Genetic Engineering and Biotechnology Yearbook 1983

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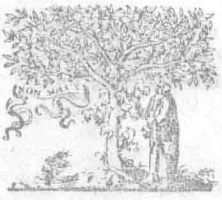

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REVIEW OF THE YEAR 1981-82

"Biotechnology" appears to have become a "buzz" word in commercial circles and is often interchanged with the terms applied genetics, bioengineering and genetic engineering. Biotechnology is the all-encompassing term referring to the derivation of chemicals or materials from natural sources and the purification, modification and methodology required for such. By this definition, biotechnology is indeed a very old science, utilized since the beginning of recorded history for producing or fermenting dyes, alcohol, etc. Likewise, applied genetics is a very old science whence mankind has been able to bring about the purposeful manipulation of heredity information in plants and animals. An understanding of the biology, or more specifically the molecular processes underlying genetics, has been realized only in the past few decades. Indeed, the ability of man to manipulate genetic information to produce required entities (genetic engineering) is very recent and represents a massive triumph of basic research in molecular biology carried out over the last three or four decades.

Other forms of biotechnology are associated with various forms of devices and engineering including medical diagnosis, fermentation processing, scale-up equipment and immobilization processes, in which the reactions produce or involve cells, bacteria and/or biological macromolecules.

This yearbook is devoted to a review of recent developments in genetic engineering, the "new bioscience" particularly relating to commercial aspects, and the many companies that are now engaged in converting the science of genetic engineering into the commerce of genetic engineering.

This book is presented in two parts. In the first part are presented significant developments during the 1981-82 year in the various fields relating to genetic engineering. These are divided into biomedical areas including pharmaceuticals, diagnostics, vaccines; agricultural progress; industrial applications, and a survey of the industrial/academic interface. This last area is included because (a) most of the major developments in genetic engineering have occurred in academia; (b) most of the fledgling biotech companies involve academic entrepreneurs and, (c) recognizing the previous facts, industry has poured massive sums into academic research in genetic engineering. In fact, during the 1981-82 academic year the N.I.H. (National Institutes of Health) in the U.S. invested approximately $100 million in genetic engineering research in universities in the medical area. The National Science Foundation (N.S.F.) placed approximately $20 million and lesser amounts were invested by the Department of Agriculture and the Department of Energy. By comparison, direct investment by industry in academia in the same period in the U.S. was

in excess of $300 million (although many of these gifts, grants or contracts were for multi-year periods). Nevertheless, a pattern appears to be emerging in which much of the basic research and development is performed in academia, being sponsored by major industry. Novel and useful innovations are then developed for production, by contrast, by small genetic engineering companies; and finally, the scaled-up product is to be marketed by the major sponsoring company. Although various methods of interplay exist between these entities, technology transfer appears to be working more actively than at any previous time. To some extent, this change has been brought about by mutual need: the large companies need the innovative capacity of universities and small biotech companies, and they, in turn, need the capital investment of the large chemical companies.

In the second part of this book is a compendium of biotech companies. The information presented is a mixture of that provided by direct contact with the principals and that found in the biotech literature. Unfortunately, information sometimes appears in the press concerning a company and its technology before that company has secured financing and in some cases the company disappears from view. Thus, it is difficult to assess which companies are doing well, which are struggling and which never really got off the ground.

There have been conflicting data published during the year concerning the pending "shakeout in biotechnology." On the one hand, the popular press has promulgated the concept that second round financing would be difficult to find and therefore a significant number of the 100-200 new biotech companies would be likely to flounder. Whereas it is probably true that realism has dulled the enthusiasm of private investors and venture capitalists for investment, particularly in start-up biotech ventures, the corporate giants appear to be investing at an increasing pace. Indeed, the fact that major profits will not be made "next year" has led to a shakeout of investors. There seems to be no diminution of the belief that genetic engineering will be the technology of the 21st century, and many large corporations are positioning themselves to take advantage of those markets. To be sure, a number of small companies will succumb in the coming years, as has happened in the past with auto and semiconductor companies. But the very wide potential of genetic engineering suggests that it is unlikely that only very few companies will survive.

