tend to dominate an industry and contribute most to product innovation. As a group, it is the small companies that have most "quickly and successfully taken new technologies from the laboratory and adapted them for large-scale production" (78). Small firms move much more aggressively to market than do established companies that have built-in disincentives to advance the state-of-the-art quickly because of existing investment in established product lines and production processes.* As a technology matures, many established companies, as later entrants, begin to play a larger role in innovation, as well as production and marketing.

That small firms contribute significantly to technological innovation is widely accepted, although there is disagreement over the amount of their contribution. Some U.S. studies suggest that small businesses play a more important role in technological innovation than do large firms. A recent study prepared for the Small Business Administration by Gellman Research Associates, Inc., for example, holds that: 1) small firms produce 2.5 times as many innovations as large firms, relative to the number of people employed; and 2) small firms bring their innovations to market much more rapidly than do large firms (32). Another study undertaken by Human Services Research for the National Science Foundation found that small firms (i.e., firms with fewer than 1,000 employees) pro-

*For example, a pharmaceutical firm with a vested interest in symptomatic treatment of colds may have little incentive to develop a vaccine against the cold-causing viruses, since it would diminish the company's sales of decongestants.

duced pharmaceuticals and MAb-based diagnostic products. Because of the large number of companies and small range of biotechnology products, most of the initial product markets are likely to be very crowded, costly to enter, and highly competitive. The sharp decline in the formation of NBFs in 1983 might be explained in part by the currently high levels of competition. How many producers the initial biotechnology product markets might ultimately accommodate is uncertain. Thus, the factors likely to affect the future commercial success of the NBFs most immediately are the timing of market introduction, product performance, and product quality. Price, and hence production costs, will be of greater importance later.

The major determinant to the commercial future of NBFs, assuming they are able to maintain a research advantage, will be their ability to obtain financing and their ability to enter the newly developing product markets. NBFs must manufacture and market their own products not only to generate sufficient revenues to fuel growth but also to be in control of the timing of their own product introduction. It remains unclear whether NBFs will have the financial resources and marketing strength to enter some of the new markets. Large established pharmaceutical companies, for example, normally employ some 500 people just to market their drugs (24), while Genentech, one of the largest NBFs, has a total of about 500 employees.

Some of the most difficult markets for NBFs to enter will be those for human therapeutics, in part because of the regulatory costs associated with product approval and in part because of the market competition posed by established U.S. pharmaceutical companies, which could control some of the early channels of distribution. Entering the markets for in vitro diagnostic products, as mentioned earlier, is relatively easy and does not require large capital investments, but because these markets are currently very crowded, survival may be difficult.

The specialty chemicals market appears relatively easy to enter, both because little competition exists at present and also because the regulatory environment does not impose high costs on product development. Research is near term for many of the products, 3 to 5 years, and an NBF would experience few production scale disadvantages in competition with larger companies.

The safety regulations applicable to animal health products are significantly less stringent than those applicable to pharmaceutical products intended for internal human use, and many market niches exist for small firm entry. Additionally, relatively little competition from established companies exists at present. However, the need for an extensive sales force to market some of the products might pose a considerable barrier to some NBFs wishing to enter animal health markets.

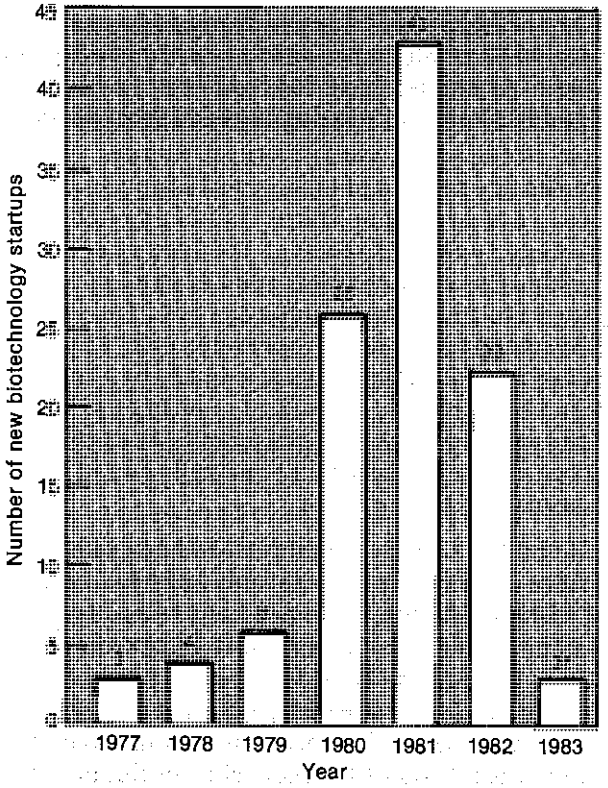
The availability of venture capital and financing for NBFs has been sufficient thus far to fuel the growth of many NBFs. The public market, particularly for new issues, and R&D limited partnerships continue to provide capital to NBFs for use in further research, pilot plant construction, clinical trials, and product development. From August 1982 to May 1983 alone, NBFs raised $200 million through R&D limited partnerships (6). One analyst estimates that R&D limited partnerships will raise a total of $500 million in 1983 (7). The public stock market has also been receptive to NBF issues. Between March and July 1983, 23 NBFs raised about $450 million (39). As long as the public market and R&D limited partnerships make financing available to NBFs, they can continue developing independent strategies, thereby reducing their reliance on established companies.

Paralleling the emerging desire by some NBFs to become integrated producers and marketers is an apparent reduction from 1982 to 1983 in the number of research contracts sponsored by established U.S. companies* and an increase in the amount of capital established U.S. companies

nostic products for detection of venereal diseases and pregnancy. Tables 18 and 23 in *Chapter 5: Pharmaceuticals* provide a list of firms engaged in cloning projects for interferon and human tissue plasminogen activator, respectively, and exhibit a rather high level of competition for the two products. Additionally, at least eight NBFs are cloning interleukin-2 (Chiron, Genex, Biogen, Cetus, Genetics Institute, Immunex, Interferon Sciences, and Quidel).

*It is impossible to quantify the number and value of all established company sponsored research contracts because not all of

Figure 11.—Emergence of New Biotechnology Firms, 1977-83



[a]As of November 1983.

SOURCE: Office of Technology Assessment.

sult in future product royalty income. Product development contracts between NBFs and established companies generally provide for periodic cash payments from the established company to the NBF during the stages of research and early product development and for additional payments to the NBF (royalties income) following product sales. Following early product development by the NBF, the established company is generally responsible for obtaining the necessary regulatory approvals, manufacturing, and marketing of the product.

In the last couple of years, more and more NBFs have begun shifting away from developing products for larger companies for reasons including the following:

- NBFs have decided to concentrate more on proprietary research,
- profit margins from licensing technology to established companies are low and may not provide sufficiently substantial earnings (26), and
- most NBFs do not want to be dependent on another company for financial survival.

Instead of relying on contract revenues many NBFs are now obtaining financing through R&D limited partnerships, public stock offerings, or private placements. By retaining the rights to produce and market some of the products they develop (rather than developing products for established companies), some NBFs are seeking to become fully integrated producers and marketers. Genentech, for example, is hoping to manufacture and market four new products (human growth hormone, tissue plasminogen activator, and two types of interferon), and a large portion of Genentech's capital expenditures since 1981 has gone into a production plant for these products (24). Similarly, the NBF Amgen is building a $10 million pilot plant in Chicago for preclinical and clinical studies, and the NBF Genex has just purchased a manufacturing plant in Kentucky to produce phenylalanine and aspartic acid (the two amino acids used to produce the sugar substitute aspartame).

COMMERCIAL PURSUITS OF NBFS

Most NBFs are applying biotechnology to the development of pharmaceutical products or products for use in animal and plant agriculture. For several reasons, the most popular area of commercial pursuit among NBFs at present is the development of MAbs for research and in vitro diagnosis of human and animal diseases.*

- MAb in vitro diagnostic products require much shorter development times than do many rDNA-produced pharmaceutical products, because the technological development of MAb products is less complex. Furthermore, FDA's premarketing approval process is less costly for in vitro products than for products intended for internal use.

*Pharmaceutical applications of MAbs are discussed in *Chapter 5: Pharmaceuticals*. The applications of MAbs in the diagnosis, prevention, and control of animal diseases are discussed in *Chapter 6: Agriculture*.

companies are effectively diffusing biotechnology across many industrial sectors.

With the help of venture capitalists, NBFs started much earlier to evaluate the commercial potential of biotechnology than did large established U.S. or foreign companies. As early as 1976, NBFs were willing to risk their very existence on the undemonstrated potential of biotechnology. A survey conducted by OTA indicated that most established U.S. companies did not begin in-house biotechnology R&D until 1981 or later.* This finding suggests that the early burden of risk was carried by NBFs. Although many established U.S. companies have now made substantial commitments to biotechnology through investments in plant and equipment for in-house biotechnology R&D programs, others are still hesitant to make such investments and many NBFs continue to function as a litmus test for the new technologies. In Europe and Japan, most companies did not make major investments in biotechnology until after 1981. Thus, it might be suggested that the early R&D activity of NBFs has given the United States a competitive lead in the early stages of biotechnology's commercialization.

The NBF initiative to commercialize biotechnology not only has spurred the development of new product markets but also is expected to expand existing markets through the introduction of products with increased effectiveness and decreased cost. For example, diagnostic kits using MAbs and DNA probes are being developed to detect venereal diseases (e.g. chlamydia and herpes) that are difficult and time-consuming to detect by existing methods. Vaccines are being developed for diseases that now have no reliable prevention (e.g., hepatitis and herpes in humans and colibacillosis in calves and pigs).

The NBFs' entry into the traditional markets served by established companies, where NBFs have taken the risks of developing new products or potentially reducing the production costs of existing ones, has prompted many established U.S. companies to explore potential applications of the new technologies. The market uncertainty created by the new firms and the perceived competition they represent to the established companies is healthy in a competitive context, because it increases the aggregate level of industrial R&D in the United States. The perceived competitive threat that NBFs pose to established companies could become even greater as NBFs such as Biogen, Genentech, and Genex begin to shift away from developing products for large corporate clients and begin to turn toward independent production and marketing of their own products.

Because of their technological expertise and early role as contract research companies, the NBFs have helped established U.S. companies evaluate the feasibility and suitability of using the new technologies in their existing lines of business. They have also helped the established companies evaluate new avenues for diversification. Frequently, the established U.S. companies maintain multiple research contracts with the NBFs to evaluate several applications simultaneously or to evaluate the same application from different perspectives. In this way, the established companies can "ride along" the NBF learning curves while minimizing expenses and risk. In a competitive context, this relationship between NBFs and established U.S. companies is important because it may help to position both types of U.S. firms in international product markets.

From the standpoint of U.S. competitiveness, the innovative lead taken by NBFs in the United States might seem to be a handicap because of the potentially adverse consequences from the transfer of technology from the United States to foreign countries. But the United States, at first through the new firms and now with the combined effort of the established companies, has the ability to maintain its lead by continuing to innovate and develop at a pace equal to or faster than its competitor countries. While competition remains mostly in research, the ability of the United States to remain competitive and in the forefront of biotechnology development rests heavily on NBFs. As biotechnology reaches production stages, the bioprocessing, regulatory, and marketing experience of the established companies will be crucial to a strong U.S. position.

*The survey questionnaire is reproduced in *Appendix E: OTA/NAS Survey of Personnel Needs of Firms in the United States.*

Table 12.—Estimates of U.S. Monoclonal Antibody Markets, 1982 and 1990 (1981 dollars in millions)

Application	1982 market size	1990 market size
Diagnostics:		
In vitro diagnostic kits	$5 to $6	$300 to $500 ($40)[a,b]
Immunohistochemical kits (examination of biopsies, smears, etc.)	Nil	$25
In vivo diagnostics (primarily imaging)	Nil	Small to $100[b,d]
Therapeutics (includes radiolabeled and toxin-labeled reagents)	Nil	$500 to $1,000[b,c]
Other:		
Research	Small	$10
Purification	Small	$10

[a]High number indicates market for total kit, number in parentheses indicates value of antibody alone for kit (includes patent licensing fees).
[b]Variation depending on industry source, although the range has been corroborated by at least two sources.
[c]This number could be much higher or lower depending on regulatory process.
[d]Based on current pricing (1981 dollars) for diagnostic tests of the same type.

SOURCE: Office of Technology Assessment.

specialized sales force may be necessary. Some NBFs do not expect to hire their own marketing force. Genentech, for example, does not expect to market its own animal vaccines. Some NBFs hope to use existing distribution networks for animal health products instead of developing their own specialized marketing force.

NBFs pursuing plant agriculture applications of biotechnology seem to have found sponsors for longer term research in areas such as enhanced protein content and nitrogen fixation, but a number of new firms are conducting proprietary research in areas such as the regeneration of inbred crop lines from tissue culture. NBFs pursuing plant biotechnology are already using cell culture technologies rather successfully to introduce new plants to the market. One firm, Ecogen, has been formed to focus exclusively on microbial and viral pesticides and other novel pest control methods. As the more frontier techniques such as gene transfer are developed, they can be incorporated into ongoing product lines (15).

FUTURE PROSPECTS OF NBFs

Almost 2 years ago, skeptics forecast a "shake-out" among the NBFs (18,31,60,66). Even though the commercialization of biotechnology now may be more time-consuming, more expensive, and less profitable than was initially hoped, such a shake-out has not yet occurred. A shake-out will occur, however, when new markets develop and present trends in financing, established firm involvement, and technical capability change.

NBFs were formed to exploit research advantages in biotechnology, and many NBFs still possess such advantages. Given their research advantages, and assuming good management and adequate financing, many NBFs may continue to compete successfully with both larger companies and other NBFs as long as competition in biotechnology remains focused in research. Eventually, however, perhaps within 2 or 3 years, most NBFs will have to manufacture and market their own products in order to finance future growth and achieve some level of commercial success. A change from a research-oriented strategy to a more production-oriented strategy will mark a new stage in development for the average NBF, because in the past (and to some extent even now) NBFs out of need for capital have sold their processes to established companies.

NBFs that are wholly dependent on biotechnology for revenues cannot spread the risk of product development over a broad range of products made by traditional methods (unlike the established companies that have several product lines to generate revenues). Many NBFs will fail if markets for the biotechnology products now being commercialized do not develop. Furthermore, many NBFs will fail if capital for production scale-up, clinical trials (if necessary), and marketing is not available when markets develop.

The commercialization of biotechnology in the United States and other countries at present is characterized by a large number of companies, many small, some medium, and many large, applying biotechnology to a very narrow range of products.* Most of the products are rDNA-pro-

*Examples of such products are interferon, interleukin-2, human growth hormone, tissue plasminogen activator, and MAb-based diag-

Table 13.—Equity Investments in New Biotechnology Firms by Established U.S. Companies, 1977-83[a]

Date	U.S. established company	New biotechnology firm	Equity (millions of dollars)
1980	Abbott Laboratories	Amgen	$5
1981	Allied Corp.	Calgene	2.5
1983		Genetics Institute	10
1981	American Cyanamid	Molecular Genetics, Inc.[b]	5.5
		Cytogen	6.75
1981	ARCO	International Genetic Engineering, Inc. (INGENE)	0.75
1982	Baxter-Travenol	Genetics Institute	5
1982	Beatrice Foods	International Genetic Engineering, Inc. (INGENE)	3.0
1980	Bendix	Engenics	1.75
1982	Bendix/Genex	Proteins Association	16.5[c]
1983	BioRad	International Plant Research Institute (IPRI)	1
1981	Campbell Soup	DNA Plant Technologies	10
1981	Continental Grain	Calgene	1
1981	Cooper Labs/Liposome Tech. Corp.	Cooper-Lipotech	2.7
1982	Corning/Genentech	Genencor	20
1983	Cutter Laboratories	Genetic Systems	9.5
1982	DeKalb	Bethesda Research Laboratories	0.6
1980	Dennison Manufacturing Corp.	Biological Technology Corp.	2
1983	Diamond Shamrock/Salk Institute Biotechnology Industrial Associates	Animal Vaccine Research Corp.	N.A.[d]
1981	Dow	Collaborative Research	5
1981	Dow	International Genetic Engineering, Inc. (Ingene)	N.A.
1981	Ethyl	Biotech Research Labs	0.95
1981	Fluor	Genentech	9
1981	FMC/Centocor	Immunorex	4.9
1980	General Foods	Engenics	0.5
1982	Getty Scientific Corp.	Synergen	4
1982	Gillette	Repligen	N.A.
1983	Hewlett-Packard Co./Genentech	HP Genenchem	N.A.
1978	INCO, Inc.	Biogen[e]	0.35
1979	INCO, Inc.	Biogen	1.25
1980	INCO, Inc.	Biogen	4.61
1981	INCO, Inc.	Biogen	2.5
1981	INCO, Inc.	Immunogen	1
1981	INCO, Inc.	Plant Genetics	N.A.
1981	INCO, Inc.	Liposome Co.	N.A.
1977	Innoven[f]	Genex; Genentech	2
1981	Johnson & Johnson	Quadroma	0.7
1982	Johnson & Johnson	Enzo Biochem	14
1983	Johnson & Johnson	Immulok[g]	18
1982	Kellogg	Agrigenetics	10
1979	Koppers	Genex	3
1980	Koppers	Genex	12
1981	Koppers	Engenics	1.25
1981	Koppers	DNA Plant Technologies	1.7
1982	Eli Lilly	International Plant Research Institute (IPRI)	5
1979	Lubrizol	Genentech	10
1980	Lubrizol	Genentech	15
1982	Lubrizol	Sungene	4
1980	McLaren Power & Paper Co.	Engenics	1.25
1982	Martin Marietta	Molecular Genetics, Inc.	9.7
1982	Martin Marietta	NPI	5
1982	Martin Marietta	Chiron	5
1983	Martin Marietta	Chiron	2
1980	Mead Co.	Engenics	1.25
1980	Monsanto	Biogen	20
1980	Monsanto	Collagen	5.5

are devoting to in-house biotechnology programs. Although the pattern is beginning to change, research contracts sponsored by established companies still provide a large portion of the NBFs' revenues.* If the decline in number of research contracts sponsored by established companies continues, which is likely, NBFs must begin finding other sources of revenue. Increases in the amount of capital established U.S. companies are devoting to in-house biotechnology programs portend greater competition in R&D from the larger companies. Equipped with greater financial and marketing resources, more regulatory and, in some cases, production expertise, many U.S. established companies will be formidable competitors in the long run as biotechnology product markets develop. Not all NBFs will survive the competition of the established companies; provided they have adequate financing, however, some NBFs will be able to commercialize their early research advantages before the established companies commercialize theirs.

As biotechnology continues to emerge, and further technical advances are made, new generations of NBFs undoubtedly will evolve to develop the technologies. Within the next several years, a second generation of NBFs is likely to emerge as the result of developments such as the following:

- intensified competition that forces some firms out and creates new opportunities for more entrants,
- a major technological advance in some area of biotechnology such as computer-assisted protein design, which encourages the entry of more new companies,
- the diffusion of advances in bioprocessing, which enables small firms to assume responsibility over their own production, and
- the development of the technologies to the point where scientists from present companies or young scientists from universities will start their own companies.

public. However, on the basis of those that have been reported, most observers would probably agree that the number of new outside research contracts sponsored by established companies in 1983 has dropped significantly from 1982 levels.

*See *Chapter 12: Financing and Tax Incentives for Firms* for further discussion of the sources of NBF revenues.

ROLE OF NBFS IN U.S. COMPETITIVENESS IN BIOTECHNOLOGY

The development of biotechnology is still at an early stage, and competition at present is predominantly in the areas of research and early product development. This early stage of biotechnology development is precisely where NBFs are playing the largest role in competition. Later, however, as the technology develops further and enters a large-scale, capital-intensive production stage, the science may become less important vis-a-vis production expertise, and the dominant role NBFs currently play in the U.S. biotechnology effort may diminish.

The launching of embryonic high-technology industries by entrepreneurial firms is a phenomenon unique to the United States. Historically, small new firms in the United States have had a major role in shaping the competitive position of the United States in emerging technologies.* As discussed further below, NBFs have thus far assumed a similar role in biotechnology:

- by contributing to the expansion of the U.S. basic and applied research base for future biotechnology development,
- by transferring the technology to several industries through joint agreements with other companies,
- by decreasing investment risk by advancing learning curves for later entrants, such as established companies or other NBFs,
- by developing markets, and
- by increasing the level of domestic competition in the United States and thereby accelerating the pace of technology advance.

The formation in the United States of over 110 NBFs that have various links to the network of university biology, chemistry, and engineering departments has extended the basic research base beyond the universities and has expanded the applied research base beyond just a few companies. While the basic and applied research base is being broadened for future biotechnology development, joint agreements and licensing arrangements between NBFs and large established U.S.

*See *Appendix C: A Comparison of the U.S. Semiconductor Industry and Biotechnology.*

viously made equity investments may have contributed to the sharp decline.

In 1982, established U.S. companies not only increased their equity investments in NBFs but they also dramatically increased their in-house investments in biotechnology R&D programs. Capital investments for in-house R&D programs generally reflect the highest level of commitment to biotechnology, as new facilities and employees are often needed to start the new effort. Several U.S. pharmaceutical companies are spending large amounts on new facilities: G.D. Searle, for example, is building a $15 million pilot plant to make proteins from rDNA organisms; DuPont is building an $85 million life sciences complex; Eli Lilly is building a $50 million Biomedical Research Center with emphasis on rDNA technology and immunology and a $9 million pilot plant and lab for rDNA products; Bristol Myers is building a new $10 million in an alpha interferon production plant in Ireland.* Companies from other sectors have also made substantial investments in biotechnology. See table 7 for a list of the 1982 biotechnology R&D budgets for some of the established U.S. and foreign companies most actively supporting biotechnology.

The product areas in which established U.S. companies have directed their biotechnology R&D efforts are as diverse as the industrial sectors they represent. Established companies, however, appear to be playing a dominant role in the development of biotechnology in the areas of plants (25) and commodity chemicals—two rather long-term and costly research areas (see table 4).

ROLE OF ESTABLISHED COMPANIES IN U.S. COMPETITIVENESS IN BIOTECHNOLOGY

Many established U.S. companies manufacture several product lines and are therefore concurrently evaluating different biotechnology application areas. DuPont, for example, is evaluating applications of biotechnology to food production, health care, and renewable resources. Broad strategies such as DuPont's will have a positive effect on the development of biotechnology in the United States by diffusing applications throughout many industrial sectors.

Unlike the many NBFs that have taken a relatively short-term approach to biotechnology in order to generate income for longer term research, many established U.S. companies have several product lines and are taking a longer term approach to biotechnology research; some established companies are not expecting commercial development for 10 to 20 years (27). The long-range research orientation of established U.S. companies will be very important to the long-term competitive position of the United States.

Established U.S. companies will play a major role in the first biotechnology product markets. Because many NBFs have licensed technology to established U.S. companies hoping to finance future growth from the royalties received from the future sale of the products, the established companies will be responsible for the production and marketing of many early biotechnology products. For example, two NBFs, Petroferm and Interferon Sciences, have already solicited the production expertise of Pfizer and Anheuser Busch, respectively. Pfizer's chemical division is the foremost producer of biopolymers and xanthan gums and will produce Petroferm's new bacterial oil emulsifier. Anheuser Busch, through beer production, has accumulated years of experience using yeast and will produce interferon using Interferon Science's genetically manipulated yeast.

The most important element in competition for pharmaceutical market acceptance and market share might be the timing of product entry. Although some NBFs have recently begun funding their own clinical trials and product development, most NBFs still have rather limited financial resources. Most NBFs also have limited production, marketing, and regulatory experience. Such limitations may hinder the ability of NBFs to become major participants in early pharmaceutical product markets. Although the U.S. competitive position in pharmaceutical markets has been declining since the mid-1970's, established U.S. companies appear strategically positioned to compete effectively in international biotechnology product markets as such markets develop.

*Schering-Plough is expected to spend more than $40 million on interferon R&D alone in 1983.

Established U.S. companies

The proliferation of many NBFs and the developments in biotechnology that have been made thus far have prompted many established U.S. companies to re-evaluate the competitive and technological environments in which they have been operating. To some extent, U.S. corporate investment in biotechnology has been both an aggressive and defensive response to the potential market threat represented by NBFs such as Biogen, Genex, Cetus, and Genentech. Although a few pharmaceutical and chemical companies such as Monsanto, DuPont, and Eli Lilly have had biotechnology research efforts underway since about 1978, most of the established U.S. companies now commercializing biotechnology did not begin to do so until about 1981.*

INVESTMENTS IN BIOTECHNOLOGY BY ESTABLISHED U.S. COMPANIES

The motivations underlying established U.S. companies' decisions to invest in biotechnology and the forms that each investment takes vary from company to company. When biotechnology first began to receive commercial attention, many established U.S. companies, particularly those without a major in-house biotechnology program, elected to gain in-house expertise by obtaining technology through research contracts with NBFs or universities,** R&D contracts with NBFs,*** or equity investments in NBFs. For some established U.S. companies, contracts with or equity positions in NBFs are still a major route by which to expand their knowledge of biotechnology.† However, several of the established U.S. companies that initially entered the field through R&D joint ventures are now increasing their commitment to biotechnology through internal expansion.

Since 1978, equity investments in NBFs, often accompanied by research contracts, have been a popular way for established U.S. companies to gain expertise in biotechnology. Table 13 lists many established U.S. companies that have made equity investments in NBFs and the NBF in which they have taken the equity position.* Although only individual corporate strategies can specifically explain why established U.S companies have taken positions in NBFs, some of the investments may have been viewed by the established companies as:

- a defensive strategy against market share losses to unknown technologies,
- an avenue for diversification and greater return on investment, and
- a means of gaining a "window on the new technology."

Figure 12 provides the aggregate equity investment figures for 1977 to 1983 based on table 13. Review of table 13 and figure 12 shows that:

- equity investments in NBFs range from $0.5 million to $20 million;
- some established companies have made multiple investments in the same NBF;
- a number of established companies have made investments in more than one NBF;
- equity investments, in some cases, have led to the formation of another firm (e.g., Genentech and Corning Glass formed Genencor, and Diamond Shamrock and Salk Institute/ Biotechnology Industrial Associates formed Animal Vaccine Research Corp.); and
- equity investments have tapered off since 1982.

The years 1978 and 1979 appear to have marked the beginning of general U.S. corporate

*This statement is based on the responses to a survey conducted by OTA and the National Academy of Sciences. The survey questionnaire is reproduced in *Appendix E: OTA/NAS Survey of Personnel Needs of Firms in the United States.*

**Major university contracts in biotechnology appear to have been declining over time. University/industry relationships in biotechnology are discussed in *Chapter 17: University/Industry Relationships*.

***For a more detailed discussion of R&D joint ventures, see the section below entitled "Collaborative Ventures Between NBFs and Established U.S. Companies."

†In 1982, Monsanto, for example, committed approximately $40 million to outside contracts in biotechnology; however, the overall number of newly formed research and licensing agreements is waning as more and more established companies commit large amounts to in-house staff and facilities.

*A much smaller number of foreign established companies have taken equity position in American NBFs. They are not included in table 13. The notable foreign investors are Sandoz (in Genetics Institute), Novo (in Zymos), a group of Japanese and Swedish investors (in Genentech), C. Itoh (in Integrated Genetics), and Bayer in Molecular Diagnostics).

**The percentage of NBFs purchased by the established companies listed in table 13 range from 1.6 to 100 percent, with 10 to 30 percent being the most common.

Table 14.—Some Collaborative Ventures Between New Biotechnology Firms and Established U.S. and Foreign Companies[a]

New biotechnology firm*—*Established company

Biogen N.V. (Netherlands Antilles)[b]:
—*Meiji Seika Kaisha, Ltd.* (Japan) has license and development agreement with Biogen N.V. for the scale-up of a still unnamed agricultural chemical which Meiji could bring to market by 1984-85.
—*International Minerals Corp.* has exclusive marketing rights to Biogen's rDNA-produced swine and bovine growth hormones. Biogen will receive royalties.
—*Shionogi & Co., Ltd.* (Japan) will conduct clinical trials and pursue the commercial development in Japan of Biogen's gamma interferon for human therapeutic use.
—*Merck* is developing Biogen's hepatitis B vaccine.
—*Shionogi* (Japan) has a license from Biogen to develop and market Biogen's human serum albumin in Japan and Taiwan.
—*Shionogi* (Japan) has a license and development agreement with Biogen to develop interleukin-2. Shionogi will conduct Japanese clinical trials.
—*INCO* has a contract with Biogen to do studies of the feasibility of bioextraction of nonferrous metals from low-grade ores and other sources of minerals.
—*Fujisawa Pharmaceutical Co.* (Japan) has an agreement to develop and produce Biogen's tissue plasminogen activator in Japan, Taiwan, and South Korea.
—*Monsanto* will fund Biogen's developments of a technique to produce and purify tissue plasminogen activator.
—*KabiVitrum* (Sweden) is collaborating with Biogen in the development of commercial products based on Factor VIII. Biogen intends to market the products in the United States and Canada, and KabiVitrum will have the right to market such products in certain other countries.
—*Green Cross* (Japan) has a license from Biogen to manufacture hepatitis B vaccine. Green Cross has exclusive license to market in Japan.
—*Suntory, Ltd.* (Japan) has an agreement with Biogen under which Biogen will develop rDNA microorganisms to produce tumor necrosis factor, to scale-up production, and to support clinical trials, and Suntory will have exclusive marketing rights in Japan and Taiwan.
—*Teijin, Ltd.* (Japan) has a license to develop and market Biogen's Factor VIII in Japan, South Korea, Taiwan, Australia, and New Zealand.

Calgene:
—*Allied Chemical Corp.* has a contract with Calgene under which Calgene will do research in nutrient efficiency in plants.

Cambridge Bioscience:
—*Virbac,* a French animal health care company, has a contract with Cambridge Bioscience under which Cambridge Bioscience will develop feline leukemia virus vaccine.

Centocor:
—*FMC Corp.* has 50/50 joint venture to develop human-derived monoclonal antibodies (MAbs).
—*Toray/Fujizoki* (Japan) have signed an agreement to manufacture and market Centocor's hepatitis diagnostic in Japan.

Cetus:
—*Roussel Uclaf* (France) has a contract with Cetus under which Cetus produces vitamin B12. Cetus is receiving royalties.
—*TechAmerica* has a contract with Cetus under which Cetus will develop a rDNA antigen to be used as a vaccine against calf bovine diarrhea. TechAmerica will perform clinical research, manufacture, and market.
—*Norden Labs, Inc.* has a contract with Cetus under which Norden will produce and market rDNA colibacillosis vaccine. Cetus receives royalties.
—*Cooper* will market a MAb from Cetus Immune that is used in tissue typing for organ transplants.
—*Shell Oil Co.* gave a research contract to Cetus under which Cetus will develop human beta-I (fibroblast) interferon.

Chiron:
Merck possesses option for exclusive worldwide license for the use, manufacture, and sale of Chiron's hepatitis B vaccine.

Collaborative Genetics:
—*Akzo N.V.* (Netherlands) gave Collaborative Genetics a research contract to develop genetically manipulated micro-organisms to produce bovine growth hormone.
—*Green Cross* (Japan) has licensed from Collaborative and Warner-Lambert the process by which urokinase is microbially produced.
—*Dow* has given a research contract to Collaborative under which Collaborative will produce rennin via genetically manipulated micro-organisms.

Cytogen:
—*American Cyanamid* has an agreement with Cytogen to develop a MAb that will deliver a chemotherapeutic agent to cancer cells.

Damon Biotech:
—*Hoffmann-La Roche* (Switz.) has contracted Damon to apply its microencapsulation system to the production of MAbs. Hoffmann-La Roche will retain the marketing rights to the interferon produced by this process.

Enzo Biochem:
—*Meiji Seika Kaisha* (Japan) obtained worldwide marketing rights to products based on Enzo's hybridoma technology, including a newly developed pregnancy test.

Genentech:
—*Monsanto* is testing Genentech's bovine and porcine growth hormones. Commercialization and production will be joint effort.
—Genentech has a joint development contract with *Hoffmann-La Roche* for the production of leukocyte and fibroblast interferons. Hoffmann-La Roche will conduct testing to determine its effectiveness. Genentech will supply part of Roche's requirements and receive royalties on sales.
—*KabiVitrum* (Sweden) has worldwide (except in the United States) marketing rights for Genentech's human growth hormone.
—*Fluor* will develop commercial production operations for Genentech to scale-up new biotechnology products.

