# PART III
# Applications of Biotechnology in Specific Industrial Sectors

Chapter 5

# Pharmaceuticals

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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 applications, 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

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 (I-ISA) rDNA projects. Other examples discussed are monoclinal 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 underde - velopment 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 hl, 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 Humu - lin[a] may be the debutant of rDNA produced drugs, the extent to which rDNA-produced hı 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.**

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

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 (l?. 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 h.1 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 maybe 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).

## *Interferon*

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:

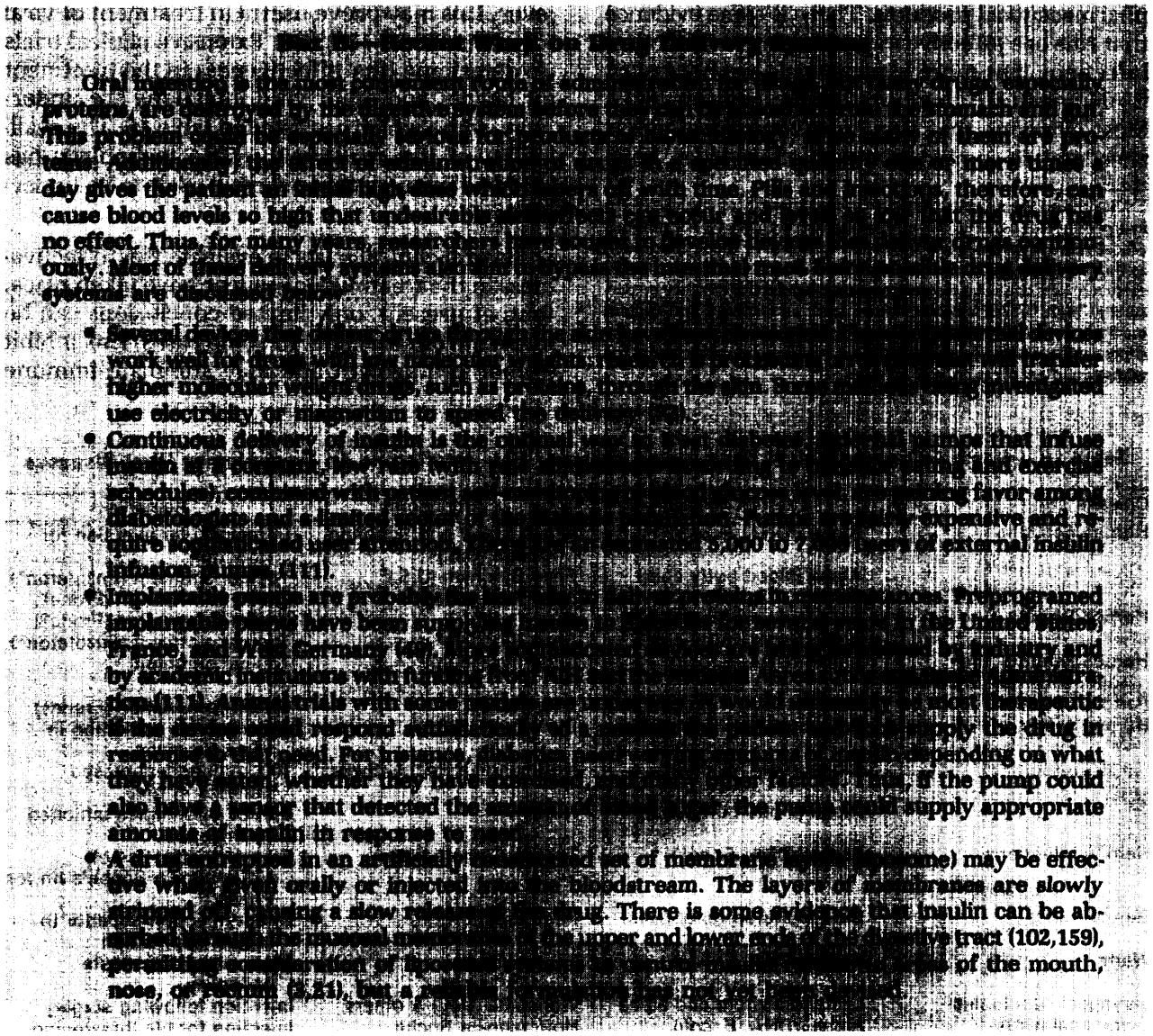
- the use of rDNA technology to produce a scarce product in quantities sufficient for research on the product's effects;
- massive, competitive scale-up campaign by

**•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.**



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 SUppofi 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.**

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 Interferon 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, Europa, 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.