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Production of Pharmaceuticals

The most accessible pharmaceuticals to be produced by gene splicing are peptides. The obvious reason is that insertion of a gene in a suitable vector codes directly for peptides and proteins. The main problems to scale-up of peptides have been choice of suitable promoters and host systems where the peptide is expressed, preferably outside of the host cell, with no enzymic degradation. In addition, separation of product from toxic metabolites can prove expensive and thus processes optimizing detection and harvesting are preferred.

Peptides are made up of a linear chain of amino acids and the number of such entities involved in the peptide drastically affects the economics of producing peptides by genetic engineering versus the conventional synthetic method.

Although it is difficult to get accurate estimates on the cost of manufacturing peptides by conventional methods, Table 1 has been compiled based on some industry estimates.

Table 1 -- Cost of Synthetic Peptides

Peptide	No. of Units (amino acids)	Cost/g
Amino acid	1	5c
Aspartame	2	55c
Brodykinin	9	$ 1,600
Leutinizing Releasing Hormone (LRH)	10	$ 2,000
Beta-Endorphin	31	$20,000

It can be seen from the previous table that the cost of peptides produced by direct synthesis increases dramatically after 10 or so amino acids are joined together. Under most circumstances, it is perceived that only peptides larger than this are logical candidates for synthesis by gene splicing. Often, small peptides produced by microorganisms are destroyed by enzymolysis, thus providing an additional reason for concentration on larger entities.

However, there are a number of companies involved in the early stages of producing various amino acids (Bethesda Research Labs: proline; Biogen: aspartic acid; W.R. Grace/M.I.T.: various) and Cetus has a process for producing the dipeptide sweetener aspartame. It remains to be seen whether this material is competitive with the synthetic aspartame now licensed in more than ten countries by G.D. Searle & Co.

These peptides and others are listed in Table 2, along with properties and corporate interest.

Table 2 -- Who Is Doing What In the Peptide Market

Peptide	Number of Residues	Company Active
Aspartame	2	Cetus, Searle
Glucagon	29	Codon
Endorphin	31	Biotech Res Labs
Calcitonin	32	Codon
HGH		Genentech, Novo, CAMR
Insulin	51, 51	Connaught, Dista
Pro Insulin	86, 91	
Interferon	130	Flow Genl, Meloy Labs, Cetus, Gen Rep Tech, Interferon Sci, Biogen, Roche/ Genentech, Searle, ICI, Interferon Sci

The two products close to commercial use are human insulin (HI) and human growth hormone (HGH). Most diabetics are treated with bovine insulin, but a significant number suffer immune reaction because of subtle differences between bovine and human insulin. In such cases, bovine insulin is replaced by the more expensive porcine (pig) insulin, which differs by only one amino acid from HI. Although relatively few people suffer adverse reactions from pig insulin, it is thought that some of the long-term side effects from non-human insulin may be related to hardening of the arteries and premature aging. Thus, the drive to produce a cheap human insulin has attracted several companies. The two most common methods for producing human insulin have been the Genentech/Eli Lilly process, which involves the use of synthetic spliced genes in E. coli and the non-engineered process, which converts pig insulin to human by chemical means. This process is being used by Novo Industries, but may prove to be more expensive in the long run than the engineered product. Novo has an agreement with Biogen to produce a genetically engineered insulin. Lilly entered Phase II clinical testing of human insulin in November

1980, and Novo in June 1981. It is believed that a product will be marketed by Lilly shortly. Dista Products is setting up to produce several hundred kg/yr. for Lilly in the United Kingdom.

Meanwhile, Genentech is in the latter stages of bringing human growth hormone (HGH) into production scale and final approval for marketing is expected in 1982-83. Various animal growth hormones have been produced or are currently being developed by Upjohn, Genentech, Ingene, and others, and include bovine growth hormone (BGH), porcine growth hormine and avian (chicken, etc.). Although the animal growth hormones do not appear to increase the size or weight of animals, they do appear to affect (increase) milk production in cows and meat quality (beef). However, because of the need to clear such agents used on feed animals with the Federal regulatory agencies, it is unlikely that genetically engineered animal hormones will be in production/use in the near future.