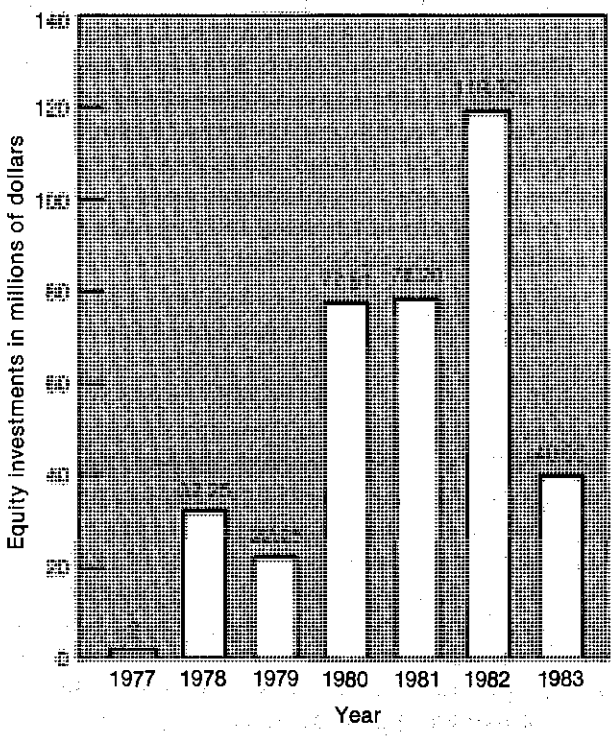
Table 13.—Equity Investments in New Biotechnology Firms by Established U.S. Companies, 1977-83[a] (Continued)

Date	U.S. established company	New biotechnology firm	Equity (millions of dollars)
1978	National Distillers	Cetus	5
1980	National Patent Development Corp.	Interferon Sciences	0.6
1980	Nuclear Medical Systems	Genetic Replication Technologies	0.95
1981	Phillips Petroleum	Salk Institute Biotechnology/Industrial Associates	10
1981	Rohm & Haas	Advanced Genetic Sciences	12
1979	Schering-Plough	Biogen	8
1980	Schering-Plough	Biogen	4
1982	Schering-Plough	DNAX[g]	29
1978	Standard Oil of California	Cetus	12.9
1978	Standard Oil of Indiana	Cetus	14
1982	Syntex[h]/Genetic Systems	Oncogen	9.5
1982	Syntex/Syva	Genetic Systems	9.5
1980	Tosco	Amgen	3.5

[a]As of May 1983.
[b]American Cynamid sold 375,000 shares of MGI to Moorman Manufacturing in 1983.
[c]Investment over a 6-year period.
[d]N.A.=Information not available.
[e]Biogen is only 80 percent U.S.-owned.
[f]Monsanto & Emerson Electric.
[g]Acquisition.
[h]Incorporated in Panama.

SOURCE: Office of Technology Assessment.

Figure 12.—Aggregate Equity Investments in New Biotechnology Firms by Established U.S. Companies, 1977-83[a]



[a]As of May 1983.

SOURCE: Office of Technology Assessment.

interest in biotechnology, with equity investments made by a number of oil and mining companies in the NBFs Biogen, Cetus, Genex, and Genentech. By 1980, commercial applications of biotechnology were advancing in industrial areas where some established companies had no prior R&D commitment, and from 1979 to 1980, there was a dramatic increase in the number and size of equity investments. Equity investments in NBFs have been made by U.S. companies from a variety of industrial sectors: Monsanto (chemicals), for example, invested $20 million in Biogen and $5.5 million in Collagen; Lubrizol (chemicals) made a second equity investment in Genentech totaling $15 million; Fluor (engineering) invested $9 million; and Koppers (mining) expanded its equity position in Genex by investing $12 million.

In 1981, the amount of equity capital invested in NBFs barely exceeded the amount invested the previous year, but in 1982, equity investments soared to a record high of $119 million, an increase of 52 percent over 1981, and the highest level of equity investments in biotechnology ever made. In 1983, the level of equity investments in NBFs dropped significantly. A growing commitment among established U.S. companies to in-house R&D programs in conjunction with pre-

Table 14.—Some Collaborative Ventures Between New Biotechnology Firms and Established U.S. and Foreign Companies[a] (Continued)

New biotechnology firm—Established company	*New biotechnology firm—Established company*
joint venture is to combine Hybritech's MAb manufacturing technique and Teijin's unique technique of binding a cytotoxic substance to an antibody for cancer therapy. —*Travenol Laboratories, Inc.* will provide $1 million for research and $1.9 million for stepwise benchmark payments to Hybritech to develop MAbs for treating major bacterial infections. Hybritech will receive royalties on Travenol's worldwide sales. ***Immunex:*** —*Diamond Shamrock* has a license to commercialize Immunex's lymphokines for use in animals. ***Integrated Genetics, Inc.:*** —*Connaught Laboratories, Ltd.* (Canada) has an R&D agreement with Integrated Genetics to produce hepatitis B surface antigen in yeast or mammalian cells. ***Interferon Sciences:*** —*Bristol-Myers* has a licensing and supply agreement with Interferon Sciences under which Bristol-Myers will commercially develop interferon for the treatment of herpes zoster. —*Green Cross* (Japan) has a $2.5 million R&D and supply agreement with Interferon Sciences under which Interferon Sciences will supply Green Cross with gamma and alpha interferon. —*Collaborative Research* is synthesizing interferon in yeast. Collaborative provides Interferon Sciences with the alpha-interferon producing clones. Interferon Sciences is involved in the product end and plans to optimize the bioprocess. ***Interferon Sciences, Inc./Collaborative Genetics:*** —Both companies have a license agreement under which *Green Cross* shares results of a study evaluating application of rDNA technology to the production of interferon by yeast or other micro-organisms. ***Molecular Genetics, Inc.:*** —*American Cyanamid* has an R&D contract and licensing agreement with Molecular Genetics under which Molecular Genetics will develop bovine growth hormone. Cyanamid is conducting scale-up and testing. —*American Cyanamid* has sponsored an R&D contract and formed a licensing agreement with Molecular Genetics to select herbicide-resistant corn in tissue culture.	—*American Cyanamid* sponsored an R&D contract and formed a licensing agreement with Molecular Genetics under which American Cyanamid will conduct human testing, secure regulatory approvals, and manufacture and market any products developed from Molecular's human herpes simplex vaccine research. *Lederle* has begun preclinical testing. —*Philips-Roxane* (subsidiary of Boehringer-Ingleheim (F.R.G.)) sponsored research and has exclusive license to manufacture and market bovine papilloma virus vaccine developed by Molecular Genetics. Philips-Roxane is responsible for obtaining government approval. ***Monoclonal Antibodies:*** —*Ortho Pharmaceuticals* has an agreement with Monoclonal Antibodies under which Monoclonal Antibodies will develop and manufacture an innovate diagnostic product that will be marketed by Ortho. ***Petrogen, Inc.:*** —*Magna Corp.* has a 10-year joint venture with Petrogen under which Magna will field test micro-organisms developed by Petrogen for use in shallow, low-pressure stripper wells. ***ARCO Plant Cell Research Institute:*** —*H. J. Heinz* and ARCO Plant Cell Research Institute have a joint venture to develop a tomato with high solids content. ***Schering-Plough:*** —*Yamanouchi* (Japan) will manufacture alpha interferon using Schering-Plough's technology. ***University Genetics:*** —*Kureha Chemical Industry* (Japan) has a license to develop bovine interferon based on University Genetics' technology. ***Worne Biotechnology:*** —*Omni Biotech* (Canada) and Worne are in a joint project to extract usable petroleum from Canadian oil sands using micro-organisms. ***Zymos, Inc.:*** —*Cooper Laboratories* funded research and has the rights to alpha-1 antitrypsin developed by Zymos for possible treatment in emphysema.

[a]Major public contracts, agreements, and ventures.
[b]Biogen is only about 80-percent U.S. owned.

SOURCE: Office of Technology Asseesment.

- NBFs in many cases are still reliant on established companies for working capital, whether it be through research contract revenue or equity investments.
- Licensing agreements diffuse technology to different industrial sectors and promote the development of biotechnology in the United States.

Typically, an NBF will enter into an R&D contract, joint venture, or licensing agreement with an established U.S. company to secure funds for proprietary R&D, or, in the case of some pharmaceutical products, to obtain a partner to do clinical evaluations, obtain regulatory approvals, and undertake marketing. Furthermore, the revenues make the new firm attractive to investors if and

Established U.S. companies also have a competitive role to play in research, because continuous technical advances will be necessary to maintain the present competitive strength of the United States. As the established U.S. multinational companies, along with the other later entrants, expand their in-house research and production facilities they will undoubtedly make substantial contributions to the U.S. commercialization of biotechnology.

Collaborative ventures between NBFs and established U.S. companies

As suggested previously, the development of biotechnology in the United States is unique from the standpoint of the dynamics of the interrelationships between NBFs and the large established U.S. companies. NBFs and established U.S. companies not only compete with one another, but they also, through joint ventures of many kinds, complement one another's skills. In addition to delaying a "shake-out" among NBFs, joint ventures between NBFs and established companies have allowed NBFs to concentrate on the research-intensive stages of product development, the area in which they have an advantage in relation to most established U.S. companies.

A joint venture is a form of association between separate business entities that falls short of a formal merger, but that unites certain agreed upon resources of each entity for a limited purpose.* Joint ventures between NBFs and established companies are attractive for at least three reasons:

- they assist NBFs and established companies in overcoming resource limitations which may prevent them from developing or marketing a product themselves;
- they offer established companies and NBFs less costly methods by which to develop expertise in areas in which they lack in-house capability; and
- they provide established companies with an opportunity to achieve economies of scale in R&D for complex technological problems that might not otherwise be obtainable.

Considerable expenditures in time and money are required to research, develop, and market biotechnologically produced products. The NBFs, started exclusively to exploit innovations in biotechnology, have initially concentrated their activities on research. As a rule, therefore, NBFs have limited financial resources with which to fund production scale-up activities beyond the laboratory or pilot plant stage, not to mention the financing required for regulatory approval and marketing should their research activities in biotechnology yield pharmaceuticals and to a lesser extent, animal drugs and biologics, food additives, chemicals, or micro-organisms for deliberate release into the environment. Established companies have an advantage over NBFs in that they have relatively more financial strength, regulatory experience, and product distribution channels that are already in place, although many established companies are at a disadvantage compared to NBFs with respect to the possession of technical expertise in biotechnology. R&D joint ventures and contracts between NBFs and established companies, therefore, reflect a mutual search for complementary skills and resources.

Examples of the collaborative agreements that are taking place between NBFs and established U.S. and foreign companies are shown in table 14.* R&D contracts accompanied by product licensing agreements form the basis for most joint ventures between NBFs and established U.S. companies in the area of pharmaceuticals. Furthermore, equity investments in NBFs by established companies are often accompanied by R&D contracts. Equity joint ventures wherein equity capital is provided by both partners (e.g., Genencor) for R&D or marketing are less common. Since research contracts and product licensing agreements characterize most joint ventures, three points should be kept in mind throughout this section:

- Licensing agreements and future royalties provide NBFs with financing to do their proprietary research.

Chapter 18: Antitrust Law explores some of the legal considerations surrounding R&D joint ventures, and *Chapter 12: Financing and Tax Incentives for Firms* highlights joint ventures from a financial perspective.

*The large proportion of pharmaceutical joint agreements presented in table 14 reflects the commercial emphasis by companies on pharmaceutical development.

the near term. Most NBFs are not assured that operating revenues from established companies will be sufficient to fund projected product development. The reliance on established firms for manufacturing and royalty incomes could also jeoprdize the future earning power of many small firms. Those NBFs that have licensed to established companies the right to manufacture and market their products do not control the timing of market entry for these products. If royalties are expected to be the major source of an NBF's operating revenue, then the NBF's correct choice of a marketing partner is crucial for financial success. It might not be wise, for example, for an NBF to choose a marketing partner whose own products stand to be displaced by the new product.

The NBF Genentech, for example, licensed Eli Lilly to produce the new human insulin product Humulin® On the one hand, because Lilly controls the insulin market in the United States, an effective distribution network is already in place and Humulin® sales could be substantial. On the other hand, Humulin® is a competitor of Eli Lilly's animal-derived insulins, and Eli Lilly holds about 85 percent of the U.S. insulin market. In other words, the pace of market development for Humulin® is controlled by the very company whose monopoly position Humulin® sales otherwise might challenge. For example, Eli Lilly could be threatened by the introduction of the new product, and delay the marketing of Humulin®, or if the costs of producing Humulin® are not competitive with Eli Lilly's existing insulin product, then Eli Lilly could also delay the market introduction of Humulin®. Other arrangements of this kind between NBFs and established companies could slow the market entry of new products and reduce the flow of royalties to NBFs.*

An obvious disadvantage common to all NBFs is the sale of technology to ensure survival. By transferring technology to established companies, some NBFs could be canceling the comparative advantage they currently possess in domestic markets. If the competitive pressures arising from the technology transfer to established companies grow too strong, many NBFs will not survive. Additionally, since the most important factor in market acceptance and market share competition may be the timing of market introduction of competitive therapeutic and diagnostic products, the correct choice of partners could be crucial to the U.S. competitive strength.

*See *Chapter 5: Pharmaceuticals* and *Appendix C: A Comparison of the U.S. Semiconductor Industry and Biotechnology* for a more general discussion of the Eli Lilly-Genentech joint agreement.

Collaborative ventures between NBFs and established foreign companies

The observations made concerning NBFs' reliance on established U.S. companies apply equally to R&D arrangements between NBFs and established foreign firms. But the same situation has greater implications for U.S. competitiveness when viewed in the context of international technology transfer.*

Joint ventures between NBFs and established foreign companies are motivated in part by a foreign need for American technology and in part by NBFs' desire to retain U.S. marketing rights—rights often ceded in joint ventures with established U.S. companies. Most observers would agree that the United States is currently the leader in developing commercial applications of biotechnology. Reflecting the strong technological position of some U.S. companies is the increasing number of established foreign companies that are seeking R&D contracts with NBFs. Between 1981 and 1982, for example, the NBF Biogen experienced a 948-percent increase ($520,000 to $5.5 million) in R&D fees from Japanese companies (3), while Genentech experienced a 504-percent increase ($2.6 million to $15.7 million) (33). NBFs often seek joint marketing agreements with established foreign companies for access to foreign markets. On the basis of publicly available R&D joint venture agreements, it appears that the United States is a net exporter of technology.

Foreign companies' joint ventures with NBFs generally take the form of licensing agreements for R&D, and few foreign companies seem to be taking equity positions in the NBFs. From the NBFs' point of view, the same advantages (e.g., the

*There are enormous difficulties in assessing the degree of technology inflow and outflow because of the many ways technology can be transferred; however, most observers would probably agree that the current net flow of biotechnology is outward from the United States.

Table 14.—Some Collaborative Ventures Between New Biotechnology Firms and Established U.S. and Foreign Companies[a] (Continued)

New biotechnology firm—*Established company*	*New biotechnology firm*—*Established company*
—*Eli Lilly* has been granted exclusive worldwide rights to manufacture and market Genentech's human insulin. —*Corning* and Genentech have a joint venture (Genencor) to manufacture and market rDNA-produced enzymes for food processing and chemical industries. Corning provides expertise in immobilization of enzymes. ***Genetics Institute:*** —*Sandoz* (Switz.) is funding research by Genetics Institute to clone monokines and lymphokines in bacteria, i.e., interleukin-2. ***Genetic Systems Corp.:*** —*Cutter Labs* and Genetic Systems have a $2.5 million joint venture to develop human MAbs for the diagnosis and treatment of *Pseudomonas* infections. For other MAb products, Genetic Systems will do R&D and market the diagnostic products, and Cutter will market therapeutic products. —*Syva* has a research, development, and marketing agreement with Genetic Systems which will finance some of Genetic Systems' R&D activities related to diagnostic tests for sexually transmitted diseases such as herpes, gonorrhea, and chlamydia. Genetic Systems receives 5 percent royalties on sales. —*Daiichi Pure Chemicals Co., Ltd.* (Japan) (a subsidiary of Daiichi Seiyaku Co.) entered into an agreement with Genetic Systems to collaborate on the R&D of a diagnostic test kit for blood disorders in the human immune system. Daiichi will receive the exclusive manufacturing and marketing rights in Japan, Taiwan, Mainland China, and Southeast Asia, for the products for treating blood disorders. Genetic Systems will receive royalties. —A separate marketing agreement with Daiichi grants the exclusive right to purchase and sell, for research products only, in Japan and other Asian countries, certain MAbs developed by Genetic Systems. —A joint venture between *Syva Co.* (a subsidiary of Syntex Corp.) and Genetic Systems to develop MAbs for the diagnosis and treatment of human cancer. —*New England Nuclear* (E. I. du Pont de Nemours & Co.) has the rights to market Genetic Systems' MAbs for the identification of different types of human blood cells to the research market throughout the world, with the exception of Japan, Taiwan, People's Republic of China, and Southeast Asia, which are covered by Daiichi Pure Chemicals Co., Ltd. ***Genex:*** —*Yamanouchi Pharmaceutical Co.* (Japan) will manufacture and sell a biological product developed by Genex which dissolves fibrin. Yamanouchi will market the product for 15 years, paying Genex a licensing fee of 8 percent of sales for development and scale-up. Genex will retain the patent rights. —*Bristol-Myers Co.* has a contract with Genex under which Genex will develop genetically modified microorganisms that will produce leukocyte (alpha) and fibroblast (beta) interferons. Bristol-Myers owns all rights. Genex receives royalties.	—A Japanese company (proprietary) has a contract with Genex under which Genex will develop a genetically modified micro-organism to produce L-tryptophan. All discoveries will be the sole property of the Japanese customer. —*Vineland Laboratories* and Genex have a joint development project to produce a vaccine against coccidiosis. —*Koppers* has a contract with Genex under which Genex will develop genetically modified micro-organisms to do biocatalytic transformations of aromatic chemicals from coal distillate derivatives. All micro-organisms and research findings are the sole property of Koppers. Genex will receive royalties. —*Schering AG (F.R.G.)* has a contract with Genex under which Genex will develop a microbe that will produce a blood plasma protein. Schering AG will receive worldwide exclusive license. —*Green Cross* (Japan) has a contract with Genex under which Genex will develop a microbial strain that produces human serum albumin (HSA). Green Cross will receive an exclusive license to sell, for at least 15 years, all microbially produced HSA under the contract in Japan, Southeast Asia, India, China, Australia, New Zealand, North America, and South America. Genex receives royalties. —*KabiVitrum* (Sweden) has a contract with Genex for HSA similar to that of Green Cross except Kabi's rights are limited to Africa, Europe, and the Middle East. —*Yoshitomi Pharmaceutical Industries* (Japan) has a contract with Genex under which Genex will develop genetically modified micro-organisms to produce interleukin-2. —*Mitsui Toatsu Chemicals Inc.* (Japan) contracted Genex to develop a microbial strain that produces human urokinase. Genex will retain the patent and Mitsui Toatsu will receive an exclusive license with the right to make, use, and sell the product for the royalty period, about 15 years. —*Mitsubishi Chemical Industries, Ltd.* (Japan) will develop and market Genex's HSA. —*Pharmacia* has a contract with Genex under which Genex will develop a nonpathogenic strain of bacteria that would produce a protein with potential therapeutic applications. ***Hana Biologics, Inc.:*** —*Recordati S.p.A.* (Italy) has an agreement with Hana under which Hana will develop and distribute biomedical research and MAb diagnostic products. —*Fujizoki Pharmaceutical Co.* (Japan) has a joint venture with Hana under which Hana will develop new immunodiagnostic tests. Also, Fujizoki has a distribution agreement with Hana under which Fujizoki will market Hana products in Japan. ***Hybritech:*** —*Teijin, Ltd.* (Japan) has an agreement with Hybritech under which Hybritech will develop human MAbs for treatment of lung, breast, colorectal, prostate, and certain leukemia-lymphoma type cancers. The goal of the

nology's commercial development in the United States through innovation, technology diffusion, product market development, and encouragement of technical advances because of the increased domestic competition they generate.

The financial constraints faced by the NBFs in the United States have led NBFs into R&D joint ventures and licensing agreements that are diffusing NBF-generated innovations to established U.S. and foreign companies. The collaborative ventures between NBFs and established U.S. companies, by broadening the U.S. technology base for future biotechnology development, in the short run have promoted competitive vigor among U.S. companies commercializing biotechnology. Increasing domestic competition arising from established company R&D, however, stands to threaten the survival of many NBFs and, consequently, the source of much of the current innovation in biotechnology. Since the established U.S. companies now have some control over the later aspects of product development, they can control the rate at which some of the early products are introduced to the marketplace. It is not clear what this situation may do to the U.S. competitive position.

Although NBFs have assumed much of the risk associated with biotechnology's early development, established U.S. companies are making substantial contributions to the U.S. commercialization effort. Through equity investments and licensing and contract agreements with NBFs, established U.S. companies are providing many NBFs with the necessary financial resources to remain solvent. Through joint development agreements with NBFs, many established companies will also provide the necessary production and marketing resources to bring many NBF products to world markets. These resources, in turn, are helping to sustain the rapid pace of technical advance spurred by NBFs. Recently, more and more established U.S. companies have been increasing their in-house investments in biotechnology research and production facilities, so the role of established U.S. companies in the U.S. biotechnology commercialization effort is expanding.

U.S. competitive strength in biotechnology will be tested when large-scale production begins and bioprocessing problems are addressed. The Japanese have extensive experience in bioprocess technology, and dozens of strong "old biotechnology" companies from a variety of industrial sectors in Japan are hoping to use new biotechnology as alever to enter profitable and expanding pharmaceutical markets. Japanese companies, which already dominate biologically produced amino acid markets, are also major competitors in new antibiotic markets; in the future, they could dominate other specialty chemical and pharmaceutical markets as well.

Pharmaceutical markets will be the first proving ground for U.S. competitive strength. International competition will be intense, and the American drug and chemical companies, as well as some NBFs, will be competing against not only the Japanese companies but also the major pharmaceutical and chemical companies of Western Europe, all of whom expect to recover their biotechnology investments through extensive international market penetration. Although there seem to be fewer European companies than Japanese companies commercializing biotechnology, the potential of European pharmaceutical companies such as Hoechst (F.R.G.), Rhone Poulenc and Elf Aquitaine (France), ICI, Wellcome, and Glaxo (U.K.), and Hoffmann-La Roche (Switzerland) is impressive. Thus, to remain competitive internationally and to compete effectively in the future, it is crucial for U.S. companies to rely on rapid innovation made possible by NBFs, rapid product development made possible by established companies, and the accumulated and combined experience of both groups of firms.

when the firm wants to use the public market as a source of financing. Typically, the research objective of the NBF in many R&D joint ventures is to develop a micro-organism and the related bioprocessing, extraction, and purification processes needed to produce the desired product in quantities sufficient to proceed with testing. The established company then organizes and implements clinical trials (if necessary) and takes responsibility for the production and marketing of the product. Joint venture partners are usually sought by NBFs to share the risk in new technological areas that appear to have significant commercial applications but that require large investments and have long development times. Joint venture partners are usually sought by established companies because they can provide a "window on the new technology" in addition to oftentimes providing products. Corporate equity investments in NBFs, in addition to providing "windows on the new technologies," can also provide the corporate investor with the possibility of a large return on its investment when (and if) the NBF goes public, or, if the NBF is already publicly held, with potential profit if the stock increases in value.

NBFs in general retain the rights to any patents resulting from the contract research performed, and should the product be marketed, the NBF obtains income through the royalties, which over a range of products may enhance the NBF's financial position so as to enable it to later enter future markets independently. The established company often obtains an exclusive license to the technology developed through the contract and also gains access to that specific product market. If the contract has been preceded by an equity investment, the established company might serve as a marketing partner to the NBF in diverse product areas.

R&D contracts also enable the established company to minimize the risks and costs associated with biotechnology R&D. Should the research not produce desirable results, the contract can be canceled and someone else has paid for the infrastructure. By sponsoring several companies at one time, as Schering-Plough, Koppers, and Martin Marietta have done, the sponsor can spread the risk of not finding the most relevant technology—in essence, portfolio diversification. Additionally, the research effort can be either short or long term depending on the desire of the contracting firm. By minimizing the front end costs and the risk, contracts serve as a kind of feasibility study (49). Successful contracts with NBFs or universities can lend credibility to the commercial potential of the new technology and can help obtain the corporate support necessary to fund future projects in the same field.

Established companies suffer no disadvantages in joint ventures with NBFs except a loss of risk capital should the research be unsuccessful. In fact, as the only buyers of the technology and the major group with the financial resources to commercialize it, established companies exert a great deal of control over the rate at which biotechnology is being developed in the United States.

NBFs do suffer disadvantages as a consequence of their own resource deficiencies, which necessitate their reliance on established companies. These financial reliances of NBFs on established companies will play a crucial role in the future viability of the entire NBF sector for three reasons:

- The low profit margins from licensing technology do not generally provide NBFs with adequate financing for growth and expansion.
- Contract relationships, and thus revenues, are very likely to be transitory. There is a strong economic incentive for established companies to exercise a high degree of "control" over their own product development efforts and to bring their own work in-house.
- The commercial success of many NBF products is reliant on the amount and timing of resources that licensees and partners (established companies) devote to clinical testing (when necessary), obtaining regulatory approval, and marketing.
- Some of the contracts with established companies are tightly written, making it difficult for some NBFs to pursue interesting research findings which might occur in the course of the contracted work.

NBFs with a heavy reliance on contract revenue could face uncertain futures unless their own proprietary research yields marketable products in

Genentech Sign Joint Venture Agreement," September/October 1983, p. 6.
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revenues) and disadvantages (e.g., reliance on royalty income instead of product sales and a loss of technological advantage) are associated with licensing agreements with foreign companies as are associated with licensing agreements with U.S. companies. From the standpoint of the U.S. competitive position in biotechnology, however, the advantages and disadvantages of such agreements are not at all the same. In the case of domestic-domestic licensing agreements, technology is diffused within the United States and U.S. biotechnology development is promoted. In the case of domestic-foreign agreements, technology is transferred out of the United States and thus contributes to the foreign development of technology.

Agreements in the pharmaceutical industry between established U.S. and foreign companies are more difficult to evaluate than agreements between NBFs and established foreign firms. Licensing in the pharmaceutical industry is standard practice to overcome the complexities of clinical testing, registration, and marketing in foreign countries. It is common for licensors to barter, so that they can obtain privileges to market in their territories some products developed by the licensee. The established U.S. companies applying biotechnology are in a position to be able to barter without a loss to their competitive position. The NBFs, if in need of financing or in pursuit of foreign markets, are not in such an advantageous position. The only bargaining chip they have is their proprietary research.

NBFs that because of their initial inability to finance development and clinical trials license some of their proprietary research to foreign companies may be ceding an indirect advantage to foreign companies. However, the licensing strategy and future royalty income may also provide some NBFs with the needed working capital to commercialize other research advantages. At this time, it remains unclear both how technology export will affect the commercial success of the NBFs and how it is likely to influence the U.S. competitive position in biotechnology.

Findings

U.S. efforts to commercialize biotechnology are currently the strongest in the world in part because of the unique dynamism and complementarity that exists between NBFs and established U.S. companies in developing biotechnology for wider commercial application and in part because of a strong U.S. support sector that supplies reagents, instrumentation, and software to the companies applying biotechnology. At present, most NBFs are still specializing in research-oriented phases of product and process development, precisely the commercial stage where they excel. The established companies, on the other hand, have assumed a major share of the responsibility for producing and marketing, and, when necessary, obtaining regulatory approval for, many of the earliest biotechnology products, the commercial stages where their resources are strongest.

Whether the dynamism arising from the competition and complementarity betwen NBFs and established companies will continue giving the United States a comparative advantage in the context of product introduction remains unclear. Since the established U.S. companies, through production and marketing agreements with NBFs, control the later stages of commercialization for many new products being developed, they will have considerable control over the pace at which these new products reach the market. Some established companies may have disincentives to market the new products that might compete with products they are already producing.

Biotechnology is still in an early stage of commercial development, and competition remains largely in research and early product development. In the current research-intensive phase of development, the new entrepreneurial firms founded specifically to exploit innovations and research advantages are providing the United States with a competitive edge in the commercial development of biotechnology. Through their R&D efforts, NBFs are contributing to biotech-

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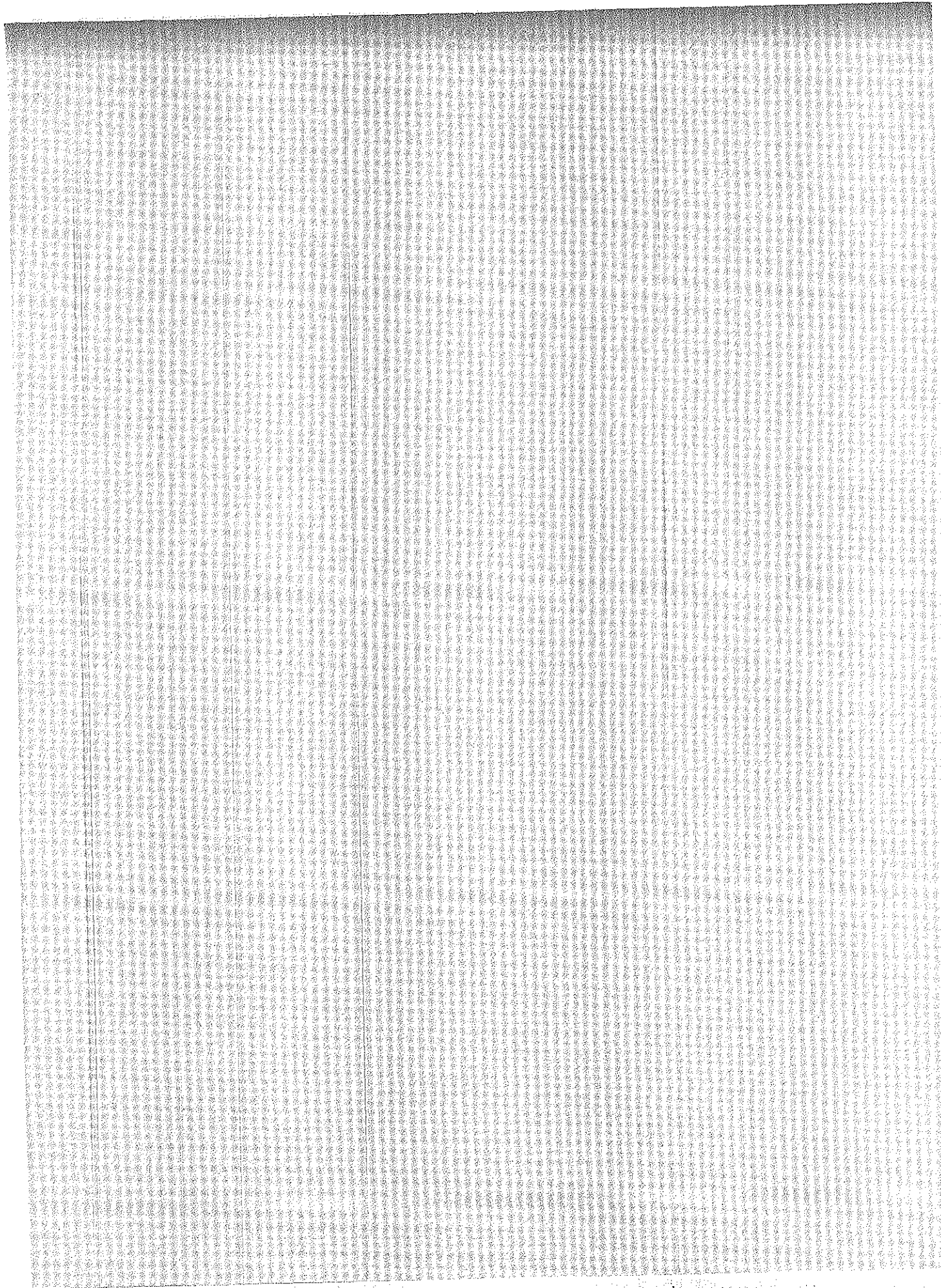

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PART III
Applications of Biotechnology in Specific Industrial Sectors

initial exploration of some compounds with biotechnology.

This chapter introduces the scientific and commercial bases of a number of pharmaceutical developments that exemplify biotechnology's promise in the pharmaceutical industry. Some examples include human insulin (hI), the first rDNA-manufactured product of biotechnology to reach the marketplace, interferon (Ifn), human growth hormone (hGH), and human serum albumin (HSA) rDNA projects. Other examples discussed are monoclonal antibodies (MAbs) and DNA hybridization probes, which are already being marketed for in vitro diagnostic use. Discussions include market profiles for each of these compounds, many of which will compete with products made by other methods.

Several important points are raised in this chapter that are discussed throughout this report. The first is that government regulation and licensing of pharmaceuticals play a major part in the development of these new products. With the rapid progress taking place in biotechnology, technical barriers may in some instances become secondary to regulatory barriers. Regulatory considerations that have shaped the use of biotechnology in the pharmaceutical industry are noted in this chapter.*

A second point is that in assessing the potential for biotechnology's use throughout the pharmaceutical industry, it is important to examine the receptivity of established companies to the adoption of new production methods. Traditionally, funding for most of the applied research and development of new pharmaceutical products in the United States has been provided by large pharmaceutical manufacturers. Since these manufacturers generally command the markets for products made by conventional means, they may have vested interests in established products that will impede the development and marketing of new products. This situation might perpetuate the problem of decreasing innovation in the pharmaceutical industry and contribute to the underdevelopment of biotechnology applications to pharmaceuticals.

