DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 11(5), 965-999 (1985)

BIOTECHNOLOGY: PRESENT AND FUTURE ROLES IN THE PHARMACEUTICAL INDUSTRY

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ABSTRACT

Biotechnology is the integration of a number of scientific disciplines including microbiology, genetics, biochemistry and chemical engineering. It uses living organisms, or systems or products from these organisms to make or modify useful products. New biotechnology comprises genetic engineering, protoplast fusion and monoclonal antibody techniques, powerful new "tools" designed to generate efficient bioprocesses and products for the pharmaceutical industry. The following areas of biotechnology are highlighted: human insulin, interferons and other growth factors, neuroactive peptides, blood products, antibiotics, enzymes, monoclonal antibodies, vaccines and oncogenes.

INTRODUCTION

Biotechnology has been defined in the Spinks Report (1980) as the application of biological organisms, systems or processes to manufacturing or service industries[1]. It is the science which integrates the application of microbiology, biochemistry, genetics and biochemical engineering for their use in present or future industrial applications. It has also been defined as the

0363-9045/85/1105-0965$3.50/0

application of commercial technology that uses living organisms, or substances from these organisms, to make or modify a product. Biotechnology develops techniques used for the improvement of the characteristics of economically important plants and animals. It also develops microbial strains which improve man's environment. Biotechnology is also used to mean "new" biotechnology; "new" referring to novel biological techniques - such as recombinant DNA (rDNA) techniques and cell fusion methodology for new bioprocesses for commercial production, including the production of monoclonal antibodies.

Biotechnology will create new industries that require little fossil energy since most biotechnological processes function at ambient temperatures, consume little energy and depend on naturally abundant, inexpensive, biodegradable substrates for biosynthesis.

The industries that will benefit most from biotechnology are agriculture, waste recycling, pollution control, new energy sources, human and animal food production and new chemical feed stocks to replace toxic petrochemical feed stocks. The veterinary and human health industry has and will continue to reap the benefits of the new biotechnology. Biotechnology is still largely an embryonic science and will require further advances in its technological development before it reaches its full growth potential.

Biotechnology is a priori multidisciplined and, therefore, biotechnologists use techniques derived from molecular biology, microbiology, biochemistry, chemical engineering and computer science. The principal objectives of biotechnologists are innovation, development and optimal process operation using biochemical catalysis as its modus operandi. Biotechnology also exercises close cooperation between related fields including medicine, nutrition, environmental protection, waste process technology and the chemical and pharmaceutical industries.

One of the major distinctions between biology and biotechnology is in their scale of operation. The biologist, using a microscope and spectrophotometer to understand basic cellular processes, works in the nanometer and nanogram scale, whereas the biotechnologist is concerned with the scale-up of successful laboratory processes yielding milligram quantities to production-scale technology yielding kilograms of pure product.

In reality, biotechnology is not a new science and has its origins in ancient and traditional fermentation processes such as the brewing of wines and other acoholic beverages and in the manufacture of vinegar, cheese, oriental food flavorings and bread. Industrial microbiology reached a new scale of operation with the large-scale commercialization of antibiotics and organic acids in the middle 1940's. The large-scale manufacture of antibiotics led to tremendous growth in industrial microbiology, microbial genetics, fermentation biochemistry, biochemical engineering and chemical separation and purification technology. Since that time, we have witnessed a dramatic expansion of the fermentation industry for the production of vaccines, polysaccharides, enzymes, hormones, etc. A close working relationship between microbiologists, biochemists, chemical engineers and natural product chemists was developed throughout this period. Thus, biotechnology is not a sudden new technological discovery, but rather, an expansion of a fermentation craft deeply rooted in man's ancient past. Fermentation developed into more of a science several decades ago and today, is rapidly becoming a true scientific discipline. Although the traditional fermentation industry will play a major role in the development of biotechnology, the hopes of present and future growth lie within applications of new discovery in the following sub-disciplines of biotechnology.

1) the development and practical application of enzyme technology and enzyme engineering;
2) the development and application of monoclonal antibody (Mab) technology, i.e. the use of homogeneous antibodies derived from a single clone of cells, and
3) genetic engineering (rDNA) technology, i.e., the process by which man can transfer and express genetic information between distantly related organisms including microorganisms, plants and animals.

The above three fields exploit the flow of discoveries made by the enzymologist, the immunologist and the molecular biologist. The three fields are now referred to as biomolecular engineering.

Biotechnology can be considered as having a two component central core in which one part is concerned with the discovery and practical utilization of

the best biological catalyst (enzyme or process) while the other component creates, by development and operation, the best possible environment for the catalyst to operate (Fig. 1). The first part concerns itself with basic science and discovery while the second is involved with reactors and systems designs usually developed by chemical, process and computer engineers[2]. Several of the major areas of application of biotechnology are summarized in Table 1. Figure 1 depicts the classical biotechnology tree showing the interaction of the major disciplines of biotechnology along with some of its major branches which together are now developing into a rapidly expanding biotechnology industry.

Biotechnology Processes

Bioprocesses are systems in which whole living cells or their components (organelles, enzymes, etc.) are used to effect desired physical or chemical changes. The basic steps in a biotechnological process are presented in Figure 2. The substrate and nutrients are prepared in a sterile medium and introduced into the process usually in the form of an immobilized cell or enzyme. Under controlled conditions, the substrate is converted to product(s) and when the desired degree of conversion has been achieved, the byproducts and waste materials are separated. The products are usually purified from dilute aqueous solutions.

Bioprocesses require a closely controlled environment as biocatalysts generally exhibit great sensitivity to changes in temperature, pH and even concentrations of certain nutrients or metal ions. Oftentimes, the success of a biotechnology process depends on the extent to which these factors are controlled in the medium where interaction between the enzyme and substrate takes place.

In addition to establishing a suitable environment, the medium must provide the essential nutrients for the living cells which contain the biocatalysts. A primary requirement of all living cells is carbon which supplies energy for overall metabolism and protein synthesis. Carbon, usually in the form of sugars, starches or glycerides, often contributes structural elements required for the synthesis of complex molecules. Other important nutrients

