Chapter 4

The Pharmaceutical Industry

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Background

The domestic sales of prescription drugs by U.S. pharmaceutical companies exceeded $7.5 billion in 1979. Of these, approximately 20 percent were products for which fermentation processes played a significant role. They included anti-infective agents, vitamins, and biological, such as vaccines and hormones. Genetics is expected to be particularly useful in the production of these pharmaceuticals and biological) which can only be obtained by extraction from human or animal tissues and fluids.

Although the pharmaceutical industry was the last to adopt traditional fermentation technologies, it has been the first industry to make widespread use of such advanced genetic technologies as recombinant DNA (rDNA) and cell fusion. Two major factors triggered the use of genetics in the pharmaceutical industry:

- The biological sources of many pharmacologically active products are micro-organisms, which are readily amenable to genetic engineering.
- The major advances in molecular genetic engineering have been made under an institutional structure that allocates funds largely to biomedical research. Hence, the Federal support system has tended to foster studies that have as their ostensible goal the improvement of health.

Two factors, however, have tended to discourage the application of genetics in the chemcal and food industries. In the former, economc considerations have not allowed biological production systems to be competitive with the existing forms of chemical conversion, with rare exceptions. And in the latter, social and institutional considerations have not favored the development of foods to which genetic engineering might make a contribution.

Past uses of genetics

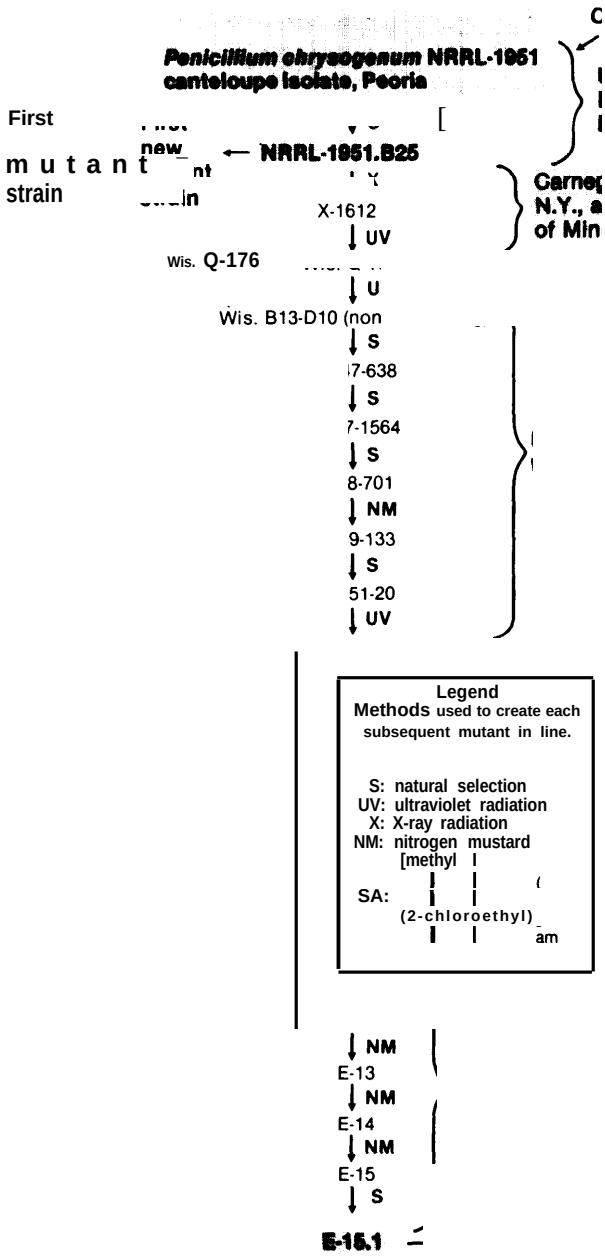
Genetic manipulation of biological systems for the production of pharmaceuticals has two general goals:

1. to increase the level or efficiency of the production of pharmaceuticals with proven or potential value; and
2. to produce totally new pharmaceuticals and compounds not found in nature.

The first goal has had the strongest influence on the industry. It has been almost axiomatic that if a naturally occurring organism can produce a pharmacologically valuable substance, genetic manipulation can increase the output. The following are three classic examples.

- The genetic improvement of penicillin production is an example of the elaborate long-term efforts that can lead to dramatic increases. The original strains of ***Penicillium chrysogenum, NRRL-1951***, were treated with chemicals and irradiation through successive stages, as shown in figure **20**, until the strain E-15.1 was developed. This strain had a 55-fold improvement in productivity over the fungus in which penicillin was originally recognized–the Fleming strain.
- Chemically induced mutations improved a strain of ***Escherichia coli*** to the point where it produced over 100 times more L-asparaginase (which is used to fight leukemia) than the original strain. This increase made the task of isolating and purifying the pharmaceutical much easier, and resulted in lowering the cost of a course of therapy from nearly $15,000 to approximately $300.
- Genetic manipulation sufficiently improved the production of the antibiotic, gentami-

Figure 20.—The Development of a High Penicillin= Producing Strain via Genetic Manipulation



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. ament f in *Genetics of Industrial Microorganisms, O.* K. Sebek and A. 1. Sebe (Washington, D.C.: American Society for Microbiology, 1979), 23.

cin, so that Schering-Plough, its producer, did not have to build a scheduled manufacturing plant, thereby saving $50 million.

Most industry analysts agree that, overall, genetic manipulation has been highly significant in increasing the availability of many pharmaceuticals or in reducing their production costs.

The second major goal of genetic manipulation, the production of new compounds, has been achieved to a lesser degree. A recent new antibiotic, deoxygentamicin, was obtained by mutation and will soon be clinically tested in man. Earlier, an important new antibiotic, amikacin, was produced through classical molecular genetic techniques. And before that, the well-known antibiotic, tetracycline, which is normally not found in nature, was produced by a strain of the bacterium, ***Streptomyces,*** after appropriate genetic changes had been carried out in that bacterium.

Potential uses of molecular genetic technologies —

Polypeptides—proteins—are the first abundant end products of genes. They include peptide hormones, enzymes, antibodies, and certain vaccines. Producing them is the goal of most current efforts to harness genetically directed processes. However, it is just a matter of time and the evolution of technology before complex nonproteins like antibiotics can also be manufactured through rDNA techniques.

Hormones

The most advanced applications of genetics today, in terms of technological sophistication and commercial development, are in the field of hormones, the potent messenger molecules that help the body coordinate the actions of various tissues. (See Tech. Note 1, p. 80.) The capacity to synthesize proteins through genetic engineering has stemmed in large part from attempts to prepare human peptide hormones (like insulin and growth hormone). The diseases caused by their deficiencies are presently treated with extracts made from animal or human glands.

The merits of engineering other peptide hormones depend on understanding their actions and those of their derivatives and analogs. Evidence that they might be used to improve the treatment of diabetes, to promote wound healing, or to stimulate the regrowth of nerves will stimulate new scientific investigations. Other relatively small polypeptides that influence the sensation of pain, appetite suppression, and cognition and memory enhancement are also being tested. If they prove useful, they will unquestionably be evaluated for production via fermentation.

While certain hormones have already attained a place in pharmacology, their testing and use has been hindered to some extent by their scarcity and high cost. Until recently, animal glands, human-cadaver glands, and urine were the only sources from which they could be drawn. Their use is also limited because polypeptide hormones must be administered by injection. They are digested if they are taken orally, a process that curtails their usefulness and causes side-effects.

There are four technologies for producing polypeptide hormones and polypeptides:

- . extraction from human or animal organs, serum, or urine;
- chemical synthesis;
- production by cells in tissue culture; and
- production by microbial fermentation after genetic engineering.

One major factor in deciding which technology is best for which hormone is the length of the hormone's amino acid chains. (See table 3.) Modern methods of chemical synthesis have made the preparation of low-molecular weight polypeptides a fairly straightforward task, and chemically synthesized hormones up to at least 32 amino acids (AA) in length—like calcitonin

Table 3.—Large Human Polypeptides Potentially Attractive for Biosynthesis

	Amino acid residues	Molecular weight
Proactin	198	
Placental lactogen	192	
● Growth hormone	191	*22,005*
Nerve growth factor	118	*13,000*
Parathyroid hormone (PTH)	84	*9,562* bovine
	82	
Insulin-like growth factors (IGF-I & IGF-2)	*70,67*	7,649,7471
Epidermal growth factor		6,100
● insulin	51	5,734
Thymopoietin	49	
Gastric inhibitory polypeptide (GIP)	43	5,104 porcine
● Corticotropin (ACTH)	39	4,567 porcine
Cholecystokinin (CCK-39)	39	
Big gastrin (BG)	34	
Active fragment of PTH	34	4,109 bovine
Cholecystokinin (CCK-33)	33	3,918 porcine
● Calcitonin	32	3,421 human 3,435 salmon
Endorphins	31	3,465
● Glucagon	29	3,483 porcine
Thymosin-a1	28	3,108
Vasoactive intestinal peptide (VIP)	28	3,326 porcine
● Secretin	27	
● Active fragment of ACTH	24	
Motilin	22	2,698

● Currently used in medical practice.

SOURCE: Office of Technology Assessment.

–have become competitive with those derived from current biological sources. Since fragments of peptide hormones often express activities comparable or sometimes superior to the intact hormone, a significant advantage of chemical synthesis for research purposes is that analogs having slight pharmacological differences from natural hormones can be prepared by incorporating different amino acids into their structures. In principle however, genetically engineered biosynthetic schemes can be devised for most desirable peptide hormones and their analogs, although the practicality of doing so must be assessed on a case-by-case basis. Ultimately, the principal factors bearing on the practicality of the competing alternatives are:

- The cost of raw materials. For genetically engineered biosynthesis, this includes the cost of the nutrient broth plus some amortization of the cost of developing the synthetic organism. In the case of chemical synthesis, it includes the cost of the pure amino acid subunits plus the chemicals used as activating, protecting, coupling, liberating, and supporting agents in the process.
- The different costs of separating the desired product from the cellular debris and the culture medium in biological production, and from the supporting resin, byproducts, and excess reagents in chemical synthesis.
- The cost of purification and freedom from toxic contaminants. The process is more expensive for biologically produced material than for materials produced by conventional chemistry, although hormones from any source can be contaminated.
- Differences in the costs of labor and equipment. Chemical synthesis involves a sequence of similar (but different) operations during a time period roughly proportional to the length of the amino acid chain (three AA per day) in an apparatus large enough to produce 100 grams (g) to 1 kilogram (kg) per batch; biological fermentations use vats —with capacities of several thousand gallons–for a few days, regardless of the length of the amino acid chain.
- The cost and suitability of comparable materials gathered from organs or fluids obtained from animals or people.