Inteferon is perhaps the third in line for commercial production. There are, in fact, many different inteferons, including fibroblast, immune and leukocyte interferons (depending on their source). For leukocyte interferons, there are several different types and subclasses. Table 3 summarizes most of the known materials. Interferons are peptides (polypeptides) with antiviral activity and initially "interferon" was thought to be a prime anticancer agent through its influence on the immune system. There are reputed to be more than 30 companies working on one or more types of interferon, many of them small companies that may have considerable difficulties in meeting regulatory criteria.

Table 3 -- Natural and Recombinant Interferons

Type	Code	Size (Amino Acids)
Fibroblast	IFF	166
Immune	IFI	
Leucocyte	IFL	
	$\alpha 1$-$\alpha 2$	144-146 (approx.)
	$\beta 1$-$\beta 3$	143-187
	$\gamma 1$-$\gamma 4$	146-187
Recombinant Leucocyte	IFLrA	165
	IFLrD	166

Preliminary results for cancer treatment show mixed results, as might be expected. Somewhat better preliminary results have been obtained in treatment of viral disorders. Experiments by Schering/Biogen in the U.K. have indicated protection against the common cold by alpha-2 interferon. Other viral conditions against which some activity has been reported include warts and herpes simplex.

Diagnostics

It has been widely assumed that one of the first areas in which genetic engineering will make an impact is that of diagnostics. Much of this optimism has been based on the ability of biotechnologists to develop quantities of monoclonal antibodies which undergo specific reaction with viral or bacterial coat proteins, thus providing a means of upgrading current methodology which often uses expensive and impure antibodies.

The production of useful monoclonals generally involves the purification of a single protein moiety that is capable of producing a specific antibody with which it will bind. Not all viral or bacterial proteins act as antigens and therefore considerable screening may be necessary before an appropriate monoclonal is developed. Furthermore, viral and bacterial strains causing the same disease may be unreactive to a standard monoclonal (i.e., they are subject to antigenic drift) and thus it may be necessary to produce a library of monoclonals to address a single disease. For example, Genetic Systems Corporation currently has a panel of 13 monoclonal antibodies that react with gonorrhea bacteria but not with other related types of bacteria. A mixture of three of these monoclonals is said to react with more than 99% of the world's 750 or so different strains of the disease. Large numbers of disease-specific monoclonals are currently developed and many are licensed. Monoclonals have been produced against most of the peptide/protein entities involved in common diseases in man and animal, but at this stage it remains to be seen how quickly genetic drift will require the development of new agents.

A common method of applying monoclonals is in an ELISA (Enzyme Linked Immunosorbent Assay) kit. This technique involves the binding of virus specific antibodies to a solid support. Test samples of antigen or standard antigen are added and reacted with the bound antisera. Then, enzyme-conjugated antibody, complementary to the antigen, is determined by addition of a substrate that results in a colorimetric reaction. Monoclonal Antibody Inc. has announced such a kit for detection human chorionic gonadotropin (HCG), indicative of pregnancy, in human urine. Similar approaches have been used by Hybritech, Summa and Biogen.

The first F.D.A.-approved monoclonal "kit" appears to be that developed by Abbott Labs for use in monitoring human cancer. Another interesting development, announced in the past year, has been the development of diagnostic kits for serum immunoglobulin IgE involved in allergenic response. Hybritech is designer of such kits that will be distributed by Mitsubishi.

Competitive methodology in the diagnostic field is that which employs synthetic or native nucleic acid fragments that are capable of combining with bacterial or viral DNA/RNA to give some photometrically detectable result. The fragment may, for example, fluoresce or be

attached to a conjugated agent that changes color when the complementary nucleic acid strand is present. Thus, the detection of disease could involve a rapid "litmus"-like test that would be cheap, rapid, and, hopefully, accurate.

Cetus is said to have such a system for testing venereal disease, and considerable experimentation is underway in this area. Once again, genetic variation in the strains of bacteria and viruses involved, or genetic drift in which spontaneous mutations in the coding nucleic acids occur, can render the development of an accurate detection system more difficult than might initially be perceived.

One particularly interesting recent observation made by Genentech scientists is that certain fragments of viral proteins can be more antigenic than the whole protein. Whereas such an observation may gladden the hearts of patent attorneys and basic scientists, it certainly renders the commercialization of new agents more difficult because of the ease of developing competitive products.