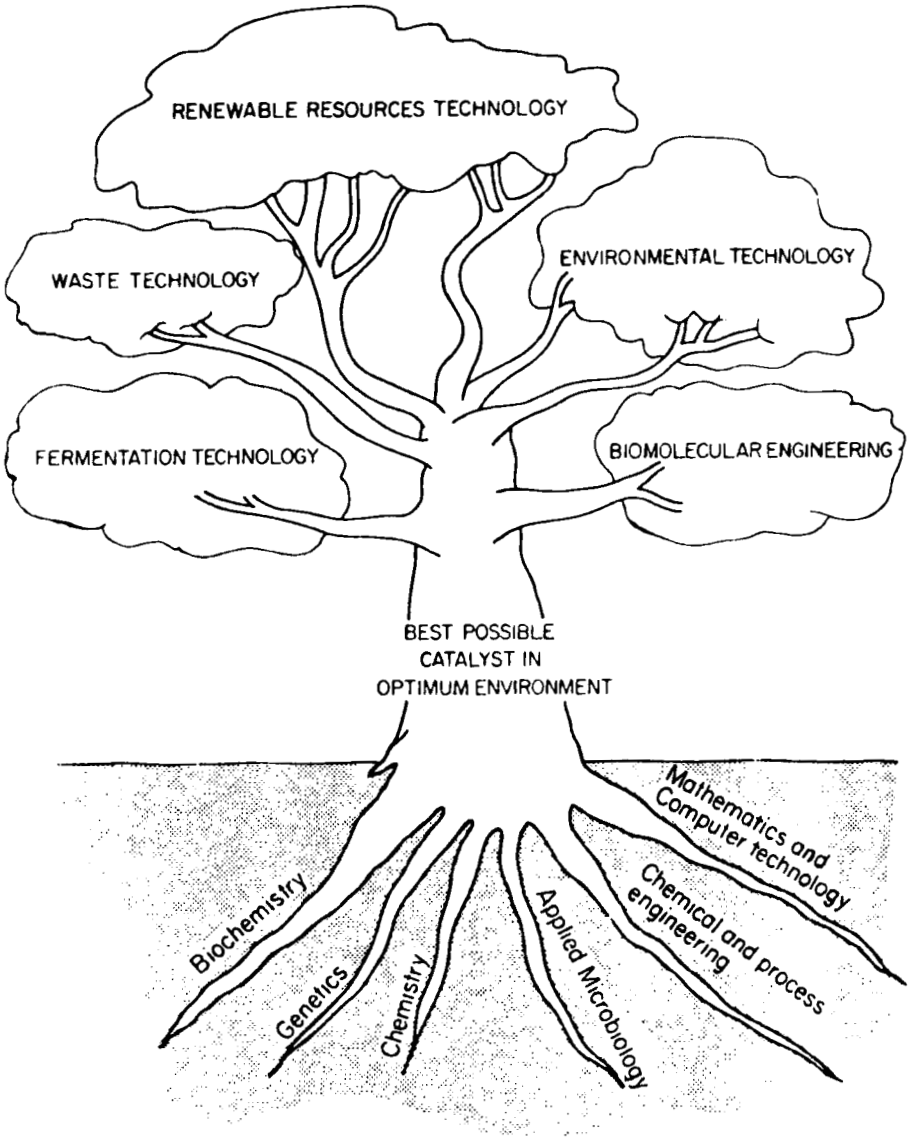


Fig. 1. The Tree of Biotechnology (from Smith[2])

required by most living cells are nitrogen, phosphorus and certain metallic ions. Oxygen is essential for all aerobic bioprocesses.

In order to make the substrate and nutrient materials accessible to the biocatalyst, mixing of the ingredients is essential. Microbial cells including bacteria and yeasts, organisms commonly employed in bioprocesses commonly grow as individual cells, whereas filamentous bacteria and fungi are grown

TABLE 1.

Some Major Areas of Biotechnology

1. Fermentation technology

   Historically, the most important area of biotechnology, viz. brewing, antibiotics, etc., extensive development in progress with new products envisaged viz. polysaccharides, medically important drugs, solvents, protein enhanced foods. Novel fermenter designs to optimize productivity.

2. Enzyme engineering

   To be used for the catalysis of extremely specific reactions; immobilization of enzymes; to create specific molecular converters (bioreactors). Products formed include L-amino acids, high fructose syrup, semi-synthetic penicillin, starch and cellulose hydrolysis, etc. Enzyme probes for analysis.

3. Waste technology

   Long historical importance but more emphasis now being made to couple these processes with the conservation and recycling of resources; foods and fertilizers, biological fuels.

4. Environmental technology

   A great scope exists for the application of biotechnological concepts for solving many environmental problems - pollution control, removing toxic wastes; recovery of metals from mining wastes and low-grade ores.

5. Renewable resources technology

   The use of renewable energy sources, in particular lignocellulose to generate new sources of chemical raw materials and energy-ethanol, methane and hydrogen. Total utilization of plant and animal material.

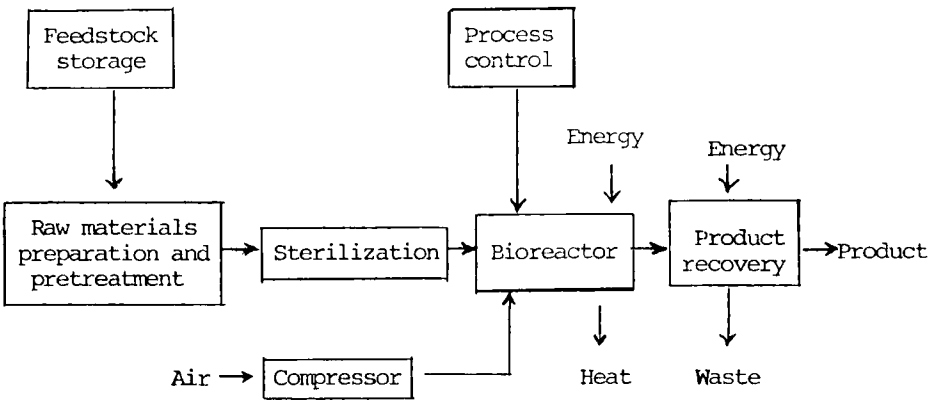


Fig. 2. Schematic Overview of a biotechnological process (from Cooney[20])

either as aggregates or as long mycelial strands. In the growth of filamentous microorganisms, this type of morphology tends to increase the viscosity of the medium often resulting in problems associated with oxygen mass transfer and nutrient accessibility to the living cells.

Most of the products of biotechnology are formed through the action of single biocatalyst, either a microorganism or an enzyme. If foreign microorganisms contaminate the system, they can interfere with the system so as to destroy the biocatalyst or the desirable byproduct. Foreign organisms may also generate undesirable metabolites which make purification of the desired product difficult.

Most biotechnological processes use pure culture techniques to avoid biocatalyst contamination. The bioreactor vessel containing the necessary nutrients must be sterilized by heat and a pure culture of the microorganism or sterile immobilized biocatalyst is introduced into the sterile system. All essential additions into the system must be appropriately sterilized.

Biotechnological processes generally use the operating modes of conventional chemical technology. These modes range from batch processing to continuous steady-state processing. In batch or fed-batch processing, the biocatalyst is added to the reactor containing the sterile nutrients and the conversion takes place over a time period ranging from several hours to many days. During this period, nutrients and other substrates, agents which effect necessary proper pH control, are supplied to the reaction vessel. Volatile waste products are usually removed from the reactor. When the conversion is complete, the reaction is stopped, the bioreactor is emptied and the purification process is initiated. In continuous steady-state processing, sterile nutrients are supplied to the bioreactor and the byproduct and spent medium are continuously withdrawn at volumetrically equal rates. Continuous bioprocessing offers advantages over batch processing, These are lower costs due to continuous reuse of the biocatalyst, greater ease of product recovery and, oftentimes, higher overall productivity rates. The simplest approach to the implementation of the continuous processing mode is to modify a batch reactor so that fresh substrate and nutrients can be added continuously while the

product stream is removed. This arrangement has a serious drawback, however, in that the biocatalyst leaves the reactor continuously. Biocatalysts can now be fixed or immobilized in a suitable matrix, thereby avoiding loss of the catalyst and allowing for continued reuse of the biocatalyst. Thus, the development of immobilized enzyme technology has greatly expanded the possibilities for continuous bioprocesses.