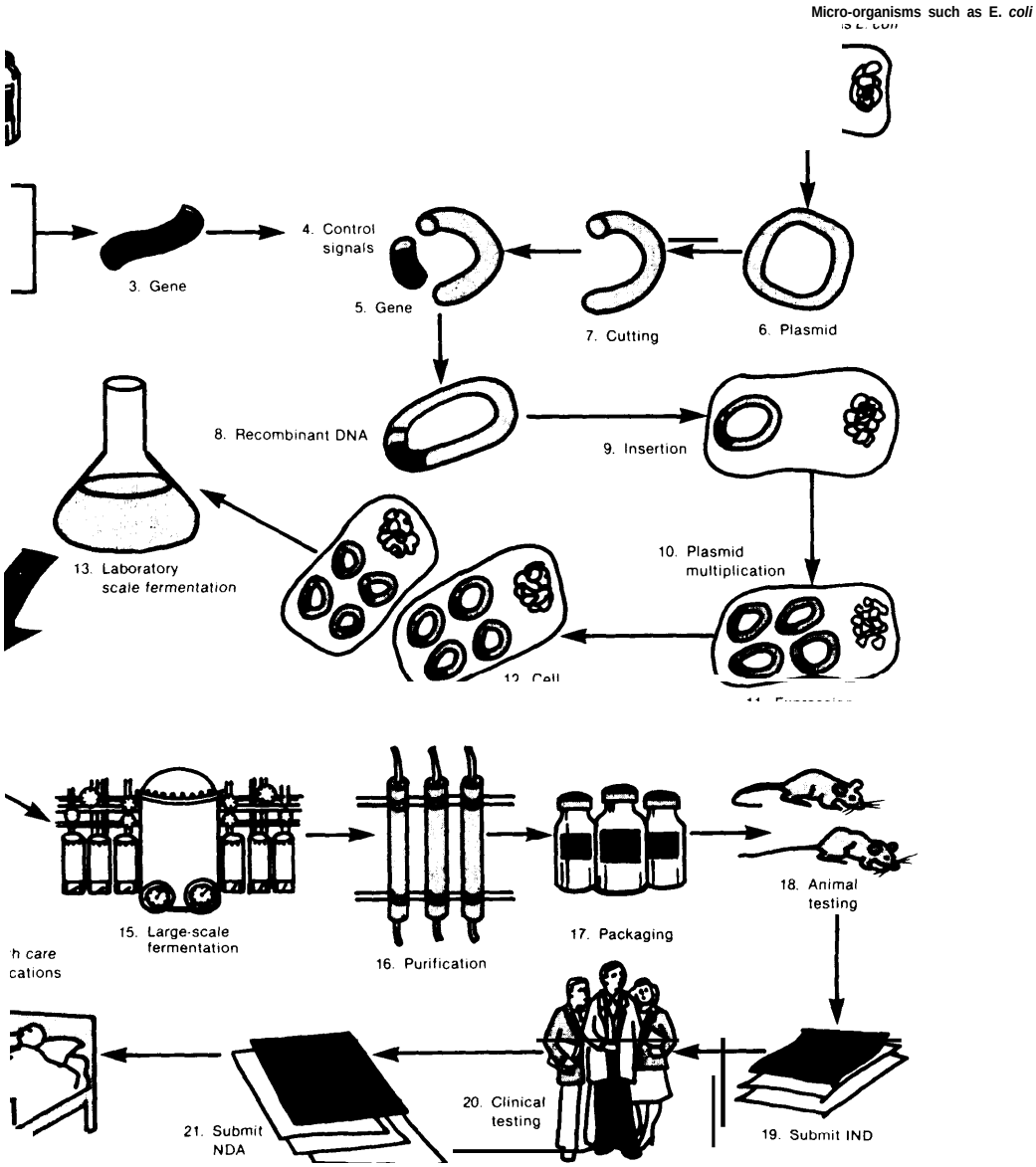
In the past decade, some simpler hormones have been chemically synthesized and a few are being marketed. However, synthesizing glycoproteins—proteins bound to carbohydrates—is still beyond the capabilities of chemists. Data obtained from companies directly involved in the production of peptides by chemical synthesis indicate that the cost of chemically preparing polypeptides of up to 50 AA in length is extremely sensitive to volume (see Tech. Note 2, p. 80,); although the costs are high, the production of large quantities by chemical synthesis offers a competitive production method.

Nevertheless, rDNA production, also known as molecular cloning, has already been used to produce low-molecular weight polypeptides. In 1977, researchers at Genentech, Inc., a small biotechnology company in California, inserted a totally synthetic DNA sequence into an E. coli plasmid and demonstrated that it led to the production of the 14 AA polypeptide sequence corresponding to somatostatin, a hormone found in the brain. The knowledge of somatostatin's amino acid sequence made the experiment possible, and the existence of sensitive assays allowed the hormone's expression to be detected. Although the primary motive for using this particular hormone for the first demonstration was simply to show that it could be done, Genentech has announced that it plans to market its genetically engineered molecule for research purposes. (See figure 21.)

Somatostatin is one of about 20 recognized small human polypeptides that can be made without difficulty by chemical synthesis. (See table 4.) Unless a sizable market is found for one of them, it is unlikely that fermentation meth ods will be developed in the foreseeable future Some small peptides that may justify the devel opment of a biosynthetic process of production are:

- The seven AA sequence known as MSH ACTH 4-10, which is reputed to influence memory, concentration, and other psyche logical-behavioral effects: should such

Figure 21.—The Product Development Process for Genetically Engineered Pharmaceuticals



The development process begins by obtaining DNA either through organic synthesis (1) or derived from biological sources such as tissues (2). The DNA obtained from one or both sources is tailored to form the basic "gene" (3) which contains the genetic information to "code" for a desired product, such as human interferon or human insulin. Control signals(4) containing plasmids (6) are isolated from micro-organisms such as E. *coli;* cut open (7) and spliced back (8) together with genes and control signals to form "recombinant DNA" molecules. These molecules are then introduced into a host cell (9).

Each plasm id is copied many times in a cell (10). Each cell then translates the information contained in these plasmids into the desired product, a process called "expression" (11). Cells divide (12) and pass on to their offspring the same genetic information contained in the parent cell.

Fermentation of large populations of genetically engineered micro-organisms is first done in shaker flasks (13), and then in small fermenters (14) to determine growth conditions, and eventually in larger fermentation tanks (15). Cellular extract obtained from the fermentation process is then separated, purified (16), and packaged (17) for health care applications.

Health care products are first tested in animal studies (18) to demonstrate a product's pharmacological activity and safety, In the United States, an investigational new drug application (19) is submitted to begin human clinical trials to establish safety and efficacy. Following clinical testing (20), a new drug application (N DA) (21) is filed with the Food and Drug Administration (FDA). When the NDA has been reviewed and approved by the FDA the product maybe marketed in the United States (22).

SOURCE: Genentech, Inc.

Table 4.-Naturally Occurring Small Peptides of Potential Medical interest

	Number of amino acids	Molecular weight
Dynorphin	17	
Little gastrin (LG)	17	2,178
Somatostatin.	14	1,639
Bombesin	14	1,620
Melanocyte stimulating hormone.	13	1,655
Active dynorphin fragment	13	
Neurotensin	13	
Mini-gastrin (G13)	13	
Substance?	11	I,347bovine
Luteinizing hormone-releasing hormone(LNRH).	10	
Active fragment of CCK.	10	
Angiotensin I	10	1,297
Caerulein	10	
Bradykinin	9	1,060
● Vasopressin(ADH)	9	
● Oxytocin	9	1,007
Facteur thymique serique(FTH)..	9	
Substance P(4-11)octapeptide.	8	
Angiotensin II	8	1,046
Angiotensin III.	7	931
MSH/ACTH4-10	7	
Enkephalins.	5	*575*
Active fragment of thymopoietin (TP5).	5	
● Thyrotropin releasing hormone (TRY)	3	362

.Currently used in medical practice.

SOURCE: Office of Technology Assessment

agents prove of value in wider testing, they have an enormous potential for use.

Both cholecystokinin (33 AA) and bombesin (10 AA), which have been shown to suppress appetite, presumably as a satiety signal from stomach to brain: there is a large market for antiobesity agents–approximately $85 million per year at the manufacturer's level.

Several hormones, such as somatostatin, which are released by nerves in the hypothalamus of the brain to stimulate or inhibit release of hormones by the pituitary gland: hormones produced by these glands are crucial in human fertility; analogs of some are being investigated as possible contraceptives.

Calcitonin (32 AA), which is currently the largest polypeptide produced by chemical synthesis for commercial pharmaceutical use: it is useful for pathologic bone disorders, such as Paget's disease, that affect up to 3 percent of the population over 40 years of age, in WesternEurope.

. Adrenocorticotropic hormone (ACTH) (39 **AA), which promotes and maintains the normal growth and development of the adrenalglands and stimulates the secretion of other hormones: in the United States, ACTH is used primarily as a diagnostic agent for adrenal insufficiency, but in principle, ACTH might be used for at least one-third of the medical indications—like rheumatic disorders, allergic states, and eye inflammation—for which about 5 million Americans annually receive corticosteroids.**

Within the last 5 years, other small polypeptides have been identified in many tissues and have been linked to a variety of activities. Some certainly bind to the same receptor sites as the pain-relieving opiates related to the morphine family. These peptides are called endogenous opiates: the smaller (5 AA) peptides are called enkephalins and the larger (3 AA), endorphins.