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 Iymphomas, benign human esophageal 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 1fns 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 immunedeficiency syndrome (AIDS). Substantial supplies of Ifns to conduct such research can now be produced with rDNA technology.

Though most rDNA-made 1fns currently under evaluation are produced in the bacterium E. ccdi, 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)OOO-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 streptococcial "vaccine," called Picibanil®, which Japanese physicians regard as an immunostimulant (118).**

**Table 17.—Some Ongoing Clinical Trials of the Use of Interferon 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<br>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<br>Duke University<br>Memorial Sloan Kettering Cancer Center |
| NCI | NCI | Kidney (renal cell) | II | Duke University |
| Wellcome Foundation | NCI | Kidney (renal cell) | II | Southwest Oncology Group<br>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<br>University of Wisconsin<br>University of Rochester<br>M. S. Hershey Medical Center<br>University of Missouri |
| s-P | S-P | Lymphoma, non-Hodgkin's | II | Roswell Park<br>University of Maryland<br>Harper Grace Hospital<br>Yale University<br>University of Chicago |
| s-P | S-P | Lymphoma, Hodgkin's | II | Yale University<br>University of Chicago<br>Wilford Hall Medical Center |
| s-P | S-P | Breast cancer | I | Bowman-Gray Hospital<br>Harper Grace Hospital<br>USC Cancer Center |
| s-P | S-P | Multiple myeloma | II | University of Texas (Galveston)<br>Roswell Park<br>Bowman-Gray<br>Dartmouth-Hitchock |
| S-P | S-P | Leukemia, acute | II | UCLA |
| S-P | S-P | Kaposi's sarcoma | II | San Francisco General Hospital<br>UCLA |
| S-P | S-P | Lung, small cell | II | USC Cancer Center<br>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<br>Mayo Clinic |

**Table 17.–Some Ongoing Clinical Trials of the Use of Interferon 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<br>Minneapolis VAH<br>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<br>Memorial Sloan Kettering Cancer Center |
| HLR | HLR | Osteogenic sarcoma | II | Mayo Clinic |
| HLR | HLR | Breast cancer | II | Georgetown University<br>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 ia 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 hypopituitariam. Another several hundred patients are treated with commercial hGH imported from abroad, which is also obtained from tissue extracts (39).**

**Table 18.—Some U.S. and Foreign Companies invoived in interferon Gene Cioning 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 seating-up to a capacity of 3- $10^{12}$ units par 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, parhaps, 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 (1 19). 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+ndorphin 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).***

## 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<br>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)<br>Japanese Cancer Institute<br>Immunex (U.S.)<br>Cetus (U.S.)<br>Chiron<br>Genex (U.S.)<br>Biogen (U.S.)<br>Genetics Institute (U.S.)<br>Interferon Sciences (U.S.)<br>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.)<br>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 |

**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 @. 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, researcher~ are attempting to recognize those compounds that might suppress coughing or diarrhea or might counteract asthenia, cerebral vascular disorders, failing memory, mental depression, Pmkinson'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 betaadorphin (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

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, 19S3.

## Blood products

Products derived from the fractionation of hu - man 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 antihemophilia factor (AHF), which accounted for 41 percent, 25 percent, and 13 percent, respective-

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 tremendous 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 amounced 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. Soosting a patient's antibody level generally is thought to help prevent infectious disease. This treatment is used especiaUy 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 | 940/0 | 870/o | 800/0 | 670/o |
| Foreign | 60/0 | 13"?/0 | 200!0 | 330!0 |
| HSA revenues: | | | | |
| Domestic (millions of dollars) | $58 | $133.4 | $168.2 | $300 |
| Foreign (millions of dollars) | | 20.3 | 43.5 | 148 |
| Total (millions of dollars) | : 2 | 153.7 | 211.7 | 448 |

SOURCE: Office of Technology Assessment, based on data and estimates in M. M. LeConey, "Who Needs Plasma?" Plasrrra C?uar?edy 2:66-93, Septembr 1960.

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.—Antlhemophilic 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 Ouarterfy 2:68-93,* September 19S0.

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 (LJ.S.)/Genetics Institute (U.S.),
- Biogen S.A. (Switzerland)fleijin (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.