Byproduct separation and purification techniques used in biotechnology are most important aspects for the production of novel products such as antibiotics, proteins, enzymes, hormones, etc. Some of the newer purification strategies used for the recovery of bioproducts include the use of ultra filtration, continuous chromatography, electrophoresis and the use of monoclonal antibodies. Although purification and separation processes have been developed for existing bioprocesses, new biotechnology is presenting new challenges. Genetic Engineering and rDNA technology have already culminated in the commercialization of human insulin derived from microbial fermentations. Considerable technology was necessary to purify this hormone from the complex microbial fermentation broths.

Examples of existing commercial biotechnology processes currently in operation are:

a) production of whole cell biomass (brewer's and baker's yeast, single-cell protein)
b) production of cell components (enzymes, nucleic acids)
c) production of chemical metabolites including primary metabolites ethanol, citric and lactic acids) and secondary metabolites (antibiotics, plant hormones)
d) catalysis of specific, single-substrate conversions [glucose to fructose, penicillins to 6-aminopenicillanic acid (6-APA)]
e) catalysis of multiple-substrate conversions (biological waste treatment)

Biotechnology processes offer a number of advantages over conventional process technology. Some advantages are milder reaction conditions (temperature, pH, pressure); use of abundant, cheap resources as raw materials; less

hazardous operation and reduced environmental impact; greater specificity of catalytic reaction (stereospecific enzymes, etc.); less complex manufacturing facilities usually requiring smaller capital investment; catalytic reactions operative in aqueous environments at ambient temperatures; improved process efficiencies through biocatalyst reuse (higher process yield and reduced energy consumption); and the potential use of genetic engineering technology to tailor-make enzymes via site-directed mutagenesis, etc.

Some of the disadvantages of bioprocess technology compared with existing chemical technology include the generation of complex products which require extensive separation and purification, especially when complex natural raw materials are used as substrates; problems arising from dilute aqueous environments in which most biocatalysts function; the susceptibility of most bioprocess systems to contamination by foreign microorganisms; the inherent variability of most biological processes due to the genetic instability of organisms, the inherent variability of most natural substrates, the necessary costly containment required for recombinant microorganisms and the need to contain waste process streams from recombinant microorganisms.

The net advantages of biotechnology processes, however, will result in an ever expanding biotechnology industry. Over the next several years, there will be an expanded research and development in the following areas:

a) Continued development on the practical use and design of bioreactors for immobilized whole cell and enzyme systems;

b) Development of a wide range of more sensitive, sterilizable sensor probes for process monitoring and controls;

c) Development of improved product recovery techniques, especially for proteinaceous products.

d) Improved bioreactor design providing for better mixing and improved mass transfer;

e) Inhibition of intracellular and extracellular protein-degrading enzymes;

f) Improved methods for heat dissipation during bioprocessing;

g) Development of genetically engineered strains with greater genetic stability;

h) Development of microbial cultures with more efficient protein-secreting mechanisms.

Applications of Biotechnology to the Pharmaceutical Industry

The domestic sales of prescription drugs by U. S. pharmaceutical companies exceeded $8.6 billion in 1982[3]. Of these sales, approximately 20% were products for which fermentation biotechnology played a significant role. The fermentation-derived products included anti-infective agents, vitamins and biologicals. The "new" biotechnology (bimolecular engineering) is expected to be particularly helpful in the production of pharmaceuticals and biologicals, which in the past, could only be obtained by extraction of animal and plant tissues, and which now can be obtained from microbial sources as well.

The pharmaceutical industry was probably the last industry to adopt traditional fermentation technologies. However, it was the first industry to make widespread use of the newer molecular biology techniques including genetic engineering and protoplast fusion. There were two major factors which accelerated the use of molecular biology in the pharmaceutical industry. First, the biological sources of many pharmacologically active products were microbial in origin and, thereby, were more amenable to directed genetic manipulation. Second, the major advances in bimolecular engineering were made under an institutional structure that allocated funding to biomedical research. In fact, the Federal support system has tended to promote studies that have as their ostensible goal, the improvement of human health (National Institutes of Health, National Cancer Institute, etc.)

Historical Uses of Genetic Technology

The genetic manipulation of biological systems for the production of pharmaceuticals has two major goals. These are

a. to increase the efficiency of pharmaceutical production with proven or potential value, and,

b. to discover potentially new useful drugs not found in nature.

The first goal has had the greatest influence on the pharmaceutical industry. Genetic manipulation has been shown to increase, in an almost dramatic

fashion, the productivity of pharmacologically active products found in nature. Three examples are outlined below:

1. The genetic improvement of penicillin production is an example of long-term efforts that lead to dramatic increases in strains of the penicillin fungus, Penicillium chrysogenum. The original Peoria isolate, NRRL-1951, was treated with toxic chemicals and ultraviolet radiation through successive stages until a very superior mutant strain was developed. This commercially valuable mutant yielded a hundredfold improvement in fermentation productivity compared to the original Fleming strain (Fig. 4).
2. Chemically induced mutations improved a valuable strain of the bacterium, Escherichia coli, which produces an enzyme, L-asparaginase, an agent used to treat leukemia. Improved mutants were isolated which produced 100-fold more enzyme, thereby resulting in easier isolation and increased purity of the enzyme preparation. Moreover, the dramatic improvements in productivity and purification resulted in lowering the cost of a course of therapy from $15,000 to $300[3].
3. Genetic manipulation of the gentamicin bacterium, Micromonospora purpurea, resulted in sufficient yield improvement that its producer, Schering-Plough Corporation, did not have to build a scheduled manufacturing plant, thereby, resulting in a savings of $50 million[4].

Current Uses of Biotechnology

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 current 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 can be improved by new biotechnology in many ways. In some instances, the production of some pharmaceutical products formerly carried out by expensive chemical synthesis or by tissue extraction methods can now be performed from cloned human genes, expressed and amplified in microbial cells. In other instances, application of rDNA technology may replace traditional bioprocesses for the production of antibiotics and other pharmaceutical agents. One of the most important aspects of biotechnology is that for the first time, techniques are now available to mass produce large amounts of compounds that in the past were extremely scarce, i.e. human growth hormone.