Certain enkephalins produce brief analgesia when injected directly into the brains of mice. Synthetic analogs that are less susceptible to enzymatic inactivation produce longer analgesia even if they are injected intravenously, as does the larger endorphin molecule. Very recently, a 17 AA polypeptide, dynorphin, was reported to be the most potent pain killer yet found—it is 1,200 times more powerful than morphine.

The preparation of new analgesic agents appears a likely outcome of the new research, but problems similar to those associated with classical opiates must be overcome. Consequently, unnatural analogs—including some made with amino acids not found in micro-organisms—might prove more useful. The value of microbial biosynthesis for these substances is questionable at this time. However, the importance of genetic technologies in clarifying the underlying mechanisms should not be underestimated.

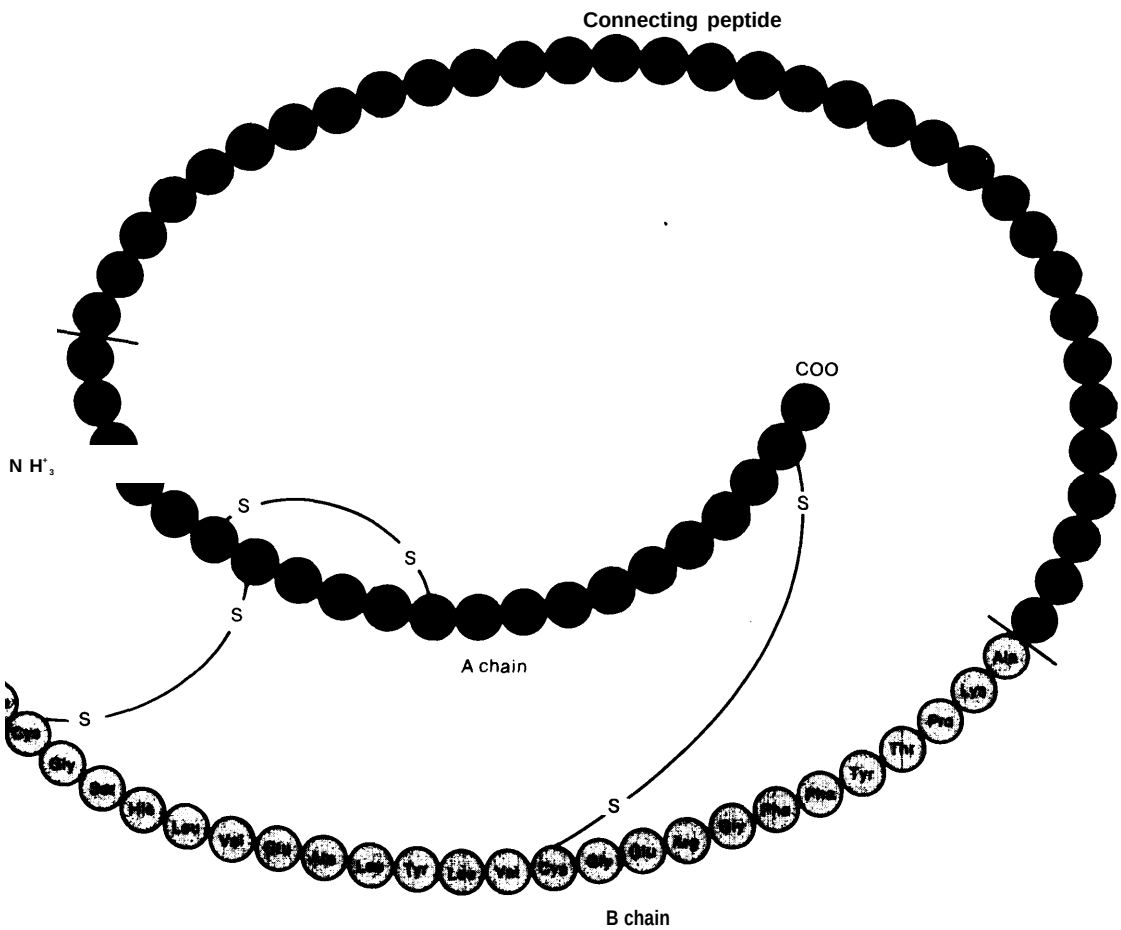
Higher molecular weight polypeptides cannot be made practically by chemical synthesis, and must be extracted from human or animal tissues or produced in cells growing in culture.

Now they can also be manufactured by fermentation using genetically designed bacteria, as has been demonstrated by the production of insulin and human growth hormone.

INSULIN

Insulin, is composed of two chains—A and B—of amino acids. It is initially produced as a single, long chain called pre-proinsulin, which is cut into a shorter chain, proinsulin. Proinsulin, in turn, is cut into the A and B chains when a piece is cleaved from the middle. (See figure 22.) Work on the genetic engineering of insulin has proceeded quickly. A year after one group reported that the insulin gene had been incorporated into E. coli without expression, a second group managed to grow colonies of E. ***coli*** that actually excreted rat proinsulin. Then, within a couple of months, workers at Genentech, in collaboration with a group at City of Hope Medical Center, announced the separate synthesis of the A (21 AA) and B (30 AA) chains of human insulin. The synthesis of the DNA sequences depended on advances in organic chemistry as well as in genetics. Six months were required simply to synthesize the necessary building blocks.

Figure 22.—The Amino Acid Sequence of Proinsulin



Proinsulin is composed of 84 amino acid residues. When the connecting peptide is removed, the retaining A and B chains form the insulin molecule. The A chain contains 21 amino acids; the B chain contains 30 amino acids.

SOURCE: Office of Technology Assessment.

A comparison with the traditional source of animal insulin is interesting. If 0.5 milligram (mg) of pure insulin can be obtained from a liter of fermentation brew, 2,000 liters (1) (roughly 500 gal) would yield 1 g of purified insulin—the amount produced by about 16 lb of animal pancreas. If, on the other hand, the efficiency of production could be increased to that achieved for asparaginase (which is produced commercially by the same organism, E. coli), 2,000 would yield 100 g of purified insulin—the amount extracted from 1,600 lb of pancreas. (The average diabetic uses the equivalent of about 2 mg of animal insulin per day.)

The extent of the actual demand for insulin is a controversial issue. Eli Lilly & Co. estimates that there are 60 million diabetics in the world (35 million in underdeveloped countries, where few are diagnosed or treated). Of the 25 million in the developed countries, perhaps 15 million have been diagnosed; according to Lilly's estimate, 5 million are treated with insulin. Only one-fourth of those diabetics treated with insulin live in the United States, but they use 40 to 50 percent of the insulin consumed in the world. A number of studies indicate that while the emphasis on diet (alone) and oral antidiabetic drugs varies, approximately 40 percent of American patients in large diabetes clinics or practices take insulin injections. In the United States, diabetes ranks as the fifth most common cause of death and second most common cause of blindness. Roughly 2 million persons require daily injections of insulin.

Today, at least, there is no real shortage of glands from slaughter houses for the production of animal (principally bovine and porcine) insulin. A study conducted by the National Diabetes Advisory Board (NDAB) concluded that a maximum demand and a minimum supply would lead to shortages in the 1990's. Eli Lilly's projection, presented in that report, also anticipates these shortages. But, Novo Industri, a major world supplier of insulin, told the NDAB that it estimates that the 1976 free-world consumption of insulin of 51 X 10^9 units constituted only 23 percent of the potential supply, and the 87X 10^9 units projected for 1996 would only equal 40 percent of the supply, assuming that the animal population stays constant.

For insulin, therefore, the limitation on bringing the fruits of genetic engineering to the marketplace is not technological but institutional. The drug must first be approved by the Food and Drug Administration (FDA) and then marketed as a product as good as or better than the insulin extracted by conventional means. Lilly has stated that it anticipates a 6-month testing period in humans. Undoubtedly, FDA will examine the evidence presented in the investigational new drug application (INDA) with special care. Its review will establish criteria that may influence the review of subsequent applications in at least the following requirements:

- evidence that the amino acid sequence of the material is identical to that of the normal human hormone;
- freedom from bacterial endotoxins that may cause fever at extremely low concentrations—an inherent hazard associated with any process using E. ***coli;*** and
- freedom from byproducts, including substances of very similar structure that may give rise to rare acute or chronic reactions of the immune system.

Furthermore, as development continues, FDA might require strict assurances that the molecules produced from batch to batch are not subject to subtle variations resulting from their genetic origin.

If the insulin obtained from rDNA techniques manages to pass FDA requirements, it must overcome a second obstacle—competition in the marketplace. The clinical rationale for using human rather than animal insulin rests on the differences in structure among insulins produced by different species. Human and porcine insulins for example, differ in a single amino acid, while human and cattle insulins differ with respect to three. As far as is known, these variations do not impair the effectiveness of the insulin, but no one has ever been in a position to conduct a significant test of the use of human insulin in a diabetic population. Many consequences of the disease, such as retinopathy (retinal disease) and nephropathy (kidney disease), are not prevented by routine injection of animal insulin. Patients also occasionally respond adversely or produce antibodies to animal insulin, with subsequent allergic or resistant reaction.

It remains to be seen how many patients will be better off with human insulin. The proof that it improves therapy will take years. Progress on the etiology of the disease—especially in identifying it in those at risk or in improving the dosage form and administration of insulin–may have far more significant effects than new developments in insulin production. Nevertheless, as long as private enterprise sees fit to invest in such developments, and as long as the cost of treating diabetics who respond properly to animal insulin is not increased, biological production of human insulin may become a kind of insurance for diabetics within the next few decades.

GROWTH HORMONE

The second polypeptide hormone currently a candidate for FDA approval is growth hormone (GH). It is one of a family of closely related, relatively large pituitary peptide hormones—single-chain polypeptides 191- to 198-AA in length. It is best known for the growth it induces in many soft tissues, cartilage, and bone, and it is a requirement for postnatal growth in man.

The growth of an organism is a highly complex process that depends on the correct balance of many variables: The action of GH in the body for example, depends on the presence of insulin, whose secretion is stimulated by GH. Under some circumstances, one or more intermediary polypeptides produced under the influence of GH by the liver (and possibly the kidneys) may actually be the proximate causes of some of the effects attributed to GH. In any case, the biological significance of GH is most clearly illustrated by the growth retardation that characterizes its absence before puberty, and by the benefits of replacement therapy.