Apart from the *in vitro* or laboratory use of monoclonals, there has been much interest in the potential of monoclonals for medicinal or pharmacologic use. In particular, it is argued that cancer cells produce specific and abnormal proteins that can act as antigens so that a "magic bullet' type of monoclonal may be produced that atacks oncogenic (cancer) cells. The direct treatment of tumors with monoclonals seems to have met with limited success so far, perhaps for two main reasons: First, the combination of antigens with antibodies on the surface of tumor cells does not necessarily lead to cell death (and phagocytosis) and, indeed, the antigens seem to be of wide spectrum with rapidly changing properties that are not readily addressed by a unique set of antibodies.

In vivo diagnostic use of monoclonals has concentrated on their use in defining the surface and extent of cancerous tumors. Thus, fluorescent or radioactive antibodies may be used to define accurately tumors for subsequent excision or for directing radiation to appropriate sites.

Perhaps most promising at this time in the field of *in vivo* use of monoclonals is their conjugation to anticancer drugs, such as methatrexate, adriamycin, etc. In this manner, the monoclonal acts as the "magic bullet" seeking the (tumor) target and the anticancer drug acts as the charge that dissipates the target.

Vaccines

Conventional vaccines are nonpathogenic suspensions of infectious agents or portions of these agents which are given for the purpose of establishing resistance to infections. Most commonly, the agent that confers resistance is proteinaceous and thus is a candidate for production by gene-splicing methodology. The proteinaceous agent (antigen) produces proteins (immunoglobulins/antibodies) that complex and inactivate the live invading agent.

Current methodology for producing vaccines involves the production of modified live organisms (polio, measles, rubella, etc.), killed whole organisms (influenza, polio), exoproduct vaccines (diphtheria toxoid, tetanus toxoid), split vaccines (influenza) and subunit vaccines (hepatitis B, etc.).

Some problems that may be encountered with the current methodology involve one or more of the following -- high cost, low potency, lot-to-lot variability, physical or immunological instability, adverse reactions, reversion to virulence and limited duration of protection.

Genetic engineering of vaccines has some advantages and disdadvantages. The advantages are that the production of a pure protein vaccine optimizes the efficiency with no danger of renewed virulence. In large scale production, genetically engineered vaccines should be cheap. However, research and development costs, scale-up and regulation costs are often such that replacement of even a relatively poor current vaccine may not be cost effective.

Several companies are known to be working on gene splicing for producing vaccines, and though no human vaccines are yet close to the marketplace, reports of progress with a flu vaccine antigen (G.D. Searle), hepatitis B (Biogen/Merck Sharp and Dohm, Enzo, Hiroshima University/Chemical-Sero, Therapeutic Research Institute, Kumamoto) and herpes simplex (MolGen, Lederle, UGEN) have been reported.

A large number of companies are working on animal vaccines, but in some cases the advantages of genetically engineered vaccines may be outweighed by the less critical requirements for pet animal uses. One company focusing on animal vaccines (Armos) filed for Chapter 11 protection during the year.

However, one of the main advantages of a genetically engineered vaccine is that it contains none of the nucleic acid content required to induce infection. Thus, such pathogenic viruses as those of foot and mouth disease cannot readily be used in "killed" form. Rather, one of the coat proteins (or a fragment thereof) may be used as an antigen to form a vaccine. The genetic engineering of such a protein or fragment is the basis of the Genentech approach. Production of the total protein antigen (VP-3 for foot and mouth disease) is not generally necessary since within the protein is a peptide of 15-30 amino acids known as an essential antigenic determinant. These peptides

can be produced by direct synthesis or by genetic engineering from synthetic or natural gene fragments. A nonapeptide (9 amino acids) has been developed by the University of California with the aid of Alpha Therapeutics for possible use as a vaccine for hepatitis B. Table 4 contains a list of the most commonly used conventional vaccines.