Whatever the intended impact of a new pharmaceutical product, profit expectations often govern the selection of products for development. Large-scale pharmaceutical manufacturers must make numerous decisions regarding the practical utility of biotechnology. These considerations include:

a) the possibility of making products superior to those already marketed for a given purpose (i.e., more effective, convenient, safe, or economical);

b) the technical feasibility of applying new methods (e.g., in rDNA applications, the feasibility of cloning DNA that directs synthesis of desired substances);

c) the cost of conventional methods (e.g., chemical synthesis, tissue extraction, or traditional bioprocessing) and the potential to reduce costs with rDNA technology or other new methods;

d) 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);

e) 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

f) 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).

Major Areas for Biotechnology in the Pharmaceutical Industry

Regulatory Proteins

Human Insulin -

The first therapeutic agent produced by rDNA technology to achieve regulatory approval and market introduction was human insulin (hI). The methodology was generated by Genentech Inc. and Eli Lilly and Company. The product now marketed in both the U.S. and United Kingdom is known as Humulin^{R} . The extent to which the new rDNA-derived products will be substituted in the marketplace for animal-derived insulin is still uncertain. Insulin derived from animals has long been the largest volume peptide hormone used in medicine. Chemically, human insulin differs only slightly from that of pigs and cows, and its incremental benefits have yet to be demonstrated. Human and porcine insulins differ in a single amino acid, while human and cattle insulins differ with respect to three. As far as is known, these slight variations do not impair the effectiveness of the insulin, but no meaningful comparative study has been undertaken because of the past insufficiency of human insulin.

In 1981, 0.75 tons of pure insulin for 1.5 million diabetics was sold in the U.S. The number of American diabetics is expected to increase to 2.1 million by 1986 (Scrip, 10/4/82). Eli Lilly dominated the U.S. market in 1981 with $133 million in sales vs a total U.S. market of $170 million. The 1985 estimated U.S. market is $345 million with Lilly's sales expected to be in excess of $200 million[3].

Interferons -

The interferons (Ifn's) represent a class of immune regulators or lymphokines that regulate the response of cells to viral infections and cancer

proliferation. These extraordinarily potent proteinaceous substances are the subject of the most widely publicized, well-funded applications of rDNA technology to date, but the basic details of their functions remain largely unknown. Until recently, the study of Ifns was limited by the extremely small amounts of Ifns that could be obtained from extracted human lymphocyte cells. However, the use of rDNA technology now allows for the large-scale production of non-glycosylated Ifns in bacteria and large clinical trials on a variety of Ifns are currently in progress.

The gene cloning and microbial production of Ifns illustrate several important aspects of the current commercialization of biotechnology. These include:

1) the use of rDNA technology to produce a scarce product in quantities sufficient for research on the product's effects;
2) a massive, competitive scale-up campaign by pharmaceutical manufacturers in advance of demonstrated uses of the product;
3) the attempt to produce economically a functional glycoprotein (protein with attached sugar molecules) in an rDNA system:
4) a pattern of international R&D investment that reflects the differing needs and medical practices of various nations; and
5) the establishment of a U.S. national effort, via research grants and procurement contracts administered through the National Cancer Institute (NCI), the American Cancer Society (ACS), and other organizations, to support testing of Ifns toward a national goal (cure of cancer).

Ifns are being considered for various health-related applications, but are not yet approved as pharmaceutical products. There is some evidence that Ifns are effective in certain viral infections, but more clinical trials are necessary. Ifns may prove useful in treatment of some viral diseases in combination with other drugs[5].

Many clinical trials are presently underway with a type of interferon, called gamma Ifn. for the treatment of certain kinds of cancer. However, at present, only limited conclusions can be drawn from the available data. In

some cases, Ifns inhibit tumor cell growth and may stimulate immune cells to destroy cancer cells; their effect on inhibiting tumor metastasis are better established than their ability to effect actual regression of primary tumors. Also, most tumors that show some response to Ifns are also quite responsive to established chemotherapeutic agents. Several problems have also been noted in clinical trials using Ifns. The occurence of fewer fatigue and flu-like symptoms in patients following injection with Ifns were once thought to be reactions to impurities contained in the drug preparation, but highly purified preparations of Ifns show similar effects[6]. Despite extensive research and vast ongoing clinical trials, numerous questions still remain concerning the anticancer potential for interferons.

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

Though most rDNA-made Ifns currently under evaluation are produced in the bacterium. E. coli, yeast is being increasingly employed as a production organism. Yeast requires less stringent culture conditions than do most bacteria, has long records of reliability and safety in large-scale bioprocessing, and is 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 biotechnology companies have reported significant progress using yeast for the manufacture of Ifns. Yeast has important advantages over bacterial synthesis in that glycosylated Ifns can be synthesized and secreted into the fermentation environment. Numerous genetic techniques are currently being employed in yeast strains to increase Ifn production. They include: 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 impeding cell growth; 3) limitation of production degradation by extracellular protease enzymes; 4) induction and enhancement of Ifn product secretion; and 5) the genetic stabilization of genetically-engineered strains.

Human Growth Hormone -

Genetic engineering technology is being used increasingly to produce large amounts of otherwise scarce biological compounds. Human growth hormone (hGH) is an excellent example of the future promise of biotechnology to produce in large amounts, hormones that are only naturally produced as several molecules per cell.

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.

Although the established pharmaceutical market for hGH is small and current supplies for the treatment are sufficient, hGH ws one of the first targets for the applications of rDNA technology. Human growth hormone is currently being evaluated for the following human maladies: 1) treatment of constitutionally delayed short stature; 2) to improve the healing of burns, wounds and bone fracture; 3) treatment of a disease condition known as cachexia or deficiency in nitrogen assimilation [7]. It has been reported that 3% of all children have constitutionally delayed short stature and that as many as one-third of these may benefit from hGH administration [3].

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 bind to the same receptors affected by opiate drugs and produce analgesic effects in the central nervous system similar to those produced by these drugs. Two of these unusual neuroactive peptide classes are known as enkephalins and endorphines and appear to be structurally related to many other polypeptides that play various roles in the nervous and endocrine system. Pancreatic endorphins may be important in providing natural analgesia during childbirth.

Another neuroactive peptide that may affect neurological processes, including attention span, is melanocyte stimulating hormone (MSH). MSH appears to enhance the ability of test animals to maintain attention span in primitive learning experiments. MSH treatment also appears to improve learning skills in mentally retarded patients[8].

In addition to screening neuroactive peptide compounds for analgesic and anesthetic activity, scientists are attempting to recognize active peptides that might suppress coughing and cerebral vascular disorders, improve failing memory, ameliorate sleep disorders and mental depression, alleviate Parkinson's disease and several forms of dementia, including senility.

Lymphokines-

Lymphokines are proteins produced by lymphocytes that convey information among lymphocytes. With the exception of interferons, lymphokines are only beginning to be characterized, but these proteins appear to be crucial to immune reactions. Some lymphocytes produce lymphokines that engage other lymphocytes to boost the immune response to a foreign antigen and repel foreign invasion. Other lymphocytes produce substances which act in conjunction with an antigen to stimulate the secretion of antibodies.