In the United States, most of the demand for human growth hormone (hGH) is met by the National Pituitary Agency, which was created in the early 1960's by the College of Pathologists and the National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD) to collect pituitary glands from coroners and private donors. Under the programs of the NIAMDD, hGH is provided without charge to treat children with hypopituitarism, or dwarfism (about 1,600 patients, each of whom receives therapy for several years), and for research.

While the National Pituitary Agency feels that it can satisfy the current demand for hGH (see Tech. Note 3, p. 80.), it welcomes the promise of additional hGH at relatively low cost to satisfy areas of research that are handicapped more by a scarcity of funds than by a scarcity of the hormone. However, if hGH is shown to be therapeutically valuable in these areas, widespread use could severely strain the present supply. At present, the potential seems greatest for patients with:

- senile osteoporosis (bone decalcification);
- other nonpituitary growth deficiencies such as Turner's syndrome (1 in 3,000 live female births);
- intrauterine growth retardation;
- bleeding ulcers that cannot be controlled by other means; and
- burn, wound, and bone-fracture healing

Two groups have already announced the preparation of micro-organisms with the capacity for synthesizing GH. (See Tech. Note 4, p. 80.) In December 1979, one of these groups—Genentech —requested and received permission from the National Institutes of Health (NIH), on the recommendation of the Recombinant DNA Advisory Committee (RAC), to scale-up its process. Its formation of a joint-venture with Kabi Gen AB is typical of the kind of alliance that develops as a result of the different expertise of groups in the multidisciplinary biomedical field. Kabi has been granted a New Drug Application (NDA) under which to market pituitary GH imported from abroad.

OTHER HORMONES

Additional polypeptide hormones targeted for molecular cloning (rDNA production) include:

- Parathyroid hormone (84 AA), which may be useful alone or in combination with calcitonin for bone disorders such as osteoporosis.
- Nerve growth factor (118 AA), which influences the development, maintenance, and

repair of nerve cells and thus could be significant for nerve restoration in surgery.
- Erythropoietin, a glycopeptide that is largely responsible for the regulation of blood cell development. Its therapeutic applications may range from hemorrhages and burns to anemias and other hematologic conditions. (See Tech. Note 5, p. 80.)

Immunoproteins

Immunoproteins include all the proteins that are part of the immune system—antigens, interferon, cytokines, and antibodies. Since polypeptides, the primary products of every molecular cloning scheme, are at the heart of immunology, developments made possible by recent breakthroughs will presumably affect the entire field. There is little doubt that applied genetics will play a critical role in developing a pharmacology for controlling immunologic functions, since it provides the only apparent means of synthesizing many of the agents that will comprise immunopharmacology.

ANTIGENS (VACCINES)

One early dramatic benefit should be in the area of vaccination, where genetic technologies may lead to the production of harmless substances capable of eliciting specific defenses against various stubborn infectious diseases.

Vaccination provides effective immunity by introducing relatively harmless antigens into the immune system thereby allowing the body to establish, in advance, adequate levels of antibody and a primed population of cells that can grow when the antigen reappears in its virulent form. Obviously, however, the vaccination itself should not be dangerous. As a result, several methods have been developed over the past two centuries to modify the virulence of micro-organisms used in vaccines without destroying their ability to trigger the production of antibodies. (See Tech. Note 6, p. 80.)

Novel pure vaccines based on antigens synthesized by rDNA have been proposed to fight communicable diseases like malaria, which have resisted classical preventive efforts. Pure vaccines have always been scarce; if they were available, they might reduce the adverse effects of conventional vaccines and change the methods and the dosages in which vaccines are administered.

Some vaccines are directed against toxic proteins (like the diphtheria toxin produced by some organisms), preparing the body to neutralize them. Molecular cloning might make it possible to produce inactivated toxins, or better nonvirulent fragments of toxins, by means of micro-organisms that are incapable of serving as disease-causing organisms.

Immunity conferred by live vaccines invariably exceeds that conferred by nonliving antigenic material–possibly because a living micro-organism creates more antigen over a longer period of time, providing continuous "booster shots." Engineered micro-organisms might become productive sources of high-potency antigen, offering far larger, more sustained, doses of vaccine without the side-effects from the contaminants found in those vaccines that consist of killed micro-organisms.

However, it is clear that formidable Federal regulatory requirements would have to be met before permission is granted for a novel living organism to be injected into human subjects. Because of problems encountered with live vaccines, the most likely application will lie in the area of killed vaccines (often using only ***parts*** of micro-organisms).

It is impossible in the scope of this report to discuss the pros, cons, and consequences of developing a vaccine for each viral disease. However, the most commercially important are the influenza vaccines, with an average of 20.8 million doses given per year from 1973 to 1975–a smaller number than the 25.0 million doses per year of polio vaccine, but more profitable.

Influenza is caused by a virus that has remained uncontrolled largely because of the frequency with which it can mutate and change its antigenic structures. It has been suggested that antigenic protein genes for influenza could be kept in a "gene bank" and used when needed. In addition, the genetic code for several antigens could be introduced into an organism such as E.

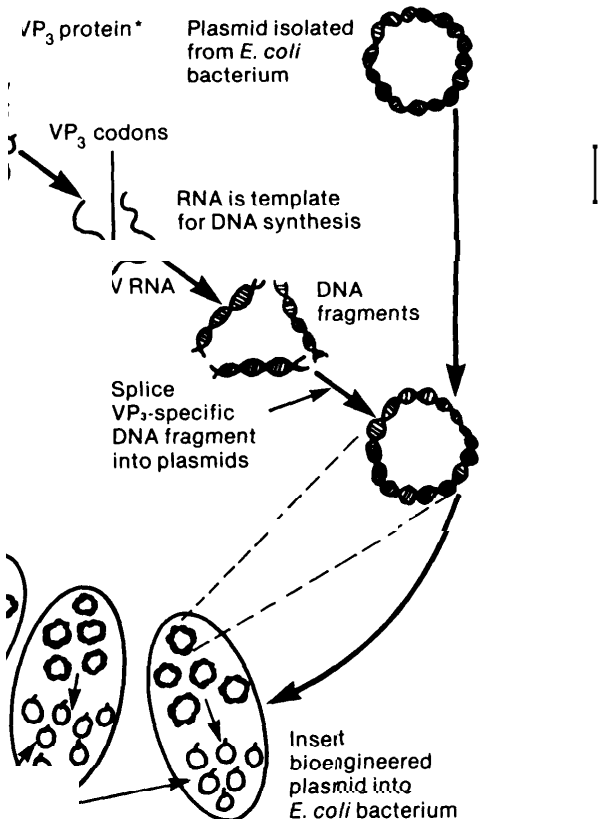
coli, so that a vaccine with several antigens might be produced in one fermentation. [1]

Two more viral diseases deserve at least brief comment. Approximately 800 million doses of foot-and-mouth disease virus (FMDV) vaccine are annually used worldwide, making it the largest volume vaccine produced. This vaccine must be given frequently to livestock in areas where the disease is endemic, which includes most of the world outside of North America. The present methods of producing the vaccine require that enormous quantities of hazardous virus be contained. Many outbreaks are attributed to incompletely inactivated vaccine or to the escape of the virus from factories. (See figure 23.)

Molecular cloning of the antigen could produce a stable vaccine at considerably less expense, without the risk of the virus escaping. On the basis of that potential, RAC has approved a joint program between the U.S. Department of Agriculture (USDA) and Genentech to clone pieces of the FMDV genome to produce pure antigen. The RAC decision marked the first exception to the NIH prohibition against cloning DNA that is derived from a virulent pathogen. [2] FMDV. vaccine made by molecular cloning will probably be distributed commercially by 1985, although not in the United States. It will be the first vaccine to achieve that status, and illustrates the potential veterinary uses of genetic technologies.

Hepatitis has also received significant attention. Vaccines against viral hepatitis, which affects some 300,000 Americans each year, may be produced by molecular cloning. This disease is second only to tuberculosis as a cause of death among reportable infectious diseases. R is extremely difficult to cultivate the causative agents. Hepatitis A has a good chance of being the first human viral disease for which the initial preparation of experimental vaccine will involve molecular cloning. A vaccine against hepatitis B, made from the blood of chronic carriers, is in the testing stage, but cloning is being investigated as a better source of an appropriate antigen. The causative agent for a third form of hepatitis has not even been identified. Since at least 16 million U.S. citizens are estimated to be at high risk of contracting hepatitis, there is keen interest in the development of vaccines among academic and industrial researchers. [3]

Figure 23.-Recombinant DNA Strategy for Making Foot-and-Mouth Disease Vaccine



Growing *E. co/i* bacteria may produce VP_3 for use as vaccine for foot-and-mouth disease. No virus or infectious RNA is produced by the harmless bacteria strain.

● VP_3 is the protein from the shell of the virus, which can act as a vaccine for immunizing livestock against foot-and-mouth disease. The idea outlined above is to make this protein without making any virus or infectious RNA.

SOURCE: Office of Technology Assessment.

[1] For **other aspects of vaccine production see: Office of Technology Assessment, U.S. Congress,** *working Papers, The impacts of* [illegible] *vol. 2.* **(Springfield, Va.: National Technical Information Service,** 1981).

[2] **Ibid.**

More hypothetically, molecular cloning may lead to three other uses of antigens as well: vaccination against parasites, such as malaria and hookworm (see Tech. Note 7, p. 80.); immunization in connection with cancer treatment; and counteracting abnormal antibodies, which are made against normal tissues in the so-called "autoimmune diseases, " such as multiple sclerosis. (See Tech. Note 8, p. 81.)

INTERFERON

Interferon are glycoproteins normally made by a variety of cells in response to viral infection. All interferon (see Tech. Note 9, p. 81) can induce an antiviral state in susceptible cells. In addition, interferon has been found to have at least 15 other biochemical effects, most of which involve other elements of the immune system.

promising preliminary studies have supported the use of interferon in the treatment of such viral diseases as rabies, hepatitis, varicella-zoster (shingles), and various herpes infections. To date, the effect of interferon has been far more impressive as a prophylactic than as a therapeutic agent. The interferon produced by Genentech, for example, has been shown to protect squirrel monkeys from infection by the lethal myocarditis virus. Once interferon is available in quantity, large-scale tests on human populations can be conducted to confirm its efficacy in man.