Table 4 -- Total Net Doses of Vaccines Distributed (U.S.A) x 1000

Biologic	1977	1978	1979	1980	1981
Influenza virus	26,949	20,411	18,171	12,391	19,829
Diphtheria toxoid	27,422	28,008	28,765	32,878	29,991
Tetanus toxoid	40,360	38,978	41,271	44,574	39,869
Pertussin	16,884	18,093	18,502	21,816	18,827
Poliomylelitis	23,212	24,579	24,579	23,775	22,806
Measles virus	10,676	8,931	8,520	7,958	6,625
Rubella virus	7,699	7,553	8,188	7,236	6,234
Mumps virus	4,093	4,649	5,285	5,208	4,781
Smallpox	4,493	4,284	3,611	2,829	2,422

Data from "Biologies Surveillance Report" CDC Report #83.

It seems unlikely that genetically engineered vaccines will enter this market. However, Harvard University has recently received NIH permission to begin work on genetically engineered diphtheria toxin. The National Research Council has recently completed a study of the new vaccines most needed in human and veterinary medicine and their accessibility by genetic engineering. Table 5 contains a list of this information.

The Center for Disease Control in Atlanta estimates that over $300 million worth of human vaccines were distributed last year and a Genentech representative puts the potential animal vaccine market at $1 billion. There are currently five major producers of conventional human vaccines in the United States, namely: Lederle, Merck, Connaught, Parke-Davis and Wyeth. Until recently, the veterinary vaccine business was divided between a few large firms (Norden, Tech America, Beecham, Fort Dodge, Pitman Moore) and scores of small firms. New entries include Schering-Plough, International Minerals and Chemical, and W.R. Grace. Johnson & Johnson and W.R. Grace appear to be relying mainly on university-based R&D to produce the initial vaccine products.

Table 5a -- National Research Council Vaccine Development Priorities for Human Disease

Disease	Pathogen	Distribution	Current Vaccine	R&D (Gen. Eng.)
1. Bacterial respiratory disease	Bacteria	Global	Fair	
2. Bacterial enteric diseases	Bacteria	Global	None	
3. Rabies	Viral	Global	Variable	Inst. Pasteur, Connaught
4. Malaria	Parasitic	Global	None	Inst. Merieux, Biogen, Cambridge Lab. Mol. Biol.
5. Chlamydial infections		Global	None	Gen Syst.
6. Dengue	Viral	Regional Epidemic	None	
7. Japanese encephalitis	Viral	Regional Epidemic	Poor	

Table 5b -- National Research Council Vaccine Development Priorities for Animal Diseases

Disease	Pathogen	Distribution	Current Vaccine	R&D (Gen. Eng.)
1. Tuberculosis	Bacterial	Global	Questionable	
2. Neonatal diarrhea	Viral	Global	Poor	Cetus
3. Bacterial respiratory disease	Bacterial	Global	Poor	
4. Hemoparasites	Parasite	Global	Poor	
5. Rabies	Viral	Global	Variable	Transgene/Wistar Inst., Connaught
6. African swine fever	Viral	Regional Epidemic	None	
7. Foot & mouth disease	Viral	Global	Medium	Genentech

Developments in Agriculture

Investor interest in genetic engineering of plants has been somewhat cyclic. Whereas the production of pharmaceuticals got off to a roaring start because of extensive accumulation of knowledge concerning the metabolic processes of E. coli and the like, there was no similar body of knowledge in the plant genetics field. Consequently, it was generally felt that at least ten years would be required before significant new properties could be encoded in plant DNA and thus there was a corresponding lack of investor interest.

The upswing in interest in agriculture genetics came in the past two years with the recognition that (a) the potential markets for engineered agriculture products was huge; (b) federal regulation and attendant costs were likely to be much less than for pharmaceuticals; and (c) methods of inserting genetic information were uncovered. The two main methods of attempting to change the genetic structure of plants have hinged around use of the pTi plasmid found in crown gall tumors induced by A. tumefaciens and the use of liposome/chloroplast transformations which attempt to insert DNA fragments directly into plant cells. Scientific news of this type, along with rumors of the successful breeding of pomato (potatoes below ground and tomatoes above), are the sunbean plant (sunflower/bean cross breed) caused a major influx of R&D funds into the area. Particularly notable was the influx of $100 million plus into Agrigenetics R&D offering in this area; some of these funds have been placed in university-based research programs.