The increasing importance of lymphokines in preventing disease and understanding cellular function (including cancerous growth) is fostering widespread research on these important immune regulator molecules. The recent establishment of discrete lymphocyte cell lines which produce various classes of lymphokines and the recent cloning of lymphokine-producing genes into rDNA systems for their large-scale production in microbial fermentation systems

will increase the amount of research in this new important branch of immunology. rDNA technology now provides for the availability of pure lymphokine preparations which will allow immunologists to answer more fundamental questions concerning cell biology and the immune system. Eventually, these efforts may lead to the use of lymphokines in medicine to stimulate the patient's own immune system to combat disease.

Interleukin-2 (IL-2) or lymphocyte growth factor has been reported to be effective in eliciting an elevated immune response in patients having an impaired immune response (compromised patients undergoing cancer chemotherapy) or patients afflicted with acquired immune deficiency syndrome (AIDS). The genes responsible for IL-2 production have now been cloned successfully into microbial systems and pure IL-2 protein is now readily available for research and clinical trial evaluation[9]. IL-2 has been shown to stimulate the body's T-cells to mature to a point where they become natural killer cells (NK-cells) which are able to attack and diminish the size of discrete tumors. In test tube and animal experiments, IL-2 has greatly increased the number of T-cells available for the destruction of tumor cells.

A number of regulatory protein "growth factors" for a number of somatic cells have been isolated and are currently being characterized (Table 2). Several of these have been sequenced as to their exact amino acid composition and soon will be candidates for production by rDNA technology. Two of these factors, epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) appear to be stimulated by cancer-causing virus genomes known as oncogenes. Oncogenes are transformed normal cellular genes found in retroviruses and other cellular tissues which have been implicated in cancer[10].

Blood Products

Products derived from the fractionation of human blood plasma represent the greatest volume of biological products marketed with an annual world market of $1 billion.. The three major blood plasma commodities are human serum albumin, gamma globulin and anti-haemophilic factor. Efforts to produce the above products via rDNA technology are underway. The gene responsible for Factor IX (an anti-haemophilic factor) has been recently cloned and expressed

TABLE 2.

Protein "Growth Factors" With Potential Pharmaceutical Applications

Factor	Function
CSF (colony stimulating factor).....	Simulate granulocyte differentiation
EGGS (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-drived growth factor).....	Stimulates cell division near inflammation
NGF (nerve growth factor)	Stimulates nerve growth and repair
PDGF (platelet-derived growth factor).....	Stimulates division of fibroblast-like cells
SGF (skeletal growth factor).....	Stimulates bone cell growth
WAF (wound angiogenesis factor).....	Stimulates wound healing
TAF (tumor angiogenesis factor).....	Stimulates blood vessel proliferation in tumors

SOURCE: Office of Technology Assessment, 1983.

in E. coli. Factor IX has been reported to be effective in treating Type B hemophilia.

Factor VIII, produced by liver cells, is important for the treatment of Type A hemophiliacs. Since the majority of hemophiliacs are of the A type, there are stronger medical and commercial reasons to clone factor VIII genes in microorganisms and, thereby, make the material in abundant quantities.

There are a number of difficulties associated with the molecular biology of factor VIII in that it is a glycoprotein and, therefore, not yet possible to be synthesized in bacteria. Also, the glycoprotein molecule is labile and extremely large (300,000 molecular weight). In fact, the molecule is about 20 times the size of most interferons.

The rDNA production of factor VIII is an elusive goal, but the implications of success are substantial. The imminent cloning of the molecule will assuredly provide for more economic treatment for hemophiliacs, but abundant quantities of the material may lead to a better understanding of the most common type of human hemophilia.

Thrombolytic and Fibrinolytic Enzymes-

Thrombosis, or the blockage of blood vessels, represents the leading cause of death in most industrialized nations. Coronary heart disease, strokes and pulmonary embolisms account for more than half of all deaths in the Western Hemisphere[3].

Therefore, the search for substances capable of dissolving blood clots is a major R&D effort in many pharmaceutical companies. The current therapy for thrombosis is the use of thrombolytic and fibrinolytic enzymes. The enzymes initiate the dissolution by converting a plasma protein called plasminogen into plasmin which, in turn, hydrolyzes the fibrin protein, a major ingredient of the blood clot.

One of the most commonly used enzymes is manufactured by a Streptomyces bacterium via a fermentation process. The enzyme is marketed generically as streptokinase. The other major enzyme, urokinase, is obtained from human urine or cultured human kidney tissue. Despite the usefulness of these enzymes, prolonged therapy with streptokinase often culminates in serious allergic reactions. In addition, both enzymes act nonspecifically throughout the body, thereby raising the risk of internal hemorrhaging. To help alleviate this serious problem, expensive, carefully placed catheters are necessary to deliver the enzyme to its target. The high costs of present enzyme manufacturing (especially, for urokinase) and enzyme administration via catheters, etc., restrain more widespread use. A typical streptokinase treatment

costs about $275, while urokinase costs about $3,000 per patient. Alternative thromobolytic enzymes and less expensive manufacturing methods are being sought via biotechnology.

A group of fibrinolytic enzymes called tissue plasminogen activators (tPAs) are currently being exploited by a number of biotechnology companies. tPA's appear to be more specific and work over longer periods of time compared to either streptokinase or urokinase. Moreover, the genes coding for their tPA synthesis have now been successfully cloned both in bacteria and in yeast so that more economic manufacture of large quantities of specific tPAs is now possible. Some of the biotechnology companies involved in the manufacture of tPAs are Genentech, Biogen, SA, Integrated Genetics, Genetics Institute and Chiron.

At present, the extent to which thromobolytic enzymes are used in different countries varies substantially. German and Japanese physicians prescribe streptokinase and urokinase extensively, often in conjunction with cancer chemotherapy. tPAs, along with streptokinase and urokinase, show promise in cancer therapy in that the substances help dissolve the fibrin membrane surrounding many tumors, thereby allowing for more effective drug entry into the solid tumor tissue. American medical practice tends to discourage the use of thromobolytic enzymes and, therefore, the current annual U.S. market is only $8 million. In Japan, urokinase is the seventh largest selling drug with total annual sales exceeding $150 million [11].

Through the use of rDNA technology and improved protein purification procedures, large quantities of scarce materials, i.e. tPAs, are now becoming available. These improvements will undoubtedly lead to substantial changes in medical practices throughout the U.S. and the world. Marketing experts currently predict that the U.S. markets for tPAs could climb to $125 million annually given a successful economic development of tPA via cost reduction (one-half the cost of urokinase production) and improved mode-of-action [11].

Monoclonal Antibody Technology(Mab)

Monoclonal antibody technology currently leads other forms of biotechnology in commercial use, as measured by numbers of *in vitro* diagnostic

products. Until recently, all antibodies were obtained from the blood of humans and animals. However, over the past nine years, it has become possible to produce antibodies from cells in culture and to achieve levels of purity previously impossible.

This high level of production and purity was attained by the development of monoclonal antibody technology by Milstein and Kohler at the Medical Research Council in England in 1975. Milstein, Kohler and co-workers fused two kinds of cells - myeloma and plasma spleen cells - to form hybridomas that mass produce specific monoclonal antibodies.