*For a further discussion of regulatory factors that affect the use of biotechnology in the pharmaceutical and other industrial sectors, see *Chapter 15: Health, Safety, and Environmental Regulation.*

Regulatory proteins

The use of biotechnology to manufacture pharmaceutical products can be viewed in several ways. First, biotechnology may be used as a substitute for conventional methods of production, which include chemical synthesis and extraction from tissue. The successful cloning projects and microbial production of the proteins hI, Ifns, and hGH in rDNA systems, outlined below, are valuable as paradigms for biotechnology's role in developing competitive pharmaceutical substitutes. Second, biotechnology may be used to produce unprecedented amounts of scarce biological compounds, of which certain regulatory proteins provide the leading examples. Finally, the use of biotechnological methods yields basic knowledge on which future research can be based.

Human insulin

The first therapeutic agent produced by means of rDNA technology to achieve regulatory approval and market introduction is hI, marketed under the name Humulin®.* Although Humulin® may be the debutant of rDNA produced drugs, the extent to which rDNA-produced hI will be substituted in the marketplace for animal insulin is uncertain. Insulin derived from animals has long been the largest volume peptide hormone used in medicine. Human insulin differs only slightly from that of pigs and cows, and its incremental benefits have yet to be demonstrated (82).

*Humulin® has been approved in both the United States and the United Kingdom.

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tracted with Biogen S.A. (Switzerland)* to develop an alternative rDNA process for the production of hI (11).

- *Refinement of process technology.* The race to supply international insulin markets has spawned further biotechnological innovation in the pharmaceutical industry. The A and B protein chains of insulin can join in several ways, only one of which is correct. Combining the two chains by nonbiological chemistry is generally regarded as the "hard way" to make insulin. In the body, a connecting peptide in proinsulin (the precursor of insulin) positions the chains appropriately for joining to make the biologically active form of insulin. The connecting peptide is deleted when proinsulin is converted to insulin within pancreatic cells. Work to design bioprocesses using immobilized enzymes** to transform rDNA-produced proinsulin into insulin and to separate the products is currently underway. Lilly has reported the production of human proinsulin in bacteria through rDNA technology and the efficient conversion of proinsulin to hI (27). The NBF Cetus (U.S.) also has an improved proinsulin process, and Hoechst (F.R.G.) is reported to be developing one (10).
- *Clarification of related problems.* The injection of insulin has saved the lives of many diabetics, but the delivery of insulin by injection is thought to cause complications.*** Initial hopes for rDNA-produced hI centered on avoiding allergic reactions to impurities in insulin preparations, but these hopes have not been realized. Although results with patients switching from animal insulin to hI are encouraging, substantial allergic responses sometimes occur in patients taking hI for the first time (79). These problems probably arise because insulin is administered by subcutaneous injection. Thus, improvements in the mode of delivering insulin to patients may be at least as important to commercial implementation as technical advances in rDNA production of hI. (See *Box B.—Recent Work on Drug Delivery Systems.)*

  Some diabetic complications may not be caused simply by insulin deficiency. Human proinsulin, for example, may have therapeutic value. Animal proinsulin, which differs significantly from its human counterpart, is considered a contaminant in preparations of animal insulin. However, some scientists hypothesize that administration of human proinsulin may be beneficial to diabetic patients. Human proinsulin's availability through rDNA technology is allowing Eli Lilly to evaluate this hypothesis (27).

Interferons

Ifns, a class of immune regulators or lymphokines, are proteins that regulate the response of cells to viral infections and cancer proliferation. These extraordinarily potent substances are the subject of the most widely publicized, well-funded applications of rDNA technology to date, but details of their functions remain unknown. Until recently, the study of Ifns was limited by the extremely small amounts of Ifn that could be obtained from cultured cells. Now, however, rDNA technology allows production of large quantities of Ifn-like proteins for testing as pharmaceutical products. Despite certain structural differences from native Ifns,* rDNA-produced Ifns appear to have identical effects on cultured cells.

The cloning and production of Ifns illustrate several aspects of the commercialization of biotechnology:

- the use of rDNA technology to produce a scarce product in quantities sufficient for research on the product's effects;
- a massive, competitive scale-up campaign by

*Biogen N.V., the parent company of the Biogen group, is registered in the Netherlands Antilles. Biogen S.A., one of Biogen N.V.'s four principal operating subsidiaries, is a Swiss corporation that conducts R&D under contract with Biogen N.V.

**Immobilized enzymes are enzymes bound to solid supports so that they can exert their catalytic effects on dissolved substances without becoming inextricably mixed up with the reactants and products. For further discussion, see *Chapter 3: The Technologies.*

***In spite of daily injection of insulin, long-term complications continue to plague many diabetics. After 20 to 30 years of disease patients often develop blindness, need for leg amputations, kidney failure, stroke, heart disease, and/or nerve damage. About 10 percent of all hospital days (21 million per year) are consequences of diabetes, and the disease accounts for 19 million physician visits per year (49).

*Ifns produced by rDNA in bacteria lack carbohydrate (sugar) groups found on native Ifns. It is not known to what extent the absence of these groups affects protein function.

Chapter 5

Pharmaceuticals

Introduction

In the United States, many industrial biotechnology developments rest on the broad base of knowledge generated by university research in the biological sciences. Such research has been funded largely by the National Institutes of Health (NIH) and other public health-oriented sponsors. As a consequence, the first areas of application of new biotechnology in the United States have been in the pharmaceutical field. As research using the new genetic techniques has progressed, the pharmaceutical industry has been the leader in industrial applications.

Perhaps the most important application of biotechnology is to facilitate further biomedical research. Among the most intriguing areas of research using biotechnology are those pertaining to the nervous system, the immune system, the endocrine system, and cancer. As research in these areas yields insight into mechanisms of disease and healthy body function, basic questions about the organization and function of the brain, the nature of behavior, and the regulation of body functions may be answered. The illumination of these phenomena, in turn, may generate new possibilities for pharmaceutical products.

Pharmaceutical production may be improved with biotechnology in many ways. In some instances, production of pharmaceutical products by chemical synthesis or tissue extraction methods may be replaced by production from cloned genes. In other instances, applications of recombinant DNA (rDNA) technology may supplant traditional bioprocess methods for the production of antibiotics and other pharmaceutical compounds. Perhaps most importantly, new biotechnology provides a means of producing for the first time large amounts of compounds that are otherwise scarce. Thus, biotechnology may give rise to the development of entirely new pharmaceutical products.

Whatever the intended impact of a new pharmaceutical product, profit expectations usually govern the selection of projects for development. In considering the use of biotechnology to produce substances by new means, manufacturers must make multifaceted decisions that include the following considerations:

- the possibility of making products superior to those already marketed for a given purpose (i.e., more effective, convenient, safe, or economical);
- the technical feasibility of applying new methods (e.g., in rDNA applicatons, the feasibility of cloning DNA that directs synthesis of desired substances);
- the cost of the conventional method (e.g., chemical synthesis, tissue extraction, or traditional bioprocessing) and the potential to reduce costs with rDNA technology or other new methods;
- the nature of the market (i.e., whether it is of high enough value or volume to justify the substantial start up costs of new production methodology and regulatory approval);
- the possible loss of production of other substances with the change in methods (e.g., substances that were coproduced in the old method), as well as the potential for developing new, useful byproducts; and
- the possibility that the new methods employed will serve as useful models for preparing other compounds (whereby the new technology may justify high startup costs and the loss of formerly coproduced products).

Although biosynthesis may eventually reduce production costs of widely used compounds by several orders of magnitude (from millions of dollars per kilogram for chemical synthesis to several thousand dollars per kilogram for biosynthesis), chemical synthesis often suffices for production of low molecular weight compounds for testing. In many cases, substantial research and development (R&D) costs and high product attrition rate in pharmaceutical development may not justify

pharmaceutical products. There is some evidence that Ifns are effective in preventing certain viral infections, but more clinical trials are necessary to demonstrate their preventive abilities (81).* Most evidence that Ifns cure viral infections is anecdotal. In combination with other drugs, however, Ifns may prove useful in treatment of viral diseases (50,81,130,157). Extensive clinical trials to determine Ifns' effectiveness in the treatment of herpes and other viral infections are underway, some which are listed in table 16. The availability of Ifns made with rDNA technology has allowed many of these clinical trials to be undertaken.

Several clinical trials to evaluate Ifns' effectiveness in the treatment of cancer have taken place, but, at present, only limited conclusions can be drawn from the data. In some cases, Ifns inhibit tumor cell growth and may stimulate immune

*Assuming the safety criterion can be satisfied for the use of Ifn in a prophylactic mode, the immediate market may be for persons whose natural defenses are weakened by illness or medication, such as those undergoing cancer therapy with drugs or radiation. Other early markets could be for patients entering elective surgery or persons at high risk of viral exposure, such as teachers and certain medical personnel. Since Ifns apparently will be available from many sources, the dosage forms or delivery systems may be crucial for widespread acceptance and efficacy.

Table 16.—Some Ongoing Clinical Trials Using Alpha or Beta Interferons To Treat Human Viral Diseases

Disease	Interferons (source)	Sponsors	Remarks
Herpes genitalis	Alpha (rDNA, *E. coli*)	NIAID (U.S.)[a] and Schering-Plough (U.S.)[b]	Intramuscular injection for infection
	Alpha (blood buffy coat)	Enzo Biochem (U.S.)[c]	Topical ointment (Enzoferon®)
	Beta (cultured fibroblasts)	Inter-Yeda (Israel)[d]	Cream formulation (Frone®)
Herpes labialis	Beta (cultured fibroblasts)	Inter-Yeda[d]	Cream formulation (Frone®)
	Alpha (blood buffy coat)	Enzo Biochem[c]	Topical ointment (Enzoferon®)
Herpes keratitis and adenovirus conjunctivitis	Alpha (rDNA, *E. coli*)	Schering-Plough[b]	Topical ointment
Periocular herpes	Beta (cultured fibroblasts)	Inter-Yeda[d]	Cream formulation (Frone®)
Herpes zoster	Beta (cultured fibroblasts)	Bioferon (F.R.G.)	Approved for marketing in West Germany
	Alpha (rDNA, *E. coli*)	Hoffmann-La Roche (Switz.)[e]	100 immunosuppressed patients in trial
	Alpha (blood buffy coat)	NIAID[f]	Spread of shingles inhibited by injection
Herpes infections	Alpha (rDNA, *E. coli*)	Takeda Chem. (Japan)	Own mfr. after use of Hoffmann-La Roche's Ifn for Phase I
Genital warts	Beta (cultured fibroblasts)	Inter-Yeda[d]	Direct injection superior to topical application
Warts	Alpha (rDNA, *E. coli*)	Takeda Chem.	Many unreported tests underway
Laryngeal papillomas[g]	Lymphoblastoid and alpha	Wellcome (U.K.) & others	Injection following surgery
Cytomegalovirus	Alpha (rDNA, *E. coli*)	Hoffmann-La Roche	Injection for life-threatening infantile infections
Hepatitis B[h]	Alpha (rDNA, *E. coli*)	NIAID[a]	Alternated with Vidarabine® in 150-patient, 5-year, wide dose range trials
	Alpha (rDNA, *E. coli*)	Takeda Chem.	
Multiple sclerosis	Alpha (blood buffy coat)	National Multiple Sclerosis Society	Subcutaneous injection of Ifn from K. Cantell, Finnish Red Cross
Amyotrophic lateral sclerosis (Lou Gehrig's disease)[i]	Alpha (rDNA, *E. coli*)	Hoffmann-La Roche	Intravenous or intrathecal injection at two U.S. centers

[a]NIAID=National Institute of Allergy and Infectious Diseases.
[b]Schering-Plough's Ifn produced for clinical trials outside of the United States is synthesized microbially from genes cloned by Biogen S.A.
[c]Enzo Biochem obtained natural alpha-Ifn from New York Blood Center and sponsors trials at Sloan Kettering.
[d]Inter-Yeda is an Israeli firm conducting clinical trials primarily in Israel, Europe, and Canada.
[e]Genentech (U.S.) cloned and produces the Ifns being evaluated by Hoffmann-La Roche (Switzerland).
[f]Phase III studies at Stanford with Ifn obtained from K. Cantell, Finnish Red Cross, completed in 1982.
[g]Regrowth of these wart-like growths, apparently caused by virus, has been inhibited by Ifns in Danish studies.
[h]NIAID-sponsored trials indicate that Ifn alone is ineffective for the carrier state in males, but combinations with other drugs show promise.
[i]Viral origin suspected but not proved.

SOURCE: Office of Technology Assessment.

A profile of insulin markets and sales by Eli Lilly & Co. (U.S.)—the dominant producer and marketer of insulin, and licensee from Genentech Corp. (U.S.) of the new rDNA product—in the United States and Europe is shown in table 15. By 1985, as indicated in that table, both U.S. and European markets for insulin are expected to double. Eli Lilly is expected to retain a sizable portion of the U.S. market, but its greatest potential lies in penetrating foreign markets with Humulin®.

The development and commercialization of Humulin® establishes several important precedents of general significance to the introduction of biotechnology to industry:

- *Liaison between industry and academic scientists.* The original bacterial production of polypeptide chains of insulin at the new biotechnology firm (NBF)* Genentech made use of nucleic acid sequences synthesized by collaborators at City of Hope Medical Center, an academic laboratory that had capabilities not otherwise available to Genentech at the time (31).
- *Collaboration between NBFs and established companies.* Early in the development of Humulin®, Genentech entered a collaborative arrangement with Eli Lilly. Under the agreement, Genentech performed the rDNA work and received financial support for the work from Lilly. Lilly, in addition to providing this financial support, was responsible for manufacturing, marketing, and obtaining regulatory approval for the hI product that resulted from Genentech's work. This arrangement capitalized on Lilly's decades of experience in large-scale bioprocessing and the purification of insulin. Most significantly, Lilly was thoroughly familiar with insulin and the procedures of regulatory agencies, marketing, and distribution. Lilly was able to satisfy the Food and Drug Administration's (FDA's) requirements for approval of Humulin® in record time—4 years after the first bacterial preparation of hI. Under their arrangement, Genentech receives royalties from Lilly on the sale of Humulin®. Lilly, in turn, has access to improvement inventions by Genentech. Proinsulin, for example, produced from genes cloned by Genentech (disclosed in March 1980), may provide a more efficient route for the production of hI or may have clinical value of its own (see below). This pattern of collaboration between NBFs and established pharmaceutical firms is common.*
- *International joint ventures.* Though Eli Lilly has had little competition in the U.S. insulin market until now, the company has been only a minor factor in insulin markets outside of the United States. Recently, however, Lilly has licensed Swedish and Japanese firms to facilitate penetration of overseas markets (121). The leading insulin supplier abroad is the Danish firm Novo Industri A/S (142). Novo countered Lilly's rDNA hI effort by commercializing an enzymatic process devised in the early 1970's to transform insulin from swine into a form identical to hI.** Novo's symisynthetic hI product was approved for marketing in the United Kingdom shortly before Lilly's Humulin® attained approval there. To compete with Lilly in the United States for insulin markets, Novo formed a joint venture with an established American pharmaceutical company, E. R. Squibb (116). Novo also con-

*NBFs, as defined in *Chapter 4: Firms Commercializing Biotechnology,* are firms that have been started up specifically to capitalize on new biotechnology. Most NBFs are U.S. firms.

Table 15.—U.S. and European Markets for Insulin: Eli Lilly's Estimated Sales (millions of dollars)

	1981	1985 estimate
U.S. market:		
Lilly's sales.....	$133	$205[a]
Total market	$170	$345
European market:		
Lilly's sales.....	$ 12	$100[a]
Total market	$140	$285

NOTE: In 1981, approximately three-quarters of a ton of pure insulin for about 1.5 million diabetics was sold in the United States. The number of American diabetics is expected to increase to 2.1 million people between 1981 and 1986 (*Scrip,* 10/4/82).

[a]Includes sales of Humulin®.

SOURCE: Office of Technology Assessment, based on estimates from D. L. Smith, *Eli Lilly and Company: A Basic Study* (New York: Smith Barney Harris Upham & Co., Inc., September 1982).

*For a further discussion of collaboration between NBFs and established firms, see *Chapter 4: Firms Commercializing Biotechnology.*

**Hoechst (F.R.G.) and Nordisk (Denmark) have subsequently introduced semisynthetic hI products, and Shionogi (Japan) has developed a significant process improvement involving an immobilized bacterial enzyme (94).

Table 17.—Some Ongoing Clinical Trials of the Use of Interferons To Treat Cancer

Interferon supplier	Sponsor	Cancer	Phase	Institution
Natural lymphoblastoid (produced from cultured cells; contains mixture of interferon types):				
National Cancer Institute (NCI)	NCI	Broad range of advanced cancers	I	University of Wisconsin
NCI	NCI	Melanoma	II	Georgetown University
Wellcome Foundation	NCI	Ovary	II	Gynecological Oncology Group East Coast Oncology Group
Wellcome Foundation	NCI	Lymphoma, non-Hodgkin's	II	Southeast Oncology Group
NCI	NCI	Breast, metastatic	II	UCLA
NCI	NCI	Breast, recurrent	II	Duke University
Wellcome Foundation	NCI	Breast, recurrent	II	National Surgical Adjuvant Breast Project
NCI	NCI	Multiple myeloma	II	UCLA Duke University Memorial Sloan Kettering Cancer Center
NCI	NCI	Kidney (renal cell)	II	Duke University
Wellcome Foundation	NCI	Kidney (renal cell)	II	Southwest Oncology Group East Coast Oncology Group
Wellcome Foundation	NCI	Leukemia, childhood acute lymphocytic	I-II	Children's Cancer Study Group
NCI	NCI	Kaposi's sarcoma	II	NCI-Clinical Oncology Program
NCI	NCI	Colorectal	II	Memorial Sloan Kettering Cancer Center
rDNA-produced alpha-interferon:				
NCI	NCI	Broad range of advanced cancers	I	NCI-Frederick Cancer Research Facility
NCI	NCI	Lymphoma, non-Hodgkin's	II	NCI-Frederick Cancer Research Facility
NCI	NCI	Lymphoma, Burkitt's	II	NCI-Frederick Cancer Research Facility
NCI	NCI	Leukemia, chronic (CLL)		NCI-Frederick Cancer Research Facility
NCI	NCI	Mycosis fungoides		NCI-Frederick Cancer Research Facility
NCI	NCI	Leukemia, acute	I-II	University of Maryland
Schering-Plough (S-P)	NCI	Multiple myeloma	II	Wake Forest University
S-P	NCI	Bladder cancer	I-II	Northern California Oncology Group
S-P	S-P	Melanoma	II	Yale University University of Wisconsin University of Rochester M. S. Hershey Medical Center University of Missouri
S-P	S-P	Lymphoma, non-Hodgkin's	II	Roswell Park University of Maryland Harper Grace Hospital Yale University University of Chicago
S-P	S-P	Lymphoma, Hodgkin's	II	Yale University University of Chicago Wilford Hall Medical Center
S-P	S-P	Breast cancer	I	Bowman-Gray Hospital Harper Grace Hospital USC Cancer Center
S-P	S-P	Multiple myeloma	II	University of Texas (Galveston) Roswell Park Bowman-Gray Dartmouth-Hitchock
S-P	S-P	Leukemia, acute	II	UCLA
S-P	S-P	Kaposi's sarcoma	II	San Francisco General Hospital UCLA
S-P	S-P	Lung, small cell	II	USC Cancer Center Bowman-Gray
S-P	S-P	Head and neck cancer	II	University of Texas (Galveston)
S-P	S-P	Colorectal	II	Lombardi Cancer Center
Hoffmann-La Roche (HLR)	HLR	Broad range of advanced cancers	II	University of Arizona
HLR	HLR	Melanoma	II	University of Arizona Mayo Clinic

Box B.—Recent Work on Drug Delivery Systems

Oral ingestion is the most convenient route of administration for drugs, but many drugs, especially proteins, are destroyed by the digestive system before they can be absorbed from the stomach and gut. This problem could be especially serious for products of biotechnology, since many of them are proteins. Additionally, the effect of administration of drugs in a specified quantity one or more times a day gives the patient an initial high dose which tapers off with time. Pills and injections, therefore, can cause blood levels so high that undesirable side effects can occur and levels so low that the drug has no effect. Thus, for many years, researchers have sought to develop devices that deliver drugs continuously. Most of these delivery systems also aim to bypass the intestinal tract. Some possible drug delivery systems are discussed below:

- Several devices that deliver drugs through the skin have been developed. These transdermal devices work well for drugs with low molecular weights; research is proceeding on devices that will transfer higher molecular weight drugs, such as proteins, through the skin. Some methods being investigated use electricity or magnetism to speed the delivery (72).
- Continuous delivery of insulin is the optimal way to treat diabetes. External pumps that infuse insulin at a constant, low rate (with rate adjustments according to patients' eating and exercise schedules), combined with patient self-monitoring of blood glucose level, are gaining favor among diabetologists and a limited sector of the diabetic population. Pumps are fairly expensive and require sophisticated user attention. There are an estimated 5,000 to 7,000 users of external insulin infusion pumps (111).
- Implantable pumps are probably the best way to deliver proteins in most instances. Preprogramed implantable plants have been supplying insulin to diabetics for several years in the United States, France, and West Germany (49). More sophisticated devices are being developed by industry and by academic institutions with funding from NIH and the National Aeronautics and Space Administration (111). Animal trials with some models are underway. It would ultimately be most therapeutic if the device could respond automatically to a need in the patient and thus supply the drug in response to that need. For instance, diabetics need varying amounts of insulin depending on what they have eaten, whether they have exercised, and many other factors. Thus, if the pump could also have a sensor that detected the amount of blood sugar, the pump could supply appropriate amounts of insulin in response to need.
- A drug entrapped in an artificially constructed set of membrane layers (liposome) may be effective when given orally or injected into the bloodstream. The layers of membranes are slowly stripped off, causing a slow release of the drug. There is some evidence that insulin can be absorbed through the mucosal membranes of the upper and lower ends of the digestive tract (102,159), permitting consideration of liposome systems to control insulin release to areas of the mouth, nose, or rectum (3,21), but a reliable formulation has not yet been devised.

pharmaceutical manufacturers in advance of demonstrated uses of the product;
- the attempt to produce economically a functional glycoprotein (protein with attached sugar molecules) in an rDNA system;
- a pattern of international R&D investment that reflects the differing needs and medical practices of various nations; and
- the establishment of a U.S. national effort, via research grants and procurement contracts administered through the National Cancer Institute, the American Cancer Society (ACS), and other organizations, to support testing of Ifns toward a national goal (cure of cancer).*

Ifns are being considered for various pharmaceutical applications, but are not yet approved as

*In general, Ifn projects in the United States have received massive public funding. Studies in Sweden, and to a limited extent in the United States, stimulated appropriations of $5.4 million by the nonprofit ACS for extended clinical trials in the early 1980's. This was by far the greatest single commitment ever made by ACS, and it was followed by a boost in NIH funding for Ifn research from $7.7 million to $19.9 million for fiscal year 1980.

Table 18.—Some U.S. and Foreign Companies Involved in Interferon Gene Cloning Projects

Alpha Interferons:
Amgen (U.S.)
Biogen S.A. (Switzerland)/Schering-Plough (U.S.)[a]
Burroughs Wellcome (U.K.)
Cetus (U.S.)
Collaborative Research (U.S.)/Green Cross (Japan)[b]
Enzo Biochem (U.S.)
Genentech (U.S.)/Hoffmann-La Roche (Switzerland)[a]
Genex (U.S.)/Bristol-Myers (U.S.)
Life Sciences (U.S.)
Meloy Labs (U.S.)
New England Enzyme Center (U.S.)

Beta Interferons:
Cetus (U.S.)/Shell Oil (U.S.)[c]
Collaborative Research (U.S.)
E. I. du Pont de Nemours (U.S.)
Genex/Bristol-Myers
Hem Research (U.S.)
Serono Labs (Italy)/ARES Applied Research Systems (Switzerland)
Toray Industries (Japan)[d]

Gamma Interferons
Advanced Biotechnologies, Inc. (U.S.)
Amgen (U.S.)
Biogen/Shionogi (Japan)
Bristol-Myers
Cetus
Collaborative Research/Green Cross
Genentech[e]/Daiichi Seiyaku & Toray Industries (Japan)
Genetics Institute (U.S.)
Genex
Hoffmann-La Roche
ImmunoModulators Labs (U.S.)
Interferon Sciences (U.S.)
Revlon (U.S.)[f]
G. D. Searle (U.S.)
Suntory (Japan)[g]
Takeda (Japan)

[a]This alpha-Ifn lacks carbohydrate groups, but lack of glycosylation does not appear to influence activity.
[b]Attempting production in yeast.
[c]Clinical trials began early in 1983.
[d]Toray is scaling-up to a capacity of $3 - 10^{12}$ units per month and expects approval from Japan's Ministry of Health and Welfare soon for beta-Ifn as an anticancer drug (122).
[e]Genentech retained all manufacturing rights and only licensed its Japanese collaborators to sell in Japan and, perhaps, other Asian markets (32).
[f]Revlon's subsidiary, Meloy Laboratories was the first firm to supply both alpha-Ifn and gamma-Ifn to the National Cancer Institute.
[g]Using Genentech's published gamma-Ifn gene sequence (450 bases long), Suntory, a Japanese beverage company, took only 3 months to synthesize and clone the gamma-Ifn gene (119). Suntory has also succeeded in producing gamma-Ifn in yeast.

SOURCE: Office of Technology Assessment; and S. Panem, *The Interferon Crusade: Public Policy and Biomedical Dreams*, Brookings Institution, Washington, D.C., in press.

KabiVitrum AB, a firm owned by the Swedish Government, is the world's largest producer of hGH from frozen human pituitaries (113). KabiVitrum owns 50 percent of KabiGen AB, which has the sole rights to manufacture and market hGH made by the Genentech process anywhere in the world, except in the United States and Canada, where Genentech has sole rights (31). KabiGen researchers are among the long-term leaders in the study of other growth-promoting hormones, especially the polypeptides known as somatomedins (30,100).

Although it is premature to judge the likelihood of success, hGH is being evaluated for: 1) treating constitutionally delayed short stature; 2) improving healing of burns, wounds, and bone fractures; and 3) treating the deficiency of nitrogen assimilation known as cachexia (9). Approximately 3 percent of all children are thought to have constitutionally delayed short stature, and Genentech advisors speculate that as many as one-third of these might benefit from hGH treatment (136).*

Neuroactive peptides

Several important biosynthetic discoveries in recent years have involved identification of polypeptides in the body that act at the same cellular receptors that are affected by drugs. Some of the body's neuroactive peptides, for example, bind to the same receptors affected by opiate drugs and produce analgesic effects in the nervous system similar to those produced by these drugs. Two of the body's own "opiates," enkephalins and endorphins, appear to be structurally related to many other polypeptides that play various roles in the nervous and endocrine (hormonal) systems (41). Another neuroactive peptide that may affect neurological processes, including attention span, is melanocyte stimulating hormone (MSH). Some evidence suggests that MSH enhances the ability of test animals to pay attention to their environment, and MSH treatment has improved the health of some mentally retarded patients as well (53). Initial hopes raised by the treatment of schizophrenic patients with beta-endorphin have not withstood more rigorous testing. Results of testing some other peptides as antidepressants, after encouraging earlier studies, are also disappointing (53).

*Genentech, Lilly, Amgen, Monsanto, and other firms are also interested in applications of rDNA-produced GHs for food production purposes, and those investigations may prove complementary to the medically oriented studies (see *Chapter 6: Agriculture*).

cells to destroy cancerous cells; their effects on inhibiting tumor metastasis are better established than their ability to cause regression of primary tumors (8). With some exceptions, the tumors that respond to Ifn treatment (certain lymphomas, benign human esophogeal papillomavirus tumors, and leukemia, in particular) are also the most responsive to established chemotherapeutic agents. Some subtypes of interferon (e.g., alpha-Ifn) occasionally induce tumor regression in patients who are resistant to radiation and multiple drug therapy (95).

Several problems have been noted in initial clinical trials designed to test Ifns' effectiveness in the treatment of cancer. For example, side effects (fever, fatigue, and influenza-like symptoms) caused by injections of Ifn made in cell cultures were thought to be toxic reactions to impurities of the culture medium, but pure rDNA-produced Ifns show similar side effects (95). Thus, despite extensive research, numerous questions remain concerning Ifns' anticancer potential. Some ongoing clinical trials for Ifns' anticancer properties are listed in table 17.

Perhaps the most enlightening results stemming from Ifn research will concern cellular function during immune responses. Such results may prove extremely valuble in medicine. Better understanding of immune mechanisms, for example, may provide insight into the etiology of the recently problematic acquired immune-deficiency syndrome (AIDS). Substantial supplies of Ifns to conduct such research can now be produced with rDNA technology.

Though most rDNA-made Ifns currently under evaluation are produced in the bacterium *E. coli*, yeast are being increasingly employed as production organisms. Yeast require less stringent culture conditions than do most bacteria, have long records of reliability and safety in large-scale bioprocessing, and are more adaptable to continuous culture production than are many bacteria. Furthermore, because yeast more closely resembles higher organisms than bacteria, yeast can add sugar molecules to protein when necessary. Thus, modified products made in yeast are more likely to be pharmaceutically useful than unmodified products made in bacteria. Several groups have recently reported progress with Ifn production from yeast, including secretion of the Ifn polypeptide into the culture medium from which it can more easily be purified (45). Academic workers funded by the British firm Celltech, Ltd., have reported yields of alpha-Ifn as high as 15 milligrams (3 billion units*) per liter of yeast culture (139). Numerous genetic techniques are being devised to increase production: 1) amplification of the number of Ifn genes, 2) enhancement of gene expression by placing it under control of regulatory elements which can be varied without hampering cell growth, 3) limitation of product degradation, 4) inducement of product secretion, and 5) stabilization of microbial strains. Additionally, the Swiss company Hoffmann-La Roche has reported a MAb system for alpha-Ifn purification that gives in excess of 1,000-fold purification with 95 percent recovery of biological activity (133).

Many U.S. and foreign companies using biotechnology are working toward large-scale Ifn production. Some of the companies with Ifn gene cloning projects are listed in table 18. The large number of companies involved in Ifn production reflects the large market potential so widely publicized in the late 1970's. Since clinical trials have not supported many of the claims made for Ifns, companies are beginning to draw back from Ifn R&D.

The international pattern of interest and investment in the use of rDNA technology to produce Ifn reflects to some extent international differences in medicine and, possibly, movements to reduce national dependence on pharmaceutical imports. Japan, for instance, has long been the largest market in the world for cancer drugs, today exceeding $375 million in annual sales (compared to $210 million in the United States) (127), and is actively investigating the production of anticancer pharmaceutical products using new biotechnology.**

*A single dose of Ifn ranges from 1 million to 100 million units.

**Protein agents are especially popular for cancer treatment in Japan. Immunotherapeutic concepts which are regarded as experimental hypotheses in the West provide the rationale for administration in Japan of hundreds of millions of dollars worth of agents, such as Krestin® (an orally administered fungal glycoprotein that accounted for Japanese sales in 1981 of $230 million) and urokinase (which is used in Japan for indications not even suggested in the United States). Sales of over $117 million were recorded in 1981 for a streptococcal "vaccine," called Picibanil®, which Japanese physicians regard as an immunostimulant (118).

Table 19.—Some Proteins With Possible Pharmaceutical Applications Being Developed With Recombinant DNA Technology (Continued)

Class/substance	Size (number of amino acids)	Function	R&D status	Project sponsors	Applications
Macrophage inhibitory factor (MIF)	N.A.	Inhibits macrophage migration	Cell fusion	Denki Kagaku (Japan)	Immunotherapy
Respiratory system regulators:					
Alpha-1-antitrypsin	45,000 molecular weight	Prevents destruction of alveolar walls by elastase	rDNA in yeast	Zymos Corp. (U.S.)/ Cooper Laboratories (U.S.)	Emphysema treatment

[a]Armor Pharmaceutical Co., the source of salmon calcitonin in the United States, does not believe that rDNA technology offers significant advantages over chemical synthesis for the production of salmon calcitonin at the present time. A New Drug Application is pending for human calcitonin, but this product is 20 times less than salmon calcitonin for the same effects. Hence, the economics of human calcitonin production are less advantageous than those of salmon calcitonin production.
[b]Most reproductive hormones thus studied are glycoproteins consisting of two polypeptide chains. All share a common (89 amino acids long) alpha chain. Biological activity is manifested in the beta chain, and most cloning efforts focus on producing the biologically active component.
[c]N.A. = Information not available.

SOURCE: Office of Technology Assessment.