Unfortunately, it is now becoming clear that certain major difficulties do indeed exist in inserting desirable coding traits into plant genes. Perhaps most serious are the aspects relating to the site of insertion and expression of any inserted gene. At present, there are no clearly understood promoter sequences in plant genes, and thus there is no definable site where new genes should be inserted. Even if such knowledge were available, the known insertion methods are incapable of positioning new genes at the desired site. Thus, one is left with a more or less random process in which a shotgun approach to screening, which is inefficient and time-consuming, must be used. There are also technical problems associated with identifying whether a gene has actually been inserted or not. Lack of expression of that gene in the modified plant is not necessarily an indication of failure to insert. At a more practical level, if a gene is inserted correctly and expresses, then a new functionability is expressed in that plant, which may detract from other functions (hardiness, reproduction, etc.).

Thus, the drive to insert genes that code for herbicide resistance, nutritional enhancement, natural pesticide production, etc., seem to be in low gear, while some fundamental biochemical questions are addressed. Several companies, including Cetus, have cut back on their agricultural R&D.

At the same time, if the term "agriculture" is used to cover development of new pesticides and phenomena external to the plant, then more aggressive progress may be expected. During the year, it was noted by the International Plant Research Institute (IPRI) and the University of California that a gene present in surface bacteria that codes for a protein involved in ice nucleation has been cloned. This protein is believed to be at the source of frost damage in a wide range of crops. However, knowing how frost damage occurs should not be equated with solving the problem.

Several interesting aspects of plant biochemistry have a feature in common with frost damage -- they are dependent upon surface adsorbed bacteria. Table 6 lists a few types of bacteria that are being examined in terms of genetic modification.

Table 6: Bacteria that Adsorb to Plant Surfaces

Bacterium	Function	Comments
Rhizobium	N_2 fixation	Found on legume roots.
A. vinelandii	N_2 fixation	Not normally adsorbed to plants.
K. pneumoniae	N_2 fixation	17 nif (nitrogen fixing) genes identified
P. putida	Fe sequester	Protects plants from certain fungi.
E. herbicola P. syringaei	Ice nucleation) Ice nucleation)	Ubiquitous surface bacteria

Rhizobium adheres only to legume roots such as soybean and integrate with the N_2 fixation process of the plant. Evidently, if rhizobium could be caused to adhere to corn or wheat, for example, it would provide a self-fertilizing system. Similarly, if other nitrogen-fixing bacteria could be caused to adhere to and interact with non-leguminous plants, drastic improvement in growth and other behavioral characteristics could be achieved. Much of the agricultural biotechnology research is aimed then at nitrogen-fixing bacteria, though no startling breakthroughs have been reported yet.

Perhaps the most interesting possibility for inserting useful genetic information into plants is the use of pollen vectors. This approach was announced almost simultaneously by researchers at the University of Illinois (Drs. DeWet and Harlan) and at Cornell University (Dr. Sanford). It is not known at this time how successful gene expression is using such a route, but the approach may be a considerable advance on the previous Ti plasmid method.

One of the most difficult factors to assess in agricultural genetics is where and when advances will happen and what their impact will be. It has already been noted that genetic engineering with plants is particularly complicated because of a lack of understanding of cell chromosome sequence structure and function, and indeed, a generally low level of understanding of all aspects of plant molecular biology. In this respect, the USDA carried out a survey of experts in maize agronomy and using a "Delphi technique" asked when they expected various techniques to impact on maize production and yield. The results are tabulated below:

Technique	Expected Introduction	Impact by Year 2000*
Photosynthetic enhancement	1995	44%
Cell tissue culture	1990	70%
Plant growth regulators	1994	89%
Nitrogen fixation	1996	66%
Genetic engineering (splicing)		80%

* Percentage of experts expecting the technique to be in use.

Although the use of long-range forcasting by the Delphi method may be questioned, it is noteworthy that most experts (at least in maize crops) expect the application of new biological methodology to be 10-25 years distant.

A new method of identifying crop disease was announced during 1982 and may lead to new tools for "plant doctors." Whereas it had proven difficult to obtain monoclonal antibodies to plant viruses, scientists at the American Type Culture Collection have announced monoclonals to prunus nectotic ring spot virus, apple mosaic virus, tobacco streak virus and alfalfa mosaic virus. Whether diagnostic kits for use by growers will be developed, remains to be seen.