## *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~cale 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

**● 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.**

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 thrornboiysis, 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.)lMitsui Toatsu Chemicals, Inc. (Japan) | Production from rDNA |
| | Genentech (U. S.)/Grunenthal (F. R.G.) | Production from rDNA |
| Human tissue plasminogen activator | GenentechlUniversity of Leuven (Belgium)l Mitsubishi Chemical Industries, Inc. (Japan)lKyowa Hakko Kogyo (Japan) | Production from rDNA |
| | Biogen S.A. (Switz.)lFujtsawa (Japan) | Production from rDNA |
| | Integrated Genetics (U.S,)l 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.

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 protoplasm 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

**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.)lMerck/University of Washington, UCSF | Production of viral surface proteins from rDNA in yeast |
| | Takeda/Osaka and Hiroshima Universities (Japan) | |
| | Amgen (US.) | |
| | Biogen/Green Cross (Japan)Wniversity of Edinburgh | |
| | Integrated Genetics (U. S.)lConnaught (Canada) | |
| Herpes viruses . . . . . | Merck | Purification of surface glycoprotein from herpes simplex viruses |
| | Molecular Genetics (U. S.)lLederle Labs (U. S.) | Production of viral proteins in bacteria |
| | Institut Merieux (France)Wniversity of Chicago | Production of nonpathogenic viruses by the deletion of specific genes |

SOURCE: Office of Technology Assessment.

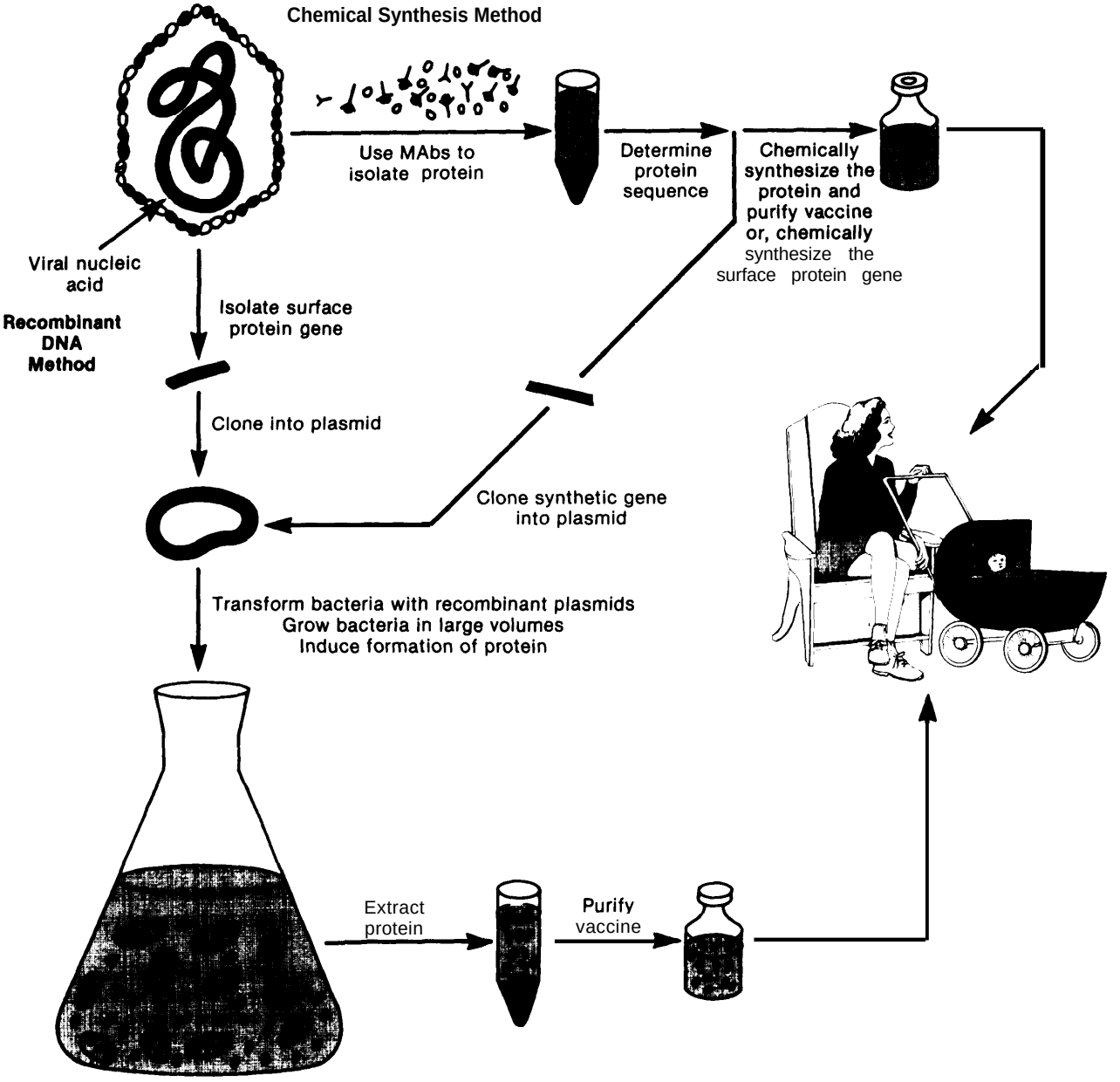
of other human viral diseases. The two main methods used to prepare subunit vaccines for viral diseases are summarized in figure 13.