Despite the setbacks noted above, many investigators are confident that neuroactive peptides are among the most promising potential advances in medicine; thus, a great deal of research is being done on synthetic analogs of neuroactive peptides (e.g., 26,41) to identify structures that may have research or pharmaceutical applications. Lilly and Burroughs-Wellcome (U.K.) are investigating the use of enkephalin analogs in clinical trials in the United States. Foreign companies with major research programs concerning neuroactive peptides include Abello (F.R.G.), Hoechst (F.R.G.), Hoffmann-La Roche (Switzerland), Organon (Netherlands), Reckitt & Colman (U.K.), Roussel Uclaf (France), Sandoz (Switzerland), and Takeda (Japan). In addition to screening neuroactive peptides compounds for analgesic and anesthetic activity, researchers are attempting to recognize those compounds that might suppress coughing or diarrhea or might counteract asthenia, cerebral vascular disorders, failing memory, mental depression, Parkinson's disease, and forms of dementia, including senility.

Much basic research remains to be done before substantial use is made of neuroactive peptides as pharmaceutical compounds in medicine (53). Studies of these substances and their chemical analogs are expected to result in the development of new drugs, some of which may be produced with biotechnology. Companies vigorously pursuing the production of neuroactive peptides with biotechnology include Amgen (U.S.), which has cloned and obtained expression of the genes for the neuroactive peptide beta-endorphin (126), and Endorphin, Inc. (U.S.), which is primarily concerned with compounds active in both the nervous and digestive systems.

Lymphokines

Lymphokines are proteins produced by lymphocytes (cells of the immune system) that convey information among lymphocytes. With the exception of Ifn, lymphokines are only beginning to be characterized, but these proteins appear to be crucial to immune reactions. Some lymphocytes, for example, produce lymphokines that engage other lymphocytes to boost the immune response to a foreign substance (antigen) and repel foreign invasion or disease. Other lymphocytes produce lymphokines that act in tandem with the antigen to stimulate the secretion of antibodies. Lymphokines may also help to ensure that only the antigen is attacked during an immune response, not the body's own tissues.

The importance of lymphokines in preventing disease and understanding cellular function (including aberrant cell function such as cancer growth) is fostering widespread research on these compounds (for review, see 47). Investigations of the complex interactions among lymphocytes have been hampered in the past by impure lymphokine preparations, which have led to ambiguous findings. Recent progress, including the establishment of lymphocyte cell lines that produce various classes of lymphokines (e.g., 37) and

Table 17.—Some Ongoing Clinical Trials of the Use of Interferons To Treat Cancer (Continued)

Interferon supplier	Sponsor	Cancer	Phase	Institution
HLR	HLR	Ovary	II	Dana Farber Cancer Institute
HLR	HLR	Lymphoma, non-Hodgkin's	II	University of Arizona Minneapolis VAH Mayo Clinic
HLR	HLR	Multiple myeloma	II	M. D. Anderson Hospital
HLR	HLR	Kidney (renal cell)	II	University of Arizona
HLR	HLR	Leukemia, chronic	II	George Washington University
HLR	HLR	Kaposi's sarcoma	II	University of Arizona Memorial Sloan Kettering Cancer Center
HLR	HLR	Osteogenic sarcoma	II	Mayo Clinic
HLR	HLR	Breast cancer	II	Georgetown University USC Cancer Center
Cultured cell-produced gamma-interferon:				
Revlon	NCI	Broad range of advanced cancers	I	NCI-Frederick Cancer Research Facility

SOURCE: Office of Technology Assessment, adapted from R. K. Oldham, U.S. National Cancer Institute, "Update on Clinical Trials With Interferon and Monoclonal Antibodies," memorandum, May 4, 1983.

Human growth hormone

As suggested by the preceding discussion, rDNA technology is increasingly being used to produce large amounts of otherwise scarce biological compounds. In addition to supplying compounds for basic research, rDNA technology is likely to contribute to the discovery of many new pharmaceutical products. Some of the promising protein compounds actively being developed with rDNA technology—human growth regulators, neuroactive peptides, and lymphokines, for instance—are listed in table 19.

The development of hGH with rDNA methods is another model for biotechnology's use in the pharmaceutical industry. Human growth hormone is one of a family of at least three, closely related, large peptide hormones secreted by the pituitary gland. These peptide hormones are about four times larger than insulin (191 to 198 amino acids in length). All three hormones possess a wider variety of biological actions than do most other hormones. The primary function of hGH is apparently the control of postnatal growth in humans. Whereas insulin derived from slaughtered animals can be used for treating diabetics, only growth hormone derived from humans is satisfactory for reversing the deficiencies of hypopituitarism in children (65).

Although the established market for hGH is small and current supplies from tissue extracts are sufficient,* hGH was one of the first targets for the applications of rDNA technology. Workers at both Genentech and the University of California, San Francisco (UCSF) reported cloning and expression of hGH in 1979 (39). Genentech's work was supported by the Swedish firm KabiGen AB, while partial funding for the UCSF work was provided by Eli Lilly, which is believed to be the licensee for the product (39). Genentech has such high aspirations of proving sufficient utility for hGH in medical applications beyond those currently treated with cadaver hGH that it has announced its intent to make the development of hGH from rDNA one of the cornerstones of its integrated pharmaceutical enterprise (9). To this end, Genentech is raising capital through an R&D limited partnership specifically to support clinical testing of hGH and is investigating a variety of possible new clinical indications for hGH use. The NIH National Pituitary Agency has been enthusiastic about these investigations, which were not practical when the supply of hGH was limited by the availability of human cadaver pituitaries (104).

*Most pharmaceutical hGH is obtained from human pituitaries removed at autopsy. In the United States, isolation and distribution of hGH has been managed primarily by the National Pituitary Agency (under the auspices of NIH and with the cooperation of the College of Pathologists). Under programs of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, hGH is provided, without charge, for approximately 1,600 children per year for treatment of hypopituitarism. Another several hundred patients are treated with commercial hGH imported from abroad, which is also obtained from tissue extracts (39).

ly, of the global plasma component market in 1978. North America and Japan each consume 25 percent of the world's blood products (106).

The United States now enjoys a favorable trade balance with respect to blood products. Because blood donation is more widely practiced in the United States than elsewhere, the United States supplies blood components to many other countries. Japan obtains 50 percent of its HSA and 60 percent of its GG* from the United States. The plasma production of Europe is about 60 percent of that of the United States (105).

The blood products industry is characterized by large markets and strong incentives for biotechnological innovation on a nationwide basis. Currently, the industry is troubled by the disease AIDS. Although the etiology of AIDS is not yet understood, the strong possibility that it can be transmitted in blood products lowers the marketability of such products. Thus, the industry is seeking new methods for the production of blood products.**

Human serum albumin

HSA, a single polypeptide chain of 585 amino acids, is the protein used in the largest quantities in medicine. HSA is used primarily during surgery and to treat shock, burns, and other physical trauma. In 1979, worldwide HSA consumption exceeded 90,000 kg, with U.S. consumption accounting for 80 percent (72,500 kg) of this amount. Although the United States consumed large amounts of HSA relative to most other countries in the past, foreign HSA consumption is rising, as shown in table 21. Worldwide HSA consumption is expected to exceed 250,000 kg by 1984 (64,106,143) with the largest increases of HSA consumption taking place in foreign countries. The United States has experienced an overcapacity of HSA production from blood fractionation since 1975 (143) and is currently the world's leading exporter of HSA.

HSA's trememdous markets make it an attractive target for production with biotechnology. However, HSA's substantial molecular size (585 amino acids) and its relatively low cost of conventional production present formidable challenges to biotechnology. In November 1981, Genentech announced successful HSA production in bacteria and yeast through rDNA manipulation (63). This achievement is a landmark in several respects:

- HSA is the largest protein (585 amino acids) yet produced by rDNA technology.
- Planners and technologists aim to manufacture tons rather than grams of injectable products using rDNA systems.
- Competitive product costs are more than an order of magnitude lower per unit weight of product than those for previously considered rDNA pharmaceuticals (e.g., less than $1/

*GG is a fraction of serum that contains antibodies. Boosting a patient's antibody level generally is thought to help prevent infectious disease. This treatment is used especially for hepatitis prevention. The ability to produce specific antibodies (MAbs) may make GG a less desirable therapy and increase the effectiveness of antibody prophylaxis.

**These efforts are to be discussed in a forthcoming OTA report, *Blood Banking Policy and Technology*.

Table 21.—Human Serum Albumin Production and Consumption in the United States

	1971	1976	1979	Forecast 1984
Plasma processed in the United States (thousands of liters)	1,950	2,910	3,950	6,920
HSA production in the United States (millions of grams)	39	67	91	159
HSA consumption:				
Domestic (millions of units)	2.9	4.6	5.8	8.5
Foreign (millions of units)	0.3	0.7	1.5	4.2
Total (millions of units)	3.2	5.3	7.3	12.7
Domestic	94%	87%	80%	67%
Foreign	6%	13%	20%	33%
HSA revenues:				
Domestic (millions of dollars)	$58	$133.4	$168.2	$300
Foreign (millions of dollars)	4	20.3	43.5	148
Total (millions of dollars)	62	153.7	211.7	448

SOURCE: Office of Technology Assessment, based on data and estimates in M. M. LeConey, "Who Needs Plasma?" *Plasma Quarterly* 2:68-93, September 1980.

Table 19.—Some Proteins With Possible Pharmaceutical Applications Being Developed With Recombinant DNA Technology

Class/substance	Size (number of amino acids)	Function	R&D status	Project sponsors	Applications
Human growth regulators:					
Growth hormone (GH)	191-198	Promotes growth	Cloned, expressed, 1979	Genentech (U.S.)/ Kabigen AB (Sweden) UCSF/Eli Lilly (U.S.)	Growth promotion; healing burns, fractures; cachexia
Somatostatin	14	Inhibits GH secretion	Cloned, expressed, 1977	UCSF/Genentech	Adjunct to insulin
Somotomedins	44-59	Mediates action of GH	Cloned, expressed, 1982	Chiron (U.S.)	Growth promotion, regulation
Growth hormone releasing factor (GRF)	44	Increases pituitary GH release	Isolated, sequenced, synthesized, 1982	Salk Institute (U.S.)	Growth promotion
Calcium regulators:					
Calmodulin	148	Mediated calcium's effects	Determined to be unprofitable[a]	None	Numerous applications in basic research; hypertension
Calcitonin	32	Inhibits bone resorption	rDNA production	Genentech, Amgen (U.S.)	Bone disease therapy
Parathyroid hormone (PTH)	84	Mobilizes calcium; prevents calcitonin excretion	Cloned, but no production	Massachusetts General Hospital	Osteoporosis therapy; calcium metabolism
Reproductive hormones:					
Luteinizing hormone (LH)	Beta chain; 115[b]	Females: induces ovulation Males: stimulates androgen secretion	Cloning in progress (glycoprotein)	Integrated Genetics (U.S.)/Serono Labs (Italy)	Antifertility
Follicle-stimulating hormone (FSH)	Beta chain; 115	Induces ovarian growth	Cloning in progress (glycoprotein)	Integrated Genetics/ Serono Labs	Reproductive services
Human chorionic gonadotrophin (HCG)	Beta chain; 147	Like LH; more potent	Cloning in progress (glycoprotein)	Integrated Genetics/ Serono Labs	Pregnancy testing
Relaxin	52	Dilation of birth canal; relaxation of uterus	Cloning in progress (non-glycoprotein)	Genentech	Soften bone connective tissue of reproductive tract; antiarthritic (?)
Neuroactive peptides:					
β-Endorphin	31	Analgesia	Cloned, expressed	Amgen, others	Analgesia
Enkephalins	5	Analgesia	Cloning in progress	Amgen, others	Analgesia
Pancreatic endorphin	N.A.[c]	Undetermined	Cloning in progress	Endorphin, Inc.	Analgesia, particularly in childbirth
Lymphokines and immunoactive peptides (other than interferons):					
Interleukin-2	133	Promotes T-cell growth, activity	Cloned, expressed	Ajinomoto Co. (Japan) Japanese Cancer Institute Immunex (U.S.) Cetus (U.S.) Chiron Genex (U.S.) Biogen (U.S.) Genetics Institute (U.S.) Interferon Sciences (U.S.) Quidel (U.S.)	Maintain T-cell cultures; immunotherapy
Thymosin (fraction 5)	10-150	Promotes maturation of bone marrow cells, T-cell differentiation	Purified, sequenced	George Washington University	Immunodeficiency diseases
Thymosin (alpha 1)	28	Promotes T-helper and T-amplifier functions	Purified, sequenced cloned, 1979	Hoffmann-La Roche (Switz.) Genentech	Systemic lupus erythmatosis; other immune disorders
Thymic hormone factor (THF)	9	Promotes T-helper and T-amplifier functions	N.A.	N.A.	Antiviral protection in immunosuppressed patients
Thymic factor (TFX)	40	Restores delayed-type hypersensitivity	N.A.	N.A.	Cancer treatment
Thymopoietins	49	Inhibits B-cell differentiation	N.A.	Ortho Pharms. (U.S.)	Reversing immunodeficiencies

Efforts to produce AHF with biotechnology are underway. The gene for factor IX has recently been cloned and expressed in *E. coli* (18,61). The availability of factor IX produced by rDNA technology facilitates studies concerning the genetic basis of type B hemophilia (e.g., 35). However, quantities of factor IX necessary to treat the relatively uncommon type B hemophilia are adequately provided by whole blood fractionation, and the rDNA product is not now a competing alternative.

Significantly stronger medical and commercial reasons motivate efforts to clone factor VIII genes, since the majority of hemophiliacs are type A. At present, difficult problems surround factor VIII gene cloning. Not only is factor VIII present in low concentrations in plasma, making its isolation and purification difficult, but this molecule is an extremely large and labile glycoprotein (over 300,000 molecular weight, about 20 times the size of Ifn). Recent progress in factor VIII research includes development of MAbs to aid in AHF isolation (86,132) and localization of AHF-producing cells in the liver (134).

The rDNA production of factor VIII is an elusive goal, but the implications of success are substantial. Apart from providing more economic treatment for hemophiliacs, results of factor VIII cloning may lead to a better understanding of the most common type of hemophilia and prove useful for prenatal screening for the disease.

Biosynthetic AHF may lower costs of treatment for the expanding population of hemophiliacs throughout the world. Furthermore, if the production of HSA from rDNA technology proves competitive with fractionation, the need to produce AHF with rDNA may be paramount, since AHF is copurified with HSA from plasma.*

Research laboratories working towards AHF microbial biosynthesis include the following (12,128):

- Armour Pharmaceutical (U.S.)/Scripps Clinic and Research Foundation (U.S.),
- Baxter Travenol Laboratories (U.S.)/Genetics Institute (U.S.),
- Biogen S.A. (Switzerland)/Teijin (Japan),
- Speywood Laboratories (U.K.)/Katherine Dormandy Hemophilia Centre and the Royal Free Hospital of London (U.K.)/Genentech (U.S.), and
- Connaught Laboratories (Canada)/Canadian Government.

*The price of factor VIII controls the price of serum albumin (64). The worldwide growth rate for AHF, about 14 percent per year (64), is twice the growth rate of HSA. Thus, any major shift of HSA production to rDNA technology with a concomitant loss of AHF production may drive the price of AHF (produced from fractionation) to higher levels.

Thrombolytic and fibrinolytic enzymes

Thrombosis, the blockage of blood vessels, is the leading cause of death in industrialized nations. Blood clots in the vessels that supply the heart (coronary heart disease), brain (stroke), or lungs (pulmonary embolism) account for more than half of all deaths in the Western Hemisphere.

The search for substances that dissolve blood clots is a major undertaking of the pharmaceutical industry. At present, the most popular compounds are thrombolytic and fibrinolytic enzymes. These substances initiate the dissolution process by converting plasminogen, a plasma protein, into plasmin, which then attacks fibrin, the protein that comprises most of the blood clot.

The two most widely used thrombolytic enzymes are streptokinase and urokinase. Streptokinase is manufactured from colonies of *Streptomyces* bacteria, while urokinase is obtained either from cultured human kidney tissue or from human urine. Recent improvements in large-scale cell culture techniques and purification methods (including the use of MAbs for the purification of protein) now yield good quantities of thrombolytic enzymes (57). Despite the great usefulness of these enzymes, however, several problems diminish their clinical value. In prolonged therapy with streptokinase, chances of allergic reactions arise. In addition, streptokinase and urokinase appear to act nonspecifically throughout the body, thus raising risks of internal hemorrhaging in patients. To circumvent this risk, carefully placed catheters must be used to deliver the enzyme to its target. Finally, high costs of manufacturing and therapy also restrain more widespread use (streptokinase treatment costs $275, while urokinase costs about $3,000 per patient) (57). Because of

cloning of lymphokine-producing genes into rDNA systems for production in bacteria (24,137), has been made possible with the use of biotechnology. The availability of pure lymphokine samples from such systems may enable researchers to answer more questions concerning cell biology and immune function. Lymphokines may also be useful in the culture of certain cell lines. Eventually, these efforts may lead to the use of lymphokines in medicine to stimulate the patient's own immune system to combat disease.

Leading commercial efforts to produce lymphokines with biotechnology are centered in Japan, Switzerland, and the United States. In Tokyo, Dr. Tadatsugi Taniguchi of the Japanese Cancer Institute is collaborating with Ajinomoto Company to produce the lymphocyte growth factor, interleukin-2 (13). In Switzerland and the United States, numerous firms using biotechnology are engaged in lymphokine research, especially in the production of interleukin-2, but their efforts are largely proprietary at this time (24).

Other regulatory proteins

In addition to hormones and other regulatory proteins, a number of protein "growth factors" for a variety of somatic (body) cells have been isolated and are currently being characterized with the possibility that they may soon be candidates for production by rDNA technology as well (see table 20). Perhaps the most important use of growth factors will be in preparing culture media for growing higher eukaryotic cells, thereby facilitating further research with more complex cells.

Table 20.—Some Protein "Growth Factors" With Potential Pharmaceutical Applications

Factor	Function
CSF (colony stimulating factor)	Stimulate granulocyte differentiation
ECGS (endothelial cell growth supplement)	Required by vascular lining cells
EDGF (endothelial-derived growth factor)	Stimulates cell division in blood vessels
EGF (epidermal growth factor)	Stimulates growth of epidermal cells and many tumors
FGF (fibroblast growth factor)	Stimulates fibroblast cell growth
FN (fibronectin)	Stimulates adhesion and proliferation of fibroblast cells
MDGF (macrophage-derived growth factor)	Stimulates cell division near inflammation
NGF (nerve growth factor)	Stimulates nerve growth and repair
PDGF (platelet-derived growth factor)	Stimulates division of fibroblast-like cells
SGF (skeletal growth factor)	Stimulates bone cell growth
WAF (wound angiogenesis factor)	Stimulates wound healing
TAF (tumor angiogenesis factor)	Stimulates blood vessel proliferation in tumors

SOURCE: Office of Technology Assessment, 1983.

Blood products

Products derived from the fractionation of human blood represent the greatest volume of biological pharmaceutical products sold today and comprise a world market of $1 billion yearly. The three main plasma commodities are human serum albumin (HSA), gamma globulin (GG), and antihemophilic factor (AHF), which accounted for 41 percent, 25 percent, and 13 percent, respective-

Through the use of improved bioprocess systems, purification methods, and rDNA technology, large quantities of scarce materials are becoming available for study, possibly leading to substantial changes in medical practices in the United States.

Given successful economic development of tPA (i.e., at one-half the cost of urokinase production) and improved mode of action, industry experts estimate that U.S. markets for tPA could climb swiftly to $125 million per year (57).

Vaccines

The combined techniques of biotechnology find perhaps no greater promise for medicine than in the preparation of vaccines and other pharmaceutical products to combat infectious diseases. There are several approaches to disease control using biotechnology, including the use of rDNA and MAb technology, artificial vaccine synthesis, and protoplast fusion to prepare novel antibiotics.

Most vaccines used at present consist of the organisms that cause the particular disease that the vaccine is intended to prevent. These organisms (pathogens) are killed or otherwise treated ("attenuated") in an effort to make them nonvirulent, and the killed or attenuated mixture is then injected into the person to be vaccinated. Ideally, the recipient's immune system responds to the introduction of the vaccine by producing antibodies that bind to particular molecules (antigens) on the surface of the vaccine organism and identifying it for destruction by other components of the immune system. The antibodies produced by the recipient remain in circulation for a period of months to years, protecting the recipient against the live pathogen should it be encountered later. Thus, the recipient becomes "immune" to the disease. Immunity thus induced, since it uses the recipient's immune system for constant surveillance and defense against the disease, is known as "active immunity." The administration of foreign antibodies or immune products that themselves protect the recipient from the disease, on the other hand, provides what is known as "passive immunity." Passive immunization usually confers only short-term protection against a disease.

Killed and attenuated vaccines represent one of the highest achievements in medicine. Nevertheless, several problems with these vaccines persist. One substantial problem is that killed and attenuated vaccines contain the complete genetic material of the pathogen. If the pathogen is not killed or attenuated completely, the vaccine itself may be capable of causing the disease it is intended to prevent. Another problem with conventional vaccines is that, in many instances, they do not immunize the recipient against all of the various strains of the pathogen. Finally, many conventional vaccines are not stable enough for use where they may be most needed, as in areas without refrigeration.

Subunit vaccines—vaccines that contain only portions of the pathogens—may solve some of the problems associated with killed and attenuated vaccines. Subunit vaccines do not contain the pathogen's genetic material, and, thus, they cannot themselves cause infection. Furthermore, subunit vaccines may be more stable for storage and of greater purity than most conventional vaccines, although these qualities remain to be demonstrated in most cases. Two new methods are being developed to prepare subunit vaccines: rDNA technology to produce all or part of a surface protein molecule of the pathogen and chemical synthesis of short polypeptides that represent surface proteins. Both of these new approaches have the added advantage that subunit vaccine manufacture does not require large-scale culture of the infectious organism.

Viral disease vaccines

Because of the relatively simple, well-understood structure of viruses, the most preeminent biotechnology efforts for the development of new vaccines are focused on viral diseases (51,135). As shown in table 24, biotechnology is being used to develop vaccines for influenza types A and B, herpes, polio, hepatitis A and B, and a number

gram, compared to somewhat less then $50/gram for insulin).
- The companies that successfully produce HSA with rDNA technology will amass knowledge of certain related processes, including purification of large amounts of product. This knowledge might allow them to dominate the production of other proteins made by similar processes.

Since cloning the HSA gene, Genentech has entered into an agreement with Mitsubishi Chemical Industries, Ltd. (Japan) to cooperate in continued R&D for manufacturing and commercialization. The partnership hopes to produce 10 metric tons (tonnes) of HSA per year by 1985 (121). Mitsubishi will probably ask Green Cross, which is the largest Japanese blood products company, to distribute the rDNA-produced product, thus avoiding discrimination against the present distributor of HSA. In 1981, HSA sales in Japan were $60 million (¥14.2 billion) (118), compared to about $200 million in the United States (64). The corporate arrangements between Genentech, Mitsubishi, and Green Cross may lead to the reduction of Japanese imports, the establishment of a blood product industry in Japan, and advances in Japanese technology for producing and purifying proteins.

Genex (U.S.) and Biogen S.A. (Switzerland) also have established arrangements with Japanese firms to conduct R&D on rDNA production of HSA (115). Genex made a contract in 1981 with Green Cross. In exchange for research funding, Genex agreed to grant Green Cross exclusive licenses to make, use, and sell all microbially produced HSA developed under the contract in the Far East, South America, and North America. Genex made a similar agreement with the Swedish firm KabiVitrum, with licensing pertaining to Europe, Africa, and the Middle East. Biogen S.A. negotiated a similar agreement in late 1981 to cooperate with Shionogi (Japan) in the development of rDNA techniques for HSA production.

Only one major American drug company, Upjohn Pharmaceuticals, shows evidence of developing a fully in-house large-scale biosynthetic HSA process. Upjohn is making HSA in both *E. coli* and yeast.

Antihemophilic factor

AHF, a class of proteins contained in the fraction of blood used to treat hemophilia (a set of hereditary disorders that prevent blood clotting), is used by approximately 14,000 hemophiliacs in the United States on a routine basis (143). Type A hemophilia, which affects about 5 people in every 100,000, is caused by a deficiency of factor VIII, and type B hemophilia (which is much rarer but equally severe) by a lack of factor IX.

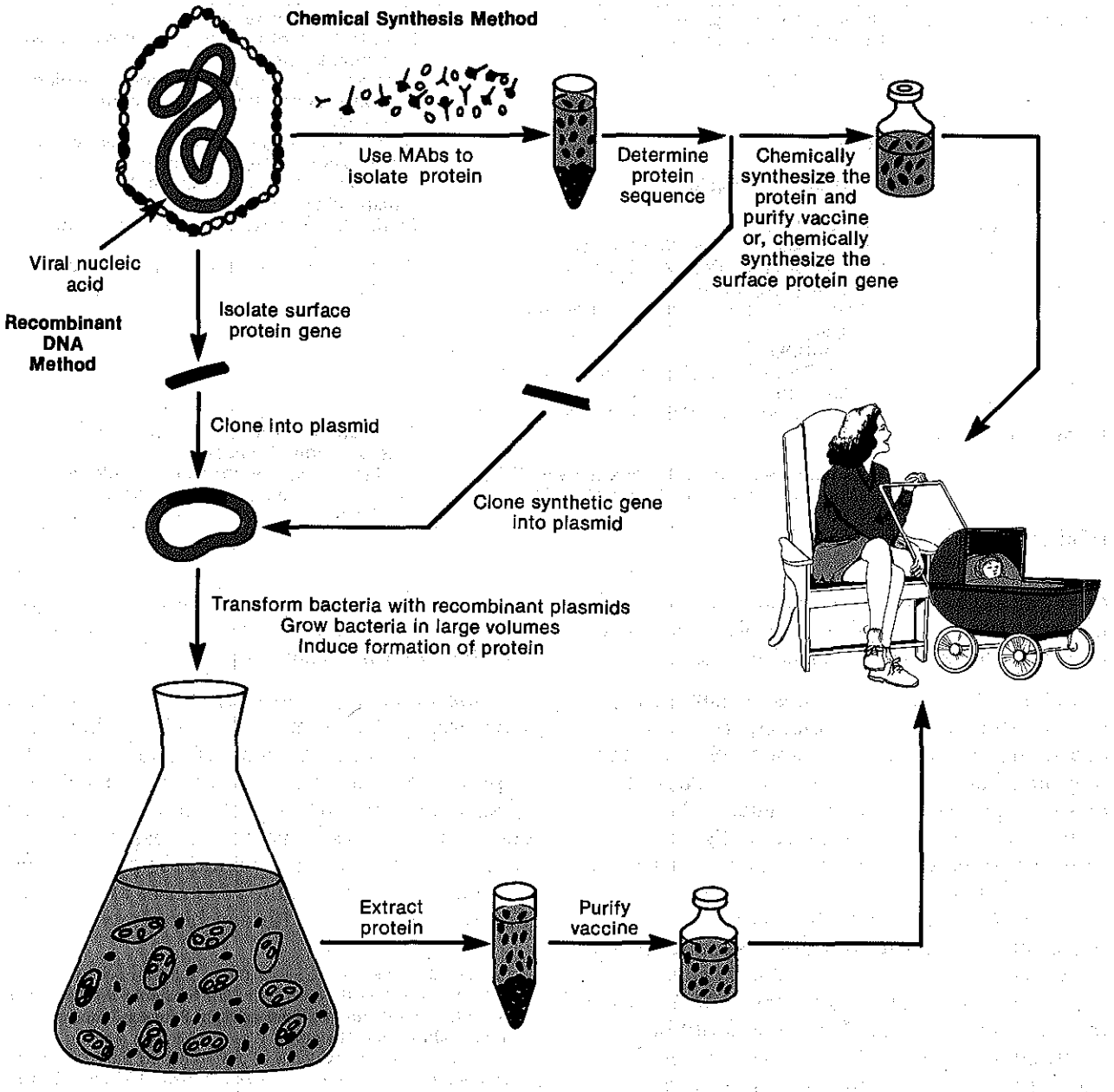
AHF is separated during the fractionation of whole blood to obtain HSA. As shown in table 22, U.S. AHF production has multiplied faster than consumption in recent years, and AHF comprises sizable exports for U.S. firms and nonprofit organizations. With AHF selling for over $1 million per gram and AHF use growing at a rate of 14 percent per year, AHF is the blood fractionation industry's most profitable product (64).

Table 22.—Antihemophilic Factor Production and Consumption in the World

	1971	1976	1979	Forecast 1984
Plasma processed globally for AHF (thousands of liters)	365	1,600	2,750	5,320
AHF units processed (millions)	80	400	688	1,330
Domestic consumption:				
Millions of units	72	300	412	648
Average price (cents/unit)	15	10	10	14
Sales (millions of dollars)	10.8	30	41.2	91
Foreign consumption:				
Millions of units	8	100	275	682
Average price (cents/unit)	40	30	30	27
Sales (millions of dollars)	3.2	30	82.5	184
Total AHF sales (million of dollars)	14	60	123.9	275

SOURCE: Office of Technology Assessment, based on data and estimates in M. M. Le Coney, "Who Needs Plasma?" *Plasma Quarterly* 2:68-93, September 1980.

Figure 13.—Methods Used to Prepare Subunit Vaccines for Viral Diseases: Recombinant DNA Technology v. Chemical Synthesis



In the **chemical synthesis method,** proteins that comprise the viral surface are isolated, often with the use of monoclonal antibodies. The protein sequence is then determined. Based on the sequencing information, large amounts of the protein or portions of the protein are made chemically for use as the vaccine; alternatively, the sequencing information may allow chemical synthesis of the gene that encodes the protein (or a small portion of the protein). This synthetic gene is cloned via rDNA techniques.
In the **recombinant DNA method,** the gene that encodes the viral surface protein is isolated and cloned into an appropriate vector (such as plasmid), transformed into a host (such as a bacterium or yeast), and the host is grown in large quantities. Formation of the protein by the rDNA and isolation of the protein results in the subunit vaccine.

SOURCE: Office of Technology Assessment.

these problems, alternative thrombolytic enzymes and more economic production methods are being sought.

A group of fibrinolytic enzymes called tissue plasminogen activators (tPAs) may solve some of the problems associated with streptokinase and urokinase. Although tPAs are generally not well characterized and are only available in limited quantities at present, they appear to work specifically at blood clots over a prolonged time (59), reducing both the risks of hemorrhage and the doses necessary for thrombolysis, thus lowering costs of treatment.

Advances in culturing tPA-secreting cells and isolating tPA using MAbs indicate that manufacturing costs may be reduced in the future. Moreover, Genentech, in collaboration with investigators at the University of Lueven (Belgium), recently succeeded in cloning the gene that produces tPA (108), and a number of other companies are working to produce tPA from rDNA systems (see table 23). Cloned genes in bacteria or yeast may provide a means for economically producing large quantities of tPA. The biochemical effectiveness and commercial viability of rDNA-produced tPAs remain to be demonstrated. In particular, questions concerning the stability of the cloned genes in bacterial strains, scale-up costs, and importance of sugar residues found on native tPA remain to be answered.

At present, the extent to which thrombolytic enzymes are used by different countries varies substantially. German and Japanese physicians prescribe streptokinase and urokinase extensively, often in conjunction with cancer chemotherapy (on the premise that fibrin shields tumors from drugs and the body's immune defenses and hence must be removed). American medical practices, on the other hand, discourage the use of streptokinase and urokinase because of the problems mentioned earlier. Thus, the annual market for thrombolytic enzymes in the United States represents a modest $8 million, whereas the annual market for urokinase in Japan, where it is the seventh largest selling drug, represents $150 million (57).

The widespread sponsorship of tPA projects by Japanese companies, as shown in table 23, reflects these national differences in thrombolytic enzyme use. In addition to underwriting clinical testing and marketing costs of enzymes produced from cultured cells, Japanese companies such as Green Cross are active in sponsoring tPA production using rDNA techniques.

The development of tPA illustrates biotechnology's role in providing new pharmaceutical agents.

Table 23.—Thrombolytic and Fibrinolytic Enzymes: Companies Involved in Development and Marketing

Protein	Company	Project description
Streptokinase	Hoechst-Roussel (F.R.G.)	Production from bacteria
	KabiVitrum (Sweden)	Production from bacteria
Urokinase	Abbott Laboratories (U.S.)	Extraction from cultured kidney cells
	Genex (U.S.)/Mitsui Toatsu Chemicals, Inc. (Japan)	Production from rDNA
	Genentech (U.S.)/Grunenthal (F.R.G.)	Production from rDNA
Human tissue plasminogen activator	Genentech/University of Leuven (Belgium)/ Mitsubishi Chemical Industries, Inc. (Japan)/Kyowa Hakko Kogyo (Japan)	Production from rDNA
	Biogen S.A. (Switz.)/Fujisawa (Japan)	Production from rDNA
	Integrated Genetics (U.S.)/ Toyobo Pharmaceutical (Japan)	Production from rDNA
	Chiron (U.S.)	Production from rDNA
	Collaborative Resarch (U.S.)/ Green Cross (Japan)	Extraction from cultured kidney cells
Anticoagulant and fibrinolytic agents	Genentech/Yamanouchi Ltd. (Japan)	Development of microbial strains that produce a fibrinolytic agent
	Genex/Yamanouchi Ltd.	