The most immediate pharmaceutical application for Mabs lies in diagnostic testing. Because monoclonal antibodies are highly specific, hybridomas will replace animals as a source for antibodies for diagnostic and monitoring purposes. Their use will dramatically improve the accuracy and reliability of tests, decrease expensive development costs and result in a more highly uniform and specific product.

Monoclonal antibody assay technology is currently being used in the following ways:

1) determine hormone levels in order to assess the proper functioning of an endocrine gland or the inappropriate production of a hormone by a tumor;
2) detect certain proteins, the presence of which has been found to correlate with a tumor or with a specific prenatal condition;
3) detect the presence of illicit drugs in a person's blood, or monitor the blood or tissue level of a drug to ensure that the dosage achieves a therapeutic level without exceeding the limits that could cause toxic effects; and
4) identify microbial pathogens (especially for venereal disease, etc.).

Other applications of monoclonal antibodies include:

1) the improvement of the acceptance of kidney and other organ transplants by injection of the recipient with antibodies against certain antigens;
2) passive immunization against an antigen involved in reproduction, as a reversible immunological approach to contraception.

3) localizing tumors with tumor-specific antibodies, and

4) targeting cancer cells with antibodies that have anticancer chemicals attached to them.

Vaccines

Biotechnology provides new techniques for the production of vaccines. Most vaccines used at present consist of killed or attenuated organisms. Ideally, the recipients' immune system responds to the introduction of the vaccine by producing antibodies that bind to specific antigen molecules on the surface of the vaccine organism. This reaction identifies the vaccine organism for destruction by other components of the immune system. The antibodies produced in response to the foreign antigen remain in circulation for a period ranging from months to years, protecting the recipient host against the live pathogen should it be encountered later. In this manner, the recipient becomes actively immune to the particular disease. The administration of foreign antibodies or other immune products that themselves protect from the disease is known as passive immunity. Such immunity usually confers only short-term protection against a disease.

Although killed and attenuated vaccines represent one of the highest achievements in medicine, there are still several serious problems associated with the technology. First, these vaccines contain the complete genetic material of the pathogen and if the pathogen is not killed or attenuated completely, the vaccine itself may be capable of causing the disease it was intended to prevent. Second, these conventional vaccines do not immunize the recipient against all strains of the pathogen and, therefore, immunized recipients are immune to only one or several strains of the pathogen. Third, many of the conventional vaccines are not sufficiently stable in 3rd world nations where they are most needed. Presently available conventional vaccines need constant refrigeration.

The new biotechnology now provides for a new type of vaccine known as subunit vaccines. Subunit vaccines do not contain the nucleic acids or genome of the pathogen and, therefore, by themselves cannot result in an infection. Furthermore, subunit vaccines are more stable and are of considerably greater purity than conventional vaccines.

Two new biotechnology methods are being developed to produce subunit vaccines:

1) rDNA technology is being used to produce all or part of a surface protein molecule of the pathogen; and
2) chemical peptide synthesis of small active portions of the polypeptide that represent the "active" recognition sequences of the complex surface proteins.

Both of these new approaches have an added advantage--subunit vaccine manufacture does not require large-scale propagation of highly infectious bacterial and viral pathogens.

Table 3 lists a number of important viral vaccine biotechnology projects currently under development by biotechnology and pharmaceutical companies. Considerable R and D is being undertaken for influenza types A & B, herpes and polio viral diseases. Hepatitis B subunit vaccines illustrate the use of rDNA technology approaches to clone genes that encode for hepatitis B surface antigens. A number of laboratories have shown that the isolated surface antigens behave similarly to the whole virus when used as a vaccine [12]. Biogen SA has successfully immunized chimpanzees against hepatitis B using its yeast-derived vaccine[3]. It has been estimated that the new Biogen subunit vaccine will sell for only $10-30 per dose compared to the $100 per dose for Merck's vaccine made from virus particles extracted from the blood of hepatitis B carriers[13].

A potential live virus vector system is being investigated with vaccine virus, a virus which is not pathogenic to humans. DNA encoding for herpes B surface antigens (HBsAg) is ligated to vaccinia virus promoters which control transcription of the HBsAg DNA. This rDNA construct is then inserted into the vaccinia virus, thereby, producing a "live" vaccine that synthesizes and excretes the specific HBsAg desired. Rabbits immunized with the live vaccine produce antibodies against the HBsAg. This subunit vaccine is currently being tested in chimpanzees. 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.

TABLE. 3

Current Viral Vaccine Biotechnology Projects

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

SOURCE: Office of Technology Assessment. (1983)

Bacterial disease vaccines -

Bacteria and other microbial pathogens have complex, dynamic cell surfaces which in the past have inhibited effective vaccine development. Most bacterial surfaces are composed of lipids, phospholipids and polysaccharides, molecules which are derived from complex biosynthetic pathways. Such biosynthetic pathways are controlled by large multiple gene complexes. This complicated genetic system is not readily available to rDNA technology at this time. Hence, bacteria are not as amenable as the more simple viruses using rDNA subunit vaccine technology. Despite these many problems, several unique proteins have been identified on the surface of some pathogenic strains of the venereal disease bacterium, Gonococcus and on several pathogenic strains of E. coli[14]. These surface proteins may provide for suitable targets for modified subunit vaccines. However, considerable additional research is necesary before effective bacterial subunit vaccines will be a reality.