Several production techniques are being explored. (See Tech. Note 10, p. 81.) Extraction of interferon from leukocytes (white blood cells), the current method of choice, may have to compete with tissue culture production as well as rDNA. (See table 5.)

Recombinant DNA is widely regarded as the key to mass production of interferon, and important initial successes have already been achieved. Each of the four major biotechnology companies is working on improved production methods, and all have reported some success.

An enormous amount remains to be learned about the interferon system. It now appears that the interferon are simply one of many families of molecules involved in physiological regulation of response to disease. Only now have molecular biology and genetics made their study—and perhaps their use—possible.

Table 5.—Summary of Potential Methods for Interferon Production

Means of Production	Types of interferon produced	Potential for scale-up	Present projected ($/10^6 units)	Problems	Potential for improvement
"Buffy coat" leukocytes	leukocyte, 95% fibroblast, 5%	No	50 –	—lack of scale up —pathogen contamination	—minimal
Lymphoblastoid cells	leukocyte, 80% fibroblast, 20%	Yes	—	—poor yields —cells derived from tumor	—improved yields —expression of fibroblast interferon
Fibroblasts	fibroblast	Yes	43-200 、 1-10	—cell culture —economic competition with recombinant DNA	—improved yields —improved cell-culture technology —expression of leukocyte-type interferon
Recombinant DNA	leukocyte or fibroblast	Yes	—	—does not produce interferon —in vitro drug stability —poor yields —drug approval —possible economic competition with fibroblast cell production	—improved yields —modified interferon

SOURCE: Office of Technology Assessment.

The interferon are presently receiving attention largely because studies in Sweden and the United States stimulated the appropriation of $5.4 million by the American Cancer Society (ACS) for expanded clinical trials in the treatment of cancer. That commitment by the nonprofit ACS–the greatest by far in its history–was followed by a boost in NIH funding for interferon research from $7.7 million to $19.9 million for fiscal year 1980. Much of the cost of interferon research is allotted to procuring the glycopeptide. Initially, the ACS bought 40 billion units of leukocyte interferon from the Finnish Red Cross for $50 per million units. In March 1980, Warner-Lambert was awarded a contract to supply the National Cancer Institute (NCI) with 50 billion units of leukocyte interferon within the next 2 years at an average price of $18 per million units. NCI is also planning to purchase 50 billion units each of fibroblast and lymphoblastoid interferon.

The bulk of the NIH funding is included in NCI's new Biological Response Modifier (BRM) program—interferon accounts for $13.9 million of the $34.1 million allocated for BRM work in fiscal year 1980. (NCI expenditures on interferon in 1979 were $2.6 million, 19 percent of the amount budgeted for 1980.) Other important elements of that BRM program concern immunoproteins known as lymphokines and thymic hormones, for which molecular genetics has major implications. The program is aimed at identifying and testing molecules that control the activities of different cell types.

LYMPHOKINES AND CYTOKINES

Lymphokines and cytokines are regulatory molecules that have begun to emerge from the obscure fringes of immunology in the past 10 years. (Interferon is generally considered a lymphokine that has been characterized sufficiently to deserve independent status.)

Lymphokines are biologically active soluble factors produced by white blood cells. Studied in depth only within the last 15 years, they are being implicated at virtually every stage in the complex series of events that make up the immune response. They now include about 100 different compounds. Cytokines, which have effects similar to lymphokines, include several compounds associated with the thymus gland, referred to as thymic hormones.[4]

In 1979, the BRM subcommittee concluded that several of these agents probably have great potential for cancer treatment. Nevertheless, adequate quantities for laboratory and clinical testing of many of them will probably not be available until the problems of producing glycoproteins by molecular cloning are overcome. No system is currently available for the industrial production of glycoproteins, although yeasts may prove to be the most useful micro-organisms.

ANTIBODIES

Antibodies are the best known and most exploited protein components of the immune system. Until recently, all antibodies were obtained from the blood of humans or animals; and they were often impure. Within the past 5 years, however, it has become possible to produce antibodies from cells in culture, and to achieve levels of purity previously unattainable. As with previous advances in antibody technology, researchers are examining ways to put this new level of purity to use. There have been hundreds, if not thousands, of examples of new diagnostic and research methods, new methods of purification, and new therapies published within the first 3 years that the technique has been available. (See Tech, Note **11, p. 81.)**

This high level of purity was attained by the development of monoclinal antibodies. These antibodies that recognize only one kind of antigen were the unanticipated fruit of fundamental immunological research conducted by Drs. Caesar Milstein and Georges Kohler at the Medical Research Council in England in 1975. They fused two types of cells–myeloma and plasma-spleen cells—to form hybridomas that produce the monoclinal antibodies. (See Tech. Note 12, p. 81.) Not only are the antibodies specific, but because the hybridomas can be grown in mass culture, a virtually limitless supply is available.

The most immediate medical application for monoclinal antibodies lies in diagnostic testing.

[4]**For *40* of the best characterized** footnote] **see footnote 1, p. 69.**

Over the past 20 years, large segments of the diagnostic and clinical laboratory industries have sprung up to detect and quantify particular substances in specimens. Because monoclonal antibodies are so specific, hybridomas seem certain to replace animals as the source of antibodies for virtually all diagnosis and monitoring. Their use will not only improve the accuracy of tests and decrease development costs, but should result in a more uniform product.

Today, such assays are used to:

determine hormone levels in order to assess the proper functioning of an endocrine gland or the inappropriate production of a hormone by a tumor;
detect certain proteins, the presence of which has been found to correlate with a tumor or with a specific prenatal condition;
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
identify microbial pathogens.

The extent of the use of antibodies and the biochemical properties that they can identify is suggested by table 6. No one assay constitutes a major market, and short product lifetime has been characteristic of this business.

Other applications of monoclinal antibodies include:

the improvement of the acceptance of kidney (and other organ) transplants by injection of the recipient with antibodies against certain antigens;
passive immunization against an antigen involved in reproduction, as a reversible immunological approach to contraception.
localizing tumors with tumor-specific antibodies (see Tech. Note 13, p. 81); and
targeting cancer cells with antibodies that have anticancer chemicals attached to them.

Enzymes and other proteins

ENZYMES

Enzymes are involved in virtually every biological process and are well-understood. Nevertheless, despite their potency, versatility, and diversity, they play a small role in the practice of medicine today. Therapeutic enzymes accounted for American sales of about $70 million (wholesale) in 1978, but one-half of those sales involved the blood-plasma-derived coagulation factors used to treat hemophilia. Although the figure is difficult to estimate, the total number of patients receiving any type of enzyme therapy in 1980 probably does not exceed 50,000.

Enzymes cannot be synthesized by conventional chemistry. Almost all those presently employed in medicine are extracted from human blood, urine, or organs, or are produced by micro-organisms. Already the possibility of using rDNA clones as the source of enzymes—primarily to reduce the cost of production—is being explored.

However, problems associated with the use of nonhuman enzymes (such as immune and febrile responses) and the scarcity of human enzymes, have hindered research, development, and clinical exploitation of enzymes for therapeutic purposes. Today, the experimental genetic technologies of rDNA and somatic cell fusion and culture open the only conceivable routes to relatively inexpensive production of compatible human enzymes.

The genetic engineering of enzymes is probably the best example of a dilemma that hampers the exploitation of rDNA: Without a clinical need large enough to justify the investment, there is no incentive to produce a product; yet without adequate supplies, the therapeutic possibilities cannot be investigated. The substances that break this cycle will probably be those that are already produced in quantity from natural tissue.

The only enzymes administered today are given to hemophiliacs–and they are actually

Table 6-Immunoassays .

- Analgesics and narcotics
  - Antipyrine
  - Codeine
  - Etorphine
  - Fentanyl
  - Meperidine
  - Methadone
  - Morphine
  - Pentazocine
- Antibiotics
  - Amikacin
  - Chloramphenicol
  - Clindamycin
  - Gentamicin
  - Isoniazid
  - Penicillin
  - Sisomycin
  - Tobramycin
- Anticonvulsants
  - Clonazepam
  - Phenytoin
  - Primidone
- Anti-inflammatory agents
  - Colchicine
  - Indomethacin
  - Phenyibutazone
- Antineoplastic agents
  - Adriamycin
  - Bleomycin
  - Daunomycin
  - Methotrexate
- Bronchodilators
  - Theophylline
- Cardiovascular drugs
  - Cardiac glycosides
    - Acetylstrophanthidin
    - Digitoxin
    - Digoxin
    - Gitoxin
    - Methyl digoxin
    - Ouabain
    - Proscillaridin
  - Dihydroergotamine
  - Propranolol
  - Quinidine
- CNS stimulants
  - Amphetamine
  - Benzoyl ecgonine (cocaine metabolize)
  - Methamphetamine
  - Pimozide
- Diuretics
  - Bumetanide
- Hallucinogenic drugs
  - Bile acid conjugates
    - Cholylglycine
    - Cholyltaurine
  - Catecholamines
    - Epinephrine
    - Norepinephrine
    - Tyramine
  - Fibrinopeptides
    - Fibrinopeptide A
    - Fibrinopeptide B
  - Indolealkylamines
    - Melatonin
    - Serotonin
  - Insect hormones
    - Ecdysone
  - Nucleosides and nucleotides
  - Cyclic AMP
  - Cyclic GMP
  - N^2-Dimethylguanosine
  - 7-Methylguanosine
  - Pseudouridine
  - Thymidine
  - Glutethimide
  - Methaqualone
- Hallucinogenic drugs
  - Mescaline
  - Tetrahydrocannabi nol
- Hypoglycemic agents
  - Butylbiguanide
  - Glibenclamid
- Insecticides
  - Aldrin
  - DDT
  - Dieldrin
- Malathion
  - Narcotic antagonists
  - Cyclazocine
  - Naloxone
- Peptide hormones
  - Angiotensin
  - Anterior pituitary
  - Bradykinin
  - Gastric
  - Hypothalamic
  - Intestinal
  - Pancreatic
  - Parathyroid
  - Posterior pituitary
  - Thyroid (calcitonin)
- Plant hormones
  - Indole-3-acetic acid
  - Gibberelilic acid
- Polyamides
  - Spermine
- Prostaglandins
- Sedatives and tranquilizers
  - Barbiturates
    - Barbital
    - Pentobarbital
    - Phenobarbital
  - Chlordiazepoxide
  - Chlorpromazine
  - Desmethylimipramine
  - Diazepam and N-desmethyldiazepam
- Steroid hormones
- Skeletal muscle relaxants e
- Synthetic peptides
  - DDAVP
- Synthetic steroids
  - Anabolic steroids
    - Trienbolone acetate
  - Androgens
    - Fluoxymesterone
  - Estrogens
    - Diethylstilbestrol
    - Ethinylestradiol
    - Mestranol
  - Glucocorticoids
    - Dexamethasone
    - Methyl prednisolone
    - Prednisolone
    - Prednisone
  - Metyrapone
  - Progestins
    - Medroxyprogesterone acetate
    - Norethindrone
    - Norethisterone
    - Norgestrel
- Toxins
  - Aflatoxin B,
  - Genistein
  - Nicotine and metabolizes A
  - Paralytic shellfish poison
- Thyroid hormones
  - Thyroxine
  - Triodothyronine
- Vitamins
  - Vitamin B_{12}
  - Vitamin D