SOURCE: Office of Technology Assessment.

A similar method involves using mutation/selection procedures on pathogenic bacteria to select bacteria that die after a short period of time in the body. For instance, a mutant of the typhoid-causing bacterium, *Salmonella typhi,* type Ty-21a, accumulates toxic amounts of galactose during growth and causes its own death. This mutant can proliferate within the body for a short time, and its presence elicits an immune response that protects against the disease. The Swiss Serum and Vaccine Institute, in association with the French Institut Pasteur, has developed an oral typhoid vaccine of this type.

Other workers have taken this typhoid vaccine strain and incorporated a plasmid with a gene encoding a protein normally produced by *Shigella sonnei,* one of the bacteria which cause dysentery. In mice, this "hybrid" strain elicits immune responses that protect against both the dysentery and typhoid organisms. Thus, it may be possible to construct a multipurpose oral, attenuated typhoid-dysentery vaccine organism that will produce "protective" antigens for both dysentery and typhoid (51).

Parasitic disease vaccines

Diseases caused by parasites, including protozoa, pose major barriers to acceptable health standards for millions of people throughout the world (see table 25). Many of these organisms exhibit even more extraordinary degrees of complexity than bacteria, however, and lack of basic knowledge restrains new vaccine development in virtually all cases (51). As basic knowledge accrues, immunization against diseases caused by parasites may eventually be the greatest breakthrough in health care provided by biotechnology.*

Progress in developing malaria vaccines exemplify efforts to realize biotechnology's potential in combating parasitic diseases. Because of the lack of a vaccine, combined with parasitic resistance to the drugs used in malaria control (e.g., chloroquine), malaria remains the most prevalent infectious disease in the world.** Historically, the search for malaria vaccines has been hampered by difficulties in growing the malarial parasite *Plasmodium* (which is transmitted by female *Anopheles* mosquitoes) in the laboratory. Other difficulties stem from *Plasmodium's* complex lifecycle and the apparent ability of the parasite to evade the body's immune system. In addition, vaccines based on killed, injected whole *Plasmodia* presently require the use of powerful adjuvants (additional components of vaccines that boost immune responses) in test animals which are too strong for human use.

The complexity of *Plasmodium's* lifecycle hints at the difficulties in developing a vaccine that protects against all forms of malaria. As shown in figure 14, the sporozoites, injected into the blood

Table 25.—Estimated Worldwide Populations Affected by Parasitic Diseases in 1971

Type of parasite	Diseased population (in millions)
Intestinal parasites:	
Ascariasis	650
Ancyclostomiasis	450
Amoebiasis	350
Trichuriasis	350
Periocular parasites:	
Trachoma	Greater than 400
Systemic parasites:	
Filariasis	250
Schistosomiasis	180
Malaria	100
Leishmaniasis	N.A.[a]
Trypanosomiasis	7

[a]N.A.=Information not available.

SOURCE: Office of Technology Assessment, based on data from World Health Organization, *Report for the Special Programme for Research and Training in Tropical Diseases,* Geneva, 1976.

*The U.S. National Academy of Sciences and the Agency for International Development convened meetings in July and December 1982 on the applications of biotechnology most significant for the developing world. Recommendations were made with respect to research priorities on the basis of applicability of the new technologies and other considerations (88,145). The only human parasitic diseases that ranked among the top priorities for development at this time were leishmaniasis and malaria. Leishmaniasis is a family of diseases, caused by sandfly-transmitted protozoa, which is considered to have grossly underestimated public health importance in South America, Africa, and the Middle East. It was identified for special attention because there is evidence that immunity can be developed by people in sandfly-infested areas over a period of time. An understanding of this immunity may provide ways to prevent leishmaniasis.

**There are now an estimated 300 million malaria cases per year and a very high mortality rate for children (1 million deaths in Africa alone per year) (158). About 850 million people live in areas where malaria continues to be transmitted despite activities to control it. An additional 345 million people reside in areas with little or no active malaria control efforts. Over half of the health budget of India is spent on malaria control. Resistance to both drugs and insecticides and the number of new malaria cases are all increasing at alarming rates (155). No vaccine is currently available.

Table 24.—Some Current Viral Vaccine Biotechnology Projects

Viral disease	Company	Project description
Influenza virus.....	Numerous investigators	Improved attenuated strains
	Numerous investigators	Modifications of viral genome through rDNA manipulations
	Scripps (U.S.)	Synthesis of short peptides corresponding to fragments of influenza virus surface proteins
	Scripps	Attachment of viral subunit to larger carrier to evoke broader immune response
Polio virus	Numerous investigators	Modifications of viral genome through rDNA manipulations
Hepatitis B virus....	Merck (U.S.)	Purification of viral particles from blood
	Institut Pasteur Production (France)	
	Chiron Corp (U.S.)/Merck/University of Washington, UCSF	Production of viral surface proteins from rDNA in yeast
	Takeda/Osaka and Hiroshima Universities (Japan)	
	Amgen (U.S.)	
	Biogen/Green Cross (Japan)/University of Edinburgh	
	Integrated Genetics (U.S.)/Connaught (Canada)	
Herpes viruses	Merck	Purification of surface glycoprotein from herpes simplex viruses
	Molecular Genetics (U.S.)/Lederle Labs (U.S.)	Production of viral proteins in bacteria
	Institut Merieux (France)/University of Chicago	Production of nonpathogenic viruses by the deletion of specific genes

SOURCE: Office of Technology Assessment.

of other human viral diseases. The two main methods used to prepare subunit vaccines for viral diseases are summarized in figure 13.

Hepatitis B subunit vaccines, in particular, illustrate the use of biotechnology in vaccine improvement. Using the rDNA approach, a number of groups have cloned genes that encode portions of the hepatitis B surface antigen (HBsAg) and have shown that isolated surface antigens behave similarly to the whole virus when used as a vaccine (25,74,131,146). Merck (U.S.), which supports work done at UCSF and Chiron Corp. (U.S.) and has built an in-house molecular genetics group of nearly 50 scientists since 1978, expects to market a hepatitis B vaccine made from rDNA in yeast by 1987 (44). Biogen S.A. (Switzerland) has successfully immunized chimpanzees against hepatitis B using its yeast-grown vaccine, and a license to Biogen's work with hepatitis vaccines has been acquired by Green Cross (Japan). It has been estimated that Biogen's hepatitis B vaccine will sell for only $10 to $30 per dose as compared with $100 per dose for Merck's vaccine made from virus particles extracted from blood of hepatitis B carriers (14,71). How well these rDNA-produced hepatitis B subunit vaccines will compete with vaccines made by traditional methods is not yet known, but the need for an effective and inexpensive hepatitis B vaccine is great.*

Using chemical synthesis, other researchers have prepared synthetic polypeptides which may be useful as subunit vaccines. These synthetic peptides are based on known amino acid sequences of virus surface proteins. The amino acid sequences and their molecular shapes are analyzed by computer, and peptide sequences that are likely to elicit immune responses are defined (for review, see 68)). Researchers have synthe-

*In the United States, there are 80,000 to 100,000 cases of hepatitis B and about 1,000 deaths each year. The incidence in some other parts of the world runs 10 times as high. Between 3 and 15 percent of healthy blood donors in Western Europe and the United States show serological evidence of past infection, and 0.1 percent are chronic carriers of the type B virus. In many African and Asian countries the majority of the adult population have been infected, and 5 to 10 percent of the population are clinically ill with hepatitis. A very strong association has recently been demonstrated between the carrier state of hepatitis and liver cancer. In areas of the world where hepatitis B is endemic, primary liver tumors account for 20 percent of cancer, in contrast to the 1 percent level of liver tumor incidence in the United States (150). A costly hepatitis B vaccine was brought to market by Merck in 1982 in the United States. Although not made with new biotechnology, this vaccine consists of natural subunits—particles of the virus coat protein which are isolated and purified from the blood of relatively rare suitable donors (34,44).

stream during the mosquito bite, infect liver cells to initiate infection. Large numbers of merozoites, the next life-stage, proliferate within the liver cells and, bursting into the blood stream, successively infect large numbers of red blood cells. Some of the merozoites remain blood-borne; other merozoites develop into gametocytes, which are picked up by mosquitoes, reproduce to form new sporozoites, and begin the cycle anew. Additionally, *Plasmodium* has the ability to evade the immune system over time.

Since the pathology of malaria is caused largely by *Plasmodia* in the merozoite stage, the merozoite appears to be the best target for vaccines. Even one sporozoite reaching a liver cell is capable of causing malaria, so vaccines against this stage must kill every sporozoite to be effective. The gametocyte itself is not pathogenic; an antigametocyte vaccine, therefore, would serve only to reduce the transmission of the disease.

Many investigators (particularly in the United States, the United Kingdom, and Switzerland) are developing MAbs that may be useful in malaria research (153). Antisporozoite and antimerozoite MAbs that inhibit the in vitro multiplication of *Plasmodia* and antigametocyte MAbs that inactivate male gametes have been developed (153). Also, MAbs that destroy merozoite-infected red blood cells have been developed. Such MAbs may prove useful as vaccines that confer passive immunity (19,87,160).

The most promising use of such MAbs is in the isolation of surface antigens which might be used for the development of subunit malarial vaccines. Though quantities of surface antigens obtained by MAb precipitation are too small for use as vaccines, these purified antigens provide a starting point for developing other MAbs with an even greater affinity for *Plasmodium* for use as passive vaccines. They may also provide a starting point for using rDNA technology to isolate large amounts of antigen. Workers at New York University (NYU) recently reported the successful cloning and expression in *E. coli* of a surface antigen from the sporozoite stage of one species of *Plasmodium* using rDNA technology (28), and similar efforts to obtain quantities of antigen from other *Plasmodium* species and life stages using rDNA technology are underway (54). These rDNA-produced surface antigens may serve as protective malarial vaccines.

NYU's "antisporozoite vaccine" has been the subject of a widely publicized dispute between NYU; Genentech (U.S.) (the proposed manufacturer of the vaccine); and the World Health Organization (WHO) (which, with the U.S. Agency for International Development, sponsored NYU's basic research with the standard provision that all WHO-funded work must be "publicly accessible").* When it became clear that Genentech would not obtain an exclusive license to commercialize the vaccine, the company bowed out of negotiations. At present, no other arrangements to pursue large-scale rDNA production of the sporozoite antigen have been made.

As mentioned earlier, a vaccine effective against only the sporozoite stage of a single *Plasmodium* species may not prove to be fully protective against malaria. Ultimately, malaria vaccines may include a variety of stage-specific antigens that result in combined sporozoite and merozoite neutralization, accelerated removal of infected red blood cells, and prevention of gametocyte transmission to the mosquito (158). The delay of further development of NYU's potential milestone sporozoite vaccine imposed by the turmoil over commercialization, however, has raised concern that, in the future, profit motivations may delay the development of urgently needed pharmaceutical products made possible by biotechnology (75,90). Despite their promise, the development of effective malarial vaccines appears to be several years away.

For a variety of reasons, biotechnology holds less promise for vaccine solutions for other parasitic diseases than for malaria. For most of the parasites, there are formidable problems related to culture of the pathogenic organisms and establishment of meaningful models of the human disease in animals. For example, the parasite that causes schistosomiasis, a disease that ranks second only to malaria as a cause of morbidity and

*A similar situation arose with regard to the cloning of several more malarial surface antigens at Walter and Eliza Hall Institute of Medical Research in Australia. This research was also partially funded by WHO (110).

sized both linear and cyclic peptides that stimulate immunity similar to the complete virus for hepatitis B and influenza (23,46,66, cf. 68). Preliminary evidence indicates that a synthetic influenza subunit vaccine adequately protects animals against several strains of the live virus, but more tests must be done before synthetic subunit vaccines are ready for clinical evaluation.

If synthetic vaccines prove effective, they may be produced in rDNA systems by cloning the DNA corresponding to the synthetic polypeptide and producing the vaccine using microbial bioprocesses. Fairly small amounts of protein may be required, with a few kilograms sufficing for millions of vaccine doses. However, it remains to be seen whether economics favor development of microbial bioprocesses over chemical synthesis. On the other hand, multivalent vaccines (vaccines that protect against several diseases) may be created by combining a number of peptide sequences to elicit responses to several different antigens and thus broaden the range of synthetic subunit vaccines. Such multivalent vaccines may be more economically produced using biotechnology.

In order for both synthetic and rDNA-produced subunit vaccines to be more effective, better immunizing systems must be devised to promote active immunity. Live (attenuated) vaccines proliferate within the body, thus sustaining immune responses that are necessary for long-term protection. On the other hand, subunit vaccines are destroyed rapidly. Delivery systems are being formulated by coupling the subunit proteins with larger carrier proteins that evoke better immune responses (e.g., 2), and by encapsulating subunit vaccines in lipid packages that allow the vaccine to diffuse slowly throughout the body and prolong exposure (92).

A potential live virus vector system is being investigated using vaccinia virus, a virus not pathogenic to humans (131). DNA encoding HBsAg is joined to DNA sequences ("vaccinia virus promotors") which control transcription of the HBsAg DNA. This rDNA construct is inserted into vaccinia virus, and a "living" vaccine that synthesizes and secretes the HBsAg is produced. Rabbits receiving injections of this live vaccine rapidly produce antibodies against HBsAg, and the vaccine is currently being tested in chimpanzees. The investigators are doing further work on the use of this live virus vector system for other vaccines. Such live vaccines may prove useful after a single, easily administered dose of the vaccine where subunit vaccines fall short in achieving a sufficient immune response.

Bacterial disease vaccines

Unlike viruses, whose surfaces are relatively simple and offer protein targets to which vaccines can be directed, bacteria and other microbial pathogens have complex, dynamic surfaces which in many cases defy vaccine development. Most bacterial surfaces are composed mainly of lipids and polysaccharides, which are molecules derived from complex biosynthetic pathways determined by many genes. Hence, bacteria are not as amenable as viruses to genetic manipulation techniques used in subunit vaccine technology.

Biotechnology is being used in several ways to create novel vaccines against bacterial infections, but the results with bacterial vaccines at present are not as extensive as those with viral vaccines. It is necessary first to identify targets that might be suitable for vaccine development. On the surface of some bacteria, such as *Gonococci* and several pathogenic *E. coli* strains, for example, there are certain proteins which perform functions essential to the disease mechanisms. Though subunit vaccine technology has not been widely explored in bacteria, these proteins may provide targets for subunit vaccines comparable to those being made against viruses.

The genes responsible for a bacterium's virulence can be genetically manipulated to create viable, harmless mutants. These mutant bacteria, which outwardly resemble the pathogenic form, can be introduced into the body, where they elicit the production of antibodies against both mutant and pathogenic bacteria.* Such mutant bacteria might be used to colonize body spaces prone to infection and to provide long-lasting immunity (51).

*As discussed in *Chapter 6: Agriculture*, such bacterial vaccines are currently being introduced to the animal agriculture industry to treat colibacillosis, a common bacterial infection in newborn farm animals.

pharmaceuticals used within the body (in vivo).* The increasing number of MAb-based products also stems from advances in knowledge about hybridoma technology and antibody functions. Further refinements of MAb technology will allow MAbs to be used in numerous applications in the pharmaceutical industry, including in vivo diagnosis, prophylaxis, and therapy.

Hybridomas (MAb-secreting cell lines) derived from human (rather than rodent) cells have only recently become available for use in the pharmaceutical industry. The use of human-cell-derived MAbs in in vivo pharmaceutical applications should give fewer adverse immune reactions than the use of mouse-derived MAbs. Though the preparation of human hybridomas is in its technical infancy, as described in *Chapter 3: The Technologies*, advances in producing MAbs from human cell lines will encourage MAb-based applications for new and replacement medicines.

Diagnostic products

IN VITRO DIAGNOSTIC PRODUCTS

The roster of MAb-based in vitro diagnostic products is growing rapidly. Table 26 provides a list of the products approved for use in the United States as of June 1983.** MAb technology is being used to make both novel diagnostic products and products to replace conventional, polyclonal diagnostic products. Although the competitive advantages of MAb products must ultimately be demonstrated in the marketplace, such products may prove superior to traditional methods used to identify infectious diseases, hormonal changes, or the presence of cancer.

Recently developed applications of MAbs for in vitro diagnosis include the following:

- *Diagnosis of human venereal diseases.* Conventional diagnosis of several common venereal diseases—gonorrhea, chlamydia, and herpes simplex virus—is hampered by time-consuming cell culture requirements. A speedy, sensitive MAb-based diagnostic kit for chlamydia has been produced by Genetic Systems Corp. (U.S.), in collaboration with Syva Co. (U.S.) and the University of Washington (93), and MAb-based diagnostic kits for all three types of infections may be used in the clinic in the near future (38,93).*
- *Diagnosis of hepatitis B and other viral infections.* MAb-based diagnosis of hepatitis B infection is reportedly 100 times more sensitive than conventional diagnosis based on polyclonal antibodies (6,151). The MAb diagnostic product, developed by Centocor (U.S.) with Massachusetts General Hospital, may benefit the blood banking industry, where unambiguous screening for hepatitis is crucial. MAbs are also proving satisfactory for diagnosing rotavirus and cytomegalovirus infections and for distinguishing between strains of influenza viruses that have until now been indistinguishable by conventional methods (80).
- *Diagnosis of bacterial infections.* The recuperation of hospitalized patients is often jeopardized by infections with bacteria such as *Pseudomonas aerouginosa,* and diagnosis may take several days before treatment is begun. Also, group B streptococcal infections are the most common serious infections of newborn infants in the United States. Prior to availability of MAbs, there was little application of immunoassays to the diagnosis of bacterial infections. Genetic Systems, in a joint venture with Cutter Laboratories (U.S.) and its parent company Bayer (F.R.G.), is developing diagnostic and therapeutic MAb products for *Pseudomonas* infections (124). Researchers at the University of Pennsylvania report that diagnosis times for streptococcal

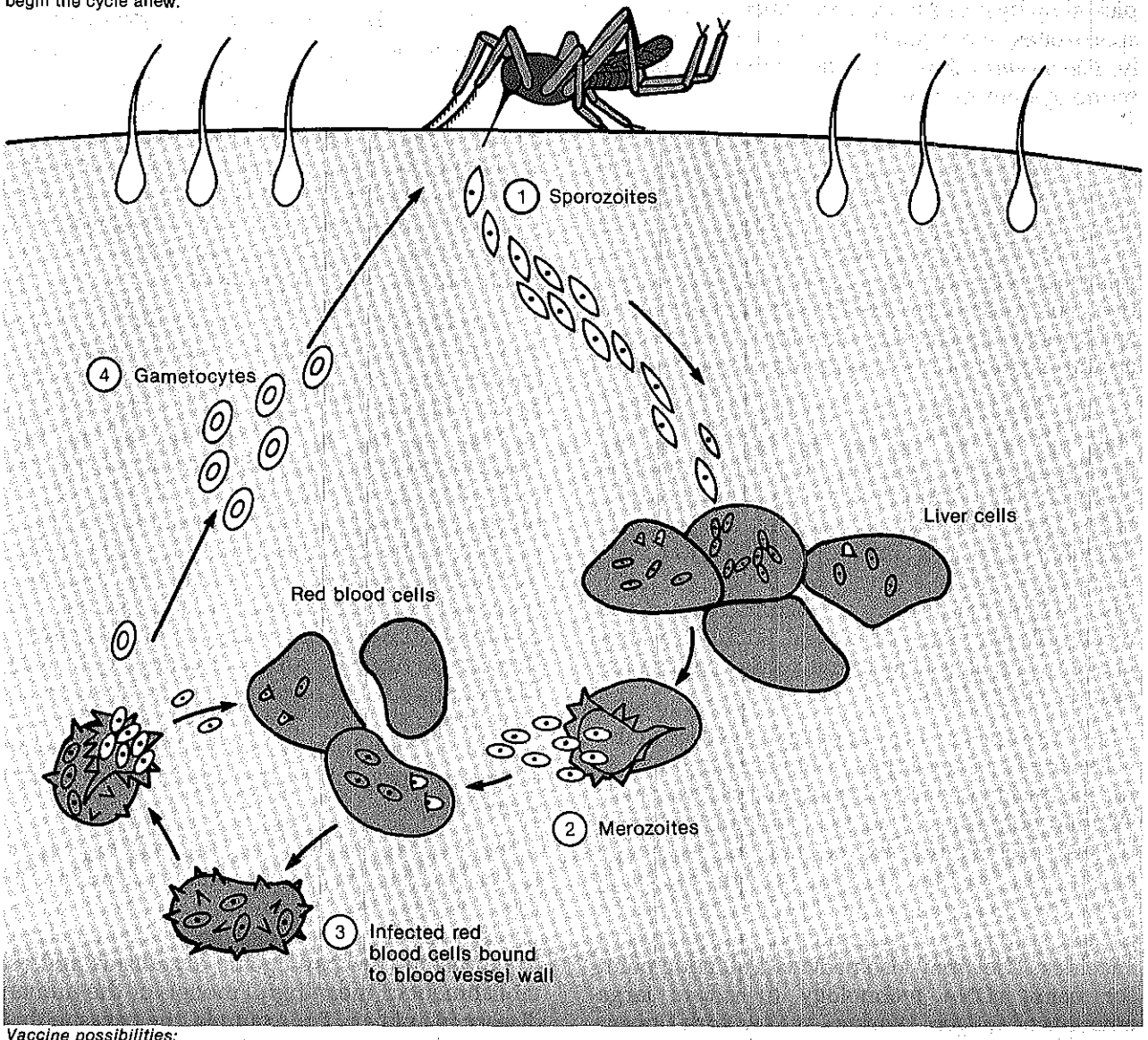
*The regulation of pharmaceutical products in the United States and other countries is discussed in *Chapter 15: Health, Safety, and Environmental Regulation.*

**A longer list of approved MAb products for research and diagnostic use appears in *Monoclonal Antibodies in Clinical Medicine* (77).

*New infections of gonorrhea, chlamydia, and herpes simplex virus type 2 (HSV2) are estimated to exceed 15 million per year in the United States. Approximately 1 million new cases of gonorrhea are reported annually to the U.S. Centers for Disease Control. It is estimated that the true prevalence of gonorrhea in the United States is 3 million cases annually. Chlamydia infections are not reported and their prevalence can only be estimated. Clinically, the infection rate is estimated to be three to four times that of gonorrhea (approximately 10 million cases annually in the United States). Separately or in combination, chlamydia and gonorrhea are responsible for an estimated 200,000 to 300,000 cases of pelvic inflammatory disease per year resulting in infertility in 50,000 to 100,000 women. HSV2 infections are becoming increasingly common, with approximately 200,000 to 300,000 new cases occurring each year. These new cases accrue on an estimated base of 10 million individuals who are already infected (38).

Figure 14.—The Lifecycle of *Plasmodium*, the Malarial Organism: Possibilities for Development of Vaccines for Malaria

The malarial infection begins when a person is bitten by an *Anopheles* mosquito that bears *Plasmodia*. Sporozoites (1) are injected into the bloodstream, where they may remain for only 30 minutes before they infect liver cells. Within the liver cells, each sporozoite divides into six to twenty-four merozoites, the next *Palsmodium* life-stage. Merozoites burst from the infected liver cell (2) destroying it, and enter the blood stream, where they infect red blood cells and proliferate. In subsequent waves of infection, merozoites burst from the red blood cells and spread to other red blood cells. Red blood cells infected with merozoites may produce new cell surface molecules which allow them to bind to blood vessel walls (3). Some of the merozoites go on to become gametophytes, the next life-stage (4). These gametophytes are picked up by another *Anopheles* mosquito in another bite; they reproduce within the mosquito and form sporozoites, which may be injected into another person to begin the cycle anew.



Vaccine possibilities:

1. *Anti-Sporozoite*—Vaccines against the sporozoite, whether antibodies that react with the sporozoite or peptides that mimic the sporozoite surface would probably be ineffective since they must kill every sporozoite to prevent infection.
2. *Anti-Merozoite*—Both passive (antibody) and active (subunit) vaccines against the merozoite might be effective in preventing malaria since the merozoite is often exposed to circulation and because the merozoite is most directly responsible for ongoing malaria infection.
3. *Anti-Malaria-infected red blood cell*—Because red blood cells infected with merozoites may be differentiated by new surface molecules, vaccines (particularly antibodies) against these surface molecules may help in reducing the spread of merozoites to other cells.
4. *Anti-Gametocyte*—Vaccines against the gametocyte would reduce the transmission of malaria since they would lower the number of gametocytes carried by mosquitoes, but such vaccines would not reduce the severity of the disease in its earlier stages.

SOURCE: Office of Technology Assessment.

immunoassays. MAb technology is an economic means of producing the high quantities of antibody required in pregnancy testing.*

- *Cancer detection.* The detection and quantitation of indicators related to malignant tumors is potentially one of the most important applications of immunoassay and MAbs. A great deal of work on tumor markers is underway, and a few MAb-based products have been approved for marketing. In some cases (e.g., prostatic acid prosphatase and CEA), MAbs are used used to detect blood-borne antigens shed by the tumor; in others, the MAb reagents are used to identify tumor cells by staining tissue specimens.

*Antibodies against human chorionic gonadotropin (hCG) were among the first products made by hybridoma technology, and several firms have made anti-hCG MAbs available commercially. To date, little advantage has been shown in comparison to polyclonal sera derived from animals. Nevertheless, Monoclonal Antibodies, Inc. (U.S.), received approval in September 1982 (based on "substantial equivalence" to previously marketed products) for ModEL, a urine hCG test for pregnancy, to be marketed to clinical labs and doctors' offices. Eventually products for consumer self-testing may be based on the same antibodies. Hybritech, Inc. (U.S.), also has an FDA-approved MAb hCG kit. It utilizes Hybritech's enzymatic amplification technology rather than radioisotopes, but an instrument is required to read the results.

IN VIVO DIAGNOSTIC PRODUCTS

Diagnosis of some diseases requires identification and localization of the disease within the body. Antibodies with detectable markers (e.g., radioactive chemicals) provide highly specific means for accomplishing these ends. Antibodies injected into the body, although used in diagnostic applications, are considered drugs; thus, they require extensive testing prior to approval for marketing.

MAb technology provides quantities of antibodies for testing, and MAbs are being evaluated in an increasing number of in vivo diagnostic appli-

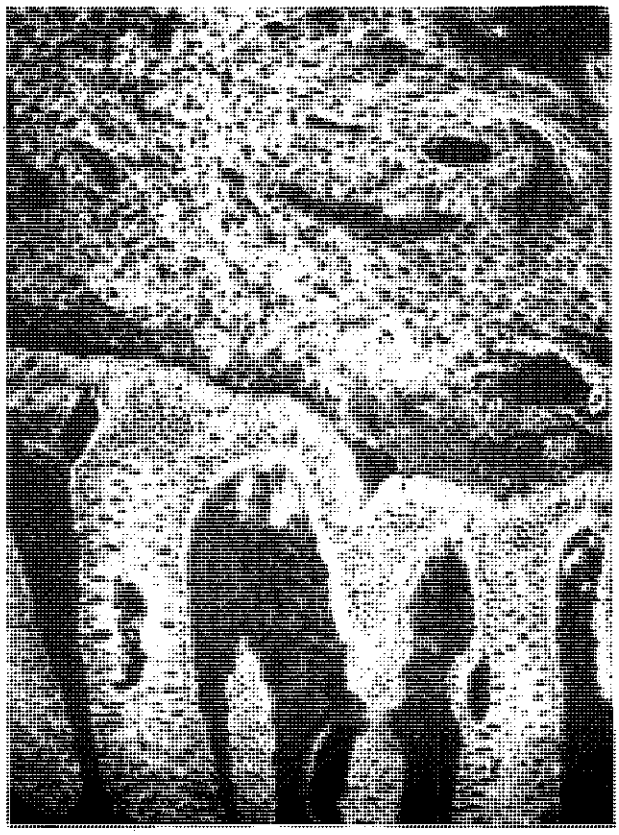


Photo credit: Science Photo Library and Porton/LH International

The spread of colonic cancer is visualized with the use of fluorescently labeled MAbs specific for cancer cells



Photo credit: Science Photo Library and Porton/LH International

Liver scan after injection of MAbs shows metastases of colonic cancer

mortality from parasitic organisms, is difficult to culture in the laboratory. The ability of this parasite to alter its susceptibility to host immunological responses and the difficulty in obtaining sufficient quantities of an antigen have hampered efforts to develop a vaccine for schistosomiasis.

Much basic research on parasites is needed in order to develop effective antiparasite vaccines using rDNA technology. The techniques of biotechnology have accelerated the study of parasitic diseases, but urgently needed pharmaceutical applications in this area are still in early stages.

Antibiotics

For the past three decades, antimicrobial agents for the treatment of infectious diseases caused by bacteria have consistently led worldwide sales of prescription pharmaceuticals. Novel antibiotics, produced mainly by traditional microbial bioprocesses, continue to be developed and introduced each year (especially in Japan in recent years). Methods of biotechnology such as the following offer strong innovative possibilities for producing new antibiotics:

- *"Sexual" recombination.* A technique known as protoplast fusion, whereby the contents of two micro-organisms are fused to give one cell, enables researchers to induce rapid improvements in bacterial germplasms. Protoplast fusion allows the rejuvenation of strains of industrial microbes that have lost vigor as a result of mutation and selection procedures that have been performed to increase their antibiotic productivity. The fusion of micro-organisms is beginning to yield new (hybrid) antibiotics (22).*
- *Recombinant DNA technology.* Gene coding for enzymes and other metabolic proteins can be cloned into antibiotic-producing micro-organisms to add steps to existing biosynthetic pathways that improve products or manufacturing processes. Ongoing research includes: 1) the rDNA-mediated transfer of acyltransferase genes among species of bacteria to obtain solvent-extractable cephalosporins (149); 2) the combination of genes via rDNA technology and transformation to obtain direct, efficient synthesis of the antibiotic amikacin (149); and 3) Eli Lilly's utilization of rDNA technology to improve the production of the antibiotic tylosin (4).

The combination of new and traditional technology in the pharmaceutical industry holds tremendous potential for improvement of micro-organisms used in antibiotic production and the isolation of new antibiotic products. Japanese pharmaceutical companies, with their extensive bioprocessing resources, are placing great emphasis on new antibiotic research (114). This emphasis may be due to the fact that antibiotics comprise 25 percent of (1981) ethical drug sales in Japan (compared to about 8 percent in the United States) and that at least 28 percent of the antibiotic sales in Japan now arise from antibiotics produced in the United States (120,125).

*Through protoplast fusion and selection, researchers at Bristol-Myers (U.S.) developed an improved method of producing purer penicillins that has accounted for 8 percent per year improvement in penicillin productivity over the past 4 years. Other genetic approaches produced 60 to 70 percent improvements in yields of cephalosporins (a class of antibiotics) in the same period. Genetic research by Pfizer, Inc., at laboratories in the United Kingdom and United States, have gradually lowered costs of producing oxytetracycline, a long established antibiotic, to costs similar to bulk chemical production, to give prices of several dollars per kilogram (73).

Monoclonal antibodies

MAb technology currently leads other forms of biotechnology in commercial use, as measured by numbers of products on the market. Its lead is largely due to MAb in vitro diagnostic products. In vitro diagnostic products do not have to undergo the same rigorous safety testing required of

DNA hybridization probes

DNA "hybridization" occurs when two single strands of DNA join to reform the double helix (see *Chapter 3: The Technologies).* The DNA strands must have exact, corresponding sequences of nucleotide bases for hybridization to occur; thus, a given strand can hybridize only with its complementary strand.

DNA hybridization is a powerful tool in molecular biology. Radioactive phosphorus is commonly incorporated into one of the DNA strands, the "probe," so that the hybridization process can be followed using the radioactive label. DNA hybridization is used to identify and isolate for further study particular DNA sequences (and cells that bear this DNA). DNA hybridization is also used to determine where certain DNA sequences are located on chromosomes. In addition, DNA probes are being tested as reagents in clinical medicine. Probe DNA obtained from a pathogenic organism such as a virus, for example, can be used to identify the presence of that virus within human cells, thus allowing specific diagnosis based on whether or not the radioactive DNA probe hybridizes with DNA in the cells.

Radioactive labeling of DNA hybridization probes raises problems of safety, handling, and disposal that in many cases limit the use of such probes to the research laboratory. Furthermore, since radioactive phosphorus loses its radioactive strength rapidly, only small batches of probes may be practically labeled with radioactivity at any given time.