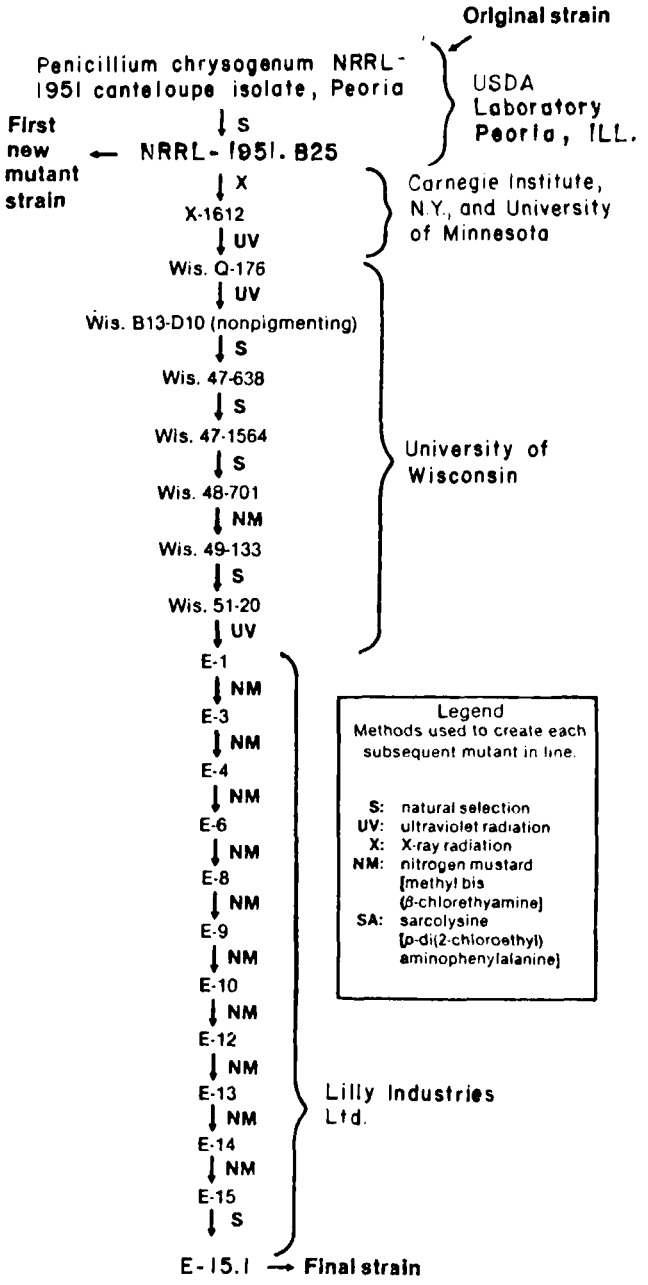
Antibiotics

Antimicrobial agents for the treatment of infectious diseases have been the largest selling prescription pharmaceuticals in the world for the past 30 years. Most of these agents are antibiotic-antimicrobial agents naturally produced by strains of microorganisms isolated from nature. Chemical synthesis and acylation of a variety of side-chain residues to naturally-occuring antibiotic nuclei is the common method of commercial manufacture for many antibiotics, many of which belong to the semisynthetic β-lactam class. Chemical synthesis of a major naturally occuring antibiotic chloramphenicol is one exception to the more common microbial biosynthesis or semi-synthesis manufacturing technology. Chemical synthesis will also probably be the more efficient technology for the manufacture of some of the future potent antibiotics of the carbapenem and monobactam classes of β-lactam antibiotics. These antibiotics are highly unstable in fermentation environments and their own yields are low via fermentation synthesis. U.S. pharmaceutical companies have been prominent in the development, production and marketing of useful antibiotics. The present annual American market share for antimicrobial agents is about $2.7 billion of the $5.8 billion annual world market. The markets expand each year with the introduction of new antibiotic compounds each year.

For 30 years, high-yielding, antibiotic-producing micro-organisms have been identified by selection from among mutant strains (Fig. 3). Initially, organisms producing new antibiotics are isolated by soil sampling and other broad screening efforts. They are then cultured in the laboratory, and efforts are made to improve their productivity.

Antibiotics are complex, usually nonprotein, substances which are generally the end products of a series of biological steps. While knowledge of molecular details in metabolism has made some difference, not a single antibiotic has had its complete biosynthetic pathway elucidated. This is partly because there is no single gene that can be isolated to produce an antibiotic. However, mutations can be induced within the original micro-organism so that the level of production can be increased.

Other methods can also increase production and possibly create new antibiotics. Microbial mating, for example, which leads to natural recombination,



An illustration of the extensive use of genetics to increase the yield of a commercially valuable substance. A variety of laboratories and methods were responsible for the successful outcome.

SOURCE: Adapted by Office of Technology Assessment from R. P. Elander in *Genetics of Industrial Microorganisms*, O. K. Sebek and A. I. Laskin (eds.) (Washington, D.C.: American Society for Microbiology, 1979), p. 23.

Fig. 3. The Development of a High Penicillin-Producing Strain via Genetic Manipulation

has been widely investigated as a way of developing vigorous, high-yielding antibiotic producers. However, its use has been limited by the mating incompatibility of many industrially important higher fungi, the presence of chromosomal aberrations in micro-organisms improved by mutation, and a number of other problems. Furthermore, natural recombination is most advantageous when strains of extremely diverse origins are mated; the proprietary secrets protecting commercial strains usually prevent the sort of divergent"competitor" strains most likely to produce vigorous hybrids from being brought together.

The technique of protoplast or cell fusion provides a convenient method for establishing a recombinant system in strains, species, and genera that lack an efficient natural means for mating. For example, as many as four strains of the antibiotic-producing bacterium Streptomyces have been fused together in a single step to yield recombinants that inherit genes from four parents. The technique is applicable to nearly all antibiotic producers. It will help combine the benefits developed in divergent lines by mutation and selection.

In addition, researchers at Glaxo Company, Ltd. in England have compared the quality of an antibiotic-producing fungus, Cephalosporium acremonium, produced by mating to one produced by protoplast fusion[15]. They concluded that protoplast fusion was far superior for that purpose. What is more, protoplast fusion can give rise to hundreds of recombinants--including one isolate that consistently produced the antibiotic cephalosporin C in 40 percent greater yield than the best producer among its parents without losing that parent strain's rare capacity to use inorganic sulfate as a source of sulfur rather than expensive methionine. It also acquired the rapid growth and sporulation characteristics of its less-productive parent. Thus, desirable attributes from different parents were combined in an important industrial organisms that had proved resistant to conventional crossing.

Even more significant are the possibilities for cell fusion between different species, thereby creating novel hybrid strains which could have unique biosynthetic properties. One group is reported to have isolated a novel

antibiotic, clearly not produced by either parent, in an organism created through fusion of actinomycete protoplasts.

The development of recombinant DNA technology and techniques for transformation and transfection of protoplasts as well as intact organisms has made possible the exploitation of these methods for gene cloning in actinomycetes and fungi. The rationale for attempting shotgun and self-cloning is that transformants may acquire new genes for enzymatic activities that can modify the chemical structure of secondary metabolites normally produced by the organism to generate new antibiotics.

The basic requirements for utilization of the recombinant DNA method to transfer and express segments of foreign DNA in a host organism include (i) an appropriate vector DNA molecule (plasmid or phage) compatible with the cell and carrying appropriately localized control elements such as promoters and ribosome binding sequences; (ii) a convenient method for preparing the foreign DNA, cleaving it to a reasonable size range, and ligating it into the vector DNA molecule such that it will have a good probability of being expressed: (iii) a method for introducing the recombinant DNA molecules into the host cell so that it is transformed to a new phenotype that includes the properties coded for by the recombinant; and (iv) a method for assaying for the expression of the desired gene products.

There are many options available for altering the genetics of antibiotic-producing microorganisms to realize particular goals. Rational selection methods based on an enhanced understanding of the biosynthetic pathways for antibiotic synthesis can lead to radical improvements in strain productivity. Protoplast fusion technology can be used to allow genetic recombination to occur among different species of antibiotic producers to generate mixed synthetic pathways that may in turn give rise to hybrid antibiotics[16]. Recombinant DNA methods can be used to clone genes for strain improvement and novel antibiotic synthesis into existing microorganisms in either a semi-random or specific manner. The coming decade promises to be filled with excitement and rewards as many of the ideas discussed here and elsewhere are implemented in the laboratories, pilot plants, and ultimately, production systems of the pharmaceutical industry.