SOURCE: f Drugs—Compr of Drugs-Comprehensive Immunology," oloav. Hidden Caffey (e l.) (New 'ork: Pl (cd.) ר Pre is. 19 Plenum Press, 1977), p. 325.

proenzymes, which are converted to active enzymes in the body when needed. The most common agents are called Factor VIII and Factor IX, which are found in serum albumin and are currently extracted from human blood plasma. Hemophilia A and Hemophilia B—accounting for over 90 percent of all major bleeding disorders—are characterized by a deficiency of these factors. Supplies of the proenzymes will exceed demand well beyond 1980 if the harvesting and processing of plasma continues as it has. Nevertheless, the risk of hepatitis associated with the use of human plasma-derived products is extremely high. One recent study found chronic hepatitis in a significant percentage of asymptomatic patients treated with Factor VIII and Factor IX.

The plasma fractionation industry, which produces the proenzymes, is currently faced with excess capacity, intense competition, high plasma costs, and tight profit margins.[5] The cost and availability of any one plasma protein is

[5]For details of the factors governing the industry. see footnote 1, p. 69.

coupled to the production of the others. Hence, the industry would still have to orchestrate the production of the other proteins even if just one of them, such as Factor VIII, becomes a target for biological production.

Another enzyme, urokinase, has been targeted for use in removing unwanted blood clots, which lead to strokes, myocardial infarctions, and pulmonary emboli. Currently, the drug is either isolated from urine or produced in tissue culture. (See Tech. Note 14, p. 81.)

Urokinase is thus far the only commercial therapeutic product derived from mammalian cell culture. Nevertheless, some calculations suggest that production by E. ***coli*** fermentation would have economic advantages. The costs implicit in having to grow cells for 30 days on fetal calf serum (or its equivalent) or in having to collect and fractionate urine–as reflected in urokinase's market price ($150/mg at the manufacturer's level) —should be enough incentive to encourage research into its production. In fact, in April 1980, Abbott Laboratories disclosed that ***E. coli*** had been induced to produce urokinase through plasmid-borne DNA.

The ***availability*** of urokinase might be guaranteed by the new genetic technologies, but its ***use is*** not. For a variety of reasons, the American medical community has not accepted the drug as readily as have the European and Japanese communities. Studies to establish the use of urokinase for deep vein thrombosis, for example, are now being conducted almost exclusively in Europe.[6]

OTHER PROTEINS

In addition to the proteins and polypeptides already mentioned, the structural proteins, such as the collagens (the most abundant proteins in the body), elastins and keratins (the compounds of extracellular structures like hair and connective tissue), albumins, globulins, and a wide variety of others, may also be susceptible to genetic engineering. Structural proteins are less likely to be suitable for molecular genetic manipulations: On the one hand, their size and complexity exceed the synthetic and analytic capabilities that will be available in the next few years; on the other, either their use in medicine has yet to be established or material derived from animals appears adequate, as is the case with collagen, for which uses are emerging.

Plasma, the fluid portion of the blood, contains about 10 percent solids, most of which are proteins. During World War II, a simple procedure was developed to separate the various components. It is still used today.

Serum albumin is the smallest of the main plasma proteins but it constitutes about half of plasma's total mass. Its major therapeutic use is to reverse the effects of shock. p It is a reasonable candidate for molecular cloning, although its relatively high molecular weight complicates purification, and its commercial value is relatively low. The market value of normal serum albumin is approximately $3/g, but the volume is such that domestic sales exceed $150 million. Including exports, annual production is in the range of 100,000 kg.

Normal serum albumin for treating shock is already regarded as too expensive compared with alternative treatments, to expand its use would require a lower price. On the other hand, the Federal Government—and especially the Department of Defense—might disregard the immediate economic prospects and conclude that having a source of human serum albumin that does not depend on payments to blood donors might be in the national interest. Since many nations import serum albumin, products derived from molecular cloning could be exported.

Serum albumin is presently the principal product of blood plasma fractionation, a change in the way it is manufactured would significantly affect that industry. Because a number of other products (such as clotting factors) are also derived from fractionation, a growth in the need for plasma-derived albumin could have a significant impact on the availability and the cost of these byproducts.

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additional information about howon about I came to play a role in therapy, see footnote 1, **p. 69.**

[7]**For a detailed discussion of the costs and benefits of using albumin and the structure of the industry, see footnote** 1, **p. 69,**

Antibiotics

Antimicrobial agents for the treatment of infectious diseases have been the largest selling prescription pharmaceuticals in the world for the past three decades. Most of these agents are antibiotics—antimicrobials naturally produced by micro-organisms rather than by chemical synthesis or by isolation from higher organisms. However, one major antibiotic, chloramphenicol—originally produced by a micro-organism, is now synthesized by chemical methods. The field of antibiotics, in fact, provides most of the precedent for employing microbial fermentation to produce useful medical substances. The United States has been prominent in their development, production, and marketing, with the result that American companies account for about half of the roughly $5 billion worth of antimicrobial agents sold worldwide each year. The American market share has been growing as new antibiotics are developed and introduced every year.

For 30 years, high-yielding, antibiotic-producing micro-organisms have been identified by selection from among mutant strains. 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, 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 protoplasm 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 recombinant 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 have compared the quality of an antibiotic-producing fungus, Cephalosporimn acremonium, produced by mating to one produced by protoplasm fusion. (See Tech. Note 15, p. 82.) They concluded that protoplasm fusion was far superior for that purpose. What is more, protoplasm 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, rather than expensive methionine, as a source of sulfur. 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 organism that had proved resistant to conventional crossing.

Even more significant are the possibilities for preparation by protoplasm fusion between different species or genera of hybrid strains, which could have unique biosynthetic capacities. 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, (See Tech. Note 16,

p. 82.) The value of protoplasm fusion, therefore, lies in potentially broadening the gene pool.

Protoplasm fusion is genetic recombination on a large scale, Instead of one or a few genes being transferred across genus and species barriers, entire sets of genes can be moved. Success is not assured, however; a weakness today is the inherited instability of the "fused" clones. The preservation of traits and long-range stability has yet to be resolved. Furthermore, it seems that one of the most daunting problems is screening—determining what to look for and how to recognize it. (See Tech. Note **17, p.** 82.)

Recombinant DNA techniques are also being examined for their ability to improve strains, Many potentially useful antibiotics do not reach their commercial potential because the micro-organisms cannot be induced to produce sufficient quantities by traditional methods. The synthesis of certain antibiotics is controlled by plasmids, and it is believed that some plasmids may nonspecifically enhance antibiotic production and excretion.

It may also be possible to transfer as a group, all the genes needed to produce an antibiotic into a new host. However, increasing the number of copies of critical genes by phage or plasmid transfer has yet to be achieved in antibiotic-producing organisms because little is known of the potential vectors. The genetic systems of commercial strains will have to be understood before the newer genetic engineering approaches can be used. Genetic maps have been published for only 3 of the 24 or more industrially useful bacteria.

Since ***2,000*** of the 2,400 known antibiotics are produced by ***Streptomyces,*** that is the genus of greatest interest to the pharmaceutical industry. Probably every company conducting research on ***Streptomyces*** is developing vectors, but little of the industrial work has been revealed to date.

Nonprotein pharmaceuticals

In both sales and quantity, over 80 percent of the pharmaceuticals produced today are not made of protein. Instead, they consist of a variety of organic chemical entities. These drugs, except for antibiotics, are either extracted from some natural plant or animal source or are synthesized chemically.

Some of the raw materials for pharmaceuticals are also obtained from plants; micro-organisms are then used to convert the material to useful drugs in one or two enzymatic steps. Such conversions are common for steroid hormones.

In 1949, when cortisone was found to be a useful agent in the treatment of arthritis, the demand for the drug could not be met since no practical method for large-scale production existed. The chemical synthesis was complicated and very expensive. In the early and mid-1950's, many investigators reported the microbial transformation of several intermediates to compounds that corresponded to the chemical synthetic scheme. By saving many chemical steps and achieving higher yields, manufacturers managed to reduce the price of steroids to a level where they were a marketable commodity. A conversion of progesterone, for example, dropped the price of cortisone from $200 to *$6/g in 1949. Through* further improvements, the price dropped to less than $1/g. The 1980 price is $0.46/g.

Developments based on genetic techniques to increase the production and secretion of key enzymes could substantially improve the economics of some presently inefficient processes. Currently, assessments are being carried out by various companies to determine which of the many nonprotein pharmaceuticals can be manufactured more readily or more economically by biological means.

Approximately 90 percent of the pharmaceuticals used in the treatment of hypertension are obtained from plants, as well as are miscellaneous cardiovascular drugs. Morphine and important vasodilators are obtained from the opium poppy, ***Papaver somniferum.*** All these chemical substances are produced by a series of enzymes that are coded by corresponding genes in the whole plant. The long-term possibility (over 10 years) of using fermentation methods will depend on identifying the important genes.