Several methods to label DNA probes with nonradioactive substances are emerging. The most predominant new method, developed and patented by Dr. David C. Ward and his colleagues at Yale University's School of Medicine, is to couple chemically the molecule biotin to DNA. Biotin-labeled DNA probes hybridize with the target DNA and the hybrids are identified using compounds that recognize biotin (62) (see fig. 15). With such detection systems, only a few hours are required to identify DNA sequences using biotin-labeled probes, whereas 1 or more days are required when radioactive phosphorus labels are used. Additionally, biotin-labeled probes have the potential to be more sensitive than radioactive probes (70).

Nonradioactively labeled DNA is stable and safe to handle, so these probes can be prepared in large (manufacturer's level) quantities and stored for long periods of time. Almost any given short DNA fragment can now be chemically synthesized for use as a probe rather than isolating the fragment from a natural source. Another method of preparing DNA for use as probes is the isolation of DNA fragments made by restriction enzymes. Several companies (e.g., Applied Biosystems (U.S.), University Genetics (U.S.)) are working toward producing a large repertoire of DNA fragments for use as probes.

The ready availability of DNA probes and the convenience of nonradioactive labeling is likely to encourage widespread use of DNA hybridization probes in the near future. While many uses for DNA probes exist in basic research, developers such as Enzo Biochemicals (U.S.) and Cetus Corp. (U.S.) are striving to produce probes for clinical use, where much larger markets exist. Some promising clinical applications of DNA probes include the following:

- *Diagnosis of infectious diseases.* DNA probes that identify and differentiate among species of bacteria that cause diarrheal diseases have been made. Other DNA probes may prove useful in diagnosing human sexually transmitted diseases. DNA probes to detect infections of rotavirus, cytomegalovirus, hepatitis, herpes, and other viruses are being developed (29). In some clinical situations, DNA probes may be more useful than MAbs for diagnosis.
- *Prenatal diagnosis of congenital abnormalities* such as sickle cell anemia (97), beta-thalassemia (101), and duchenne muscular dystrophy.
- *Diagnosis of disease susceptibility.* Researchers in several laboratories are developing DNA probes that recognize DNA abnormalities leading to such conditions as atherosclerosis, the leading cause of death in the United States (5).

Table 26.—In Vitro Monoclonal Antibody Diagnostic Products Approved in the United States[a]

Manufacturer	Analyte	Date approved
Hybritech, Inc.	IgE	5/29/81
Hybritech, Inc.	PAP	9/1/81
Hybritech, Inc.	HCG	10/13/81
Hybritech, Inc.	T Cell	7/26/81
Hybritech, Inc.	Ferritin	10/19/81
Abbott	PAP	1/19/82
Abbott	CEA	3/3/82
Abbott	CEA	3/29/82
Ortho III	OKT-11	4/6/82
Centocor	Anti-Rabies	4/16/82
Hybritech, Inc.	HCG	4/23/82
Hybritech, Inc.	HGH	6/8/82
Mallinckrodt, Inc.	Total T_4	6/9/82
Hybritech, Inc.	Prolactin	6/10/82
Clinical Assays	^{125}I-IgE	6/18/82
Biogenex Laboratories	β-HCG	7/13/82
Hybritech, Inc.	HCG (EIA)	7/22/82
New Horizons	Gonogen	8/4/82
Monoclonal Antibodies, Inc.	UCG	9/24/82
Hybritech, Inc.	TSH	10/8/82
Allergenetics (Div. of Axonics)	IgE-Fast® (Specific IgE)	11/10/82
Becton Dickinson & Co.	T_4	12/7/82
Syva Co.	Chlamydia	12/10/82
Miles Laboratories	Gentamicin	12/14/82
Allergenetics (Div. of Axonics)	Total IgE-FAST	1/13/83
Carter-Wallace, Inc.	β-HCG	1/20/83
Hybritech, Inc.	Tandem-E® Ferritin	2/24/83
Ortho	Rubella	3/15/83
PCL-RIA	HCG	4/5/83
Quidel Home	HCG[b]	4/14/83
Ventrex Labs, Inc.	Enzyme TSH	4/26/83
Quidel Home	HCG[b]	4/26/83
Diagnon	Ferritin	4/28/83
BTC Diagnostics	HCG	4/28/83
Immunlok	Chlamydia	4/29/83
Monoclonal Antibodies	HCG	5/25/83
Ventrex Labs., Inc.	IgE (total)	5/25/83
Organon Inc.	HCG	5/26/83
BioGenex Laboratories	RIA Gen β-HCG RIA Kit	5/26/83
Micromedia System, Inc.	Micromedia β-HCG RIA	6/1/83
Organon Inc.	Neo-Pregnosticon Duoclon Tube Kit	6/3/83

[a]As of 6/14/83.
[b]These kits are for home use.

SOURCE: U.S. Department of Health and Human Services, Food and Drug Administration, National Center for Devices and Radiological Health, 1983.

infections may be reduced to 2 hours using MAb-based products. Additionally, Becton Dickinson (U.S.) has introduced a MAb kit that detects the bacteria responsible for meningitis infection. The bacterial strains can be detected in 10 minutes, and the company's price for each test is $2 (17).

- *Pregnancy testing.* Products composed of polyclonal antibodies have long been used to detect high levels of human chorionic gonadotropin (hCG) in the blood as an indicator of pregnancy. Large amounts of antisera are required to circumvent the need for radioactive isotope labels, which often accompany

Commercial aspects of biotechnology in the pharmaceutical industry

The path leading from the concept for a drug to a marketable product is arduous, costly, and extremely speculative. Discovery and development costs alone in the United States are estimated to range from $54 million to over $70 million per drug (43). Despite the generally low returns on the majority of potential drugs, however, high investments in pharmaceutical R&D continue. With an average of 11.5 percent of annual sales invested in R&D (99), the U.S. pharmaceutical industry ranks only below the information processing and semiconductor industries in terms of R&D as a percentage of annual sales (16).

During the past 40 years, the pharmaceutical industry has given increasing attention to R&D, and extensive government regulation of pharmaceutical products has evolved. Despite the increasing R&D commitments, however, recent trends indicate that the rate of innovative return to pharmaceutical companies throughout the world has declined (89). In short, fewer new drug introductions are emanating from larger research commitments by the public and industry (40).

Reasons most often cited for this decline in the United States center on the burdens imposed by Government legislation, including high costs of obtaining FDA approval, brevity and insufficiency of patent protection for new drugs, sponsorship of competition and product undercutting by State substitution laws and maximum allowable cost programs, and other regulatory factors that act as disincentives for renewed industrial R&D for new drugs. Other popular hypotheses for lower pharmaceutical innovation are decentralization of R&D resources by pharmaceutical companies to other industries such as specialty chemicals, cosmetics, and agricultural products, and increased displacement of R&D in industrial countries by R&D in less developed countries, emphasizing substitution rather than innovation.

Although biotechnology should not be viewed as a panacea for the problem of diminishing innovation in the pharmaceutical industry, it does offer promise in augmenting existing technologies in the pharmaceutical industry. In addition to allowing improvements in pharmaceuticals themselves, the adoption of biotechnology may provide ways for companies to streamline R&D costs for such things as biological screening, pharmacological testing, and clinical evaluation of new products. To a large degree, pharmaceutical development involves the correlation of function and molecular structure, and biotechnology may aid in making such correlations. Prior knowledge about the structure of drug receptor molecules, as gained partially from gene cloning and DNA sequencing research, for example, could supply investigators with information about which structures of new drugs might be effective in reacting with these receptors. This predictive ability may be increased by the use of computers to select appropriate drugs for development, as has been done in the development of synthetic subunit vaccines (67,68).

The costs of applying biotechnology to the development of new pharmaceutical entities cannot be readily determined at this time. In most instances, however, biotechnological methods of production are probably not yet cost-competitive with conventional methods. With biotechnology, as with other new technologies, there are costs associated with learning the technology that will diminish as facilities and skills are acquired. Achieving the limited goal of supplying MAbs successfully to manufacturers of in vitro diagnostic products, it has been estimated, will require a cumulative 3-year investment of $3.5 million to $4 million, and final immunodiagnostic product development may require 5 to 10 times this amount (138). The costs of commercial rDNA work are considerably higher. Although expenditures are rarely disclosed, indications of the cost of production for rDNA produced products can be gleaned from Schering-Plough's (U.S.) $6 million investment in a pilot-scale bioprocessing and purification facility (52), Genentech's drive to raise $32 million to sponsor clinical testing and development of its rDNA produced tPA (32), and Eli Lilly's $60 million investment in facilities to produce hI (129).

cations. One application involves radioisotope-labeled MAbs that bind to cardiac myosin (a major heart muscle protein) to locate and characterize myocardial infarcts (the most common type of "heart attack") (55,56). Another application involves the use of radioisotope-labeled MAbs that bind to antigens on cancer cells, but results to date have not been encouraging. As yet, no antigen that occurs on cancer cells exclusively has been found. A few clinical trials of in vivo diagnosis using MAbs have been undertaken, but experts agree that clinically useful products will require 5 or more years of further development (48). Success in this work could provide useful information prior to and following surgery.

In certain types of malignancies, such as plasmacytomas whose surface immunoglobulins are homogeneous and particular to the tumor, MAbs can be made against these proteins and then used as diagnostic or therapeutic agents. The therapeutic approach has been used in clinical trials for some types of cancer with encouraging results (20,109).

Preventive and therapeutic products

Applications of MAbs to prevent or treat diseases are being pursued on two fronts: 1) administration of MAbs as passive vaccines to protect against specific diseases, and 2) coupling cytotoxic agents (e.g., diptheria toxin, ricin, or cobra venom) to MAbs that direct the agents to diseased cells (7).

Much of the technology being developed that uses MAbs as diagnostic reagents may lead to development of MAb-based (passive) vaccines. This is especially true in the case of the viral diseases hepatitis B, herpes, and cytomegalovirus. Until recently, no cell culture system for hepatitis B has been available; however, a human liver tumor has been adapted to cell culture, and these tumor cells secrete the HBsAg (23). The availability of this HBsAg may make MAb preparation possible, leading to MAbs that neutralize the virus and are effective as a passive vaccine. Infants born to women with hepatitis B apparently benefit from treatment with human serum that contains antibodies against hepatitis B (78), and such serum is used prophylactically in many parts of the world. MAb technology provides a means for producing large quantities of antibodies against hepatitis B.

Scientists at Genetic Systems have produced human MAbs against *Pseudomonas, Klebsiella,* and *E. coli,* all gram negative bacteria which account for serious problems in patients with depressed immune system function (83). Clinical trials of these MAbs as passive vaccines are underway.

Trials of MAb-directed cytotoxic agents against tumor cells indicate that while cytotoxic agents such as cobra venom factor can be made to direct their activity in a very specific fashion against their targets, problems with finding cancer-specific antigens noted earlier restrain such applications of MAbs (36,60,147,148,161). Other problems associated with the use of MAbs in either chemotoxic or direct anticancer therapy include the following:

- toxicity problems associated with rapid administration of antibodies,
- cancer defense mechanisms that apparently involve shielding of target antigens by tumor cells (109),
- the difficulty of getting the cytotoxic agents inside the tumor cells, and
- the difficulty of getting the agent to the majority of cells of a solid tumor.

MAbs will undoubtedly play a major role in the pharmaceutical industry in the future, both as products and reagents for pharmaceutical research. R&D in the use of MAbs as pharmaceuticals is proceeding rapidly in the United States, where several MAb-based biotechnology companies have emerged, in the United Kingdom, where MAb technology was invented, and in Japan.

- investigation into the clinical use of neuroactive peptides and thrombolytic and fibrinolytic peptides,
- development of improved drug delivery systems,
- clarification of the mechanisms of acquired immunity leading to better vaccine development procedures,
- development of the ability to culture and an increased understanding of the lifecycle of the world's more debilitating protozoan parasites, and
- acquisition of a better understanding of the physiology and genetics of cancer.

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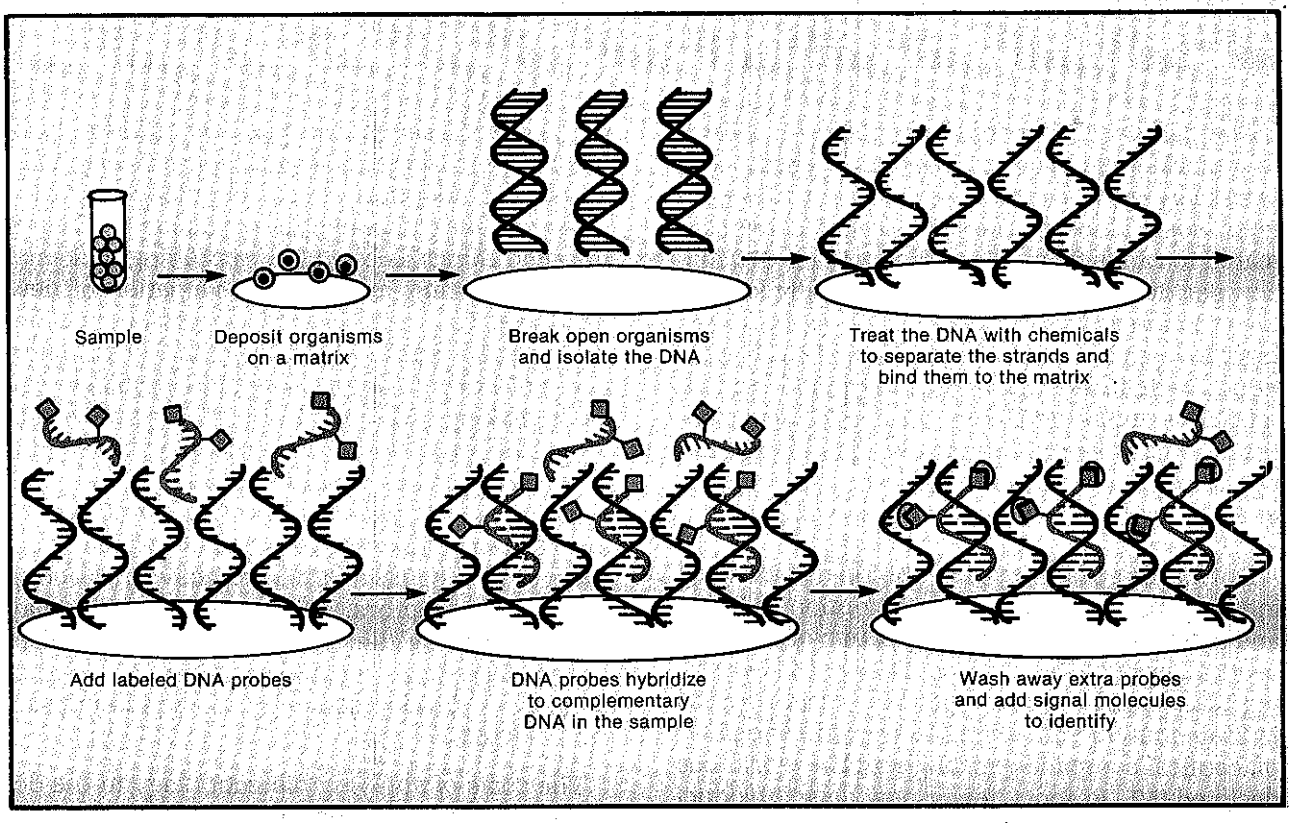
Figure 15.—DNA Probe Filter Assay



SOURCE: A. Klausner and T. Wilson, "Gene Detection Technology Opens Doors for Many Industries," *Biotechnology*, August 1983; Ron Carboni, N.Y., N.Y., artist.

The success of DNA probes for clinical use probably depends most on convenience and safe labeling of the DNA. Enzo Biochem (U.S.), capitalizing on Ward's biotin labeling technique, markets kits for labeling any given DNA sequence with biotin for use as a probe. Enzo has granted Ortho Diagnostics, a subsidiary of Johnson & Johnson (U.S.), exclusive worldwide marketing rights for its human diagnostic products. Cetus (U.S.), the exclusive licensee of a patent that involves diagnostic applications of DNA probes stemming from work at the University of Washington, is also emphasizing diagnostic applications of probes (91). Other NBFs that have announced their intentions to develop commercial diagnostic products based on DNA probe technology are Amgen (with backing by Abbott Laboratories) and Integrated Genetics (in collaboration with the University of California, San Diego).

The development of DNA hybridization probes represents a challenge to MAb technology for clinical diagnostic applications. MAb kits for diagnosing human venereal diseases are now on the market, but proponents of DNA hybridization probes claim that DNA hybridization offers an even more specific method of diagnosing infections (58). DNA hybridization can be performed with a minimum of tissue handling and may be used on some fixed tissues that are not amenable to MAb use. Ultimately, the relative strengths of DNA hybridization probes and other diagnostic products must be assessed on an individual basis.

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The international pharmaceutical market represents a major source of trade between nations, and foreign sales are comprising increasing percentages of total sales in the developed countries. From 1975 to 1981, for example, U.S. companies' control of their domestic market fell to 73 percent from 85 percent, and Japanese companies' share of their domestic market fell to 69 percent from 87 percent (120). Foreign sales account for 43 percent of total sales by U.S. ethical drug manufacturers. West German and Swiss companies are even more oriented toward foreign markets than their U.S. counterparts (40).

Many companies conducting biotechnology R&D are considering markets on a global scale, and for that reason, international market differences are likely to have strong effects on the pattern of biotechnology's introduction to the pharmaceutical industry. These differences are suggested by the fact that the most widely used pharmaceuticals in the U.S. market are neuroactive drugs, while those most widely used in foreign markets are anti-infective compounds. Thus, national preferences lead to differences in the choice of R&D ventures among leading companies, as exemplified by Japanese companies' interest in thrombolytic compounds. The potential of these new agents is more readily appreciated by Japanese drug firms than their U.S. counterparts, and thrombolytic agent R&D efforts by U.S. NBFs are underwritten largely by Japanese companies.

International differences of pharmaceutical use may also make the high costs associated with developing new methods such as biotechnology more acceptable in certain nations. In Japan, where blood products are imported because of cultural barriers to domestic collection, the Government may choose to subsidize the costs for domestic production of HSA by rDNA technology (which is likely to exceed the current price of HSA on the world market) rather than perpetuate the import trade. Such an action might enable firms involved with HSA biotechnology in Japan to move more rapidly along the manufacturing learning curve with generally applicable technology. Ultimately, this could reverse Japan's substantial pharmaceutical trade debt with the United States.

Biotechnology is likely to augment the international stature of the pharmaceutical industry through international corporate arrangements that combine research, production, and licensing in ways that best satisfy market needs in various countries. Because biotechnology offers possibilities of creating novel pharmaceutical compounds in large quantities and at reduced costs (e.g., Ifns, growth hormones, vaccines, and other biological response modifiers) and because many small new companies are involved in pharmaceutical R&D, the demands of "less glamorous" markets for products such as parasitic vaccines may have greater chances of being met than they have in previous years. Thus, biotechnology provides the pharmaceutical industry with a variety of new sources of R&D possibilities.

Priorities for future research

Funding from NIH has been and will continue to be instrumental in developing biotechnology for pharmaceutical use. The new biological techniques have dramatically increased the understanding of many disease mechanisms. Areas of research that would benefit pharmceutical innovation in biotechnology including the following:

- clarification of the functions and mechanisms of action of immune regulators such as Ifn and interleukin-2,

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Animal agriculture

The commercial use of biotechnology in animal agriculture is affected by several often-contradictory forces. Favorable forces include the extensive use of animals as test models in basic research and, as is discussed in *Chapter 15: Health, Safety, and Environmental Regulation,* less stringent regulatory approval processes for animal health products than for pharmaceutical products intended for human use. Because animals are used during the development of pharmaceutical and biologic products for humans, veterinary medicine stands to benefit from biotechnology research and development (R&D) such as that described in *Chapter 5: Pharmaceuticals.* Biotechnologically made products for use in animal agriculture, such as MAb diagnostic products, growth hormones (GHs), and vaccines, are becoming available on a limited basis.

Among the factors that inhibit commercial applications of biotechnology in animal agriculture is the fact that the low value-added nature of individual farm animals limits veterinary costs per animal, veterinary medicine sales, and funding for veterinary R&D. In addition, some biotechnologically made products do not suit current animal husbandry practices. Commercialization of at least one rDNA-made vaccine, the vaccine for foot-and-mouth disease (FMD), for example, awaits successful applied research results to achieve protection against several strains of the disease, fewer dosage requirements, lower costs, and other improvements that make the vaccine amenable to animal husbandry practices in the developing nations where FMD exists (20).

Biotechnological developments in the areas of animal disease control, animal nutrition and health, and genetic improvement of animal breeds are discussed further below. Distinctions between the use of biotechnology to expand fundamental knowledge and to develop specific products for commercial use are noted.

Diagnosis, prevention, and control of animal diseases

Losses due to animal diseases exceed hundreds of millions of dollars yearly in the United States,* giving strong impetus to efforts to improve animal health. A combination of the techniques of biotechnology is being used to better understand viral, bacterial, protozoan, and parasitic infections that affect animal productivity throughout the world. MAbs, for example, are being used as research tools to gain a better understanding of the molecular biology of animal diseases. MAbs may also be used for diagnosis of diseases, for monitoring the efficacy of drugs, and for providing short-term passive immunity against animal diseases. In addition, recombinant DNA technology and polypeptide synthesis may be used to develop vaccines for long-term immunization against certain animal diseases.

MONOCLONAL ANTIBODY DIAGNOSTIC PRODUCTS

The diagnosis of animal diseases can be accomplished by the identification in the laboratory of specific antigens displayed by the infectious agent. As discussed in *Chapter 3: The Technologies,* MAbs that recognize specific antigens can be prepared readily. MAbs for several animal diseases are now being made, and in vitro MAb diagnostic products for a number of animal diseases may be used in the near future. MAb-based diagnostic tests are currently being developed for bluetongue, equine infectious anemia, and bovine leukosis virus. Furthermore, diagnostic MAbs are be-

*"Animal losses" are described by a number of parameters, including dollar value of animals lost, losses in productivity due to morbidity, and value of potential progeny lost due to sickness or death of breeders. In this report, the dollar value of animals actually lost to disease (as a primary cause of death) is used for the sake of comparison in describing animal diseases. These estimates are based on data collected for U.S. Department of Agriculture's Animal and Plant Health Inspection Service, Veterinary Services, Hyattsville, Md., and by Doane Agricultural Services, Inc., St. Louis, Mo.

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Table 27.—Viral Animal Diseases and Potential Vaccine Production

Disease	Potential for new biotechnology	Company	Current vaccine status	Potential for new vaccine
Viral diseases:				
Foot-and-mouth disease	+	Genentech (U.S.)/USDA (U.S.) Pirbright (U.K.) Biotech Gen (Israel) MGI (U.S.)[a]	Medium	Relacement
Rabies	+	Wistar Transgene (France) Genentech (U.S.) Inst. Pasteur (France)	Variable	Replacement
Parvovirus:				
Swine	+	MGI	Poor	Replacement
Canine	+	TechAmerica (U.S.)	Medium	Replacement
Bovine leukosis virus	+	MGI	N.A.[b]	Replacement
Bovine papilloma virus	+	MGI	N.A.	Export animals
Rift Valley fever	+	MGI/U.S. Department of Defense	Good	Replacement
Marek's disease (fowl)	+	BRL (U.S.)[c]	Medium	Replacement
Infectious bovine rhinotracheitis	+	MGI	Medium	Replacement
Pseudorabies	+	MGI	Medium	Replacement
African swine fever	+	Spanish Government	None	New product
Rota viruses	−	Vido Institute University of Saskatchewan	None	New product
Bluetongue	+	Bio-Tech Gen. (Israel) USDA	Poor to medium	Export animals
Hog cholera	−	N.A.	Good	Replacement
Newcastle disease	+	USDA	Poor in some areas	Replacement
Bacterial diseases:				
Tuberculosis	N.A.	N.A.	None	New product
Neonatal diarrhea	+	Cetus (U.S.)/Norden (U.S.) Intervet (Netherlands/ Akza (U.S.) MGI	Poor	Replacement
Bacterial respiratory disease	N.A.	N.A.	Poor	Replacement
Anaplasmosis	N.A.	N.A.	None	New Product
Parasitic diseases:				
Babesiosis	+	IMC (U.S.)[d]	None	Replacement
Trypanosomiasis	+	American Cyanamid (U.S.) Genex (U.S.) Hoffmann-La Roche (Switz.)	None	New product
Coccidiosis	+	Eli Lilly (U.S.)	Good	Replacement
Helminithic diseases	+	Merck (U.S.)	Fair	Replacement

[a] MGI = Molecular Genetics, Inc.
[b] N.A. = Information not available.
[c] BRL = Bethesda Research Laboratories.
[d] IMC = International Minerals & Chemicals Corp.

SOURCE: Board of Science and Technology for International Development, et al., "Priorities in Biotechnology Research for International Development—Proceedings of a Workshop" (Washington, D.C.: National Academy Press); and the Office of Technology Assessment.

Genentech Corp. (U.S.), in collaboration with the U.S. Department of Agriculture (USDA), cloned the DNA that encodes the protein of one strain of FMD into bacteria, made the protein product in large enough quantities for field trials, and tested it at USDA's Plum Island Animal Disease Facility (14). The FMD subunit vaccine protected animals against infection by the particular strain against which the vaccine was made (although the field trial was not extensive), but it was less effective than the whole inactivated vaccine. The two other research groups working on a subunit FMD virus vaccine are a Swiss-West German team (University of Heidelberg, Federal Research Institute for Animal Virus Diseases at Tubingen, Max Planck Institute for Biochemistry, and Biogen S.A.) and a British team (Animal Virus Research Institute and Wellcome Research Laboratories) (9).

Chapter 6

Agriculture

Introduction

As the world population grows, agriculture will need to provide more and more food. Biotechnology may yield methods and products that improve agriculture in many ways. In animal agriculture, biotechnology offers promise in the following areas:

- *diagnosis, prevention, and control of animal diseases* with the use of monoclonal antibody (MAb) technology to diagnose, monitor, and better understand disease and the use of recombinant DNA (rDNA) to expand the pharmacopoeia of vaccines and other animal health products;
- *animal nutrition and growth promotion* through the use of growth hormones and feed additives to improve animal feed usage and animal health; and
- *genetic improvement of animal breeds* by using MAb and rDNA technology to better understand the bases of animal productivity and disease resistance and by the direct transfer of "beneficial" genes from one animal breed to another.

Though the potential for using biotechnology to improve animal agriculture is exciting, the commercial feasibility and actual impacts of using biotechnology in this area at present remain largely speculative. In some cases, existing animal health products may be replaced by improved, biotechnologically made materials. In other cases, entirely new products may become available to solve formerly intractable problems. In almost all instances, efficacy, safety, and practicality must be demonstrated for each new product. Only a few products for practical use in animal agriculture have been produced to date, so the success of biotechnologically produced compounds compared with conventionally made products remains to be demonstrated. For many animal agriculture products made with new biotechnology, the speed and scale of adoption by producers will be determined by the ease with which the products can be integrated into existing production systems (20).

In addition to the potential applications of biotechnology in animal agriculture, there are several potential applications in the area of crop improvement. The potential applications generally fall into two categories:

- *improvement of specific plant characteristics,* for example, through the introduction or manipulation of genes that confer resistance to disease and environmental factors, that increase the amount and quality of primary and secondary products from plants, that enhance plant growth rate, or that increase photosynthetic efficiency; and
- *genetic manipulation of micro-organisms,* for example, to enhance the process of nitrogen fixation, to produce insecticides, or to suppress disease or promote growth in plants.

The genetic manipulation and modification of plants presents some special challenges, but research is proceeding rapidly. There is a great deal of research interest at present in the use of biotechnology to improve plant resistances to disease and environmental factors. If plants are made more resistant to disease and certain environmental factors, greater crop yields or a reduction in the cost of crop production may result. Furthermore, unlike most plant traits, some of these specific crop improvements may be accomplished with only one or a few gene modifications. It is likely that there will be considerable research progress in this area in the next 5 to 10 years.

The applications and commercial aspects of biotechnology in animal and plant agriculture, respectively, are discussed in more detail in the next two sections of this chapter. A separate section at the end of the chapter indicates priorities for future research in each of these areas.

ing made with biotechnology. Recombinant DNA technology is used to change bacterial plasmids found in pathogenic strains of enteric bacteria from a virulent to a harmless state. This approach is used by both Intervet (Netherlands) and Cetus Corp. (U.S.) to prepare vaccines against colibacillosis. These vaccines have been successfully tested in pregnant cows, which transferred immunity against colibacillosis to their offspring, and the products are now available commercially.*

Using another approach to fight colibacillosis, the NBF Molecular Genetics, Inc. (U.S.) uses hybridomas to produce MAbs against the attachment antigens of the bacteria responsible for the disease. Incorporating these MAbs in milk fed to young calves within 36 hours of birth protects the animals through the period for which they are most susceptible (36). This product is approved for use in the United States and Canada.

The development of biotechnological solutions to bacterial animal diseases, as well as viral infections, will require much basic research. Pasteurellosis (a lower respiratory tract infection in cows, sheep, and pigs) and swine dysentery (which causes annual losses of $75 million in the United States) are among the major animal bacterial infections about which more knowledge is needed before applications of biotechnology are possible. The potential for biotechnological production of new bacterial vaccines and the development of successful delivery systems is largely unexplored.

Protozoan and Parasitic Infections of Animals.—Coccidiostats (compounds that prevent coccidiosis in poultry) and anthelmintics (substances that fight helminthic parasites such as roundworms, tapeworms, lung worms, and liver flukes) constitute large, rapidly expanding animal health product markets. In 1985, the global market for coccidiostats is expected to be $500 million (compared to $300 million in 1981), and the global market for anthelmintics may exceed $900 million (compared to $450 million in 1981) (35). At present, coccidiostats and anthelmintics are synthesized by either chemical synthesis or microbial bioprocess methods. These agents, many of which have been discovered serendipitously, are commonly administered in animal feed (10).

The widespread use of coccidiostats, anthelmintics, and antibacterials in animal feed raises concerns about the nurturing of drug resistance among populations of micro-organisms. These risks are outlined in a 1979 OTA report entitled *Drugs in Livestock Feed* (30). As described in that report, the genes in bacteria that encode resistance to most drugs are located on plasmids. Resistance to drugs may be shuttled via these plasmids into pathogenic micro-organisms such as *Salmonella*. Widespread use of antibacterials selects for bacteria, including *Salmonella,* that contain resistance genes, perpetuating drug resistance among bacteria. Thus, wide use of antibacterials in animal feed eventually may compromise the effectiveness of the same drugs in treatment of human diseases. Drug resistance among the protozoa and parasites is even less well understood than is resistance among bacteria. Such resistance is difficult to quantify but may be increasing (13,30).

Fundamental knowledge may be gained by using rDNA technology to explore the structure and function of genes that confer resistance to drugs. MAb technology and other conventional methods may be used to isolate, purify, and better understand antigens found on parasitic cells, perhaps resulting in vaccines effective against these parasites. The increased use of vaccines would decrease the use of feed additives and presumably lessen the problems of drug resistance.

Strong needs, large market potentials, and safety considerations characterize the further development of compounds effective against protozoa and parasites that afflict animal populations. Because of the complexity of most parasitic infections, however, biotechnological solutions may not be forthcoming immediately. In addition, the recent introduction of potent new antiparasitic feed additive compounds, such as the avermectins (which are microbially produced) (8), may lower incentives to explore new antiparasitic possibilities with biotechnology in the near term.

*These bacterial vaccines were made by replacing a "virulence gene" (a gene which encodes a protein that regulates cellular water loss and is responsible for the diarrhea) located on a plasmid with a harmless gene and "infecting" animals with bacteria containing these harmless plasmids. The bacteria continue to produce surface antigens, but they do not produce the virulence protein. The surface antigens stimulate an immune response that prevents adherence of natural virulent bacteria (18).

ing sought for canine parvovirus, canine rotavirus (a potentially fatal viral diarrhea in puppies), feline leukemia virus, and canine heartworm disease. For MAb diagnostic products to be effective diagnostic tools and hence commercially viable, they must recognize the large variety of disease strains likely to be encountered (20).

The acceptance of MAbs for field use by veterinarians and animal owners remains to be demonstrated. Whether MAb products will have a large role in the diagnosis of specific animal diseases is unclear. Since livestock producers and poultry growers attempt to spend as little money as possible per animal raised, the markets for individual MAb diagnostic tests initially may be limited. Applications of MAb diagnostic as well as therapeutic products initially may be restricted to high-profit animals, animal products for export, and companion animals such as dogs, cats, and horses. Although individual diagnostic kits are not costly, the farmer's narrow margin of return on other animals may prevent the routine use of diagnostic products.

In the future, diagnostic MAbs could substantially assist large-scale disease control programs in both developed and less developed countries (16). Such reagents might be used to detect disease in order to select an appropriate vaccine and monitor the level of disease during the course of a control program.

Apart from potentially being used as diagnostic reagents by animal producers, MAbs can be used as purification tools to isolate compounds (antigens) that may prove to be effective animal vaccines. They can also be used to provide "passive immunity" to certain animal diseases. The applications of biotechnology to the development of animal vaccines is described further below.