Oncogenes and Cancer-

The notion that our own cells actually contain genes, now referred to as oncogenes or cancer-causing genes, having the potential of causing cancerous transformation of those cells is not a comfortable one. Nevertheless, some 20 oncogenes have now been identified that can, when appropriately activated, produce cancer in animals and cause the malignant transformation of special lines of human cultured tissue.

The recent advances in oncogene research and their implications in cancer come from studies on RNA-containing viruses that induce cancers. Such viruses are termed retroviruses. The oncogenes contained in certain retroviruses are not native to viruses nor are they even native to cancer cells. Rather, these genes are present and functioning in normal cells of vertebrates. In fact, they may be essential for the normal cellular function and development, as well as for the unrestrained growth of cancer tissue. The cellular counterparts of the viral oncogenes have been closely conserved throughout evolution. Indeed, they are found in species as diverse as fruit flies, fish, and mammals, including humans and now have been recently discovered in common baker's yeast. Oncogenes have been defined as simply wayward copies of genes found in all metazoan organisms and probably evolved long before the advent of human kind.

Oncogenes code for proteins which appear to be capable of triggering a cascade of biochemical events that ultimately lead to a malignant transformation, eventually resulting in neoplasia. One oncogene product is known as ppbov-src protein which consists of some 520 amino acid subunits. The protein is an enzyme (protein kinase) which adds phosphate groups to amino acids and other protein subunits. The protein is bound to the plasma membrane of cells.

Cellular elements such as these oncogene proteins appear to be at the heart of the cancer process, but just how these proteins work remains unclear at this time. They appear to regulate the growth of cells, with the protein encoded by the normal cellular gene (proto-oncogene) controlling normal growth whereas the altered version of the gene (oncogene) forces cancerous growth.

The cancer-gene concept, supported by oncogene data presently suggests a unifying explanation for various forms of carcinogenesis (Fig. 4). The common

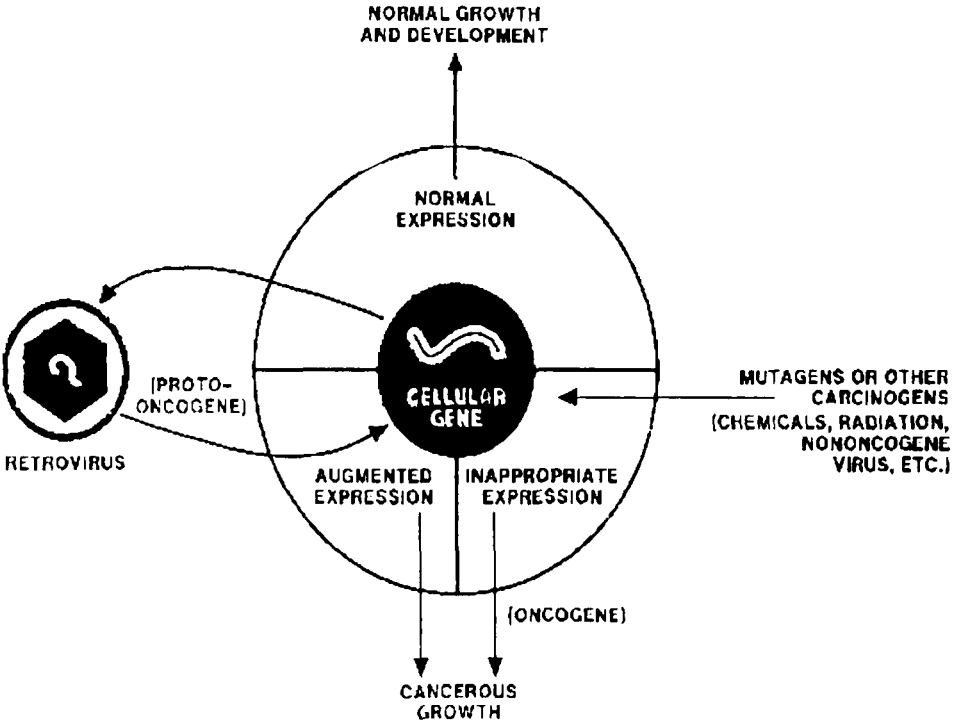


Fig. 4. Oncogene-Cancer Concept (from Bishop[18])

central element is a group of cellular genes required for normal growth and development. When the cellular gene is transplanted into a retrovirus genome, such a gene becomes an oncogene. Cancer can also result if the cellular gene is changed by any of a wide variety of mutagens and other carcinogens. A single point mutation in a single oncogene segment consisting of only 350 nucleotides can result in a gene transformation of a proto-oncogene which when transformed develops into a EJ-bladder carcinoma tissue [17]. Growth factors also appear to be involved in the transformation process and appear to stimulate the rapid cell division process typical of cancerous growth.

An understanding of the precise function of oncogene proteins and their exact involvement in the phosphorylation cascade and combined growth factor involvement may make it possible to develop drug antagonists that inhibit key steps in this intricate biochemical interaction, so that a new therapy could be targeted to the few central defects of a transformed cancerous cell[18,19].

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

SOURCE: Office of Technology Assessment.

Fig. 5. Major Events in the Commercialization of Biotechnology

Future Roles for Biotechnology in the Pharmaceutical Industry

The past decade of biotechnology has resulted in a rapid development of a new biotechnology industry. A number of the major events important for the expansion of this technology are summarized in Figure 5. Future expanded government training grants for biotechnologists and increased funding for molecular biology programs at the NIH, NCI and other government laboratories will accelerate biotechnology and its numerous applications for the improvement of man's well-being. The increased government biotechnology funding coupled with the greatly expanded pharmaceutical R&D budgets for molecular

biology and biotechnology will culminate in an increased understanding of many disease mechanisms. Hopefully, this understanding will result in the discovery and development of many new, more effective pharmaceutical products. Areas of research that will benefit pharmaceutical innovation in biotechnology are highlighted below:

1) Clarification of the functions and mechanisms of action of immune regulators such as the interferons, interleukins and related growth factors,
2) Investigation into the clinical use of neuroactive, thrombolytic and fibrinolytic peptides,
3) Development of improved drug delivery systems,
4) Clarification of mechanisms of acquired immunity leading to better vaccine development procedures,
5) Development of monoclonal antibody technology, ultrafiltration and reverse osmosis technology for the purification of enzymes and other biological response modifier proteins,
6) Development of the use of monoclonal antibody technology for the early detection of disease through use of diagnostic kits, tests, etc.
7) Development of the ability to culture and increase man's understanding of the infection cycle of debilitating fungal pathogens and protozoan disease parasites,
8) Use of monoclonal antibody technology for the targeting of existing and new non-toxic anticancer molecules to tumor foci; and
9) An increased understanding on how oncogene proteins ultimately trigger the transformation of normal cells to neoplastic cells via a protein phosphorylation cascade, growth factor involvement, etc. Such understanding may lead to more rational, non-toxic cancer therapy.

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