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).**

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




IIn the chemlcal synthesis method, proteins that comprise the viral surface are Isolated, often with the use of monoclinal 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 wa 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.

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 promoters") 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.**

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, Sahnonella 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 ***Shige]la 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 ty - phoiddysentery 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 Plasnmdium (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 ***Plasmocfia*** 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 |
| Try~anosomiasis | 7 |

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

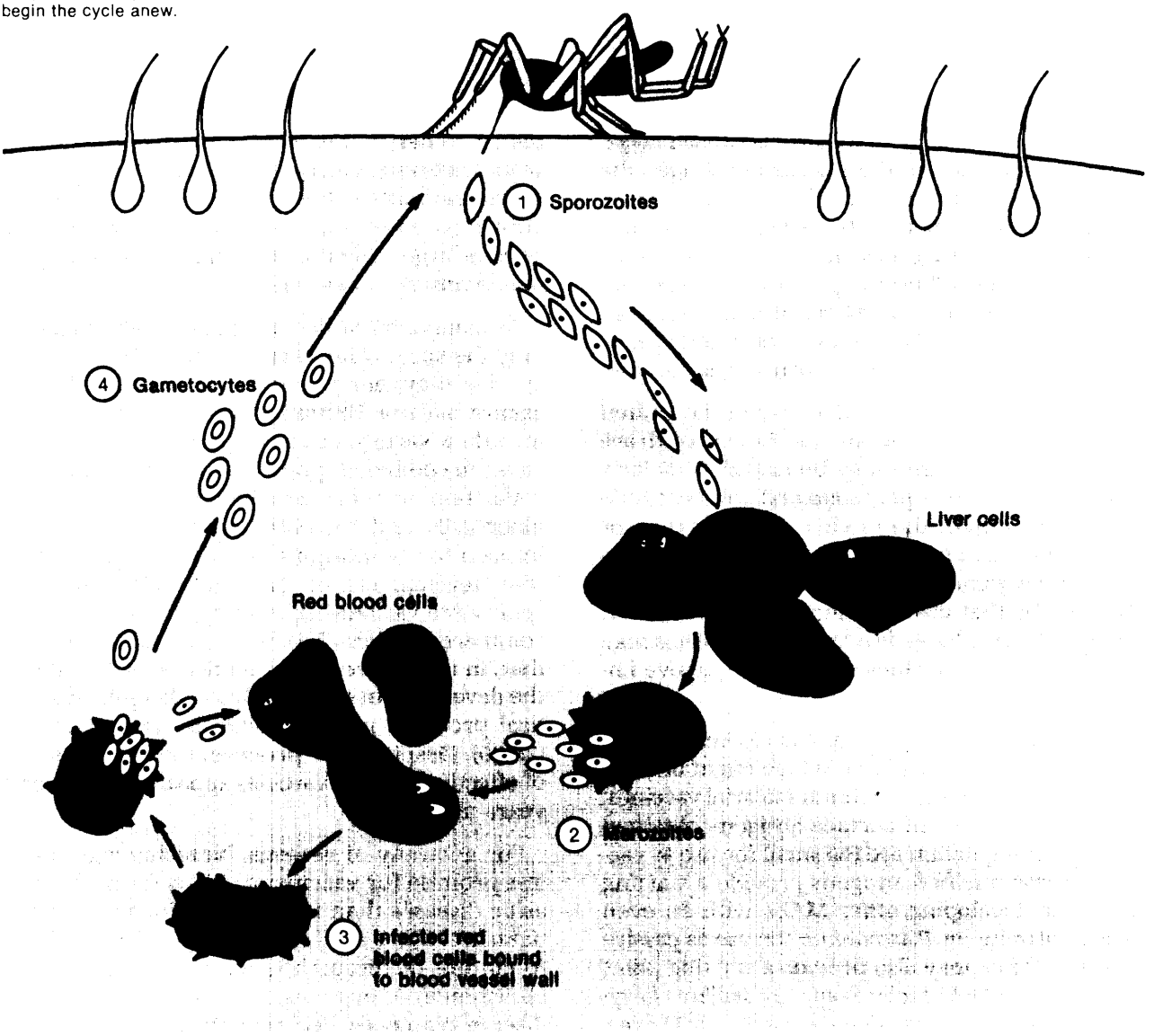
SOURCE: Office of Technology Assessment, based on data from World Health Organization, *Report for the Special Programme for Research and Training in Tropical Diseases,* Geneva, 1976.