ANIMAL VACCINES

Prevention of a number of animal diseases is being sought with rDNA subunit vaccines in efforts similar to human vaccine programs described in *Chapter 5: Pharmaceuticals*. Subunit vaccines may solve some of the problems associated with conventional vaccines. One problem, for example, is that "attenuated" and killed whole vaccines contain the genetic material of the pathogen and therefore have the potential to cause the infection they are supposed to prevent. Subunit vaccines do not contain the pathogen's genetic material and therefore cannot cause infection. Subunit vaccines may also be more stable, more easily stored, and of greater purity than conventional vaccines, but these qualities remain to be demonstrated. Despite their potential advantages, subunit vaccines raise new technical problems, as mentioned above, and these must be overcome if the vaccines are to prove useful in the field (20).

Viral Animal Diseases.—The development of improved vaccines may allow the prevention of several problematic animal diseases caused by viruses (34). Most subunit vaccine research for animals to date has been focused on viral diseases, particularly FMD and rabies, but some of the findings can be generalized to other viral diseases. Table 27 shows some viral diseases in animals against which subunit vaccines may prove effective and economic.

The development of subunit vaccines for FMD is currently receiving much attention from researchers (2). Although the disease is nonexistent in the United States, FMD affects livestock productivity and exportability throughout South America, Africa, and the Far East. The world market for FMD vaccine is larger than that of any other vaccine, either animal or human. In 1981, 800 million doses of inactivated FMD virus vaccine worth $250 million were used (36). Vaccines for all types of FMD commonly encountered exist at present, but these vaccines vary in effectiveness against different FMD field strains. Evolution of new field strains is a continuing problem, because a vaccine may lose its effectiveness against such strains. The impetus for developing a subunit vaccine for FMD is the hope that such a vaccine will offer enhanced protection with greater safety than conventional vaccines. The degree of protection offered, however, will only become clear over the next few years as research and field evaluations progress (9).

Three research groups have cloned the gene that codes for the major FMD viral surface protein (5,14,15). The new biotechnology firm (NBF)*

*NBFs, as defined in *Chapter 4: Firms Commercializing Biotechnology*, are firms that have been started up specifically to capitalize on new biotechnology.

tivity. The results of experiments pertaining to GHs' mode of action to date have yielded results that suggest caution. Previous observations that injections of bovine pituitary gland extracts enhance lactation in cows led to the finding that purified GHs increase milk yield by 10 to 17 percent, without a concurrent change in feed intake (24). Other experiments with sheep and pigs have shown rapid growth following GH treatment (36). However, other evidence indicates that GHs stimulate growth and feed-use efficiency at the expense of body-fat deposition (24). Thus, critics argue, GHs may impair long-term animal health and productivity (24).

Substantial hurdles must be overcome before rDNA-produced GHs become commercially available. In addition to requiring regulatory approval, the commercial success of GHs requires an adequate drug delivery system that introduces GH slowly to animals. Oral administration of GHs, although most convenient and marketable, is an inadequate system of delivery because polypeptides such as GHs are degraded by digestive enzymes. The hormones must be made available to the body's circulation, where they can reach endocrine organs. Slow-releasing ear implants may be used as alternatives to frequent injections (injections are not amenable to most husbandry practices except those for dairy cattle), but, at present, dose requirements are too high for such implants to be practical (21). Eli Lilly (U.S.) is developing a long-lasting bolus to be used in the rumen. Presumably, enough GH is released directly through gastrointestinal tract walls to avoid the problem of enzymatic degradation. With the development of convenient delivery systems, better field trials to investigate the efficacy of GH may result.

Genetic improvement of animal breeds

Throughout the history of animal agriculture, breeders have sought to improve animal productivity by selecting animals with desirable traits for breeding. Recent increases in the understanding of animal reproductive biology and the genetic basis of traits have fostered new animal breeding technologies (31). As a result of increased knowledge due to biotechnology, the identification of genes and gene products that influence traits of productivity, vigor, and resistance to certain diseases may be possible.

In the future, animal breeding programs may be augmented by biotechnology to achieve desired changes with unprecedented speed and selectivity. Biotechnology may be used in ongoing breeding programs first to identify animals with desirable genes (e.g., genes that make the animals resistant to certain infections), and second, to transfer these genes directly into the germ line (cells that contain the genes that will be passed on to future generations) of other animals. Possible applications of biotechnology include the use of MAbs to identify and isolate gene products correlated with certain traits, the use of rDNA technology to produce large quantities of desired gene products for further study, gene transfer (micro-injection of isolated DNA into embryo cells), and implantation of the embryo cells to which genes have been transferred into surrogate mothers.

The technology of gene transfer is in its infancy. To date, it has been used only in laboratory animals. In most instances, the gene(s) to be studied is fused within a plasmid to a gene with a known "housekeeper" function required for growth. The plasmid is injected into a host cell that is deficient in the housekeeper function. Only host cells whose chromosomes incorporate the foreign DNA have the restored housekeeping activity and survive. These cells then are screened for activity of the desired gene. The GH gene has been the subject of many recent gene transfer experiments.

Thus far, gene transfer experiments in animals have increased fundamental understanding in several areas. Scientists have made great gains in preparing receptive host cells, transferring genes from one animal cell to another, and recognizing the successful recombination of foreign DNA in host chromosomes (1,6,32,33). Fundamental understanding of mechanisms of gene control in mammals has also burgeoned in recent years. Several investigations have revealed that the host tissues surrounding the cells that contain implanted genes affect expression of the foreign genes (as surrounding tissue may regulate gene

Cloning of the genes that code for the surface proteins of viruses of fowl plague, influenza, vesicular stomatitis, herpes simplex, and rabies also has been achieved, and the cloned genes may lead to the development of effective subunit vaccines for these animal diseases (2). Cloning projects for virus proteins that cause gastroenteritis, infectious bovine rhinotracheitis, Rift Valley fever, and paramyxovirus currently are underway (2). Different challenges are associated with each project. Rabies projects, for example, have encountered problems with the consistent expression of the surface protein from rDNA plasmids (34). Influenza virus projects, among others, face problems in that the natural viruses spontaneously change their surface proteins to evade the immune system, making the choice of optimal genes for cloning difficult.

Another method being used to prepare new subunit vaccines for animals, aside from the use of rDNA technology, is chemical synthesis of peptides. Synthetic peptides corresponding to part of one viral surface protein of FMD protect test animals against live FMD virus (3), and efforts are underway to prepare synthetic rabies vaccines (28). As noted in *Chapter 5: Pharmaceuticals,* most synthetic vaccines are prepared with the use of MAbs as purification tools. Chemically synthesized peptides may prove useful in rapid screening programs to determine which peptides act as the best vaccines; subsequently, the DNA corresponding to these fragments may be cloned for large-scale production in microbial systems.

Whether produced from rDNA or chemical synthesis, subunit vaccines for viral animal diseases must satisfy several requirements to be effective. In most instances, subunit vaccines must contain antigens from a sufficient number of different strains of virus to offer comprehensive protection against field challenge. The new vaccines must induce a protective immune response to the same or greater degree than conventional vaccines if they are to compete for market shares. Proper dosage and timing of vaccination must be determined. Ideally, the vaccines should be administered in a single injection to be amenable to most husbandry practices throughout the world where animals are dispersed over wide tracts of land. Also, long shelf storage life and stability when stored at room temperature are desirable features of the new vaccines for use in all the countries affected by the particular diseases.*

In addition to subunit vaccines that provide active immunity, MAbs may be used to provide passive immunity against a variety of viral animal diseases. Several MAb-based products currently are being developed. For instance, antirabies MAbs that protect mice from active rabies virus have been made (19). The use of these products, however, is likely to be limited to herds (e.g., dairy animals) where the passive vaccines can be readily and repeatedly administered.

Bacterial Animal Diseases.—The potential for biotechnology in fighting bacterial diseases in animals is less clear than its potential in fighting viral diseases, but several promising advances are currently being made. In developing new methods to prevent these diseases, an understanding of the natural and pathogenic roles bacteria play in domestic animals is important. Numerous types of bacteria are normal inhabitants of both human and animal gut. In general, disease may result when animals, especially those predisposed to infection (e.g., young, weak, or stressed animals), either succumb to pathogenic bacteria or suffer from overgrowths of their own native bacteria. Bacterial infections often occur simultaneously with other infections, including viral invasions. Because of the complexity of most of the currently important animal diseases in which bacteria are involved, the effectiveness of bacterial vaccines produced by biotechnology is difficult to predict.

Bacterial vaccines against colibacillosis (scours), a widespread disease that causes diarrhea, dehydration, and death in calves and piglets, are be-

*At present, the rabies subunit vaccine is most promising in meeting the criteria for becoming a competitive vaccine. There appear to be only slight variations in surface protein sequences between rabies virus strains, and these surface proteins elicit large immune responses. The RNA encoding several viral surface proteins has been cloned and expressed in *E. coli* (34). Questions that remain concerning the efficacy of this vaccine include: 1) the need for glycosylation of the rDNA-product for proper functioning (see *Chapter 5: Pharmaceuticals);* and 2) proper delivery systems, primarily to wild animal reservoirs such as skunks and foxes, where rabies proliferates, and to dispersed animal herds such as those in South America, where the death of cattle infected by the bites of rabid vampire bats results in an estimated yearly loss of more than $29 million (34).

Table 29.—Global Animal Health Product Markets

	Estimated sales, 1981 (millions of dollars)	Estimated annual growth, 1981-85
Nutritional products	$2,500	10-15%
Medicinal products:		
Biologics/Vaccines	1,000	20-25%
Antibacterials	2,000	10-15%
Anthelmintics	450	25-30%
Ectoparasiticides	400	10-15%
Coccidiostats	300	15-20%
Growth promotants	200	24-30%
Other	650	15-20%
Subtotal	5,000	15-20%
Total	$7,500	15-20%

SOURCE: S. J. Zimmer and R. B. Emmitt, "Industry Report: Animal Health Products Market" (New York: F. Eberstadt & Co., Inc., 1981).

Table 30.—U.S. Producers of Animal Health Products

	Estimated animal health sales, 1981 (millions of dollars)	Percent of corporate sales	Percent of corporate operating income	Estimated animal health sales annual growth, 1981-85
Pfizer	$ 440	13%	13%	10%
Eli Lilly	365	13%	15%	20%
American Cyanamid	265	7%	7%	11%
Merck	200	7%	7%	27%
SmithKline	155	7%	5%	17%
Upjohn	134	7%	7%	11%
Syntex	83	12%	N.A.[a]	11%

[a] N.A. = Information not available.

SOURCE: S. J. Zimmer and R. B. Emmitt, "Industry Report: Animal Health Products Market" (New York: F. Eberstadt & Co., Inc., 1981).

product sales by the U.S. companies that produce such products constitute a fairly low percentage (an average of 11 percent) of the companies' total sales. Investments in animal-related biotechnology R&D in those companies probably average about the same or less than the investments by the leading NBFs that are applying biotechnology to animal agriculture (22).

As noted in *Chapter 4: Firms Commercializing Biotechnology,* most major pharmaceutical and veterinary medicine companies are investing in biotechnology R&D, but there is some question as to their motivation for producing new products for large, established animal health care markets. Such markets include those for antibiotics, anthelmintics, and coccidiostats. Established companies with existing lines of conventionally made, widely marketed animal health products may have strong interests in maintaining these products. In many cases, therefore, their primary interests do not lie in R&D to produce new animal health products. As described earlier, applications of biotechnology to the production of animal products involve a substantial investment in basic research. In some cases, healthy sales of conventionally made products may dissuade a company from pursuing basic research that could lead to the development of a competing product. In other cases, corporate developers may choose to pursue human pharmaceutical innovations of new biotechnology, rather than applications of biotechnology to animal health. Because of these considerations, innovation and new product development in animal agriculture might be slowed.

Innovation in smaller product market areas, such as animal vaccines and diagnostic products, however, is widespread. New or replacement animal vaccines are among the most promising applications of biotechnology, as are MAb-based diagnostic products. Much of the innovation in

One serious worldwide rickettsial disease that requires urgent attention is anaplasmosis. Anaplasmosis, which is caused by blood-borne micro-organisms transmitted to cattle by ticks, causes severe anemia and subsequent death in afflicted animals. In the United States, annual losses due to anaplasmosis are estimated to exceed tens of millions of dollars. At present, an unsatisfactory attenuated vaccine exists, and attempts to culture the micro-organism and prepare better vaccines have been only marginally effective (36).

Animal nutrition and growth promotion

Practices and products that promote animal nutrition and growth have the potential to produce direct, substantial returns on investments. Animal scientists seek better animal nutrition and feed-use efficiency in several ways, including the study of gut bacteria that participate in animal digestion, feed additives that enhance absorption of nutrients, and substances such as GHs that may directly stimulate growth and animal productivity.

Synthetic steroids and natural hormones are used widely to promote animal growth, as indicated in table 28. Furthermore, as noted above, health- and growth-promoting compounds from industrially grown micro-organisms constitute a large share of feed additives (30). Some of these compounds act by enhancing the growth of beneficial micro-organisms in the gut, others by reducing the prevalence of harmful micro-organisms and parasites throughout the gastrointestinal tract; still other compounds directly provide animal nutrition. In cases where microbial metabolic pathways and products are known, biotechnology may augment the production of compounds used as feed additives by increasing the production of specific microbial metabolites.* At present, however, applications of biotechnology to the production of metabolites largely remain unexploited (10).

GHs produced by rDNA technology, in contrast, currently are undergoing trials in humans and animals in efforts to demonstrate safety and effectiveness in stimulating growth. Several U.S. NBFs, including Genentech Corp. (in collaboration with Monsanto Corp.), Molecular Genetics, Inc. (for American Cyanamid), Bio-Technology General, Amgen, and Genex Corp., are producing GHs for various animal species. In addition to yielding potential commercial products, rDNA GH projects are stimulating widespread research into the nature of growth, development, and animal produc-

*The production of compounds used as feed additives is discussed in *Chapter 7: Specialty Chemicals and Food Additives.*

Table 28.—World and U.S. Sales of Growth Promotants (millions of dollars)

	Sales								
	1979		1980		1981E[a]		1985E[a]		Compound annual growth
Products	World	U.S.	World	U.S.	World	U.S.	World	U.S.	1981-85E[a]
Hormones:									
Synovex (Syntex)	$ 14	$ 8	$ 16	$ 8	$ 19	$ 8	$ 23	$ 6	9%
MGA (Upjohn)	12	11	12	10	12	9	12	0	No change
Ralgro (IMC)	16	15	24	22	32	29	55	45	15%
Compudose (Eli Lilly)	—	—	—	—	4	—	100	50	N.A.[b]
Other:									
Rumerain (Eli Lilly)	$ 60	$ 55	$ 65	$ 55	$ 75	$ 60	$200	$125	28%
Feed	60	55	65	55	75	60	125	100	14%
Bolus	—	—	—	—	—	—	75	25	N.A.
Avoparcin ("Avotan") (American Cyanamid)	15	—	20	—	25	—	50	10	19%
Other	33	—	38	—	43	—	75	—	15%
Total	$150	$ 29	$175	$ 95	$210	$106	$515	$246	25%

[a]E = estimated.
[b]N.A. = information not available.

SOURCE: S. J. Zimmer and R. B. Emmitt, "Industry Report: Animal Health Products Market" (New York: F. Eberstadt & Co., Inc., 1981). Modified by the Office of Technology Assessment.

Plant agriculture

There are hundreds of forms of crop improvement whose purpose generally falls into one of three categories. The first is to increase crop yields by increasing resistances to pests or environmental conditions such as drought or soil salinity or by developing more productive plants. The second is to improve crop quality by enhancing such features as nutritional value, flavor, or processability. The third is to reduce agricultural production costs by reducing a crop's dependence on chemicals or by making harvesting easier (55, 56).

During the last century, plant breeders have been efficiently and successfully addressing all of these goals. The use of new biotechnology in crop improvement, as in other areas, is not a new beginning, but an extension of previously evolved skills. New biotechnology alone will not produce better crop plants, but combined with knowledge from other plant science and microbiological disciplines, biotechnology will develop techniques that could be very powerful in improving agriculturally important crops. Thus, the greatest advances in crop improvement are likely to be made using an interdisciplinary approach.

The genetic manipulation and modification of plants presents some special challenges. Most molecular genetics to date has been done with simple unicellular organisms and, to a lesser extent, with laboratory animals. The application of molecular genetics to plants is relatively more recent and consequently at an earlier stage of technical development. Furthermore, there are fewer studies of the physiology and biochemistry of plants than there are studies of these aspects of animals. The recent application of the new techniques of molecular biology to plants has produced astoundingly rapid results, however, and these techniques are sure to have an impact on crop improvement in the next several years.

Of the several hundred domesticated plants in the world today, only about 30 are of great economic significance. Of these, eight domesticated grains, including rice, corn, and wheat, produce most of the calories and protein consumed by humans and agriculturally important animals. The legumes, which include soybeans, represent the second most common source of food for human and animal consumption. There are two philosophies, which are not incompatible, with regard to improving crop plants. One is that there should be diversification of crop plants and attention given to the domestication and breeding of new major crop plants. Another philosophy is that plant breeding, tissue culture, and biotechnology efforts should be devoted to the most successful crop plants. The genetic diversity of some of the world's current crop plants is not great. Consequently, even if the major crop plants are the focus of research efforts, some genetic material from exotic sources may be required to effect the desired improvements. In any case, the techniques discussed here are equally applicable to the improvement of both common and exotic species.

Research on plants has shown that the genetic organization of plants exhibits striking similarity to that of animals. The universal genetic code is used, and most genes contain intervening sequences and are surrounded by very similar regulatory sequences. Unlike animals, however, plants have a characteristic called totipotency that, for many species, indicates the potential for regeneration of a single cell into a complete plant. Because plants have this totipotent characteristic, certain genetic manipulations can be done in cell culture, and, after selection of cells with the appropriate qualities, the cells can be regenerated into parental plants (for breeding programs). Adjusting the laboratory variables to achieve regeneration from single cells is evolving from an art to a science and has yet to be accomplished consistently for the principle cereal grains (monocots*), but regeneration research is proceeding at a rapid rate. It is likely that many important crop plants will be able to be regenerated from single cells in the next few years.

There are several potential applications of new biotechnology for plants that may help in the im-

*Early in the evolutionary history of flowering plants, two main types of plants, monocots and dicots, diverged. Cereal grains (corn, wheat, rye, barley, rice, etc.) are monocots, whereas legumes (soybeans, etc.) are dicots.

expression in normal cells) and that this "tissue-specific gene regulation" continues through successive generations (7,12,23,25,27). Finally, gene transfer experiments have allowed the study of the expression of single genes that, with other genes, comprise traits that might be too complex for study by other methods.

Gene transfer studies may reveal much about the function of single gene products. For instance, the transfer of genes implicated in immune responses and resistance/susceptibility to disease are being studied (some of these genes encode immunological cell-surface proteins called the HLA antigens) (11). The ability to transfer such genes into foreign cells to distinguish the production and function of their products may lead to valuable knowledge about animal diseases.

In the future, gene transfer may prove to be the sole means of overcoming certain animal diseases that defy preventive vaccine technology and/or veterinary treatment. An example of such a disease is trypanosomiasis ("nagana" in cattle and "sleeping sickness" in humans). Trypanosomiasis is caused by parasites borne in the saliva of certain insects and impedes livestock productivity throughout Africa (where the disease is transmitted by tsetse flies). Strains of cattle and sheep with resistance to trypanosomiasis (trypanotolerance) exist, and their resistance may be traceable to several distinct genes (26,29). Gene transfer may prove useful in better identifying these genes and selecting animals for breeding programs designed to encourage trypanotolerance in affected areas. In the future, transfer of these genes into cattle germ lines may rapidly foster widespread trypanotolerance where most other programs to control trypanosomiasis have failed. The application of knowledge gained from gene transfer experiments to animal agriculture will not be immediate, but such knowledge eventually may lead to considerable agricultural advances.

Commercial aspects of biotechnology in animal agriculture

Although field trials of several biotechnology products for animals are underway and a few products (e.g., vaccines for colibacillosis) have been approved for use, it is not yet clear to what extent biotechnologically made products will be adopted for use in animal agriculture. Most of the nascent products will require more convenient, cost-effective delivery systems, greater demonstration of effectiveness, and appropriate publicity before they are used widely.

If these challenges are successfully met, biotechnology may affect animal agriculture in numerous ways. Some novel products, such as rDNA and synthetic vaccines, in addition to lowering the costs of animal health care, may create new markets. Other products, such as diagnostic MAbs, may replace conventional diagnostic tests. At present, the animal health product markets are skewed against biologics such as vaccines in favor of pharmaceuticals such as antibiotics, mostly because biologics have not demonstrated high levels of efficacy. Until recently, commercial investment in vaccine research has been relatively small, but the wide-ranging applications of biotechnology to animal agriculture is prompting increasing amounts of investment in vaccine research. Applications of biotechnology to products for highly valued animals, such as companion animals and breeding stock, may help support substantial R&D and licensing costs associated with the first new animal drugs and biologics made using biotechnology.

Existing global markets for animal health products are shown in table 29, which differentiates major markets for nutritional products, antibacterials, and other compounds from the market for vaccines. As shown in that table, the markets for vaccines, anthelmintics, and growth promotants are expanding the most rapidly.

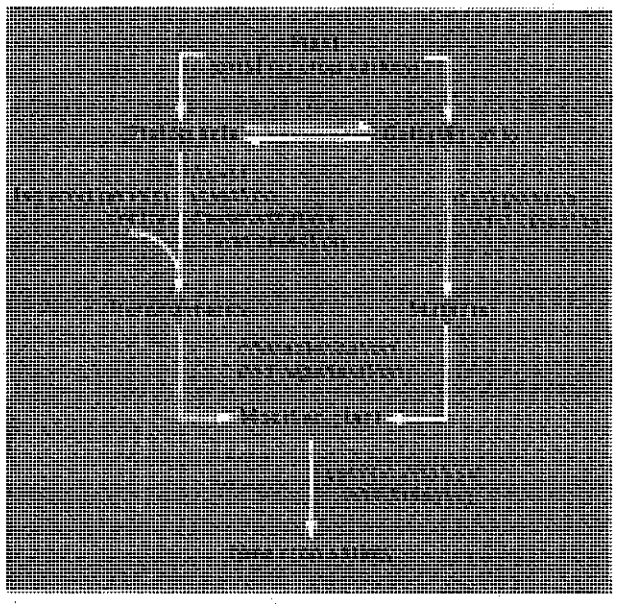
The companies that dominate the production of most animal health products are primarily major chemical and pharmaceutical manufacturers.* Most of these companies possess global marketing and distribution networks and undertake animal drug production as a diversification of their principal activities. As shown in table 30, animal health

*Major U.S. producers include Syntex, Pfizer, Eli Lilly, Upjohn, SmithKline Beckman, American Cyanamid, Merck, American Home Products, Johnson & Johnson, Tech America, and Schering-Plough. Major foreign producers include Burroughs-Wellcome (U.K.), Rhone-Merieux (France), Hoechst AG (F.R.G.), Bayer AG (F.R.G.), Connaught (Canada), Beecham (U.K.), Solvay (Belgium), Boehringer Ingelheim (F.R.G.), Intervet (Netherlands), and Elf Aquitaine (France).

provement of crop species, as shown in figure 16. New technologies for testing for the presence or absence of traits, for example, will save years of plant breeding time. Many applications to plant agriculture will be in the regulation of endogenous genes, and other improvements will be made using techniques such as the following, which transfer DNA from one species to another:

- The fusion of cells from two different plant species can be used to overcome species hybridization barriers. In order to be useful, the resulting cell fusion product must be regenerated to form a whole plant. To date, regenerated plants have only resulted from fusions between closely related genera (62). The regenerated plants are selected to express beneficial characteristics of both parents (94). As yet, no economically important variety has been produced using this method (62).
- Transferring subcellular organelles such as nuclei or chloroplasts from one plant species to another can be accomplished by a variety of techniques. One of these, liposome transfer, involves surrounding the organelle with a lipid membrane. Because chloroplasts carry many of the genes important in photosynthesis, liposome transfer may be instrumental in improving photosynthetic efficiency.
- Vector-mediated DNA transfer (and microinjection of DNA) is the most specific, and potentially the most versatile, of the genetic manipulation techniques. Recently, foreign plant genes have been inserted and expressed in plants.

Figure 16.—Steps To Create a New Variety of Plant by Using Biotechnology



SOURCE: Office of Technology Assessment.

Recent advances in the methods of plant cell culture and the techniques for introducing DNA from one plant species to another are discussed in *Box C.—Methodology Important in Plant Agriculture*. The applications of these methods to specific problems in plant agriculture, such as disease resistance, photosynthetic efficiency, and nitrogen fixation and the commercial aspects of biotechnology in plant agriculture are discussed in the sections that follow.

Improvement of specific plant characteristics

Greater crop yields or a reduction in the cost of crop production would be possible if plants were resistant to disease and certain environmental factors and contained a larger amount of higher quality product. In the United States, there is great research interest in crop resistance and crop quality improvement in academic, Federal, and industrial laboratories. Unlike most other plant traits, some resistances and specific improvements may be accomplished with one or a few gene modifications. This area, therefore, is probably the most active area of industrial research, and it is likely that considerable research progress in this area will be made in the next 5 to 10 years.

PLANT RESISTANCE FACTORS

Disease and environmental resistances are important to most crops in most areas of the world. Important plant resistances are shown in table 33. Productivity losses often can be attributed to the lack of resistance to one or more factors (see tables 34 and 35). Thus, the study of resistances could lead to greatly improved productivity and an increased realization of genetic potential (42).

Numerous single gene resistance factors are known in higher plants. The most common re-

developing these products is attributable to NBFs in the United States.

At present, the extent to which biotechnology will be used for the development of animal vaccines is uncertain. Most individual vaccine markets are relatively small—as shown in table 31, most U.S. vaccine markets for animals range from $5 million to $10 million per year—and sales of a single vaccine line would probably be insufficient to sustain a company. Therefore, most companies must market a broad range of vaccines to be competitive. The practice of maintaining a diverse selection of these products may facilitate the development of vaccines for diseases that alone might be unprofitable, such as diseases endemic to developing countries (16). Ultimately, the application of biotechnology to animal vaccine development depends on technical feasibility and the ability of vaccine developers (currently, mostly NBFs) to obtain funding for further work.

In addition to improving vaccines for a broad range of animal diseases, biotechnology may shift the sites of vaccine production from several large foreign producers (e.g., Rhone-Merieux (France) and Burroughs-Wellcome (U.K.))* to smaller U.S. producers. Currently, as shown in table 32, three foreign manufacturers control approximately 25 percent of the U.S. veterinary vaccine market (which accounts for one-fourth of the world's yearly $1 billion veterinary vaccine market). With the successful development of subunit veterinary vaccines by U.S. NBFs, competition may result in a redistribution of worldwide vaccine production. Collaborative arrangements between NBFs and local producers for the development of safe subunit vaccines effective against local strains of animal diseases may increase in the future.

Conclusion

The use of biotechnology to improve animal feed, nutrition, and health promise to improve production of food from animals. MAb-based diagnostic products exemplify this promise. Other products, such as new vaccines, may face technical problems of dosage, formulation, and delivery before they are suitable to animal husbandry practices. Until these problems successfully are resolved, the impact of biotechnology on improving animal productivity will not be realized. Applications of biotechnology such as gene transfer experiments and investigations into the nature of growth using rDNA-produced GHs currently serve to increase basic knowledge about animal biology.

*Rhone-Merieux and Wellcome command the international markets for rabies and FMD vaccines. Together these two vaccines comprise 30 to 35 percent of global animal vaccine sales. Other leading FMD vaccine producers are Bayer (F.R.G.), Pfizer (U.S.), Hoechst (F.R.G), and Rosenbusch (Argentina). State agencies serve about one-half of the rabies market, and Rhone-Merieux, Wellcome, and Connaught (Canada) dominate the rest.

Table 31.—Sales of Major U.S. Animal Vaccine Products, 1981 (millions of dollars)

	Sales
Cattle products:	
Clostridium	$ 16.0
Infectious bovine rhinotracheitis and bovine leukosis virus	13.0
Leptospirosis and combinations	6.0
Vibriosis and combinations	3.0
Swine products:	
Atrophic rhinitis (Bordetella)	$ 6.0
Pseudorabies	5.0
Erysipelas	3.5
Pet products:	
3-way feline virus disease	$ 4.5
Rabies	12.0
Canine parvovirus and combinations	9.0
Poultry products:	
Marek's disease	$ 12.0
Newcastle disease and combinations	9.0

SOURCE: S. J. Zimmer, "The Impacts of Applied Genetics in Animal Agriculture," contract report prepared for the Office of Technology Assessment, August 1982.

Table 32.—Major Producers of Animal Vaccines Sold in the United States

Company	1981 sales (millions of dollars)	Market share
Norden (SmithKline) (U.S.)	$40	27%
Philips-Roxane (Boehringer Ingelheim) (F.R.G.)	18	12
Fort Dodge (American Home Products) (U.S.)	14.5	10
Beecham (U.K.)	11	7
Jensen Salsbery (Wellcome) (U.K.)	9	6
Dellen (TechAmerica) (U.S.)	8.6	6
Pitman-Moore (Johnson & Johnson) (U.S.)	1.8	1
Syntex Agribusiness (U.S.)	1.5	1

SOURCE: S. J. Zimmer, "The Impacts of Applied Genetics in Animal Agriculture," contract report prepared for the Office of Technology Assessment, August 1982.

in culture has provided a great diversity of useful plant cell lines with altered properties. This variation found in cultured cells is called somaclonal variation (46,74). Somaclonal variation is an important source of genetic diversity for the plant breeder. Several naturally occurring disease-resistant plants have been isolated from cell culture (89).

Vector Construction and Transformation

Remarkable progress has been made in perfecting vectors for delivery of novel genetic information to plants. Most of the research excitement during the past few years has focused on understanding how certain plant pathogens transfer their DNA into a host plant. The bacterial plant pathogen genus *Agrobacterium*, for example, is able to transfer a portion of its plasmid DNA to infected dicot plant cells. *A. tumefaciens* carries the tumor-inducing (Ti) plasmid, and *A. rhizogenes* carries the root-inducing (Ri) plasmid. The portions of each of the plasmids that become stably integrated into plant chromosomal DNA are the transferred-DNA (T-DNA). Because of the relative ease of doing bacterial genetics, much progress has been made in inactivitating the disease-causing portions of the Ti and Ri plasmids to provide vectors for the introduction of novel DNA without causing plant disease during regeneration (64,75). There have been several announcements of the successful introduction and expression of bacterial genes in plants using nonpathogenic forms of T-DNA (49,70,91). The inserted genes are for antibiotic resistance, allowing the transformed plant cells to be identified easily in culture. In one case, these cells have been regenerated into whole plants, and the foreign genes are still expressed (63). One group reported the introduction and expression of a seed storage protein from beans in both sunflower and tobacco cells (73). This result is the first demonstration of the directed transfer and expression of a plant gene from one species in another plant species. The next challenge is to regenerate the transformed plant cells and show that the foreign genes are expressed in a heritable form in the mature plant and transmitted to its offspring.

Vector systems for the agriculturally important monocots do not yet exist. However, natural vectors, like the plasmids of *Agrobacterium*, are being looked for, and synthetic vectors are being constructed. More basic research in plant pathogens will be required to discover whether appropriate monocot vectors already exist in nature. The advantage of using natural vectors such as *Agrobacterium* is that the prokaryotic DNA has acquired appropriate regulatory signals for effective gene expression inside a eukaryotic cell.

Another potential vector, known as a transposable element, may prove useful for introducing DNA into monocots. A transposable element is a segment of DNA that is capable of inserting itself into the DNA of a cell. Transposable elements appear to be universal, but their natural function is unknown. An isolated transposable element can be attached to a gene to be inserted into a plant. This construct is microinjected into plant cells, and the transposable element carries the gene of interest into the cellular DNA. Experiments using transposable elements as vectors have been done with corn cells (87).

sistance genes are those that confer decreased susceptibility to disease (54,71,79). In maize, for example, there are resistance genes to several diseases such as northern corn-leaf blight (51). Because most of the single gene resistance factors confer resistance to a single pathogenic organism, it is thought that a single characteristic of the host and pathogen determine the outcome of an infection.