The genes that are transferred from plant to bacteria must obviously be determined on a case-by-case basis. The case study on acetaminophen (the active ingredient in analgesics such as Tylenol) demonstrates the steps in such a feasibility study. (See app. I-A.)

The first step in such a study is to determine whether and where enzymes exist to carry out the necessary transformation for a given product. Acetaminophen for instance, can be made from aniline, a relatively inexpensive starting material. The two necessary enzymes can be found in several fungi. Either the enzymes can be isolated and used directly in a two-step conversion or the genes for both enzymes can be transferred into an organism that can carry out the entire conversion by itself.

Given the cost assumptions outlined in the case study and the assumptions on the efficiency of converting aniline to acetaminophen, the cost of producing the drug by fermentation could be 20 percent lower than production by chemical synthesis.

Impacts

Genetic technologies can help provide a variety of pharmaceutical products, many of which have been identified in this report. But the technologies cannot guarantee how a product will be used or even whether it will be used at all. The pharmaceuticals discussed have illustrated the kinds of major economic, technical, social, and legal constraints that will play a role in the application of genetic technologies.

Clearly, the major direct impacts of genetic technologies will be felt primarily through the type of products they bring to market. Nevertheless, each new pharmaceutical will offer its own spectrum and magnitude of impacts. Technically, genetic engineering may lead to the production of growth hormone and interferon with equal likelihood; but if the patient population is a thousandfold higher for interferon, and if its therapeutic effect is to alleviate pain and lower the cancer mortality rate, its impact will be significantly greater.

Many hormones and human proteins cannot be extensively studied because they are still either unavailable or too expensive. Until the physiological properties of a hormone are understood, its therapeutic values remain **unknown. Recombinant DNA techniques are being used to overcome this circular problem. In one laboratory, somatostatin is being used as a research tool to study the regulation of the hormonal milieu of burn patients. A single experiment may use as much as 25 mg of the hormone, which, as a product of solid state chemical synthesis, costs as much as $l2,000. Reducing its cost would allow for more extensive research on its physiological and therapeutic qualities.**

By making a pharmaceutical available, genetic engineering can have two types of impacts. First, pharmaceuticals that already have medical promise will be available for testing. For example, interferon can be tested for its efficacy in cancer and viral therapy, and human growth hormone can be evaluated for its ability to heal wounds. For these medical conditions, the indirect, societal impact of applied genetics could be widespread.

Second, other pharmacologically active substances that have no present use will be available in sufficient quantities and at a low enough cost to enable researchers to explore their possibilities, thus creating the potential for totally new therapies. Genetic technologies can make available for example, cell regulatory proteins, a class of molecules that control gene activity and that is found in only minute quantities in the body. The cytokines and lymphokines typify the countless rare molecules involved in regulation, communication, and defense of the body to maintain health. Now, for the first time, genetic technologies make it possible to recognize, isolate, characterize, and produce these proteins.

The potential importance of this class of pharmaceuticals—the new cell regulatory mole-

cules—is underscored by the fact that half of the 22 active INDs for new molecular entities that have been rated by FDA as promising important therapeutic gains are in the Metabolic and Endocrine Division, which oversees such drugs. It is reasonable to anticipate that they will be employed to treat cancer, to prevent or combat infections, to facilitate transplantation of organs and skin, and to treat allergies and other diseases in which the immune system has turned against the organism to which it belongs. (See table 7.)

At the very least, even if immediate medical uses cannot be found for any of these compounds, their indirect impact on medical research is assured. For the first time, almost any biological phenomenon of medical interest can be explored at the ***cellular level*** by the application of available scientific tools. These new molecules are valuable tools for dissecting the structure and function of the cell. The knowledge gained may lead to the development of new therapies or preventive measures for diseases.

The increased availability of new vaccines might also have serious consequences. But the extent to which molecular cloning will provide useful vaccines for intractable diseases is still unknown. For some widespread diseases, such as amebic dysentery, not enough is known about the interaction between the micro-organism and the patient to help researchers design a rational plan of attack. For others, such as trachoma, malaria, hepatitis, and influenza, there is only preliminary experimental evidence that a useful vaccine could be produced. (See table 8.) To date, the vaccine that is most likely to have an immediate impact combats foot-and-mouth disease in veterinary medicine. There is little doubt however, that should any one of the vaccines for human diseases become available, the societal, economic, and political consequences of a decrease in morbidity and mortality would be significant. Many of these diseases are particularly prevalent in less-developed countries. The effects of developing vaccines

cable 7.–Diseases Amenable to Drugs Produced by Genetic Engineering in the Pharmaceutical Industry

Disease or condition	Drug potentially produced by genetically engineered organism
Diabetese	Insulin
Atherosclerosis	Platelet-derived growth factor
Virus diseases	Interferon
Influenza	
Hepatitis	
Polio	
Herpes	
Common cold	
Cancer	Interferon
	Hodgkin's disease
	Leukemia
	Breast cancer
Anovulation	Human Hu gonadotropin
Dwarfism [a]	Human growth hormone
Pain	and endorphins
Wounds and burns	Human growth hormone
Inflammation, rheumatic diseases[a]	Adrenocorticotrophic hormone
Bone disorders, e.g., Paget's disease[a]	Calcitonin and parathyroid hormone
Nerve damage	Nerve growth factor growt
Anemia, hemorrhage	morrhage
Hemophilia	Factor VIII and Factor IX
Blood clots[a]	a
Shock[a]	Serum albumin
Immune disorders	sorders

[a] currently treated by urrently treated

SOURCE: Office of Technology Assessment.

Table 8.-Major Diseases for Which Vaccines Need To Be Developed

Parasitic diseases
Hookworm
Trachoma
Malaria
Schistosomiasis (river blindness)
Sleeping sickness
viruses
Hepatitis
Influenza
Foot-and-mouth disease (for cloven-hoofed animals)
Newcastle disease virus (for poultry)
Herpes simplex
Mumps
Measles
Common cold rhinoviruses
Varicella-zoster (shingles)
Dysentery
Typhoid fever
Cholera
Traveller's diarrhea

SOURCE: Office of Technology Assessment.

for them will be felt on an international scale and will involve hundreds of millions of people.

The new technologies may also lower the risks of vaccine production. For example, the FMDV vaccine produced by Genentech is constructed out of 17 of the 20 genes in the entire virus—enough to confer resistance, but too few to develop into a viable organism.

The new technology may also supply pharmaceuticals with effects beyond therapy. At least two promise impacts with broad consequences: MSH/ACTH 4-10 can be expected to be used on a wide scale if it is shown to improve memory; and bombesin and cholecystokinin might expand the appetite suppression market. But neither of these compounds has yet been found to be useful. While genetic technologies may provide large supplies of the drugs, they do not guarantee their value.

Antibody-based diagnostic tests, developed through genetic engineering, may eventually include early warning signals for cancer; they should be able to recognize any one of the scores of cancers that cause about a half-million deaths per year in the United States. If antibodies prove successful as diagnostic screening agents to predict disease, large-scale screening of the population can occur, accelerating the trend toward preventive medicine in the United States.

In addition to drugs and diagnostic agents, proteins could be produced for laboratory use. Expensive, complex media such as fetal calf serum are presently required for growing most mammalian tissue cells. Genetic cloning could make it possible to synthesize vital constituents cheaply, and could markedly reduce the costs of cell culture for both research and production. Ironically, genetic cloning could make economically competitive the very technology that offers an alternative production method for many drugs: tissue culture.

Nevertheless, the mere availability of a pharmacologically active substance does not ensure its adoption in medical practice. Even if it is shown to have therapeutic usefulness, it may not succeed in the marketplace. Consumer resistance limits the use of some drugs. The American aversion to therapies that require frequent injection, for instance, is illustrated by the opinion of some that a drug like ACTH offers few, if any, advantages over steroids.

The use of ACTH is somewhat greater abroad than in the United States. This is due in part because physicians in other cultures make far less use of systemic steroids than their American counterparts, and in part because frequent injections are more acceptable hence more common. Sales of ACTH in Great Britain—with its much smaller population—equal American sales.

At present, the need for injection is a far more likely deterrent to the wider use of ACTH than the cost of the drug itself. Reports that it can be applied by nasal spray suggest that its use may grow. Implantable controlled-release dosages may also become available within the next 5 years. This dependence on appropriate drug delivery mechanisms may lead to another line of research–increased attempts to develop technologies for drug-delivery.

As new pharmaceuticals become available, disruption can be expected to occur in the supply of some old ones. Pharmaceuticals whose production is tied to the production of others might become increasingly expensive to produce. Clotting factors, for example, are extracted with other blood components from plasma. Nevertheless, producing any of the **14** currently approved blood plasma products by rDNA would reduce the incidence of hepatitis caused by contamination from natural blood sources.

Whether new pharmaceuticals are produced or new production methods for existing pharmaceuticals are devised, future ***sources*** for the drugs may change. Currently, the sources are diverse, including many different plants, numerous animal organs, various tissue culture cells, and a wide range of raw materials used for chemical synthesis. A massive shift to fermentation would narrow the selection. The impacts on present sources can only be judged on a case-by-case basis. The new sources—microorganisms and the materials that feed them—offer the guarantee that the raw materials won't dry up. If one disappears, another can be found.

Clearly, there is no simple formula to identify all the impacts of applied genetics on the pharmaceutical industry. Even projections of economic impacts must remain crude estimates. Nevertheless, the degree to which genetic engineering and fermentation technologies might potentially account for drug production in specific categories is projected in appendix I-B. Given the assumptions described, the immediate direct economic impact of using genetic manipulation in the industry, measured as sales, can be estimated in the billions of dollars, with the indirect impacts (sales for suppliers, savings due to decreased sick days, etc.) reaching several times that value.