● **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 deatha 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.**

**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.

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 ***Wasmodia*** 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. COLZ" 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; Gmentech (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 ***(7'5,90).*** 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 antigena at Walter and Eliza Hall Institute of Medical Research in Australia. This research was also partially funded by WHO (110).

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 applacations 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 protoplasm fusion, whereby the contents of two micro-organisms are fused to give one cell, enables researchers to induce rapid improvements in bacterial germplasms. Protoplasm 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 microorganisms 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 microorganisms 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+xtractable 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 microorganisms 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 protoplasm 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 coats similar to bulk chemical production, to give prices of several dollars per kilogram (73).**

## Monoclinal 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

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 maybe 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 immunoassay 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).**

**Table 26.—In Vitro Monocionai Antibody Diagnostic Products Approved in the United States'**

| Manufacturer | Analyte | Date approved |
|---|---|---|
| Hybritech, Inc. | IgE | 5/29/81 |
| Hybritech, Inc. | PAP | 9/1/81 |
| Hybritech, Inc. | HCG | 10I13I81 |
| Hybritech, Inc. | T Cell | 7126J81 |
| Hybritech, Inc. | Ferritin | 10I19I81 |
| Abbott | PAP | 1}19182 |
| Abbott | CEA | 313182 |
| Abbott | CEA | 3/29/82 |
| Ortholil | OKT-11 | 416/82 |
| Centocor | Anti-Rabies | 4116182 |
| Hybritech, Inc. | HCG | 4/23/82 |
| Hybritech, Inc. | HGH | 618182 |
| Mallinckrodt, inc. | Total Ti | 6/9/82 |
| Hybritech, Inc. | Prolactin | 6I1OI82 |
| Clinical Assays | ''sI-IgE | 6118/82 |
| Biogenex Laboratories | @-HCG | 7/13/82 |
| Hybritech, Inc. | HCG (EIA) | 7122182 |
| New Horizons | Gonogen | 8/4/82 |
| Monoclinal Antibodies, Inc. | UCG | 9/24/82 |
| Hybritech, inc. | TSH | 10/8/82 |
| Afiergenefics (Div.of Axonics). | IgFast@t® (Specific IgE) | 11/10/82 |
| Becton Dickinson&Co. | T4 | 1217182 |
| Syva Co. | Chlamydia | 12I1OI82 |
| Miles Laboratories | Gentamicin | 12/14/82 |
| Allergenetics (Div.of Axonics). | TotalIgFASTST | 1113/83 |
| Carter-Wallace, inc. | @HCG | 1/20/83 |
| Hybritech, Inc. | Tandem-E® Ferritin | 2/24183 |
| Ortho | Rubefia | 3/15/83 |
| PCL-RIA | HCG | 4/5/83 |
| Quidel Home | HCG[b] | 4/14/83 |
| Ventrex Labs, Inc. | Enzyme TSH | 4126/83 |
| Quidel Home | HCG[b] | 4/26183 |
| Diagnon | Ferritin | 4/28/83 |
| BTC Diagnostics | HCG | 4/28/83 |
| Immunlok | Chlamydia | 4129183 |
| Monoclinal Antibodies | HCG | 5125/83 |
| Ventrex Labs, inc. | IgE (total) | 5125183 |
| Organon Inc. | HCG | 5/26/83 |
| BioGenex Laboratories | RIAGen~-HCGRIA Kit | 5/26/83 |
| Micromedia System, Inc. | Micromedia @-HCG RIA | 6/1/83 |
| Organon Inc. | Neo-Presmosticon 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 maybe reduced to 2hours 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 polyclonalantibodies have long beenusedto 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

immunoassay. 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.