Most of the existing disease-resistance genes have been introduced into economically important lines of interbreeding plant species by traditional plant breeding. Currently, however, there is interest in cloning disease-resistance genes from plants in order to study the nature of resistance and to determine the possibility of transferring resistance factors among species that do not normally interbreed. It is not known in most cases

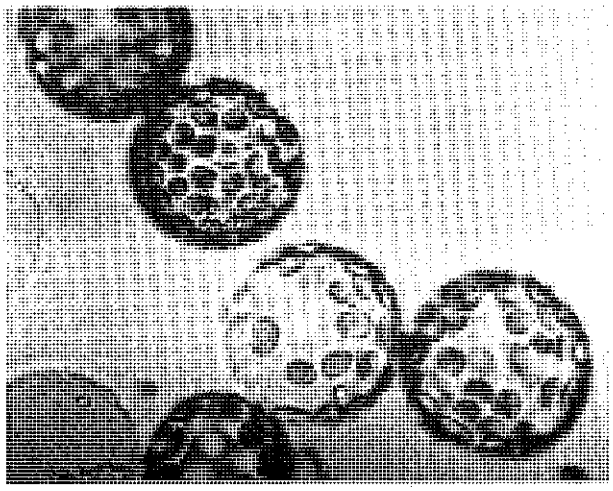


Photo Credit: Dean Engler, Agrigenetics Corp.

Freshly isolated plant protoplasts

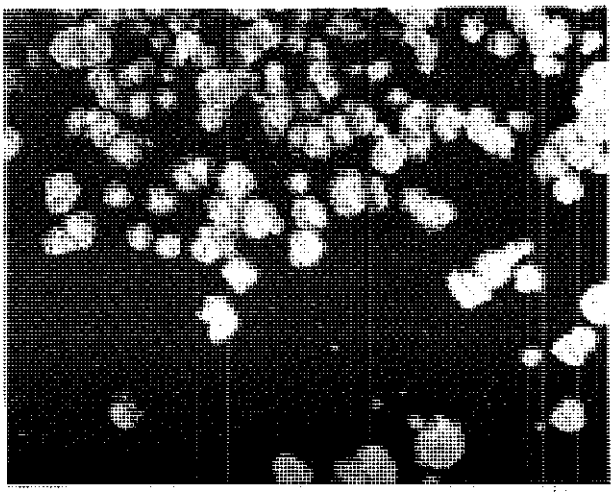


Photo Credit: Dean Engler, Agrigenetics Corp.

Masses of plant cells (calli) resulting from the divisions of individual protoplasts

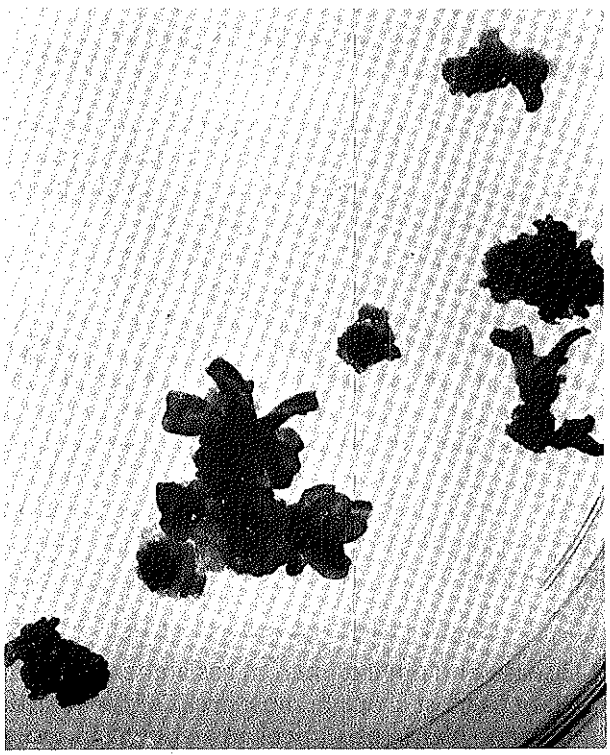


Photo Credit: Dean Engler, Agrigenetics Corp.

Plant shoots arising from protoplast-derived calli



Photo Credit: Dean Engler, Agrigenetics Corp.

Complete plants, each regenerated from a single protoplast

ic adaptations. Consequently, numerous regenerated plants will be required to determine if a particular selection procedure yields whole plants with important agronomic traits. On the other hand, pollen or embryo manipulation may circumvent some of these problems.

Genetic manipulations can make plants resistant to chemicals or can enhance their response to chemicals. These traits are of particular importance to the agricultural chemical industry. For instance, various plant growth regulators are produced by this industry. These chemicals can affect many stages of the growth or reproduction activities of plants to give a crop with increased yield. Enhanced response to these chemicals allows the crop to be grown at a lower cost.

Producing herbicide-resistant plants can have definite benefits, especially in crop rotation. For instance, corn is naturally resistant to triazine herbicides, whereas soybeans are not. Occasionally, soybeans do not grow well in a field the year after triazine-sprayed corn was grown there. In this case, one solution would be to introduce triazine resistance into soybeans. This particular resistance is due to a modified protein in the chloroplast membrane. Therefore, resistance between dissimilar species could be transferred by protoplast fusion or liposome-mediated chloroplast transfer (38,66). It should be noted, though, that increased use of agricultural chemicals could have serious environmental consequences.

PRIMARY PLANT PRODUCTS

The largest research effort in the modification of plant products using biotechnology is concentrated on the improvement of seeds and seed proteins. Seeds serve a dual role in agriculture. They are the major source of food for people and animals and represent an easily stored form of plant material, and they are also the material for propagating the next plant generation. The storage materials of seeds contain all of the materials necessary to nourish a plant, because each seed must support the initial phases of germination and seedling establishment until the plant is self-sufficient. During domestication, various crops have undergone an enormous exaggeration of the normal storage reserves. Today, far more material is stored in agricultural crop seeds than in the seeds of wild relatives; sometimes the increase is as much as tenfold (68).

Although the agronomic (applied) research effort is devoted primarily to increasing the amount of seeds and seed protein, current basic research efforts are devoted both to increasing the quality of the stored materials and to exploring plant gene structure. Because plants are capable of synthesizing all of the amino acids required for protein synthesis from simple carbon- and nitrogen-containing precursor molecules, the exact amino acid composition of the stored protein in seeds may not matter to a plant, and seed proteins often have an unbalanced amino acid composition. Because humans and most animals are unable to synthesize eight amino acids (the essential amino acids), the composition of ingested protein matters very much in their nutrition.

Much is known about the structure of the storage-protein products and the genes encoding these proteins in the major crop plants. In all cases studied so far, the storage-protein genes are found in small gene families with 3 to 30 members. Typically, a few genes of the multigene family contribute a significant fraction of the total protein. There is not much genetic variation among the seed storage-protein genes of a given species, although this low variation might be due to the limited diversity of the varieties currently studied. These crops may have lost much of the original diversity present in the progenitor species during the intensive plant breeding activities that have occurred throughout history.

DNA clones of storage proteins are available from several crop species: soybean, garden bean, corn, wheat, and other less significant crop plants. Changes in these genes can be made readily in vitro to improve the balance of amino acids in the protein. The difficult part is reintroducing the altered storage-protein gene back into the crop plant and ensuring that this novel gene is expressed appropriately. Most storage proteins are present only in seeds. Retention of this tissue specificity is important; storage proteins' presence in other plant cells may be detrimental. Another important consideration is that the storage-protein genes are found in families. Introduction of a new gene may change only a fraction of the total protein produced. To modify the overall amino

Box C.—Methodology Important in Plant Agriculture

Methods of Plant Cell Culture

Plant cell culture is important commercially today. Several species of plants can be cultured and regenerated, allowing many identical plants, including trees, to be grown. For example, cultured plant cells are used for selection of virus-free cells. In the past, it has been typical for yields in potato crops to be greatly reduced by several potato viruses. Virus-free potato plants are now obtained from regenerated cell-cultured potatoes, and, as a consequence, the yield of these plants has increased substantially (98).

In the research laboratory, plant cell culture provides a bridge between molecular genetics and plant breeding. Most schemes involving the transfer of genetic information require that cells from the recipient plant be cultured, although it should be noted that many genetic experiments can now be done by the direct manipulation of pollen or embryos, which circumvents the necessity for regeneration. If single plant cells or embryos can be cultured, selected, and regenerated, the number of cells in any experiment can be large, contributing to the overall potential success of an experiment. Many millions of plant cells can be studied in a small laboratory; if the same number of whole plants were to be used in an experiment, acres of greenhouses or fields would be required. The development of cell culture conditions is far from routine, and even though the recent successes of plant cell culture are notable, there are still only a limited number of species that are cultured routinely (53).

Plant cells are useful to geneticists, but to be useful agriculturally, whole plants must be able to be regenerated from these cells. Regeneration is accomplished by placing the cells under appropriate conditions of light, temperature, nutrition, and growth-regulating chemicals. An early stage of regeneration is the formation of an undifferentiated clump of cells known as a callus. From the callus arise the differentiated cells of roots and stems. Alternatively, the formation of embryos can be induced from the callus. The crop species for which regeneration from cells can be accomplished routinely include asparagus, rapeseed, cabbage, citrus, sunflower, carrot, cassava, alfalfa, millet, clover, endive, tomatoes, potatoes, and tobacco (62). Currently, there is considerable effort in industry and academia to regenerate corn, wheat, and soybeans from single cells routinely. There is as yet, however, no routine method published for regeneration of these major U.S. crop plants, not because there are any inherent differences in these species, but because for many years scientists worked with only a few model systems. Now that direct genetic manipulations can be done on agriculturally important plants, there is renewed interest in developing regeneration schemes for these plants.

One recent innovation is the introduction of methods for freezing plant cells and calli while retaining cell viability. Several laboratories have devised specific successful freezing regimes to preserve materials (101). One application for this technology would be to freeze germplasm or plant embryos of rare mutants for later use in breeding, tissue culture, or molecular biology programs. A side benefit of the methods of freezing plant cells would be the ability to preserve germplasm of endangered species—tropical trees, for example—until ecological niche destruction has been halted. Also, frozen embryo banks might replace or supplement some seed storage facilities. The initial applications of these methods will simplify laboratory maintenance of cell cultures.

The ultimate goal of most genetics programs utilized in conjunction with molecular biology is to create improved plant varieties (47). The process may involve the transformation of plant cells where the cells are receptive to added DNA. After treatment with DNA and selection for the desired trait, a high percentage of the cells (or embryo or pollen) must proliferate to form a whole reproductive plant. Although many pieces of the transformation, selection, and regeneration procedure have been demonstrated, the outlined scenario is far from routine. Within 5 years, however, technical innovations could provide suitable cell culture and regeneration protocols for many important crop species.

One important fact recognized in the past few years is that there sometimes is extreme variation in the phenotype and genotype of cells in tissue culture. In the past, mutants were sought after mutagenesis treatment. Beginning with work on sugarcane, however, the variability uncovered or created

gascar periwinkle cells in order to isolate anticancer compounds (58). In fact, the Japanese Government is spending $150 million over 10 years for research on obtaining secondary compounds from plants. It is argued by some, though, that plant cell culture for producing secondary products is necessary only when good farm land is not abundant (65).

PLANT GROWTH RATE

The rate at which plants grow can limit both the amount of harvestable biomass (food, fiber, secondary products) and the length of time between planting and harvesting. Traditional plant breeding has been quite successful in modifying and improving plants to respond to modern agricultural practices of herbicide, pesticide, irrigation, cultivation, and high-fertilizer application. These breeding programs have established that there is no single gene for yield. On the other hand, much is known about the genetics of harvestable products such as seed size. Additionally, there are single gene mutants, such as that for gibberellic acid, that can affect plant growth dramatically. Increased understanding of these areas of genetics may have an impact on this area of plant biology. For instance, a plant can be imagined that had a decreased amount of total biomass but an increased amount of harvestable product.

PHOTOSYNTHETIC EFFICIENCY

Photosynthesis is the basis for most life on Earth. Higher green plants, algae, and some bacteria can utilize the energy in sunlight to split water molecules; in this process, energy is generated and utilized to combine atmospheric carbon dioxide (CO_2) into an organic form as well as to drive other energy requiring processes of plants. A byproduct of this reaction is molecular oxygen (O_2). Thus, photosynthesis is not only the ultimate source of fixed carbon we use as food and fiber, but also of the oxygen we breathe.

Because photosynthesis is so important to food production, much research has focused on the mechanism of photosynthetic action. The photosynthetic system is very complex, combining enzymatic activities, key roles played by cellular organelles, and plant anatomy as well as environmental factors such as light, water, and temperature. Many proposals have been made to improve the efficiency of this system by genetic manipulation.

The critical step of the photosynthetic CO_2 fixation cycle is catalyzed by the enzyme ribulose bisphosphate carboxylase (RuBPCase), probably the most abundant protein on Earth. This enzyme is sequestered in chloroplasts, the cellular organelles where photosynthesis occurs. It is a complex molecule synthesized from both chloroplast genes and nuclear genes (50,51).

When photosynthesis originated, the Earth's atmosphere is postulated to have been nearly devoid of oxygen. The oxygen we have today is a byproduct of photosynthesis, and oxygen comprises about 20 percent of our atmosphere. RuBPCase initially evolved in a low oxygen atmosphere but now must fix CO_2 with a large excess of O_2 present. RuBPCase can utilize this O_2 in what appears to be a nonproductive enzymatic reaction. This process is called photorespiration and results in a net loss of fixed CO_2 (45). Photorespiration can decrease crop yields by as much as 50 percent (82). It is ironic that RuBPCase activity over the past millions of years has produced the O_2 that now decreases the efficiency of photosynthesis. On the other hand, it has been postulated that the ubiquitous and continued presence of photorespiratory activity implies some natural selection advantage (61).

Suggestions have been made for modifying RuBPCase or other enzymes involved in the photosynthetic system. For instance, genetic manipulations that would increase the affinity of RuBPCase for CO_2 or decrease its affinity for O_2 could substantially increase net CO_2 fixation. It has yet to be determined what effects these changes would have on the survivability of plants.

In addition to manipulating the enzymatic system, changing the plant's anatomy, such as the types of cells in leaves, might be possible. Several groups of higher plants have increased rates of CO_2 fixation that correlate with modified anatomy and physiology. Very little is known about the genetic control of leaf and cellular anatomical development, so near-term success in modifying these aspects of plant anatomy is unlikely.

Table 33.—Plant Resistances of Economic Value

Resistance to:	Relevance in United States
Disease	All crops
Saline	Irrigated soils, particularly in California and Southwest
Alkaline earth metals	Southeastern United States and West
Anaerobic soil conditions	Areas subject to flooding
Drought	All crops
Herbicides	All crops
Pesticides	All crops
Soil pH	Low pH on acid mine tailings and soil affected by acid rain; high pH on most Western soils

SOURCE: Office of Technology Assessment.

Table 34.—U. S. Soils With Environmental Limitations

Environmental limitation	Percentage of U. S. soil affected
Drought	25.3%
Shallowness	19.6
Cold	16.5
Wet	15.7
Saline or no soil	4.5
Alkaline salts	2.9
Other	3.4
None	12.1

SOURCE: J. S. Boyer, "Plant Productivity and Environment," *Science* 218:443-448, 1982.

Table 35.—Distribution of Insurance Indemnities From Crop Losses in the United States From 1939 to 1978

Cause of crop loss	Proportion of payments (%)
Drought	40.8%
Excess water	16.4
Cold	13.8
Hail	11.3
Wind	7.0
Insect	4.5
Disease	2.7
Flood	2.1
Other	1.5

SOURCE: J. S. Boyer, "Plant Productivity and Environment," *Science* 218:443-448, 1982.

what the disease-resistance genes in plants actually do to plant metabolism or structure. By understanding how the products of plant disease-resistance genes work, better screening programs for enhanced resistance can be designed. Environmentally, it is desirable to develop pest-resistant plants, because such plants would reduce the need for spraying crops with pesticide* chemicals, and disease control would be more effective. It should be kept in mind, however, that much of the agricultural research effort is being made by the agricultural chemical industry, and this industry may see the early opportunity of developing pesticide-resistant plants rather than undertaking the longer term effort of developing pest-resistant plants.

Resistance to environmental conditions probably depends on both single and multigenic inheritance. These traits, as well as disease resistance, can be selected for in tissue culture. If analogs of the disease or detrimental environment conditions can be applied to plant cells in culture, the entire procedure can take place in a few test tubes or petri dishes in a laboratory setting. Millions of individual cells can be treated simultaneously and then examined for survivors. Stepwise selections under gradually more stringent conditions (e.g., a gradual increase in the salinity of the medium) are accomplished readily.

Some of the traits that could be selected in tissue culture are listed in table 33. Many of the traits are resistance factors that confer protection against disease and salinity. In selection schemes for these factors, the test organism is exposed either to normally lethal doses of the toxins produced by a disease organism or to high doses of salt (to mimic salinity), and the surviving cells are identified by their growth under these normally toxic conditions. This protocol holds great promise for identifying rare cells that have spontaneously acquired a novel resistance. Somaclonal variation probably supplies much of the variation seen in tissue culture (see box C) (47).

A rate-limiting step in applying selection techniques more widely is the present inability to regenerate major cereal and legume crops from individual cells or small cell clumps on a routine basis. Furthermore, some of the traits selected in tissue culture for resistance to a specific factor may not be manifested in the whole plant, because it is possible for cells to develop nongenet-

*A pesticide is an agent that prevents the growth or propagation of deleterious organisms, including weeds and insects. Both herbicides and insecticides are pesticides.

an appropriate concern, but this cost should be compared with the true cost of nitrogen nutrition in field-grown plants (i.e., the cost of chemical fertilizer synthesis and other biological costs to the plant).

It may be possible to decrease the energy required for nitrogen fixation by 30 to 50 percent by preventing the evolution of hydrogen during nitrogen fixation. Some bacteria have a set of genes that allow for hydrogen recycling. These genes have been cloned and inserted into less efficient nitrogen-fixing bacteria. The recipient bacteria showed increased nitrogen-fixing efficiency (37).

Agriculturally important nitrogen-fixation systems discussed below are nonlegume nitrogen fixation and symbiotic nitrogen fixation in legumes.

Nonlegume Nitrogen Fixation.—Nitrogen fixation is performed by several groups of bacteria and blue-green algae that live free in soil or in aquatic habitats. The best studied nitrogen-fixing bacterium is the free-living *Klebsiella pneumonia*, which can easily be grown in the laboratory.* The gene complex coding for the nitrogen-fixing function in *Klebsiella pneumonia* is comprised of 17 genes, and the regulation and activities of these genes now are being studied extensively. Still, the nitrogen-fixing function is extremely complex and not well understood.

Algae have been used to fix nitrogen in Asian rice paddies for many years. Recently, research has produced strains of algae that could be used in soil to fix nitrogen for domestic crops. Algae are inexpensive compared to nitrogen fertilizer, and because they release nitrogen slowly into the soil, algae bypass the problem of nitrogen leaching (60). Furthermore, algae are being considered the botanical equivalent of yeast for genetic manipulation, and vector systems for algae transformation are in development (41).

Symbiotic Nitrogen Fixation in Legumes.—The legume-*Rhizobium* symbiosis is the most agriculturally significant biological source of fixed nitrogen. Both grain and forage legumes have large amounts of nitrogen fixed by *Rhizobium*. Recent work on legume-*Rhizobium* symbiosis has focused on several areas, including the determination of energy costs, pathways of nitrogen assimilation and transport, the biochemistry of symbiotic nodule development, and the genetics of the bacterial partner.

Symbiotic nitrogen fixation can be a significant source of nitrogen nutrition for legume crops, but its practical application can be limited by several sets of factors, some environmental, others intrinsic to the plant-bacterial partners. Soil conditions and environmental levels of fixed nitrogen have significant effects on rhizobial survival, nodule formation, and levels of nitrogen fixation. One crucial area, poorly understood at present, is the role played in symbiotic nitrogen fixation of soil micro-organisms other than *Rhizobium*. Understanding nodule formation in detail will help explain environmental effects on infection that may relate to competitiveness and effectiveness of various *Rhizobium* inocula. In addition, an understanding of why legumes, and not other plants, can nodulate would be essential for attempting to extend host range.

Another nitrogen-fixing micro-organism, the actinomycete *Frankia*, is of interest because it nodulates a number of unrelated plant genera. This ability suggests a simpler genetic symbiosis than that of *Rhizobium* and legumes. If this is true, it may be easier to extend genetically the host range of the symbiotic relationship of *Frankia* than to extend that of *Rhizobium* (41).

Specific host proteins are produced in nodules. One of these is leghemoglobin, which controls the oxygen content of the infected nodule cells. This protein is produced in high quantities in nodules. Two research groups have cloned the genes for soybean leghemoglobin (77,96), but their mechanism of action is not understood. Other new proteins appear when nodules develop (76). These are called "nodulins" and are likely to be essential for symbiotic nitrogen fixation; however, their exact role is not known. Some of these might be enzymes, such as those for ammonium assimilation (67). When nodulins and their functions are better understood, a logical extension of current research will be to move cloned nodulation genes into other plants. This may make it possible to extend nitrogen fixation to other plant species.

*Two other free-living nitrogen-fixing bacteria, *Azosporillum* and *Azotobacter*, also are important agriculturally.

acid composition, several genes may have to be introduced or the natural genes deleted and replaced by novel genes. In some crops such as corn, there are mutations that reduce the production of zein, the storage protein. These mutant genes can be used to reduce the zein concentration, thus allowing an introduced gene to have a greater impact on overall amino acid composition.

An alternative to the modification of existing crop species' genes is the introduction of completely novel genes isolated from other organisms. Genes whose products are very rich in the amino acids that are deficient in a particular seed type could be introduced to increase the concentration of specific amino acids. One promising example of this type is the storage protein of the Brazil nut (97). This protein is composed of 25 percent sulfur-containing amino acids (methionine and cysteine). Legume seed protein usually is deficient in these amino acids. Introduction of a few copies of the Brazil nut storage-protein gene into legume species might overcome the sulfur amino acid deficiency. Proteins of unusual composition may offer the quickest method of preparing a gene to complement deficiencies in major crop storage proteins.

SECONDARY COMPOUNDS FROM PLANTS*

Table 36 lists some of the desirable secondary products from plants. Very little research has been done on the tissue culture production of these compounds, yet it should be possible to produce important high-value plant products using culture systems instead of gathering plants from nature. Cell culture offers the advantages of reproducibility and control over production where seasonal variations, weather changes, or disease are not problems (40,57,95). On the other hand, a difficulty in the production of some products is maintaining the plant cell culture in a differentiated state such that compound production occurs.

Biotechnology offers many opportunities for the production of secondary plant compounds. The transfer of the plant metabolic pathway for a compound to a bacterial or fungal cell, for instance, could offer an opportunity for providing a steady supply of these compounds, although much more knowledge concerning the genetics and biochemistry of the pathways that produce these compounds is necessary. Another possibility is identifying and modifying the gene coding for the enzyme responsible for the rate-limiting step in product production. Overproduction of the products could result from the plants being grown in culture or in the field.

There is little current U.S. research effort to improve the yield of secondary plant compounds from cultured cells or whole plants. The Federal Republic of Germany, Canada, India, and Japan, on the other hand, have large research programs, as measured by the number of papers presented at the 1982 International Congress of Plant Tissue and Cell Culture (58). Japan, for instance, has scaled up the growth of tobacco cells to 7,000 liters, and researchers at the University of British Columbia are growing 100 liter batches of Mada-

Table 36.—Examples of Secondary Plant Products of Economic Value

Agricultural chemicals:
Pyrethrins
Rotenone
Nicotine
Allelopathic compounds
Antibiotics against soil microbes
Pharmaceutical drugs:
Codeine
Morphine
Steroids
Cardiac glycosides
Alkaloids
Reserpine
Retinoic acid
Caffeine
Cannabinoids
Antitumor compounds
Flavorings and salts:
Licorice
Coumarin
Colorings and pigments:
Anthocyanins and betacyanins
Carotenoids
Industrial intermediates:
Latex
Lignin
Dye bases
Steroid and alkaloids products

SOURCE: Office of Technology Assessment, adapted from E. A. Bell and B. V. Charlwood (eds.) *Secondary Plant Products* (New York: Springer-Verlag, 1980).

*This topic was covered by a recent OTA workshop entitled "Plants: The Potential for Extracting Protein, Medicines, and Other Useful Chemicals" (99).

gen-fixing plants, pest-resistant plants), the development of biological pesticides, or the development of plants with enhanced responses to chemicals. Another major industrial sector investing in plant biotechnology in the United States is the petroleum industry. The firms in this sector may see plants as the next source of energy, either in the form of biomass or photosynthesis itself. Pharmaceutical and food companies also are investing in plant agriculture. How the large chemical and petroleum corporations, the existing seed companies, and the NBFs will compete for market shares is yet to be seen.

The seed and vegetative cutting market is very large, and it appears that U.S. companies are oriented mainly toward domestic markets because of the transportation costs and the expense and inconvenience of field trials in other countries. Probably because of large domestic markets, many new entrepreneurial firms are directing their efforts toward plant agriculture. In fact, the number of NBFs in plant agriculture is third only to the number in pharmaceuticals and animal agriculture (see *Chapter 4: Firms Commercializing Biotechnology*).

Priorities for future research

Animal agriculture

The prospects for the application of biotechnology in the areas of animal and plant agriculture are truly exciting. To encourage the introduction and progress of biotechnology in animal agriculture, however, several persistent problems must be overcome. These problems include the following:

- developing effective delivery systems for almost all products of new biotechnology to be used in animals;
- achieving consistent expression of polypeptides such as those used for subunit vaccines from rDNA systems;
- developing host/vector systems that yield products more closely resembling maminalian molecules (e.g., glycosylated proteins) and that secrete products for easier purification.
- demonstrating product stability under the climactic and handling conditions where these products (e.g., subunit vaccines) will be implemented; and
- achieving higher immune responses with subunit vaccines, for example, by developing delivery systems that prolong exposure to the vaccine.

More basic knowledge about biological processes in animals and about the cellular and molecular biology of pathogenic bacteria and animal parasites is required before many biotechnological applications are realistic. Advances in basic knowledge about metabolic pathways in beneficial bacteria may lead to useful growth-enhancing compounds. Finally, more basic knowledge concerning the actions of nascent products such as rDNA-produced GH is needed to discern effectiveness and safety.

Given the novelty of disciplines such as molecular genetics and cellular biology in animal science, there is some question as to whether sufficient communicative links are established yet between basic and applied scientists. The efforts of applied scientists usually are communicated to animal growers in the United States through the land grant universities' State Agricultural Experiment Stations and extension services, supported by USDA. A corollary to the productiveness of future research rests in encouraging the establishment of communication between basic and applied scientists to encourage biotechnological applications in animal agriculture.

*Plant agriculture**

Because interest in plant molecular biology is fairly recent, the most important research priority is an increased understanding of DNA structure

*Research goals similar to those outlined in this section were published recently by the National Research Council (83) and the National Academy of Sciences (82).

Genetic manipulations to increase photosynthetic efficiency, and consequently food production, are very difficult now because of the complexity of the system. It will be several years before rDNA technology will aid in producing agriculturally important plants with increased inherent photosynthetic efficiency.

PLANT-PRODUCED PESTICIDES

Some species of plants are highly resistant to potentially damaging insects. Although not very much is understood about this phenomenon, it appears that certain plants can produce compounds that are toxic to specific species of insects or that interfere with the insects' normal reproductive or growth functions (86). An African plant, for example, produces a compound that interferes with a particular caterpillar's molting, and, as a result, the insect cannot eat (48). Other plants are known that produce chemicals that cause potentially harmful insects to avoid those plants for feeding or egg laying (59). The specificity of these plant-produced insecticides and nonpreference chemicals allows the control of pests while permitting potentially useful insects to survive. Many applied chemical pesticides do not have this specificity. It may be feasible soon to clone and transfer the genes that code for these naturally occurring chemicals, allowing them to be expressed in other plants. The result of these gene transfers could be to reduce greatly the amount of agricultural chemicals needed and, hence, the cost of production.

Investigations into chemicals released by some plants that adversely affect neighboring plants is receiving an increased amount of attention (90). These herbicides, known as allelopathic chemicals, may influence another plant directly or may act by inhibiting the micro-organisms normally associated with that plant. Allelopathic chemicals consist of a wide variety of chemical types, and their actions range from inhibiting cell division to protein synthesis to photosynthesis. Much more still is to be learned about these naturally occurring chemicals, including the factors influencing their production and how best to use them agriculturally. A goal of biotechnology is to identify the genes responsible for the synthesis and release of the plant pesticides and to transfer them to nonresistant plants. Biotechnology also could aid in the understanding of their production and possibly help develop their production in controlled laboratory culture systems.

Uses of micro-organisms for crop improvement

Applications of biotechnology in the area of crop improvement include genetic manipulations of micro-organisms that interact with plants in nitrogen fixation, for example, or that produce substances such as insecticides of potential benefit to plants. These applications are discussed further below.

NITROGEN FIXATION

Plants have a universal need for metabolically usable nitrogen in the form of ammonia (NH_3), which can originate either from the air or from applied ammonia fertilizer. Biological nitrogen fixation, the process by which living systems convert nitrogen gas in air to NH_3, is catalyzed in living systems by the enzyme nitrogenase. Nitrogenase, and consequently the capacity to fix nitrogen, is found only in prokaryotes, either bacteria or blue-green algae. Some nitrogen-fixing prokaryotes are free-living and can be either anaerobic or aerobic; other prokaryotes fix nitrogen only when they coexist symbiotically with a higher plant host. The application of biotechnology to nitrogen fixation may result in more efficient prokaryotic nitrogen fixation or the transfer of nitrogen-fixing ability to plants themselves.

Nitrogen-fixing prokaryotes share some common physiologic features. First, nitrogen fixation typically does not occur in cells already supplied with usable nitrogen. Second, nitrogenase is oxygen-sensitive, so all nitrogen-fixing organisms have mechanisms for limiting oxygen. Third, NH_3, which is toxic at high concentration, must be converted readily into organic nitrogen.

Biological nitrogen fixation is energy intensive (84,88,93), and in plant-microbe associations, this energy is derived from the plant. Estimating the energy expenditures for biological nitrogen fixation is difficult, and few reliable numbers are available. The energy cost of nitrogen fixation is

Summary.—Individual nitrogen-fixing systems can be improved or extended by a knowledge of how they work and by techniques that permit the genes for nitrogen fixation to be altered and moved. One line of research will be the improvement of existing systems. Some new nitrogen-fixing systems have been proposed, as well. Proposals have been made, for example, to insert directly the genes for nitrogen fixation into the plant genome. Success of these as well as other systems in the end will be measured by the practicality of the new association. The problems of specificity, oxygen regulation, and effect on yield must be considered, and these will require broad-based knowledge of biochemistry, genetics, and physiology in a variety of nitrogen-fixing organisms (see table 37). There is a considerable amount of research being done in the area of nitrogen fixation, and genetically manipulated *Rhizobium* may be field tested soon (85).

MICROBIALLY PRODUCED INSECTICIDES

Problems or drawbacks associated with chemical insecticides, including their increasing cost and environmental hazards, their lack of specificity, and the ease with which insect resistances to such insecticides are developed, have sparked renewed interest in microbially induced insect control to improve crop yield. Microbial insecticides, because of their narrow host ranges, can control specific pests while allowing natural predators and beneficial insects to survive. Furthermore, the few characterized microbial pesticides do not appear to harm humans or animals, and they are biodegradable.

There are three natural sources of microbial insecticides: bacterial, viral, and fungal. About 100 bacteria have been reported to synthesize toxins that are insecticidal. Very few of these bacteria have been studied extensively, but in one case (*Bacillus thuringiensis kurstaki*), the gene that controls the synthesis of a toxin has been cloned using rDNA technology (69). The cellular mechanism of the toxin's insecticidal activity is not yet well understood. Genes for bacterial toxins could be put into other bacteria that normally exist on the surface of plants (48).

Viruses also can be insecticidal by virtue of their ability to cause disease in various insects. Several families of viruses have been identified as potentially pathogenic to insects, but the family *Baculoviridae* has received the most attention. The U.S. Environmental Protection Agency (EPA) has registered, or is considering registering, several baculoviruses for the treatment of such diseases as cotton bollworm, Douglas fir tussock moth, gypsy moth, and alfalfa looper (81). One particular baculovirus (*Autographa californica* nuclear polyhedrosis virus (AcNPV)) has been genetically and molecularly well characterized, making the use of rDNA techniques with this virus feasible.

In contrast to bacterial and viral insecticides, fungal pathogens need not be ingested; they can disable or kill the insect by colonizing its surface. More than 500 fungal species can infect insects,

Table 37.—Importance of Basic Research (Model Systems) on Nitrogen Fixation

Research area	Organisms used in research	Importance
Cloning nitrogenase genes	*Klebsiella pneumoniae*	Direct study of genes Introduction of nitrogen-fixing genes into other organisms
Physiology of nitrogen fixation	*Azotobacter* *Anbaena* *Klebsiella*	Improving energy efficiency of nitrogen fixation in the cell Understanding role of ammonia in nitrogen fixation
Biochemistry of nitrogenase	*Clostridium* *Azotobacter* *Klebsiella* *Rhodospirillum*	Understanding oxygen sensitivity of nitrogenase Improving energy efficiency of nitrogenase enzyme
Cell and developmental biology of nodulation	*Rhizobium*	Bacterial/plant recognition process Nodulation process

SOURCE: Office of Technology Assessment.