Technical notes

1. Many hormones are simply chains of amino acids (poly - peptides); some are polypeptides that have been modified by the attachment of carbohydrates (glycopeptides). Hormones usually trigger events in cells remote from the cells that produced them. Some act over relatively short distances—between segments in the brain, or in glands closely linked to the brain, others act on distant sites in tissues throughout the body.
2. For peptides about 30 AA in length, the cost may appreach $1 per mg as the volume approaches the kilogram level–a level of demand rarely existing today but likely to be generated by work in progress. Today, the cost of the 32 AA polypeptide, calcitonin, which is synthesized chemically and marketed as a pharmaceutical product by Armour, is probably in the range of$20 per mg, since the wholesale price in vials containing approximately 0.15 mg is about $85/mg. (That price is an educated guess, since such costs are closely guarded secrets and since the price of a pharmaceutical includes so many variables that the cost of the agent itself is a small consideration.)
3. In addition to those helped by the National Pituitary Agency, another 100 to 400 patients are treated with hGH from comercial sources. The commercial price is approximately $15 per unit (roughly $30/mg). The production cost at the National Pituitary Agency is about $0.75/unit ($1.50/mg). The National Pituitary Agency produces 650,000 international units iona (about 325 g) of hGH, along with the thyroid-stimulating hormone, prolactin, and other hormones, from about 60,000 human pituitaries collected each year. That is enough hGH both for the current demand and for perhaps another 100 hypopituitary patients.
4. Workers at the Howard Hughes Medical Institute of the University of California, San Francisco, isolated messenger RNA from a human pituitary tumor and converted it into a DNA-sequence that could be put into *E. coli.* The sequence, however, was a mixture of hGH and non-hGH material. It has been reported that Eli Lilly & Co., which has provided some grant money to the Institute, has obtained a license to the patents relating to this work. Grants from the National Institutes of Health and the National Science Foundation were also acknowledged in the publication.

   At practically the same time, researchers at Genentech, in conjunction with their associates at City of Hope National Medical Center disclosed the production of an hGH analog. This was the first time that a human polypeptide was directly expressed in *E. coli* in functional form. The work was supported by Kabi Gen AB, and Kabi has the marketing rights.

   The level of hGH production reported in the scientific account of the Genentech work was on the same order as that reported for the insulin fragments—approximately 186,000 hGH molecules per cell—a level that might be competitive even before efforts are made to increase yield. Genentech stresses the point that design, rather than classical mutation and selection, is the logical way to improve the system, since the hormone's "blueprint" is incorporated in a plasmid that can be moved between strains of E. coli, between species, or even from simple bacteria into more complex organisms, such as yeast.
5. Since erythropoietin is a glycoprotein, it may not be feasible to synthesize the active hormone with presently available rDNA techniques.
6. Antigens are surface components of pathogenic organisms, toxins, or other proteins secreted by pathogenic micro-organisms. They are also the specific counterparts of antibodies: antibodies are formed by the body's immune system in response to their presence. Antibodies are synthesized by white blood cells and are created in such a way that they are uniquely structured to bind to specific antigens.
7. Many of the most devastating infectious diseases involve complex parasites that refuse to grow under laboratory conditions. The first cultivation of the most malignant of the species of protozoa that causes malaria, using human red blood cells, was described in 1976 by a Rockefeller University parasitologist, William Rager. Experimental immunogens were prepared and showed promise in monkeys, but concern about the existence of the red blood cell remnants—which could give rise to autoimmune reactions–curtailed the prospect for making practical vaccines by that route. Several biotechnology firms are currently trying to synthesize malaria antigens by molecular cloning. This effort may produce technical solutions to such scourges as

schistosomiasis (bilharzia), filariasis (onchocerciasis and elephantiasis), Ieshmaniasis, hookworm, amebic infections, and trypanosomiasis (sleeping sickness and Chagas disease).

8. Another potential use of antigens is suggested by the experimental treatment of stage I lung cancer patients with vaccines prepared from purified human lung cancer antigens, which appears to substantially prolong survival. And the Salk Institute is expanding clinical trials in which a procine myelin protein prepared by Eli Lilly & Co. is injected into multiple sclerosis patients to mop up the antimyelin antibodies that those patients are producing. Fifteen to forty-two g of myelin have been injected without adverse effects, suggesting a new therapeutic approach to auto-immune diseases. The protein appears to suppress the symptoms of experimental allergic encephalomyelitis, an animal disease resembling multiple sclerosis. Should this research succeed, the use of molecular clones to produce human protein antigens seems inevitable.
9. There are at least two distinct kinds of "classical" interferons—leukocyte interferon and fibroblast interferon, so-called for the types of cells from which they are obtained. A third kind, called lymphoblastoid because it is produced from cells derived from a Burkitt's lymphoma, appears to be a mixture of the other two interferon. All produce the antiviral state and are induced by viruses. A fourth kind, known as "immune" interferon, is produced by lymphocytes. Some evidence indicates that it may be a more potent antitumor agent than the classical types. Currently, interferon is obtained chiefly from white blood cells (leukocytes) from the blood bank in Helsinki that serves all of Finland, or from fibroblasts grown in cell culture.
10. Recently, G. D. Searle & Co. announced that new technology developed at its R&D facility in England has increased the yield of fibroblast interferon by a factor of 60. On the basis of this process, Searle expects to supply material for the first large-scale clinical trial of fibroblast interferon. Abbott Laboratories also recently announced plans to resume production of limited quantities of fibroblast interferon for clinical studies it plans to sponsor.

    Unlike leukocytes and specially treated fibroblasts, which can be used only once, lymphoblasts derived from the tumor Burkitt's lymphoma grow freely in suspension and produce the least costly interferon presently obtainable. However, they also produce a disadvantageous mixture of both leukocyte and fibroblast interferon. The Burroughs-Wellcome Co. produces lymphoblastoid interferon in 1,000-1 fermenters and has begun clinical trials in England, but the U.S. FDA has generally resisted efforts to make use of products derived from malignant cells. It is used extensively in research, and FDA is considering evidence from Burroughs-Wellcome that may lead to a relaxation of the prohibition, under pressure from the National Cancer Institute.
11. What may be a landmark patent has been issued to Hilary Koprowski and Carlos Croce of the Wistar Institute (for work done under the then Department of Health, Education, and Welfare funding) on the production of monoclinal antibodies against tumor cells. In a number of examples, these researchers demonstrated that an animal can be immunized with tumor cells, and that hybridomas derived from that animal will produce antibodies that demonstrate a specificity for the tumor.

    The final sentence of the patent text provides the rationale for the use of antibodies in both cancer and infectious disease therapies: "If the (tumor) antigen is present, the patient can be given an injection of an antibody as an aid to react with the antigen. " (U.S. 4,172,124.)
12. Myeloma cells grow vigorously in culture and have the unique characteristic of producing large quantities of antibodies. Each spleen cell of the immune type, on the other hand, produces an antibody that recognizes a single antigen, but these do not grow well in culture. When normal immune spleen cells are fused with myeloma cells, the resulting mixture of genetic capacities forms a cell, called a "hybridoma," which displays the desired characteristics of the parent cells: 1) it secretes the antibody specified by the genes of the spleen cell; and 2) it displays the vigorous growth, production, and longevity that is typical of the myeloma cell.
13. The use of high-correlation antibody assays in cancer studies has only just begun. Antibodies that have been treated so they can be seen with X-rays and that are specific for a tumor, can be used early to detect the occurrence or spread of tumor cells in the body. Because some 785,000 new cancer cases will be detected in 1980 with current diagnostic methods, because cancer will cause 405,000 deaths, and because early detection is the major key to improving survival, the implications are indeed enormous.
14. In the late 1950's, Lederle Laboratories marketed a preparation of 95-percent pure streptokinase (a bacterially produced enzyme that dissolves blood clots) for intravenous administration. They withdrew the product from the market around 1960 because it caused allergic reactions, which dampened clinical enthusiasm for its therapeutic potential.

    The presence in human urine of urokinase, an enzyme also capable of removing blood clots, was also discovered in the early 1950's. Urokinase was purified, crystallized, and brought into clinical use in the mid-1960's. From the beginning it was apparent that "an intense thrombolytic state could be achieved with a much milder coagulation defect than occurred with streptokinase; no pyrogenic or allergic reactions were noted, and no antibodies resulted from its administration . . . There did not appear to be as great variation in patient responsiveness." In 1967-68 and 1970-73, the National Heart and Lung Institute organized clinical trials that compared urokinase with streptokinase and heparin, an anticoagulant, in the treatment of pulmonary embolism. The trials indicated that streptokinase and urokinase were equivalent and superior to heparin over the short term, although their long-

term benefits were not established. Since then, clinical investigation of urokinase has been hampered by domestic regulatory problems, which have raised the cost of production and restricted its availability in the United States.

In January 1978, Abbott Laboratories obtained a new drug application for urokinase and introduced the product Abbokinase; by that time, however, the sales of urokinase in Japan were already pushing $90 million per year. Recently, Sterling Drug has begun marketing a urokinase product (Breokinase) manufactured by Green Cross of Japan: "According to Japanese reports, urokinase is the first Japanese-made drug formulation to receive production and sales approval from FDA. Green Cross estimates that within 3 years of the start of Sterling's marketing activities, the value of urokinase exports will reach Yen 500 million ($2.12 million) per month, and considers that its profits from exporting a finished product will probably be better than those from bulk drug sales or the licensing of technology. " The Green Cross product is made from human urine collected throughout Korea and Japan, and takes advantage of technology licensed from Sterling. Abbott's product, on the other hand, is derived from kidney-cell culture.

15. Intergeneric hybrids have extremely interesting possibilities. For example, it would be beneficial to cephalosporin-process technology to combine in one organism the acyltransferase from *Penicillium chrysogenum* and the enzymes of C. *acremonium,* which does not incorporate side chain precursors onto cephalosporin like *P. chrysogenum* does for penicillins.
16. Another example of recombination between species is that reported for two species of fungi, *Aspergillus* ;i, *As* lans and *A. rugulosus,* subsequent to protoplasm fusion.

    The only report of a successful cross between genera using protoplasm fusion technology has been between the yeasts *Candida tropicalis* and *Saccharomycopsis fibuligera,* which took place at low frequency and gave rise to types intermediate between the parents.
17. An example of screening is provided by the new B-lactam (penicillin-like) antibiotics. Using older screening methods, no new B-lactams were found from 1956 until 1972 when a new method was devised. A new series of these antibiotics was thus found. Within the past year, 6 new B-lactams have been commercialized and at least 12 more are in clinical trials around the world. The sales forecasts for these new agents are estimated to be over $1 billion.