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### *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*

Liver scan after injection of MAbs shows metastases of colonic cancer

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 MAbdirected 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 MAbsas 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.

# 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.

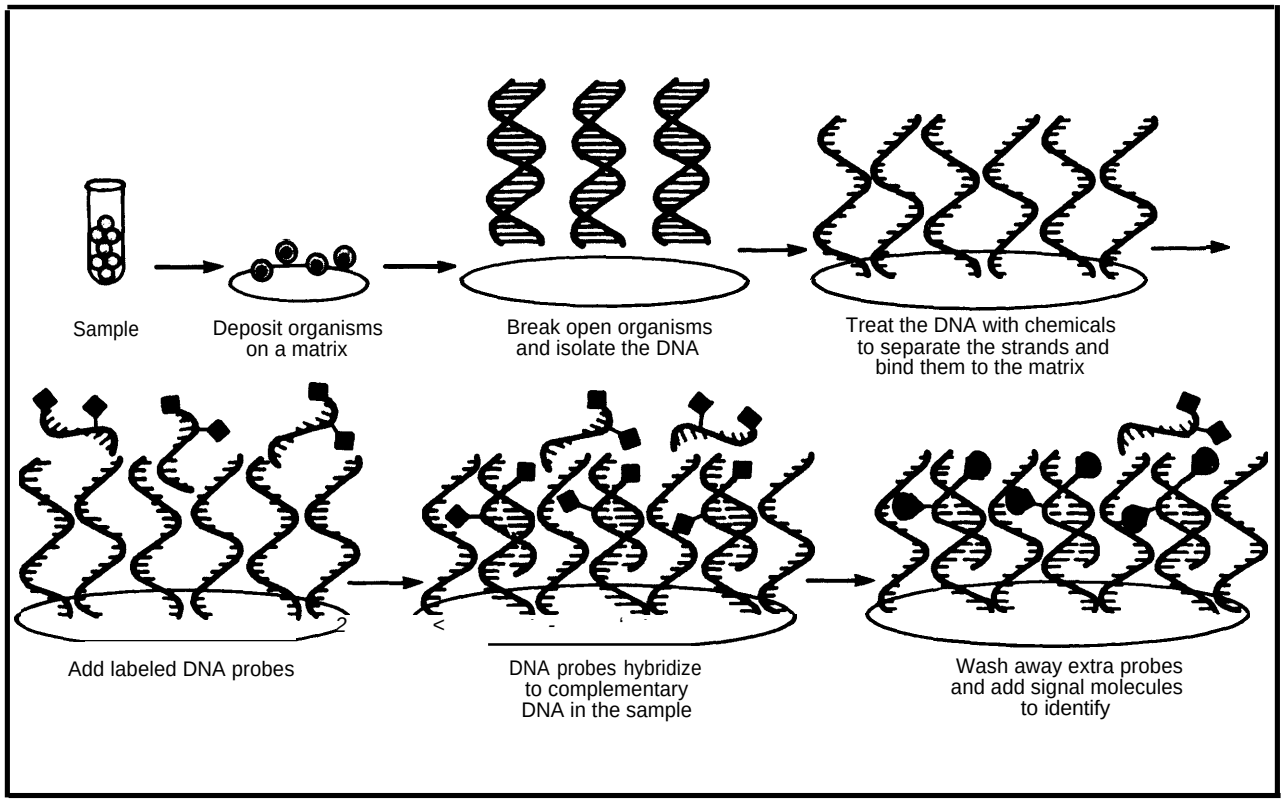
Several methods to label DNA probes with nonradioactive substances are emerging. The most predominant new method, developed and patented by Dr. David C. Ward and his colleagues at Yale University's School of Medicine, is to couple chemically the molecule biotin to DNA. Biotin-labeled DNA probes hybridize 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 Biochemical (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).

**Figure 15.—DNA Probe Filter Assay**




SOURCE: A. Klausner and T. Wilson, "Gene Detection Technology Opena Doors for Many Industries," *Wotechnology,* Auguat 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 (US,), 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 amounced 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.

# 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&Din 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).

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 largeIy 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 pharmaceutical innovation in biotechnology including the following:

- clarification of the functions and mechanisms of action of immune regulators such as Ifn and interleukin-2,

- 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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